Spring 3-21-2023

GABAergic interneurons and prenatal ethanol exposure: from development to aging

Adelaide R. Tousley
adelaide.r.tousley.gr@dartmouth.edu

GABAergic interneurons and prenatal ethanol exposure: from development to aging

A Thesis Submitted to the Faculty in partial fulfillment of the requirements for the degree

of

Doctor of Philosophy in Integrative Neuroscience at Dartmouth

by Adelaide R. Tousley

Guarini School of Graduate and Advanced Studies

Dartmouth College

Hanover, New Hampshire

March 2023

Examining Committee:

_____

(chair) Hermes Yeh, Ph.D.

_____

Kyle Smith, Ph.D.

_____

Wilder Doucette, M.D., Ph.D

_____

Brian Mathur, Ph.D.

_____

F. Jon Kull, Ph.D.

Dean of the Guarini School of Graduate and Advanced Studies

Abstract

Fetal Alcohol Spectrum Disorders are the most common non-genetic cause of neurodevelopmental disability worldwide. Individuals with Fetal Alcohol Spectrum Disorder experience clinical symptoms including differences in physical, cognitive and behavioral development beginning in early childhood, but continue to face challenges into adulthood. There is a critical need to examine the effects of prenatal ethanol exposure across early development, and to establish how the developmental effects of prenatal ethanol exposure may or may not progress in aging individuals. To contribute to these two areas, I asked how a binge-type prenatal ethanol exposure might affect: (1) early postnatal development of striatal neurons and, relate to the development of early motor behaviors over time, and (2) synaptic function in the medial prefrontal cortex, and affect the onset and severity of cognitive deficits in a transgenic mouse model of familial Alzheimer's disease. I used whole-cell patch clamp electrophysiology to assess the functional and synaptic maturation of two populations of striatal neurons: striatal GABAergic interneurons and spiny striatal projection neurons, and the excitatory-inhibitory balance in deep layer medial prefrontal cortex pyramidal neurons. I found that prenatal ethanol exposure altered the postnatal developmental trajectory of striatal neurons in a sex-dependent manner, that coincided with sex-differences in the development of early motor behaviors, and morphological differences in striatal projection neurons. I also determined that prenatal ethanol exposure resulted in an earlier onset of deficits in GABAergic synaptic activity in cortical pyramidal neurons, that was an associated with a decreased number of parvalbumin expressing GABAergic interneurons, and an increase in intraneuronal APP/β-amyloid. These findings highlight the dynamic effects of prenatal ethanol exposure on synaptic function and behavioral outcomes during early development, and the lasting effects of prenatal ethanol exposure on neural circuits, modifying the aging process.

Acknowledgments

I would like to thank my mentor, Dr. Hermes Yeh, for his patience, his unending willingness to give constructive feedback, and for taking so many small, but deliberate steps to help me gain confidence as a researcher. Also for always making sure the snack cabinet is well stocked. I couldn't have asked for a better mentor, I feel tremendously lucky to have completed my PhD work in the Yeh lab.

I would like to thank my committee members Drs. Kyle Smith, Wilder Doucette, and Brian Mathur for their guidance and support throughout this process.

I would like to thank Pam Yeh her tremendous hard work. I'm so grateful have gotten to share the last four years with you! Thank you for making the Yeh Lab a welcoming place, and for sharing your love of pets, dessert and science.

Thank you to Stephanie Lee and Laurie Delatour, for making my start in the lab so outstanding, I would never have patched a cell or measured an osmolarity if it weren't for you both! I'm tremendously grateful for the time you took to teach me, and for being the best lab mates I could have imagined.

I'd also like to thank the outstanding Dartmouth undergraduates I've had the chance to work with over the past four years, particularly acknowledging: Alyssa Fayerman, Giulia Barbella, Betul Koc, Ilana Deykin, Zoe Enright, Avery Schuldt, and Megan Ungerman for their outstanding commitment to their projects, hard work and willingness to learn I'm so grateful to have gotten the chance to know each of you!

I would also like to thank my past research mentors: Dr. Kim Cronise, Dr. Jie Liu, Dr. Marian DiFiglia, and Dr. Kimberly Kegel-Gleason for giving me the chance to make mistakes, inspiring me, and supporting me in pursuing a career in science.

Thank you to the administrative staff of the MSB department for answering every question even at inconvenient times, making the grant application process approachable, and working so hard to make sure all of our lives run smoothly.

I'd like to thank my mom Elizabeth, and brother Elias for helping me survive this adventure.

I acknowledge support by F30 AA029261, RO1 AA027754-02

Contents

List of Figures

CHAPTER 2

CHAPTER 1

Introduction

1.1 Outline of Thesis

Investigations of the causative mechanisms underlying neurodevelopmental disorders using rodent models frequently rely on data collected from a single developmental timepoint, cell population, or brain region to draw broad conclusions about the etiology of these disorders across development. A growing body of work suggests that both environmental and genetic exposures during embryonic development can result in structural and functional phenotypes that undergo dynamic changes from early childhood to adolescence and into adulthood.

One such example is found in recent work examining the etiology for fetal alcohol spectrum disorders (FASD), a series of clinical diagnoses that may occur following a prenatal exposure to ethanol. Longitudinal clinical imaging studies of individuals with FASD suggest that while differences in imaging findings and functional readouts are present in both early development and adolescence, the severity and or direction (increase or decrease in function) of these phenotypes may evolve during development (Jacobson et al., 2021). The relationship between these dynamic changes in the expression of phenotypes related to prenatal ethanol exposure and critical events during early neural development is not yet known and warrants further investigation.

In addition, increasing evidence from epidemiological studies suggests that early life exposures can modify the risk for and severity of neurodegenerative diseases in aging adults, and alternatively, that neurodevelopmental disorders can contribute to risk of later developing neurodegenerative diseases (Ballard et al., 2016; Becker et al., 2021; Lott and Head, 2019; Seifan et al., 2015; Vivanti et al., 2021; Yu et al., 2020). These

data highlight the critical need to investigate the impact of prenatal ethanol exposure across the lifetime, and led me to ask two overarching questions in my thesis research: **(1) how do early neural circuits respond to prenatal exposure to ethanol over the course of early development, and (2) can the effects of a prenatal exposure to ethanol modify the aging process?**

To begin answering these questions I made use of a mouse model of prenatal ethanol exposure, previously used in the lab to identify deficits in the developing medial prefrontal cortex (mPFC) and somatosensory cortex, and related changes in reversal learning and tactile sensitivity behaviors (Delatour et al., 2019b, 2019a; Skorput et al., 2019, 2015). We exposed pregnant dams to 5% (w/w) ethanol in a liquid diet on embryonic days (E) 13.5- 16.5 and made comparisons between their offspring, as well as the progeny of pregnant dams fed a control lab chow diet. We assessed the effects of this brief binge ethanol exposure in the striatum of developing mice, and in the mPFC of aging transgenic mice, modelling Alzheimer disease (AD). We concurrently assessed the behavioral effects of prenatal ethanol exposure on early motor behavior and spatial learning and memory in aging.

In my investigation of the functional changes associated with our prenatal ethanol exposure, my thesis work focused on the contributions of GABAergic interneurons (GINs), which play a critical role in developing circuits, contributing to a variety of developmental processes including: neuronal migration, synapse formation, morphological maturation and early network synchrony (Ben-Ari et al., 2012; Cellot and Cherubini, 2013; Sernagor et al., 2010). GINs have also previously been shown to contribute motor, and executive function behaviors, similar to those we assess in mice exposed prenatally to ethanol (Abbas et al., 2018; Cho et al., 2020; Gage et al., 2010; Goodwill et al., 2018; Gritton et al., 2019; Lee et al., 2017; Martiros et al., 2018; Owen et

al., 2018; Pinto and Dan, 2015; Roberts et al., 2019). Furthermore, past work from our lab and others suggests that GINs may be vulnerable to the effects of pre- and perinatal ethanol exposures (Bird et al., 2021, 2018; Cuzon et al., 2008; De Giorgio et al., 2012; Kenton et al., 2020; Lee et al., 2022; Léger et al., 2020; Marguet et al., 2020; Saito et al., 2019; Shenoda, 2017; Skorput et al., 2019, 2015; Smiley et al., 2015).

In the following sections, I will describe the clinical data characterizing alterations to motor behaviors and executive function that may result from prenatal ethanol exposure, how the two brain regions, namely the striatum and mPFC, that I investigate in this thesis are structured and contribute to animal behavior, as well as the developmental events occurring during the ethanol-exposure period and the weeks to months that follow, and will also provide evidence for the effects of acute and chronic exposures to ethanol subpopulations of neurons within each brain region, with a particular focus on GINs.

In Chapter 2, I explore the rapid changes in synaptic inputs, functional maturation, and morphology in two populations of striatal neurons: spiny striatal projection neurons (SPNs) and GINs, which occur during early postnatal development, and how they may be altered by and relate to differences early motor development following a brief binge prenatal ethanol exposure.

In Chapter 3, I set out to answer the question as to whether a brief exposure to ethanol prenatally could modify the risk and severity of memory deficits, changes in mPFC composition and/or synaptic function using a transgenic mouse model (3xTg-AD) of familial Alzheimer's disease (AD).

These studies will improve our understanding of the changes at the level of neural circuits that underlie behavioral differences in individuals with FASD and may contribute

to both efforts to identify individuals with FASD early, as well as those to ensure appropriate access to necessary resources for individuals with FASD throughout the aging process.

1.2 Diagnosis and Intervention in Fetal Alcohol Spectrum Disorder (FASD)

In the United States prenatal ethanol exposures are common. 1 in 9 pregnant women reports drinking in the last 30 days, and of those 1 in 3 reports binge drinking (Denny, 2019). Prenatal ethanol exposure may result in diagnosis with a Fetal Alcohol Spectrum Disorder (FASD), an umbrella term encompassing several clinical diagnoses: Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE), Alcohol-Related Neurodevelopmental Disorder (ARND), Fetal Alcohol Syndrome (FAS) and Partial Fetal Alcohol Syndrome (PFAS), and Alcohol-Related Birth Defects (ARBD), which vary based on the degree and range of physical, intellectual and behavioral clinical symptoms. FASD is the most common non-genetic cause of neurodevelopmental disability worldwide, estimated to affect 2-5% of school-aged children in the US (Lange et al., 2017; May et al., 2014). Individuals with FASD experience varied clinical symptoms, depending on the dose and timing of ethanol exposure, that make diagnosing FASD in early childhood challenging (Bandoli et al., 2019; Chasnoff et al., 2015; Garrison et al., 2019).

As a result, although FASDs result from an *in utero* exposure, diagnoses are often not made until after early childhood (Flannigan et al., 2019). Individuals with FASD are also frequently misdiagnosed (Chasnoff et al., 2015). Access to appropriate intervention is also constrained by limitations in healthcare providers' knowledge about FASD, a lack of consensus on appropriate diagnostic criteria for clinical diagnoses within the FASD spectrum, and the number of available diagnostic clinics, as well as by challenges in

qualifying for supportive services within healthcare systems (Elliott et al., 2006; Peadon et al., 2008; Petrenko et al., 2014).

Evidence-based interventions for FASD are still evolving. Currently, commonly used interventions include psychosocial approaches focused on early childhood intervention, supporting caregivers in managing challenging behaviors associated with FASD diagnosis, and improving access to supportive resources for school-aged children (Flannigan et al., 2020; Petrenko and Alto, 2017). Pre and perinatal nutritional supplementation to address nutritional deficits in pregnant women and young children with known prenatal ethanol exposures have also been demonstrated to mitigate the negative effects of ethanol-exposure in early childhood (Szewczyk et al., 2021; Wozniak et al., 2020, 2019; Young et al., 2014). However, success of these interventions is contingent on early identification of individuals with FASD and their subsequent access to intervention (May et al., 2014).

While psychosocial interventions are the first line approach to managing the behavioral differences associated with FASD, efforts to provide more targeted pharmacological approaches to manage specific behavioral symptoms in older children and adults where other therapeutic approaches prove insufficient are also ongoing. Current approaches to pharmacological management of FASD symptoms are informed both by past reports of the considerable negative consequences of overmedication on individuals with FASD and their caregivers, as well as by the therapeutic approaches employed in the management of other neurodevelopmental disorders (Ipsiroglu et al., 2015).

Recent advances include an effort to develop an appropriate treatment ‘algorithm’ for managing the behavioral symptoms associated with FASD (Mela et al., 2020). Researchers first identified four categories of behavioral symptoms: hyperarousal, hyperactivity, cognitive inflexibility and emotional dysregulation, and pharmacological

interventions to address each category of symptom, employing short-acting benzodiazepines acutely to facilitate sleep hygiene, stimulants such as methylphenidate in cases of hyperactivity, anticonvulsants that also act as mood stabilizers such as lamotrigine, and carbamazepine for emotional dysregulation (Durr et al., 2021; Mela et al., 2020). While developing more consistent approaches to pharmacological management of FASD will likely add to ongoing efforts to identify novel psychosocial interventions in improving the quality of life of individuals with FASD and their caregivers, it remains a challenging set of clinical diagnoses to manage. Improving our understanding of how prenatal ethanol exposure may alter neural development and contribute to early behavioral differences will advance our ability to identify and provide early interventions to individuals affected by FASD.

Alternatively, while the negative influence of prenatal ethanol exposure on quality of life in adulthood is well documented, how the physical, cognitive and behavioral challenges associated with a prenatal ethanol exposure may or may not progress in the aging process is poorly understood (Moore and Riley, 2015; Streissguth et al., 2004). Clinical management of individuals with FASD after young adulthood has not been well investigated (Moore and Riley, 2015), and also warrants further consideration.

1.3 Modelling of prenatal ethanol exposure: dose, timing and delivery method?

In our model of prenatal ethanol exposure, we attempted to simulate the type of ethanol exposure that may result in FASD diagnoses in the progeny. To this end we exposed pregnant dams to 5% (w/w) ethanol in a liquid diet on embryonic days (E)13.5 to 16.5. Delivery of ethanol in a liquid diet avoids the rapid rise in blood ethanol content (BEC), and maternal stress observed in models using oral gavage or IP injection (Patten et al., 2014). However, it allows for an increase in variability in the pattern/degree of ethanol exposure as access to the liquid diet is voluntary and varies between mice. Additionally,

Although our model reliably results in blood ethanol levels of ~80 mg/dL in mice on a C57/BL6 background, it resulted in considerably lower BEC in 3xTg-AD mice which were created using a B6129 background strain as discussed in Chapter 3 (Skorput et al., 2015). Although no animal model can completely and accurately recapitulate the effects of prenatal ethanol in humans, I will highlight how I have used this model system to ask questions about the effects of prenatal ethanol exposure on early development and aging in Chapters 2 and 3.

1.4 Motor dysfunction in individuals with and animal models of FASD

Individuals with FASD may have challenges with either, or both gross and fine motor tasks, and in tasks that require sensorimotor integration (Doney et al., 2014; Lucas et al., 2014; Williams et al., 2014). While poorer performance on clinical assessment of motor development is one of the earliest clinical signs of a FASD, expression of this phenotype varies between individuals such that assessments of motor performance are poorly sensitive to differences (Johnston et al., 2019; May et al., 2014). Though the contributions of early motor deficits in FASD to quality of life measures, or their relationship to later developmental outcomes has not been well investigated, clinical study of children with developmental delays and autism spectrum disorders suggests that deficits in early motor development may be predictive of altered development of more complex behaviors such as: language, later in childhood (Sack et al., 2021)

Past work from several groups using different ethanol exposure paradigms but similar methods of behavioral assessment as described in Chapter 2, have established that prenatal ethanol exposure can alter the maturation of early motor behaviors of neonatal rodents. For example, prenatal ethanol exposure (E14-18) delayed the onset of neonatal grasping behavior, though exposure at an earlier embryonic timepoint (E8-12) did not result in any obvious deficits (Fish et al., 1981). A single binge exposure to ethanol at E8

resulted in delayed onset of righting reflex and a loss of negative geotaxis behavior, while low or moderate dose exposures on E7 or E8 resulted in delays in developing quadruped walking, surface righting and cliff aversion behaviors (Molina et al., 1987; Schambra et al., 2015).

Motor deficits have also been identified in adolescent and adult mice following a prenatal ethanol exposure. Adolescent mice demonstrated deficits changes to: motor coordination as assessed by a decreased latency to fall on the rotarod task, and decreased ability to perform parallel bar, Suok and ledge tests, as well as in fine motor abilities based upon impaired acquisition of a skilled reaching (Abbott et al., 2016; Breit et al., 2022, p. 2; Cuzon et al., 2008; El Shawa et al., 2013; Hamilton et al., 2014; Heck et al., 2008; Mohammad et al., 2020; Tong et al., 2013). Adult animals also show differences in fine motor abilities, again measured by skilled reaching task, as well as in the formation of motor habits, and in the performance of orofacial movements (Cuzon Carlson et al., 2020; Hamilton et al., 2014; Heck et al., 2008). Although the identification of motor deficits in rodent models may depend on both the degree and timing of ethanol exposure, in addition to the behavioral task employed, this body of work strongly suggests that prenatal ethanol exposure may disrupt the development of motor systems (Boehm et al., 2008; Mantha et al., 2013; Wang et al., 2021).

In Chapter 2, I assess the early motor development of neonatal mice using a series of brief behavioral tasks comparable to those previously used to demonstrate motor deficits resulting from early gestational and late gestational binge ethanol exposures, allowing for comparison to behavioral outcomes to our brief mid-gestational (E13.5-16.5) binge ethanol exposure paradigm. In Chapter 3, I assess the distance travelled in 3xTG-AD mice exposed prenatally to ethanol, as they complete the modified Barnes Maze task,

assessing age-associated differences in motor behavior in mice predisposed to develop AD pathology.

1.5 Organization, function and development of the striatum

Striatal organization and function

The striatum, the input nucleus of the basal ganglia, plays a critical role in early motor behaviors, as well as motor learning and behavior in adulthood (Cataldi et al., 2021; Dehorter et al., 2011; Grillner and El Manira, 2020). Ninety-five percent of striatal neurons are SPN, which send projections to deeper basal ganglia nuclei via two pathways: direct, which forms synapses on the substantia nigra and generally increases movements via disinhibition of the thalamus, and indirect, which includes additional synapses on the globus pallidus externus and subthalamic nucleus, and generally functions to decrease movements by facilitating substantia nigra inhibition of the thalamus (Cui et al., 2013; Gerfen et al., 1990; Kemp et al., 1997a; Lee et al., 2016)

Direct and indirect pathway SPNs are classically identified by their differential expression of dopamine receptors. Direct pathway SPNs express D1 receptors, while indirect pathway SPNs express D2 receptors (Deng et al., 2006; Gerfen et al., 1990). However, SPNs have been demonstrated to frequently co-express both types of dopamine receptor early in development (Thibault et al., 2013). The two populations of SPNs also demonstrate differences in morphology and AP firing properties, with indirect pathway SPNs found to have more complex dendritic arbors, and increased excitability, beginning early in development (Cazorla et al., 2014; Gertler et al., 2008; Krajeski et al., 2019) These are important considerations in formulating the design of my experiments assessing the effects of prenatal ethanol exposure on the disposition of SPNs, especially at early postnatal ages.

SPNs are also organized into two functional compartments: the striosomal (or patch) and matrix compartments, defined embryonically, and into adulthood by: differential expression of neurochemical markers including opioid receptors, preference for dopaminergic inputs as well as differing functional roles (Brimblecombe and Cragg, 2017; Crittenden and Graybiel, 2011; Gerfen et al., 1987). SPNs in each compartment are not highly connected but receive inputs from the remaining five percent of striatal neurons, namely the GINs and cholinergic interneurons, which tend to reside on the borders between patch and striosomal compartments (Bernácer et al., 2012, 2007; Lopez-Huerta et al., 2016). Striatal GINs can be further divided into 5 major subtypes: fast-spiking (FS), parvalbumin (PV)-expressing GINs, low threshold-spiking (LTS), neuropeptide Y(NPY)/somatostatin (SST)-expressing GINs, calretinin (CR)-expressing GINs, and tyrosine hydroxylase (TH)-expressing GINs (Muñoz-Manchado et al., 2018; Tepper et al., 2018, 2010)

All striatal neurons receive extensive convergent excitatory input from the cortex, thalamus and limbic regions including hippocampus and amygdala, although different subtypes of neurons preferentially receive inputs from different regions, with effects mediated through differing glutamate receptors (Arias-García et al., 2018; Gittis et al., 2010; Johansson and Silberberg, 2020; Kemp et al., 1997b; Kress et al., 2013; Reiner et al., 2010; Wall et al., 2013). The striatum can also be considered as functional distinct subregions based on the distribution of excitatory afferents from distinct distant sites to the four striatal quadrants: dorsolateral, dorsomedial, ventrolateral, and ventromedial (Alloway et al., 2017; Tsutsui-Kimura et al., 2017). In addition to feedforward inhibition from GABAergic inputs from striatal GINs, also receive long-range inhibitory projections from deep cortical layers (Bertero et al., 2020; Gittis et al., 2010; Straub et al., 2016; Tepper et al., 2008)

Adding to the complexity of the striatal circuit, different subpopulations of striatal neurons preferentially connect to each. For example, indirect pathway SPNs form stronger connections to direct pathway SPNs, while direct pathway SPNs are under stronger inhibitory control from PV-expressing(+) GINs (Planert et al., 2010; Taverna et al., 2008) Feed forward inhibition from GABAergic interneurons to SPNs may result from either shunting or feed-forward mechanisms based the relative depolarization or hyperpolarization of recorded SPN (Monteiro et al., 2018; Tepper et al., 2008). PV+ neurons receive afferent inputs from both cholinergic interneurons and SST+ GINs, and also form autapses but do not themselves form afferent connections with SST+ GINs or (Straub et al., 2016; Wang et al., 2022)

Additionally, while acetylcholine tonically released from CINs directly modulates synaptic inputs and excitability of SPNs via muscarinic receptors acetylcholine (AChRs), it also acts to modify SPN function indirectly via binding to nicotinic receptors on the soma of striatal GINs, increasing the frequency of GABAergic synaptic inputs from PV+ GINs and TH+ GINs onto SPNs, as well as on dopaminergic afferents to facilitate dopamine release, and thus increase SPN excitability (Goldberg et al., 2012; Kocaturk et al., 2022; Matityahu et al., 2022). Striatal neurons also receive neuromodulatory inputs from dopaminergic, and serotonergic neurons that differentially modify the glutamatergic afferents to and excitability of the subtypes of striatal neurons (Bamford et al., 2018; Blomeley and Bracci, 2009; Clarke and Adermark, 2015; Pommer et al., 2021; Virk et al., 2016). The complicated interconnectedness between striatal neurons may contribute to the cell-type specific effects of prenatal ethanol exposure that I will describe in Chapter 2.

Striatal Development

SPNs are born in the lateral ganglionic eminence, a periventricular bulge comprised of neural plate-derived neural precursor cells between E11 and birth, with a small population of neurons born postnatally (Deacon et al., 1994; Sheth and Bhide, 1997). In contrast with MGE-derived GINs which form from intermediate progenitors, LGE-derived SPNs form directly from less differentiated radial glial cells (Pilz et al., 2013). While the earliest born neurons (born prior to E18) eventually form the patch or striosomal compartment of the striatum, later born neurons form the matrix compartment (Kelly et al., 2018; Matsushima and Graybiel, 2020; Newman et al., 2015; van der Kooy and Fishell, 1987). SPNs born in the LGE migrate radially, then tangentially to populate the striatum (Tinterri et al., 2018).

Early born striosomal SPNs project to substantia nigra (SN) beginning at E17, while later born matrix cells begin to make projections during the first postnatal week (Fishell and Kooy, 1987; van der Kooy and Fishell, 1987).Increasing the output of SPNs to relieve SN inhibition of the thalamus by increasing the activity of direct pathway SPNs or decreasing the activity of indirect pathway SPNs facilitates the formation glutamatergic afferents to the developing striatum during the first two postnatal weeks (Dehorter et al., 2011; Kozorovitskiy et al., 2012; Krajeski et al., 2019; Tepper et al., 1998). Dopaminergic inputs from substantia nigra and ventral tegmental area reach the striatum during the first two postnatal weeks and facilitate glutamatergic synaptic activity during early development (Antonopoulos et al., 2002).

MGE-derived GABAergic interneurons migrate to both the developing cortex and striatum, and eventually give rise to three subpopulations of interneurons: PV+ GINs, SST+ GINs, and cholinergic interneurons (Xu et al., 2008). The different subpopulations of interneurons preferentially populate different regions of the striatum with PV+ GINs

and cholinergic interneurons populating the lateral and SST+ GINs observed at a higher density in the medial striatum (Fino et al., 2018; Kawaguchi, 1993). While early work suggests that each interneuron population from MGE-derived neurons is born during the same time period (E12-18), striatal interneurons migrate along a medial to lateral path such that the earliest born interneurons eventually populate the lateral striatum, while later born neurons are found medially in the adult striatum (Marin et al., 2000; Sadikot and Sasseville, 1997; Semba et al., 1988).

Excitatory inputs to the striatum are first apparent during embryonic development. In rodents, after the thalamus forms from cells born E10-E16.5, thalamic afferents extend grown into the prethalamus, including both MGE and LGE (E11-13), and begin to target the developing striatum (E13-16) guided by cues expressed by radially and tangentially migrating neurons (Angevine, 1970; López-Bendito et al., 2006; Métin and Godement, 1996; Molnár et al., 1998). While thalamic inputs have been demonstrated to be critical to the appropriate functional organization and development of the cortex, how they contribute to early striatal development is not yet known (Antón-Bolaños et al., 2019). In contrast with the early embryonic onset of thalamostriate projections, anterograde tracing studies and immunohistochemical studies suggest that corticostriatal afferents first reach the developing striatum E18 and steadily increase during the first postnatal month (Sheth et al., 1998; Sohur et al., 2014). It first becomes possible to record thalamically and cortically-evoked glutamatergic post-synaptic potentials in striatal GINs and SPNs during the first two postnatal weeks (Krajeski et al., 2019; Plotkin et al., 2005; Tepper et al., 1998).

Inputs from local cholinergic interneurons and dopaminergic neurons in the substantia nigra and ventral tegmental area also increase in number and strength during postnatal development. Cholinergic interneurons develop spontaneous activity during early

postnatal development which increases into adulthood (McGuirt et al., 2021). The postnatal function of striatal cholinergic interneurons is modified by the changing strength of thalamic inputs, and these neurons develop the ability to modify striatal dopamine release by P28 (McGuirt et al., 2022, 2021). Dopaminergic release in turn increases in early postnatal development, contributing to glutamatergic afferent formation and the maturation of SPNs (Kozorovitskiy et al., 2015; Lieberman et al., 2018).

The earliest network activity in the striatum begins embryonically with the onset of asynchronous calcium spikes in SPNs (E12), followed by synchronous plateau calcium events (E14) (Dehorter et al., 2011). These events gradually increased until (P4), and disappear by the end of the second postnatal week (Dehorter et al., 2011). Early synchronous activity occurs independent of synaptic inputs from GABAergic and glutamatergic neurons, which are present embryonically (E16-18) but do not begin to drive the coordinated network activity of SPNs until the end of the first postnatal week (P5-P7) (Dehorter et al., 2011).

During early postnatal development, increasing glutamatergic and dopaminergic input is associated with the functional maturation of SPNs, which gradually become less intrinsically excitable, as characterized by an increased action potential (AP) threshold, decreased input resistance (IR), and decreased AP firing rate (Krajeski et al., 2019; Lieberman et al., 2018; Peixoto et al., 2016; Sohur et al., 2014; Tepper et al., 1998). The onset of mature functional properties has been associated with increased expression of Kir2.1 mediated inwardly rectifying potassium currents (Dehorter et al., 2011; Shen et al., 2007; Wilson and Kawaguchi, 1996). Maturing SPNs also demonstrate differences in short-term and long-term plasticity in response to excitatory inputs (Fino and Venance, 2010; Partridge et al., 2000).

In Chapter 2, our prenatal ethanol exposure occurs during a period critical to the development of both SPNs and GINs, when SPNs are first forming efferent connections, and the striatum first receives glutamatergic and dopaminergic inputs. Further, the early postnatal time period during which we assess the development of early motor behaviors and the functional maturation of striatal neurons is one significant changes to striatal network activity.

1.6 The impact of ethanol exposure on the developing and adult striatum

Clinical imaging studies have identified changes in striatal structure and function in individuals with FASD. These include decreased striatal volume, altered functional connectivity with the cortex, and decreased frontostriatal fMRI BOLD activation during a response inhibition task (Cortese et al., 2006; Donald et al., 2016; Fryer et al., 2007; Sarah N. Mattson et al., 1996).

The effects of prenatal ethanol exposure on striatal structure and function have also been observed in animal models. A chronic moderate prenatal ethanol exposure resulted in enhanced AMPA receptor-mediated currents in D1-receptor+ SPNs of adolescent mice (Cheng et al., 2018). A similar chronic prenatal ethanol exposure model altered the timing of the normal developmental shift of long term plasticity from potentiation to depression, and facilitated glutamatergic inputs to the dorsolateral striatum due to an increase in D1-receptor function (Zhou et al., 2012).Chronic prenatal ethanol exposure has also been observed to alter the excitability of neurons in the dorsal striatum during a reversal learning task performed by adult animals (Marquardt et al., 2020).

Additionally, while a chronic low-dose prenatal ethanol exposure resulted in increased dendritic complexity in the dorsolateral striatum of adult animals of both sexes, a model employing a comparable dose and timing of *in utero* exposure resulted in decreased

dendritic complexity in the nucleus accumbens shell of adult male animals (Cheng et al., 2018; Rice et al., 2012). Prenatal ethanol exposure has also been demonstrated to alter the function and morphology of striatal GINs in adult animals: prenatal ethanol exposure resulted in increased SPN excitability associated with decreased GABAergic inputs to SPNS, and decreased GIN dendritic complexity (Cuzon Carlson et al., 2020; De Giorgio et al., 2012).

Both acute and chronic exposure to ethanol have also been observed to modify the function of striatal neurons in adult animals (Lovinger and Alvarez, 2017). Ethanol exposure tends to inhibit glutamatergic inputs and facilitate GABAergic signaling in the striatum (Adermark et al., 2013; Cheng et al., 2017; Wang et al., 2012). However, the effects of exposure vary depending on the striatal neuronal subtype (Blomeley et al., 2011; Patton et al., 2016).

Together, these data indicate that both the local development and excitatory inputs to the striatum may be susceptible to the effects of prenatal ethanol exposure. However, efforts to understand the potential contribution of striatal dysfunction to clinical phenotypes observed in individuals with FASD have largely focused on the structure and function of SPN and relied on evidence from adult animals. How both early striatal development and the function of striatal GINs may be altered by prenatal ethanol exposure remains underexplored, inspiring my experimental approach in Chapter 2.

1.7 Cognitive and executive function: FASD and AD

Executive function is generally defined as the skills required to plan and execute a goal directed behavior. Differences in executive function have been identified in individuals with FASD based on a battery of neuropsychological tests. Differences in individuals with FASD included: differences in response inhibition, perseverative behaviors during learning tasks, planning, attention, and set shifting behavior (Green et al., 2009; Kingdon

et al., 2016; S. N. Mattson et al., 1996; Mattson et al., 1999; Pei et al., 2011). Young children and adolescents with FASD demonstrate differences in IQ, working memory, short-term memory, as well as spatial learning and memory (Becker et al., 1990; Hamilton et al., 2003; Kodituwakku et al., 1995; Streissguth et al., 1990; Willoughby et al., 2008). Recent work suggests differences in spatial memory ability may be age-dependent; when compared with younger children, adolescents with FASD are less able to develop adaptive strategies in a spatial memory task than their peers (Moore et al., 2021). Investigation of executive and cognitive function in aging adults following a prenatal ethanol exposure has been limited, though it suggests that cognitive differences may persist into adulthood (Manji et al., 2009; Moore and Riley, 2015).

Differences in executive and cognitive function are observed in rodent models following a variety of prenatal ethanol-exposure paradigms, and behavioral tasks to assess adult animals (Marquardt and Brigman, 2016, 2016; Skorput et al., 2015). Spatial memory is frequently assayed using Morris Water or Y-Maze tasks, while reversal learning is assessed using a variety of modalities, but following a general pattern of pairing, then unpairing a sensory cue with a reward, mimicking the Wisconsin Card Sorting Task, a task used in clinical neuropsychiatric assessment (Marquardt and Brigman, 2016; Skorput et al., 2015). In Chapter 3, we use a modified Barnes Maze task to assess spatial learning, memory and behavioral flexibility. This testing paradigm was previously used to identify differences in reversal learning following our brief binge prenatal ethanol exposure paradigm, and differences in learning and memory in 3xTg-AD mice following two months of voluntary ethanol consumption (Muñoz et al., 2015; Skorput et al., 2015).

Early life risk factors developing AD have not been well explored, but include childhood differences in cognitive and executive function, and related challenges in school performance (Seifan et al., 2015; Yu et al., 2020). The cognitive differences, and related

differences educational achievements that may result from a prenatal ethanol exposure are markedly similar to the early developmental characteristics that are associated with increased AD risk Wozniak et al., 2019). Adverse childhood experiences (ACEs) have also recently been shown to contribute to AD risk later in life (Corney et al., 2022). Individuals with FASD are considerably more likely to have experience an ACE; when compared to non-FASD controls. A recent study determined that individuals aged 2-20 years with FASD experience on average 3.7 more ACEs than age-matched non-FASD controls (Kambeitz et al., 2019).

Alcohol use during adulthood has also been shown to modify AD risk (Heymann et al., 2016; Huang et al., 2016; Koch et al., 2020; Peng et al., 2020). Alcohol use in aging populations is highly prevalent and increasing more than among younger drinkers (White et al., 2022). While low levels of alcohol use are associated with decreased risk of developing AD, alcohol misuse including heavy or chronic alcohol use may result in a predisposition for AD (Xie and Feng, 2022). Studies modeling adult alcohol use in rodents have identified convergent effects of alcohol use and aging on oxidative stress and neuroimmune activation pathways as potential contributors to the association between AD risk and alcohol use (Barnett et al., 2022; Carlson et al., 2022; Hoffman et al., 2019; León et al., 2022; Marsland et al., 2022; Tucker et al., 2022). Consistent with our results in Chapter 3, one recent study assessing the influence of prenatal ethanol exposure E8.5-17.5 on 3xTg-AD mice identified an exacerbation of memory deficits and conditioned fear responses in animals exposed prenatally to ethanol, and associated with an increase in hippocampal microglial numbers (Walter et al., 2022).

Alzheimer's disease is currently estimated to affect more than 30 million individuals worldwide, with cases expected to increase in the coming decades, and affect more than 150 million by 2050 (Alzheimer's Disease International and Patterson, 2018; Gustavsson

et al., 2023). Identifying early life risk factors, and how they may modify the pathological progression will be critical in managing the rising number of AD cases. AD is characterized clinically by problems with episodic memory, and diagnosed post-mortem with the accumulation of pathological proteins: β-amyloid and tau proteins, and loss of synaptic proteins (Braak and Braak, 1996; DeTure and Dickson, 2019; Lane et al., 2018; Selkoe, 2002). Clinical diagnosis of AD is made by ruling out alternative explanations of neurological dysfunction, and is supported by imaging findings using amyloid positron emission tomography (Lane et al., 2018).

The etiology of AD is still under investigation, the best characterized pathway involves the pathological production of β-amyloid, resulting from: increased levels of amyloid precursor protein, increased production of toxic amyloid cleavage products, and failure to clear those products ultimately resulting in amyloid plaques (Hardy and Selkoe, 2002). Alternative pathways identified as contributing to AD pathology include: the pathological hyperphosphorylation of tau, mitochondrial dysfunction resulting in oxidative stress, lipid dyshomeostasis, chronic neuroimmune activation and neurovascular changes (Verma et al., 2022).Synaptic pathology is also present in AD patients prior to the onset of neuropathological protein accumulation, and has been observed to be more predictive of cognitive symptom progression (Arendt, 2009; DeKosky and Scheff, 1990; Scheff et al., 2006; Selkoe, 2002).

Current available pharmacological treatments for AD target two main pathways: medial forebrain cholinergic dysfunction and amyloid accumulation. Clinical trials are underway assessing the safety and effectiveness of treatment strategies to decrease oxidative stress and CNS inflammatory responses (Liu et al., 2022; Sang et al., 2022; Trushina et al., 2022). However, despite considerable recent efforts, therapeutic options for AD patients remain limited (Yiannopoulou et al., 2019).

In Chapter 3, I provide the first preclinical evidence that a prenatal ethanol exposure may advance the onset of AD pathology and related challenges in spatial learning and memory, and reversal learning. The findings in this chapter provide a first indication that prenatal ethanol exposure may influence the clinical trajectory of individuals predisposed to develop Alzheimer's disease, suggesting that increased efforts should be made to identify the changing needs of individuals with FASD during the aging process. Additionally, further investigation of the mechanisms underlying increased AD risk following prenatal ethanol exposure may result in novel therapeutic targets.

1.8 Mouse model of prenatal ethanol exposure and AD

To model the potential contribution of prenatal ethanol exposure to AD risk, we exposed 3xTg-AD mice to ethanol, using a brief binge ethanol exposure paradigm, described in both Chapters 2 and 3. 3xTg-AD mice bear three dementia-associated transgenes encoding: presenilin1 ($PS1_{M146V}$), human amyloid precursor protein (APP_{Swe}) and human tau (Tau_{P301S}) under the Thy1 promoter, which results in transgene expression in excitatory neurons(Campsall et al., 2002; Oddo et al., 2003).

The 3xTg-AD line has both advantages and limitations when compared to other available transgenic mouse models of AD (Jankowsky and Zheng, 2017). In contrast to other mouse models of AD, 3xTg-AD mice develop both amyloid and tau pathology beginning with the formation of intraneuronal amyloid inclusions at the end of the first postnatal month, while tau pathology is not apparent until animals age to 6-12 months (Oddo et al., 2003; Oh et al., 2010). 3xTg-AD mice also have been shown to develop cognitive deficits, including spatial memory deficits by 6 months of age and develop sex differences in cognitive phenotypes relevant to the epidemiology of AD (Billings et al., 2005; Dennison et al., 2021; Oddo et al., 2003). This in combination with past work in the lab, assessing the impact of prenatal ethanol exposure on the developing brain led

us to focus our investigation the function and disposition of neurons within the mPFC of 3xTg-AD mice in Chapter 3 (Lee et al., 2022; Skorput et al., 2019, 2015).

While 3xTg-AD mice reliably develop both neuropathological and memory phenotypes consistent with AD progression in humans, this mouse model relies on dramatic overexpression of human APP making it challenging to discern pathological changes mediated by disease processes relevant to AD, rather than those resulting from the physiological challenge of managing ectopic protein overexpression (Elder et al., 2010). In addition, transgene expression is limited to pyramidal neurons, while pathological proteins have been localized to GIN populations in patient post-mortem samples (Kurucu et al., 2021). Our findings in Chapter 3 should be considered, in light of these limitations to our model system.

1.9 Organization, function and development of the medial prefrontal cortex (mPFC)

Organization and function of the mPFC

While the mPFC is organized into distinct layers comparable to other neocortical regions, unlike the classical six layered sensory cortices it lacks typical agranular layer IV (Harris and Shepherd, 2015). In the mPFC, Layer I contains the apical dendrites of deeper layer pyramidal neurons, and local GINs, Layer II/III is composed of intratelencephalic tract glutamatergic neurons projecting within the cortex, Layer V is largely made up of pyramidal tract neurons which form connections with subcortical regions, while Layer VI neurons project to the thalamus (Abs et al., 2018; Baker et al., 2018; Ueta et al., 2019). Information flows between cortical layers with Layer 2/3 neurons sending projections to Layer V neurons (Collins et al., 2018).

GINs are found throughout all cortical layers and include three major subpopulations: PV+, SST+, and cholecystokinin (CCK)+ GINs, which all provide inhibitory connections to neighboring neurons but differ in their role at a circuit level based on their preferential afferent and efferent connections. Both CCK+ and PV+ GINs provide strong feed-forward inhibition only to neighboring pyramidal neurons, while SST+ GINs directly inhibit both pyramidal neurons and PV+ GINs, resulting in a net inhibition or disinhibition of pyramidal neurons (Cummings and Clem, 2020; Tremblay et al., 2016). The facilitation or inhibition of pyramidal neuron activity by SST+ GINs differs depending on the source of their afferent excitatory inputs (McGarry and Carter, 2016; Tremblay et al., 2016)

As observed in the striatum, the mPFC can also be organized into subregions based on contributions to different behaviors and associated differences in afferent and efferent connections (Anastasiades and Carter, 2021; Heidbreder and Groenewegen, 2003). The mPFC is generally described as including three subregion: the dorsal anterior cingulate (ACC), prelimbic (PL), and the most ventral infralimbic (IL). The ACC mPFC contributes to action selection in reward-mediated behaviors (Gabbott et al., 2005; Rushworth et al., 2011). Both IL and PL mPFC have been associated with performance of: perseverative behavior in spatial memory tasks, conditioned fear and anxiety-like behavior (Capriles et al., 2003; Dias and Aggleton, 2000; Euston et al., 2012; Milad and Quirk, 2002; Morgan et al., 1993)

Neurons within the mPFC make reciprocal connections with: thalamus, hippocampus, basolateral amygdala, serotonergic, dopaminergic, cholinergic, and noradrenergic nuclei. They also connect with other cortical regions, and project to spinal cord, hypothalamus and striatum (Ferino et al., 1987; Gabbott et al., 2005). The dorsal mPFC (ACC) bears stronger reciprocal connections with motor/sensory cortices, in contrast,

with increased ventral region (PL/IL) connectivity with limbic and association cortices and medial forebrain cholinergic and dopaminergic neurons (Gaykema et al., 1991; Heidbreder and Groenewegen, 2003; Thierry et al., 1973).

Reciprocal connections between the mPFC and hippocampus play a critical role in behaviors requiring spatial memory (Floresco et al., 1997; Wirt and Hyman, 2017; S.-T. Yang et al., 2014). Recent work suggests that neurons in the mPFC provide critical information about the environmental context during a behavioral task (Wirt and Hyman, 2017). The mPFC also plays a functional role in reversal learning behaviors, particularly when they require a high degree of spatial information, or require consideration of multiple factors in behavioral decision-making (Izquierdo et al., 2017). We assess both types of behavior using the modified Barnes Maze task in Chapter 3.

Development of the mPFC

The mPFC first develops with asymmetric divisions of ventricular zone radial glial cells, beginning postnatal day around E10 in rodents (Kolk and Rakic, 2022). This early proliferation of cells gives rise to a subventricular zone, intermediate zone, subplate and cortical plate (Noctor et al., 2004). Cortical layers are discernible in the mPFC at the end of the first postnatal week, while layers continue to become more differentiated throughout the first postnatal month (van Eden and Uylings, 1985).

Neurons in the mPFC first receive afferent connections from the mid dopaminergic neurons at E15, followed by inputs from: serotoninergic neurons in the raphe nuclei, noradrenergic neurons in the locus coeruleus by E18, cholinergic neurons in the basal forebrain and glutamatergic projections from the hippocampus and thalamus during the first postnatal week, and glutamatergic projections from the amygdala at the end of the second postnatal week (Arruda-Carvalho et al., 2017; Bang et al., 2012; Brockmann et al., 2011; Ferguson and Gao, 2015; Janiesch et al., 2011; Kalsbeek et al., 1988; Levitt

and Moore, 1979). Long range efferent connections from mPFC neurons begin to form in the first postnatal week, reaching the thalamus at P1, and basolateral amygdala at P10, and increase in number until P30 (Arruda-Carvalho et al., 2017; Hartung et al., 2016). In addition, as observed in the developing striatum, neurons within the mPFC develop early synchronous firing mediated by both glutamatergic and GABAergic neurotransmission (Pires et al., 2021).

In Chapter 3, I identify layer and region-specific alterations in inhibitory inputs to pyramidal neurons in the mPFC associated with changes in PV+ interneuron density in 3xTg-AD animals. Our ethanol exposure occurs during a prenatal period when pyramidal neurons and GINs are migrating to the developing cortex, the mPFC has begun to receive its first afferent connections, but prior to the formation of efferent connections from mPFC pyramidal neurons to their long-range projection sites.

1.10 Contributions of GABAergic interneurons to developing neural circuits

GINs that populate the developing cortex and striatum are born in several brain regions including the MGE, the source of ~60% of cortical GINs in rodents, caudal ganglionic eminence (CGE), a source of calretinin, 5HT3A and VIP-expressing GINs, the preoptic area of the developing hypothalamus, and to a small degree ventricular zone radial glial cells (Anderson et al., 2001; Gelman et al., 2011; Kelsom and Lu, 2013; Wichterle et al., 2001). Similar to during striatal development, embryonic cortical GINs migrate tangentially to the populate the developing cortex, with the earliest born GINs eventually residing in deeper cortical layers, while later born GINs populate superficial cortical layers (Marín and Rubenstein, 2001; Miller, 1985). The postnatal subtype of GABAergic interneurons is specified during embryonic development (Butt et al., 2005; Fogarty et al., 2007; Lodato et al., 2011).

Both cortical and striatal GINs are identifiable in early development prior to the expression of classical protein markers using promoters specific to cell lineages born in the MGE, including: Nkx2.1 and Lhx6 (Alifragis et al., 2004; Xu et al., 2008). In Chapter 2, we identify developing GABAergic interneurons prior to their expression of traditional protein markers: somatostatin during the first postnatal week, and parvalbumin (PV) during the second postnatal week in the cortex, and striatum using the Nkx2.1-cre mouse line, crossed with the Ai14 (a cre-dependent TdTomato reporter line) (Sreenivasan et al., 2022; Xu et al., 2008). PV+ and SST+ GINs can be distinguished during electrophysiological experiments based on their distinct firing properties, and morphological differences. SST+ neurons fire action potentials at a lower threshold current and at a decreased frequency and have smaller and rounder cell bodies with fewer projections. Alternatively, PV+ neurons have slightly larger and more oblong cell bodies, with many more projections and demonstrate both a much higher firing threshold and firing rate. The Nkx2.1-Cre/tdTomato line also results in the fluorescent labeling of a small population of cholinergic interneurons, which can be differentiated from GABAergic interneurons given their significantly larger cell body size.

Cortical and striatal GINs release GABA which acts on GABA-A receptors, ligand-gated ion channels, and GABA-B receptors, G-protein coupled receptors. In adult animals, GABA binding to both GABA-A and GABA-B receptors results in inhibition of the post-synaptic cell, resulting from chloride influx in case of GABA-A receptors, and potassium channel activation along with calcium channel inhibition in GABA-B receptors (Ghit et al., 2021). Alternatively, in some populations of neurons in adult animals, including SPNs, GABA action on somatic GABA-A receptors is both depolarizing and inhibitory as it results in depolarizing shifts membrane potential that are still more hyperpolarizing than

the post synaptic neuron's resting membrane potential. GABA binding to dendritic GABA-A receptors can also be depolarizing and excitatory (Gulledge and Stuart, 2003).

Both GABA-A and GABA-B receptors also undergo dynamic changes during development, and differentially localize both specific cell compartments (axon, dendrite, or soma) and subpopulations of neurons (Bassetti, 2022; Wu and Sun, 2015). GABA-A receptors also differ in their subunit expression across brain regions, development and between subpopulations of neurons (Boccalaro et al., 2019; Laurie et al., 1992; Santhakumar et al., 2010).

During early postnatal development, GABA binding to somatic GABA-A (GABA-AR) receptors is depolarizing and excitatory (Ben-Ari et al., 2012; Luhmann and Prince, 1991). Depolarizing GABA contributes to synchronized firing of neurons characteristic of immature network activity, peaking at the end of the first postnatal week in the striatum and mPFC, and related to neuronal survival during the early postnatal period (Allene and Cossart, 2010; Dehorter et al., 2011; Duan et al., 2020; Kalemaki et al., 2022; Modol et al., 2020; Pires et al., 2021). Loss of cortical GABAergic signaling during early postnatal development can result in persistent network hypersynchrony (Duan et al., 2020). Persistence of depolarizing GABA has also been observed in animal models of autism, Down's syndrome, schizophrenia and epilepsy (Amin et al., 2017; He et al., 2019; Parrini et al., 2021; Yuan et al., 2019). Both SST+ and PV+ GINs contribute to early network synchrony, while PV+ GINs contribute to local coordinated firing SST+ GINs determine long range network synchrony (Modol et al., 2020)

In addition to the contribution of depolarizing GABA to the coordinated firing of neurons in early network development, it has also been shown to alter gene expression, morphology, and migration in cortical neurons. Blocking the depolarizing effects of GABA in the embryonic cortex has been shown to increase the number of neurons

synthesizing DNA in the ventricular zone (LoTurco et al., 1995). Increased expression of KCC2, a chloride co-transporter, and the related switch in the action of GABA from depolarizing to hyperpolarizing resulted in the conclusion of GIN migration (Bortone and Polleux, 2009). Inhibiting the early depolarizing actions of GABA altered the dendritic morphology of cortical pyramidal neurons (Cancedda et al., 2007). GABA release from GINs also facilitates both synapse formation and elimination depending on the maturational state (ie. depolarizing vs. hyperpolarizing action) of GABA-AR mediated currents (Baho and Cristo, 2012; Oh et al., 2016; Salmon et al., 2020; Wang and Kriegstein, 2008).

Following the ‘GABA switch’, which occurs between P10 and 20 in the mPFC, cortical PV+ GINs develop adult-like functional properties (Kalemaki et al., 2022). PV+ GINs first develop their characteristic fast-spiking firing properties at the end of the second postnatal week coinciding with the increased expression of the Kv3.1 voltage-gated potassium channel and voltage gated sodium channels, and the decreased expression of Kv2.2 precipitating the loss of immature spike adaptation phenotype, and onset of PV expression (Goldberg et al., 2011; Okaty et al., 2009; J.-M. Yang et al., 2014).The maturation of GABAergic neurons continues into the end of the first postnatal month with increasing expression protein markers distinguishing GIN subtypes, including: PV and SST (Du et al., 2018).

The maturation of cortical GINs has been shown to play an essential role in closing ‘critical periods’ in both the sensory and associative cortex (Amegandjin et al., 2021; Deidda et al., 2015; Fagiolini et al., 2004; Takesian et al., 2018). Critical periods in development refer to the times during which developing networks display a high degree of plasticity response to sensory experiences, associated with highly synchronous neuronal firing (Reh et al., 2020; Rice and Barone, 2000) Critical periods ‘close’ when

developing neuronal networks demonstrate decreased plasticity in response to sensory inputs. This has been shown to coincide with the onset of hyperpolarizing GABA activity and the formation of perineuronal nets, a morphological feature of PV+ GINs derived from extracellular matrix (Carulli and Verhaagen, 2021; Deidda et al., 2015)

The contribution of GINs to the function of developing striatal circuits remains uncertain. While striatal GINs demonstrate similar patterns functional maturation as those in the developing cortex and GABA has been shown to contribute to synchronous network activity during early postnatal development, how striatal GINs contribute to the migration, synaptic or morphological development or critical plastiticy in striatum-mediated behavior is not yet known (Chesselet et al., 2007).

The role of striatal GINs in network activity and adult behaviors has been better described. Though striatal GINs make up a small proportion of the total striatal cell population, parvalbumin expressing (PV+) GINs (<1% total population), which provide feed forward inhibition onto SPNs, contribute to a significant proportion of striatal neuronal activity (6-11%) (Duhne et al., 2020). PV+ GIN-activation has been shown to alter the cell populations that participate in co-active ensembles, and may contribute to ensemble formation during motor learning (Duhne et al., 2020; Martiros et al., 2018). Alternatively, striatal SST+ GINs provide long range in inhibitory inputs to both PV+ GINs and FSIs, and exert stronger control over SPNs in the dorsomedial striatum (Fino et al., 2018; Straub et al., 2016). Recent studies using in vivo recording and optogenetic approaches in have identified roles for PV+ striatal GINs in modifying the speed, direction, and precision of motor behaviors in response to sensory inputs, and motor habit formation (Gage et al., 2010; Gritton et al., 2019; Lee et al., 2017; Martiros et al., 2018; O'Hare et al., 2016; Owen et al., 2018; Roberts et al., 2019). The role SST+ striatal GINs has been less well documented but includes control over reward mediated

behaviors (Holly et al., 2021). These support previous lesion and pharmacological inhibition-based studies that indicated a critical role of striatal GINs in adult motor behaviors (Gazan et al., 2019; Gittis et al., 2011; Owen et al., 2018; Xu et al., 2016).

In the adult mPFC, though GINs are located in all cortical layers, they provide feedforward inhibition in response to excitatory inputs from distant sites that preferentially form in Layer I-III: ventral hippocampus, basolateral amygdala and mediodorsal thalamus, and hippocampus, regulating the inhibitory excitatory balance (Delevich et al., 2015; Floresco and Grace, 2003; Gabbott et al., 2006; Gigg et al., 1994) Using targeted optogenetic approaches to increase and decrease the function of mPFC neurons, and *in vivo* recording experiments, researchers have identified distinct functional changes in PV+ and SST+ GINs during working memory tasks, suggesting differential roles of PV+ and SST+ GINs in working memory performance (Abbas et al., 2018; Pinto and Dan, 2015). PV+ GINs in the mPFC have also been shown to contribute to set-shifting behaviors characteristic of behavioral flexibility in goal directed behaviors driven by sensory stimuli, and reversal learning behaviors, similar to the reversal learning behavior we assessed using the modified Barnes Maze in Chapter 3 (Cho et al., 2020; Goodwill et al., 2018).

Alternatively, mPFC PV+ and SST+ GINs have been shown to modify the expression of conditioned fear behaviors (Courtin et al., 2014; Cummings and Clem, 2020). Pharmacologically activating or inhibiting GABAergic signaling in the IL mPFC has also been shown to facilitate or prevent the extinction of conditioned fear responses (Marek et al., 2018). PV+ interneurons in the dmPFC have also been shown to mediate the development of adult social behaviors, with optogenetically and chemogenetically increasing the activity of PV+ GIN increases social behavior, and juvenile social isolation

disrupts the maturation of PV+ GINs resulting to similar social deficits in adult animals as observed with chemogenetic inhibition of PV+ GIN function (Bicks et al., 2020).

1.11 GABAergic interneurons in Alzheimer's disease

GINs were first implicated in the etiology of AD when a mouse model of AD (hAPP) was determined to have an increased susceptibility to seizure activity, that coincided with a decrease in EEG-recorded gamma oscillations, and decreased expression of the Nav1.1 sodium channel in PV+ cortical GINs (Verret et al., 2012). Consistent with this mouse model, individuals with AD also have a higher risk of seizures than the general population, while individuals with epilepsy are at increased risk of developing Alzheimer's disease (Yang et al., 2022).

Since this initial finding, decreased expression of protein markers of GINs, including PV, as well as alterations in GIN synaptic function have been identified in several mouse models of AD (Albuquerque et al., 2015; Andrews-Zwilling et al., 2010; Cheng et al., 2019; Kiss et al., 2016, 2016; Leung et al., 2012; Li et al., 2021; Mitew et al., 2013; Murray et al., 2011; Prince et al., 2021). Differences in interneuron number also been observed in post-mortem tissue from AD patients (Kurucu et al., 2021)

The mechanism underlying the susceptibility of GINs to AD pathology and how differences in GIN function may contribute to cognitive changes in AD is still subject to debate. Current theories posit that GINs are susceptible to either the direct action of accumulated amyloid, resulting in their dysfunction, contributing to an excitation/inhibition (E/I) imbalance, or an indirect effect on GINs resulting from excitotoxicity mediated by amyloid accumulation in neighboring glutamatergic neurons (Palop and Mucke, 2016; Xu et al., 2020). In addition to the direct effects of accumulated beta-amyloid on GINs, or indirect effects of on the excitability of glutamatergic neurons, dysfunctional GABAergic signaling in AD may relate to the pathological presence of

depolarizing GABA activity, which may contribute to network hyperactivity and memory impairment in aging adult animals (Bakker et al., 2012; Capsoni et al., 2022). In support of this potential mechanism, ectopically increasing amyloid precursor protein expression in cultured neurons results neuronal hyperactivity associated with decreased KCC2 expression, while NKCC1 levels are unaltered (Doshina et al., 2017)

In Chapter 3, I focus on the potential contribution of PV+ mPFC GINs to the early emergence of memory deficits in 3xTg-AD mice following a brief binge exposure to ethanol. I find that prenatal ethanol exposure exacerbates the loss of GABAergic synaptic currents observed in 3xTg-AD mice as well as the decreased numbers of PV+ GINs in the mPFC.

1.12 The effects of prenatal ethanol exposure on the developing cortex and GABAergic interneurons

Past work in the lab demonstrated that prenatal ethanol exposure can disrupt the development of pyramidal neurons in the somatosensory cortex, resulting in early postnatal and adolescent synaptic dysfunction as well deficits in tactile sensitivity (Delatour et al., 2019a, 2019b). This work added to data from clinical imaging studies, as well as studies focused on adolescent and adult animals that implicate prenatal ethanol exposure detrimental effects on cortical development as potential contributors to clinical symptoms in FASD (El Shawa et al., 2013; Kodali et al., 2017; Leigland et al., 2013; Louth et al., 2018, 2016; Miller, 2017; Olateju et al., 2019; Treit et al., 2014; Wong et al., 2018)

We have also previously shown that prenatal ethanol exposure enhances the migration of GINs to the developing cortex, resulting in an embryonic increase in the number of cortical Nkx2.1+ GINs, that persists into adulthood resulting in an increase in PV+ GINs

in the mPFC in young adult animals (Cuzon et al., 2008; Lee et al., 2022; Skorput et al., 2019, 2015; Skorput and Yeh, 2016). This change in the number of GINs resulted in an E/I imbalance in the mPFC of young adult animals, coincided with deficits in reversal learning and hyperactivity (Skorput et al., 2015). Recent work from the lab also suggests that the ethanol's effects on the embryonic migration of cortical GINs may be the result ethanol-induced enhancement of GABA-induced depolarization of migrating neurons, via potentiation of L-type calcium channels (Lee et al., 2022; Skorput et al., 2019).

Others have also shown that both prenatal and neonatal ethanol exposure can alter the number, function and morphological maturation of GINs in the cortex and striatum, as well as other brain regions including the hippocampus (Bird et al., 2021, 2018; De Giorgio et al., 2012; Kenton et al., 2020; Léger et al., 2020; Marguet et al., 2020; Saito et al., 2019, 2019; Smiley et al., 2015). These data highlight the potential contributions of the dysfunctional development of GINs to functional and behavioral outcomes following prenatal ethanol exposure, and ultimately the etiology of FASD, which I will continue to explore throughout this thesis.

References

Abbas, A.I., Sundiang, M.J.M., Henoch, B., Morton, M.P., Bolkan, S.S., Park, A.J., Harris, A.Z., Kellendonk, C., Gordon, J.A., 2018. Somatostatin Interneurons Facilitate Hippocampal-Prefrontal Synchrony and Prefrontal Spatial Encoding. Neuron 100, 926-939.e3. https://doi.org/10.1016/j.neuron.2018.09.029

Abbott, C.W., Kozanian, O.O., Kanaan, J., Wendel, K.M., Huffman, K.J., 2016. The Impact of Prenatal Ethanol Exposure on Neuroanatomical and Behavioral Development in Mice. Alcohol: Clinical and Experimental Research 40, 122–133. https://doi.org/10.1111/acer.12936

Abs, E., Poorthuis, R.B., Apelblat, D., Muhammad, K., Pardi, M.B., Enke, L., Kushinsky, D., Pu, D.-L., Eizinger, M.F., Conzelmann, K.-K., Spiegel, I., Letzkus, J.J., 2018. Learning-Related Plasticity in Dendrite-Targeting Layer 1 Interneurons. Neuron 100, 684-699.e6. https://doi.org/10.1016/j.neuron.2018.09.001

Adermark, L., Jonsson, S., Söderpalm, B., Ericson, M., 2013. Region-specific depression of striatal activity in Wistar rat by modest ethanol consumption over a ten-month period. Alcohol 47, 289–298. https://doi.org/10.1016/j.alcohol.2013.03.003

Albuquerque, M.S., Mahar, I., Davoli, M.A., Chabot, J.-G., Mechawar, N., Quirion, R., Krantic, S., 2015. Regional and sub-regional differences in hippocampal GABAergic neuronal vulnerability in the TgCRND8 mouse model of Alzheimer's disease. Frontiers in Aging Neuroscience 7.

Alifragis, P., Liapi, A., Parnavelas, J.G., 2004. Lhx6 Regulates the Migration of Cortical Interneurons from the Ventral Telencephalon But Does Not Specify their GABA Phenotype. J Neurosci 24, 5643–5648. https://doi.org/10.1523/JNEUROSCI.1245-04.2004

Allene, C., Cossart, R., 2010. Early NMDA receptor-driven waves of activity in the developing neocortex: physiological or pathological network oscillations? J Physiol 588, 83–91. https://doi.org/10.1113/jphysiol.2009.178798

Alloway, K.D., Smith, J.B., Mowery, T.M., Watson, G.D.R., 2017. Sensory Processing in the Dorsolateral Striatum: The Contribution of Thalamostriatal Pathways. Front Syst Neurosci 11, 53. https://doi.org/10.3389/fnsys.2017.00053

Alzheimer's Disease International, Patterson, C., 2018. World Alzheimer Report 2018: The state of the art of dementia research: New frontiers.

Amegandjin, C.A., Choudhury, M., Jadhav, V., Carriço, J.N., Quintal, A., Berryer, M., Snapyan, M., Chattopadhyaya, B., Saghatelyan, A., Di Cristo, G., 2021. Sensitive period

for rescuing parvalbumin interneurons connectivity and social behavior deficits caused by TSC1 loss. Nat Commun 12, 3653. https://doi.org/10.1038/s41467-021-23939-7

Anastasiades, P.G., Carter, A.G., 2021. Circuit organization of the rodent medial prefrontal cortex. Trends in Neurosciences 0. https://doi.org/10.1016/j.tins.2021.03.006

Anderson, S.A., Marín, O., Horn, C., Jennings, K., Rubenstein, J.L., 2001. Distinct cortical migrations from the medial and lateral ganglionic eminences. Development 128, 353–363.

Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S.Y., Zwilling, D., Yan, T.X., Chen, L., Huang, Y., 2010. Apolipoprotein E4 Causes Age- and Tau-Dependent Impairment of GABAergic Interneurons, Leading to Learning and Memory Deficits in Mice. J. Neurosci. 30, 13707–13717. https://doi.org/10.1523/JNEUROSCI.4040-10.2010

Angevine, J.B., 1970. Time of neuron origin in the diencephalon of the mouse. An autoradiographic study. Journal of Comparative Neurology 139, 129–187. https://doi.org/10.1002/cne.901390202

Antón-Bolaños, N., Sempere-Ferràndez, A., Guillamón-Vivancos, T., Martini, F.J., Pérez-Saiz, L., Gezelius, H., Filipchuk, A., Valdeolmillos, M., López-Bendito, G., 2019. Prenatal activity from thalamic neurons governs the emergence of functional cortical maps in mice. Science 364, 987–990. https://doi.org/10.1126/science.aav7617

Antonopoulos, J., Dori, I., Dinopoulos, A., Chiotelli, M., Parnavelas, J.G., 2002. Postnatal development of the dopaminergic system of the striatum in the rat. Neuroscience 110, 245–256. https://doi.org/10.1016/S0306-4522(01)00575-9

Arendt, T., 2009. Synaptic degeneration in Alzheimer's disease. Acta Neuropathol 118, 167–179. https://doi.org/10.1007/s00401-009-0536-x

Arias-García, M.A., Tapia, D., Laville, J.A., Calderón, V.M., Ramiro-Cortés, Y., Bargas, J., Galarraga, E., 2018. Functional comparison of corticostriatal and thalamostriatal postsynaptic responses in striatal neurons of the mouse. Brain Struct Funct 223, 1229–1253. https://doi.org/10.1007/s00429-017-1536-6

Arruda-Carvalho, M., Wu, W.-C., Cummings, K.A., Clem, R.L., 2017. Optogenetic Examination of Prefrontal-Amygdala Synaptic Development. J Neurosci 37, 2976–2985. https://doi.org/10.1523/JNEUROSCI.3097-16.2017

Baho, E., Cristo, G.D., 2012. Neural Activity and Neurotransmission Regulate the Maturation of the Innervation Field of Cortical GABAergic Interneurons in an Age-Dependent Manner. J. Neurosci. 32, 911–918. https://doi.org/10.1523/JNEUROSCI.4352-11.2012

Baker, A., Kalmbach, B., Morishima, M., Kim, J., Juavinett, A., Li, N., Dembrow, N., 2018. Specialized Subpopulations of Deep-Layer Pyramidal Neurons in the Neocortex: Bridging Cellular Properties to Functional Consequences. J. Neurosci. 38, 5441–5455. https://doi.org/10.1523/JNEUROSCI.0150-18.2018
Bakker, A., Krauss, G.L., Albert, M.S., Speck, C.L., Jones, L.R., Stark, C.E., Yassa, M.A., Bassett, S.S., Shelton, A.L., Gallagher, M., 2012. Reduction of Hippocampal Hyperactivity Improves Cognition in Amnestic Mild Cognitive Impairment. Neuron 74, 467–474. https://doi.org/10.1016/j.neuron.2012.03.023

Ballard, C., Mobley, W., Hardy, J., Williams, G., Corbett, A., 2016. Dementia in Down's syndrome. The Lancet Neurology 15, 622–636. https://doi.org/10.1016/S1474-4422(16)00063-6
Bamford, N.S., Wightman, R.M., Sulzer, D., 2018. Dopamine's effects on corticostriatal synapses during reward-based behaviors. Neuron 97, 494–510. https://doi.org/10.1016/j.neuron.2018.01.006

Bandoli, G., Coles, C.D., Kable, J.A., Wertelecki, W., Yevtushok, L., Zymak-Zakutnya, N., Wells, A., Granovska, I.V., Pashtepa, A.O., Chambers, C.D., Cifasd, T., 2019. Patterns of Prenatal Alcohol Use That Predict Infant Growth and Development. Pediatrics 143. https://doi.org/10.1542/peds.2018-2399

Bang, S.J., Jensen, P., Dymecki, S.M., Commons, K.G., 2012. Projections and interconnections of genetically defined serotonin neurons in mice. European Journal of Neuroscience 35, 85–96. https://doi.org/10.1111/j.1460-9568.2011.07936.x

Barnett, A., David, E., Rohlman, A., Nikolova, V.D., Moy, S.S., Vetreno, R.P., Coleman, L.G., 2022. Adolescent Binge Alcohol Enhances Early Alzheimer's Disease Pathology in Adulthood Through Proinflammatory Neuroimmune Activation. Frontiers in Pharmacology 13.

Bassetti, D., 2022. Keeping the Balance: GABAB Receptors in the Developing Brain and Beyond. Brain Sciences 12, 419. https://doi.org/10.3390/brainsci12040419

Becker, M., Warr-Leeper, G.A., Leeper, H.A., 1990. Fetal alcohol syndrome: a description of oral motor, articulatory, short-term memory, grammatical, and semantic abilities. J Commun Disord 23, 97–124. https://doi.org/10.1016/0021-9924(90)90016-r

Becker, S., Sharma, M.J., Callahan, B.L., 2021. ADHD and Neurodegenerative Disease Risk: A Critical Examination of the Evidence. Front Aging Neurosci 13, 826213. https://doi.org/10.3389/fnagi.2021.826213

Ben-Ari, Y., Khalilov, I., Kahle, K.T., Cherubini, E., 2012. The GABA Excitatory/Inhibitory Shift in Brain Maturation and Neurological Disorders. Neuroscientist 18, 467–486. https://doi.org/10.1177/1073858412438697

Bernácer, J., Prensa, L., Giménez-Amaya, J.M., 2012. Distribution of GABAergic interneurons and dopaminergic cells in the functional territories of the human striatum. PLoS One 7, e30504. https://doi.org/10.1371/journal.pone.0030504
Bernácer, J., Prensa, L., Giménez-Amaya, J.M., 2007. Cholinergic Interneurons Are Differentially Distributed in the Human Striatum. PLOS ONE 2, e1174. https://doi.org/10.1371/journal.pone.0001174

Bertero, A., Zurita, H., Normandin, M., Apicella, A.J., 2020. Auditory Long-Range Parvalbumin Cortico-Striatal Neurons. Front Neural Circuits 14. https://doi.org/10.3389/fncir.2020.00045

Bicks, L.K., Yamamuro, K., Flanigan, M.E., Kim, J.M., Kato, D., Lucas, E.K., Koike, H., Peng, M.S., Brady, D.M., Chandrasekaran, S., Norman, K.J., Smith, M.R., Clem, R.L., Russo, S.J., Akbarian, S., Morishita, H., 2020. Prefrontal parvalbumin interneurons require juvenile social experience to establish adult social behavior. Nat Commun 11, 1003. https://doi.org/10.1038/s41467-020-14740-z

Billings, L.M., Oddo, S., Green, K.N., McGaugh, J.L., LaFerla, F.M., 2005. Intraneuronal Aβ Causes the Onset of Early Alzheimer's Disease-Related Cognitive Deficits in Transgenic Mice. Neuron 45, 675–688. https://doi.org/10.1016/j.neuron.2005.01.040

Bird, C.W., Chavez, G.J., Barber, M.J., Valenzuela, C.F., 2021. Enhancement of parvalbumin interneuron-mediated neurotransmission in the retrosplenial cortex of adolescent mice following third trimester-equivalent ethanol exposure. Sci Rep 11, 1716. https://doi.org/10.1038/s41598-021-81173-z

Bird, C.W., Taylor, D.H., Pinkowski, N.J., Chavez, G.J., Valenzuela, C.F., 2018. Long-term Reductions in the Population of GABAergic Interneurons in the Mouse Hippocampus following Developmental Ethanol Exposure. Neuroscience 383, 60–73. https://doi.org/10.1016/j.neuroscience.2018.05.003

Blomeley, C.P., Bracci, E., 2009. Serotonin excites fast-spiking interneurons in the striatum. Eur J Neurosci 29, 1604–1614. https://doi.org/10.1111/j.1460-9568.2009.06725.x

Blomeley, C.P., Cains, S., Smith, R., Bracci, E., 2011. Ethanol affects striatal interneurons directly and projection neurons through a reduction in cholinergic tone. Neuropsychopharmacology 36, 1033–1046. https://doi.org/10.1038/npp.2010.241

Boccalaro, I.L., Cristiá-Lara, L., Schwerdel, C., Fritschy, J.-M., Rubi, L., 2019. Cell type-specific distribution of GABAA receptor subtypes in the mouse dorsal striatum. J Comp Neurol 527, 2030–2046. https://doi.org/10.1002/cne.24665

Boehm, S.L., Moore, E.M., Walsh, C.D., Gross, C.D., Cavelli, A.M., Gigante, E., Linsenbardt, D.N., 2008. Using Drinking in the Dark to model prenatal binge-like exposure to ethanol in C57BL/6J mice. Dev Psychobiol 50, 566–578. https://doi.org/10.1002/dev.20320

Bortone, D., Polleux, F., 2009. KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. Neuron 62, 53–71. https://doi.org/10.1016/j.neuron.2009.01.034
Braak, H., Braak, E., 1996. Evolution of the neuropathology of Alzheimer's disease. Acta Neurologica Scandinavica 94, 3–12. https://doi.org/10.1111/j.1600-0404.1996.tb05866.x

Breit, K.R., Rodriguez, C.G., Lei, A., Hussain, S., Thomas, J.D., 2022. Effects of prenatal alcohol and delta-9-tetrahydrocannabinol exposure via electronic cigarettes on motor development. Alcohol Clin Exp Res 46, 1408–1422. https://doi.org/10.1111/acer.14892

Brimblecombe, K.R., Cragg, S.J., 2017. The Striosome and Matrix Compartments of the Striatum: A Path through the Labyrinth from Neurochemistry toward Function. ACS Chem. Neurosci. 8, 235–242. https://doi.org/10.1021/acschemneuro.6b00333

Brockmann, M.D., Pöschel, B., Cichon, N., Hanganu-Opatz, I.L., 2011. Coupled oscillations mediate directed interactions between prefrontal cortex and hippocampus of the neonatal rat. Neuron 71, 332–347. https://doi.org/10.1016/j.neuron.2011.05.041

Butt, S.J.B., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J.G., Fishell, G., 2005. The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron 48, 591–604. https://doi.org/10.1016/j.neuron.2005.09.034

Campsall, K.D., Mazerolle, C.J., De Repentigy, Y., Kothary, R., Wallace, V.A., 2002. Characterization of transgene expression and Cre recombinase activity in a panel of Thy-1 promoter-Cre transgenic mice. Developmental Dynamics 224, 135–143. https://doi.org/10.1002/dvdy.10092

Cancedda, L., Fiumelli, H., Chen, K., Poo, M., 2007. Excitatory GABA Action Is Essential for Morphological Maturation of Cortical Neurons In Vivo. J. Neurosci. 27, 5224–5235. https://doi.org/10.1523/JNEUROSCI.5169-06.2007

Capriles, N., Rodaros, D., Sorge, R.E., Stewart, J., 2003. A role for the prefrontal cortex in stress- and cocaine-induced reinstatement of cocaine seeking in rats. Psychopharmacology 168, 66–74. https://doi.org/10.1007/s00213-002-1283-z

Capsoni, S., Arisi, I., Malerba, F., D'Onofrio, M., Cattaneo, A., Cherubini, E., 2022. Targeting the Cation-Chloride Co-Transporter NKCC1 to Re-Establish GABAergic Inhibition and an Appropriate Excitatory/Inhibitory Balance in Selective Neuronal Circuits: A Novel Approach for the Treatment of Alzheimer's Disease. Brain Sci 12, 783. https://doi.org/10.3390/brainsci12060783

Carlson, E.R., Guerin, S.P., Nixon, K., Fonken, L.K., 2022. The neuroimmune system - Where aging and excess alcohol intersect. Alcohol S0741-8329(22)00093–3. https://doi.org/10.1016/j.alcohol.2022.08.009

Carulli, D., Verhaagen, J., 2021. An Extracellular Perspective on CNS Maturation: Perineuronal Nets and the Control of Plasticity. International Journal of Molecular Sciences 22, 2434. https://doi.org/10.3390/ijms22052434
Cataldi, S., Stanley, A.T., Miniaci, M.C., Sulzer, D., 2021. Interpreting the role of the striatum during multiple phases of motor learning. FEBS J. https://doi.org/10.1111/febs.15908

Cazorla, M., de Carvalho, F.D., Chohan, M.O., Shegda, M., Chuhma, N., Rayport, S., Ahmari, S.E., Moore, H., Kellendonk, C., 2014. Dopamine D2 Receptors Regulate the Anatomical and Functional Balance of Basal Ganglia Circuitry. Neuron 81, 153–164. https://doi.org/10.1016/j.neuron.2013.10.041

Cellot, G., Cherubini, E., 2013. Functional role of ambient GABA in refining neuronal circuits early in postnatal development. Front. Neural Circuits 7. https://doi.org/10.3389/fncir.2013.00136

Chasnoff, I.J., Wells, A.M., King, L., 2015. Misdiagnosis and Missed Diagnoses in Foster and
Adopted Children With Prenatal Alcohol Exposure. Pediatrics 135, 264–270. https://doi.org/10.1542/peds.2014-2171

Cheng, A., Wang, J., Ghena, N., Zhao, Q., Perone, I., King, M.T., Veech, R.L., Gorospe, M., Wan, R., Mattson, M.P., 2019. SIRT3 Haploinsufficiency Aggravates Loss of GABAergic Interneurons and Neuronal Network Hyperexcitability in an Alzheimer's Disease Model. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.1446-19.2019

Cheng, Y., Huang, C.C.Y., Ma, T., Wei, X., Wang, X., Lu, J., Wang, J., 2017. Distinct Synaptic Strengthening of the Striatal Direct and Indirect Pathways Drives Alcohol Consumption. Biol. Psychiatry 81, 918–929. https://doi.org/10.1016/j.biopsych.2016.05.016

Cheng, Y., Wang, X., Wei, X., Xie, X., Melo, S., Miranda, R.C., Wang, J., 2018. Prenatal Exposure to Alcohol Induces Functional and Structural Plasticity in Dopamine D1 Receptor-Expressing Neurons of the Dorsomedial Striatum. Alcohol Clin Exp Res. https://doi.org/10.1111/acer.13806

Chesselet, M.-F., Plotkin, J.L., Wu, N., Levine, M.S., 2007. Development of striatal fast-spiking GABAergic interneurons, in: Tepper, J.M., Abercrombie, E.D., Bolam, J.P. (Eds.), Progress in Brain Research, Gaba and the Basal Ganglia. Elsevier, pp. 261–272. https://doi.org/10.1016/S0079-6123(06)60015-0

Cho, K.K.A., Davidson, T.J., Bouvier, G., Marshall, J.D., Schnitzer, M.J., Sohal, V.S., 2020. Cross-hemispheric gamma synchrony between prefrontal parvalbumin interneurons supports behavioral adaptation during rule shift learning. Nat Neurosci 23, 892–902. https://doi.org/10.1038/s41593-020-0647-1

Clarke, R., Adermark, L., 2015. Dopaminergic regulation of striatal interneurons in reward and addiction: Focus on alcohol. Neural Plasticity 2015. https://doi.org/10.1155/2015/814567
Collins, D.P., Anastasiades, P.G., Marlin, J.J., Carter, A.G., 2018. Reciprocal Circuits Linking the Prefrontal Cortex with Dorsal and Ventral Thalamic Nuclei. Neuron 98, 366-379.e4. https://doi.org/10.1016/j.neuron.2018.03.024

Corney, K.B., West, E.C., Quirk, S.E., Pasco, J.A., Stuart, A.L., Manavi, B.A., Kavanagh, B.E., Williams, L.J., 2022. The Relationship Between Adverse Childhood Experiences and Alzheimer's Disease: A Systematic Review. Front Aging Neurosci 14, 831378. https://doi.org/10.3389/fnagi.2022.831378
Cortese, B.M., Moore, G.J., Bailey, B.A., Jacobson, S.W., Delaney-Black, V., Hannigan, J.H., 2006. Magnetic resonance and spectroscopic imaging in prenatal alcohol-exposed children: Preliminary findings in the caudate nucleus. Neurotoxicology and Teratology 28, 597–606. https://doi.org/10.1016/j.ntt.2006.08.002

Courtin, J., Chaudun, F., Rozeske, R.R., Karalis, N., Gonzalez-Campo, C., Wurtz, H., Abdi, A., Baufreton, J., Bienvenu, T.C.M., Herry, C., 2014. Prefrontal parvalbumin interneurons shape neuronal activity to drive fear expression. Nature 505, 92–96. https://doi.org/10.1038/nature12755

Crittenden, J., Graybiel, A., 2011. Basal Ganglia Disorders Associated with Imbalances in the Striatal Striosome and Matrix Compartments. Frontiers in Neuroanatomy 5.

Cui, G., Jun, S.B., Jin, X., Pham, M.D., Vogel, S.S., Lovinger, D.M., Costa, R.M., 2013. Concurrent activation of striatal direct and indirect pathways during action initiation. Nature 494, 238–242. https://doi.org/10.1038/nature11846

Cummings, K.A., Clem, R.L., 2020. Prefrontal somatostatin interneurons encode fear memory. Nat Neurosci 23, 61–74. https://doi.org/10.1038/s41593-019-0552-7
Cuzon Carlson, V.C., Gremel, C.M., Lovinger, D.M., 2020. Gestational alcohol exposure disrupts cognitive function and striatal circuits in adult offspring. Nature Communications 11, 2555. https://doi.org/10.1038/s41467-020-16385-4

Cuzon, V.C., Yeh, P.W.L., Yanagawa, Y., Obata, K., Yeh, H.H., 2008. Ethanol Consumption during Early Pregnancy Alters the Disposition of Tangentially Migrating GABAergic Interneurons in the Fetal Cortex. J. Neurosci. 28, 1854–1864. https://doi.org/10.1523/JNEUROSCI.5110-07.2008

De Giorgio, A., Comparini, S.E., Intra, F.S., Granato, A., 2012. Long-term alterations of striatal parvalbumin interneurons in a rat model of early exposure to alcohol. J Neurodev Disord 4, 18. https://doi.org/10.1186/1866-1955-4-18

Deacon, T.W., Pakzaban, P., Isacson, O., 1994. The lateral ganglionic eminence is the origin of cells committed to striatal phenotypes: neural transplantation and developmental evidence. Brain Research 668, 211–219. https://doi.org/10.1016/0006-8993(94)90526-6

Dehorter, N., Michel, F.J., Marissal, T., Rotrou, Y., Matrot, B., Lopez, C., Humphries, M.D., Hammond, C., 2011. Onset of Pup Locomotion Coincides with Loss of NR2C/D-

Mediated Cortico-Striatal EPSCs and Dampening of Striatal Network Immature Activity. Front. Cell. Neurosci. 5. https://doi.org/10.3389/fncel.2011.00024

Deidda, G., Allegra, M., Cerri, C., Naskar, S., Bony, G., Zunino, G., Bozzi, Y., Caleo, M., Cancedda, L., 2015. Early depolarizing GABA controls critical period plasticity in the rat visual cortex. Nat Neurosci 18, 87–96. https://doi.org/10.1038/nn.3890

DeKosky, S.T., Scheff, S.W., 1990. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 27, 457–464. https://doi.org/10.1002/ana.410270502

Delatour, L.C., Yeh, P.W., Yeh, H.H., 2019a. Ethanol Exposure In Utero Disrupts Radial Migration and Pyramidal Cell Development in the Somatosensory Cortex. Cereb Cortex 29, 2125–2139. https://doi.org/10.1093/cercor/bhy094

Delatour, L.C., Yeh, P.W.L., Yeh, H.H., 2019b. Prenatal Exposure to Ethanol Alters Synaptic Activity in Layer V/VI Pyramidal Neurons of the Somatosensory Cortex. Cereb. Cortex. https://doi.org/10.1093/cercor/bhz199

Delevich, K., Tucciarone, J., Huang, Z.J., Li, B., 2015. The Mediodorsal Thalamus Drives Feedforward Inhibition in the Anterior Cingulate Cortex via Parvalbumin Interneurons. J. Neurosci. 35, 5743–5753. https://doi.org/10.1523/JNEUROSCI.4565-14.2015

Deng, Y.-P., Lei, W.-L., Reiner, A., 2006. Differential perikaryal localization in rats of D1 and D2
dopamine receptors on striatal projection neuron types identified by retrograde labeling. J Chem Neuroanat 32, 101–116. https://doi.org/10.1016/j.jchemneu.2006.07.001

Dennison, J.L., Ricciardi, N.R., Lohse, I., Volmar, C.-H., Wahlestedt, C., 2021. Sexual Dimorphism in the 3xTg-AD Mouse Model and Its Impact on Pre-Clinical Research. Journal of Alzheimer's Disease 80, 41–52. https://doi.org/10.3233/JAD-201014

Denny, C.H., 2019. Consumption of Alcohol Beverages and Binge Drinking Among Pregnant Women Aged 18–44 Years — United States, 2015–2017. MMWR Morb Mortal Wkly Rep 68. https://doi.org/10.15585/mmwr.mm6816a1

DeTure, M.A., Dickson, D.W., 2019. The neuropathological diagnosis of Alzheimer's disease. Molecular Neurodegeneration 14, 32. https://doi.org/10.1186/s13024-019-0333-5

Dias, R., Aggleton, J.P., 2000. Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential involvement of the prelimbic–infralimbic and anterior cingulate cortices in providing behavioural flexibility. European Journal of Neuroscience 12, 4457–4466. https://doi.org/10.1046/j.0953-816X.2000.01323.x

Donald, K.A., Ipser, J.C., Howells, F.M., Roos, A., Fouche, J.-P., Riley, E.P., Koen, N., Woods, R.P., Biswal, B., Zar, H.J., Narr, K.L., Stein, D.J., 2016. Interhemispheric Functional Brain Connectivity in Neonates with Prenatal Alcohol Exposure: Preliminary Findings. Alcohol Clin Exp Res 40, 113–121. https://doi.org/10.1111/acer.12930

Doney, R., Lucas, B.R., Jones, T., Howat, J., Sauer, K., Elliott, E.J., 2014. Fine Motor Skills in Children With Prenatal Alcohol Exposure or Fetal Alcohol Spectrum Disorder [WWW Document]. Journal of developmental and behavioral pediatrics : JDBP. https://doi.org/10.1097/DBP.0000000000000107

Doshina, A., Gourgue, F., Onizuka, M., Opsomer, R., Wang, P., Ando, K., Tasiaux, B., Dewachter, I., Kienlen-Campard, P., Brion, J.-P., Gailly, P., Octave, J.-N., Pierrot, N., 2017. Cortical cells reveal APP as a new player in the regulation of GABAergic neurotransmission. Sci Rep 7, 370. https://doi.org/10.1038/s41598-017-00325-2

Du, X., Serena, K., Hwang, W.J., Grech, A.M., Wu, Y.W.C., Schroeder, A., Hill, R.A., 2018. Prefrontal cortical parvalbumin and somatostatin expression and cell density increase during adolescence and are modified by BDNF and sex. Mol Cell Neurosci 88, 177–188. https://doi.org/10.1016/j.mcn.2018.02.001

Duan, Z.R.S., Che, A., Chu, P., Modol, L., Bollmann, Y., Babij, R., Fetcho, R.N., Otsuka, T., Fuccillo, M.V., Liston, C., Pisapia, D.J., Cossart, R., De Marco García, N.V., 2020. GABAergic Restriction of Network Dynamics Regulates Interneuron Survival in the Developing Cortex. Neuron 105, 75-92.e5. https://doi.org/10.1016/j.neuron.2019.10.008

Duhne, M., Lara-González, E., Laville, A., Padilla-Orozco, M., Ávila-Cascajares, F., Arias-García, M., Galarraga, E., Bargas, J., 2020. Activation of parvalbumin-expressing neurons reconfigures neuronal ensembles in murine striatal microcircuits. European Journal of Neuroscience 00, 1–6. https://doi.org/10.1111/ejn.14670

Durr, M.-R.R., Petryk, S., Mela, M., DesRoches, A., Wekerle, M., Newaz, S., 2021. Utilization of psychotropic medications in children with FASD: a retrospective review. BMC Pediatr 21, 512. https://doi.org/10.1186/s12887-021-02986-5

El Shawa, H., Abbott, C.W., Huffman, K.J., 2013. Prenatal Ethanol Exposure Disrupts Intraneocortical Circuitry, Cortical Gene Expression, and Behavior in a Mouse Model of FASD. J. Neurosci. 33, 18893–18905. https://doi.org/10.1523/JNEUROSCI.3721-13.2013

Elder, G.A., Gama Sosa, M.A., De Gasperi, R., 2010. Transgenic Mouse Models of Alzheimer's Disease. Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine 77, 69–81. https://doi.org/10.1002/msj.20159

Elliott, E.J., Payne, J., Haan, E., Bower, C., 2006. Diagnosis of foetal alcohol syndrome and alcohol use in pregnancy: a survey of paediatricians' knowledge, attitudes and practice. J Paediatr Child Health 42, 698–703. https://doi.org/10.1111/j.1440-1754.2006.00954.x

Euston, D.R., Gruber, A.J., McNaughton, B.L., 2012. The role of medial prefrontal cortex in memory and decision making. Neuron 76, 1057–1070. https://doi.org/10.1016/j.neuron.2012.12.002

Fagiolini, M., Fritschy, J.-M., Löw, K., Möhler, H., Rudolph, U., Hensch, T.K., 2004. Specific GABAA circuits for visual cortical plasticity. Science 303, 1681–1683. https://doi.org/10.1126/science.1091032

Ferguson, B.R., Gao, W.-J., 2015. Development of thalamocortical connections between the mediodorsal thalamus and the prefrontal cortex and its implication in cognition. Frontiers in Human Neuroscience 8.

Ferino, F., Thierry, A.M., Saffroy, M., Glowinski, J., 1987. Interhemispheric and subcortical collaterals of medial prefrontal cortical neurons in the rat. Brain Research 417, 257–266. https://doi.org/10.1016/0006-8993(87)90450-1

Fino, E., Vandecasteele, M., Perez, S., Saudou, F., Venance, L., 2018. Region-specific and state-dependent action of striatal GABAergic interneurons. Nature Communications 9, 1–17. https://doi.org/10.1038/s41467-018-05847-5

Fino, E., Venance, L., 2010. Spike-timing dependent plasticity in the striatum. Front. Synaptic Neurosci. 2. https://doi.org/10.3389/fnsyn.2010.00006
Fish, B.S., Rank, S.A., Wilson, J.R., Collins, A.C., 1981. Viability and sensorimotor development of mice exposed to prenatal short-term ethanol. Pharmacology Biochemistry and Behavior 14, 57–65. https://doi.org/10.1016/0091-3057(81)90103-9

Fishell, G., Kooy, D. van der, 1987. Pattern formation in the striatum: developmental changes in the distribution of striatonigral neurons. J. Neurosci. 7, 1969–1978. https://doi.org/10.1523/JNEUROSCI.07-07-01969.1987

Flannigan, K., Coons-Harding, K.D., Anderson, T., Wolfson, L., Campbell, A., Mela, M., Pei, J., 2020. A Systematic Review of Interventions to Improve Mental Health and Substance Use Outcomes for Individuals with Prenatal Alcohol Exposure and Fetal Alcohol Spectrum Disorder. Alcohol: Clinical and Experimental Research 44, 2401–2430. https://doi.org/10.1111/acer.14490

Flannigan, K., Gill, K., Pei, J., Andrew, G., Rajani, H., McFarlane, A., O'Riordan, T., Symes, B., Rasmussen, C., 2019. Deferred diagnosis in children assessed for fetal alcohol spectrum disorder. Appl Neuropsychol Child 8, 213–222. https://doi.org/10.1080/21622965.2018.1427094

Floresco, S.B., Grace, A.A., 2003. Gating of Hippocampal-Evoked Activity in Prefrontal Cortical
Neurons by Inputs from the Mediodorsal Thalamus and Ventral Tegmental Area. J. Neurosci. 23, 3930–3943. https://doi.org/10.1523/JNEUROSCI.23-09-03930.2003

Floresco, S.B., Seamans, J.K., Phillips, A.G., 1997. Selective Roles for Hippocampal, Prefrontal Cortical, and Ventral Striatal Circuits in Radial-Arm Maze Tasks With or Without a Delay. J. Neurosci. 17, 1880–1890. https://doi.org/10.1523/JNEUROSCI.17-05-01880.1997

Fogarty, M., Grist, M., Gelman, D., Marín, O., Pachnis, V., Kessaris, N., 2007. Spatial Genetic Patterning of the Embryonic Neuroepithelium Generates GABAergic Interneuron Diversity in the Adult Cortex. J. Neurosci. 27, 10935–10946. https://doi.org/10.1523/JNEUROSCI.1629-07.2007

Fryer, S.L., Tapert, S.F., Mattson, S.N., Paulus, M.P., Spadoni, A.D., Riley, E.P., 2007. Prenatal Alcohol Exposure Affects Frontal–Striatal BOLD Response During Inhibitory Control. Alcohol: Clinical and Experimental Research 31, 1415–1424. https://doi.org/10.1111/j.1530-0277.2007.00443.x

Gabbott, P.L.A., Warner, T.A., Busby, S.J., 2006. Amygdala input monosynaptically innervates parvalbumin immunoreactive local circuit neurons in rat medial prefrontal cortex. Neuroscience 139, 1039–1048. https://doi.org/10.1016/j.neuroscience.2006.01.026

Gabbott, P.L.A., Warner, T.A., Jays, P.R.L., Salway, P., Busby, S.J., 2005. Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. Journal of Comparative Neurology 492, 145–177. https://doi.org/10.1002/cne.20738

Gage, G.J., Stoetzner, C.R., Wiltschko, A.B., Berke, J.D., 2010. Selective Activation of Striatal Fast Spiking Interneurons during Choice Execution. Neuron 67, 466–479. https://doi.org/10.1016/j.neuron.2010.06.034

Garrison, L., Morley, S., Chambers, C.D., Bakhireva, L.N., 2019. Forty Years of Assessing Neurodevelopmental and Behavioral Effects of Prenatal Alcohol Exposure in Infants: What Have We Learned? Alcoholism: Clinical and Experimental Research 43, 1632–1642. https://doi.org/10.1111/acer.14127

Gaykema, R.P.A., Van Weeghel, R., Hersh, L.B., Luiten, P.G.M., 1991. Prefrontal cortical projections to the cholinergic neurons in the basal forebrain. Journal of Comparative Neurology 303, 563–583. https://doi.org/10.1002/cne.903030405

Gazan, A., Rial, D., Schiffmann, S.N., 2019. Ablation of striatal somatostatin interneurons
affects MSN morphology and electrophysiological properties, and increases cocaine-induced hyperlocomotion in mice. European Journal of Neuroscience 51. https://doi.org/10.1111/ejn.14581

Gelman, D., Griveau, A., Dehorter, N., Teissier, A., Varela, C., Pla, R., Pierani, A., Marín, O., 2011. A Wide Diversity of Cortical GABAergic Interneurons Derives from the Embryonic Preoptic Area. J. Neurosci. 31, 16570–16580. https://doi.org/10.1523/JNEUROSCI.4068-11.2011

Gerfen, C., Baimbridge, K., Thibault, J., 1987. The neostriatal mosaic: III. Biochemical and developmental dissociation of patch-matrix mesostriatal systems. J Neurosci 7, 3935–3944. https://doi.org/10.1523/JNEUROSCI.07-12-03935.1987

Gerfen, C.R., Engber, T.M., Mahan, L.C., Susel, Z., Chase, T.N., Monsma, F.J., Sibley, D.R., 1990. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250, 1429–1432. https://doi.org/10.1126/science.2147780

Gertler, T.S., Chan, C.S., Surmeier, D.J., 2008. Dichotomous anatomical properties of adult striatal medium spiny neurons. J. Neurosci. 28, 10814–10824. https://doi.org/10.1523/JNEUROSCI.2660-08.2008

Ghit, A., Assal, D., Al-Shami, A.S., Hussein, D.E.E., 2021. GABAA receptors: structure, function, pharmacology, and related disorders. Journal of Genetic Engineering and Biotechnology 19, 123. https://doi.org/10.1186/s43141-021-00224-0

Gigg, J., Tan, A.M., Finch, D.M., 1994. Glutamatergic hippocampal formation projections to prefrontal cortex in the rat are regulated by GABAergic inhibition and show convergence with glutamatergic projections from the limbic thalamus. Hippocampus 4, 189–198. https://doi.org/10.1002/hipo.450040209

Gittis, A.H., Leventhal, D.K., Fensterheim, B.A., Pettibone, J.R., Berke, J.D., Kreitzer, A.C., 2011. Selective Inhibition of Striatal Fast-Spiking Interneurons Causes Dyskinesias. J Neurosci 31, 15727–15731. https://doi.org/10.1523/JNEUROSCI.3875-11.2011

Gittis, A.H., Nelson, A.B., Thwin, M.T., Palop, J.J., Kreitzer, A.C., 2010. Distinct roles of GABAergic interneurons in the regulation of striatal output pathways. J. Neurosci. 30, 2223–2234. https://doi.org/10.1523/JNEUROSCI.4870-09.2010

Goldberg, E.M., Jeong, H.-Y., Kruglikov, I., Tremblay, R., Lazarenko, R.M., Rudy, B., 2011. Rapid Developmental Maturation of Neocortical FS Cell Intrinsic Excitability. Cerebral Cortex 21, 666–682. https://doi.org/10.1093/cercor/bhq138

Goldberg, J.A., Ding, J.B., Surmeier, D.J., 2012. Muscarinic Modulation of Striatal Function and Circuitry, in: Fryer, A.D., Christopoulos, A., Nathanson, N.M. (Eds.), Muscarinic Receptors, Handbook of Experimental Pharmacology. Springer, Berlin, Heidelberg, pp. 223–241. https://doi.org/10.1007/978-3-642-23274-9_10

Goodwill, H.L., Manzano-Nieves, G., LaChance, P., Teramoto, S., Lin, S., Lopez, C., Stevenson, R.J., Theyel, B.B., Moore, C.I., Connors, B.W., Bath, K.G., 2018. Early Life Stress Drives Sex-Selective Impairment in Reversal Learning by Affecting Parvalbumin Interneurons in Orbitofrontal Cortex of Mice. Cell Reports 25, 2299-2307.e4. https://doi.org/10.1016/j.celrep.2018.11.010

Green, C. r., Mihic, A. m., Nikkel, S. m., Stade, B. c., Rasmussen, C., Munoz, D. p., Reynolds, J. n., 2009. Executive function deficits in children with fetal alcohol spectrum disorders (FASD) measured using the Cambridge Neuropsychological Tests Automated Battery (CANTAB). Journal of Child Psychology and Psychiatry 50, 688–697. https://doi.org/10.1111/j.1469-7610.2008.01990.x

Grillner, S., El Manira, A., 2020. Current Principles of Motor Control, with Special Reference to Vertebrate Locomotion. Physiological Reviews 100, 271–320. https://doi.org/10.1152/physrev.00015.2019

Gritton, H.J., Howe, W.M., Romano, M.F., DiFeliceantonio, A.G., Kramer, M.A., Saligrama, V., Bucklin, M.E., Zemel, D., Han, X., 2019. Unique contributions of parvalbumin and cholinergic interneurons in organizing striatal networks during movement. Nat Neurosci 22, 586–597. https://doi.org/10.1038/s41593-019-0341-3

Gulledge, A.T., Stuart, G.J., 2003. Excitatory actions of GABA in the cortex. Neuron 37, 299–309. https://doi.org/10.1016/s0896-6273(02)01146-7

Gustavsson, A., Norton, N., Fast, T., Frölich, L., Georges, J., Holzapfel, D., Kirabali, T., Krolak-Salmon, P., Rossini, P.M., Ferretti, M.T., Lanman, L., Chadha, A.S., van der Flier, W.M., 2023. Global estimates on the number of persons across the Alzheimer's disease continuum. Alzheimer's & Dementia 19, 658–670. https://doi.org/10.1002/alz.12694

Hamilton, D.A., Barto, D., Rodriguez, C.I., Magcalas, C.M., Fink, B.C., Rice, J.P., Bird, C.W., Davies, S., Savage, D.D., 2014. Effects of moderate prenatal ethanol exposure and age on social behavior, spatial response perseveration errors and motor behavior. Behavioural Brain Research 269, 44–54. https://doi.org/10.1016/j.bbr.2014.04.029

Hamilton, D.A., Kodituwakku, P., Sutherland, R.J., Savage, D.D., 2003. Children with Fetal Alcohol Syndrome are impaired at place learning but not cued-navigation in a virtual Morris water task. Behavioural Brain Research 143, 85–94. https://doi.org/10.1016/S0166-4328(03)00028-7

Hardy, J., Selkoe, D.J., 2002. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297, 353–356. https://doi.org/10.1126/science.1072994

Harris, K.D., Shepherd, G.M.G., 2015. The neocortical circuit: themes and variations. Nat Neurosci 18, 170–181. https://doi.org/10.1038/nn.3917

Hartung, H., Brockmann, M.D., Pöschel, B., Feo, V.D., Hanganu-Opatz, I.L., 2016. Thalamic and Entorhinal Network Activity Differently Modulates the Functional Development of Prefrontal–Hippocampal Interactions. J. Neurosci. 36, 3676–3690. https://doi.org/10.1523/JNEUROSCI.3232-15.2016

Heck, D.H., Roy, S., Xie, N., Waters, R.S., 2008. Prenatal alcohol exposure delays acquisition and use of skilled reaching movements in juvenile rats. Physiology & Behavior 94, 540–544. https://doi.org/10.1016/j.physbeh.2008.03.011

Heidbreder, C.A., Groenewegen, H.J., 2003. The medial prefrontal cortex in the rat: evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neuroscience & Biobehavioral Reviews 27, 555–579. https://doi.org/10.1016/j.neubiorev.2003.09.003

Heymann, D., Stern, Y., Cosentino, S., Tatarina-Nulman, O., Dorrejo, J.N., Gu, Y., 2016. The association between alcohol use and the progression of Alzheimer's disease. Curr Alzheimer Res 13, 1356–1362.

Hoffman, J.L., Faccidomo, S., Kim, M., Taylor, S.M., Agoglia, A.E., May, A.M., Smith, E.N., Wong, L.C., Hodge, C.W., 2019. Alcohol drinking exacerbates neural and behavioral pathology in the 3xTg-AD mouse model of Alzheimer's disease. Int Rev Neurobiol 148, 169–230. https://doi.org/10.1016/bs.irn.2019.10.017

Holly, E.N., Davatolhagh, M.F., España, R.A., Fuccillo, M.V., 2021. Striatal low-threshold spiking interneurons locally gate dopamine. Current Biology. https://doi.org/10.1016/j.cub.2021.06.081

Huang, W.-J., Zhang, X., Chen, W.-W., 2016. Association between alcohol and Alzheimer's disease (Review). Experimental and Therapeutic Medicine 12, 1247–1250. https://doi.org/10.3892/etm.2016.3455

Ipsiroglu, O., Berger, M., Lin, T., Elbe, D., Stockler, S., Carleton, B., 2015. Chapter 6 - Pathways to Overmedication and Polypharmacy: Case Examples from Adolescents with Fetal Alcohol Spectrum Disorders, in: Di Pietro, N., Illes, J. (Eds.), The Science and Ethics of Antipsychotic Use in Children. Academic Press, San Diego, pp. 125–148. https://doi.org/10.1016/B978-0-12-800016-8.00006-4

Izquierdo, A., Brigman, J.L., Radke, A.K., Rudebeck, P.H., Holmes, A., 2017. The neural basis of reversal learning: An updated perspective. Neuroscience 345, 12–26. https://doi.org/10.1016/j.neuroscience.2016.03.021

Jacobson, J.L., Akkaya-Hocagil, T., Ryan, L.M., Dodge, N.C., Richardson, G.A., Olson, H.C., Coles, C.D., Day, N.L., Cook, R.J., Jacobson, S.W., 2021. Effects of prenatal alcohol exposure on cognitive and behavioral development: Findings from a hierarchical meta-analysis of data from six prospective longitudinal U.S. cohorts. Alcohol Clin Exp Res 45, 2040–2058. https://doi.org/10.1111/acer.14686

Janiesch, P.C., Krüger, H.-S., Pöschel, B., Hanganu-Opatz, I.L., 2011. Cholinergic control in developing prefrontal-hippocampal networks. J Neurosci 31, 17955–17970. https://doi.org/10.1523/JNEUROSCI.2644-11.2011

Jankowsky, J.L., Zheng, H., 2017. Practical considerations for choosing a mouse model of Alzheimer's disease. Mol Neurodegener 12, 89. https://doi.org/10.1186/s13024-017-0231-7

Johansson, Y., Silberberg, G., 2020. The Functional Organization of Cortical and Thalamic Inputs onto Five Types of Striatal Neurons Is Determined by Source and Target Cell Identities. Cell Reports 30, 1178-1194.e3. https://doi.org/10.1016/j.celrep.2019.12.095

Johnston, D., Branton, E., Rasmuson, L., Schell, S., Gross, D.P., Pritchard-Wiart, L., 2019. Accuracy of motor assessment in the diagnosis of fetal alcohol spectrum disorder. BMC Pediatr 19, 1–9. https://doi.org/10.1186/s12887-019-1542-3

Kalemaki, K., Velli, A., Christodoulou, O., Denaxa, M., Karagogeos, D., Sidiropoulou, K., 2022. The developmental changes in intrinsic and synaptic properties of prefrontal neurons enhance local network activity from the second to the third postnatal weeks in mice. Cereb Cortex 32, 3633–3650. https://doi.org/10.1093/cercor/bhab438

Kalsbeek, A., Voorn, P., Buijs, R.M., Pool, C.W., Uylings, H.B., 1988. Development of the dopaminergic innervation in the prefrontal cortex of the rat. J Comp Neurol 269, 58–72. https://doi.org/10.1002/cne.902690105

Kambeitz, C., Klug, M.G., Greenmyer, J., Popova, S., Burd, L., 2019. Association of adverse childhood experiences and neurodevelopmental disorders in people with fetal alcohol spectrum disorders (FASD) and non-FASD controls. BMC Pediatrics 19, 498. https://doi.org/10.1186/s12887-019-1878-8

Kawaguchi, Y., 1993. Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. J. Neurosci. 13, 4908–4923.

Kelly, S.M., Raudales, R., He, M., Lee, J.H., Kim, Y., Gibb, L.G., Wu, P., Matho, K., Osten, P., Graybiel, A.M., Huang, Z.J., 2018. Radial Glial Lineage Progression and

Differential Intermediate Progenitor Amplification Underlie Striatal Compartments and Circuit Organization. Neuron 99, 345-361.e4. https://doi.org/10.1016/j.neuron.2018.06.021

Kelsom, C., Lu, W., 2013. Development and specification of GABAergic cortical interneurons. Cell Biosci 3, 19. https://doi.org/10.1186/2045-3701-3-19
Kemp, J.M., Powell, T.P.S., Harris, G.W., 1997a. The structure of the caudate nucleus of the cat: light and electron microscopy. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 262, 383–401. https://doi.org/10.1098/rstb.1971.0102

Kemp, J.M., Powell, T.P.S., Harris, G.W., 1997b. The site of termination of afferent fibres in the caudate nucleus. Philosophical Transactions of the Royal Society of London. B, Biological Sciences 262, 413–427. https://doi.org/10.1098/rstb.1971.0104

Kenton, J.A., Ontiveros, T., Bird, C.W., Valenzuela, C.F., Brigman, J.L., 2020. Moderate prenatal alcohol exposure alters the number and function of GABAergic interneurons in the murine orbitofrontal cortex. Alcohol. https://doi.org/10.1016/j.alcohol.2020.06.001
Kingdon, D., Cardoso, C., McGrath, J.J., 2016. Research Review: Executive function deficits in fetal alcohol spectrum disorders and attention-deficit/hyperactivity disorder – a meta-analysis. J Child Psychol Psychiatry 57, 116–131. https://doi.org/10.1111/jcpp.12451

Kiss, E., Gorgas, K., Schlicksupp, A., Groß, D., Kins, S., Kirsch, J., Kuhse, J., 2016. Biphasic Alteration of the Inhibitory Synapse Scaffold Protein Gephyrin in Early and Late Stages of an Alzheimer Disease Model. Am J Pathol 186, 2279–2291. https://doi.org/10.1016/j.ajpath.2016.05.013

Kocaturk, S., Guven, E.B., Shah, F., Tepper, J.M., Assous, M., 2022. Cholinergic control of striatal GABAergic microcircuits. Cell Rep 41, 111531. https://doi.org/10.1016/j.celrep.2022.111531
Koch, M., Costanzo, S., Fitzpatrick, A.L., Lopez, O.L., DeKosky, S., Kuller, L.H., Price, J., Mackey, R.H., Jensen, M.K., Mukamal, K.J., 2020. Alcohol Consumption, Brain Amyloid-β Deposition, and Brain Structural Integrity Among Older Adults Free of Dementia. Journal of Alzheimer's Disease 74, 509–519. https://doi.org/10.3233/JAD-190834

Kodali, V.N., Jacobson, J.L., Lindinger, N.M., Dodge, N.C., Molteno, C.D., Meintjes, E.M., Jacobson, S.W., 2017. Differential Recruitment of Brain Regions During Response Inhibition in Children Prenatally Exposed to Alcohol. Alcohol Clin Exp Res 41, 334–344. https://doi.org/10.1111/acer.13307

Kodituwakku, P.W., Handmaker, N.S., Cutler, S.K., Weathersby, E.K., Handmaker, S.D., 1995. Specific Impairments in Self-Regulation in Children Exposed to Alcohol Prenatally. Alcohol: Clinical and Experimental Research 19, 1558–1564. https://doi.org/10.1111/j.1530-0277.1995.tb01024.x

Kolk, S.M., Rakic, P., 2022. Development of prefrontal cortex. Neuropsychopharmacology 47, 41–57. https://doi.org/10.1038/s41386-021-01137-9

Kozorovitskiy, Y., Peixoto, R., Wang, W., Saunders, A., Sabatini, B.L., 2015. Neuromodulation

of excitatory synaptogenesis in striatal development. eLife 4, e10111. https://doi.org/10.7554/eLife.10111

Kozorovitskiy, Y., Saunders, A., Johnson, C.A., Lowell, B.B., Sabatini, B.L., 2012. Recurrent network activity drives striatal synaptogenesis. Nature 485, 646–650. https://doi.org/10.1038/nature11052

Krajeski, R.N., Macey-Dare, A., Heusden, F. van, Ebrahimjee, F., Ellender, T.J., 2019. Dynamic postnatal development of the cellular and circuit properties of striatal D1 and D2 spiny projection neurons. The Journal of Physiology 597, 5265–5293. https://doi.org/10.1113/JP278416

Kress, G.J., Yamawaki, N., Wokosin, D.L., Wickersham, I.R., Shepherd, G.M.G., Surmeier, D.J., 2013. Convergent cortical innervation of striatal projection neurons. Nat Neurosci 16, 665–667. https://doi.org/10.1038/nn.3397

Kurucu, H., Colom-Cadena, M., Davies, C., Wilkins, L., King, D., Rose, J., Tzioras, M., Tulloch, J.H., Smith, C., Spires-Jones, T.L., 2021. Inhibitory synapse loss and accumulation of amyloid beta in inhibitory presynaptic terminals in Alzheimer's disease. Eur J Neurol. https://doi.org/10.1111/ene.15043

Lane, C.A., Hardy, J., Schott, J.M., 2018. Alzheimer's disease. European Journal of Neurology 25, 59–70. https://doi.org/10.1111/ene.13439
Lange, S., Probst, C., Gmel, G., Rehm, J., Burd, L., Popova, S., 2017. Global Prevalence of Fetal Alcohol Spectrum Disorder Among Children and Youth. JAMA Pediatr 171, 948–956. https://doi.org/10.1001/jamapediatrics.2017.1919

Laurie, D.J., Wisden, W., Seeburg, P.H., 1992. The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development. J. Neurosci. 12, 4151–4172. https://doi.org/10.1523/JNEUROSCI.12-11-04151.1992

Lee, H.J., Weitz, A.J., Bernal-Casas, D., Duffy, B.A., Choy, M., Kravitz, A.V., Kreitzer, A.C., Lee, J.H., 2016. Activation of direct and indirect pathway medium spiny neurons drives distinct brain-wide responses. Neuron 91, 412–424. https://doi.org/10.1016/j.neuron.2016.06.010
Lee, K., Holley, S.M., Shobe, J.L., Chong, N.C., Cepeda, C., Levine, M.S., Masmanidis, S.C., 2017. Parvalbumin Interneurons Modulate Striatal Output and Enhance Performance during Associative Learning. Neuron 93, 1451-1463.e4. https://doi.org/10.1016/j.neuron.2017.02.033

Lee, S.M., Yeh, P.W.L., Yeh, H.H., 2022. L-Type Calcium Channels Contribute to Ethanol-Induced Aberrant Tangential Migration of Primordial Cortical GABAergic Interneurons in the Embryonic Medial Prefrontal Cortex. eNeuro 9, ENEURO.0359-21.2021. https://doi.org/10.1523/ENEURO.0359-21.2021

Léger, C., Dupré, N., Laquerrière, A., Lecointre, M., Dumanoir, M., Janin, F., Hauchecorne, M., Fabre, M., Jégou, S., Frébourg, T., Cleren, C., Leroux, P., Marcorelles, P., Brasse-Lagnel, C., Marret, S., Marguet, F., Gonzalez, B.J., 2020. In utero alcohol exposure exacerbates endothelial protease activity from pial microvessels and impairs GABA interneuron positioning. Neurobiology of Disease 105074. https://doi.org/10.1016/j.nbd.2020.105074

Leigland, L.A., Ford, M.M., Lerch, J.P., Kroenke, C.D., 2013. The influence of fetal ethanol exposure on subsequent development of the cerebral cortex as revealed by magnetic resonance imaging. Alcohol Clin Exp Res 37, 924–932. https://doi.org/10.1111/acer.12051

León, B.E., Kang, S., Franca-Solomon, G., Shang, P., Choi, D.-S., 2022. Alcohol-Induced Neuroinflammatory Response and Mitochondrial Dysfunction on Aging and Alzheimer's Disease. Frontiers in Behavioral Neuroscience 15.

Leung, L., Andrews-Zwilling, Y., Yoon, S.Y., Jain, S., Ring, K., Dai, J., Wang, M.M., Tong, L., Walker, D., Huang, Y., 2012. Apolipoprotein E4 causes age- and sex-dependent impairments of hilar GABAergic interneurons and learning and memory deficits in mice. PLoS One 7, e53569. https://doi.org/10.1371/journal.pone.0053569

Levitt, P., Moore, R.Y., 1979. Development of the noradrenergic innervation of neocortex. Brain Res 162, 243–259. https://doi.org/10.1016/0006-8993(79)90287-7
Li, Y., Zhu, K., Li, N., Wang, Xiaotong, Xiao, X., Li, Linying, Li, Lijuan, He, Y., Zhang, Jinglan, Wo, J., Cui, Y., Huang, H., Zhang, Jianliang, Wang, W., Wang, Xiaomin, Zheng, Y., 2021. Reversible GABAergic dysfunction involved in hippocampal hyperactivity predicts early-stage Alzheimer disease in a mouse model. Alz Res Therapy 13, 1–14. https://doi.org/10.1186/s13195-021-00859-8

Lieberman, O.J., McGuirt, A.F., Mosharov, E.V., Pigulevskiy, I., Hobson, B.D., Choi, S., Frier, M.D., Santini, E., Borgkvist, A., Sulzer, D., 2018. Dopamine Triggers the Maturation of Striatal Spiny Projection Neuron Excitability during a Critical Period. Neuron 99, 540-554.e4. https://doi.org/10.1016/j.neuron.2018.06.044

Liu, P., Wang, Y., Sun, Y., Peng, G., 2022. Neuroinflammation as a Potential Therapeutic Target in Alzheimer's Disease. Clinical Interventions in Aging 17, 665–674. https://doi.org/10.2147/CIA.S357558

Lodato, S., Rouaux, C., Quast, K.B., Jantrachotechatchawan, C., Studer, M., Hensch, T.K., Arlotta, P., 2011. Excitatory Projection Neuron Subtypes Control the Distribution of Local Inhibitory Interneurons in the Cerebral Cortex. Neuron 69, 763–779. https://doi.org/10.1016/j.neuron.2011.01.015

López-Bendito, G., Cautinat, A., Sánchez, J.A., Bielle, F., Flames, N., Garratt, A.N., Talmage, D.A., Role, L.W., Charnay, P., Marín, O., Garel, S., 2006. Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation. Cell 125, 127–142. https://doi.org/10.1016/j.cell.2006.01.042

Lopez-Huerta, V.G., Nakano, Y., Bausenwein, J., Jaidar, O., Lazarus, M., Cherassse, Y., Garcia-Munoz, M., Arbuthnott, G., 2016. The neostriatum: two entities, one structure? Brain Struct Funct 221, 1737–1749. https://doi.org/10.1007/s00429-015-1000-4

Lott, I.T., Head, E., 2019. Dementia in Down syndrome: unique insights for Alzheimer disease research. Nat Rev Neurol 15, 135–147. https://doi.org/10.1038/s41582-018-0132-6

LoTurco, J.J., Owens, D.F., Heath, M.J.S., Davis, M.B.E., Kriegstein, A.R., 1995. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 15, 1287–1298. https://doi.org/10.1016/0896-6273(95)90008-X

Louth, E.L., Bignell, W., Taylor, C.L., Bailey, C.D.C., 2016. Developmental Ethanol Exposure
Leads to Long-Term Deficits in Attention and Its Underlying Prefrontal Circuitry. eNeuro 3, ENEURO.0267-16.2016. https://doi.org/10.1523/ENEURO.0267-16.2016

Louth, E.L., Luctkar, H.D., Heney, K.A., Bailey, C.D.C., 2018. Developmental ethanol exposure alters the morphology of mouse prefrontal neurons in a layer-specific manner. Brain Res 1678, 94–105. https://doi.org/10.1016/j.brainres.2017.10.005

Lovinger, D.M., Alvarez, V.A., 2017. Alcohol and basal ganglia circuitry: Animal models. Neuropharmacology 122, 46–55. https://doi.org/10.1016/j.neuropharm.2017.03.023
Lucas, B.R., Latimer, J., Pinto, R.Z., Ferreira, M.L., Doney, R., Lau, M., Jones, T., Dries, D., Elliott, E.J., 2014. Gross motor deficits in children prenatally exposed to alcohol: a meta-analysis. Pediatrics 134, e192-209. https://doi.org/10.1542/peds.2013-3733

Luhmann, H.J., Prince, D.A., 1991. Postnatal maturation of the GABAergic system in rat neocortex. Journal of Neurophysiology 65, 247–263.
https://doi.org/10.1152/jn.1991.65.2.247

Manji, S., Pei, J., Loomes, C., Rasmussen, C., 2009. A review of the verbal and visual memory impairments in children with foetal alcohol spectrum disorders. Dev Neurorehabil 12, 239–247. https://doi.org/10.1080/17518420902980118

Mantha, K., Kleiber, M., Singh, S., 2013. Neurodevelopmental Timing of Ethanol Exposure May Contribute to Observed Heterogeneity of Behavioral Deficits in a Mouse Model of Fetal Alcohol Spectrum Disorder (FASD). Journal of Behavioral and Brain Science 3, 85–99. https://doi.org/10.4236/jbbs.2013.31009

Marek, R., Jin, J., Goode, T.D., Giustino, T.F., Wang, Q., Acca, G.M., Holehonnur, R., Ploski, J.E., Fitzgerald, P.J., Lynagh, T., Lynch, J.W., Maren, S., Sah, P., 2018. Hippocampus-driven feed-forward inhibition of the prefrontal cortex mediates relapse of extinguished fear. Nat Neurosci 21, 384–392. https://doi.org/10.1038/s41593-018-0073-9

Marguet, F., Friocourt, G., Brosolo, M., Sauvestre, F., Marcorelles, P., Lesueur, C., Marret, S., Gonzalez, B.J., Laquerrière, A., 2020. Prenatal alcohol exposure is a leading cause of interneuronopathy in humans. Acta Neuropathologica Communications 8, 208. https://doi.org/10.1186/s40478-020-01089-z

Marin, O., Anderson, S.A., Rubenstein, J.L., 2000. Origin and molecular specification of striatal interneurons. J. Neurosci. 20, 6063–6076.

Marín, O., Rubenstein, J.L.R., 2001. A long, remarkable journey: Tangential migration in the telencephalon. Nature Reviews Neuroscience 2, 780.
https://doi.org/10.1038/35097509

Marquardt, K., Brigman, J.L., 2016. The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: Insights from rodent models. Alcohol 51, 1–15. https://doi.org/10.1016/j.alcohol.2015.12.002

Marquardt, K., Cavanagh, J.F., Brigman, J.L., 2020. Alcohol exposure in utero disrupts cortico-striatal coordination required for behavioral flexibility. Neuropharmacology 162, 107832. https://doi.org/10.1016/j.neuropharm.2019.107832

Marsland, P., Vore, A.S., DaPrano, E., Paluch, J.M., Blackwell, A.A., Varlinskaya, E.I., Deak, T., 2022. Sex-specific effects of ethanol consumption in older Fischer 344 rats on microglial dynamics and Aβ(1-42) accumulation. Alcohol S0741-8329(22)00097–0. https://doi.org/10.1016/j.alcohol.2022.08.013

Martiros, N., Burgess, A.A., Graybiel, A.M., 2018. Inversely Active Striatal Projection Neurons and Interneurons Selectively Delimit Useful Behavioral Sequences. Current Biology 28, 560-573.e5. https://doi.org/10.1016/j.cub.2018.01.031
Matityahu, L., Malgady, J.M., Schirelman, M., Johansson, Y., Wilking, J.A., Silberberg, G.,

Goldberg, J.A., Plotkin, J.L., 2022. A tonic nicotinic brake controls spike timing in striatal spiny projection neurons. eLife 11, e75829. https://doi.org/10.7554/eLife.75829

Matsushima, A., Graybiel, A.M., 2020. Combinatorial Developmental Controls on Striatonigral Circuits. Cell Reports 31, 107778. https://doi.org/10.1016/j.celrep.2020.107778

Mattson, S.N., Goodman, A.M., Caine, C., Delis, D.C., Riley, E.P., 1999. Executive Functioning in Children With Heavy Prenatal Alcohol Exposure. Alcohol: Clinical and Experimental Research 23, 1808–1815. https://doi.org/10.1111/j.1530-0277.1999.tb04077.x

Mattson, S. N., Riley, E.P., Delis, D.C., Stern, C., Jones, K.L., 1996. Verbal learning and memory in children with fetal alcohol syndrome. Alcohol Clin Exp Res 20, 810–816. https://doi.org/10.1111/j.1530-0277.1996.tb05256.x

Mattson, Sarah N., Riley, E.P., Sowell, E.R., Jernigan, T.L., Sobel, D.F., Jones, K.L., 1996. A Decrease in the Size of the Basal Ganglia in Children with Fetal Alcohol Syndrome. Alcohol: Clinical and Experimental Research 20, 1088–1093. https://doi.org/10.1111/j.1530-0277.1996.tb01951.x

May, P.A., Baete, A., Russo, J., Elliott, A.J., Blankenship, J., Kalberg, W.O., Buckley, D., Brooks, M., Hasken, J., Abdul-Rahman, O., Adam, M.P., Robinson, L.K., Manning, M., Hoyme, H.E., 2014. Prevalence and Characteristics of Fetal Alcohol Spectrum Disorders. Pediatrics 134, 855–866. https://doi.org/10.1542/peds.2013-3319

McGarry, L.M., Carter, A.G., 2016. Inhibitory Gating of Basolateral Amygdala Inputs to the Prefrontal Cortex. J. Neurosci. 36, 9391–9406. https://doi.org/10.1523/JNEUROSCI.0874-16.2016

McGuirt, A., Pigulevskiy, I., Sulzer, D., 2022. Developmental regulation of thalamus-driven pauses in striatal cholinergic interneurons. iScience 25, 105332. https://doi.org/10.1016/j.isci.2022.105332

McGuirt, A., Post, M., Pigulevskiy, I., Sulzer, D., Lieberman, O., 2021. Coordinated postnatal maturation of striatal cholinergic interneurons and dopamine release dynamics in mice. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.0755-20.2021

Mela, M., Hanlon-Dearman, A., Ahmed, A.G., Rich, S.D., Densmore, R., Reid, D., Barr, A.M., Osser, D., Anderson, T., Suberu, B., Ipsiroglu, O., Rajani, H., Loock, C., 2020. Treatment
algorithm for the use of psychopharmacological agents in individuals prenatally exposed to alcohol and/or with diagnosis of fetal alcohol spectrum disorder (FASD). J Popl Ther Clin Pharmacol 27, e1–e13. https://doi.org/10.15586/jptcp.v27i3.681

Métin, C., Godement, P., 1996. The ganglionic eminence may be an intermediate target for
corticofugal and thalamocortical axons. J Neurosci 16, 3219–3235. https://doi.org/10.1523/JNEUROSCI.16-10-03219.1996

Milad, M.R., Quirk, G.J., 2002. Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420, 70–74. https://doi.org/10.1038/nature01138

Miller, M.W., 2017. Effect of prenatal exposure to ethanol on the pyramidal tract in developing rats. Brain Res 1672, 122–128. https://doi.org/10.1016/j.brainres.2017.07.028

Miller, M.W., 1985. Cogeneration of retrogradely labeled corticocortical projection and GABA-immunoreactive local circuit neurons in cerebral cortex. Developmental Brain Research 23, 187–192. https://doi.org/10.1016/0165-3806(85)90040-9

Mitew, S., Kirkcaldie, M.T.K., Dickson, T.C., Vickers, J.C., 2013. Altered synapses and gliotransmission in Alzheimer's disease and AD model mice. Neurobiol Aging 34, 2341–2351. https://doi.org/10.1016/j.neurobiolaging.2013.04.010

Modol, L., Bollmann, Y., Tressard, T., Baude, A., Che, A., Duan, Z.R.S., Babij, R., De Marco García, N.V., Cossart, R., 2020. Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex. Neuron 105, 93-105.e4. https://doi.org/10.1016/j.neuron.2019.10.007

Mohammad, S., Page, S.J., Wang, L., Ishii, S., Li, P., Sasaki, T., Basha, A., Salzberg, A., Quezado, Z., Imamura, F., Nishi, H., Isaka, K., Corbin, J.G., Liu, J.S., Kawasawa, Y.I., Torii, M., Hashimoto-Torii, K., 2020. Kcnn2 blockade reverses learning deficits in a mouse model of fetal alcohol spectrum disorders. Nature Neuroscience 23, 533–543. https://doi.org/10.1038/s41593-020-0592-z

Molina, J.C., Hoffmann, H., Spear, L.P., Spear, N.E., 1987. Sensorimotor maturation and alcohol responsiveness in rats prenatally exposed to alcohol during gestational day 8. Neurotoxicology and Teratology 9, 121–128. https://doi.org/10.1016/0892-0362(87)90088-2

Molnár, Z., Adams, R., Blakemore, C., 1998. Mechanisms Underlying the Early Establishment of Thalamocortical Connections in the Rat. J. Neurosci. 18, 5723–5745. https://doi.org/10.1523/JNEUROSCI.18-15-05723.1998

Monteiro, P., Barak, B., Zhou, Y., McRae, R., Rodrigues, D., Wickersham, I.R., Feng, G., 2018. Dichotomous parvalbumin interneuron populations in dorsolateral and dorsomedial striatum. The Journal of Physiology 596, 3695–3707. https://doi.org/10.1113/JP275936

Moore, E.M., Glass, L., Infante, M.A., Coles, C.D., Kable, J.A., Jones, K.L., Riley, E.P., Mattson, S.N., 2021. Cross-Sectional Analysis of Spatial Working Memory Development in Children with Histories of Heavy Prenatal Alcohol Exposure. Alcohol Clin Exp Res 45, 215–223. https://doi.org/10.1111/acer.14506

Moore, E.M., Riley, E.P., 2015. What Happens When Children with Fetal Alcohol Spectrum Disorders Become Adults? Curr Dev Disord Rep 2, 219–227. https://doi.org/10.1007/s40474-015-0053-7

Morgan, M.A., Romanski, L.M., LeDoux, J.E., 1993. Extinction of emotional learning: Contribution of medial prefrontal cortex. Neuroscience Letters 163, 109–113. https://doi.org/10.1016/0304-3940(93)90241-C

Muñoz, G., Urrutia, J.C., Burgos, C.F., Silva, V., Aguilar, F., Sama, M., Yeh, H.H., Opazo, C., Aguayo, L.G., 2015. Low concentrations of ethanol protect against synaptotoxicity induced by Aβ in hippocampal neurons. Neurobiology of Aging 36, 845–856. https://doi.org/10.1016/j.neurobiolaging.2014.10.017

Muñoz-Manchado, A.B., Bengtsson Gonzales, C., Zeisel, A., Munguba, H., Bekkouche, B., Skene, N.G., Lönnerberg, P., Ryge, J., Harris, K.D., Linnarsson, S., Hjerling-Leffler, J., 2018. Diversity of Interneurons in the Dorsal Striatum Revealed by Single-Cell RNA Sequencing and PatchSeq. Cell Reports 24, 2179-2190.e7. https://doi.org/10.1016/j.celrep.2018.07.053

Murray, A.J., Sauer, J.-F., Riedel, G., McClure, C., Ansel, L., Cheyne, L., Bartos, M., Wisden, W., Wulff, P., 2011. Parvalbumin-positive CA1 interneurons are required for spatial working but not for reference memory. Nat Neurosci 14, 297–299. https://doi.org/10.1038/nn.2751

Newman, H., Liu, F.-C., Graybiel, A.M., 2015. Dynamic ordering of early generated striatal cells destined to form the striosomal compartment of the striatum. J. Comp. Neurol. 523, 943–962. https://doi.org/10.1002/cne.23725

Noctor, S.C., Martínez-Cerdeño, V., Ivic, L., Kriegstein, A.R., 2004. Cortical neurons arise in symmetric and asymmetric division zones and migrate through specific phases. Nat Neurosci 7, 136–144. https://doi.org/10.1038/nn1172
Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R.,

Mattson, M.P., Akbari, Y., LaFerla, F.M., 2003. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39, 409–421. https://doi.org/10.1016/s0896-6273(03)00434-3

Oh, K.-J., Perez, S.E., Lagalwar, S., Vana, L., Binder, L., Mufson, E.J., 2010. Staging of Alzheimer's pathology in triple transgenic mice: a light and electron microscopic analysis. Int J Alzheimers Dis 2010, 780102. https://doi.org/10.4061/2010/780102

Oh, W.C., Lutzu, S., Castillo, P.E., Kwon, H.-B., 2016. De novo synaptogenesis induced by GABA in the developing mouse cortex. Science 353, 1037–1040. https://doi.org/10.1126/science.aaf5206

O'Hare, J.K., Ade, K.K., Sukharnikova, T., Van Hooser, S.D., Palmeri, M.L., Yin, H.H., Calakos, N., 2016. Pathway-Specific Striatal Substrates for Habitual Behavior. Neuron 89, 472–479. https://doi.org/10.1016/j.neuron.2015.12.032

Okaty, B.W., Miller, M.N., Sugino, K., Hempel, C.M., Nelson, S.B., 2009. Transcriptional and Electrophysiological Maturation of Neocortical Fast-Spiking GABAergic Interneurons. J. Neurosci. 29, 7040–7052. https://doi.org/10.1523/JNEUROSCI.0105-09.2009

Olateju, O.I., Ihunwo, A.O., Manger, P.R., 2019. Changes to the somatosensory barrel cortex in C57BL/6J mice at early adulthood (56 days post-natal) following prenatal alcohol exposure. J Chem Neuroanat 96, 49–56. https://doi.org/10.1016/j.jchemneu.2018.12.006

Owen, S.F., Berke, J.D., Kreitzer, A.C., 2018. Fast-Spiking Interneurons Supply Feedforward Control of Bursting, Calcium, and Plasticity for Efficient Learning. Cell 172, 683-695.e15. https://doi.org/10.1016/j.cell.2018.01.005

Palop, J.J., Mucke, L., 2016. Network abnormalities and interneuron dysfunction in Alzheimer disease. Nat Rev Neurosci 17, 777–792. https://doi.org/10.1038/nrn.2016.141

Partridge, J.G., Tang, K.-C., Lovinger, D.M., 2000. Regional and Postnatal Heterogeneity of Activity-Dependent Long-Term Changes in Synaptic Efficacy in the Dorsal Striatum. Journal of Neurophysiology 84, 1422–1429. https://doi.org/10.1152/jn.2000.84.3.1422

Patten, A.R., Fontaine, C.J., Christie, B.R., 2014. A Comparison of the Different Animal Models of Fetal Alcohol Spectrum Disorders and Their Use in Studying Complex Behaviors. Frontiers in Pediatrics 2.

Patton, M.H., Roberts, B.M., Lovinger, D.M., Mathur, B.N., 2016. Ethanol Disinhibits Dorsolateral Striatal Medium Spiny Neurons Through Activation of A Presynaptic Delta Opioid Receptor. Neuropsychopharmacology 41, 1831–1840. https://doi.org/10.1038/npp.2015.353

Peadon, E., Fremantle, E., Bower, C., Elliott, E.J., 2008. International survey of diagnostic services for children with Fetal Alcohol Spectrum Disorders. BMC Pediatr 8, 12. https://doi.org/10.1186/1471-2431-8-12

Pei, J., Job, J., Kully-Martens, K., Rasmussen, C., 2011. Executive function and memory in children with Fetal Alcohol Spectrum Disorder. Child Neuropsychol 17, 290–309. https://doi.org/10.1080/09297049.2010.544650

Peixoto, R.T., Wang, W., Croney, D.M., Kozorovitskiy, Y., Sabatini, B.L., 2016. Early hyperactivity and precocious maturation of corticostriatal circuits in Shank3B −/− mice. Nature Neuroscience 19, 716–724. https://doi.org/10.1038/nn.4260
Peng, B., Yang, Q., B Joshi, R., Liu, Y., Akbar, M., Song, B.-J., Zhou, S., Wang, X., 2020. Role of Alcohol Drinking in Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis. International Journal of Molecular Sciences 21, 2316. https://doi.org/10.3390/ijms21072316

Petrenko, C.L.M., Alto, M.E., 2017. Interventions in fetal alcohol spectrum disorders: An international perspective. European Journal of Medical Genetics, Special issue on Environmental Teratogens 60, 79–91. https://doi.org/10.1016/j.ejmg.2016.10.005

Petrenko, C.L.M., Tahir, N., Mahoney, E.C., Chin, N.P., 2014. Prevention of Secondary Conditions in Fetal Alcohol Spectrum Disorders: Identification of Systems-Level Barriers. Matern Child Health J 18, 1496–1505. https://doi.org/10.1007/s10995-013-1390-y

Pilz, G.-A., Shitamukai, A., Reillo, I., Pacary, E., Schwausch, J., Stahl, R., Ninkovic, J., Snippert, H.J., Clevers, H., Godinho, L., Guillemot, F., Borrell, V., Matsuzaki, F., Götz, M., 2013. Amplification of progenitors in the mammalian telencephalon includes a new radial glial cell type. Nat Commun 4, 2125. https://doi.org/10.1038/ncomms3125

Pinto, L., Dan, Y., 2015. Cell-Type-Specific Activity in Prefrontal Cortex during Goal-Directed Behavior. Neuron 87, 437–450. https://doi.org/10.1016/j.neuron.2015.06.021
Pires, J., Nelissen, R., Mansvelder, H.D., Meredith, R.M., 2021. Spontaneous Synchronous Network Activity in the Neonatal Development of mPFC in Mice. Developmental Neurobiology 81, 207–225. https://doi.org/10.1002/dneu.22811

Planert, H., Szydlowski, S.N., Hjorth, J.J.J., Grillner, S., Silberberg, G., 2010. Dynamics of Synaptic Transmission between Fast-Spiking Interneurons and Striatal Projection Neurons of the Direct and Indirect Pathways. J. Neurosci. 30, 3499–3507. https://doi.org/10.1523/JNEUROSCI.5139-09.2010

Plotkin, J.L., Wu, N., Chesselet, M.-F., Levine, M.S., 2005. Functional and molecular development of striatal fast-spiking GABAergic interneurons and their cortical inputs. European Journal of Neuroscience 22, 1097–1108. https://doi.org/10.1111/j.1460-9568.2005.04303.x
Pommer, S., Akamine, Y., Schiffmann, S.N., d'Exaerde, A. de K., Wickens, J.R., 2021. The Effect of Serotonin Receptor 5-HT1B on Lateral Inhibition between Spiny Projection Neurons in the Mouse Striatum. J. Neurosci. 41, 7831–7847. https://doi.org/10.1523/JNEUROSCI.1037-20.2021

Prince, S.M., Paulson, A.L., Jeong, N., Zhang, L., Amigues, S., Singer, A.C., 2021. Alzheimer's pathology causes impaired inhibitory connections and reactivation of spatial codes during spatial navigation. Cell Reports 35. https://doi.org/10.1016/j.celrep.2021.109008

Reh, R.K., Dias, B.G., Nelson, C.A., Kaufer, D., Werker, J.F., Kolb, B., Levine, J.D., Hensch, T.K., 2020. Critical period regulation across multiple timescales. Proceedings of the National Academy of Sciences 117, 23242–23251. https://doi.org/10.1073/pnas.1820836117

Reiner, A., Hart, N.M., Lei, W., Deng, Y., 2010. Corticostriatal Projection Neurons – Dichotomous Types and Dichotomous Functions. Front. Neuroanat. 4. https://doi.org/10.3389/fnana.2010.00142

Rice, D., Barone, S., 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environmental Health Perspectives 108, 511–533. https://doi.org/10.1289/ehp.00108s3511

Rice, J.P., Suggs, L.E., Lusk, A.V., Parker, M.O., Candelaria-Cook, F.T., Akers, K.G., Savage, D.D., Hamilton, D.A., 2012. Effects of exposure to moderate levels of ethanol during prenatal brain development on dendritic length, branching, and spine density in the nucleus accumbens and dorsal striatum of adult rats. Alcohol 46, 577–584. https://doi.org/10.1016/j.alcohol.2011.11.008

Roberts, B.M., White, M.G., Patton, M.H., Chen, R., Mathur, B.N., 2019. Ensemble encoding of action speed by striatal fast-spiking interneurons. Brain Struct Funct 224, 2567–2576. https://doi.org/10.1007/s00429-019-01908-7

Rushworth, M.F.S., Noonan, M.P., Boorman, E.D., Walton, M.E., Behrens, T.E., 2011. Frontal Cortex and Reward-Guided Learning and Decision-Making. Neuron 70, 1054–1069. https://doi.org/10.1016/j.neuron.2011.05.014

Sack, L., Dollaghan, C., Goffman, L., 2021. Contributions of early motor deficits in predicting language outcomes among preschoolers with developmental language disorder. Int J Speech Lang Pathol 1–13. https://doi.org/10.1080/17549507.2021.1998629

Sadikot, A.F., Sasseville, R., 1997. Neurogenesis in the mammalian neostriatum and nucleus accumbens: parvalbumin-immunoreactive GABAergic interneurons. J. Comp. Neurol. 389, 193–211.

Saito, Mariko, Smiley, J.F., Hui, M., Masiello, K., Betz, J., Ilina, M., Saito, Mitsuo, Wilson, D.A., 2019. Neonatal Ethanol Disturbs the Normal Maturation of Parvalbumin Interneurons Surrounded by Subsets of Perineuronal Nets in the Cerebral Cortex: Partial Reversal by Lithium. Cereb Cortex 29, 1383–1397. https://doi.org/10.1093/cercor/bhy034

Salmon, C.K., Pribiag, H., Gizowski, C., Farmer, W.T., Cameron, S., Jones, E.V., Mahadevan,
V., Bourque, C.W., Stellwagen, D., Woodin, M.A., Murai, K.K., 2020. Depolarizing GABA Transmission Restrains Activity-Dependent Glutamatergic Synapse Formation in the Developing Hippocampal Circuit. Front. Cell. Neurosci. 14. https://doi.org/10.3389/fncel.2020.00036

Sang, Z., Wang, K., Dong, J., Tang, L., 2022. Alzheimer's disease: Updated multi-targets therapeutics are in clinical and in progress. European Journal of Medicinal Chemistry 238, 114464. https://doi.org/10.1016/j.ejmech.2022.114464

Santhakumar, V., Jones, R.T., Mody, I., 2010. Developmental regulation and neuroprotective effects of striatal tonic GABAA currents. Neuroscience 167, 644–655. https://doi.org/10.1016/j.neuroscience.2010.02.048

Schambra, U.B., Goldsmith, J., Nunley, K., Liu, Y., Harirforoosh, S., Schambra, H.M., 2015. Low and moderate prenatal ethanol exposures of mice during gastrulation or neurulation delays neurobehavioral development. Neurotoxicology and Teratology 51, 1–11. https://doi.org/10.1016/j.ntt.2015.07.003
Scheff, S.W., Price, D.A., Schmitt, F.A., Mufson, E.J., 2006. Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiol Aging 27, 1372–1384. https://doi.org/10.1016/j.neurobiolaging.2005.09.012

Seifan, A., Schelke, M., Obeng-Aduasare, Y., Isaacson, R., 2015. Early Life Epidemiology of Alzheimer's Disease--A Critical Review. Neuroepidemiology 45, 237–254. https://doi.org/10.1159/000439568

Selkoe, D.J., 2002. Alzheimer's Disease Is a Synaptic Failure. Science 298, 789–791. https://doi.org/10.1126/science.1074069

Semba, K., Vincent, S., Fibiger, H., 1988. Different times of origin of choline acetyltransferase- and somatostatin-immunoreactive neurons in the rat striatum. J Neurosci 8, 3937–3944. https://doi.org/10.1523/JNEUROSCI.08-10-03937.1988

Sernagor, E., Chabrol, F., Bony, G., Cancedda, L., 2010. GABAergic control of neurite outgrowth and remodeling during development and adult neurogenesis: general rules and differences in diverse systems. Frontiers in Cellular Neuroscience 4.

Shen, W., Tian, X., Day, M., Ulrich, S., Tkatch, T., Nathanson, N.M., Surmeier, D.J., 2007. Cholinergic modulation of Kir2 channels selectively elevates dendritic excitability in striatopallidal neurons. Nat Neurosci 10, 1458–1466. https://doi.org/10.1038/nn1972

Shenoda, B.B., 2017. An Overview of the Mechanisms of Abnormal GABAergic Interneuronal Cortical Migration Associated with Prenatal Ethanol Exposure. Neurochem Res 42, 1279–1287. https://doi.org/10.1007/s11064-016-2169-5

Sheth, A.N., Bhide, P.G., 1997. Concurrent cellular output from two proliferative populations in the early embryonic mouse corpus striatum. Journal of Comparative Neurology 383, 220–230. https://doi.org/10.1002/(SICI)1096-9861(19970630)383:2<220::AID-CNE8>3.0.CO;2-2

Sheth, A.N., McKee, M.L., Bhide, P.G., 1998. The Sequence of Formation and Development of Corticostriate Connections in Mice. DNE 20, 98–112. https://doi.org/10.1159/000017306

Skorput, A., Gupta, V.P., Yeh, P.W.L., Yeh, H.H., 2015. Persistent Interneuronopathy in the Prefrontal Cortex of Young Adult Offspring Exposed to Ethanol In Utero. J. Neurosci. 35, 10977–10988. https://doi.org/10.1523/JNEUROSCI.1462-15.2015

Skorput, A., Lee, S.M., Yeh, P.W., Yeh, H.H., 2019. The NKCC1 antagonist bumetanide mitigates interneuronopathy associated with ethanol exposure in utero. eLife 8, e48648. https://doi.org/10.7554/eLife.48648

Skorput, A., Yeh, H.H., 2016. Chronic Gestational Exposure to Ethanol Leads to Enduring Aberrances in Cortical Form and Function in the Medial Prefrontal Cortex. Alcohol Clin Exp Res 40, 1479–1488. https://doi.org/10.1111/acer.13107

Smiley, J.F., Saito, M., Bleiwas, C., Masiello, K., Ardekani, B., Guilfoyle, D.N., Gerum, S., Wilson, D.A., Vadasz, C., 2015. Selective reduction of cerebral cortex GABA neurons in a late gestation model of fetal alcohol spectrum disorder. Alcohol 49, 571–580. https://doi.org/10.1016/j.alcohol.2015.04.008
Sohur, U.S., Padmanabhan, H.K., Kotchetkov, I.S., Menezes, J.R.L., Macklis, J.D., 2014. Anatomic and Molecular Development of Corticostriatal Projection Neurons in Mice. Cerebral Cortex 24, 293–303. https://doi.org/10.1093/cercor/bhs342

Sreenivasan, V., Serafeimidou-Pouliou, E., Exposito-Alonso, D., Bercsenyi, K., Bernard, C., Bae, S.-E., Oozeer, F., Hanusz-Godoy, A., Edwards, R.H., Marín, O., 2022. Input-specific control of interneuron numbers in nascent striatal networks. Proc. Natl. Acad. Sci. U.S.A. 119, e2118430119. https://doi.org/10.1073/pnas.2118430119

Straub, C., Saulnier, J.L., Bègue, A., Feng, D.D., Huang, K.W., Sabatini, B.L., 2016. Principles of Synaptic Organization of GABAergic Interneurons in the Striatum. Neuron 92, 84–92. https://doi.org/10.1016/j.neuron.2016.09.007

Streissguth, A.P., Barr, H.M., Sampson, P.D., 1990. Moderate prenatal alcohol exposure: effects on child IQ and learning problems at age 7 1/2 years. Alcohol Clin Exp Res 14, 662–669. https://doi.org/10.1111/j.1530-0277.1990.tb01224.x

Streissguth, A.P., Bookstein, F.L., Barr, H.M., Sampson, P.D., O'Malley, K., Young, J.K., 2004. Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. J Dev Behav Pediatr 25, 228–238. https://doi.org/10.1097/00004703-200408000-00002

Szewczyk, Z., Holliday, E., Dean, B., Collins, C., Reeves, P., 2021. A systematic review of economic evaluations of antenatal nutrition and alcohol interventions and their associated implementation interventions. Nutrition Reviews 79, 261–273. https://doi.org/10.1093/nutrit/nuaa015

Takesian, A.E., Bogart, L.J., Lichtman, J.W., Hensch, T.K., 2018. Inhibitory circuit gating of auditory critical-period plasticity. Nat Neurosci 21, 218–227. https://doi.org/10.1038/s41593-017-0064-2

Taverna, S., Ilijic, E., Surmeier, D.J., 2008. Recurrent Collateral Connections of Striatal Medium Spiny Neurons Are Disrupted in Models of Parkinson's Disease. J. Neurosci. 28, 5504–5512. https://doi.org/10.1523/JNEUROSCI.5493-07.2008

Tepper, J.M., Koós, T., Ibanez-Sandoval, O., Tecuapetla, F., Faust, T.W., Assous, M., 2018. Heterogeneity and Diversity of Striatal GABAergic Interneurons: Update 2018. Frontiers in Neuroanatomy 12.

Tepper, J.M., Sharpe, N.A., Koós, T.Z., Trent, F., 1998. Postnatal development of the rat neostriatum: electrophysiological, light- and electron-microscopic studies. Dev. Neurosci. 20, 125–145. https://doi.org/10.1159/000017308

Tepper, J.M., Tecuapetla, F., Koós, T., Ibáñez-Sandoval, O., 2010. Heterogeneity and diversity of striatal GABAergic interneurons. Front Neuroanat 4, 150. https://doi.org/10.3389/fnana.2010.00150

Tepper, J.M., Wilson, C.J., Koós, T., 2008. Feedforward and Feedback inhibition in Neostriatal GABAergic Spiny Neurons. Brain Res Rev 58, 272–281. https://doi.org/10.1016/j.brainresrev.2007.10.008
Thibault, D., Loustalot, F., Fortin, G.M., Bourque, M.-J., Trudeau, L.-É., 2013. Evaluation of D1 and D2 dopamine receptor segregation in the developing striatum using BAC transgenic mice. PLoS One 8, e67219. https://doi.org/10.1371/journal.pone.0067219

Thierry, A.M., Blanc, G., Sobel, A., Stinus, L., Glowinski, J., 1973. Dopaminergic Terminals in the Rat Cortex. Science 182, 499–501. https://doi.org/10.1126/science.182.4111.499

Tong, M., Ziplow, J., Chen, W.C., Nguyen, Q.-G., Kim, C., de la Monte, S.M., 2013. Motor Function Deficits Following Chronic Prenatal Ethanol Exposure are Linked to Impairments in Insulin/IGF, Notch and Wnt Signaling in the Cerebellum. J Diabetes Metab 4, 238.

Treit, S., Zhou, D., Lebel, C., Rasmussen, C., Andrew, G., Beaulieu, C., 2014. Longitudinal MRI reveals impaired cortical thinning in children and adolescents prenatally exposed to alcohol. Hum Brain Mapp 35, 4892–4903. https://doi.org/10.1002/hbm.22520

Tremblay, R., Lee, S., Rudy, B., 2016. GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. Neuron 91, 260–292. https://doi.org/10.1016/j.neuron.2016.06.033

Trushina, E., Trushin, S., Hasan, M.F., 2022. Mitochondrial complex I as a therapeutic target for Alzheimer's disease. Acta Pharmaceutica Sinica B 12, 483–495. https://doi.org/10.1016/j.apsb.2021.11.003

Tsutsui-Kimura, I., Natsubori, A., Mori, M., Kobayashi, K., Drew, M.R., de Kerchove d'Exaerde, A., Mimura, M., Tanaka, K.F., 2017. Distinct Roles of Ventromedial versus Ventrolateral Striatal Medium Spiny Neurons in Reward-Oriented Behavior. Curr Biol 27, 3042-3048.e4. https://doi.org/10.1016/j.cub.2017.08.061

Tucker, A.E., Alicea Pauneto, C. del M., Barnett, A.M., Coleman, L.G., 2022. Chronic Ethanol Causes Persistent Increases in Alzheimer's Tau Pathology in Female 3xTg-AD Mice: A Potential Role for Lysosomal Impairment. Frontiers in Behavioral Neuroscience 16.

Ueta, Y., Sohn, J., Agahari, F.A., Im, S., Hirai, Y., Miyata, M., Kawaguchi, Y., 2019. Ipsi- and contralateral corticocortical projection-dependent subcircuits in layer 2 of the rat frontal cortex. Journal of Neurophysiology 122, 1461–1472. https://doi.org/10.1152/jn.00333.2019
van der Kooy, D., Fishell, G., 1987. Neuronal birthdate underlies the development of striatal compartments. Brain Research 401, 155–161. https://doi.org/10.1016/0006-8993(87)91176-0

van Eden, C.G., Uylings, H.B.M., 1985. Cytoarchitectonic development of the prefrontal cortex in the rat. Journal of Comparative Neurology 241, 253–267. https://doi.org/10.1002/cne.902410302

Verma, A., Kumar Waiker, D., Bhardwaj, B., Saraf, P., Shrivastava, S.K., 2022. The molecular mechanism, targets, and novel molecules in the treatment of Alzheimer's disease. Bioorganic Chemistry 119, 105562. https://doi.org/10.1016/j.bioorg.2021.105562

Verret, L., Mann, E.O., Hang, G.B., Barth, A.M.I., Cobos, I., Ho, K., Devidze, N., Masliah, E., Kreitzer, A.C., Mody, I., Mucke, L., Palop, J.J., 2012. Inhibitory Interneuron Deficit Links Altered Network Activity and Cognitive Dysfunction in Alzheimer Model. Cell 149, 708–721. https://doi.org/10.1016/j.cell.2012.02.046

Virk, M.S., Sagi, Y., Medrihan, L., Leung, J., Kaplitt, M.G., Greengard, P., 2016. Opposing roles for serotonin in cholinergic neurons of the ventral and dorsal striatum. Proceedings of the National Academy of Sciences 113, 734–739. https://doi.org/10.1073/pnas.1524183113

Vivanti, G., Tao, S., Lyall, K., Robins, D.L., Shea, L.L., 2021. The prevalence and incidence of early-onset dementia among adults with autism spectrum disorder. Autism Res. https://doi.org/10.1002/aur.2590

Wall, N.R., De La Parra, M., Callaway, E.M., Kreitzer, A.C., 2013. Differential Innervation of Direct- and Indirect-Pathway Striatal Projection Neurons. Neuron 79, 347–360. https://doi.org/10.1016/j.neuron.2013.05.014

Walter, K.R., Ricketts, D.K., Presswood, B.H., Smith, S.M., Mooney, S.M., 2022. Prenatal alcohol exposure causes persistent microglial activation and age- and sex-specific effects on cognition and metabolic outcomes in an Alzheimer's Disease mouse model. The American Journal of Drug and Alcohol Abuse 0, 1–19. https://doi.org/10.1080/00952990.2022.2119571

Wang, D.D., Kriegstein, A.R., 2008. GABA Regulates Excitatory Synapse Formation in the Neocortex via NMDA Receptor Activation. J Neurosci 28, 5547–5558. https://doi.org/10.1523/JNEUROSCI.5599-07.2008

Wang, J., Hamida, S.B., Darcq, E., Zhu, W., Gibb, S.L., Lanfranco, M.F., Carnicella, S., Ron, D., 2012. Ethanol-Mediated Facilitation of AMPA Receptor Function in the Dorsomedial Striatum: Implications for Alcohol Drinking Behavior. J Neurosci 32, 15124–15132. https://doi.org/10.1523/JNEUROSCI.2783-12.2012

Wang, R., Martin, C.D., Lei, A.L., Hausknecht, K.A., Ishiwari, K., Oubraim, S., Wang, A.-L., Richards, J.B., Haj-Dahmane, S., Shen, R.-Y., 2021. Moderate prenatal ethanol exposure leads to attention deficits in both male and female rats. Alcohol: Clinical and Experimental Research 45, 1122–1135. https://doi.org/10.1111/acer.14599

Wang, X., Shu, Z., He, Q., Zhang, Xiaowen, Li, Luozheng, Zhang, Xiaoxue, Li, Liang, Xiao, Y., Peng, B., Guo, F., Wang, D.-H., Shu, Y., 2022. Functional Autapses Form in Striatal Parvalbumin Interneurons but not Medium Spiny Projection Neurons. Neurosci Bull. https://doi.org/10.1007/s12264-022-00991-x

White, A.M., Orosz, A., Powell, P.A., Koob, G.F., 2022. Alcohol and aging – An area of increasing concern. Alcohol. https://doi.org/10.1016/j.alcohol.2022.07.005

Wichterle, H., Turnbull, D.H., Nery, S., Fishell, G., Alvarez-Buylla, A., 2001. In utero fate mapping reveals distinct migratory pathways and fates of neurons born in the mammalian basal forebrain. Development 128, 3759–3771. https://doi.org/10.1242/dev.128.19.3759

Williams, L., Jackson, C.P.T., Choe, N., Pelland, L., Scott, S.H., Reynolds, J.N., 2014. Sensory-Motor Deficits in Children with Fetal Alcohol Spectrum Disorder Assessed Using a Robotic Virtual Reality Platform. Alcohol: Clinical and Experimental Research 38, 116–125. https://doi.org/10.1111/acer.12225

Willoughby, K.A., Sheard, E.D., Nash, K., Rovet, J., 2008. Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. J Int Neuropsychol Soc 14, 1022–1033. https://doi.org/10.1017/S1355617708081368
Wilson, C.J., Kawaguchi, Y., 1996. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J. Neurosci. 16, 2397–2410. https://doi.org/10.1523/JNEUROSCI.16-07-02397.1996

Wirt, R.A., Hyman, J.M., 2017. Integrating Spatial Working Memory and Remote Memory: Interactions between the Medial Prefrontal Cortex and Hippocampus. Brain Sci 7, 43. https://doi.org/10.3390/brainsci7040043

Wong, E.L., Lutz, N.M., Hogan, V.A., Lamantia, C.E., McMurray, H.R., Myers, J.R., Ashton, J.M., Majewska, A.K., 2018. Developmental alcohol exposure impairs synaptic plasticity without overtly altering microglial function in mouse visual cortex. Brain Behav Immun 67, 257–278. https://doi.org/10.1016/j.bbi.2017.09.003

Wozniak, J.R., Fink, B.A., Fuglestad, A.J., Eckerle, J.K., Boys, C.J., Sandness, K.E., Radke, J.P., Miller, N.C., Lindgren, C., Brearley, A.M., Zeisel, S.H., Georgieff, M.K., 2020. Four-year follow-up of a randomized controlled trial of choline for neurodevelopment in fetal alcohol spectrum disorder. Journal of Neurodevelopmental Disorders 12, 9. https://doi.org/10.1186/s11689-020-09312-7

Wozniak, J.R., Riley, E.P., Charness, M.E., 2019. Clinical presentation, diagnosis, and management of fetal alcohol spectrum disorder. The Lancet Neurology 18, 760–770. https://doi.org/10.1016/S1474-4422(19)30150-4

Wu, C., Sun, D., 2015. GABA receptors in brain development, function, and injury. Metab Brain Dis 30, 367–379. https://doi.org/10.1007/s11011-014-9560-1

Xie, C., Feng, Y., 2022. Alcohol consumption and risk of Alzheimer's disease: A dose-response meta-analysis. Geriatr Gerontol Int 22, 278–285. https://doi.org/10.1111/ggi.14357

Xu, M., Li, L., Pittenger, C., 2016. Ablation of fast-spiking interneurons in the dorsal striatum, recapitulating abnormalities seen post-mortem in Tourette syndrome, produces anxiety and elevated grooming. Neuroscience 324, 321–329. https://doi.org/10.1016/j.neuroscience.2016.02.074

Xu, Q., Tam, M., Anderson, S.A., 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. Journal of Comparative Neurology 506, 16–29. https://doi.org/10.1002/cne.21529

Xu, Y., Zhao, M., Han, Y., Zhang, H., 2020. GABAergic Inhibitory Interneuron Deficits in Alzheimer's Disease: Implications for Treatment. Frontiers in Neuroscience 14.

Yang, F., Chen, Liling, Yu, Y., Xu, T., Chen, Lu, Yang, W., Wu, Q., Han, Y., 2022. Alzheimer's disease and epilepsy: An increasingly recognized comorbidity. Front. Aging Neurosci. 14. https://doi.org/10.3389/fnagi.2022.940515

Yang, J.-M., Zhang, J., Yu, Y.-Q., Duan, S., Li, X.-M., 2014. Postnatal development of 2 microcircuits involving fast-spiking interneurons in the mouse prefrontal cortex. Cereb Cortex 24, 98–109. https://doi.org/10.1093/cercor/bhs291

Yang, S.-T., Shi, Y., Wang, Q., Peng, J.-Y., Li, B.-M., 2014. Neuronal representation of working memory in the medial prefrontal cortex of rats. Mol Brain 7, 61. https://doi.org/10.1186/s13041-014-0061-2

Yiannopoulou, K.G., Anastasiou, A.I., Zachariou, V., Pelidou, S.-H., 2019. Reasons for Failed Trials of Disease-Modifying Treatments for Alzheimer Disease and Their Contribution in Recent Research. Biomedicines 7, 97. https://doi.org/10.3390/biomedicines7040097

Young, J.K., Giesbrecht, H.E., Eskin, M.N., Aliani, M., Suh, M., 2014. Nutrition Implications for Fetal Alcohol Spectrum Disorder. Advances in Nutrition 5, 675–692. https://doi.org/10.3945/an.113.004846

Yu, J.-T., Xu, W., Tan, C.-C., Andrieu, S., Suckling, J., Evangelou, E., Pan, A., Zhang, C., Jia, J., Feng, L., Kua, E.-H., Wang, Y.-J., Wang, H.-F., Tan, M.-S., Li, J.-Q., Hou, X.-H., Wan, Y., Tan, Lin, Mok, V., Tan, Lan, Dong, Q., Touchon, J., Gauthier, S., Aisen, P.S., Vellas, B., 2020. Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. J Neurol Neurosurg Psychiatry 91, 1201–1209. https://doi.org/10.1136/jnnp-2019-321913

Zhou, R., Wang, S., Zhu, X., 2012. Prenatal Ethanol Exposure Alters Synaptic Plasticity in the Dorsolateral Striatum of Rat Offspring via Changing the Reactivity of Dopamine Receptor. PLOS ONE 7, e42443. https://doi.org/10.1371/journal.pone.0042443

CHAPTER 2

Prenatal ethanol exposure results in cell-type, age, and sex-dependent differences in the early postnatal development of the striatum, coinciding with developmental motor deficits

AR Tousley and HH Yeh contributed to experimental design and writing of the manuscript. AR Tousley gathered and analyzed all data with the help of two Dartmouth undergraduate students I Deykin and B Koc contributed to morphological analyses of striatal neurons. PW Yeh contributed to animal care and breeding.

2.1 Abstract

Developmental motor delays are a common early clinical sign of Fetal Alcohol Spectrum Disorders (FASD). Individuals with FASD can develop a range of motor symptoms including problems with both gross and fine motor function, and sensorimotor integration. However, the neural circuit level changes that underlie early motor deficits remain underexplored. The striatum, the input nucleus of the basal ganglia, plays an important role in motor learning in adult animals, while the maturation of the striatal circuit has been associated with the development of early motor behaviors. Here we demonstrate that a brief binge exposure to ethanol (5% w/w) in a liquid diet on embryonic days (E)13.5-16.5, results in developmental motor deficits concurrent with alterations in synaptic activity, passive/active properties and morphology in two populations of striatal neurons: GABAergic interneurons (GINs) and striatal projection neurons (SPNs), in a sex and developmental age-dependent manner. We performed a series of 9 brief motor behavior tasks on postnatal days (P)2-14. Behaviorally tested animals were then used to complete whole cell-voltage and current clamp recordings of GINs and SPNs in order to assess GABAergic/glutamatergic synaptic activity and their passive/active properties. Cells were filled with biocytin during recording for morphological analysis. We found that prenatal ethanol exposure developmental motor delays that were more severe in male mice and coincided with sex-dependent differences in the functional, synaptic and morphological properties of striatal neurons, that also varied depending upon the postnatal day assessed. Our findings indicate that prenatal ethanol exposure results in dynamic changes to the developmental trajectories of striatal GINs and SPNs, that differ between male and female animals, may contribute to differences in the development of early motor behaviors.

2.2 Introduction

Developmental motor delays are among the earliest clinical symptoms observed in individuals diagnosed with Fetal Alcohol Spectrum Disorders (FASD) (Wozniak et al., 2019). FASD, is an umbrella term encompassing the range of clinical diagnoses that may result from a prenatal exposure to ethanol, are the most common non-genetic cause of neurodevelopmental disorder worldwide (Lange et al., 2017). Individuals with FASD can develop a range of motor symptoms including challenges with both gross and fine motor function, as well as sensorimotor integration (Doney et al., 2016, 2014; Lucas et al., 2014). However, the neural circuit level changes that underlie early motor deficits in individuals with FASD remain underexplored.

The striatum, the input nucleus of the basal ganglia, contributes to motor learning in adult animals, while the maturation of the striatal circuit has been associated with the development of early motor behaviors (Cataldi et al., 2021; Dehorter et al., 2011; Graybiel and Grafton, 2015). Imaging studies indicate that prenatal ethanol exposure may modify both the size and functional connectivity of the developing striatum in individuals with FASD (Cortese et al., 2006; Donald et al., 2016; Mattson et al., 2011). Here, we investigated the effects of prenatal ethanol exposure on two populations of GABAergic striatal neurons: GABAergic interneurons (GINs) and medium-spiny striatal projection neurons (SPNs), asking how the altered maturation of these two populations of striatal neurons might relate to the onset of early motor behaviors during the first two postnatal weeks.

Though striatal GINs make up a only a small proportion of striatal neurons (<1%), they play a critical role in modifying striatal network activity and striatal-mediated motor behaviors (Duhne et al., 2020; Gazan et al., 2019; Gritton et al., 2019; Holly et al., 2021; Jin et al., 2014; Lee et al., 2017; Martiros et al., 2018; O'Hare et al., 2016; Owen et al.,

2018; Rueda-Orozco and Robbe, 2015; Xu et al., 2016). Prenatal ethanol exposure has been shown to alter the disposition and function of GINs in the striatum of adult animals, and across the lifespan in several brain regions including cortex and hippocampus, and human post-mortem tissue (Bengtsson Gonzales et al., 2020; Cuzon Carlson et al., 2020; De Giorgio et al., 2012; Léger et al., 2020; Madden et al., 2020; Marguet et al., 2020; Skorput et al., 2019, 2015). Alternatively, direct pathway and indirect pathway SPNs make up ~95% of striatal neurons, increase and decrease motor behaviors respectively and collectively facilitate the onset of movement (Cui et al., 2013; Ferguson et al., 2011; Kravitz et al., 2012). Past work suggests that prenatal ethanol exposure can have a lasting impact on the morphology and function of SPNs in adult animals (Cheng et al., 2018; Marquardt et al., 2020; Rice et al., 2012; Roselli et al., 2020; Zhou et al., 2012). Though acute ethanol exposure has been shown to modify the function of striatal neurons in a subtype specific manner in adult animals, how prenatal ethanol exposure may differentially affect striatal GINS and SPNs during early postnatal development has yet to be investigated (Blomeley et al., 2011; Marty and Spigelman, 2012; Patton et al., 2016).

We hypothesized that a brief binge exposure to ethanol in a liquid diet (5% w/w) from embryonic day (E) 13.5-16.5, a period of significant migration of striatal GINs and SPNs to the developing striatum, would alter the development of early sensorimotor behaviors in neonatal mice, and associated with changes to the functional, synaptic and morphological development of striatal GINs and SPNs (Deacon et al., 1994; Marin et al., 2000; Olsson et al., 1998; Villar-Cerviño et al., 2015). We demonstrate that prenatal ethanol exposure results in sex-dependent developmental motor differences concurrent with alterations in synaptic activity, passive/active properties and morphology of striatal neurons during the first two postnatal weeks.

2.3 Methods

2.3.1 Mice and prenatal ethanol exposure paradigm

All procedures involving mice were conducted per the National Institutes of Health Guide for the Care and Use of Laboratory Animals with the approval of the Dartmouth Institutional Animal Care and Use Committee (Protocol #: 00002109). Mice were housed on a 12h/12h light/dark cycle from 7 a.m. to 7 p.m. Nkx2.1Cre mice (The Jackson Laboratory, Bar Harbor, ME, #008661) were crossed with Ai14Cre reporter mice (The Jackson Laboratory #007914), resulting in offspring which expressing a tdTomato reporter in medial ganglionic eminence (MGE)-derived GINs beginning during embryonic development (Xu et al., 2008) (**Figure 2.2a,b**). Pregnant dams were fed 5% (w/w) ethanol in a liquid diet (L10251A, Research Diets, New Brunswick, NJ) or a lab chow diet (5V5M, ScottPharma Solutions, Marlborough, MA) from E13.5-16.5 with water available ad libitum, which routinely resulted in blood ethanol levels of ~80 mg/dL (Skorput et al., 2015). Following the date of birth, designated P0, pups were co-housed with littermates and their female parent, and maintained until behavioral testing occurring: P2, P4, P6, P8, P10 or P14 then sacrificed for electrophysiology experiments (**Figure 2.1a**).

2.3.2 Behavior

Mice chosen at random on a given postnatal day were subjected to a series of brief behavioral tasks to assess sensorimotor development based on those first employed by Fox (1965), in a 14.5 cm x 25.5 cm x 10.5 cm clear plastic testing arena, completed between 9 am-1pm (**Table *2.*1**). 3 trials were completed for each task, excepting the assessment of quadruped walking which was completed twice as animals dramatically decreased exploratory behavior of the testing arena between the first and second trials. Mice were allowed a 15-30 second break on a warming plate between each task and a

one-minute break between each trial. The trial arena and testing materials were cleaned with Peroxigard (Virox Technologies Inc, Oakville, Ontario, #29101) between mice. For behavioral tasks assessed for a time to completion: surface righting time and negative geotaxis time, animals that failed to perform either behavior were assigned the maximum time for analysis: 30 sec and 45 sec, respectively. To assess the overall performance of each animal on all tasks a total motor score (TMS) was computed as the sum of scores from the 3 (forepaw grasp, hindpaw grasp, tactile startle, auditory startle, horizontal screen, vertical screen, negative geotaxis, cliff avoidance) or 2 trials (quadruped walking) completed for each task. Performance on each task was then analyzed separately by postnatal day to determine which tasks contributed to the differences observed in TMS.

2.3.3 Electrophysiology

Immediately following completion of behavioral experiments, mice were weighed, then asphyxiated with isoflurane for electrophysiology experiments. Acute coronal slices (250 μM thick) for whole-cell patch clamp recording of striatal GINs and SPNs were prepared using a Leica VT1200s Vibratome (Leica Biosystems, Deer Park, IL) in oxygenated (95% O2, 5% CO2) cutting solution: ([in mM] 3 KCl, 7 MgCl2, 0.5 CaCl2, 1.25 NaH2PO4, 28 NaHCO3, 8.3 d-glucose, 110 sucrose, pH 7.4 [adjusted with 1 N NaOH]), then maintained in artificial cerebrospinal fluid (aCSF): ([in mM] 124 NaCl, 5.0 KCl, 2.0 MgCl2, 2.0 CaCl2, 1.25 NaH2PO4, 26 NaHCO3, 10 d-glucose, pH 7.4 [adjusted with 1 N NaOH]), for a minimum of 1-h prior to electrophysiological recordings as previously described (Delatour et al., 2019b, 2019a; Skorput et al., 2015; Tousley et al., 2022). Coronal slices were collected beginning when the corpus callosum could be visualized connecting both hemispheres, until the appearance of a fused anterior commissure posteriorly, 4-6 slices per mouse depending on the developmental age. For P2-P10

mice: slices were prepared in ice cold cutting solution, then incubated 20-min at ~32ºC followed by 1-h at room temperature as previously described (Delatour et al., 2019b). To improve the survival of acute slices from P14 mice, slices were prepared in ~32ºC cutting solution, and maintained in ~32ºC aCSF for at least 1-h prior to recording (Huang and Uusisaari, 2013).

During recording, slices were placed in an acrylic recording chamber continuously perfused with oxygenated aCSF (0.5-1 mL/minute) and maintained at ~32ºC. Striatal neurons were visualized using a fixed-stage upright fluorescence microscope (Olympus BX41WI, Evident Corporation, Tokyo, Japan) with Hoffman modulation optics. Recording pipettes with resistances 8-10 MΩ were fabricated from borosilicate glass (Sutter Instrument, Novato, CA; 1.5 mm; ID 0.86 mm) using a Flaming Brown Micropipette Puller (Sutter Instrument, Model P80 PC). Whole-cell patch clamp recordings were performed using a Multiclamp 700b amplifier (Molecular Devices, San Jose, CA), with signals low pass-filtered at 10kHz (Clampex, Version 9.2, Molecular Devices) and digitized at 25 kHz (Digidata 1320A, Molecular Devices).

Current clamp recordings were completed using a potassium gluconate internal solution (in mM): 100 K-gluconate, 2 MgCl2, 1 CaCl2, 11 EGTA, 10 HEPES, 30 KCl, 3 Mg+2 ATP, 3 Na+ GTP (adjusted to pH 7.3 with 1N KOH). Resting membrane potential (RMP) was determined immediately upon breaking into the cell membrane. AP firing rate was determined from a series of 8-10, 500 ms depolarizing current steps: P2 and P4-6 (0 to 80 pA by 10 pA), P8-10 (0-160 pA by 20 pA), and P14 (0 to 500 pA by 50 pA). Input resistance (IR) was calculated from a series of 8-10, 500 ms hyperpolarizing current steps: P2 and P4-6 (0 to -80pA by 10 pA), P8-10 (0 to -160 pA by 20pA), and P14 (0 to -500 pA by 50 pA). AP half-width and amplitude were determined from a single action potential (AP) for each neuron: the second AP evoked by the threshold current was

compared between neurons. AP threshold was determined as the point when the slope (dV/dT) was greater than 10 mV/ms. Capacitance and membrane time constant were calculated using a Multiclamp 700b commander with a 10 mV voltage step and a sampling rate of 0.4 kHz. Analysis of current clamp recordings was completed using Clampex 9.2 software (Molecular Devices).

Spontaneous post-synaptic current (sPSC) recordings were performed in whole-cell voltage clamp using a cesium methanesulfonate internal solution: 30 Cs-methanesulfonate, 10 HEPES, 0.5 EGTA, 8 NaCl, 10 Na-phosphocreatine, 4 Mg2+ ATP, and 0.4 Na+ GTP adjusted to pH 7.3 with 1 N CsOH, isolating glutamatergic currents by recording at a holding potential of -70 mV and GABAergic currents at a holding potential of 0 mV. Analysis of average frequency, amplitude and charge of sPSCs from 2-min epochs was performed using MiniAnalysis software with manual confirmation of each event (Version 6.0.7, Synaptosoft).

2.3.4 SPN morphology

Internal solutions for both voltage clamp and current clamp recordings were prepared with 2% Neurobiotin tracer (SP1120, Vector Labs), filling neurons during 2-15 min recording periods. Filled cells were prepared for imaging and tracing as previously described (Delatour et al., 2019b, 2019a; Tousley et al., 2022). Briefly, following electrophysiological recordings 250 µM slices were fixed in 4% paraformaldehyde (PFA)/0.1 M phosphate-buffered saline (PBS) overnight, then maintained in 30% sucrose/0.1 M PBS prior to processing at 4 ºC. Slices were washed in 0.1 M PBS, then incubated for 30 minutes in 30% hydrogen peroxide (H2O2) in 0.1 M PBS followed by a 0.1M PBS wash, and a second 30-minute incubation in 30% H2O2 in 0.1M PBS. Slices were then permeabilized and blocked in 10% NGS in 0.4% Triton X 100/ 0.1 M PBS for two hours, then placed overnight at 4°C in 1:1000 Dylight-488 streptavidin (#SA-5488-

1,Vector Biosciences, Newark, CA). Z-stack images of filled cells were captured at 20x magnification using a Zeiss LSM 510 laser-scanning confocal microscope (Zeiss, with a HENE 543 Laser using a plan-apochromat 20x/0.75 NA objective 0.7x zoom at 1024 x 1024 for striatal GINs (x, y, z at 0.621 x 0.621 x 1.5 μM) and 1x at 1024 x 1024 zoom for SPNs (x, y, z at 0.439 x 0.439 x 1.5 μM). Filled cells were traced and analyzed for soma area (μM2) and measures of dendritic morphology including: numbers of dendrites, mean nodes per dendrite, and mean dendritic length (μM). Sholl analysis was conducted using Neuroleucida360 software, assessing the number of intersections per 10 μM radius extending from the soma (Version 2021.1.3), MBF Bioscience LLC, Williston, VT USA).

2.3.5 Immunohistochemistry

Nkx2.1Cre x tdTomato mice were transcardially perfused with 4% PFA/0.1M PBS. Brains were dissected and immersed overnight in 4% PFA/0.1M PBS, followed by incubation for 1 day in 15% sucrose/0.1M PBS, and then 1 day in 15% sucrose/0.1M PBS. 30 μM cryosections were prepared using a sliding microtome and incubated overnight in 0.1M PBS. Sections were then permeabilized and blocked with 10% NGS in in 0.25% Triton X 100.1M PBS for 30-min, then incubated overnight at 4ºC in 1:200 CTIP2 [25B6] (ab18465, Abcam) primary antibody in 1XPBS. The following day after two 30-min washes, sections were incubated overnight in 1:1000 goat anti-rat AlexaFluor-488 secondary antibody (A110006, Molecular Probes) at 4ºC. Following an overnight incubation in 0.1 M PBS, sections were mounted and counterstained with 4′,6-diamidino-2-phenylindole (DAPI) the cover-slipped with FluoroSave Reagent (#345789, Calbiochem). Images of fluorescent tdTomato+ striatal GINs, and CTIP2+ SPNs were obtained using a CCD camera (Hamamatsu) mounted on an upright spinning disk confocal microscope with a 10x0.30 NA objective (Olympus BX61WI, Evident

Corporation) and digitized using Olympus CellSens software (Version 1.18, Evident Corporation), and pseudocolored using FIJI (NIH, Bethesda, MD) (Schindelin et al., 2012).

2.3.6 Statistical Analysis

Three-way ANOVAs with experimental exposure (control vs. ethanol), sex (female vs. male), and postnatal day (for behavioral analyses: P2, P4, P6, P8, P10 and P14, for analyses of function and morphology: P2, P4-6, P8-10 and P14) as factors, were performed using IBM SPSS (IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY). Where significant main effects or interactions were indicated, one-way ANOVAs comparing groups: control female, ethanol female, control male, ethanol male were performed for each postnatal day, followed by Bonferroni post-hoc analyses or Kruskall-Wallis tests, with Dunn's post-hoc analyses in cases where data were not normally distributed using GraphPad Prism software (GraphPad Prism, version 5.03, San Diego, CA). Normality was assessed using Shapiro-Wilk tests. For scored behavioral tasks and dendritic number, ordinal logistic regressions were performed using SPSS following assessment for multicollinearity, with Wald post-hoc tests. Where significant exposure, sex, or postnatal main effects or interactions were determined comparisons between groups made at each postnatal day with Kruskall-Wallis tests, and Dunn's post-hoc analyses (IBM SPSS Statistics for Windows).

Data are presented throughout as mean ± standard error of mean (SEM). For all experiments, experimenters were blinded to experimental group. No more than one male and female animal per litter was used at a given time point, with animals from minimum of 3 litters used per time point. For electrophysiological recording experiments, cells were evenly sampled from all four striatal quadrants: dorsolateral, dorsomedial, ventrolateral, ventromedial with no more than one cell per quadrant per animal from at

least 3 animals included for analysis. For behavioral experiments, 4-19 animals per group were assessed at each time point. Sample size for each experiment was determined based upon power analysis of preliminary data produced by our lab, and previously published literature with the minimal number of animals used to obtain an $\alpha = 0.05$ and $1-\beta = 0.8$ (G*Power 3.1, Heinrich Heine University, Dusseldorf, Germany).

2.4 Results

2.4.1 A brief binge exposure to ethanol E13.5 to 16.5 delays the development of sensorimotor behaviors in a sex-dependent manner

Previous work has demonstrated that both chronic and acute binge exposures to ethanol prenatally may result in developmental motor deficits in rodent models (Fish et al., 1981; Molina et al., 1987; Schambra et al., 2015). We first asked if our model of a brief binge exposure to ethanol might result in deficits in the development of early motor responses to sensory stimuli. To evaluate early motor development, we employed a series of 9 brief behavioral tasks first developed by Fox (1965), and widely used to identified developmental motor deficits in rodent models of prenatal exposures and neurodevelopmental disorders (Bignami, 1996; Crawley, 2012; Michetti et al., 2022). On P2, P4, P6, P8, P10, or P14, behavioral performance was assessed in male and female neonates immediately prior to sacrifice for whole-cell patch clamp electrophysiology experiments assessing the functional, synaptic and morphological properties of striatal neurons (control: female: N=63 mice, male: N= 70 mice; ethanol: female: N= 42, male: N=46 mice). Mice were assessed between P2-P14, as P2 was the first time point we observed some but not all mice to exhibit forelimb driven pivoting behavior, while mice aged older than P14 were reluctant to complete several of the behavioral tasks, as previously described (Armstrong et al., 2019). Animals were scored by blinded experimenters with higher scores indicative of more mature behaviors (**Table *2.1***).

We first determined if there was an overall effect of prenatal ethanol exposure on motor task performance, and if that effect differed between female and male mice. We calculated a total motor score (TMS) for each tested animal as the sum of scores for each task over the course of 3 trials (forepaw grasp, hindpaw grasp, tactile startle, auditory startle, horizontal screen, vertical screen, negative geotaxis, cliff avoidance), or 2 trials (quadruped walking). Prenatal ethanol exposure resulted in significantly lower TMS indicative of delayed motor development (ordinal logistic regression, Wald $\chi2(1) = 11.069$, $p < 0.001$) (**Figure *2.*1c**). Prenatal ethanol exposure did not alter body weight (g) of behaviorally tested mice of either sex over the first two postnatal weeks (**Figure *2.*1b**). These data suggest that prenatal ethanol exposure results in developmental motor differences in the absence of gross differences in physical development. Additionally, while we did not observe an effect of biological sex on TMS (ordinal logistic regression, Wald $\chi2(1) = 0.386$, $p= 0.534$), interestingly, we identified a significant exposure x sex x postnatal day interaction (ordinal logistic regression, Wald $\chi2(1) = 323.717$, $p<0.0001$) suggesting that performance of male and female neonates may be differentially altered by prenatal ethanol exposure depending on the developmental timepoint assessed.

Analysis of animal performance by task indicated that behavioral differences in mice exposed prenatally to ethanol were present in more complex tasks requiring coordinated movement and or the integration of sensory and motor information: quadruped walking score (**Figure *2.*1d**), vertical screen score (**Figure *2.*1e**), negative geotaxis score (**Figure *2.*2g**), rather than those driven be simple reflex loops: tactile startle score (Figure 2a), auditory startle score (**Figure *2.*2d**), forepaw grasp score (**Figure *2.*2b**), hindpaw grasp score (**Figure *2.*2e**), horizontal screen score (**Figure *2.*2c**), and cliff avoidance score (**Figure *2.*2f**) (ordinal logistic regression, quadruped walking: Wald $\chi2(1) = 14.364$, $p<0.001$, vertical screen: Wald $\chi2(1) = 2.724$, $p=0.099$, negative

geotaxis: Wald $\chi2(1) = 10.848$, $p<0.001$, tactile startle: Wald $\chi2(1) = 2.623$, $p=0.105$, auditory startle: Wald $\chi2(1) = 1.842$, $p=0.175$, forepaw grasp: Wald $\chi2(1) = 0.000$, $p=1.000$, hindpaw grasp: Wald $\chi2(1) = 0.000$, $p=0.998$, horizontal screen: Wald $\chi2(1) = 1.885$, $p=0.170$). While prenatal ethanol exposure resulted in decreases in quadruped walking score in both male and female mice, the effects of prenatal ethanol exposure on vertical screen and negative geotaxis tasks were sex-dependent, with male mice tending to demonstrate less mature motor behaviors following prenatal ethanol exposure when compared to control-fed male, control-fed female and ethanol-exposed female neonates (ordinal logistic regressions, quadruped walking: sex: Wald $\chi2(1) = 0.022$, $p=0.882$, group x sex: Wald $\chi2(1) =0.454$, $p=0.500$, vertical screen score: sex: Wald $\chi2(1) = 0.002$, $p=0.963$, group x sex: Wald $\chi2(1) =5.582$, $p=0.018$, negative geotaxis: sex: Wald $\chi2(1)$ 0.001= 0.972, group x sex: Wald $\chi2(1) = 4.108$, $p=0.043$).

Having identified a significant effect of prenatal ethanol exposure on performance during several behavioral tasks that differed between male and female animals, we next asked when during the first two postnatal weeks behavioral differences might be most pronounced between groups: control female, ethanol female, control male, ethanol male. We found that prenatal ethanol exposure resulted in significantly lower TMS in male mice at P8 relative to control-fed male mice (**Figure 2.1c**). While no significant between group differences were identified at a single postnatal day in scoring of quadruped walking behavior, the most distinct between group differences were observed at P14, when animals are making the progression from immature crawling behavior involving all four limbs (score = 2), to running, indicated by an elevated trunk, decreased hindlimb slips, more synchronous fore and hind limb movements, and a faster overall speed (score = 3), with fewer ethanol-exposed male mice demonstrating running behavior (Figure 1d) (Altman and Sudarshan, 1975; Fox, 1965). Differences were less

pronounced during the onset of early postnatal pivoting behavior, involving only the use of forelimbs (score=1), or in the transition from pivoting to crawling (**Figure *2.*1d**). Trends towards between group differences in vertical screen task performance were also the most pronounced at P4 and P6, when animals are first able to grasp and hold their position on the vertical screen (score = 1) but before they are able to climb the vertical screen (score = 2), with ethanol-exposed male mice again demonstrating the least mature behaviors (**Figure *2.*1e**).

In contrast with scored behavioral tasks which allowed us to assess the absence or presence of early motor behaviors across development, we also assessed the time it took mice complete negative geotaxis and surface righting behaviors to determine if more subtle alterations in motor behavior might be present and not accounted for by observer scoring. (**Figure *2.*2g,h**). Unlike the sex-dependent effects of prenatal ethanol exposure on negative geotaxis score, we determined that prenatal ethanol exposure significantly increased negative geotaxis times in both female and male mice, though analysis of between group differences at individual postnatal days suggested that the prenatal ethanol exposure resulted in significantly increased negative geotaxis times in male mice relative to control-fed male mice at P8 (**Figure *2.*1g**) (3-way ANOVA , exposure: $F(1,200) = 6.660$, $p=0.011$), sex: $F(1, 200) = 0.016$, $p=0.899$), exposure x sex postnatal day: $F(5,200) = 1.046$, $p=0.392$, exposure x sex: $F(1,200) = 2.537$, $p=0.113$, exposure x postnatal day: $F(5,200) = 0.964$, $p=0.441$, sex x postnatal day $F(5, 200) = 0.204$, $p=0.961$).

Additionally, while surface righting score was not significantly affected by prenatal ethanol exposure or sex, it was differentially altered by prenatal ethanol exposure in female and male mice depending on the postnatal day with female mice generally demonstrating less mature behaviors than male mice (score: ordinal logistic regression,

exposure: Wald $\chi 2(1) = 0.268$, $p=0.604$; sex: Wald $\chi 2(1) = 2.522$, $p=0.112$; sex; time: 3-way ANOVA: main effect: exposure: $F(1,200) = 1.245$, $p=0.266$ sex: $F(1,200) = 9.424$, $p=0.002$; interactions: exposure x sex x postnatal day: $F(5,200) = 4.128$, $p=0.001$, exposure x sex: $F(1, 200) = 4,242$, $p=0.041$, exposure x postnatal day: $F(5,200)$, $p= 0.025$, sex x postnatal day: $F(5, 200)=3.791$, $p =0.003$) (**Figure *2.*1f, *2.*2h***)***

Alternatively, comparisons of surface righting times between groups suggested that prenatal ethanol exposure may differentially alter developmental trajectories of surface righting behavior in male and female mice. Prenatal ethanol exposure resulted in increased surface righting times in male mice relative to control-fed male mice at P2. However, the effects of prenatal ethanol exposure on surface righting time in female mice were more complex (**Figure *2.*1f**). Although ethanol-exposed female mice demonstrated increased surface righting times relative to control-fed and ethanol-exposed male mice at P6, this was not consistent across early postnatal development. At P4, ethanol-exposed female mice, as well as ethanol-exposed and control-fed male mice demonstrated decreased surface righting times when compared to those of control-fed female mice (**Figure *2.*1f**). These data suggest that prenatal ethanol can both improve performance (P4) and result in deficits (P6) in surface righting behavior in female mice depending on the postnatal day.

2.4.2 Prenatal ethanol exposure differentially alters the maturation of active and passive properties of striatal neurons in male and female mice

Concurrent with the onset of increasingly complex motor behaviors during the first postnatal month, striatal GINs and SPNs gradually develop adult-like firing and membrane properties (Belleau and Warren, 2000; Chesselet et al., 2007; Plotkin et al., 2005; Tepper et al., 1998). These include changes in the characteristics of APs: increased AP firing rate, and decreased AP half-width, as well as shifts in membrane

properties conferring a decrease in neuronal excitability: more depolarized RMP and AP threshold, and decreased IR. The development of mature quadruped walking behavior has been closely associated with the functional maturation of SPNs in neonates during this postnatal time period (Dehorter et al., 2011). How striatal GINs mature during this period, and how the development of striatal GINs and SPNs may be altered in early postnatal development following a prenatal ethanol exposure has yet to be explored. Thus, we next asked if prenatal ethanol exposure might alter the functional development of striatal GINs and SPNs. To differentiate striatal GINs and SPNs in acute slices during whole-cell patch clamp recording experiments, we crossed the Nkx2.1Cre mouse line with a Ai14Cre reporter mice, resulting in tdTomato expression in MGE-derived striatal GINs (Xu et al., 2008). In addition, we were able to further confirm the identity of SPNs and GINs based their distinctive morphological properties by filling cells with 2% neurobiotin dye during whole-cell recording, and finally by their distinctive firing properties (**Figure 2.3a-d, 2.4b,f**).

2.4.2a Striatal GINs- AP firing properties

We first asked if prenatal ethanol exposure altered the firing rate, or AP characteristics: AP threshold, AP half-width or AP peak amplitude of striatal GINs or SPNs in response to a series of depolarizing current steps: P2 and P4-6 (0 to 80 pA by 10 pA), P8-10 (0 to 160 pA by 20 pA), and P14 (0 to 500 pA by 50 pA). At P2, GINs from female mice demonstrated an increased firing rate regardless of group, however, only GINs from ethanol-exposed female mice had firing rates that significantly differed from ethanol-exposed or control-fed male mice. The firing rate of striatal GINs from ethanol-fed female mice did not differ from control-fed female mice (**Figure 2.4a**). At P4-6, firing rate significantly differed between groups, with the highest firing rates observed in striatal GINs from control-fed male mice which were increased relative ethanol-exposed males

and females, as well as control females (**Figure 2.4a**). At P8-10, GIN firing rate again significantly differed between groups, with the largest differences in firing rate observed between striatal GINs between ethanol-exposed and control-fed female and male mice.

Prenatal ethanol exposure also differentially altered the AP threshold and AP half-width of developing striatal GINs from male and female mice depending on the postnatal day (3-way ANOVAs, AP threshold: main effects: exposure: $F(1,231) = 6.504$, $p=0.011$, sex: $F(1,231) = 10.088$, $p =0.002$; interactions: exposure x sex x postnatal day: $F(3, 231) = 3.316$, $p=0.021$, exposure x sex: $F(1,231) = 0.976$, $p=0.324$, exposure x postnatal day: $F(3,231) = 3.178$, $p=0.025$, sex x postnatal day: $F(3,231) = 5.538$, $p=0.001$; (3-way ANOVA, main effects: exposure: $F(1,230) = 7.020$, $p=0.019$, sex: $F(1,230) = 7.268$, $p =0.017$; interactions: exposure x sex x postnatal day: $F(3, 230) = 7.781$, $p<0.001$, exposure x sex: $F(1,230 = 12.062$, $p<0.001$, exposure x postnatal day: $F(3,230) = 1.977$, $p=0.118$, sex x postnatal day: $F(3,230) = 5.826$, $p<0.001$) (**Figure 2.4c,d**). Having identified a postnatal day dependent effect of prenatal ethanol exposure, we next compared differences between groups: control F, ethanol F, control M and ethanol M, by postnatal day (P2, P4-6, P8-10 and P14).

At P2, prenatal ethanol exposure significantly altered both AP half-width and AP threshold (**Figure 2.4c,d**). Prenatal ethanol exposure significantly increased AP half-width in striatal GINs recorded from male mice, relative to control-fed male mice (**Figure 2.4d**). AP threshold was more depolarized in striatal GINs recorded from ethanol-exposed female mice relative to control-fed female mice, while there was only a trend towards a similar depolarization of AP threshold in striatal GINs from ethanol-exposed male mice, relative to those from control-fed male mice ($p=0.061$). (**Figure 2.4c**). Alternatively, at the end of the second postnatal week (P14), prenatal ethanol exposure resulted in hyperpolarized AP thresholds in striatal GINs recorded from both female and

male mice relative to control-fed female and male mice, respectively (**Figure 2.4c**).The peak amplitude of APs recorded from striatal GINs was unaffected by prenatal ethanol exposure (3-way ANOVA, main effects: exposure: $F(1,231) = 0.646$, $p=0.422$, sex: $F(1,231) = 0.542$, $p =0.463$; interactions: exposure x sex x postnatal day: $F(3,231) = 1.197$, $p=0.312$, exposure x sex: $F(1,231) = 0.021$, $p=0.884$, exposure x postnatal day: $F(3,231) = 1.405$, $p=0.242$, sex x postnatal day: $F(3, 231) = 0.300$, $p=0.826$) (**Figure 2.6a**).

These data suggest that prenatal ethanol exposure differentially alters the development of striatal GINs in male and female mice. During early postnatal development, striatal GINs in female exposed prenatally to ethanol developed early increases in AP firing, despite more depolarized AP thresholds that transiently resolved at P4-6, but were again present at P8-10 and P14 (**Figure 2.4a,c**). In contrast, striatal GINs from male mice failed to appropriately mature following prenatal ethanol exposure during as indicated by wider APs at P2, and a failure to demonstrate of increased GIN firing rate at P4 (**Figure 2.4a,d**). However, by the end of the first postnatal week striatal GINs from both male and female mice demonstrate an increased excitability with increased firing rates and more hyperpolarized AP thresholds (**Figure 2.4a,c**).

2.4.2b SPNs- AP firing properties

As we found in striatal GINs, prenatal ethanol exposure resulted in differential effects on SPN AP firing rate depending on sex and postnatal age (**Figure 2.4e**). At P2, prenatal ethanol exposure had no significant effect on AP firing rate when SPNs from control-fed and ethanol-exposed cohorts of either sex were compared (**Figure 2.4e**). Alternatively, significant between group differences were observed at P4-6, P8-10 and P14 (**Figure 2.4e**). At P4-6, prenatal ethanol exposure resulted in an increased firing rate in SPNs from female mice, relative to those from ethanol-exposed male mice (**Figure 2.4e**). By

contrast, at P8-10 SPNs from ethanol-exposed male mice demonstrated an increased firing rate relative to those from ethanol-exposed female mice (**Figure 2.4e**). At P14 the effects of prenatal ethanol exposure differed between groups depending on the size of the current step. At low levels of current input SPNs from ethanol-exposed female mice demonstrated a higher firing rate. With increasing depolarizing current inputs, SPNs from male mice exposed-prenatally to ethanol demonstrated lower firing rates relative to control-fed male mice. Both SPNs from both control-fed and ethanol-exposed female mice also had lower firing rates compared to those from control-fed male mice at P14.

SPNs also significantly differed in AP threshold following prenatal ethanol exposure depending on the postnatal day, with a trend toward differences in the effects of prenatal ethanol exposure on APs recorded from SPNs from male and female animals depending on the postnatal day (3-way ANOVA, main effects: exposure: $F(1,271) = 0.034$, $p=0.853$, sex: $F(1,271) = 0.016$, $p =0.899$; interactions: exposure x sex x postnatal day: $F(3, 271) = 2.620$, $p=0.051$, exposure x sex: $F(1,271) = 1.698$, $p=0.194$,exposure x postnatal day: $F(3,271) = 7.306$, $p<0.001$, sex x postnatal day: $F(3,271) = 0.454$, $p=0.715$) (Table 3, Figure 4g). At P2 prenatal ethanol exposure resulted in depolarized AP firing threshold in SPNs from male mice relative to those from control-fed male mice, while SPN AP threshold was unaffected in female mice (**Figure 2.4g**). At P14, prenatal ethanol exposure resulted in a hyperpolarized AP threshold in both male and female mice relative their control-fed male and female counterparts (**Figure 2.4g**). The half-width of APs recorded from SPNs was also affected by prenatal ethanol exposure (3-way ANOVA, main effects: exposure: $F(1,271) = 13.193$, $p<0.001$, sex: $F(1,271) = 0.035$, $p =0.852$; interactions: exposure x sex x postnatal day: $F(3, 230) = 0.020$, $p= 0.996$, exposure x sex: $F(1,271 = 1.165$, $p=0.281$, exposure x postnatal day: $F(3,271) = 2.193$, $p=0.089$, sex x postnatal day: $F(3,271) = 0.326$, $p=0.807$). SPN half-widths tended to be

increased from animals exposed prenatally to ethanol, however, no significant differences in AP half-width were observed between groups on any postnatal day (**Figure 2.4h**). As observed in striatal GINs, AP peak amplitude was also unaltered by prenatal ethanol exposure regardless of postnatal day in SPNs from male or female mice (3-way ANOVA, main effects: exposure: $F(1,271) = 0.006$, $p=0.939$, sex: $F(1,271) = 0.397$, $p =0.529$; interactions: exposure x sex x postnatal day: $F(3, 230) = 0.349$, $p= 0.790$, exposure x sex: $F(1,271 = 0.980$, $p=0.323$, exposure x postnatal day: $F(3,271) = 1.544$, $p=0.203$, sex x postnatal day: $F(3,271) = 1.424$, $p=0.236$) (**Figure 2.6b**).

Together, these data again indicate differences in the effects of prenatal ethanol exposure on the development of SPNs in female and mice. While SPNs from both male and female mice demonstrated increased AP half-widths and firing rates during early postnatal develop following prenatal ethanol exposure, increases in SPN AP firing rate occur later (P8-10) in male mice relative to female mice (P4-6) (**Figure 2.4e,h**). Consistent with what we observed in striatal GINs, SPNs from male mice also demonstrated more depolarized AP thresholds following prenatal ethanol exposure at P2 (**Figure 2.4g**). Alternatively, by the end of the second postnatal week SPNs from both demonstrated more hyperpolarized AP thresholds (**Figure 2.4g**). This shift in AP threshold coincides with increased firing rates in response to lower current inputs in SPNs from female mice following prenatal ethanol exposure, while SPNs from male mice demonstrate decreased firing rates regardless of the degree of depolarizing current input (**Figure 2.4e**).

2.4.2c Striatal GINs- Intrinsic properties

Given the significant differences in the firing properties of striatal GINs following prenatal ethanol exposure, we next asked if the intrinsic properties: RMP, IR, membrane capacitance and membrane time constant, of developing striatal GINs and SPNs were

also changed. Prenatal ethanol exposure significantly altered the resting membrane potential of striatal GINs, with effects that varied depending on the sex and postnatal day assessed (3-way ANOVA, main effects: exposure: $F(1,230) = 3.728$, $p=0.055$, sex: $F(1,230) = 4.940$, $p =0.027$; interactions: exposure x sex x postnatal day: $F(3, 230) = 2.917$, $p=0.035$, exposure x sex: $F(1,230 = 6.523$, $p = 0.019$, exposure x postnatal day: $F(3,230) = 3.671$, $p=0.013$, sex x postnatal day: $F(3,230) = 4.065$, $p<0.001$) (Table 3, Figure 5 a-h). Though no significant differences between groups were observed on individual postnatal days, several trends emerged. At P2 and P4, the RMP of striatal GINs from ethanol-exposed female mice tended to more hyperpolarized those of both control-fed females, and GINs from male mice regardless of exposure, which resolves at P8-10, but is again present at P14 (**Figure 2.5b**). In contrast, at P8-10, prenatal ethanol resulted in depolarized RMP in striatal GINs from male mice relative to those from control-fed male mice (**Figure 2.5b**).

IR, membrane time constant and capacitance of striatal GINs were unaltered by prenatal ethanol exposure (3-way ANOVAs, IR: main effects: exposure: $F(1,232) = 1.254$, $p=0.264$, sex: $F(1,232) = 0.530$, $p =0.467$; interactions: exposure x sex x postnatal day: $F(3,232) = 1.083$, $p=0.357$, exposure x sex: $F(1,232) = 1.404$, $p=0.237$, exposure x postnatal day: $F(3,232) = 0.791$, $p=0.500$, sex x postnatal day: $F(3,232) = 1.378$, $p=0.250$; membrane time constant: main effects: exposure: $F(1,197) = 0.249$, $p=0.619$, sex: $F(1,197) = 0.718$, $p =0.398$; interactions: exposure x sex x postnatal day: $F(3, 197) = 2.120$, $p=0.099$, exposure x sex: $F(1,197) = 0.257$, $p=0.613$, exposure x postnatal day: $F(3,197) = 0.362$, $p=0.781$, sex x postnatal day: $F(3,197) = 0.412$, $p=0.745$ capacitance: main effects: exposure: $F(1,197) = 0.642$, $p=0.424$, sex: $F(1,197) = 0.187$, $p =0.666$; interactions: exposure x sex x postnatal day: $F(3,197) = 1.440$, $p=0.232$, exposure x sex:

F(1,197) = 0.646, p=0.422, exposure x postnatal day: F(3,197) = 0.463, p=0.708, sex x postnatal day: F(3,197) = 1.950, p=0.123 (**Figure 2.5c-d, 2.6b**).

These data indicate that increases in striatal GIN AP firing rates following prenatal ethanol exposure (**Figure 2.4a,c**) occur in the presence of both subtly more hyperpolarized and depolarized RMPs, depending on the postnatal day and in the absence of obvious differences in other intrinsic properties of striatal GINs (**Figure 2.5b**).

2.4.2c Striatal SPNs- Intrinsic Properties

Prenatal ethanol exposure resulted in significant differences in the RMP of developing SPNs in both male and female mice (3-way ANOVA, main effects: exposure: F(1,271) = 10.503, p=0.001, sex: F(1,271) = 0.831, p =0.363; interactions: exposure x sex x postnatal day: F(3,271) = 0.670, p=0.571, exposure x sex: F(1,271) = 1.418, p=0.235, exposure x postnatal day: F(3,271) = 1.295, p=0.276, sex x postnatal day: F(3,271) = 1.339, p=0.262) (**Figure 2.5f**). At P4-6 prenatal ethanol exposure resulted in depolarized RMP in SPNs from male mice relative to control-fed male and female mice, while prenatal ethanol exposure did not result in differences in SPNs from female mice (**Figure 2.5f**). A similar trend was observed at P2, though differences were not significant (1-way ANOVA, F(1,62) = 3.390, p=0.070). Significant differences in SPN RMP were not present during the second postnatal week (**Figure 2.5f**). Alternatively, prenatal ethanol exposure also resulted in more depolarized RMP in SPNs from female mice relative to those in SPNs from control-fed female mice at both P2 and P4-6, though these differences were not significant (**Figure 2.5f**).

Unlike striatal GINs, the IR of SPNs was also significantly affected by prenatal ethanol exposure, independent of sex or postnatal day (3-way ANOVA: main effects: exposure: F(1,271) = 8.100, p=0.005, sex: F(1,271) = 0.009, p =0.923; interactions: exposure x sex

x postnatal day: F(3,271) = 0.369, p=0.775, exposure x sex: F(1,271) = 0.484, p=0.487, exposure x postnatal day: F(3,271) = 1.555, p=0.201, sex x postnatal day: F(3,271) = 0.038, p=0.990). Though significant differences were not observed on individual postnatal days, we observed a trend towards an increase in IR of SPN at P2 and P4-6 in SPNs from both male and female mice relative those fed a control diet (**Figure 2.5g**). Membrane time constant of SPNs also significantly differed following a prenatal ethanol exposure (3-way ANOVA: main effects: exposure: F(1,204) = 5.814, p=0.017, sex: F(1,204) = 0.017, p =0.897; interactions: exposure x sex x postnatal day: F(3,204) = 0.815, p=0.487, exposure x sex: F(1,204) = 0.086, p=0.770, exposure x postnatal day: F(3,204) = 0.937, p=0.424, sex x postnatal day: F(3,204) = 4.081, p=0.008.) On postnatal day 8-10: SPNs recorded from control-fed males demonstrated a significantly increased membrane time constant relative to those from ethanol-exposed male mice (**Figure 2.5h**).The membrane capacitance of SPNs was unaffected by prenatal ethanol exposure and did not differ between sexes (3-way ANOVA, main effects: exposure: F(1,204) = 2.525, p=0.114, sex: F(1,204) = 0.020, p =0.888; interactions: exposure x sex x postnatal day: F(3,204) = 1.645, p=0.180, exposure x sex: F(1,204) = 2.706, p=0.102, exposure x postnatal day: F(3,204) = 0.680, p=0.565, sex x postnatal day: F(3,204) = 0.284, p=0.837 (**Figure 2.6d**).

Consistent with observed increases in SPN AP half-width during early postnatal development (P2-6), the more depolarized RMP and increased IR of developing SPNs suggest that prenatal ethanol exposure results in a delayed functional maturation of SPNs in mice of both sexes, although these effects are more pronounced in SPNs from male mice. These data also suggest that alterations to the intrinsic properties precede increases in SPN firing rate observed following prenatal ethanol exposure in SPNs from female mice (P4-6), and male mice (P8-10) (**Figure 2.5e**).

In addition to the effects of prenatal ethanol exposure on striatal neurons in female and male mice, we also observed differences in the functional and firing properties of striatal GINs and SPNs between control-fed male and female mice. At P2, striatal GINs and SPNs from control-fed male mice tended to have a more depolarized RMP relative control-fed female mice (**Figure 2.4b,f**). This coincided with a more depolarized AP threshold and lower firing rate in striatal GINs from male mice, relative to those from female mice (**Figure 2.4a,c**). No sex differences were observed in the AP threshold or firing rate of SPNs at P2 (**Figure 2.4e,g**). Sex differences were also present in control-fed male and female mice were also apparent at P14, where SPNs demonstrated an increased firing rate in male mice, and striatal GINs demonstrated a trend towards an increased firing rate in male mice that coincided with a more depolarized AP threshold (**Figure 2.4a,c,e**). In addition, SPNs from male and female mice differed in their membrane time constant depending on the postnatal day, regardless of prenatal ethanol exposure (3-way ANOVA, interaction: sex x postnatal day: $F(3,204) = 4.081$, $p=0.008$.) Time constant tended to be increased in male mice at P2 and P14, but decreased relative to female mice at P4-6 and P8-10, though these differences were not significant.

2.4.3 Prenatal ethanol exposure alters the development of glutamatergic and synaptic GABAergic connections to striatal GINs and SPNs in a sex-dependent manner during the first two postnatal weeks

The intrinsic excitability and firing properties of striatal neurons is modified by synaptic input in both developing and adult animals (Lahiri and Bevan, 2020; Lieberman et al., 2018; Wilson and Kawaguchi, 1996). Thus, we next asked how differences in the functional properties might relate to the development of glutamatergic and synaptic afferents of striatal GINs and SPNs. Striatal GINs and SPNS receive convergent glutamatergic input from the cortex, thalamus and limbic regions including the

hippocampus and amygdala, and share local GABAergic inputs from GINs and SPNs, and distant inhibitory inputs from cortex and pallidum (Bertero et al., 2020; Klug et al., 2018; Melzer et al., 2017; Tepper et al., 2008; Wall et al., 2013). Glutamatergic and GABAergic synaptic currents have been observed in embryonic SPNs, and gradually increase over the first two postnatal weeks coinciding with the onset of mature functional properties in SPNs (Dehorter et al., 2011; Kozorovitskiy et al., 2012; Krajeski et al., 2019; Peixoto et al., 2016; Sohur et al., 2014; Tepper et al., 1998). When striatal GINs first receive input from synaptic afferents is not yet known. We again performed whole-cell patch clamp recordings using acute coronal sections from behaviorally tested neonates to record GABAergic and glutamatergic spontaneous post-synaptic currents (sPSCs) from developing striatal GINs and SPNs. We asked if prenatal ethanol exposure altered the frequency and amplitude of glutamatergic sPSCs during the first two postnatal weeks.

2.4.3a Striatal GINs- Glutamatergic sPSCs

Though we did not identify significant effects of prenatal ethanol exposure or sex on glutamatergic sPSC frequency in striatal GINs, we observed a trend towards an exposure x sex x postnatal day interaction suggesting the prenatal ethanol exposure alters the frequency of glutamatergic sPSCs in striatal GINs in a sex-dependent manner that varies across early postnatal development (3-way ANOVA, exposure x sex x postnatal day: $F(3,182) = 2.536$, $p = 0.058$; main effects: exposure: $F(1,182) = 0.883$, $p=0.349$, sex: $F(1,182) = 0.883$, $p =0.473$, postnatal day: $F(1,182) = 168.705$, $p<0.001$; interactions: exposure x sex: $F(1,182 = 0.909$, $p =0.342$, exposure x postnatal day: $F(3,182) = 0.789$, $p=0.501$; sex x postnatal day: $F(3,182) = 0.807$, $p = 0.492$) (**Figure 2.7c**). We then compared glutamate sPSC frequency in striatal GINs from control female, ethanol-exposed female, control-fed male and ethanol-exposed male mice by

postnatal day (P2, P4-6, P8-10, and P14). At P2, prenatal ethanol exposure also resulted in an increased glutamatergic sPSC frequency in control-fed male mice relative to control-fed female mice, though no differences were observed between ethanol-exposed male and female mice (**Figure 2.7c**). At P14, we determined that prenatal ethanol exposure decreased the frequency of glutamatergic PSCs in female mice relative to control-female mice (**Figure 2.7c**).

Prenatal ethanol exposure differentially altered the amplitude of glutamatergic sPSCs recorded from striatal GINs depending on the postnatal day assessed (3-way ANOVA, main effects: exposure: $F(1,182) = 0.164$, $p=0.686$, postnatal day: $F(1,182) = 6.263$, $p<0.001$, sex: $F(1,182) = 0.770$, $p =0.381$; interactions: exposure x sex x postnatal day: $F(3,182) = 0.603$, $p=0.614$), exposure x sex: $F(1,182 = 0.026$, $p = 0.872$, exposure x postnatal day: $F(3,182) = 3.885$, $p = 0.010$; sex x postnatal day: $F(3,182) = 0.692$, $p = 0.058$) (**Figure 2.7d**). At P14, prenatal ethanol exposure resulted in decreased glutamatergic sPSC amplitude in striatal GINs from female mice relative to those from control-fed female mice (**Figure 2.7d**).

2.4.3b SPNs- Glutamatergic sPSCs

Alternatively, we observed that prenatal ethanol exposure significantly changed the frequency of glutamatergic sPSCs recorded from SPNs, and found a trend towards an towards an exposure x sex x postnatal day interaction suggesting that similar to GINs, prenatal ethanol exposure alters the frequency of glutamatergic sPSCs in SPNs in a sex-dependent manner that varies across early postnatal development (3-way ANOVA, main effects: exposure: $F(1,193) = 12.003$, $p<0.001$, postnatal day: $F(1,193)= 18.882$, $p<0.001$, sex: $F(1,193) = 0.305$, $p =0.581$, interactions: exposure x sex x postnatal day: $F(3,193) = 0.526$, $p = 0.665$, exposure x sex: $F(1,193 = 2.621$, $p =0.528$, exposure x postnatal day: $F(3,193) = 0.052$, sex x postnatal day: $F(3,193) = 3.487$, $p = 0.017$. We

determined that at P2 prenatal ethanol exposure results in a decreased frequency of glutamatergic sPSCs in female mice, relative to control-fed male mice, and observed a trends toward decrease relative to control-fed female mice (p=0.061) (**Figure 2.7e**). At P14, we identified significant differences in ethanol-exposed male and control-fed female mice. (**Figure 2.7e**).

Prenatal ethanol exposure did not alter the amplitude of glutamatergic sPSCs recorded from SPNs, regardless of sex and postnatal day (3-way ANOVA, main effects: exposure: $F(1,182) = 0.253$, $p=0.615$, postnatal day: $F(1,182) = 12.194$, $p<0.001$, sex: $F(1,182) = 3.265$, $p =0.072$; interactions: exposure x sex x postnatal day: $F(3,182) = 0.706$, $p=0.549$, exposure x sex: $F(1,182 = 0.946$, $p = 0.332$, exposure x postnatal day: $F(3,182) = 1.199$, $p = 0.311$, sex x postnatal day: $F(3,182) = 1.805$, $p = 0.148$). (**Figure 2.7f**).

In summary, in female mice, prenatal ethanol exposure results in an early postnatal (P2) increase the frequency of glutamatergic sPSCs recorded from striatal GINs, suggesting an increase in glutamatergic synaptic inputs to striatal GINs coinciding with a decrease in glutamatergic inputs to SPNs. These differences resolve by the end of the first postnatal week, however, by P14 are replaced by decreases in both sPSC frequency of striatal GINs suggesting that prenatal ethanol exposure later results in diminished glutamatergic neurotransmission via both pre and postsynaptic mechanisms (**Figure 2.7b,c,e,f**). Contrastingly, prenatal ethanol exposure though prenatal ethanol exposure also results in an early postnatal decrease glutamatergic sPSC frequency in SPNs from male mice, this difference is not significant, and does not coincide with an increased glutamatergic sPSC frequency in striatal GINs frommale mice (**Figure 2.7b,c,e,f**). Following prenatal ethanol exposure, striatal GINs from male mice also fail to demonstrate decreases in glutamatergic sPSC frequency and amplitude observed in

GINs from female mice at P14, but unlike female mice, develop a more pronounced decrease in SPN glutamatergic sPSC frequency at P14.

2.4.3c Striatal GINs- GABAergic sPSCs

We next asked if the differences we observed in glutamatergic sPSC frequency and amplitude might coincide with changes to GABAergic sPSCs following prenatal ethanol exposure. In striatal GINs, we found an overall trend towards a sex-dependent effect of prenatal ethanol exposure on the frequency of GABAergic sPSCs that was not significant (3-way ANOVA, main effects: exposure: $F(1,182) = 2.973$, $p= 0.476$, postnatal day: $F(1,182) = 88.952$, $p<0.001$, sex: $F(1,182) = 6.748$, $p =0.01$; interactions: exposure x sex x postnatal day: $F(3,182) = 0.171$, $p=0.196$, exposure x sex: $F(1,182 =0.064$, $p =0.801$, exposure x postnatal day: $F(3,182) = 1.859$, $p=0.138$, sex x postnatal day: $F(3,182) = 2.581$, p 0.055) (**Figure 2.8c**). We did identify a specific group-dependent differences in glutamatergic sPSC frequency at P14, striatal GINs from control-fed female mice had a significantly increased GABAergic sPSC frequency relative to control-fed and ethanol-exposed male mice, while striatal GINs from ethanol-exposed female mice failed to demonstrate a similar increase in frequency (**Figure 2.8c**).

The amplitude of GABAergic sPSCs recorded from striatal GINs as significantly altered by prenatal ethanol exposure, depending on the postnatal day assessed regardless of sex (3-way ANOVA, main effects: exposure: $F(1,172) = 0.751$, $p=0.387$, postnatal day: $F(1,172) = 9.632$, $p<0.001$, sex: $F(1,172) = 0.851$, $p =0.358$; interactions: exposure x sex x postnatal day: $F(3,172) = 1.638$, $p=0.182$, exposure x sex: $F(1,172 = 0.311$, $p = 0.578$, exposure x postnatal day: $F(3,172) = 4.467$, $p = 0.005$, sex x postnatal day: $F(3,172) = 0.775$, $p = 0.509$). At P14, Striatal GINs from control-fed female mice had a decreased GABAergic sPSC amplitude relative to control-fed and ethanol-exposed male mice,

while ethanol-fed female mice failed to demonstrate a comparable decrease in GABAergic sPSC amplitude (**Figure 2.8d**).

2.4.3d SPNs- GABAergic sPSCs

Prenatal ethanol exposure significantly altered the frequency of GABAergic sPSCs in SPNs from both male and female mice across the first two postnatal weeks (3-way ANOVA, main effects: exposure: $F(1,193) = 9.687$, $p=0.002$, postnatal day: $F(1,193) = 38.104$, $p<0.001$, sex: $F(1,193) = 0.009$, $p =0.923$, interactions: exposure x sex x postnatal day: $F(3,193) = 2.099$, $p=0.102$, exposure x sex: $F(1,193 = 0.105$, $p = 0.747$, exposure x postnatal day: $F(3,193) = 0.365$, $p = 0.778$, sex x postnatal day: $F(3,193) = 2.107$, $p = 0.101$) (**Figure 2.8e**). At P2, SPNs from control fed-male mice demonstrated a significantly higher GABAergic sPSC frequency relative to all other groups, SPNs from male mice exposed prenatally to ethanol failed to demonstrate a similarly increased GABAergic sPSC frequency (Figure 8e). Although no significant differences in SPN GABAergic sPSC frequency were observed female or male mice P4-P14, both SPNs from both male and female mice tended to demonstrate a decreased GABAergic SPN frequency following prenatal ethanol exposure in both male and female mice during this time period (**Figure 2.8e**).

The amplitude of GABAergic sPSCs recorded from SPNs was also significantly altered by prenatal ethanol exposure depending on the postnatal day (3-way ANOVA, main effect: exposure: $F(1,192) = 0.009$, $p=0.924$, postnatal day: $F(1,192)= 9.302$, $p<0.001$, sex: $F(1,192) = 7.388$, $p=0.007$; interactions: exposure x sex x postnatal day: $F(3,192) = 0.324$, $p=0.808$, exposure x sex: $F(1,192 = 0.276$, $p = 0.600$, exposure x postnatal day: $F(3,192) = 2.836$, $p = 0.039$, sex x postnatal day: $F(3,192) = 1.155$, $p = 0.328$). We determined that at P8-10, prenatal ethanol exposure resulted in a decrease in SPN

GABAergic sPSC amplitude in male mice, relative to control-fed male mice. No differences were observed in GABAergic sPSC amplitude in female mice at P8-10.

In summary, prenatal ethanol exposure differentially affected GABAergic synaptic activity in striatal neurons of female and male mice during the first two postnatal weeks, including both pre and post-synaptic GABAergic neurotransmission in striatal GINs and SPNs, with more pronounced differences observed in striatal neurons from male mice.

Sex differences in synaptic inputs to striatal GINs and SPNs were also observed in control-fed mice. We determined that GABAergic sPSC frequency was significantly different in in striatal GINs from male and female animals, with a trend toward specific effects depending on the postnatal day assessed (3-way ANOVA, main effect: sex: $F(1,182) = 6.748$, $p = 0.01$, interaction: sex x postnatal day: $F(3,182) = 2.581$, p 0.055). GABAergic sPSC frequency tended to be decreased in striatal GINs from control-fed male relative to those from control-fed females at P8-10 and P14, though these differences were not significant (**Figure 2.8b**). Alternatively, at P14 both the amplitude GABAergic sPSCs recorded from SPNs was significantly different between control-fed male and female animals (3-way ANOVA, main effect: sex: $F(1,192) = 7.388$, $p = 0.007$) (**Figure 2.8f**).

2.4.4 Prenatal ethanol exposure results in transient alterations in SPN morphology in both female and male mice

Concurrent with rapid changes in functional properties, and synaptic inputs during the first two weeks of life postnatally, SPNs undergo considerable morphological development (Dehorter et al., 2011; Krajeski et al., 2019; Peixoto et al., 2016; Tepper et al., 1998). The dendritic complexity of SPNs has been closely associated with their excitability both during development and in adulthood, with less excitable SPNs neurons observed to bear longer and more complex dendritic trees (Cazorla et al., 2014; Gertler

et al., 2008; Krajeski et al., 2019; Lieberman et al., 2018). Thus, we next asked if differences in functional maturation and synaptic connectivity of SPNs might coincide with altered SPN morphology. To assess SPN morphology we filled SPNs with a neurobiotin tracer during patch-clamp electrophysiology experiments, then traced and reconstructed SPN dendrites from fixed tissue (**Figure 2.9**).

At P2, SPNs from female mice exposed prenatally to ethanol have more complex dendrites as measured by Sholl analysis (ie. an increased number of intersections/10 μM radius) when compared to control-fed female mice, while increases in the dendritic complexity of SPNs from ethanol-exposed male mice relative to SPNs from control-fed male mice are less pronounced (**Figure 2.9c**). Observed increases in dendritic complexity in female mice diminished over time, with significant differences only apparent at P4-6 between SPNs from ethanol-exposed female mice and those of control and ethanol-exposed male mice (**Figure 2.9c**). Alternatively, at both P2 and P8-10 SPNs from ethanol-exposed male mice demonstrated increased complexity relative to those from control-fed female mice but not control-fed male mice, while complexity of SPNs from control-fed male and female mice did not differ (**Figure 2.9c**).

Differences in the dendritic complexity as measured by Sholl analysis could result from changes in the number of dendrites, dendritic length, or dendritic branchpoints(number of nodes), and may coincide with changes in the growth of other neuronal compartments, including the soma. We found that prenatal ethanol exposure differentially altered the number of dendrites in SPNs of female and male mice depending on the postnatal day (ordinal logistic regression, exposure x sex x postnatal day: Wald $\chi2(3) = 10.425$, $p=0.015$; main effects: exposure: Wald $\chi2(1) = 8.575$, $p=0.003$, sex: Wald $\chi2(1) = 2.505$, $p=0.113$, postnatal day: Wald $\chi2(1) = 0.268$, $p=0.604$) (**Figure 2.9e**). An increase in dendritic number was observed in SPNs from

ethanol-exposed female mice relative to SPNs from control-fed female, and ethanol-exposed male mice at P2 and persisted to P4-6.

Prenatal ethanol exposure also differentially modified mean length/dendrite (μM) (mean length/dendrite: 3-way ANOVA, exposure x sex x postnatal day: $F(3,232) = 5.453$, $p = 0.001$; main effects: exposure: $F(1,232) = 1.030$, $p = 0.311$, sex: $F(1,232) = 0.055$, $p = 0.055$, postnatal day: $F(3,232) = 29.118$, $p < 0.001$; interactions: exposure x postnatal day: $F(3,232) = 1.503$, $p = 0.214$, exposure x sex $F(1,232) = 1.162$, $p = 0.282$, postnatal day x sex: $F(3,232) = 11.774$, $p < 0.001$) (**Figure 2.9d**). SPNs from ethanol-exposed female mice demonstrated a decreased mean length/dendrite relative to SPNs from control-fed male and female mice at both P2 and P4-6. Differences in dendritic complexity as measured by Sholl analysis resolved completely by P14 in both male and female mice, while an increase in the mean length of dendrites in SPNs from male mice exposed-prenatally to ethanol relative to ethanol-exposed female mice was observed at both P8-10, while decreased mean dendritic length was observed in ethanol exposed at P14 (**Figure 2.9c,d**).

The effects of prenatal ethanol exposure on the mean of nodes per dendrite and soma area (μM2) were also sex-dependent (mean nodes per dendrite: 2-way ANOVA, exposure x sex: $F(1,232) = 7.159$, $p = 0.008$; main effects: exposure: $F(1,232) = 0.007$, $p = 0.933$, sex: $F(1,232) = 1.362$, $p = 0.244$; soma area: 2-way ANOVA, exposure x sex: $F(1,232) = 10.014$, $p = 0.002$; main effects: exposure: $F(1,232) = 0.674$, $p = 0.412$, sex: $F(1,232) = 0.141$, $p = 0.707$) (Figure 9f,g). We were unable to resolve differences in the mean number of dendritic branchpoints (nodes) per dendrite following prenatal ethanol exposure in male or female mice at specific postnatal time points (**Figure 2.9f**). Prenatal ethanol exposure increased the soma area (μM2) if SPNs from F mice, relative to those from control-fed F mice at P4, while ethanol-exposure did not alter soma area in SPNs

from male mice No differences in soma area were observed at P2, P8-10 or P14 (**Figure 2.9g**).

All in all, though SPNs from both female and male mice demonstrate increases in dendritic complexity during the first two postnatal weeks following prenatal ethanol, increases in complexity are delayed in SPNs from male mice, and result from changes to different aspects of dendritic growth with SPNs from male mice increasing dendritic length, while SPNs from female mice were observed to have an increased number of dendrites.

In addition to effects of prenatal ethanol exposure, we also observed sex differences in the dendritic morphology of SPNs from control-fed male and female animal during the first postnatal week: control-fed female mice demonstrated an increase in mean nodes/dendrite at P2, that resolved by P4 (**Figure 2.9f**). SPNs from control-fed female mice also had a decreased soma area relative to control-fed male mice (**Figure 2.9g**).

2.5 Discussion

The clinical effects of prenatal ethanol exposure first become apparent during early child development and persist into adolescence and adulthood despite the significant growth and change that occurs during postnatal neural development. However, investigations of changes at the circuit-level in animal models of prenatal ethanol exposure have frequently focused on a single developmental time point or a single population of neurons in adolescent or adult animals. Here, we explored the differential effects of prenatal ethanol exposure on two principal populations of striatal neurons: GINs and SPNs, during the first two postnatal weeks, a time period during which there are dramatic changes in the functional properties of and synaptic inputs to striatal neurons, as well as the development of early motor behaviors. We identify: (1) sex-dependent

differences in the development of early motor behaviors and concurrent differences in the maturation of two populations of striatal neurons: striatal GINs and SPNs, (2) differential susceptibility of striatal GINs and SPNs to the effects of a brief binge exposure to ethanol in male and female mice, and (3) effects of prenatal ethanol exposure on both motor behaviors and neuronal maturation that differ across the first two postnatal weeks.

2.5.1 Sex differences in the development of early motor behaviors following a brief binge prenatal ethanol exposure.

We determined that male neonates exposed prenatally to ethanol demonstrate developmental motor delays, detectable in several motor tasks throughout the first two postnatal weeks that are independent of differences in gross physical development (**Figure 2.1b, d-g**). Alternatively, changes in the motor development of female mice following prenatal ethanol exposure were less pronounced; subtle behavioral differences were observed in the onset of quadruped walking behavior and surface righting time (**Figure 2.1d,f**). We also observed a period (P4-6) when female appeared to develop more rapidly than control-fed females in surface righting behavior (**Figure 2.1f**).

Male vulnerability to deficits related to gestational and perinatal exposures, as well as neurodevelopmental disorders, is well documented (Bölte et al., 2023; DiPietro & Voegtline, 2017). While several cohort studies have identified higher rates of FASD in male children, they have not been recapitulated in recent epidemiological studies seeking to estimate the global prevalence of FASD (Astley, 2010; Fox et al., 2015; May et al., 2014, 1983; Thanh et al., 2014). However, the clinical presentation of FASD has been observed to differ between sexes, with individuals identifying as male demonstrating more dramatic differences in early development and measures of neurodevelopmental impairment, including the onset of early motor behaviors, while

those identifying as female experienced higher rates of co-diagnosed endocrine, mood and anxiety disorders (Flannigan et al., 2023; May et al., 2017). Increased susceptibility of male offspring to the functional and behavioral effects of prenatal ethanol exposure has been observed in rodent models assessing adolescent and adult animals, including sex-dependent differences in both motor behaviors and the function of striatal neurons (Blanchard et al., 1993; Mooney and Varlinskaya, 2011; Rice et al., 2012; Rodriguez et al., 2016; Rouzer and Diaz, 2022; Schambra et al., 2016). We find that a brief binge prenatal ethanol exposure model results in temporally concurrent changes in neonatal motor behavior and subtype-specific differences in the function, synaptic connections and morphology of striatal neurons, suggesting that differences in the development of striatal neurons could contribute to observed developmental motor delays.

The temporal association between sex-dependent differences in behavior and the maturation of striatal neurons is apparent across the first two postnatal weeks. For example, at postnatal day 2, we determined that prenatal ethanol exposure results in a male-specific increase in surface righting time (**Figure *2*.1f**). At this postnatal time point, though SPNs from both male and female mice tended to receive fewer glutamatergic synaptic afferents and had less mature/more excitable functional properties: increased IR, decreased AP half-width following prenatal ethanol exposure, the effects of prenatal ethanol exposure on synaptic inputs to, and functional properties of striatal GINs in female and male animals significantly differed (**Figure *2*.4d, *2*.5g, *2*.7e**). In female mice, prenatal ethanol exposure resulted in an increase in glutamatergic sPSC frequency in striatal GINs, suggesting they receive an increased number or strength afferent inputs from glutamatergic neurons, as well as an increased GIN AP firing rate (**Figure *2*.4a, *2*.7b**). Alternatively, in male mice, prenatal ethanol exposure did not alter the GIN glutamatergic sPSC frequency or GIN AP firing rate, while action potentials recorded

from striatal GINs increased half-widths, suggesting a maturational delay (Plotkin et al., 2005) (**Figure 2.4a,d, 2.7b**). SPNs in male mice also receive fewer or weaker GABAergic inputs and failed to develop increases in dendritic complexity comparable to those observed in female after prenatal ethanol exposure (**Figure 2.7e, 2.9a,c**). Taken together, our findings lead us to hypothesize that prenatal ethanol exposure delays the maturation of striatal GINs in male mice, resulting in the formation of fewer GABAergic inputs to developing SPNs. GABAergic signaling has previously been shown to facilitate the formation of neuronal processes in multiple brain regions during early development, thus we further hypothesize that the decrease in GABAergic synaptic activity we observe in SPNs from male mice after prenatal ethanol exposure may result in a failure to increase SPN dendritic complexity (Sernagor et al., 2010).

Sex differences in the morphological development of SPNs following prenatal ethanol exposure are also apparent at later developmental time points. While SPNs from female mice develop an early (P2-4) increase in dendritic complexity, resulting from an increased number of shorter and less complex dendrites, male mice show fewer morphological differences early on (P2), but develop a longer lasting increase in dendritic complexity (P4-10), associated with longer, more highly branched dendrites (**Figure 2.9a-f**). Differences in dendritic complexity coincide with changes in SPN AP firing rate that differ between female and male mice. Though both female and male mice demonstrate transient increases in SPN AP firing rate between P4-P10 the increase observed at P4-6 in SPNs from female mice exposed prenatally to ethanol, precedes a similar elevation in firing rate at P8-10 in SPNs from male mice exposed prenatally to ethanol (**Figure 2.4e**). These changes in SPN firing properties occur simultaneously with increases in GABAergic activity: while at P4-6 the firing rate of striatal GINs from ethanol-exposed female mice does not differ from that of control-fed females, striatal

GINs do demonstrate a higher GABAergic sPSC frequency (**Figure *2*.4a, 8a**). Alternatively, at P8-10, an increase in the amplitude of GABAergic sPSC frequency is increased in SPNs recorded from male mice exposed prenatally to ethanol (**Figure *2*.8f**). Interestingly, these changes in SPN firing and GABAergic signaling parallel time points where behavioral testing revealed developmental motor delays unique to one experimental group: at P4-6 only prenatal ethanol exposure only results in increased surface righting time in female mice, while only male mice exposed prenatally to ethanol demonstrate increased negative geotaxis time and increased TMS at P8-10 (**Figure *2*.1c, 1g**).

Finally, at P14 when minimal differences in motor behavior are apparent (**Figure *2*.1d**), we again observe pronounced differences in the effects of prenatal ethanol exposure on striatal GINs and SPNs from female and male neonates. While prenatal ethanol exposure resulted in more hyperpolarized AP thresholds and increased AP firing rates in striatal GINs from both female and male mice, GINs from female mice also received smaller amplitude GABAergic sPSCs (**Figure *2*.4a,c, *2*.8c**). Differences in firing rate following prenatal ethanol exposure were less pronounced in GINs from male mice, and coincided with decreases in the frequency and amplitude of glutamatergic sPSCs (F**igure *2*.4a,c, *2*.7b-c**). Prenatal ethanol exposure also resulted in more hyperpolarized AP thresholds in SPN from both female and male mice. SPN from female mice exposed prenatally to ethanol demonstrated differences in SPN firing rate depending on the size of the current input with increased firing in response to smaller currents, and a decreased firing rate at larger currents, as a well as a decrease in the frequency and amplitude of glutamatergic sPSCs (**Figure *2*.4e,g, *2*.7e,f**). Prenatal ethanol exposure resulted in decreased AP firing rates in SPN in male mice and a decrease in the frequency of glutamatergic synaptic inputs (**Figure *2*.4e,g, *2*.7e**).

Overall, the time-dependence of the behavioral and functional phenotypes we observe suggests that prenatal ethanol exposure may differentially alter the developmental trajectories of motor behavior in female and male mice, and may be related to differences in the maturation of striatal neurons. Though the parallels we observe between differences in behavioral outcomes and the development of striatal neurons are internally consistent and, therefore, highly suggestive. However, the effects of prenatal ethanol exposure on motor development of female and male mice could also be due to altered function of other local and distant afferent inputs to the striatum, including those from cholinergic and dopaminergic neurons or altered development of other CNS structures that contribute to early motor behavioral development, which have been shown to demonstrate sexually dimorphic features (Blanchard et al., 1993; Boggan et al., 1996; Giacometti and Barker, 2020; Miller, 1983; Supekar and Menon, 2015; Zachry et al., 2021). Further investigation will be necessary to rule out alternative sources of sex differences in early motor behavior following prenatal ethanol exposure.

2.5.2 Differential susceptibility of striatal GINs and SPNs to the effects of prenatal ethanol exposure during early postnatal development

We found that prenatal ethanol exposure differentially affected the functional and synaptic properties of striatal GINs and SPNs, adding to previous evidence that these two populations of neurons are differentially affected by exposures to ethanol in adult animals (Blomeley et al., 2011; Patton et al., 2016) (**Figures 2.4-8**). Overall, we observed that in both female and male mice exposed-prenatally to ethanol, events that facilitate the GABAergic signaling in the striatum, notably increased excitatory inputs to striatal GINs, increased AP firing rate of striatal GINs, increased number or strength of GABAergic inputs to striatal GINs and SPNs, or differences in number or subtype of GABA receptors that facilitate larger amplitude GABAergic synaptic events, are more

obvious in female mice, which demonstrate fewer behavioral differences (**Figure 2.1c-g, 2.4a-d, 2.7b-c, 2.8**). Increases in GABAergic signaling are also apparent during periods of behavioral recovery in male mice, while evidence of diminished disrupted maturation of GABAergic signaling coincide with periods when prenatal ethanol exposure results in marked developmental motor delays in male mice (**Figure 2.1c-g, 2.4a-d, 2.7b-c, 2.8**).

Changes in GABAergic signaling also coincide sex and developmental age-mediated differences in the effects of prenatal ethanol exposure SPN function and morphology. Female mice develop an increase in GIN firing rate and concurrent increase in SPN dendritic complexity (P2), this is followed by period of transient relief from elevated GIN firing rates concurrent with an increased SPN firing rate (P4-6), after which elevated GIN firing rates return and coincide with current input-dependent effects of prenatal ethanol exposure on SPN firing rate, with higher firing rates with smaller current inputs, and lower firing rates at with larger currents (P14) (**Figure 2.4a,e, 2.9c,e**). Alternatively, male mice demonstrate initial deficits in GIN maturation, and no increase in firing rate (P2), followed by a delayed increase in GIN firing rate (P8-10) that coincides with a later increase in dendritic complexity and SPN firing rate, followed by an increased GIN firing rate that coincides with decreased SPN firing rate (**Figure 2.4a,e, 2.9c-d,f**). The temporal association of changes in GABAergic signaling, and differences in the development of motor behaviors and striatal neurons in male and female mice following prenatal ethanol exposure highlights the potential importance of striatal GINs in recovering from the effects of a prenatal insult.

Differences in the number, subtype and function of GABAergic interneurons are frequently observed in developing circuits following early genetic or environmental insults, and contribute to behavioral differences in neurodevelopmental disorders (Penzes et al., 2013; Ruden et al., 2021; Yang et al., 2021). We have previously

demonstrated that our brief binge exposure alters the migration of GINs to the developing cortex, and contributes to an excitatory/inhibitory imbalance in the mPFC of young adult mice coinciding with hyperactivity and reversal learning deficits (Skorput et al., 2015). GABA facilitates early neuronal network development in a variety of ways: controlling gene expression, proliferation, growth, migration, synapse formation and the coordinating firing of developing neurons (Baho and Cristo, 2012; Bortone and Polleux, 2009; LoTurco et al., 1995; Sernagor et al., 2010; Wang and Kriegstein, 2008). Further investigation is necessary to determine how prenatal ethanol exposure may contribute to differences in GABAergic signaling in the developing striatum.

Potential mechanisms contributing to observed differences in the effects of prenatal ethanol exposure on striatal GINs and SPNs include both direct effects of ethanol on striatal neurons, indirect effects on the synaptic inputs to striatal neurons. Recent investigation of the acute effects of ethanol on cortical cells in utero suggests that ethanol's effects on gene expression in embryonic neurons may be both subtype-specific and sexually dimorphic (Salem et al., 2021; Sambo et al., 2022). The differing effects of prenatal ethanol exposure on striatal GINs and SPNs may result from either the acute ethanol exposure effects of ethanol prenatally, or the downstream effects of the prenatal exposure during early development. In either case, prenatal ethanol exposure could differentially modify the function or expression of ion channels in striatal GINs and SPNs, contributing to observed differences in membrane and firing properties (**Figure 2.4,2.5**). Differences in the expression and subtype of potassium channels contributes to the maturation of firing properties in striatal GINs and SPNs, an confer differential susceptibility to the effects of ethanol exposure in adult rodents (Aryal et al., 2009; Bates, 2013; Brodie et al., 2007; Cazorla et al., 2014; Dehorter et al., 2011; Lenz et al., 1994; Lieberman et al., 2018; Plotkin et al., 2005; Prüss et al., 2003; Tavian et al.,

2011). Sodium and calcium channels can both contribute to the excitability of striatal neurons, are modified by ethanol exposure, and may also differ between striatal neuronal subtypes during early development (Hunt and Mullin, 1985; Lee et al., 2022; Walter and Messing, 1999). Alternatively, given the sex-dependence of the effects of prenatal ethanol exposure on the functional developmental of striatal neurons, the unique effects on striatal GINs and SPNs could also be due to differences in the embryonic or postnatal expression of estrogen receptors or aromatase in these neurons (Fan et al., 2006; Küppers and Beyer, 1998; MacLusky et al., 1979).

Apart from differences in ion channel expression or function, prenatal ethanol exposure may result in cell-subtype specific differences in afferents to striatal neurons contributing to differences in firing properties. Striatal GINs and SPNs differ in their connectivity to the thalamus, cortex, dopaminergic neuron and cholinergic neurons, and demonstrate different forms of short-term plasticity in response to excitatory stimulations from motor, somatosensory and prefrontal cortex (Arias-García et al., 2018; Clarke and Adermark, 2015; Gittis et al., 2010; Johansson and Silberberg, 2020; Kocaturk et al., 2022; Lim et al., 2014; Smith et al., 2004). Prenatal ethanol exposure has been shown to alter the function of pyramidal neurons in the thalamus, as well as in motor, somatosensory and prefrontal cortex (Delatour et al., 2019a, 2019b; Granato et al., 1995; Mohammad et al., 2020; Mooney and Miller, 2010; Skorput et al., 2015). Furthermore, the relative contribution of AMPA and NMDA post-synaptic glutamate receptors mediating glutamatergic inputs differs between striatal GINs and SPNs (Gittis et al., 2010; Vizcarra-Chacón et al., 2013). Glutamate receptor subunit expression also varies in subpopulations of striatal neurons (Chen et al., 1996; Götz et al., 1997). Ethanol acutely inhibits glutamate receptor function to varying degrees depending on glutamate receptor

subunit expression (Allgaier, 2002; Lovinger, 1993; Lovinger et al., 1989; Möykkynen and Korpi, 2012).

Striatal GIN and SPNs also vary in their GABAergic inputs, demonstrating differences in the strength and source of GABAergic inputs, their post-synaptic GABA receptor subunit expression, and the net effect of GABA to depolarize or hyperpolarize post-synaptic neurons (Boccalaro et al., 2019; Dehorter et al., 2011; Misgeld et al., 1982; Straub et al., 2016; Tapia et al., 2019). Furthermore, past work also suggests that the firing properties and synaptic inputs are highly related in striatal neurons; genetic manipulation of post-synaptic SPNs, and their excitability can alter the strength of their glutamatergic inputs, while diminishing glutamatergic inputs to SPNs can modify their excitability (Benthall et al., 2021; Kozorovitskiy et al., 2012; Mowery et al., 2017). Differences in the distribution and subunit composition of post-synaptic glutamate and GABA receptors, connections to cortical and thalamic regions, and GABAergic neurons, mechanisms short-term synaptic plasticity, and differences in neuromodulation by dopamine or acetylcholine could all contribute to observed differences in the effects of prenatal ethanol exposure on the developing GABAergic and glutamatergic synaptic connections of striatal GINs and SPNs (**Figure 2.7, 2.8**).

2.5.3 Dynamic change in the effects of prenatal ethanol exposure on early striatal development

In addition to differences in based on biological sex, and striatal neuron subtype, we also determined that the effects of prenatal ethanol exposure across behavioral, functional and morphological phenotypes varied depending on the developmental period assessed. We observed an increase in SPN complexity after prenatal ethanol exposure at early developmental time points (P2-6) in female mice that were slightly delayed in male mice (P4-10) but had largely resolved in both male and female mice by P14 (**Figure 2.9**). This

transient alteration in neuronal morphology is consistent with the response of pyramidal neurons in the somatosensory cortex using the same ethanol exposure paradigm, which demonstrated increased dendritic complexity at P6-8, resolving by P28-30, despite persistent changes in synaptic connectivity (Delatour et al., 2019a, 2019b). Our findings stand in contrast with previous investigations which found that prenatal ethanol exposure resulted in increases or reductions in SPN dendritic complexity in adolescent or adult mice (Cheng et al., 2018; Rice et al., 2012). The contrasting results we observe may be due to differences in the timing and dose of the prenatal ethanol exposure which may contribute to the degree and persistence of morphological changes. Alternatively, the resolution of morphological differences we observe in SPN may be brief, and differences in dendritic complexity may return in adolescent and adult mice.

We also observed that while deficits in glutamatergic and GABAergic synapses were apparent at both P2, and P14, there was a transient resolution of early GABAergic and glutamatergic synaptic deficits from P4-10, that may occur via several possible mechanisms (**Figure 2.7 and 2.8**). Glutamatergic synaptic activity detectable in the embryonic striatum is likely driven by thalamic inputs which form E10-16.5, rather than those from the developing cortex which form later during embryonic and early postnatal development (Angevine, 1970; Dehorter et al., 2011; Molnár et al., 1998; Nakamura et al., 2005; Sohur et al., 2014). Thalamo- and corticostriate afferents also differ in the timing of their developmental shift in the contributions of post-synaptic AMPA and NMDA receptors to glutamatergic currents (Krajeski et al., 2019). Early postnatal glutamatergic inputs to the striatum largely rely on NMDA receptors, while levels of AMPA receptors increase during the first postnatal week at thalamostriate synapses, and later (P12-28) in corticostriate synapses (Krajeski et al., 2019). Considering these differences in the context of the timing of our in utero ethanol exposure, it is possible that the early

changes we observed in glutamatergic synaptic inputs to SPN are due to alterations in the development of thalamic inputs, while the transient recovery we occur during from P4-10 occurs due to formation of novel corticostriate connections or a developmental increase in thalamostriate activity. Alternatively, early glutamatergic synaptic events are largely due to spontaneous rather than action-potential mediated vesicle release mechanisms, which are first detectable through whole-cell patch clamp recordings of electrically evoked glutamatergic currents at (Dehorter et al., 2011; Krajeski et al., 2019; Mozhayeva et al., 2002). The transient resolution in synaptic activity we observed from P4-10 could also result from compensatory increases in action-potential mediated currents, which increase as development progresses (Dehorter et al., 2011; Krajeski et al., 2019).

In addition to developmental alterations to striatal glutamatergic afferents, the nature of GABAergic signaling also changes in early development and may contribute to the temporal variation we observe in the effects of prenatal ethanol exposure on developing striatal GINs and SPNs. In the cortex and hippocampus, early GABAergic action on post-synaptic GABA-A receptors results in a net depolarization of the post-synaptic neuron (Ben-Ari et al., 2012). This depolarizing action of GABA persists until the end of the first postnatal week, after which there is a developmental shift from depolarizing to hyperpolarizing action of GABA, which occurs due to differences in the concentration of intracellular chloride, mediated by changing expression levels of two chloride co-transporters: NKCC1 and KCC2 (Ben-Ari et al., 2012; Kalemaki et al., 2022; Leonzino et al., 2016). Alternatively, GABA-A receptor mediated activity continues to result in depolarizing post-synaptic currents in SPN into adulthood due to a lack of KCC2 expression, and may result in a net excitation or inhibition depending on SPN membrane potential (Bartos et al., 2007; Dehorter et al., 2011, 2009; Misgeld et al., 1982; Tapia et

al., 2019). However, when during development the ‘GABA switch’ occurs in striatal GINs, and if SPNs demonstrate from excitatory to inhibitory action of depolarizing GABA is not yet known. Determining if and when these developmental changes in GABAergic signaling occur, and how they may be altered following prenatal ethanol exposure will enhance our understanding of the differential effects of prenatal ethanol exposure on developing striatal neurons.

Additionally, though differences in performance on our series of motor tasks are nearly imperceptible between ethanol-exposed and control -fed mice by P14, we observed persistent differences in the firing properties of and synaptic inputs to striatal neurons (**Figure 2.7,2.8**). These data raise the question of how the effects of prenatal ethanol exposure on the function of striatal neurons may continue to evolve into adolescence and adulthood, and ultimately contribute to differences in motor behavior in adolescents and adults diagnosed with FASD (Connor et al., 2006). Understanding the effects of prenatal ethanol exposure on major developmental events in the striatum, including: the developmental increase in thalamic and cortical glutamatergic afferents, increased strength of action-potential mediated synaptic events, and the shift in GABA’s relative excitatory vs. inhibitory action, will be critical in interpreting the potential contribution of altered striatal circuit development to the etiology of FASDs.

2.5.4 Implications for FASD

This study identifies sexually dimorphic effects of prenatal ethanol exposure on early motor behaviors that coincide with sex-dependent differences in the development of striatal GINs and SPNs, and vary during the first two postnatal weeks. These data support recent evidence from studies employing both clinical imaging and rodent models that suggest males may be more vulnerable to certain effects of prenatal ethanol exposure, and particularly the early clinical presentation of FASDs. Our findings also

highlight potential age-dependent changes in neuronal function resulting from a prenatal ethanol exposure, that may modify the expression of clinical symptoms in FASD. Differences in functional and behavioral phenotypes depending on the postnatal time point have been previously observed in several rodent models of neurodevelopmental disorders (Chung et al., 2022; Kim et al., 2022; Peixoto et al., 2019, 2016). Reports from longitudinal clinical imaging studies suggest that prenatal ethanol exposure can also result in differential alterations the volume and functional connectivity of cortical structures and white matter, across development (Hendrickson et al., 2018; Kar et al., 2022; Lebel et al., 2012; Long et al., 2018; Moore and Xia, 2022; Treit et al., 2014). Our findings further indicate that shifts in the effects of prenatal ethanol exposure during early development occur at the level of differences in the functional and morphological development of individual neurons. Considering the changing impact of prenatal ethanol exposure across the developmental trajectory in the future may inform care management decisions for individuals with FASDs, beginning with early childhood diagnosis and intervention, and extending into adulthood.

2.6 References

Allgaier, C., 2002. Ethanol sensitivity of NMDA receptors. Neurochemistry International 41, 377–382. https://doi.org/10.1016/S0197-0186(02)00046-3

Altman, J., Sudarshan, K., 1975. Postnatal development of locomotion in the laboratory rat. Animal Behaviour 23, 896–920. https://doi.org/10.1016/0003-3472(75)90114-1

Angevine, J.B., 1970. Time of neuron origin in the diencephalon of the mouse. An autoradiographic study. Journal of Comparative Neurology 139, 129–187. https://doi.org/10.1002/cne.901390202

Arias-García, M.A., Tapia, D., Laville, J.A., Calderón, V.M., Ramiro-Cortés, Y., Bargas, J., Galarraga, E., 2018. Functional comparison of corticostriatal and thalamostriatal postsynaptic responses in striatal neurons of the mouse. Brain Struct Funct 223, 1229–1253. https://doi.org/10.1007/s00429-017-1536-6

Armstrong, E.C., Caruso, A., Servadio, M., Andreae, L.C., Trezza, V., Scattoni, M.L., Fernandes, C., 2019. Assessing the developmental trajectory of mouse models of neurodevelopmental disorders: Social and communication deficits in mice with Neurexin 1α deletion. Genes, Brain and Behavior n/a. https://doi.org/10.1111/gbb.12630

Aryal, P., Dvir, H., Choe, S., Slesinger, P.A., 2009. A discrete alcohol pocket involved in GIRK channel activation. Nat Neurosci 12, 988–995. https://doi.org/10.1038/nn.2358

Astley, S.J., 2010. Profile of the first 1,400 patients receiving diagnostic evaluations for fetal alcohol spectrum disorder at the Washington State Fetal Alcohol Syndrome Diagnostic & Prevention Network. Can J Clin Pharmacol 17, e132-164.

Baho, E., Cristo, G.D., 2012. Neural Activity and Neurotransmission Regulate the Maturation of the Innervation Field of Cortical GABAergic Interneurons in an Age-Dependent Manner. J. Neurosci. 32, 911–918. https://doi.org/10.1523/JNEUROSCI.4352-11.2012

Bartos, M., Vida, I., Jonas, P., 2007. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. Nat Rev Neurosci 8, 45–56. https://doi.org/10.1038/nrn2044

Bates, E.A., 2013. A potential molecular target for morphological defects of fetal alcohol syndrome: Kir2.1. Current Opinion in Genetics & Development, Molecular and genetic bases of disease 23, 324–329. https://doi.org/10.1016/j.gde.2013.05.001

Belleau, M.L., Warren, R.A., 2000. Postnatal development of electrophysiological properties of nucleus accumbens neurons. J. Neurophysiol. 84, 2204–2216. https://doi.org/10.1152/jn.2000.84.5.2204

Ben-Ari, Y., Khalilov, I., Kahle, K.T., Cherubini, E., 2012. The GABA Excitatory/Inhibitory Shift in Brain Maturation and Neurological Disorders. Neuroscientist 18, 467–486. https://doi.org/10.1177/1073858412438697

Benthall, K.N., Cording, K.R., Agopyan-Miu, A.H.C.W., Wong, C.D., Chen, E.Y., Bateup, H.S., 2021. Loss of Tsc1 from striatal direct pathway neurons impairs endocannabinoid-LTD and enhances motor routine learning. Cell Rep 36, 109511. https://doi.org/10.1016/j.celrep.2021.109511

Bertero, A., Zurita, H., Normandin, M., Apicella, A.J., 2020. Auditory Long-Range Parvalbumin Cortico-Striatal Neurons. Front Neural Circuits 14. https://doi.org/10.3389/fncir.2020.00045

Bignami, G., 1996. Economical test methods for developmental neurobehavioral toxicity. Environmental Health Perspectives 104, 285–298. https://doi.org/10.1289/ehp.96104s2285

Blanchard, B.A., Steindorf, S., Wang, S., LeFevre, R., Mankes, R.F., Glick, S.D., 1993. Prenatal Ethanol Exposure Alters Ethanol-Induced Dopamine Release in Nucleus Accumbens and Striatum in Male and Female Rats. Alcohol: Clinical and Experimental Research 17, 974–981. https://doi.org/10.1111/j.1530-0277.1993.tb05651.x

Blomeley, C.P., Cains, S., Smith, R., Bracci, E., 2011. Ethanol affects striatal interneurons directly and projection neurons through a reduction in cholinergic tone. Neuropsychopharmacology 36, 1033–1046. https://doi.org/10.1038/npp.2010.241

Boccalaro, I.L., Cristiá-Lara, L., Schwerdel, C., Fritschy, J.-M., Rubi, L., 2019. Cell type-specific distribution of GABAA receptor subtypes in the mouse dorsal striatum. J Comp Neurol 527, 2030–2046. https://doi.org/10.1002/cne.24665

Boggan, W.O., Xu, W., Shepherd, C.L., Middaugh, L.D., 1996. Effects of prenatal ethanol exposure on dopamine systems in C57BL/6J mice. Neurotoxicology and Teratology 18, 41–48. https://doi.org/10.1016/0892-0362(95)02027-6

Bortone, D., Polleux, F., 2009. KCC2 expression promotes the termination of cortical interneuron migration in a voltage-sensitive calcium-dependent manner. Neuron 62, 53–71. https://doi.org/10.1016/j.neuron.2009.01.034

Brodie, M.S., Scholz, A., Weiger, T.M., Dopico, A.M., 2007. Ethanol Interactions With Calcium-Dependent Potassium Channels. Alcohol: Clinical and Experimental Research 31, 1625–1632. https://doi.org/10.1111/j.1530-0277.2007.00469.x

Cazorla, M., de Carvalho, F.D., Chohan, M.O., Shegda, M., Chuhma, N., Rayport, S., Ahmari, S.E., Moore, H., Kellendonk, C., 2014. Dopamine D2 Receptors Regulate the Anatomical and Functional Balance of Basal Ganglia Circuitry. Neuron 81, 153–164. https://doi.org/10.1016/j.neuron.2013.10.041

Chen, Q., Veenman, C.L., Reiner, A., 1996. Cellular expression of ionotropic glutamate receptor subunits on specific striatal neuron types and its implication for striatal vulnerability in glutamate receptor-mediated excitotoxicity. Neuroscience 73, 715–731. https://doi.org/10.1016/0306-4522(96)00011-5

Cheng, Y., Wang, X., Wei, X., Xie, X., Melo, S., Miranda, R.C., Wang, J., 2018. Prenatal Exposure to Alcohol Induces Functional and Structural Plasticity in Dopamine D1 Receptor-Expressing Neurons of the Dorsomedial Striatum. Alcoholism: Clinical and Experimental Research 42, 1493–1502. https://doi.org/10.1111/acer.13806

Chesselet, M.-F., Plotkin, J.L., Wu, N., Levine, M.S., 2007. Development of striatal fast-spiking GABAergic interneurons, in: Tepper, J.M., Abercrombie, E.D., Bolam, J.P. (Eds.), Progress in Brain Research, Gaba and the Basal Ganglia. Elsevier, pp. 261–272. https://doi.org/10.1016/S0079-6123(06)60015-0

Chung, C., Shin, W., Kim, E., 2022. Early and Late Corrections in Mouse Models of Autism Spectrum Disorder. Biological Psychiatry 91, 934–944. https://doi.org/10.1016/j.biopsych.2021.07.021

Clarke, R., Adermark, L., 2015. Dopaminergic Regulation of Striatal Interneurons in Reward and Addiction: Focus on Alcohol. Neural Plast. 2015, 814567. https://doi.org/10.1155/2015/814567

Connor, P.D., Sampson, P.D., Streissguth, A.P., Bookstein, F.L., Barr, H.M., 2006. Effects of prenatal alcohol exposure on fine motor coordination and balance: A study of two adult samples. Neuropsychologia 44, 744–751. https://doi.org/10.1016/j.neuropsychologia.2005.07.016

Crawley, J.N., 2012. Translational animal models of autism and neurodevelopmental disorders. Dialogues in Clinical Neuroscience 14, 293–305. https://doi.org/10.31887/DCNS.2012.14.3/jcrawley

Dehorter, N., Guigoni, C., Lopez, C., Hirsch, J., Eusebio, A., Ben-Ari, Y., Hammond, C., 2009. Dopamine-Deprived Striatal GABAergic Interneurons Burst and Generate Repetitive Gigantic IPSCs in Medium Spiny Neurons. J. Neurosci. 29, 7776–7787. https://doi.org/10.1523/JNEUROSCI.1527-09.2009

Dehorter, N., Michel, F.J., Marissal, T., Rotrou, Y., Matrot, B., Lopez, C., Humphries, M.D., Hammond, C., 2011. Onset of Pup Locomotion Coincides with Loss of NR2C/D-Mediated Cortico-Striatal EPSCs and Dampening of Striatal Network Immature Activity. Front. Cell. Neurosci. 5. https://doi.org/10.3389/fncel.2011.00024

Delatour, L.C., Yeh, P.W., Yeh, H.H., 2019a. Ethanol Exposure In Utero Disrupts Radial Migration and Pyramidal Cell Development in the Somatosensory Cortex. Cereb Cortex 29, 2125–2139. https://doi.org/10.1093/cercor/bhy094

Delatour, L.C., Yeh, P.W.L., Yeh, H.H., 2019b. Prenatal Exposure to Ethanol Alters Synaptic Activity in Layer V/VI Pyramidal Neurons of the Somatosensory Cortex. Cereb. Cortex. https://doi.org/10.1093/cercor/bhz199

Fan, X., Warner, M., Gustafsson, J.-Å., 2006. Estrogen receptor β expression in the embryonic brain regulates development of calretinin-immunoreactive GABAergic interneurons. Proceedings of the National Academy of Sciences 103, 19338–19343. https://doi.org/10.1073/pnas.0609663103

Fish, B.S., Rank, S.A., Wilson, J.R., Collins, A.C., 1981. Viability and sensorimotor development of mice exposed to prenatal short-term ethanol. Pharmacology Biochemistry and Behavior 14, 57–65. https://doi.org/10.1016/0091-3057(81)90103-9

Flannigan, K., Poole, N., Cook, J., Unsworth, K., 2023. Sex-related differences among individuals assessed for fetal alcohol spectrum disorder in Canada. Alcohol: Clinical and Experimental Research 47, 613–623. https://doi.org/10.1111/acer.15017

Fox, D.J., Pettygrove, S., Cunniff, C., O'Leary, L.A., Gilboa, S.M., Bertrand, J., Druschel, C.M., Breen, A., Robinson, L., Ortiz, L., Frías, J.L., Ruttenber, M., Klumb, D., Meaney, F.J., 2015. Fetal Alcohol Syndrome Among Children Aged 7–9 Years — Arizona, Colorado, and New York, 2010. MMWR Morb Mortal Wkly Rep 64, 54–57.

Fox, W.M., 1965. Reflex-ontogeny and behavioural development of the mouse. Animal Behaviour 13, 234-IN5. https://doi.org/10.1016/0003-3472(65)90041-2

Gertler, T.S., Chan, C.S., Surmeier, D.J., 2008. Dichotomous anatomical properties of adult striatal medium spiny neurons. J. Neurosci. 28, 10814–10824. https://doi.org/10.1523/JNEUROSCI.2660-08.2008

Giacometti, L., Barker, J., 2020. Sex differences in the glutamate system: Implications for addiction. Neuroscience & Biobehavioral Reviews 113, 157–168. https://doi.org/10.1016/j.neubiorev.2020.03.010

Gittis, A.H., Nelson, A.B., Thwin, M.T., Palop, J.J., Kreitzer, A.C., 2010. Distinct roles of GABAergic interneurons in the regulation of striatal output pathways. J. Neurosci. 30, 2223–2234. https://doi.org/10.1523/JNEUROSCI.4870-09.2010

Götz, T., Kraushaar, U., Geiger, J., Lübke, J., Berger, T., Jonas, P., 1997. Functional Properties of AMPA and NMDA Receptors Expressed in Identified Types of Basal Ganglia Neurons. J. Neurosci. 17, 204–215. https://doi.org/10.1523/JNEUROSCI.17-01-00204.1997

Granato, A., Santarelli, M., Sbriccoli, A., Minciacchi, D., 1995. Multifaceted alterations of the thalamo-cortico-thalamic loop in adult rats prenatally exposed to ethanol. Anat Embryol (Berl) 191, 11–23. https://doi.org/10.1007/BF00215293

Hendrickson, T.J., Mueller, B.A., Sowell, E.R., Mattson, S.N., Coles, C.D., Kable, J.A., Jones, K.L., Boys, C.J., Lee, S., Lim, K.O., Riley, E.P., Wozniak, J.R., 2018. Two-year cortical trajectories are abnormal in children and adolescents with prenatal alcohol exposure. Dev Cogn Neurosci 30, 123–133. https://doi.org/10.1016/j.dcn.2018.02.008

Huang, S., Uusisaari, M., 2013. Physiological temperature during brain slicing enhances the quality of acute slice preparations. Frontiers in Cellular Neuroscience 7.

Hunt, W.A., Mullin, M.J., 1985. Effects of ethanol exposure on brain sodium channels. Alcohol Drug Res 6, 419–422.

Johansson, Y., Silberberg, G., 2020. The Functional Organization of Cortical and Thalamic Inputs onto Five Types of Striatal Neurons Is Determined by Source and Target Cell Identities. Cell Reports 30, 1178-1194.e3. https://doi.org/10.1016/j.celrep.2019.12.095

Kalemaki, K., Velli, A., Christodoulou, O., Denaxa, M., Karagogeos, D., Sidiropoulou, K., 2022. The developmental changes in intrinsic and synaptic properties of prefrontal neurons enhance local network activity from the second to the third postnatal weeks in mice. Cereb Cortex 32, 3633–3650. https://doi.org/10.1093/cercor/bhab438

Kar, P., Reynolds, J.E., Gibbard, W.B., McMorris, C., Tortorelli, C., Lebel, C., 2022. Trajectories of brain white matter development in young children with prenatal alcohol exposure. Hum Brain Mapp 43, 4145–4157. https://doi.org/10.1002/hbm.25944

Kim, S., Oh, H., Choi, S.H., Yoo, Y.-E., Noh, Y.W., Cho, Y., Im, G.H., Lee, C., Oh, Y., Yang, E., Kim, G., Chung, W.-S., Kim, H., Kang, H., Bae, Y., Kim, S.-G., Kim, E., 2022. Postnatal age-differential ASD-like transcriptomic, synaptic, and behavioral deficits in Myt1l-mutant mice. Cell Reports 40, 111398. https://doi.org/10.1016/j.celrep.2022.111398

Klug, J.R., Engelhardt, M.D., Cadman, C.N., Li, H., Smith, J.B., Ayala, S., Williams, E.W., Hoffman, H., Jin, X., 2018. Differential inputs to striatal cholinergic and parvalbumin interneurons imply functional distinctions. eLife 7, e35657. https://doi.org/10.7554/eLife.35657

Kocaturk, S., Guven, E.B., Shah, F., Tepper, J.M., Assous, M., 2022. Cholinergic control of striatal GABAergic microcircuits. Cell Rep 41, 111531. https://doi.org/10.1016/j.celrep.2022.111531

Kozorovitskiy, Y., Saunders, A., Johnson, C.A., Lowell, B.B., Sabatini, B.L., 2012. Recurrent network activity drives striatal synaptogenesis. Nature 485, 646–650. https://doi.org/10.1038/nature11052

Krajeski, R.N., Macey-Dare, A., Heusden, F. van, Ebrahimjee, F., Ellender, T.J., 2019. Dynamic postnatal development of the cellular and circuit properties of striatal D1 and D2 spiny projection neurons. The Journal of Physiology 597, 5265–5293. https://doi.org/10.1113/JP278416

Küppers, E., Beyer, C., 1998. Expression of aromatase in the embryonic and postnatal mouse striatum. Molecular Brain Research 63, 184–188. https://doi.org/10.1016/S0169-328X(98)00279-4

Lahiri, A.K., Bevan, M.D., 2020. Dopaminergic Transmission Rapidly and Persistently Enhances Excitability of D1 Receptor-Expressing Striatal Projection Neurons. Neuron. https://doi.org/10.1016/j.neuron.2020.01.028

Lebel, C., Mattson, S.N., Riley, E.P., Jones, K.L., Adnams, C.M., May, P.A., Bookheimer, S.Y., O'Connor, M.J., Narr, K.L., Kan, E., Abaryan, Z., Sowell, E.R., 2012. A Longitudinal Study of the Long-Term Consequences of Drinking during Pregnancy: Heavy In Utero Alcohol Exposure Disrupts the Normal Processes of Brain Development. J. Neurosci. 32, 15243–15251. https://doi.org/10.1523/JNEUROSCI.1161-12.2012

Lee, S.M., Yeh, P.W.L., Yeh, H.H., 2022. L-Type Calcium Channels Contribute to Ethanol-Induced Aberrant Tangential Migration of Primordial Cortical GABAergic Interneurons in the Embryonic Medial Prefrontal Cortex. eNeuro 9, ENEURO.0359-21.2021. https://doi.org/10.1523/ENEURO.0359-21.2021

Lenz, S., Perney, T.M., Qin, Y., Robbins, E., Chesselet, M.F., 1994. GABA-ergic interneurons of the striatum express the Shaw-like potassium channel Kv3.1. Synapse 18, 55–66. https://doi.org/10.1002/syn.890180108

Leonzino, M., Busnelli, M., Antonucci, F., Verderio, C., Mazzanti, M., Chini, B., 2016. The Timing of the Excitatory-to-Inhibitory GABA Switch Is Regulated by the Oxytocin Receptor via KCC2. Cell Reports 15, 96–103. https://doi.org/10.1016/j.celrep.2016.03.013

Lieberman, O.J., McGuirt, A.F., Mosharov, E.V., Pigulevskiy, I., Hobson, B.D., Choi, S., Frier, M.D., Santini, E., Borgkvist, A., Sulzer, D., 2018. Dopamine Triggers the Maturation of Striatal Spiny Projection Neuron Excitability during a Critical Period. Neuron 99, 540-554.e4. https://doi.org/10.1016/j.neuron.2018.06.044

Lim, S.A.O., Kang, U.J., McGehee, D.S., 2014. Striatal cholinergic interneuron regulation and circuit effects. Front Synaptic Neurosci 6, 22. https://doi.org/10.3389/fnsyn.2014.00022

Long, X., Little, G., Beaulieu, C., Lebel, C., 2018. Sensorimotor network alterations in children and youth with prenatal alcohol exposure. Human Brain Mapping 39, 2258–2268. https://doi.org/10.1002/hbm.24004

LoTurco, J.J., Owens, D.F., Heath, M.J.S., Davis, M.B.E., Kriegstein, A.R., 1995. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. Neuron 15, 1287–1298. https://doi.org/10.1016/0896-6273(95)90008-X

Lovinger, D.M., 1993. High ethanol sensitivity of recombinant AMPA-type glutamate receptors expressed in mammalian cells. Neurosci Lett 159, 83–87. https://doi.org/10.1016/0304-3940(93)90804-t

Lovinger, D.M., White, G., Weight, F.F., 1989. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 243, 1721–1724. https://doi.org/10.1126/science.2467382

MacLusky, N.J., Chaptal, C., McEwen, B.S., 1979. The development of estrogen receptor systems in the rat brain and pituitary: Postnatal development. Brain Research 178, 143–160. https://doi.org/10.1016/0006-8993(79)90094-5

May, P.A., Baete, A., Russo, J., Elliott, A.J., Blankenship, J., Kalberg, W.O., Buckley, D., Brooks, M., Hasken, J., Abdul-Rahman, O., Adam, M.P., Robinson, L.K., Manning, M., Hoyme, H.E., 2014. Prevalence and Characteristics of Fetal Alcohol Spectrum Disorders. Pediatrics 134, 855–866. https://doi.org/10.1542/peds.2013-3319

May, P.A., Hymbaugh, K.J., Aase, J.M., Samet, J.M., 1983. Epidemiology of fetal alcohol syndrome among American Indians of the southwest. Social Biology 30, 374–387. https://doi.org/10.1080/19485565.1983.9988551

May, P.A., Tabachnick, B., Hasken, J.M., Marais, A.-S., de Vries, M.M., Barnard, R., Joubert, B., Cloete, M., Botha, I., Kalberg, W.O., Buckley, D., Burroughs, Z.R., Bezuidenhout, H., Robinson, L.K., Manning, M.A., Adnams, C.M., Seedat, S., Parry, C.D.H., Hoyme, H.E., 2017. Who is most affected by prenatal alcohol exposure: Boys or girls? Drug Alcohol Depend 177, 258–267. https://doi.org/10.1016/j.drugalcdep.2017.04.010

Melzer, S., Gil, M., Koser, D.E., Michael, M., Huang, K.W., Monyer, H., 2017. Distinct Corticostriatal GABAergic Neurons Modulate Striatal Output Neurons and Motor Activity. Cell Rep 19, 1045–1055. https://doi.org/10.1016/j.celrep.2017.04.024

Michetti, C., Falace, A., Benfenati, F., Fassio, A., 2022. Synaptic genes and neurodevelopmental disorders: From molecular mechanisms to developmental strategies of behavioral testing. Neurobiology of Disease 173, 105856. https://doi.org/10.1016/j.nbd.2022.105856

Miller, J.C., 1983. Sex differences in dopaminergic and cholinergic activity and function in the nigro-striatal system of the rat. Psychoneuroendocrinology 8, 225–236. https://doi.org/10.1016/0306-4530(83)90059-8

Misgeld, U., Wagner, A., Ohno, T., 1982. Depolarizing IPSPs and depolarization by GABA of rat neostriatum cells in vitro. Exp Brain Res 45, 108–114. https://doi.org/10.1007/BF00235769

Mohammad, S., Page, S.J., Wang, L., Ishii, S., Li, P., Sasaki, T., Basha, A., Salzberg, A., Quezado, Z., Imamura, F., Nishi, H., Isaka, K., Corbin, J.G., Liu, J.S., Kawasawa, Y.I., Torii, M., Hashimoto-Torii, K., 2020. Kcnn2 blockade reverses learning deficits in a mouse model of fetal alcohol spectrum disorders. Nature Neuroscience 23, 533–543. https://doi.org/10.1038/s41593-020-0592-z

Molina, J.C., Hoffmann, H., Spear, L.P., Spear, N.E., 1987. Sensorimotor maturation and alcohol responsiveness in rats prenatally exposed to alcohol during gestational day 8. Neurotoxicology and Teratology 9, 121–128. https://doi.org/10.1016/0892-0362(87)90088-2

Molnár, Z., Adams, R., Blakemore, C., 1998. Mechanisms Underlying the Early Establishment of Thalamocortical Connections in the Rat. J. Neurosci. 18, 5723–5745. https://doi.org/10.1523/JNEUROSCI.18-15-05723.1998

Mooney, S.M., Miller, M.W., 2010. Prenatal exposure to ethanol affects postnatal neurogenesis in thalamus. Experimental Neurology, Beta-amyloid and tau protein abnormalities in Alzheimer's disease 223, 566–573. https://doi.org/10.1016/j.expneurol.2010.02.003

Mooney, S.M., Varlinskaya, E.I., 2011. Acute prenatal exposure to ethanol and social behavior: Effects of age, sex, and timing of exposure. Behavioural Brain Research 216, 358–364. https://doi.org/10.1016/j.bbr.2010.08.014

Moore, E.M., Xia, Y., 2022. Neurodevelopmental Trajectories Following Prenatal Alcohol Exposure. Frontiers in Human Neuroscience 15.

Mowery, T.M., Penikis, K.B., Young, S.K., Ferrer, C.E., Kotak, V.C., Sanes, D.H., 2017. The Sensory Striatum Is Permanently Impaired by Transient Developmental Deprivation. Cell Reports 19, 2462–2468. https://doi.org/10.1016/j.celrep.2017.05.083

Möykkynen, T., Korpi, E.R., 2012. Acute Effects of Ethanol on Glutamate Receptors. Basic & Clinical Pharmacology & Toxicology 111, 4–13. https://doi.org/10.1111/j.1742-7843.2012.00879.x

Mozhayeva, M.G., Sara, Y., Liu, X., Kavalali, E.T., 2002. Development of Vesicle Pools during Maturation of Hippocampal Synapses. J Neurosci 22, 654–665. https://doi.org/10.1523/JNEUROSCI.22-03-00654.2002

Nakamura, K., Hioki, H., Fujiyama, F., Kaneko, T., 2005. Postnatal changes of vesicular glutamate transporter (VGluT)1 and VGluT2 immunoreactivities and their colocalization in the mouse forebrain. Journal of Comparative Neurology 492, 263–288. https://doi.org/10.1002/cne.20705

Patton, M.H., Roberts, B.M., Lovinger, D.M., Mathur, B.N., 2016. Ethanol Disinhibits Dorsolateral Striatal Medium Spiny Neurons Through Activation of A Presynaptic Delta Opioid Receptor. Neuropsychopharmacology 41, 1831–1840. https://doi.org/10.1038/npp.2015.353

Peixoto, R.T., Chantranupong, L., Hakim, R., Levasseur, J., Wang, W., Merchant, T., Gorman, K., Budnik, B., Sabatini, B.L., 2019. Abnormal Striatal Development Underlies the Early Onset of Behavioral Deficits in Shank3B-/- Mice. Cell Rep 29, 2016-2027.e4. https://doi.org/10.1016/j.celrep.2019.10.021

Peixoto, R.T., Wang, W., Croney, D.M., Kozorovitskiy, Y., Sabatini, B.L., 2016. Early hyperactivity and precocious maturation of corticostriatal circuits in Shank3B −/− mice. Nature Neuroscience 19, 716–724. https://doi.org/10.1038/nn.4260

Penzes, P., Buonanno, A., Passafaro, M., Sala, C., Sweet, R.A., 2013. Developmental vulnerability of synapses and circuits associated with neuropsychiatric disorders. Journal of Neurochemistry 126, 165–182. https://doi.org/10.1111/jnc.12261

Plotkin, J.L., Wu, N., Chesselet, M.-F., Levine, M.S., 2005. Functional and molecular development of striatal fast-spiking GABAergic interneurons and their cortical inputs. European Journal of Neuroscience 22, 1097–1108. https://doi.org/10.1111/j.1460-9568.2005.04303.x

Prüss, H., Wenzel, M., Eulitz, D., Thomzig, A., Karschin, A., Veh, R.W., 2003. Kir2 potassium channels in rat striatum are strategically localized to control basal ganglia function. Brain Res Mol Brain Res 110, 203–219. https://doi.org/10.1016/s0169-328x(02)00649-6

Rice, J.P., Suggs, L.E., Lusk, A.V., Parker, M.O., Candelaria-Cook, F.T., Akers, K.G., Savage, D.D., Hamilton, D.A., 2012. Effects of exposure to moderate levels of ethanol during prenatal brain development on dendritic length, branching, and spine density in the nucleus accumbens and dorsal striatum of adult rats. Alcohol 46, 577–584. https://doi.org/10.1016/j.alcohol.2011.11.008

Rodriguez, C.I., Magcalas, C.M., Barto, D., Fink, B.C., Rice, J.P., Bird, C.W., Davies, S., Pentkowski, N.S., Savage, D.D., Hamilton, D.A., 2016. Effects of sex and housing on social, spatial, and motor behavior in adult rats exposed to moderate levels of alcohol during prenatal development. Behavioural Brain Research 313, 233–243. https://doi.org/10.1016/j.bbr.2016.07.018

Rouzer, S.K., Diaz, M.R., 2022. Moderate prenatal alcohol exposure modifies sex-specific CRFR1 activity in the central amygdala and anxiety-like behavior in adolescent

offspring. Neuropsychopharmacology 47, 2140–2149. https://doi.org/10.1038/s41386-022-01327-z

Ruden, J.B., Dugan, L.L., Konradi, C., 2021. Parvalbumin interneuron vulnerability and brain disorders. Neuropsychopharmacol. 46, 279–287. https://doi.org/10.1038/s41386-020-0778-9

Salem, N.A., Mahnke, A.H., Konganti, K., Hillhouse, A.E., Miranda, R.C., 2021. Cell-type and fetal-sex-specific targets of prenatal alcohol exposure in developing mouse cerebral cortex. iScience 24, 102439. https://doi.org/10.1016/j.isci.2021.102439

Sambo, D., Gohel, C., Yuan, Q., Sukumar, G., Alba, C., Dalgard, C.L., Goldman, D., 2022. Cell type-specific changes in Wnt signaling and neuronal differentiation in the developing mouse cortex after prenatal alcohol exposure during neurogenesis. Frontiers in Cell and Developmental Biology 10.

Schambra, U.B., Goldsmith, J., Nunley, K., Liu, Y., Harirforoosh, S., Schambra, H.M., 2015. Low and moderate prenatal ethanol exposures of mice during gastrulation or neurulation delays neurobehavioral development. Neurotoxicology and Teratology 51, 1–11. https://doi.org/10.1016/j.ntt.2015.07.003

Schambra, U.B., Nunley, K., Harrison, T.A., Lewis, C.N., 2016. Consequences of low or moderate prenatal ethanol exposures during gastrulation or neurulation for open field activity and emotionality in mice. Neurotoxicology and Teratology 57, 39–53. https://doi.org/10.1016/j.ntt.2016.06.003

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K., Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676–682. https://doi.org/10.1038/nmeth.2019

Sernagor, E., Chabrol, F., Bony, G., Cancedda, L., 2010. GABAergic control of neurite outgrowth and remodeling during development and adult neurogenesis: general rules and differences in diverse systems. Front. Cell. Neurosci. 4. https://doi.org/10.3389/fncel.2010.00011

Skorput, A., Gupta, V.P., Yeh, P.W.L., Yeh, H.H., 2015. Persistent Interneuronopathy in the Prefrontal Cortex of Young Adult Offspring Exposed to Ethanol In Utero. J. Neurosci. 35, 10977–10988. https://doi.org/10.1523/JNEUROSCI.1462-15.2015

Smith, Y., Raju, D.V., Pare, J.-F., Sidibe, M., 2004. The thalamostriatal system: a highly specific network of the basal ganglia circuitry. Trends in Neurosciences 27, 520–527. https://doi.org/10.1016/j.tins.2004.07.004

Sohur, U.S., Padmanabhan, H.K., Kotchetkov, I.S., Menezes, J.R.L., Macklis, J.D., 2014. Anatomic and Molecular Development of Corticostriatal Projection Neurons in Mice. Cerebral Cortex 24, 293–303. https://doi.org/10.1093/cercor/bhs342

Straub, C., Saulnier, J.L., Bègue, A., Feng, D.D., Huang, K.W., Sabatini, B.L., 2016. Principles of Synaptic Organization of GABAergic Interneurons in the Striatum. Neuron 92, 84–92. https://doi.org/10.1016/j.neuron.2016.09.007

Supekar, K., Menon, V., 2015. Sex differences in structural organization of motor systems and their dissociable links with repetitive/restricted behaviors in children with autism. Molecular Autism 6, 50. https://doi.org/10.1186/s13229-015-0042-z

Tapia, D., Suárez, P., Arias-García, M.A., Garcia-Vilchis, B., Serrano-Reyes, M., Bargas, J., Galarraga, E., 2019. Localization of chloride co-transporters in striatal neurons. Neuroreport 30, 457–462. https://doi.org/10.1097/WNR.0000000000001234

Tavian, D., De Giorgio, A., Granato, A., 2011. Selective underexpression of Kv3.2 and Kv3.4 channels in the cortex of rats exposed to ethanol during early postnatal life. Neurol Sci 32, 571–577. https://doi.org/10.1007/s10072-010-0446-7

Tepper, J.M., Sharpe, N.A., Koós, T.Z., Trent, F., 1998. Postnatal development of the rat neostriatum: electrophysiological, light- and electron-microscopic studies. Dev. Neurosci. 20, 125–145. https://doi.org/10.1159/000017308

Tepper, J.M., Wilson, C.J., Koós, T., 2008. Feedforward and Feedback inhibition in Neostriatal GABAergic Spiny Neurons. Brain Res Rev 58, 272–281. https://doi.org/10.1016/j.brainresrev.2007.10.008

Thanh, N.X., Jonsson, E., Salmon, A., Sebastianski, M., 2014. Incidence and prevalence of fetal alcohol spectrum disorder by sex and age group in Alberta, Canada. J Popul Ther Clin Pharmacol 21, e395-404.

Tousley, A.R., Yeh, P.W.L., Yeh, H.H., 2022. Precocious emergence of cognitive and synaptic dysfunction in 3xTg-AD mice exposed prenatally to ethanol. Alcohol S0741-8329(22)00073–8. https://doi.org/10.1016/j.alcohol.2022.08.003

Treit, S., Zhou, D., Lebel, C., Rasmussen, C., Andrew, G., Beaulieu, C., 2014. Longitudinal MRI reveals impaired cortical thinning in children and adolescents prenatally exposed to alcohol. Hum Brain Mapp 35, 4892–4903. https://doi.org/10.1002/hbm.22520

Vizcarra-Chacón, B.J., Arias-García, M.A., Pérez-Ramírez, M.B., Flores-Barrera, E., Tapia, D., Drucker-Colin, R., Bargas, J., Galarraga, E., 2013. Contribution of different classes of glutamate receptors in the corticostriatal polysynaptic responses from striatal direct and indirect projection neurons. BMC Neurosci 14, 60. https://doi.org/10.1186/1471-2202-14-60

Wall, N.R., De La Parra, M., Callaway, E.M., Kreitzer, A.C., 2013. Differential Innervation of Direct- and Indirect-Pathway Striatal Projection Neurons. Neuron 79, 347–360. https://doi.org/10.1016/j.neuron.2013.05.014

Walter, H.J., Messing, R.O., 1999. Regulation of neuronal voltage-gated calcium channels by ethanol. Neurochemistry International 35, 95–101. https://doi.org/10.1016/S0197-0186(99)00050-9

Wang, D.D., Kriegstein, A.R., 2008. GABA Regulates Excitatory Synapse Formation in the Neocortex via NMDA Receptor Activation. J Neurosci 28, 5547–5558. https://doi.org/10.1523/JNEUROSCI.5599-07.2008

Wilson, C.J., Kawaguchi, Y., 1996. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J. Neurosci. 16, 2397–2410. https://doi.org/10.1523/JNEUROSCI.16-07-02397.1996

Xu, Q., Tam, M., Anderson, S.A., 2008. Fate mapping Nkx2.1-lineage cells in the mouse telencephalon. Journal of Comparative Neurology 506, 16–29. https://doi.org/10.1002/cne.21529

Yang, J., Yang, X., Tang, K., 2021. Interneuron development and dysfunction. The FEBS Journal n/a. https://doi.org/10.1111/febs.15872

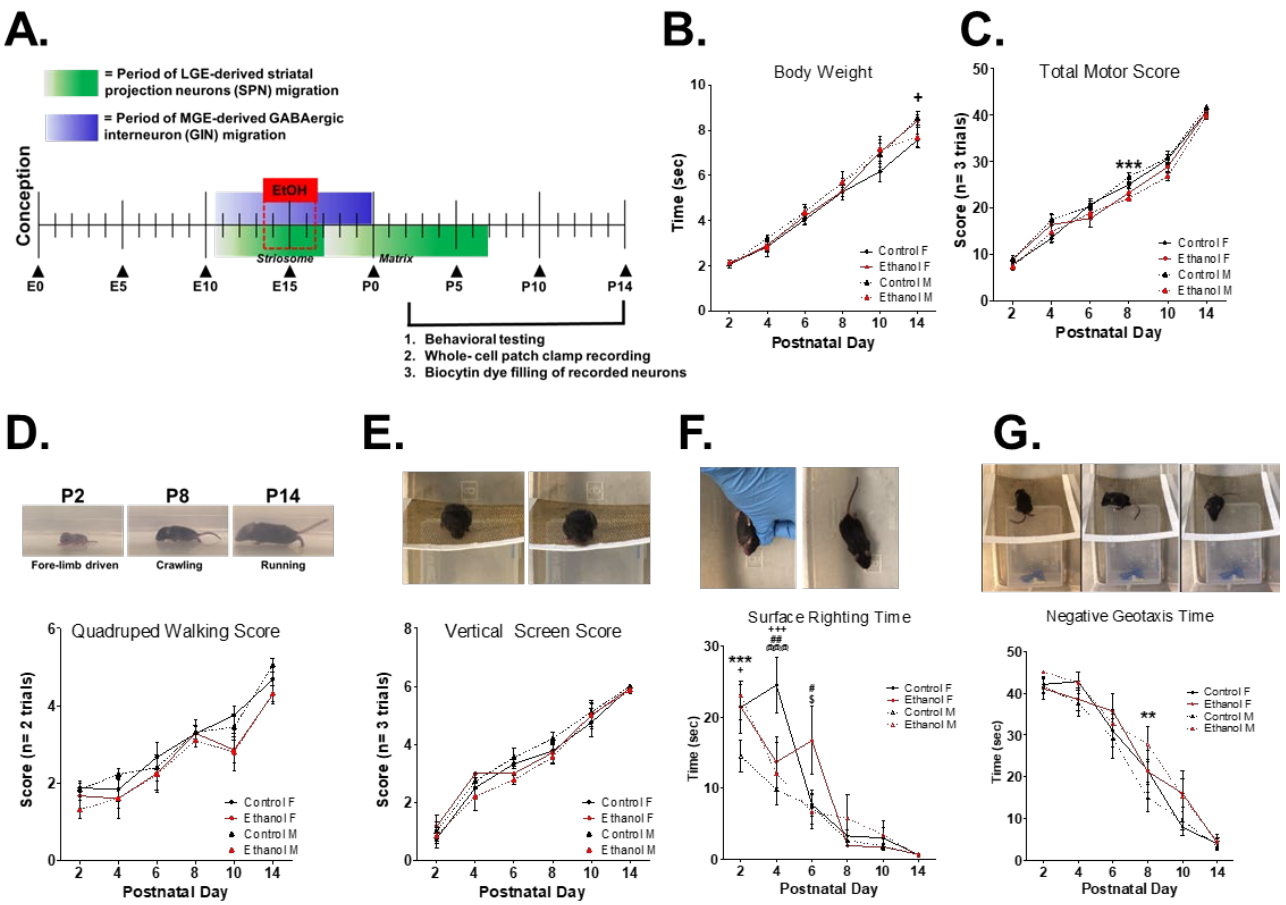
Zachry, J.E., Nolan, S.O., Brady, L.J., Kelly, S.J., Siciliano, C.A., Calipari, E.S., 2021. Sex differences in dopamine release regulation in the striatum. Neuropsychopharmacol. 46, 491–499. https://doi.org/10.1038/s41386-020-00915-1

Table 2.1. Scoring parameters and description of neonatal motor behavioral testing tasks

Behavioral Task	Trials	Scoring	Description
Surface Righting	3	0= absent, 1 =present; Time to complete task (s)	Pup held on back for 5 sec then given 30 sec to right itself
Auditory Startle	3	0= absent, 1 =present	Pup presented with loud tone, observed for startle
Tactile Startle	3	0= absent, 1 =present	Pup presented with air puff, observed for startle
Grasp Reflex	3	0= absent, 1 =present	Pup stimulated on forepaw or hindpaw with dull side of metal blade, observed for grasp reflex
Horizontal Screen Test	3	0= absent, 1 =grasp screen, 2 = lift screen	Pup pulled against horizontal wire mesh
Vertical Screen Test	3	0= absent, 1 =grasp screen, 2 = climb screen	Pup pulled against vertical wire mesh
Negative Geotaxis	3	0= absent, 1 = orients to horizontal, 2 = orients to vertical, Time to complete task (s)	Pup placed on 45° wire mesh head facing down, given 45 sec to reverse direction and climb
Cliff Avoidance	3	0= absent, 1= present	Mouse placed with snout and forepaw digits over a ledge (box), given up to 30 sec to remove
Quadruped walking	2	0 = no forward movement, 1 = crawling, asymmetric movements, 2 = crawling, symmetric movements, 3 = fast crawling/walking;	Mouse recorded for 3 min in an empty cage

Figure 2.1. Experimental timeline and the influence of prenatal ethanol exposure on early postnatal sensorimotor development

(A) Pregnant dams were exposed to 5% (w/w) ethanol in a liquid diet, or a control (lab chow) diet from embryonic day (E)13.5-16.5, a period of significant migration of early-born striosomal striatal projection neurons (SPNs) and striatal GABAergic interneurons (GINs), to the developing striatum. After birth at postnatal day (P)0 mice were maintained to postnatal timepoints: P2, P4, P6, P8, P10 or P14, and assessed for the development of a set of 9 sensorimotor behaviors, then sacrificed for whole-cell patch clamp recordings and morphological analysis of biocytin dye-filled cells. (B) A brief binge prenatal ethanol exposure does not alter the postnatal growth: body weight (g), of neonatal mice (two-way ANOVA: group: $F(3,197)= 2.309$, $p= 0.0777$, postnatal day: $F(5,197)= 223.7$, $p< 0.0001$, group x postnatal day $F(15,197)= 0.6217$, $p=0.8551$). However, control-fed male (M) mice were larger than control-fed female (F) mice at P14 (Bonferroni post-hoc test, $t = 3.021$, $p<0.05$). (C) Prenatal ethanol exposure resulted in decreased total motor score (TMS) in ethanol-exposed M mice, which significantly differed from control M mice at P8(Kruskall-Wallis test, P2: $H(3) = 3.602$,$p=0.308$, P4: $H(3) = 4.883$, $p=0.181$, P6: $H(3) = 2.778$, $p =0.427$, P8: $H(3) = 11.343$, $p =0.010$, Dunn's post-hoc test: ethanol M vs. ethanol F: $p = 0.009$, P10: $H(3) = 0.000$, $p =1.000$, P14 $H(3) = 2.893$, $p =0.408$. (D) Prenatal ethanol exposure delayed the transition from fore-limb driven pivoting behavior, to crawling, and eventually running in ethanol-exposed M mice resulting in decreased quadruped walking scores (Kruskall Wallis tests, P2: $H(3)= 4.509$, $p=0.211$, P4: $H(3)=3.647$, $p=0.301$, P6: $H(3) = 1.407$, $p=0.704$, P8: $H(3)=0.937$, $p=0.817$, P10: $H(3) = 3.568$, $p=0.312$, P14: $H(3)= 8.728$, $p=0.033$). (E) Prenatal ethanol exposure delayed the development of mature vertical screen task behavior in M mice (Kruskall Wallis tests, P2: $H(3)= 0.731$, 0.866, P4: $H(3)=6.436$, $p=0.096$, P6: $H(3)= 7.248$, $p=0.064$, P8: $H(3)=4.311$, $p=0.230$, P10: $H(3)=0.602$, $p=0.896$, P14: $H(3)= 1.422$, $p=0.700$). (F) Prenatal ethanol exposure altered surface righting times in sex-dependent manner (2-way ANOVA, group: $F(3,200)=6.307$, p 0.0004, group x postnatal day: $F(15,200)=3.502$, $p<0.0001$, postnatal day: $F(5, 200) = 81.71$, $p<0.0001$, Bonferroni post-hoc tests: control M vs ethanol M: P2 : $t=3.856$, $p<0.001$, ethanol M vs. ethanol F: P6: $t=3.400$, $p<0.01$, control F vs. control M: P2: $t=2.956$, $p<0.05$, P4: $t=5.498$, $p<0.001$, control M vs. ethanol F: P4: $t=3.443$, $p<0.01$, P6: $t=3.226$, $p<0.01$, control F vs. ethanol M: P4: $t=3.969$, $p<0.001$). (G) Prenatal ethanol exposure increased negative geotaxis time in M mice (2-way ANOVA, group: $F(3,200)=3.502$, p 0.0164, group x postnatal day: $F(15,200)=3.502$, $p<0.0001$, postnatal day: $F(5, 200) = 131.3$, $p<0.0001$, Bonferroni post-hoc tests: control M vs ethanol M: $t=5.178$, $p<0.01$). Data are presented as mean score or time, error bars are standard error of the mean (SEM). **$p<0.01$, control male vs. ethanol male, #$p<0.05$, ##$p<0.01$: control female vs. ethanol +$p<0.05$, +++$p<0.001$: control male vs. control female, @@@$p<0.001$: control female vs. ethanol


A.
= Period of LGE-derived striatal projection neurons (SPN) migration
= Period of MGE-derived GABAergic interneuron (GIN) migration
Conception
EtOH
Striosome
Matrix
E0
E5
E10
E15
P0
P5
P10
P14
1. Behavioral testing
2. Whole- cell patch clamp recording
3. Biocytin dye filling of recorded neurons
B.
Body Weight
Time (sec)
Postnatal Day
Control F
Ethanol F
Control M
Ethanol M
C.
Total Motor Score
Score (n= 3 trials)
***
Postnatal Day
D.
P2
P8
P14
Fore-limb driven
Crawling
Running
Quadruped Walking Score
Score (n= 2 trials)
Postnatal Day
E.
Vertical Screen Score
Score (n= 3 trials)
Postnatal Day
F.
Surface Righting Time
Time (sec)
***
Postnatal Day
G.
Negative Geotaxis Time
Time (sec)
**
Postnatal Day

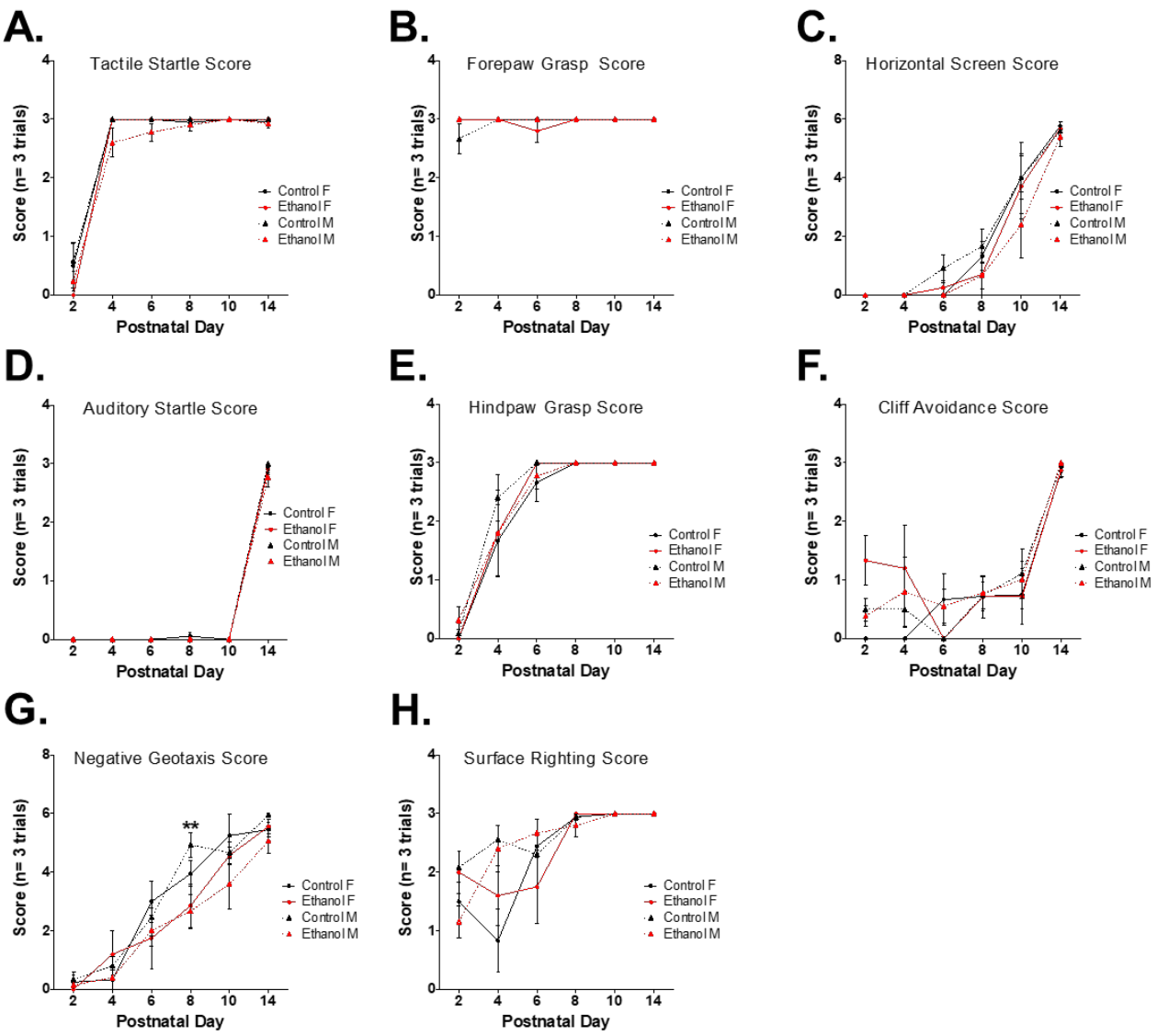


Figure 2.2. Prenatal ethanol exposure does not alter the development of reflexive behaviors.

No differences were observed between groups in (A) tactile startle score, (B) forepaw grasp score, (C) horizontal screen test score, (D) auditory startle score, (E) hindpaw grasp score, (F) cliff avoidance score, or (H) surface righting score, while (G) negative geotaxis score significantly differed between ethanol exposed male and female mice on postnatal day 8 (Kruskall-Wallis test, P2: H(3) = 1.319,p=0.725, P4: H(3) = 1.566,p=0.667, P6: H(3) = 1.633, p =0.652, P8: H(3) = 12.926, p =0.005, Dunn's post-hoc tests: ethanol M vs. ethanol F, p = 0.007, P10: H(3) = 3.064, p =0.382, P14 H(3) = 2.893, p =0.408. Data are presented as mean score or time, error bars are standard error of the mean (SEM). **p<0.01, control male vs. ethanol male,

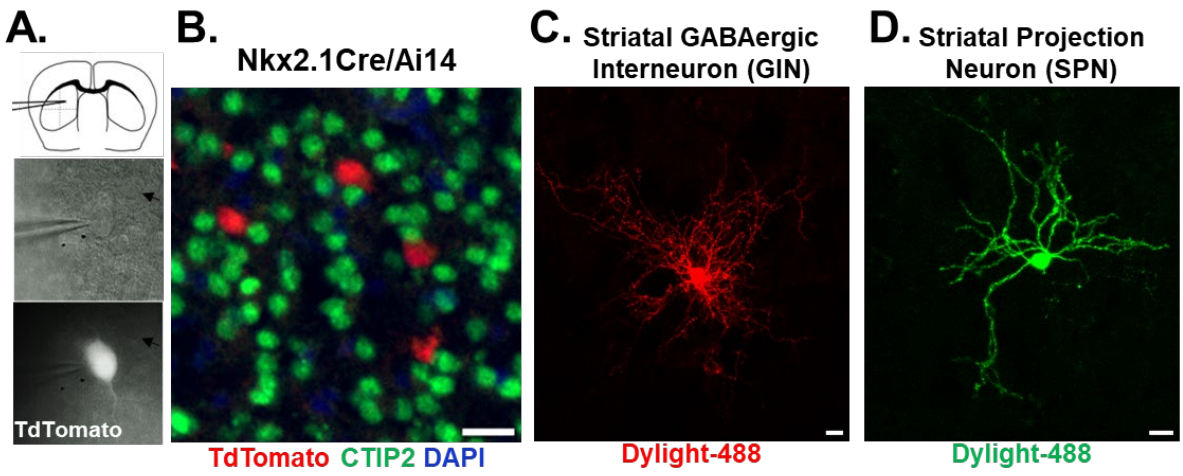


Figure 2.3. Identifying striatal GABAergic interneurons (GINs) and striatal projection neurons (SPNs) in acute cortical slices from Nkx2.1Cre x TdTomato mice.

(A) Striatal GINs and SPNs were differentiated during whole cell patch clamp recordings from 250 μM acute coronal slices from Nkx2.1Cre x Tdtomato mice based on tdTomato (red) expression in MGE-derived GABAergic interneurons. *Top*: schematic of whole-cell patch clamp recordings from striatal neurons, *Middle*: 40× magnification Hoffman modulated contrast image of acute slice during recording from a striatal GIN (white), and a neighboring SPN (arrow), *Bottom*: fluorescent tdTomato+ striatal GIN (white) and a neighboring SPN (arrow). **(B)** a SPN-specific nuclear immunomarker CTIP2 (green) does not co-label td-Tomato+ GINs (red) in the dorsal striatum of a P6 Nkx2.1Cre mice, with a DAPI-counterstain (blue) **(C)** Image of a neurobiotin-filled P14 striatal GIN after recording, pseudocolored (red). **(D)** Image of a neurobiotin-filled P14 SPN after recording (green).

Figure 2.4. Prenatal ethanol exposure alters the functional development of striatal GABAergic interneurons (GINs) and striatal projection neurons (SPNs) in early postnatal mice in an cell-type, age and sex-dependent manner.

(A) Prenatal ethanol exposure modifies the firing rate (Hz) of striatal GIN in an age and sex-dependent manner. At P2, striatal GINs from ethanol-exposed F mice had higher firing rates relative to those from ethanol-exposed and control-fed M mice (2-way ANOVA, group: $F(3,450) = 10.56$, $p < 0.0001$, current: $F(3,450) = 24.52$, group x current $F(24,450) = 1.282$, $p = 0.16910$, Bonferroni post-hoc tests: ethanol F vs. ethanol M: 60 pA: $t=2.981$, $p<0.05$, 70 pA: $t=3.474$, $p<0.01$, 80 pA: $t = 3.620$, $p<0.01$; ethanol F vs. control M: 70 pA: $t=3.242$, $p<0.05$, 80 pA: $t = 3.574$, $p<0.0$. At P4-6, firing rate significantly differed between groups with striatal GINs: control-fed M mice demonstrated higher firing rates relative to those from ethanol-exposed M and F, and control-fed M mice (2-way ANOVA, group: $F(3,468) = 14.26$, $p < 0.0022$, current: $F(8,468) = 14.26$, $p < 0.001$, group x current: $F(24,468) = 0.4322$. $p=0.9922$). At P8-10 and P14, firing rate again significantly differed between groups with striatal GINs from ethanol-fed mice demonstrating higher firing rates relative to those from control-fed mice (2-way ANOVA, P8-10: group: $F(3, 621) = 14.26$, $p < 0.0022$, current: $F(8,621) = 28.31$, $p<0.0001$, group x current: $F(8,621)= 0.4459$, $p= 0.0023$; P14: $F(3, 726) = 4.871$, $p < 0.0022$, current: $F(8,726) = 56.96$ $p<0.0001$, group x current: $F(8,726)= 0.3229$, $p= 0.9998$. **(B)** Example traces of voltage responses of striatal GINs following depolarizing current steps from control-fed female mice at P2 and P14. **(C)** Prenatal ethanol exposure significantly depolarized AP threshold in GINs from F mice relative to those from control-fed F mice at P2, hyperpolarized AP threshold in GINs from F and M mice relative to control-fed F and M mice at P14, and control-fed F relative to control fed M mice, but did not alter AP threshold from P4-10 (1-way ANOVAs, P2: $F(3, 50) = 4.298$, $p=0.005$, Bonferroni post-hoc tests: ethanol F vs. control F, $p=0.005$, P4-6: $F(3, 50) = 0.949$, $p=0.424$, P8-10: $F(3, 69) = 2.859$, $p=0.043$, P14: $F(3, 69) = 5.352$, $p=0.002$ Bonferroni post-hoc tests: ethanol F vs. control F, $p=0.003$, ethanol M vs. control M: p 0.027, control F vs. control M: p 0.023. **(D)** Prenatal ethanol exposure significantly increased GIN AP half-width in M mice relative to those from control-fed M or F, or ethanol-exposed F mice at P2, but did not affect GIN AP half-width P4-14 (1-way ANOVAs, P2: $F(3, 50) = 5.639$, $p=0.002$, Bonferroni post-hoc tests: ethanol M vs. control M, $p=0.002$, ethanol M vs. ethanol F, $p= 0.014$, ethanol M vs. control F, $p=0.014$; P4-6 $F(3, 50) = 1.335$, $p=0.273$; P8-10 $F(3, 69) = 0.699$, $p=0.556$; P14: $F(3, 61) = 1.086$, $p=0.362$). **(E)** Prenatal ethanol exposure modifies the firing rate of SPN in an age and sex-dependent manner. While no significant differences were observed between groups: control F, ethanol F, control M and ethanol M at P2, group-dependent differences were observed at P4-6, P8-10 and P14, with a group differences varying based on current input at P14 (2-way ANOVAs, P2:$F(3, 531) = 0.6138$, $p=0.6063$, current: $F(8,531) = 44.82$ $p<0.0001$, group x current: $F(8,531)= 0.7447$, $p= 0.8059$; P4-6: group: 2-way ANOVA, $F(3,567) = 6.923$, $p=0.0001$, current: $F(8,567) = 81.60$, $p<0.0001$, group x current: $F(24,677) = 0.7128$, $p=0.8409$; P8-10: group: $F(3,729) = 12.97$, $p<0.0001$, current: $F(8, 729) = 99.70$, $p<0.0001$, group x current: $F(24, 729) = 0.6896$, $p=0.8647$; P14: group: $F(3,803) = 7.874$, $p<0.0001$, current: $F(8, 803) = 104.4$, $p<0.0001$, group x current: $F(24, 803) = 1.676$, $p=0.0136$).At P8, prenatal ethanol exposure significantly increased SPN firing rate in M mice relative to ethanol-exposed F mice. At P14, prenatal ethanol exposure significantly decreased

SPN firing rate in M mice relative to control-fed M mice, while control-fed M mice demonstrated higher firing rates than both control-fed and ethanol exposed F mice (Bonferroni post-hoc tests, ethanol M vs. control M: 500 pA: t = 2.864, p<0.05, control M vs. control F:: 450 pA: t=2.854, p<0.05, 500 pA: t=3.856, p <0.01, control M vs. ethanol F: 500 pA: t=4.050, p<0.0001). **(F)** Example traces of voltage responses of striatal GINs following hyperpolarizing and depolarizing current steps from control-fed female mice at P2 and P14. **(G)** Prenatal ethanol exposure significantly depolarized AP threshold at P2 and hyperpolarized at P14 in SPNs from M mice, relative to those from control-fed M mice, and in depolarized AP threshold in SPNs from F mice relative to control-fed M mice at P14, but did not alter AP threshold between P4-10 (1-way ANOVAs, P2:F(3,59) = 3.862, p=0.014, Bonferroni post-hoc test: ethanol M vs. control M: p 0.009; P4-6: F(3,66) =1.039, p=0.381; P8-10: F(3, 81) = 1.180, p=0.323; P14: F(3,73) = 5.055, p=0.003, Bonferroni post-hoc tests: ethanol M vs. control M: p 0.020, ethanol F vs. control M: p=0.010. **(H)** Prenatal ethanol exposure did not alter the SPN AP half-width. (1-way ANOVAs, P2: F(3, 59) = 1.384, p=0.257, P4-6: F(3,66) = 1.979, p=0.126, P8-10: F(3,81) = 1.017, p=0.389, P14: F(3,73) = 0.419, p=0.740.Data are presented as means (bars), error bars are standard error of the mean (SEM), dots are individual neurons from at least 3 animals per group. *p<0.05, **p<0.01 control male vs. ethanol male, $^{\#\#}$p<0.01 control female vs. ethanol female, $^{+}$p<0.05 control male vs. control female, $^{@@}$p<0.01 control female vs. ethanol male $^{\$}$p<0.05 control male vs. ethanol female, ^{x}p<0.05 ethanol male vs. ethanol female

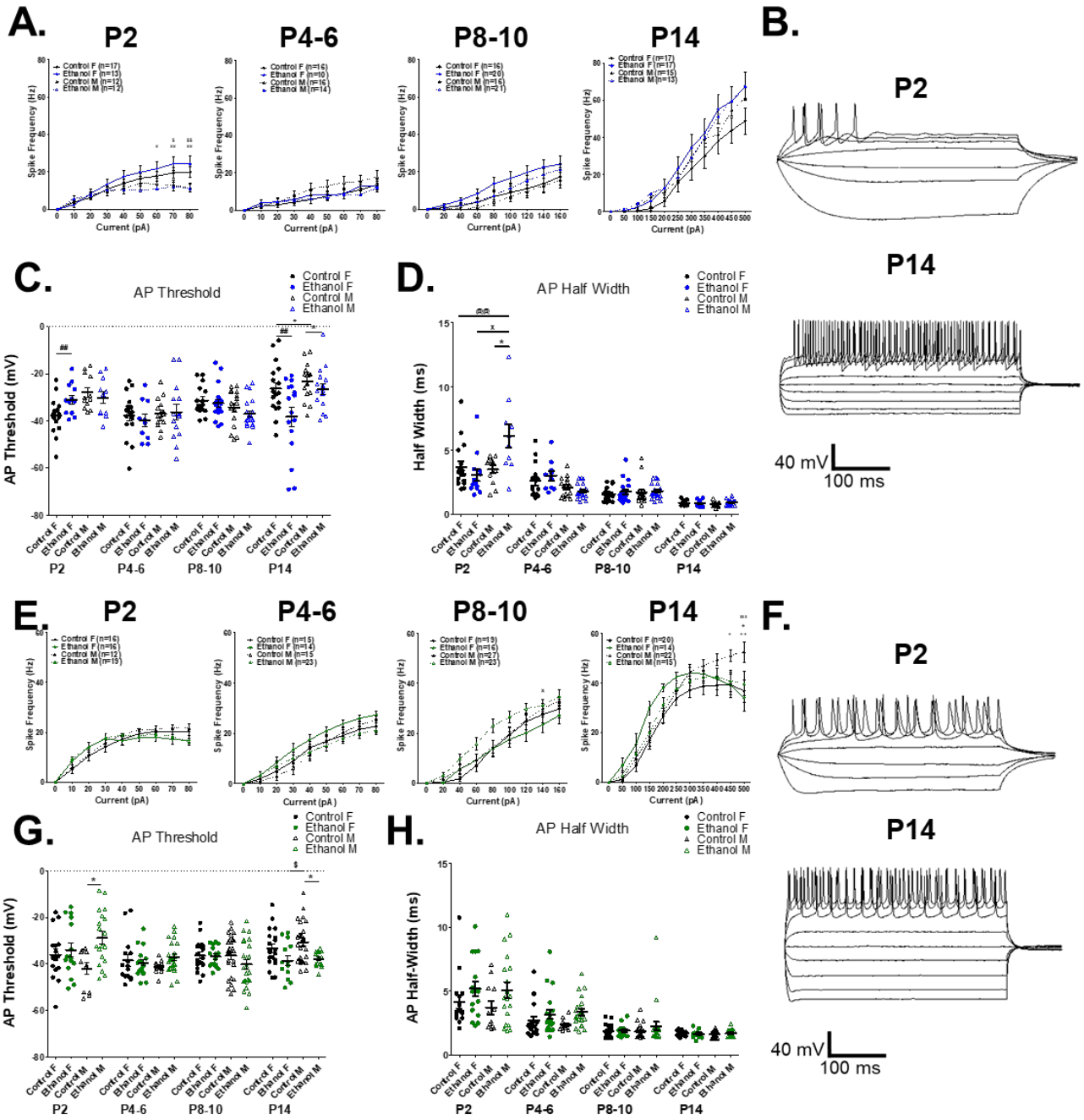
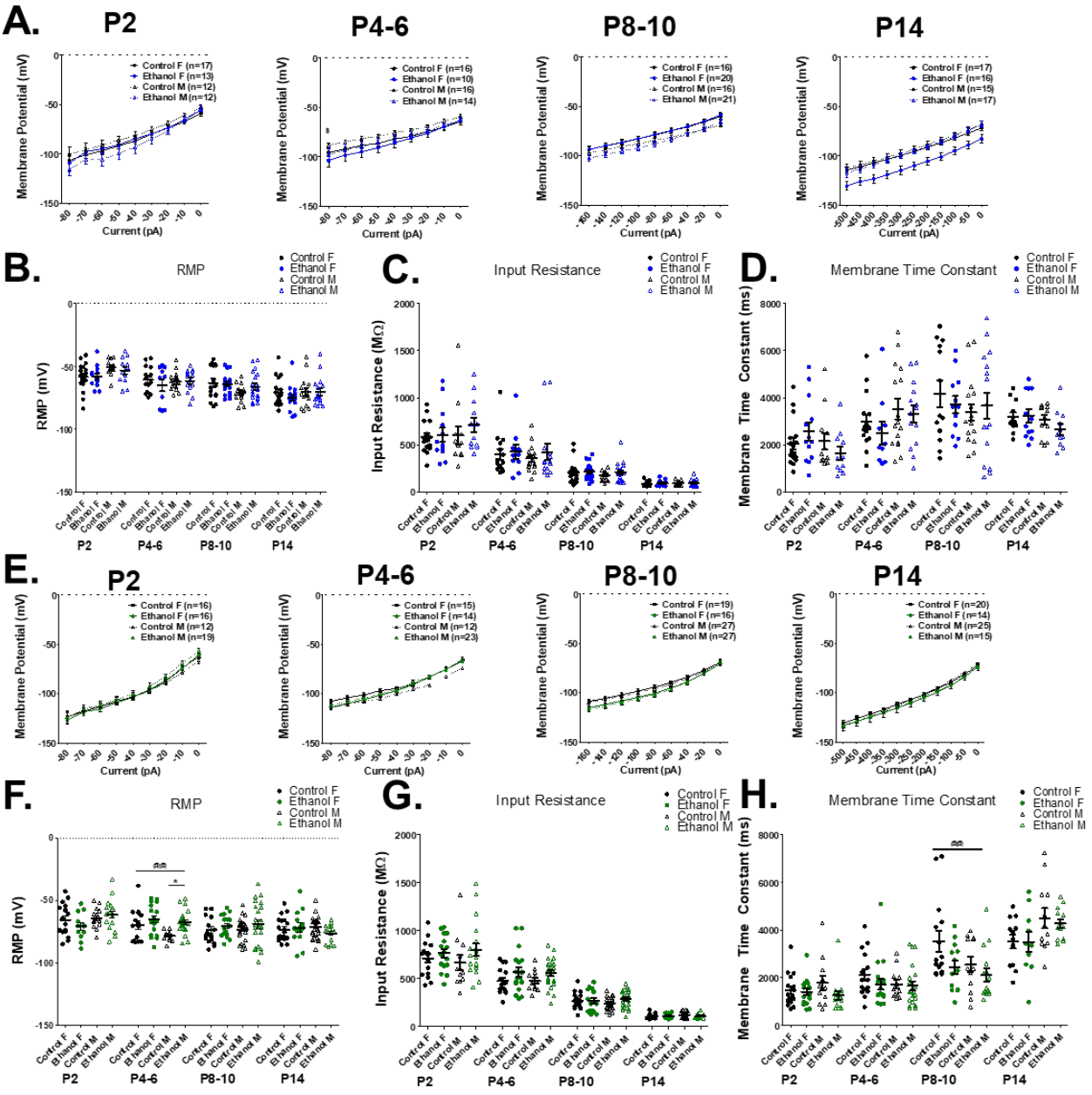

A.
P2
P4-6
P8-10
P14
Spike Frequency (Hz)
Current (pA)
B.
P2
P14
40 mV
100 ms
C.
AP Threshold
AP Threshold (mV)
D.
AP Half Width
Half Width (ms)
Control F
Ethanol F
Control M
Ethanol M
E.
F.
G.
AP Threshold
H.
AP Half Width
AP Half-Width (ms)

Figure 2.5. Prenatal ethanol exposure differentially effects the intrinsic properties of striatal GINs and SPN from female and male mice, depending on the postnatal day.

(A) IV curves for responses to hyperpolarizing current steps during whole-cell current clamp recordings of striatal GINs during the first postnatal week. **(B)** Prenatal ethanol exposure resulted in sex-dependent differences in resting membrane potential (RMP) that varied based on the postnatal day, though no significant differences were observed between groups on individual postnatal days. **(C)** Input resistance (IR) (mΩ) and **(D)** membrane time constant (ms) were unaffected in striatal GINs were unaffected by prenatal ethanol exposure, sex or postnatal day (1-way ANOVAs, GIN: IR: P2: $F(3,50) = 0.611$, $p = 0.649$ P4-6: $F(3,50) = 0.219$, $p = 0.883$; P8-10: $F(3,69) = 0.557$, $p = 0.302$; P14: $F(3,61) = 0.611$, $p = 0.610$; membrane time constant: P2: $F(3,50) = 1.318$, $p=0.279$; P4-6: $F(3,61) = 0.689$, $p = 0.563$; P8-10: $F(3,69) = 1.239$, $p = 0.302$; P14: $F(3,61) = 1.277$, $p = 0.294$. **(E)** IV curves for responses to hyperpolarizing current steps during whole-cell current clamp recordings of SPNs during the first postnatal week. **(F)** Prenatal ethanol exposure resulted in sex-dependent differences in SPN RMP that varied based on the postnatal day: At P4-6: prenatal ethanol exposure resulted in significantly depolarized RMP in male mice relative to control-fed male and female mice (1-way ANOVA: $F(3,66) = 4.632$, Bonferroni post-hocs: ethanol M vs. control F: $^{*}p=0.023$**,** ethanol M vs control F: $^{@@}p=0.004$ SPN RMP was unaltered by prenatal ethanol exposure at P2, 8-10 or P14 (1-way ANOVAs, P2: $F(3,59) = 1.790$, $p = 0.159$; P8-10: $F(3,81) = 0.796$, $p = 0.499$; P14: $F(3,73) = 1.042$, $p = 0.379$). **(G)** Prenatal ethanol exposure did not alter the IR of SPNs (1-way ANOVAs, P2: $F(3,59) = 0.946$, $p = 0.424$; P4-6: $F(3,66) = 1.039$, $p = 0.202$; P8-10: $F(3,81) = 1.182$, $p = 0.322$; P14: $F(3,73) = 1.034$, $p=0.383$). **(H)** Prenatal ethanol exposure results in decreased membrane time constant in SPNs from ethanol-exposed M mice at P8-10, relative to control-fed F mice, but did not alter membrane time constant in F mice. (1-way ANOVAs, P2: $F(3,59) = 1.175$, $p = 0.327$; P4-6: $F(3,66) = 0.934$, $p = 0.430$; P8-10: $F(3,81) = 3.405$, $p = 0.024$; P14: $F(3,73) = 2.283$, $p = 0.093$.Data are presented as means (bars), error bars are standard error of the mean (SEM), dots are individual neurons from at least 3 animals per group. $^{*}p<0.05$ control male vs. ethanol male, $^{@@}p<0.01$ control female vs. ethanol male. $^{*}p<0.05$, $^{**}p<0.01$ control male vs. ethanol male, $^{\#\#}p<0.01$ control female vs. ethanol female, $^{+}p<0.05$ control male vs. control female, $^{\$}p<0.05$ control male vs. ethanol female, $^{x}p<0.05$ ethanol male vs. ethanol female


A.
P2
P4-6
P8-10
P14
Membrane Potential (mV)
Current (pA)
Control F (n=17)
Ethanol F (n=13)
Control M (n=12)
Ethanol M (n=12)
Control F (n=16)
Ethanol F (n=10)
Control M (n=16)
Ethanol M (n=14)
Control F (n=16)
Ethanol F (n=20)
Control M (n=16)
Ethanol M (n=21)
Control F (n=17)
Ethanol F (n=16)
Control M (n=15)
Ethanol M (n=17)
B.
RMP
RMP (mV)
C.
Input Resistance
Input Resistance (MΩ)
D.
Membrane Time Constant
Membrane Time Constant (ms)
Control F
Ethanol F
Control M
Ethanol M
E.
P2
P4-6
P8-10
P14
Control F (n=16)
Ethanol F (n=16)
Control M (n=12)
Ethanol M (n=19)
Control F (n=15)
Ethanol F (n=14)
Control M (n=12)
Ethanol M (n=23)
Control F (n=19)
Ethanol F (n=16)
Control M (n=27)
Ethanol M (n=27)
Control F (n=20)
Ethanol F (n=14)
Control M (n=25)
Ethanol M (n=15)
F.
RMP
G.
Input Resistance
H.
Membrane Time Constant

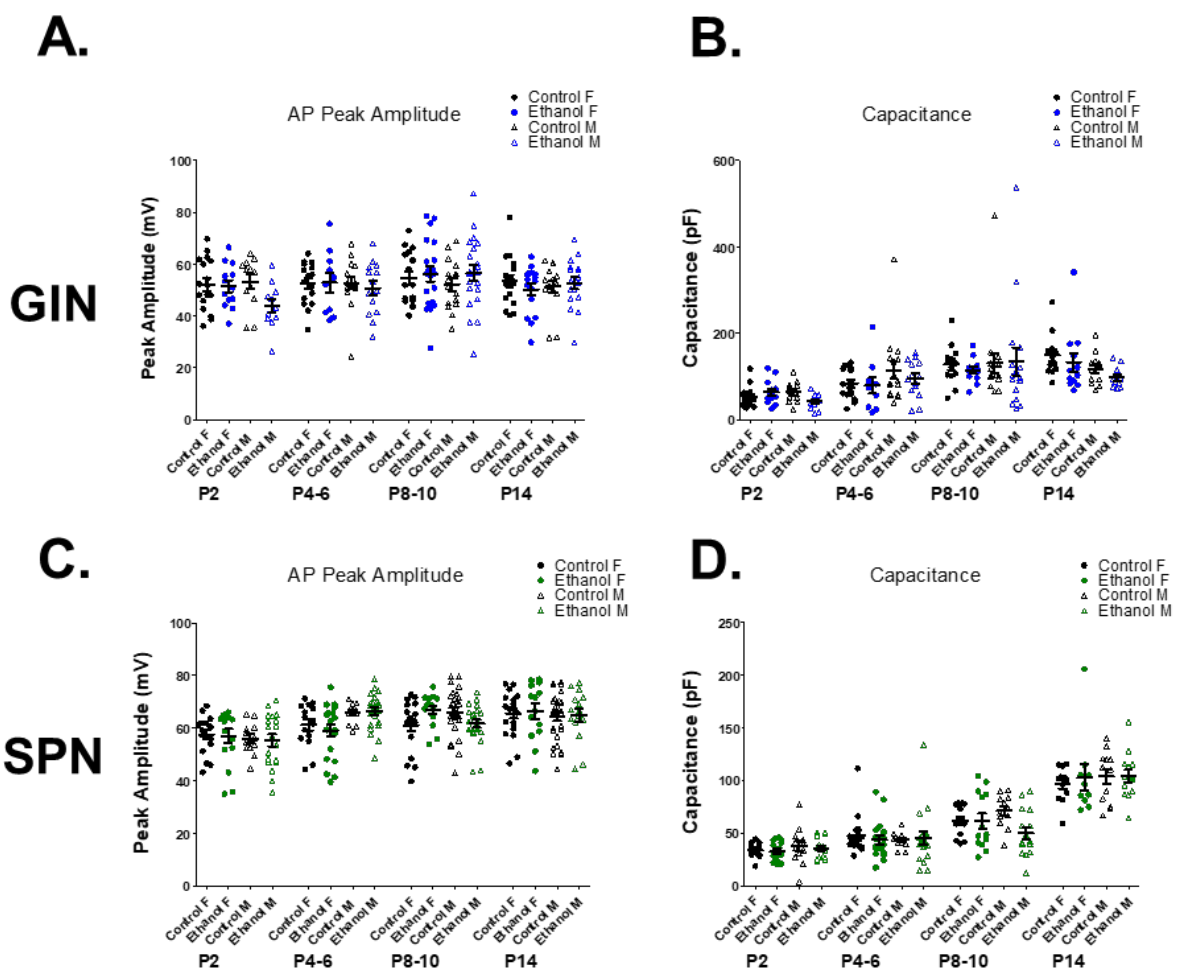


Figure 2.6. Prenatal ethanol exposure does not alter the action potential (AP) amplitude or membrane capacitance of developing striatal GABAergic interneurons (GINs) or striatal projection neurons (SPNs)

(A) The peak amplitude of APs (pA) did not differ in striatal GINs or **(C)** SPNs following prenatal ethanol exposure. Membrane capacitance was also unaffected by prenatal ethanol exposure in **(C)** striatal GINs or **(D)** SPNs. Data are presented as means (bars), error bars are standard error of the mean (SEM), dots are individual neurons from at least 3 animals per group.

Figure 2.7. Glutamatergic synaptic activity in the developing striatum is disrupted by prenatal ethanol exposure depending on biological sex and neuronal subtype: striatal GABAergic interneurons (GINs) and striatal projection neurons (SPNs).

(A) Example whole-cell voltage clamp recordings of spontaneous glutamatergic post synaptic current (sPSC) recordings from striatal GINs during the first two postnatal weeks. **(B)** Example glutamatergic sPSC recordings from SPNs during the first two postnatal weeks. **(C)** Prenatal ethanol exposure differentially effects the frequency of glutamatergic sPSCs recorded in striatal GIN from female and male mice depending upon their postnatal age: at P2, control-fed male mice demonstrate a higher glutamatergic sPSC frequency than control-fed female mice, while frequency does not differ in GINs from ethanol-exposed male vs. female mice (1-way ANOVA, $F(3,49) = 3.884$, p 0.014; Bonferroni post-hoc tests: control M vs. control F: p =0.017). At P14, prenatal ethanol exposure results in a decreased frequency of glutamatergic sPSCs in female mice relative to control-fed female mice (1-way ANOVA, $F(3,47) =4.007$, $p=0.025$, Bonferroni post-hoc tests: ethanol F vs. control F: $p=0.037$). Prenatal ethanol exposure does not affect glutamatergic sPSC frequency in striatal GINs from P4-6 or P8-10 mice (1-way ANOVAs, P4-6: $F(3,47) = 0.530$, $p=0.664$; P8-10: $F(3,47) = 0.092$, $p=0.964$.**(D)** Prenatal ethanol exposure decreases glutamatergic sPSC amplitude in striatal GINs from P14 female mice relative to control-fed female mice, but does not alter glutamatergic sPSC frequency in striatal GINs from female between P2-P10, or from male mice (1-way ANOVAs, P2: $F(3,49) = 2.283$, $p=0.091$; P4-6: $F(3,47) = 0.666$, $p=0.577$; P8-10: $F(3,47) = 1.266$, $p=0.298$; P14: $F(3,47) = 0.666$, $p=0.012$, Bonferroni post-hoc tests: ethanol F vs. control: $p=0.012$). (**E)** Prenatal ethanol exposure results in an early postnatal (P2) decrease in glutamatergic sPSC frequency in SPNs from female mice, relative to control-fed male mice (1-way ANOVA, $F(3,41) = 4.852$, p 0.006; Bonferroni post-hoc tests: control M vs. ethanol F: p =0.005). At P14, prenatal ethanol exposure resulted in significantly decreased glutamatergic sPSC frequency in SPNs from male mice relative to control-fed female mice (1-way ANOVA, $F(3,47) = 4.853$, p 0.005, Bonferroni post-hoc tests: control F vs. ethanol M: $p=0.005$). Prenatal ethanol exposure did not affect glutamatergic sPSC frequency in SPNS from male and female mice at P4-6 or P8-10 (1-way ANOVAs, P4-6: $F(3,56) = 0.537$, $p=0.659$; P8-10: $F(3,52) = 0.094$, $p=0.963$. **(F)** Prenatal ethanol exposure did not alter amplitude of sEPSCs recorded from SPN during the first two postnatal weeks (One-way ANOVAs, P2: $F(3,41) = 0.861$, p 0.469; P4-6: $F(3,56) = 1.332$, $p=0.273$; P8-10: $F(3,52) = 1.341$, $p=0.271$; P14: $F(3,47) = 1.093$, p 0.362). Data are presented as means (bars), error bars are standard error of the mean (SEM), dots are individual neurons from at least 3 animals per group. For all: $^{+}p<0.05$, $^{++}p<0.01$, control M vs. control F, $^{\#}p<0.05$, control F vs. ethanol F, $^{@@}p<0.01$, control F vs. ethanol M, $^{\$\$}p<0.01$, control M vs. ethanol F.

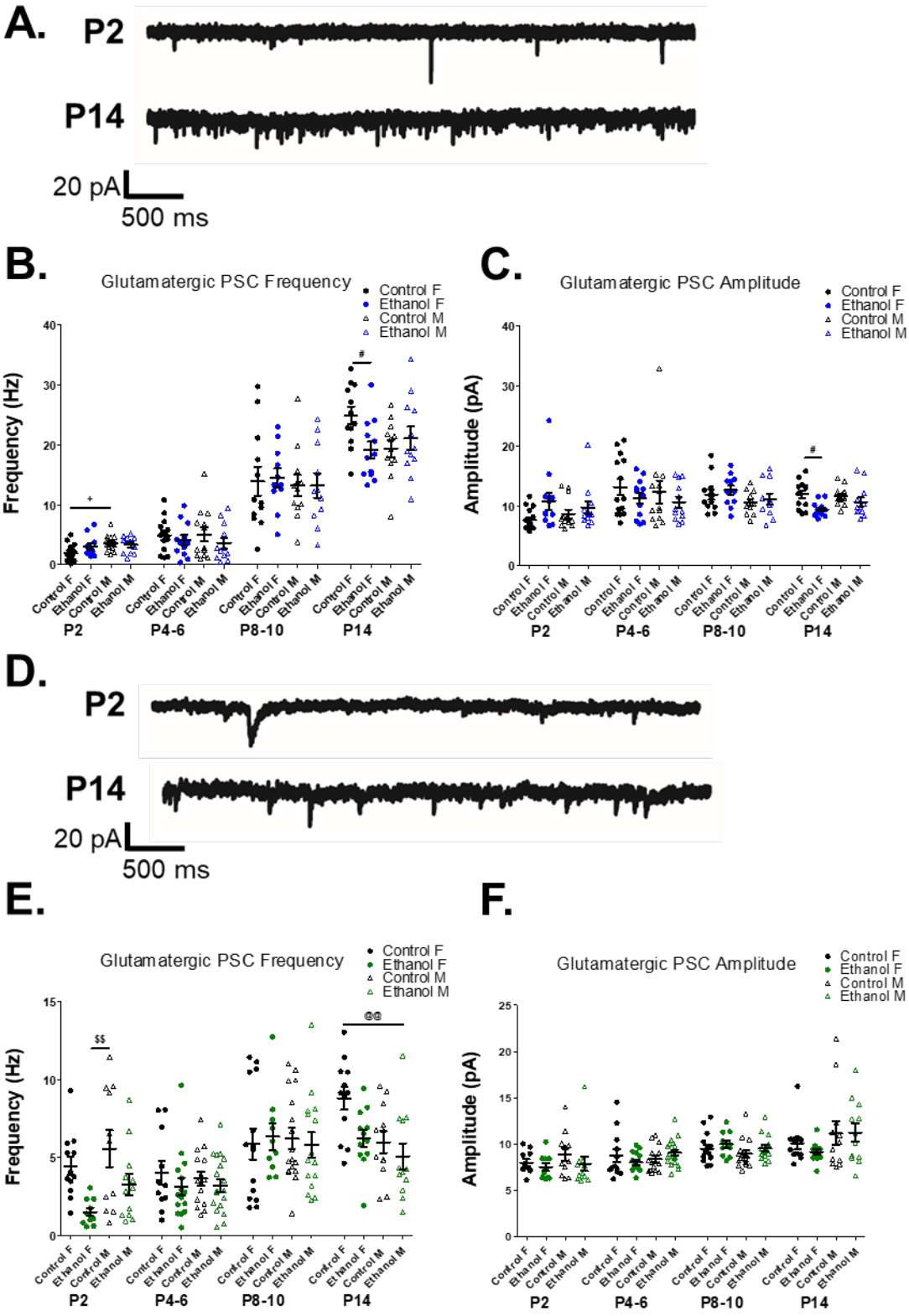

A.
P2
P14
20 pA
500 ms
B.
Glutamatergic PSC Frequency
Control F
Ethanol F
Control M
Ethanol M
Frequency (Hz)
P2
P4-6
P8-10
P14
C.
Glutamatergic PSC Amplitude
Amplitude (pA)
D.
P2
P14
20 pA
500 ms
E.
Glutamatergic PSC Frequency
Frequency (Hz)
F.
Glutamatergic PSC Amplitude
Amplitude (pA)

Figure 2.8. GABAergic synaptic activity in the developing striatum is disrupted by prenatal ethanol exposure depending on biological sex and neuronal subtype: striatal GABAergic interneurons (GINs) and striatal projection neurons (SPNs).

(A) Example whole-cell voltage clamp recordings of spontaneous GABAergic post synaptic current (sPSC) recordings from striatal GINs during the first two postnatal weeks.**(B)** Example GABAergic sPSC recordings from SPNs during the first two postnatal weeks.**(C)** Prenatal ethanol exposure increases the frequency of GABAergic sPSC recorded from striatal GINs in female mice relative to control-fed F mice at P4-6, but does not alter GABAergic sPSC frequency in F mice at P8-10 or P14, and does not affect GABAergic sPSC frequency in male mice (1-way ANOVAs, P2: $F(3,48) = 2.919$, p 0.043; P4-6: $F(3,52) = 3.307$, p 0.028; Bonferroni post-hoc tests: control F vs. ethanol F: $p=0.023$; P8-10: $F(3,41) = 0.699$, p 0.558; P14: $F(3,47) = 2.005$, p 0.127). **(D)** Striatal GINs from control-fed F mice had significantly increased GABAergic sPSC amplitude relative to those from control-fed M and ethanol-exposed M P14 mice (1-way ANOVA, $F(3,47) = 3.150$, p 0.034, Bonferroni post-hoc tests: control M vs. control F: $p=0.028$, control F vs. ethanol M: $p = 0.028$). Prenatal ethanol exposure did not alter GABAergic sPSC amplitude between P2-P10 (one-way ANOVAs, P2: $F(3,40) = 1.461$, p 0.240; P4-6: $F(3,52) = 2.253$, p 0.094; P8-10:$F(3,41) = 0.160$, p 0.923) **(E)** Prenatal ethanol exposure significantly decreased the frequency of GABAergic sPSC in SPNs from M mice at P2, relative to control-fed M and F mice, and ethanol-exposed F mice (1-way ANOVA, $F(3,42) = 6.383$, $p=0.001$; Bonferroni post-hoc test: control M vs. ethanol M: $p=0.008$, control M vs. control F: $p =0.004$, control M vs. ethanol F: $p =0.004$). Prenatal ethanol exposure did not alter GABAergic sIPSC frequency in SPNs P4-14 (1-way ANOVAs, P4-6: $F(3,57) = 0.921$, $p=0.437$; P8-10: $F(3,54) = 0.921$, $p=0.437$; P14: $F(3,46) = 1.344$, p 0.272). **(F)** At P8-10, prenatal ethanol exposure increases the amplitude of GABAergic SPCs in SPNs from M mice relative to control-fed M (1-way ANOVA, $F(3,54) = 1.341$, $p=0.019$, Bonferroni post-hoc: control M vs. ethanol M $p = 0.027$). Prenatal ethanol exposure does not affect GABAergic sPSC amplitude at P2, P4-6 or P14 (1-way ANOVAs, P2: $F(3,38) = 0.587$, p 0.627; P4-6:$F(3,57) = 1.920$, $p=0.137$, P14: $F(3,46) = 1.865$, p 0.149). Data are presented as means (bars), error bars are standard error of the mean (SEM), dots are individual neurons from at least 3 animals per group. For all: $^{+}p<0.05$, $^{++}p<0.01$, control M vs. control F, $^{\#}p<0.05$, control F vs. ethanol F, $^{*}p<0.05$, $^{**}p<0.01$, control M vs. ethanol M, $^{@}p<0.05$, control F vs. ethanol M, $^{\$\$}p<0.01$ control M vs. ethanol F.

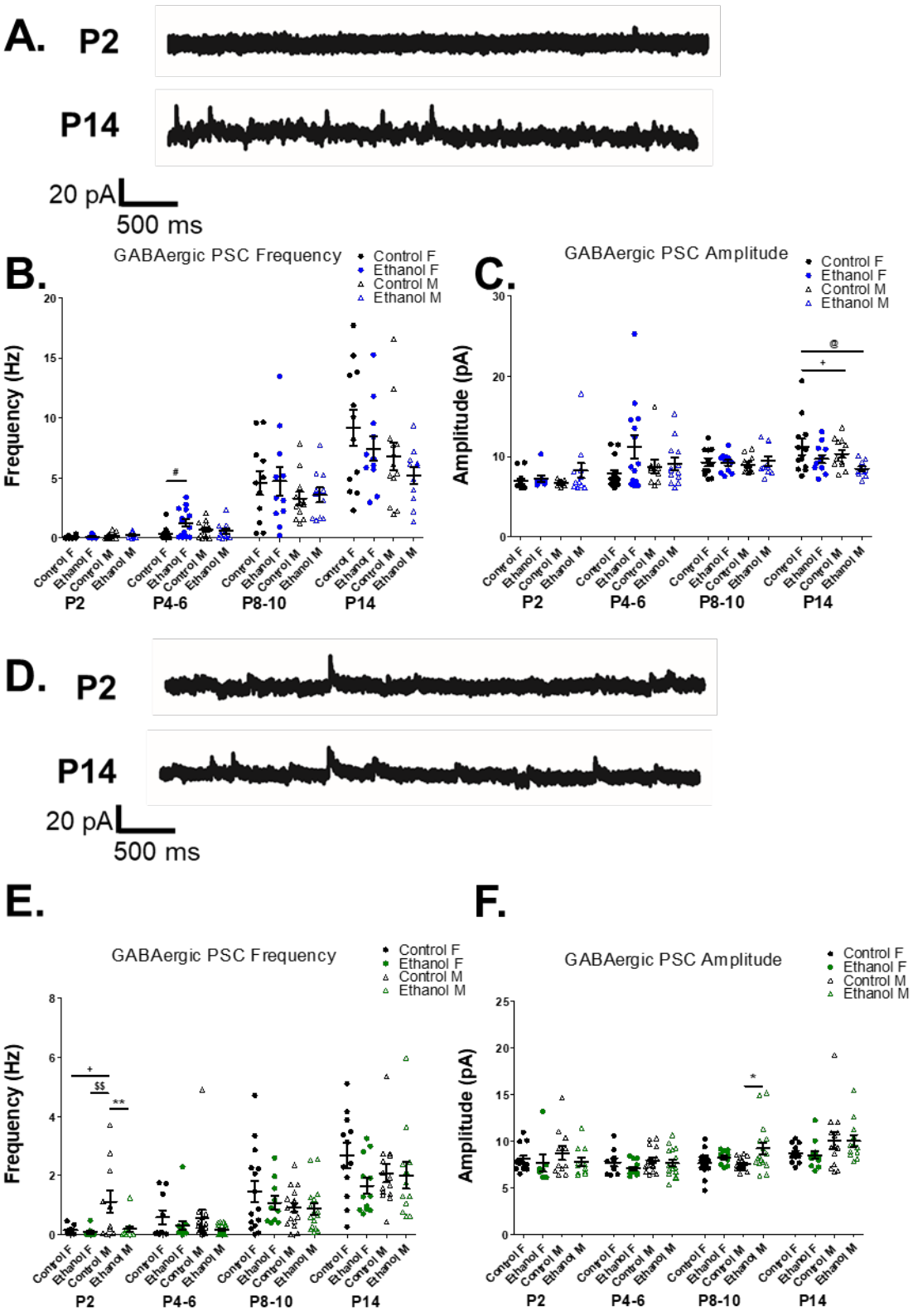
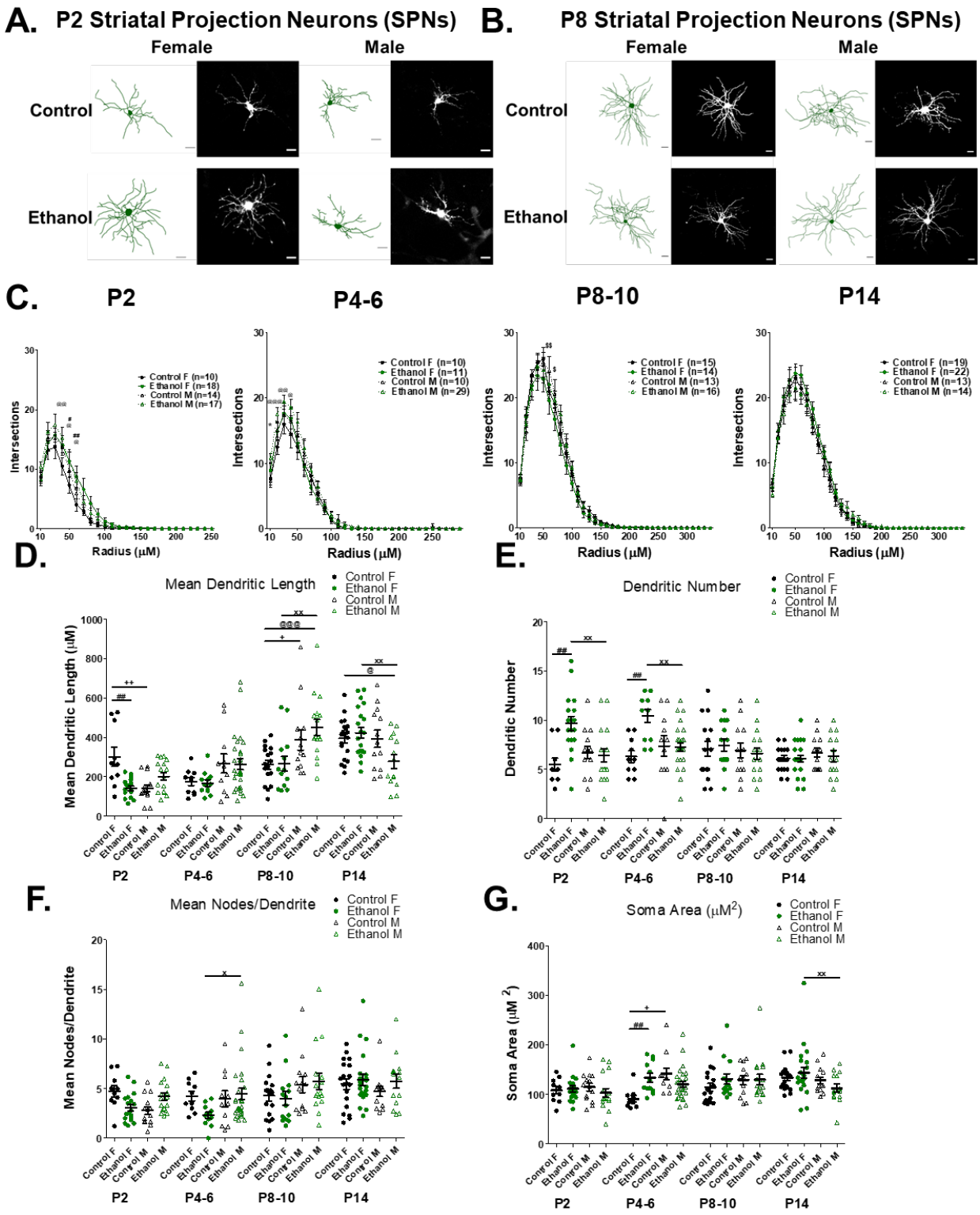

A.
P2
P14
20 pA
500 ms
B.
GABAergic PSC Frequency
Control F
Ethanol F
Control M
Ethanol M
Frequency (Hz)
P2
P4-6
P8-10
P14
C.
GABAergic PSC Amplitude
Amplitude (pA)
D.
E.
F.

Figure 2.9. Prenatal ethanol exposure results in early postnatal increases in striatal projection neuron (SPN) dendritic morphology

(A) Representative reconstructions (right) and images (left) of neurobiotin-filled SPNs from female (F) control, male (M) control (top), F ethanol-exposed, M ethanol-exposed (bottom) postnatal day (P)2 neonates. **(B)** Representative reconstructions (right) and images (left) of neurobiotin-filled SPNs from F control, M control (top), F ethanol-exposed, M ethanol-exposed (bottom) postnatal day (P)2 neonates. **(C)** Sholl analysis indicates that prenatal ethanol exposure significantly increases the dendritic complexity (number of intersections/increasing 10 μM radius) in a radius-dependent manner of SPNs from P2 F and M mice relative to control-fed F mice, P4-6 M mice relative control-fed F mice, and decreased in P8-10 F mice relative to ethanol-exposed M and control-fed M, while differences have resolved by P14 (one-way repeated measures ANOVA, P2: $F(72,1320)= 1.241$, $p=0.0878$, Bonferroni post-hoc tests: ethanol F vs. control F: radius = 50 μM, $t= 3.348$, $p <0.05$, radius = 60 μM, $t = 3.866$, $p <0.01$); ethanol M vs. control F: radius = 40 μM, $t =3.837$, $p<0.01$, radius = 50 μM, $t= 3.189$, $p<0.05$, radius = 60 μM, $t = 3.241$, $p<0.05$; P4-6: $F(84,1596) =1.093$ $p=0.2684$, Bonferroni post-hoc tests: ethanol M vs. control F: radius = 20 μM, $t=4.540$, $p<0.0001$, 40 μM, $t= 3.692$, $p<0.01$, 50 μm, $t =3.412$, $p<0.05$.P8-10: $F(99,1782) = 0.8764$, Bonferroni post-hoc tests: ethanol F vs. ethanol M: radius = 80 μM, $t =4.061$, ethanol F vs. control M: radius = 60 μM, $t=3.694$, $p<0.01$, 70 μM, $t = 3.207$, $p<0.05$. P14: $F(99,2112) = 0.6128$. **(D)** Prenatal ethanol exposure decreased the mean length/dendrite (μM) in SPN from both M and F mice relative to control-fed F, resulted in no significant differences at P4-6. At P8-10 prenatal ethanol exposure increased the mean length/dendrite in SPNs from M mice relative to ethanol-exposed and control-fed F mice, while a persistent increase in mean length/dendrite in SPNs from ethanol-exposed M mice relative to ethanol-exposed F mice. (P2: (Kruskall-Wallis test, $H(3) = 13.49$, $p<0.0039$, Dunn's post-hoc tests: ethanol F vs. control F: $p<0.05$, ethanol F vs. control M, $p>0.05$; P4-6: (Kruskal-Wallis test, $H(3) = 6.905$; P8-10: one-way ANOVA, $F(3,59) = 6.276$, $p = 0.001$, Bonferroni post-hoc tests: ethanol M vs. ethanol F, $t= 3.427$, $p<0.01$, ethanol M vs. control F, $t=3.710$, $p<0.01$; P14: one-way ANOVA, $F(3,64) = 3.962$, $p = 0.0118$, Bonferroni post-hoc tests: ethanol M vs. ethanol F, $t= 3.308$, $p<0.01$). **(E)** Prenatal ethanol exposure results in a transient increase in the number of dendrites in SPNs from P2 and P4-6 F mice relative to those from control-fed F and ethanol-exposed M mice of the same ages, that resolves by P8-10 (Kruskal-Wallis tests, P2: $H(3)= 12.832$, $p=0.005$, Dunn's post-hoc tests: ethanol F vs. control F: $p< 0.05$, ethanol F vs. ethanol M, $p<0.05$; P4-6: $H(3) = 14.116$, $p=0.003$, Dunn's post-hoc tests: ethanol F vs. control F: $p<0.01$, ethanol F vs. ethanol M, $p<0.01$; P8-10: $H(3)= 0.897$, $p=0.826$; P14: $H(3)= 0.747$, $p=0.862$). **(F)** Prenatal ethanol exposure resulted in trend towards a decreased mean number of nodes/dendrite in SPNs from P4-6 F mice relative to control-fed F mice, and ethanol-exposed M mice. Control-fed F mice had increased mean nodes/dendrite relative to control-fed M mice at P2. No differences in mean nodes/dendrite mean nodes per dendrite were observed in SPNs at P8-10, or P14 (P2: one-way ANOVA, $F(3,58)=4.596$, $p= 0.0061$, Bonferroni post-hoc tests, control F vs. control M, $p<0.05$; P4-6: Kruskal-Wallis test, $H(3) = 8.619$. $p=0.0348$; P8-10: $F(3,59) = 1.340$, P14: $F(3,67) = 0.6633$). **(G)** Prenatal ethanol exposure increased the soma area (μM^2) if SPNs from F mice, relative to those from control-fed F mice at P4. SPNs from control-fed F mice also had decreased soma area relative to those from

control-fed male mice at P4. No differences in soma area were observed at P2, P8-10 or P14.P2: one way ANOVA, $F(3,58) = 0.4028$, $p = 0.7515$; P4: one-way ANOVA, $F(3,60) = 5.002$, $p<0.0038$, Bonferroni post- hoc tests: ethanol F vs. control F, $t=3.121$, $p<0.05$, control F vs. control M, $t = 3.591$, $p<0.01$; P8-10: one-way ANOVA, $F(3,59) = 0.7238$, $p= 0.5420$; P14: Kruskall-Wallis test, $H(3) = 6.232$. For all images, scale bar = 20 μM. Data are presented as means (bars), error bars are standard error of the mean (SEM), dots are individual neurons from at least 3 animals per group. For all: +$p<0.05$, ++$p<0.01$, control M vs. control F, #$p<0.05$, control F vs. ethanol F, *$p<0.05$, **$p<0.01$, control M vs. ethanol M, @$p<0.05$, control F vs. ethanol M, $$ $p<0.01$ control M vs. ethanol F.


A.
P2 Striatal Projection Neurons (SPNs)
Female
Male
Control
Ethanol
B.
P8 Striatal Projection Neurons (SPNs)
Female
Male
Control
Ethanol
C.
P2
P4-6
P8-10
P14
Intersections
Radius (μM)
Control F (n=10)
Ethanol F (n=18)
Control M (n=14)
Ethanol M (n=17)
Control F (n=10)
Ethanol F (n=11)
Control M (n=10)
Ethanol M (n=29)
Control F (n=15)
Ethanol F (n=14)
Control M (n=13)
Ethanol M (n=16)
Control F (n=19)
Ethanol F (n=22)
Control M (n=13)
Ethanol M (n=14)
D.
Mean Dendritic Length
Mean Dendritic Length (μM)
E.
Dendritic Number
Dendritic Number
F.
Mean Nodes/Dendrite
Mean Nodes/Dendrite
G.
Soma Area (μM2)
Soma Area (μM 2)
Control F
Ethanol F
Control M
Ethanol M
P2
P4-6
P8-10
P14

CHAPTER 3

Precocious emergence of cognitive and synaptic dysfunction in 3xTg-AD mice exposed prenatally to ethanol

This work was published as: Tousley AR, Yeh PW, Yeh HH (2021) Precocious emergence of cognitive and synaptic dysfunction in 3xTg-AD mice exposed prenatally to ethanol. *Alcohol*. AT and HH Yeh contributed to experimental design. AT collected and analyzed all data. PW Yeh cared for animals. This is in compliance with all copyright requirements.

3.1 Abstract

Alzheimer's disease (AD) is the most common cause of dementia, affecting approximately 50 million people worldwide. Early life risk factors for AD, including prenatal exposures, remain underexplored. Exposure of the fetus to alcohol (ethanol) is not uncommon during pregnancy, and may result in physical, behavioral, and cognitive changes that are first detected during childhood but result in lifelong challenges. Whether or not prenatal ethanol exposure may contribute to Alzheimer's disease risk is not yet known. Here we exposed a mouse model of Alzheimer's disease (3xTg-AD), bearing three dementia-associated transgenes, presenilin1 (PS1M146V), human amyloid precursor protein (APPSwe), and human tau (TauP301S), to ethanol on gestational days 13.5–16.5 using an established binge-type maternal ethanol exposure paradigm. We sought to investigate whether prenatal ethanol exposure resulted in a precocious onset or increased severity of AD progression, or both. We found that a brief binge-type gestational exposure to ethanol during a period of peak neuronal migration to the developing cortex resulted in an earlier onset of spatial memory deficits and behavioral inflexibility in the progeny, as assessed by performance on the modified Barnes maze task. The observed cognitive changes coincided with alterations to both GABAergic and glutamatergic synaptic transmission in layer V/VI neurons, diminished GABAergic interneurons, and increased β-amyloid accumulation in the medial prefrontal cortex. These findings provide the first preclinical evidence for prenatal ethanol exposure as a potential factor for modifying the onset of AD-like behavioral dysfunction and set the groundwork for more comprehensive investigations into the underpinnings of AD-like cognitive changes in individuals with fetal alcohol spectrum disorders.

3.2 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative condition characterized clinically by cognitive impairment, including memory and language deficits, and pathologically by synaptic loss, tau neurofibrillary tangles, amyloid β plaques, and glial activation (Hardy & Selkoe, 2002; Knopman et al., 2021; Naseri, Wang, Guo, Sharma, & Luo, 2019). Alzheimer's disease is the most common cause of dementia, currently affecting an estimated 50 million people worldwide, and cases are expected to triple by 2050 (Patterson, 2018). Both genetic and environmental factors have been reported to increase the risk for developing AD, though studies of environmental risk factors have largely focused on lifestyle choices and comorbidities in adulthood (Edwards, Gamez, Escobedo, Calderon, & Moreno-Gonzalez, 2019; Pimenova, Raj, & Goate, 2018). In this light, early risk factors for AD remain largely underexplored (Seifan, Schelke, Obeng-Aduasare, & Isaacson, 2015; Yu et al., 2020). Identifying early risk factors for AD may contribute to extending the period for modifying disease progression. In the least, it will increase the time that affected individuals and their potential caregivers have to plan for the future (Rasmussen & Langerman, 2019).

Prenatal exposure to ethanol is common: One in 9 pregnant women in the United States report having consumed ethanol in the last 30 days and, of those, approximately one-third report binge drinking (Denny, 2019). This exposure can result in considerable physical, cognitive, and behavioral impairments in the progeny that persist into adulthood (Wozniak, Riley, & Charness, 2019). Outcomes following a prenatal exposure to ethanol depend upon factors such as drinking pattern, dose, and timing of ethanol exposure, all of which can contribute to developing fetal alcohol spectrum disorder (FASD) (Jacobson et al., 2021; May & Gossage, 2011; Wozniak et al., 2019). Individuals

with FASD experience challenges in learning, memory, language skills, and comprehension, which contribute to difficulties in academic performance, as well as social and professional challenges across their lifespans (Davis, Gagnier, Moore, & Todorow, 2013; Mattson, Crocker, & Nguyen, 2011; Rasmussen, 2005).

Alcohol exposure during adulthood is a risk factor for AD pathology in humans (Heymann et al., 2016; Huang, Zhang, & Chen, 2016; Koch et al., 2020; Peng et al., 2020). Remarkably, many cognitive impairments identified in FASD individuals are reminiscent of those identified as early-life risk factors for AD. Indeed, there is a growing appreciation that prenatal ethanol exposure may also increase risk for developing adult-onset diseases, including heart disease, type II diabetes, autoimmune diseases, and certain cancers (Lunde et al., 2016; Moore & Riley, 2015). However, whether prenatal ethanol exposure may influence any aspect of AD pathogenesis during aging is not yet known. In this study, we asked whether and how prenatal exposure to ethanol might be a risk factor for contributing to the development or progression, or both, of AD.

We examined the effects of a brief ethanol exposure in utero on spatial learning/memory, behavioral flexibility, synaptic function, interneuron number, and the disposition of β-amyloid histopathology in a transgenic mouse model of AD (3xTg-AD). We have previously shown that an exposure to ethanol during embryonic days (E) 13.5–16.5 results in altered neuronal migration, synaptic activity, and behavior in neonatal, adolescent, and young adult mice (Delatour, Yeh, & Yeh, 2019a, 2019b; Skorput, Gupta, Yeh, & Yeh, 2015; Skorput, Lee, Yeh, & Yeh, 2019). Here we applied the same prenatal ethanol exposure paradigm to pregnant 3xTg-AD mice, which harbor three dementia-associated transgenes encoding presenilin1 (PS1M146V), human amyloid precursor protein (APPSwe), and human tau (TauP301S), under the Thy1 promoter (Campsall, Mazerolle, De Repentingy, Kothary, & Wallace, 2002; Oddo et al., 2003). In contrast to

other mouse models of AD, 3xTg-AD mice develop A-b and tau pathology concurrently with the onset of spatial memory deficits by 6 months of age (Billings, Oddo, Green, McGaugh, & LaFerla, 2005; Mastrangelo & Bowers, 2008; Oddo et al., 2003). Notably, ethanol exposure has been reported to modify both the AD neuropathology and behavior of adult 3xTg-AD mice (Barnett et al., 2022; Hoffman et al., 2019; Muñoz et al., 2015). Based on findings in this study, we report that a brief prenatal exposure to ethanol during a period of peak neuronal migration in the embryonic cortex (embryonic day [E] 13.5–16.5) results in a precocious emergence of spatial memory deficits and behavioral inflexibility in 4-month-old 3xTg-AD mice, but does not exacerbate their severity in 6-month-old 3xTg-AD mice. In addition, this precocious onset of behavioral abnormality is associated with changes in synaptic connectivity and number of parvalbumin-expressing (PV+) GABAergic interneurons, and degree of β-amyloid deposition in the medial prefrontal cortex (mPFC). Thus, our findings suggest that prenatal alcohol exposure may modify the susceptibility of 3xTg-AD mice to the pathological progression of AD.

3.3 Methods

3.3.1 Transgenic mice

B6129SF1/J (B6129) (Jackson Labs #101045) and B6;129-Tg(APPSwe,tauP301L)1Lfa Psen1tm1Mpm/Mmjax (3xTg-AD) mice (MMRC# 034830-JAX) were bred and maintained following the guidelines and procedures prescribed by NIH Guide for Animal Care and Use, and approved by Dartmouth College's Institutional Care and Use Committee. Animals were housed on a 12-h light/dark cycle with light periods occurring between 7:00 AM and 7:00 PM.

3.3.2 Ethanol exposure paradigm and experimental timeline

In line with a previously described binge-type ethanol exposure paradigm (Delatour et al., 2019a, 2019b; Skorput et al., 2015, 2019), single pairs of male and female mice were housed overnight for breeding with the following day designated as embryonic day (E) 0.5 (**Figure 3.1a**). Pregnant dams were allowed access ad libitum to either 5% (w/w) ethanol in liquid diet or lab chow (5V5M, ScottPharma Solutions) from E13.5 to E16.5. We have previously compared control groups fed ad libitum with those pair-fed an isocaloric maltose liquid diet and detected no significant differences (Cuzon, Yeh, Yanagawa, Obata, & Yeh, 2008). Thus, for the sake of efficiency, the pair-fed diet control group was excluded. Dams were weighed and liquid diet was replaced daily, with water available ad libitum. E13.5 to 16.5 is a critical gestational time span for the peak of migration of primordial GABAergic interneurons and pyramidal neurons into the cortical plate of the embryonic cortex (Batista-Brito & Fishell, 2009; Clancy, Darlington, & Finlay, 2001).

3xTg-AD dams drank to a mean blood ethanol concentration (BEC) of 49.17 mg/dL ± 14.03 mg/dL, as measured at 11:00 PM on E15.5 using an Analox AM1 series III analyzer (Analox Instruments) (**Figure 3.1b**). Our previous assessment of transgenic

mice on congenic C57BL6J background using the same drinking paradigm resulted in binge levels of ~80 mg/dL (Skorput et al., 2015). Background strain dramatically affects ethanol preference, sensitivity, and metabolism (Bachmanov, Tordoff, & Beauchamp, 1996; Downing, Balderrama-Durbin, Broncucia, Gilliam, & Johnson, 2009; Lim, Zou, Janak, & Messing, 2012). In contrast to C57BL6J mice, 129/J mice tend to have lower alcohol preference and are less susceptible to the teratogenic effects of prenatal ethanol exposure, suggesting potential differences in ethanol metabolism or genetic disposition. Hybrid B6129 mice also show decreased ethanol preference relative to C57BL6J mice (Bachmanov et al., 1996; Downing et al., 2009; Lim et al., 2012). Differences in ethanol preference, sensitivity or metabolism could also account for the decreased BEC measured in 3xTg-AD dams, given their hybrid B6129 background, relative to that attained by congenic C57BL6J transgenic mice.

After birth, mice were maintained to 4 months or 6 months of age, then subjected to the 10-day Barnes maze behavioral testing paradigm (see below). Following the 2-week behavioral testing, the mice were sacrificed for electrophysiology and immunohistochemistry experiments at ~4.5 and ~6.5 months, respectively (**Figure 3.1a**). Each animal was used for behavioral testing at a single time point: 4 months or 6 months. Body weight did not differ between B6129 control or 3xTg-AD or 3xTg-AD + EtOH experimental cohorts at either 4-month or 6-month time points (two-way ANOVA, $F(2,50) = 0.5151, 0.6006$) (**Figure 3.1c**).

3.3.3 Modified Barnes maze testing

Barnes maze testing was conducted as described by Skorput et al. (2015, 2019), based on a modified Barnes maze protocol (Koopmans, Blokland, van Nieuwenhuijzen, & Prickaerts, 2003). The Barnes maze consists of 12 holes equally spaced around a circumferential circular wall (diameter = 95 cm), with spatial cues (large red letters)

between each hole. Briefly, mice were single-housed for 3–7 days prior to initiation of the 10-day behavioral testing paradigm. Mice underwent 4 days of training during which they learned to associate escape from the maze through one of the 12 holes, referred to as the “escape hole”, while all other holes were plugged. This acquisition phase was followed by 2 days of rest, then 2 days of testing for memory of the original escape hole, followed by 2 days of testing for reversal learning. In reversal learning, the escape hole was reversed to the quadrant of the maze opposite from the original escape hole (**Figure 3.1b**). Memory of the escape hole was reinforced by the anxiolytic effects of returning mice to their home cage following completion of each trial. Escape holes were assigned randomly to each individual mouse and changed between mice completing testing during the same time period. Escape latency and number of errors (nose pokes in a non-escape hole) were recorded by blinded observers over 4 trials per mouse per day, lasting until a mouse poked its nose into the escape hole or a maximum of 4 min. An overhead video camera driven by video tracking software (Videomex-one, Colburn Instruments) was used to determine total distance traveled during each trial. At the beginning of each trial, the mouse was placed at the center of the modified Barnes maze and covered, with the cover removed at the initiation of a given trial. Between trials, the maze was cleaned with Clidox (Pharmacal Research). Testing was completed daily between 9:00 AM and 12:00 PM at the beginning of the light period.

3.3.4 Electrophysiology

Behaviorally tested mice were anesthetized with 4% isoflurane and euthanized by decapitation. The cerebral cortex was hemisected, with the left half used for electrophysiology experiments and the right half immerse fixed in 4% paraformaldehyde (PFA)/0.1 M phosphate-buffered saline (PBS) for immunohistochemistry experiments. The left hemisphere was cut to 250-μm sections with a Leica vibratome in oxygenated

(95% O2, 5% CO2) ice-cold cutting solution ([in mM] 3 KCl, 7 MgCl2, 0.5 CaCl2, 1.25 NaH2PO4, 28 NaHCO3, 8.3 d-glucose, 110 sucrose, pH 7.4 [adjusted with 1 N NaOH]), followed by at least a 1-h incubation at room temperature in oxygenated artificial cerebrospinal fluid (aCSF) ([in mM] 124 NaCl, 5.0 KCl, 2.0 MgCl2, 2.0 CaCl2, 1.25 NaH2PO4, 26 NaHCO3, 10 d-glucose, pH 7.4 [adjusted with 1 N NaOH]) prior to electrophysiological recording.

Acute brain slices containing mPFC were placed in a custom-made acrylic recording chamber maintained at 32 °C and continuously perfused (0.5–1.0 mL/min) with oxygenated aCSF. Slices were visualized using a fixed-stage upright fluorescence microscope (Olympus BX51W1) with Hoffman Modulation Contrast optics. Whole-cell recordings were conducted using a MultiClamp 700B amplifier (Axon Instruments), and borosilicate glass (Sutter Instrument; OD: 1.5 mm; ID: 0.86 mm) recording electrodes were pulled with a Flaming Brown Micropipette Puller (Sutter Instrument Model P80 PC). The resistance of the recording pipettes ranged between 7 and 10 MΩ as measured in bath aCSF.

A cesium methanesulfonate-based internal solution ([in mM] 130 Cs-methanesulfonate, 10 HEPES, 0.5 EGTA, 8 NaCl, 10 Na-phosphocreatine, 1 QX-314, 4 Mg2+ ATP, and 0.4 Na + GTP adjusted to pH 7.3 with 1 N CsOH) was used for whole-cell recording of spontaneous excitatory (sEPSC) and inhibitory (sIPSC) postsynaptic currents from Layer V/VI pyramidal neurons in the mPFC. sEPSCs and sIPSCs were isolated at holding potentials of −70 mV and 0 mV, respectively. Recordings were filtered with a 10-kHz low pass filter (Clampex Version 9.2) and digitized at 25 kHz (Digidata 1320A; Molecular Devices). Holding potential was corrected for junction potential.

Average frequency, amplitude, and charge from 2-min epochs after a 2-min baseline stabilization period at each holding potential were determined using MiniAnalysis

Software (Version 6.0, Synaptosoft). Pyramidal neurons were identified in acute live brain slices by the relatively large size and laminar location of their soma. Each electrophysiologically recorded cell was also filled with recording solution containing 2% neurobiotin (Vector Laboratories) during recording (**Figure 3.3A**, right panel). The slices containing neurobiotin-filled neurons were fixed after processing as described previously (Delatour et al., 2019b). Briefly, acute slices were fixed overnight in 4% PFA/0.1 M PBS, cryopreserved overnight in 30% sucrose/0.1 M PBS, permeabilized with 30% H2O2/0.1 M PBS, blocked in 10% normal goat serum (NGS)/0.4% Triton X 100, and incubated overnight at 4 °C in 10 µg/mL Streptavidin DyLight 594 (Vector Labs).

3.3.5 Immunohistochemistry, imaging, and image analysis

Following immersion fixation overnight in 4% PFA/0.1 M PBS, the right hemispheres were cryopreserved overnight first in 15% sucrose/0.1 M PBS, then 30% sucrose/0.1 M PBS. Thirty-micron sections were obtained using a sliding microtome and collected into 0.1 M PBS. Sections were permeabilized for 30 min by incubating in 0.25% Triton X-100/10% NGS solution then overnight at 4 °C in primary antibody: rabbit anti-parvalbumin (Abcam, ab11427, 1:1000) in 0.1 M PBS, or mouse anti-β amyloid (Aβ), amino acid residue 11–16/amyloid precursor protein (APP) (6E10, BioLegend #803001, 1:200) in 0.25% Triton X-100/10% NGS. The following day, sections underwent two 30-min washes in 1X PBS, followed by overnight incubation in secondary antibody: goat anti-rabbit Alexa Fluor-488 or goat anti-mouse Alexa Fluor-555 (Invitrogen, 1:1000) in 0.1 M PBS at 4 °C. Prior to mounting, sections were washed overnight in PBS and counterstained with DAPI (Roche, 10236276001). Sections were coverslipped with FluorSave reagent (Millipore, 345789).

Images were captured by a CCD camera (Hamamatsu) using a 10 × 0.3 NA objective (Olympus) fit to a spinning disk confocal microscope (BX61WI, Olympus) and digitized

using cellSens software (Olympus). Images were then montaged using Photoshop CS2 software for a complete view of the mPFC, and cells were counted using FIJI ImageJ Cell Counter analysis plug-ins by researchers blinded to experimental group (Schindelin et al., 2012). Cells were counted in 10 equivalent serial sections from the mPFC defined rostrally by the slice where all five cortical layers of the mPFC were first observable and caudally by the presence of the corpus callosum. Relative APP/Aβ fluorescence intensity in the mPFC was assessed again from 10 serial sections from the mPFC, using FIJI ImageJ software. Calculations of relative fluorescence intensity were made as follows: relative fluorescence intensity = (APP/Aβ fluorescence intensity − Baseline fluorescence intensity)/Baseline fluorescence intensity, where fluorescence intensity = raw integrated density/area or region of interest (μm2). Baseline fluorescence intensity measurements were made per slice for 10 equivalent serial sections.

3.3.6 Data analysis and statistics

Data derived from both male and female mice were pooled, and analyses were performed by experimenters blinded to genotype, exposure, and age of mice. Multifactor ANOVAs were performed using IBM SPSS statistics (version 28.0.1.0), followed by one- or two-way ANOVAs with Bonferroni post hoc tests where Bartlett's test for equal variances indicated data experimental groups had equal variances, and Kolmogorov–Smirnov tests demonstrated that data were normally distributed, while Kruskall–Wallis tests with Dunn's multiple comparisons post hoc tests were used when unequal variances were identified using GraphPad Prism (version 5.03). Repeated-measure ANOVAs were used to compare the performance (escape latency, errors, and total distance traveled) of individual mice over the course of the 4-day training, 2-day testing, and 2-day reversal periods. For behavioral experiments and counts of parvalbumin-immunoreactive cells, comparisons were made between mice from at least three litters.

For electrophysiology experiments, comparisons were made between neurons from mice from at least three litters, with no more than three neurons per mouse. $p < 0.05$ was considered significant and group means presented as mean ± standard error of the mean for all analyses.

3.4 Results

3.4.1 Prenatal ethanol exposure results in precocious onset of spatial memory deficits and alters reversal learning in 3xTg-AD mice without exacerbating AD-like behavioral symptoms

The modified Barnes maze is a behavioral task that assesses spatial learning, memory, and behavioral flexibility over the course of three testing phases: training, testing, and reversal (Fowler et al., 2013; Koopmans et al., 2003; Vetreno & Crews, 2012). During the preliminary 4-day training phase, mice are asked to use spatial cues to identify one of the 12 holes within the Barnes maze setup as an “escape hole” (**Figure 3.2 a-b**). Following a 2-day break from exposure to the maze, memory of the original escape hole is assessed during the 2-day testing period. Mice are then asked during the reversal phase to learn to identify a novel escape hole in the maze placed on the opposite side of the original escape hole. Previous work using the same 3-day ethanol exposure paradigm uncovered a deficit in reversal learning in young adult (~2-month-old) mice in the modified Barnes maze task (Skorput et al., 2015, 2019). Here, we tested 4- and 6-month-old B6129 (control), 3xTg-AD, and 3xTg-AD + EtOH (experimental) cohorts to assess age- and ethanol exposure-dependent persistent deficits in spatial learning and memory, and behavioral flexibility (**Table 3.1; Figure 3.2c-f**).

At 4 months of age, neither the control (B6129) nor the experimental groups (3xTg-AD or 3xTg-AD + EtOH) displayed altered escape latencies, total distance traveled (cm), or number of incorrect nose pokes (mean errors/trial) during the 4-day training (acquisition)

phase of the modified Barnes maze paradigm (two-way repeated-measures ANOVA, escape latency: $F(2,147) = 2.630$, $p = 0.0822$; total distance traveled: $F(2,147) = 2.064$, $p = 0.1379$; errors: $F(2,147) = 3.067$, $p = 0.0556$) (**Figure 3.2c,e; Supplementary Figure 1a**). Although the B6129 mice escaped the Barnes maze more quickly than 3xTg-AD mice on Training Day 1 (B6129: 132.9 ± 15.39 s; 3xTg-AD: 184.6 ± 11.3 s, $t = 2.806$, $p < 0.05$) and trended toward an improved initial performance vs. 3xTg-AD + EtOH mice (179.9 ± 12.58 s), these data suggested that, regardless of control or experimental groups, 4-month-old mice do not differ significantly in their overall ability to learn to perform the Barnes maze task.

Figure 3.2C illustrates that group-dependent differences in escape latency became evident in 4-month-old mice during both testing and reversal phases (two-way repeated-measures ANOVA, testing: $F(2,49) = 4.881$, $p = 0.0117$; reversal: $F(2,49) = 4.397$, $p = 0.0175$). Bonferroni post hoc tests revealed that, while B6129 and 3xTg-AD mice differed on Testing Day 1 alone (B6129: 117.1 ± 11.38 s; 3xTg-AD: 161.2 ± 13.52 s, $t = 2.565$, $p < 0.01$), 3xTg-AD + EtOH mice required significantly more time to escape the Barnes maze on both testing days relative to B6129 mice (Test Day 1: B6129:117.1 ± 11.38 s;, 3xTg-AD + EtOH: 165.9 ± 11.61 s, $t = 3.574$, $p < 0.05$; Test Day 2: B6129:112.3 ± 10.85 s; 3xTg-AD + EtOH: 162.1 ± 10.57 s, $t = 2.583$, $p < 0.05$) (**Figure 3.2C**). In addition, the 3xTg-AD + EtOH cohort displayed increased escape latency relative to B6129 mice on Reversal Day 1, pointing to a deficit in reversal learning (Bonferroni post hoc, B6129: 88.74 ± 8.914 s; 3xTg-AD + EtOH: 152.9 ± 17.81 s, $t = 4.397$, $p < 0.01$) (**Figure 3.2C**). This increase in escape latency was not accompanied by an overall increase in the number of errors ($F(2,49) = 1.155$, $p = 0.3234$) (**Figure 3.2e**), but coincided with a decrease in total distance traveled by 3xTg-AD + EtOH mice relative to B6129 mice on Test Day 1 (two-way repeated-measures ANOVA, testing: $F(2,49) = 3.529$, $p = 0.037$;

Bonferroni post hoc, B6129: 300.2 ± 25.72 cm; 3xTg-AD + EtOH: 218.0 ± 33.23 cm, t = 2.621, p < 0.05). No differences in total distance traveled were observed during the reversal phase (F(2,49) = 1.929, p = 0.1562) (**Supplementary Figure 3.1a**). These data suggest that, in contrast to the unexposed 3xTg-AD mice, the ethanol-exposed 3xTg-AD mice have more severe spatial memory deficits and a deficit in reversal learning at 4 months.

Consistent with the performance of 4-month-old mice, no group differences in escape latency were identified in 6-month-old mice during the training phase (two-way repeated-measures ANOVA, F(2,147) = 2.064, p = 0.1379) or mean errors/trial (F(2,159) = 1.364, p = 0.2645). This suggests that the ability to learn the Barnes maze task was not altered in the 6-month-old experimental groups over the 4-day acquisition phase (**Figure 3.2d**). In addition, although they did not differ in escape latency, 6-month-old mice did demonstrate group-specific differences in total distance traveled during the training phase (F(2,159) = 13.82, p < 0.0001). Bonferroni post hoc tests indicated that B6129 mice traveled significantly more than 3xTg-AD mice during Training Day 1 and 2 (Training Day 1: B6129, 550.4 ± 82.50 cm; 3xTg-AD, 240.9 ± 28.61 cm, t = 5.726, p < 0.001, t = 2.565, p < 0.01; Training Day 2: B6129, 370.2 ± 37.88 cm; 3xTg-AD, 222.6 ± 39.49 cm, t = 2.731, p < 0.05), and 3xTg-AD + EtOH mice on Training Day 1 (3xTg-AD + EtOH: 256.1 ± 36.38 cm, t = 4.982, p < 0.001) (**Supplementary Figure 3.1B**).

In contrast to the 4-month-old mice, no significant differences were detected in escape latency between B6129, 3xTg-AD, and 3xTg-AD + EtOH mice during the testing and reversal phases, although 3xTg-AD + EtOH mice tended to display longer escape latencies during testing and reversal phases (Testing Day 1: 138.5 ± 18.54 s; Testing Day 2: 155.7 ± 21.17 s, Reversal Day 1: 131.4 ± 16.88 s; Reversal Day 2: 122.9 ± 12.04 s) relative to B6129 mice (Testing Day 1: 127.5 ± 14.29 s; Testing Day 2: 127.3 ± 15.32

s; Reversal Day 1: 93.39 ± 13.59 s; Reversal Day 2: 95.34 ± 13.24 s) and 3xTg-AD (Testing Day 1: 138.5 ± 18.54 s; Testing Day 2: 132.1 ± 14.57 s; Reversal Day 1: 116.8 ± 12.04 s; Reversal Day 2: 126.4 ± 12.04 s) mice (Testing: $F(2,53) = 0.8528$, $p = 0.5619$; Reversal: $F(2,53) = 2.136$, $p = 0.1282$) (**Figure 3.2d**). No group-dependent differences in total distance traveled or number of errors were identified during the testing or reversal phases (Errors: Testing: $F(2,53) = 0.8881$, $p = 0.4175$; Reversal: $F(2,53) = 1.069$, $p = 0.3505$; Total distance traveled: Testing: $F(2,53) = 1.363$, $p = 0.2646$; Reversal: $F(2,53) = 0.1803$, $p = 0.8355$) (**Figure 3.2f; Supplementary Figure 3.1b**).

Overall, these data suggest that, although 6-month-old 3xTg-AD + EtOH mice tend to have the poorest performance during the testing and reversal phases of the Barnes maze task, the differences are less dramatic at 6 months than at 4 months of age. These findings are consistent with the notion of a precocious appearance of spatial memory deficits without exacerbating their severity in 3xTg-AD mice exposed prenatally to ethanol.

3.4.2 Ethanol exposure results in age-dependent changes in glutamatergic and GABAergic synaptic inputs to Layer V/VI pyramidal neurons in the mPFC

Synaptic loss and dysfunction precede the onset of the protein-based biomarkers of AD and have been reported to be more predictive of cognitive performance than levels of tau or β-amyloid deposition (Arendt, 2009; DeKosky & Scheff, 1990; Scheff, Price, Schmitt, & Mufson, 2006; Selkoe, 2002). Our ethanol exposure paradigm results in altered synaptic connectivity in the mPFC of young adult mice (Skorput et al., 2015). Thus, we sought to determine whether there were differences in synaptic function between B6129, 3xTg-AD, and 3xTg-AD + EtOH mice. We recorded spontaneous postsynaptic glutamatergic (sEPSC) and GABAergic (sIPSC) currents from layer V/VI pyramidal neurons in the mPFC of the same 4-month-old and 6-month-old mice that

were tested behaviorally. Pyramidal neurons were identified online under Hoffman Modulation Contrast optics based on their laminar location and large size of the cell bodies relative to neighboring cells. Their morphology was also confirmed post hoc following intracellular filling with neurobiotin (**Figure 3.3a**). Glutamatergic and GABAergic sPSCs were isolated from individual cells by recording at holding potentials of −70 mV and 0 mV, respectively, and analyzed separately by age to explore the effects of prenatal ethanol on the onset vs. progression of AD-related changes in synaptic transmission.

Both mean sEPSC and sIPSC frequency were significantly affected in pyramidal neurons recorded in the mPFC of 4-month-old mice (one-way ANOVA, sEPSC frequency: $F(2,40) = 4.702$, $p = 0.0147$; sIPSC frequency: $F(2,40) = 3.842$, $p = 0.0298$). However, only sIPSC frequency was significantly affected at 6 months of age (one-way ANOVA, sEPSC frequency: $F(2,40) = 1.984$, $p = 0.1523$; sIPSC frequency: $F(2,40) = 5.822$, $p = 0.0063$) mice (**Figure 3.3, 3.4, 3.5, 3.5a**). Bonferroni post hoc tests indicated that cells from 4-month-old 3xTg-AD + EtOH mice displayed decreased sEPSC frequency and a distinct rightward shift in the cumulative frequency distribution, reflecting increased inter-stimulus interval (sEPSC frequency: 4.649 ± 0.8420 Hz) relative to those recorded from B6129 mice (sEPSC frequency: 8.044 ± 1.110 Hz, $t = 2.517$, $p < 0.05$) and 3xTg-AD mice (sEPSC frequency: 8.356 ± 0.9183 Hz, $t = 2.749$, $p < 0.05$) (**Figure 3.3a**). sIPSC frequency differed between cells from 4-month-old B6129 and 3xTg-AD + EtOH mice, with cells from 3xTg-AD + EtOH mice showing a decrease in frequency and a rightward shift in the cumulative distribution of interstimulus interval (B6129: 8.766 ± 0.8418 Hz; 3xTg-AD + ETOH: 5.973 ± 0.5527 Hz, $t = 2.758$, $p < 0.05$). sEPSC and sIPSC frequency did not differ in cells recorded from 3xTg-AD (sEPSC frequency: 8.356

± 0.9183; sIPSC frequency: 7.570 ± 0.7561 Hz) and B6129 mice (sEPSC frequency: 8.044 ± 1.110 Hz; sIPSC frequency: 8.766 ± 0.8418 Hz) (**Figure 3.4A**).

In the mPFC of 6-month-old 3xTg-AD + EtOH mice, Layer V/VI pyramidal neurons trended toward decreased sEPSC frequency (3.889 ± 09879 Hz) relative to B6129 (5.962 ± 0.9879 Hz) and 3xTg-AD mice (6.433 ± 0.1.028 Hz), but did not reach statistical significance (**Figure 3.5A**). sIPSC frequency significantly decreased in 3xTg-AD and 3xTg-AD + EtOH mice (5.692 ± 0.6343 Hz and 5.23 ± 0.8729 Hz, respectively) relative to those monitored in 6-month-old B6129 mice (9.055 ± 0.9925 Hz) (**Figure 3.6a**). These data suggest an age-dependent diminution of presynaptic GABAergic, but not glutamatergic, inputs to mPFC pyramidal neurons in 3xTg-AD mice at both 4 and 6 months of age (**Supplementary Figure 3.4**).

Experimental condition significantly affected mean sIPSC amplitude in 4-month-old mice (one-way ANOVA, $F(2,40) = 5.788$, $p = 0.0063$), with cells from 3xTg-AD mice demonstrating an increase in sIPSC amplitude and leftward shift in the cumulative distribution of amplitude relative to cells recorded from both B6129 and 3xTg-AD + EtOH mice (Bonferroni post hoc, 3xTg-AD vs. B6129: $t = 2.903$, $p < 0.05$; 3xTg-AD vs. 3xTg-AD + EtOH: $t = 2.989$, $p < 0.05$) (**Figure 3.4b**). By 6 months of age, though sIPSCs amplitudes recorded from 3xTg-AD mice tended to be larger (16.35 ± 1.380 pA), differences from B6129 (12.53 ± 0.5269 pA) and 3xTg-AD + EtOH (12.49 ± 0.6462 pA) mice were no longer significant (Kruskall–Wallis test, $p = 0.0610$) (**Figure 3.6b**). sEPSC amplitude did not differ in cells from either 4-month-old (B6129: 8.871 ± 0.4151; 3xTg-AD: 9.697 ± 0.5884 pA; 3xTg-AD + ETOH: 8.808 ± 0.2695 pA) or 6-month-old mice (B6129: 8.539 ± 0.4814 pA; 3xTg-AD: 8.526 ± 0.4833 pA; 3xTg-AD + ETOH: 8.76 ± 0.5294 pA) (one-way ANOVA, 4-month-old: $F(2,40) = 1.276$, $p = 0.2903$; 6-month-old: $F(2,40) = 0.070$, $p = 0.9329$) (**Figure 3.3, Figure 3.5b**). The increase in sIPSC amplitude

in 3xTg-AD mice relative to B6129 and 3xTg-AD + EtOH mice suggests that prenatal ethanol exposure may prevent layer V/VI pyramidal neurons in 3xTg-AD mice from compensating for pre-synaptic deficits related to AD pathology with an increase in the number or function of postsynaptic GABAergic receptors.

Experimental condition also significantly affected the mean sEPSC charge in layer V/VI mPFC pyramidal neurons from 4-month-old mice (Kruskall–Wallis test, $p < 0.0001$). Layer V/VI pyramidal neurons recorded from 4-month-old 3xTg-AD + EtOH mice displayed a significant increase in charge relative to those from 3xTg-AD mice and B6129 mice (3xTg-AD + EtOH:, 36.67 ± 2.497 pA·ms; 3xTg-AD: 23.03 ± 2.497 pA·ms; B6: 19.65 ± 2.110 pA·ms; Dunn's multiple comparison test, B6129 vs. 3xTg-AD + EtOH, $p < 0.001$; 3xTg-AD vs. 3xTg-AD + EtOH, $p < 0.01$). This increase in charge was associated with significant increases in both rise and decay time in cells from 4-month-old 3xTg-AD + EtOH mice (rise time: 2.887 ± 0.1267 ms; decay time: 2.981 ± 0.1817 ms) relative to those from B6129 mice (rise time: 1.946 ± 0.1771 ms; decay time: 1.644 ± 0.1843 ms) and 3xTg-AD mice (rise time: 2.160 ± 0.1764 ms; decay time: 1.798 ± 0.1484 ms), while sEPSC amplitude was unaffected by experimental conditions at 4 months of age (one-way ANOVA, rise time: $F(2,40) = 1.364$, $p = 0.2683$; decay time: $F(2,40) = 2.428$, $p = 0.1160$; amplitude: $F(2,40) = 1.276$, $p = 0.2903$) (**Figure** 3D and Supplementary Figs. 2A and B). No group-dependent effects were observed in sIPSC charge at 4 months (one-way ANOVA, $F(2,40) = 1.364$, $p = 0.1190$), nor sEPSC or sIPSC charge at 6 months of age (Kruskall–Wallis test, sEPSC charge: $p = 0.0625$, one-way ANOVA; sIPSC charge: $p = 0.0574$) (**Figure 3.4, Figure 3.5, Figure 3.6c**). The differences in mean glutamatergic and GABAergic frequency, amplitude, and charge were not accompanied by changes in the overall excitatory:inhibitory ratio at either the 4-month or 6-month timepoints (one-way ANOVAs, 4-month-old: frequency: $F(2,40) =$

2.512, p = 0.0938; amplitude: F(2,40) = 1.856, p = 0.1679; charge: F(2,40) = 1.480, p = 0.2398; 6-month-old: frequency: F(2,40) = 2.102, p = 0.1366; amplitude: F(2,40) = 3.079, p = 0.0579; charge: F(2,40) = 0.8732, p = 0.4260) (Supplementary **Figure 3.3**).

Our results suggest that prenatal ethanol exposure hastens the onset of deficits in presynaptic GABAergic inputs to Layer V/VI pyramidal neurons in the mPFC of 3xTg-AD mice (**Supplementary Figure 3.4**). In addition, ethanol-exposed 3xTg-AD + EtOH mice lack the postsynaptic changes to GABAergic synapses observed in 3xTg-AD mice. The changes to GABAergic synaptic properties coincided with a decrease in glutamatergic synaptic inputs.

3.4.3 3xTg-AD mice exposed prenatally to ethanol have fewer PV+ GABAergic interneurons in the mPFC

Given the decrease in GABAergic synaptic inputs to Layer V/VI mPFC pyramidal neurons in 3xTg-AD + EtOH mice, we next asked whether the number of GABAergic interneurons in the mPFC might be affected by AD genotype and whether the differences would be exacerbated by prenatal exposure to ethanol. We focused our assessment on parvalbumin-expressing (PV+) GABAergic interneurons that play an essential role in cortical network activity and cognitive function (Nahar, Delacroix, & Nam, 2021). GABAergic interneurons are susceptible to both AD pathology and prenatal ethanol exposure (Bird, Taylor, Pinkowski, Chavez, & Valenzuela, 2018; Cuzon et al., 2008; Madden et al., 2020; Marguet et al., 2020; Skorput et al., 2015; Xu, Zhao, Han, & Zhang, 2020). In this light, we assessed the disposition of PV+ interneurons in the mPFC of 4- and 6-month-old behaviorally tested B6129, 3xTg-AD, and 3xTg-AD + EtOH mice.

Both AD transgenes and prenatal ethanol exposure significantly decreased the number of PV+ GABAergic interneurons in 4-month-old mice (one-way ANOVA $F(2,21) = 8.426$, $p < 0.01$). While a 29.1% decrease in the number of PV+ GABAergic interneurons was

observed in 4-month-old 3xTg-AD mice (83.10 ± 8.1 cells) when compared to their B6129 counterparts (117.3 ± 12.4 cells; Bonferroni post hoc, $t = 2.668$, $p < 0.05$), 3xTg-AD + EtOH mice displayed an even more dramatic decrease of more than 50% of PV+ cells (58.52 ± 5.3 cells; Bonferroni post hoc, $t = 4.025$, $p < 0.01$) (**Figure 3.7b**).

By 6 months of age, both 3xTg-AD (59.4 ± 5.3 cells, 24.2% decrease) and 3xTg-AD + EtOH (50.71 ± 4.8 cells, 35.3% decrease) mice displayed decreases in PV+ GABAergic interneurons relative to B6129 mice at 4 months (78.4 ± 9.6 cells; one-way ANOVA, $F(2,21) = 4.514$, $p < 0.05$). Differences at 6 months of age were less pronounced, with only 3xTg-AD + EtOH mice displaying significant differences relative to control mice (Bonferroni post hoc, $t = 2.974$, $p < 0.05$) (**Figure 3.8a and b**).

Our findings point to an earlier decrease in the number of PV+ GABAergic interneurons in the mPFC of 3xTg-AD mice exposed prenatally to ethanol. In addition, while the control B6129 mice demonstrated a 33% loss of PV+ GABAergic interneurons in the mPFC between 4 and 6 months of age (Bonferroni post hoc, $t = 3.247$, $p < 0.01$), this age-dependent decrease is not significant in the 3xTg-AD (28.5% decrease) and 3xTg-AD + EtOH (13.3% decrease) mice (**Supplementary Figure 3.4**).

3.4.4 Prenatal ethanol exposure results in region-specific and cortical layer-specific decreases in PV+ GABAergic interneurons in 3xTg-AD mice

Different layers and regions of the mPFC differ dramatically in cell composition and function, and afferent and efferent connections (Anastasiades & Carter, 2021). To assess whether AD pathology and the effects of prenatal ethanol exposure on the number of PV+ GABAergic interneurons in the mPFC might be specific to a particular cortical layer (Layer I, II/II, V, or VI), or region (anterior cingulate [ACC], prelimbic [PL], and infralimbic [IL]), as identified by DAPI counterstaining, we first performed a three-way ANOVA with experimental group, region, and layer as factors. We identified a

significant experimental group × layer × region interaction ($F(30,504) = 11.196$, $p < 0.05$). Thus, cortical layer-specific differences were assessed in each region of the mPFC at 4 months and 6 months using two-way ANOVAs with group and layer as factors.

A significant effect of experimental group was found in the PL and IL regions of the mPFC in 4-month-old mice (PL: $F(2,84) = 7.338$, $p = 0.0012$; IL: $F(2,84) = 14.46$, $p < 0.0001$), but not in the ACC ($F(2,84) = 2.199$, $p = 0.1173$). Bonferroni post hoc analyses of 4-month-old mice revealed significant differences in Layer V of the IL region between both 3xTg-AD (7.608 ± 1.075 cells, $t = 3.450$, $p < 0.01$) and 3xTg-AD + EtOH (6.333 ± 1.537 cells, $t = 3.896$, $p < 0.001$) relative to B6129 mice (12.07 ± 1.668 cells). Similarly, at 4 months, there were significantly fewer cells in Layer VI of the IL region in both 3xTg-AD (5.219 ± 0.88 cells, $t = 2.658$, $p < 0.05$) and 3xTg-AD + EtOH (4.567 ± 1.061 cells, $t = 2.778$, $p < 0.05$) relative to B6129 controls (8.657 ± 1.243 cells) (**Figure 3.7c**). Significant differences were also observed between 4-month-old B6129 and 3xTg-AD + ETOH mice in Layer II/III of the ACC (B6129: 29.52 ± 10.05 cells; 3xTg-AD + EtOH: 13.82 ± 5.135 cells, $t = 2.577$, $p < 0.05$), PL (B6129: 15 ± 3.933 cells; 3xTg-AD + EtOH: 7.517 ± 2.256 cells, $t = 2.840$, $p < 0.05$), and IL (B6129: 7.235 ± 0.6739 cells; 3xTg-AD + EtOH: 3.45 ± 0.8902 cells, $t = 2.571$, $p < 0.05$) as well as Layer V of the PL regions (B6129: 14.08 ± 1.976 cells; 3xTg-AD + EtOH: 7.233 ± 1.575, $t = 2.598$, $p < 0.05$) (**Figure 3.7c**).

In 6-month-old mice, consistent with observations in their 4-month-old counterparts, a significant effect of experimental group was observed only in the PL and IL mPFC regions (PL: $F(2,84) = 11.18$, $p < 0.0001$; IL: $F(2,84) = 9.577$, $p = 0.0002$). Again, no differences were observed between groups in the ACC ($F(2,84) = 2.004$, $p = 0.1412$). Post hoc analyses identified differences between both 3xTg-AD and 3xTg-AD + EtOH

mice relative to B6129 controls in Layers V and VI of the PL mPFC (Layer V, B6129: 12.74 ± 2.146 cells; 3xTg-AD: 8.175 ± 1.095 cells; 3xTg-AD + EtOH: 7.7 ± 0.9245; B6129 vs. 3xTg-AD: $t = 3.578$, $p < 0.01$; B6129 vs. 3xTg-AD + EtOH: $t = 4.067$, $p < 0.001$; Layer VI, B6129: 8.829 ± 0.9564 cells; 3xTg-AD: 5.425 ± 0.4617 cells; 3xTg-AD + EtOH: 5.256 ± 0.5242 cells; B6129 vs. 3xTg-AD: $t = 2.673$, $p < 0.05$; B6129 vs. 3xTg-AD + EtOH: $t = 2.882$, $p < 0.05$) (**Figure 3.8c**). Differences specific to 3xTg-AD + EtOH vs. B6129 mice were observed in Layer V and VI of IL mPFC (Layer V, B6129: 12.86 ± 1.724 cells; 3xTg-AD + EtOH: 7.289 ± 1.386 cells, $t = 4.060$, $p < 0.001$; Layer VI, B6129: 10.96 ± 1.2 cells; 3xTg-AD + EtOH: 6.056 ± 1.109 cells, $t = 3.574$, $p < 0.01$) (**Figure 3.8c**).

Overall, 3xTg-AD mice displayed a trend toward decreased PV+ interneurons in the PL and IL of the mPFC independent of prenatal ethanol exposure. However, with prenatal ethanol exposure, the decrease in PV+ interneurons was more widespread and severe, particularly in the deeper layers V and VI in the mPFC of 3xTg-AD mice. These differences were most apparent in 4-month-old mice, whereas fewer regions were affected in 3xTg-AD + EtOH mice at 6 months.

3.4.5 Prenatal ethanol exposure results in an earlier onset of β-amyloid pathology in 3xTg-AD mice

Pathological accumulation of β-amyloid and tau proteins coincides with the development of GABAergic interneuron dysfunction in animal models of AD and in human AD patients (Ali, Baringer, Neal, Choi, & Kwan, 2019; Li et al., 2016; Tampellini, 2015). In 3xTg-AD mice, accumulation of intraneuronal β-amyloid occurs by 4 months of age, coinciding with onset of cognitive deficits and preceding the formation of amyloid plaques by 6 months of age, as well as the onset of pathological tau neurofibrillary tangles, the latter not detected until 12–18 months of age (Billings et al., 2005; España et al., 2010). Here

we asked whether PAE altered the accumulation of intraneuronal β-amyloid pathology in 4-month-old and 6-month-old 3xTg-AD mice. We measured the relative intensity of APP/Aβ immunofluorescence with 6E10, a mouse monoclonal antibody to human APP/Aβ driven by transgene expression (**Figure 3.9**).

A two-way ANOVA with age and group as factors revealed a significant effect of group on APP/Aβ relative fluorescence intensity ($F(2,12) = 10.49$, $p < 0.01$). In 4-month-old mice, post hoc analysis revealed both diffuse and intraneuronal APP/Aβ fluorescence in 3xTg-AD (0.1436 ± 0.0252) and 3xTg-AD + EtOH (0.1655 ± 0.010) mice. However, only 3xTg-AD + EtOH mice had significantly increased APP/Aβ fluorescence relative to B6129 animals (0.1094 ± 0.0174) (Bonferroni post hoc, B6129 vs. 3xTg-AD + EtOH: $t = 2.724$, $p < 0.05$). Alternatively, at 6 months of age, both 3xTg-AD (0.1738 ± 0.011) and 3xTg-AD + EtOH (0.1059 ± 0.007) mice showed significantly increased APP/Aβ fluorescence relative to B6129 animals (Bonferroni post hoc, B6129 vs. 3xTg-AD: $t = 3.303$, $p < 0.05$; B6129 vs. 3xTg-AD + EtOH: $t = 3.361$, $p < 0.05$). This again suggested that PAE may hasten the onset of AD phenotypes in 3xTG-AD mice. In addition, β-amyloid staining did not appear to co-localize with parvalbumin + GABAergic interneurons, consistent with previous reports that intraneuronal human β-amyloid is preferentially expressed in pyramidal neurons, likely due to Thy1.2 promoter-driven transgene expression in 3xTg-AD mice (España et al., 2010) (**Supplementary Figure 3.5**).

3.6 Discussion

In this study, we provide the first preclinical evidence that prenatal ethanol exposure can contribute to AD pathology. Prenatal ethanol exposure alters developing neurocircuits and can result in differences in behavior and cognition in children that persist into adulthood (Delatour & Yeh, 2017; Jacobson et al., 2021; Wozniak et al., 2019). How

prenatal ethanol exposure may contribute to neurocircuit-level and cognitive changes during aging in general, and specifically to the risk and/or progression of AD, is uncertain. The major finding of this study is that prenatal ethanol exposure resulted in a precocious onset of spatial memory deficits and reversal learning deficits in 4-month-old 3xTg-AD mice, while differences were less severe in 6-month-old mice. This is consistent with the notion that prenatal ethanol exposure may alter the onset but not the severity of AD symptoms. These changes coincided with age-dependent alterations in glutamatergic and GABAergic synaptic activity, as well as a progressive decrease in PV+ GABAergic interneurons and an accumulation of β-amyloid in the mPFC.

3.6.1 3xTg-AD mice exposed to ethanol in utero develop cognitive deficits earlier, but do not show differences in the progression or severity of the deficits

We demonstrated that while 4-month-old 3xTg-AD mice displayed some spatial memory deficits, 3xTg-AD + EtOH mice had more severe spatial memory impairment and behavioral flexibility. Alternatively, while 6-month-old 3xTg-AD + EtOH mice continued to perform the most poorly in the modified Barnes maze task, group-dependent differences in task performance were no longer significant. The lack of observed differences between B6129 and 3xTg-AD mice stands in contrast to previous work that demonstrated spatial memory deficits using the modified Barnes maze task in 6-month-old 3xTg-AD animals (Muñoz et al., 2015; Stover, Campbell, Van Winssen, & Brown, 2015). The more subtle differences between 6-month-old 3xTg-AD and 3xTg-AD + EtOH groups suggests that mice may employ different strategies at different ages that may account for the observed decrease in severity in spatial memory.

The potential for age-dependent differences in behavioral task strategy is supported by differences in the onset of cognitive deficits in 3xTg-AD mice, observed depending on the method of behavioral assessment and the type of cognition assessed (Billings et al.,

2005; Clinton et al., 2007; Pietropaolo, Feldon, & Yee, 2008; Stevens & Brown, 2015). The possibility cannot be ruled out that prenatal ethanol exposure could have increased the severity of AD symptoms later in disease progression had we employed a different learning and memory behavioral paradigm. Indeed, given the continued progression of decrease in PV+ GABAergic interneurons, synaptic deficits, and subtle differences in spatial memory behavior in 6-month-old mice, future work should assess 3xTg-AD mice using more complex spatial memory tasks, testing alternate types of memory, and at older ages.

In addition, given the lack of observed differences in ethanol-exposed or ethanol-unexposed 6-month-old 3xTg-AD mice relative to control mice, or between 3xTg-AD and 3xTg-AD + EtOH groups at either time point, it is possible that the differences we observed in the modified Barnes maze task performance of 4-month-old 3xTg-AD + EtOH mice represents an effect of prenatal ethanol exposure on cognition rather than a modification of the progression of AD pathology. Our past work suggests that young adult mice of a C57B6 background strain exposed prenatally to ethanol demonstrated reversal learning deficits but no alterations in spatial learning or memory on the modified Barnes maze task (Skorput et al., 2015). Prenatal ethanol exposure to mice of the B6129 background strain may result in spatial memory deficits in addition to reversal learning deficits in modified Barnes maze task performance, or alternatively memory deficits may appear in older adult animals exposed prenatally to ethanol regardless of background strain.

While strain differences in the physical effects of prenatal ethanol exposure have been observed, less is known about whether these differences alter cognitive and behavioral performance (Chen, Ozturk, Ni, Goodlett, & Zhou, 2011; Downing et al., 2009; Ogawa, Kuwagata, Ruiz, & Zhou, 2005). Future work should address whether strain differences

extend to differences in spatial learning and memory, and alternatively explore the progression of cognitive differences in aging mice exposed prenatally to ethanol. However, given the parallels in age-related differences of 3xTg-AD and 3xTg-AD + EtOH mice relative to control mice, in cognitive performance, presence of synaptic dysfunction, changes in PV+ GABAergic interneuron numbers, and the degree of amyloid deposition, we believe it is likely that these cognitive deficits we observed in 4-month-old mice reflect an earlier onset of AD pathology (**Figure 3.9, Supplementary Figure 3.5**). Alternatively, employing a heavier binge or longer time course of prenatal ethanol exposure may amplify subtle differences we observed between 3xTg-AD and 3xTg-AD + EtOH mice.

3.6.2 Spatial memory deficits and behavioral inflexibility in ethanol-exposed 3xTg-AD mice coincide with age-dependent changes in synaptic connectivity

We found precocious progression of presynaptic GABAergic deficits in 3xTg-AD + EtOH mice. Dysfunction of inhibitory synapses has been reported in mouse models of AD as well as postmortem tissue from human AD patients (Kiss et al., 2016; Kurucu et al., 2021; Li et al., 2021; Mitew, Kirkcaldie, Dickson, & Vickers, 2013; Palop & Mucke, 2016; Prince et al., 2021; Verret et al., 2012). Conversely, recent work suggests that modifying GABAergic function can improve cognitive outcomes in mouse models of AD (Hijazi et al., 2019; Martinez-Losa et al., 2018). Several mechanisms could account for the differences in sPSC frequency, including change in the number of synapses, in the release probability of vesicles in response to changes in intracellular calcium levels, or in the number of vesicles present presynaptically. These may result from differences in action potential-driven or spontaneous vesicle release events (Ramirez & Kavalali, 2011). Both ethanol exposure and AD pathology have been shown to alter presynaptic function and intracellular calcium dynamics (Barthet & Mulle, 2020; Catlin, Guizzetti, &

Costa, 1999; Lee, Yeh, & Yeh, 2022; Leslie, Brown, Dildy, & Sims, 1990; Lovinger, 2018; Siggins, Roberto, & Nie, 2005; Tong, Wu, Li, & Cheung, 2018). Our findings underscore the importance of considering changes in GABAergic interneuron function as a potential mechanism underlying early AD pathology in general, and specifically the effects of prenatal ethanol exposure on AD onset.

The enhanced postsynaptic GABA responses observed in 4-month-old 3xTg-AD mice are suggestive of increased postsynaptic receptor number or function. Altered postsynaptic GABA responses have been observed in young 3xTg-AD mice and other mouse models of AD (Chen et al., 2021; Hijazi et al., 2019; Li et al., 2021). Whether or not 3xTg-AD + EtOH mice develop postsynaptic GABAergic changes at earlier timepoints, and how postsynaptic changes may be affected at later timepoints, remains to be elucidated.

Changes in glutamatergic event kinetics could reflect alterations in composition of glutamate receptor subunits or glutamate receptor modulators (Hansen, Yuan, & Traynelis, 2007; Iacobucci & Popescu, 2018; Koike, Tsukada, Tsuzuki, Kijima, & Ozawa, 2000; Milstein & Nicoll, 2008; Stincic & Frerking, 2015). Both acute and chronic ethanol exposure have been shown to alter presynaptic glutamate release and glutamate receptor function in adult mice. However, further investigation into the mechanisms contributing to altered glutamatergic synaptic transmission in 3xTg-AD + EtOH is needed (Lovinger, 2018; Möykkynen & Korpi, 2012; Roberto et al., 2006; Roberto & Varodayan, 2017; Siggins et al., 2005). Overall, continued inquiry into the mechanisms underlying the pre- and post-synaptic changes in GABAergic and glutamatergic synaptic activity – could shed light on possible novel therapeutic targets for intervention at early stages of AD progression.

3.6.3 Altered GABAergic synaptic activity coincides with decreased number of PV+ GABAergic interneurons in ethanol-exposed 3xTg-AD mice

A diminution in PV+ GABAergic interneuron number and synaptic inputs was already present in 3xTg-AD mice at 4 months of age, but was more severe and widespread in 3xTg-AD + EtOH mice. It remains to be determined when changes in interneuron number first appear, and relatedly whether such changes are due to differences in the embryonic proliferation, migration of GABAergic interneurons in the mPFC, or the result of cell loss in adulthood. Migrating embryonic cortical neurons are susceptible to the effects of prenatal ethanol exposure (Cuzon et al., 2008; Delatour et al., 2019a; Lee et al., 2022; Skorput et al., 2015, 2019). Decreased numbers of GABAergic interneurons have also been identified in post mortem tissue from human AD patients (Arai, Emson, Mountjoy, Carassco, & Heizmann, 1987; Brady & Mufson, 1997; Sanchez-Mejias et al., 2020). In addition, several AD mouse models, including 3xTg-AD mice, have age-, region-, and mouse strain-dependent changes in interneuron number. As a case in point, loss of GABAergic interneurons in the hippocampus in particular has been associated with spatial memory deficits (Albuquerque et al., 2015; Andrews-Zwilling et al., 2010; Cheng et al., 2019; Leung et al., 2012; Murray et al., 2011; Sanchez-Mejias et al., 2020; Takahashi & Yamanaka, 2006; Verret et al., 2012). Neuroanatomical information regarding whether the loss of GABAergic interneurons in the 3xTg-AD mice is limited to the mPFC or may be manifest in other brain areas will provide insight into the pathological changes underlying observed spatial memory deficits.

3.6.4 Synaptic dysfunction and diminished numbers of PV+ GABAergic interneurons parallel increased accumulation of β-amyloid pathology in the mPFC of 3xTg-AD + EtOH mice

We observed an increased severity of β-amyloid pathology in 4-month-old 3xTg-AD mice exposed to ethanol when compared to unexposed animals. At 6 months of age, both 3xTg-AD and 3xTg-AD + EtOH mice demonstrated differences from control mice. Consistent with previous work, we observed the deposition of both diffuse and intraneuronal β-amyloid immunofluorescence in 4-month-old 3xTg-AD mice that appears to progress in 6-month-old mice regardless of ethanol exposure. These data provide further support for the hypothesis that prenatal ethanol exposure may modify the progression of AD phenotype in 3xTg-AD mice. Our findings also concur with work suggesting that prenatal stress can exacerbate early β-amyloid pathology in mouse models of AD (Jafari et al., 2019).

Intraneuronal β-amyloid accumulation has been demonstrated to precede that of phosphorylated tau and development of neurofibrillary tangles in the cortex of 3xTg-AD mice and post mortem brain tissue from individuals with AD (Oddo et al., 2003; Welikovitch et al., 2018). Intraneuronal β-amyloid deposition has been observed in the cortex of 3xTg-AD mice as young as 3 weeks old, while phosphorylated tau is not observed until animals reach 12 months of age (Billings et al., 2005; Oddo et al., 2003; Oh et al., 2010). Future work will address whether prenatal ethanol exposure alters the timing of onset or severity of tau pathology in older 3xTg-AD mice, and how this may be connected to the onset of β-amyloid pathology.

Progressive increases in intraneuronal β-amyloid have also been shown to coincide with the onset of cognitive deficits and synaptic dysfunction in several rodent models of AD (Billings et al., 2005; Chui et al., 1999; Gouras, Tampellini, Takahashi, & Capetillo-

Zarate, 2010; Iulita et al., 2014; Leon et al., 2010; Takahashi et al., 2002). In considering how this accumulation of β-amyloid may relate to synaptic changes, diminution of GABAergic interneurons, and spatial memory performance of 3xTg-AD + EtOH mice, it is essential to consider when transgenic amyloid, presenilin, and tau proteins may first be present during development. The Thy1 promoter is first expressed embryonically in dividing neural precursor cells in the ventricular zone (Campsall et al., 2002; Caroni, 1997). However, reports vary in the developmental onset of Thy1-driven transgene expression (Campsall et al., 2002; Caroni, 1997). To begin to understand the complex relationship between network level dysfunction and β-amyloid proteinopathy in the 3xTg-AD model after prenatal ethanol exposure, it is prerequisite to determine when transgene expression, and related β-amyloid/tau pathology, are first detectable during development and how this may relate to the synaptic dysfunction as well as changes in cognition and behavioral flexibility later in life.

3.6.5 Implications in FASD

This study provided the first preclinical evidence of an association between prenatal alcohol exposure and AD risk. However, clinical and epidemiological studies have yet to explore how this may relate to the experiences of aging individuals with FASD. The clinical association between AD and other neurodevelopmental disorders, including autism spectrum disorder (ASD) and Down syndrome (DS), is already under investigation (Ballard, Mobley, Hardy, Williams, & Corbett, 2016; Head et al., 2003; Vivanti, Tao, Lyall, Robins, & Shea, 2021). It has been nearly 50 years since the first diagnosis of fetal alcohol syndrome (FAS), and appreciation of the diverse presentation of disorders with the FASD spectrum is still evolving and growing (Benz, Rasmussen, & Andrew, 2009; Jones & Smith, 1973; Wozniak et al., 2019). As increasing numbers of individuals with FASD begin to reach ages for assessing the onset of dementia and AD

progression, further studies will enlighten and identify clinical needs to provide support throughout the aging process.

3.6.6 Conclusion

The experimental model and investigative approaches employed in this study provide a propitious opportunity to begin to understand how prenatal ethanol exposure may accelerate the onset of cognitive deficits in AD. Our findings reinforce the need to consider the long-term health outcomes of developmental exposures in general, and specifically the implications of in utero ethanol exposure on long-term cognitive function and AD risk.

3.7 References

Albuquerque, M. S., Mahar, I., Davoli, M. A., Chabot, J.-G., Mechawar, N., Quirion, R., et al. (2015). Regional and sub-regional differences in hippocampal GABAergic neuronal vulnerability in the TgCRND8 mouse model of Alzheimer's disease. Frontiers in Aging Neuroscience, 7, 30.

Ali, F., Baringer, S. L., Neal, A., Choi, E. Y., & Kwan, A. C. (2019). Parvalbumin-Positive Neuron Loss and Amyloid-β Deposits in the Frontal Cortex of Alzheimer's Disease-Related Mice. Journal of Alzheimer's Disease, 72(4), 1323–1339.

Anastasiades, P. G., & Carter, A. G. (2021). Circuit organization of the rodent medial prefrontal cortex. Trends in Neurosciences, 44(7), 550–563.

Andrews-Zwilling, Y., Bien-Ly, N., Xu, Q., Li, G., Bernardo, A., Yoon, S.Y., et al. (2010). Apolipoprotein E4 Causes Age- and Tau-Dependent Impairment of GABAergic Interneurons, Leading to Learning and Memory Deficits in Mice. The Journal of Neuroscience, 30(41), 13707–13717.

Arai, H., Emson, P. C., Mountjoy, C. Q., Carassco, L. H., & Heizmann, C. W. (1987). Loss of parvalbumin-immunoreactive neurones from cortex in Alzheimer-type dementia. Brain Research, 418(1), 164–169.

Arendt, T. (2009). Synaptic degeneration in Alzheimer's disease. Acta Neuropathologica, 118(1), 167–179.

Bachmanov, A. A., Tordoff, M. G., & Beauchamp, G. K. (1996). Ethanol Consumption and Taste Preferences in C57BL/6ByJ and 129/J Mice. Alcoholism: Clinical and Experimental Research, 20(2), 201–206.

Ballard, C., Mobley, W., Hardy, J., Williams, G., & Corbett, A. (2016). Dementia in Down's syndrome. The Lancet Neurology, 15(6), 622–636.

Barnett, A., David, E., Rohlman, A., Nikolova, V. D., Moy, S. S., Vetreno, R. P., et al. (2022). Adolescent Binge Alcohol Enhances Early Alzheimer's Disease Pathology in Adulthood Through Proinflammatory Neuroimmune Activation. Frontiers in Pharmacology, 13, 884170.

Barthet, G., & Mulle, C. (2020). Presynaptic failure in Alzheimer's disease. Progress in Neurobiology, 194, 101801.

Batista-Brito, R., & Fishell, G. (2009). The developmental integration of cortical interneurons into a functional network. Current Topics in Developmental Biology, 87, 81–118.

Benz, J., Rasmussen, C., & Andrew, G. (2009). Diagnosing fetal alcohol spectrum disorder: History, challenges and future directions. Paediatrics & Child Health 14(4), 231–237.

Billings, L. M., Oddo, S., Green, K .N., McGaugh, J. L., & LaFerla, F. M. (2005). Intraneuronal Abeta Causes the Onset of Early Alzheimer's Disease-Related Cognitive Deficits in Transgenic Mice. Neuron, 45(5), 675–688.

Bird, C. W., Taylor, D. H., Pinkowski, N. J., Chavez, G. J., & Valenzuela, C. F. (2018). Long-term Reductions in the Population of GABAergic Interneurons in the Mouse Hippocampus following Developmental Ethanol Exposure. Neuroscience, 383, 60–73.

Brady, D. R., & Mufson, E. J. (1997). Parvalbumin-immunoreactive neurons in the hippocampal formation of Alzheimer's diseased brain. Neuroscience, 80(4), 1113–1125.

Campsall, K. D., Mazerolle, C. J., De Repentingy, Y., Kothary, R., & Wallace, V. A. (2002). Characterization of transgene expression and Cre recombinase activity in a panel of Thy-1 promoter-Cre transgenic mice. Developmental Dynamics, 224(2), 135–143.

Caroni, P. (1997). Overexpression of growth-associated proteins in the neurons of adult transgenic mice. Journal of Neuroscience Methods, 71(1), 3–9.

Catlin, M. C., Guizzetti, M., & Costa, L. G. (1999). Effects of ethanol on calcium homeostasis in the nervous system. Molecular Neurobiology, 19(1), 1–24.

Chen, M., Chen, Y., Huo, Q., Wang, L., Tan, S., Misrani, A., et al. (2021). Enhancing GABAergic signaling ameliorates aberrant gamma oscillations of olfactory bulb in AD mouse models. Molecular Neurodegeneration, 16(1), 1–23.

Chen, Y., Ozturk, N .C., Ni, L., Goodlett, C., & Zhou, F. C. (2011). Strain Differences in Developmental Vulnerability to Alcohol Exposure Via Embryo Culture in Mice. Alcoholism: Clinical and Experimental Research, 35(7), 1293–1304.

Cheng, A., Wang, J., Ghena, N., Zhao, Q., Perone, I., King, M. T., et al. (2019). SIRT3 Haploinsufficiency Aggravates Loss of GABAergic Interneurons and Neuronal Network Hyperexcitability in an Alzheimer's Disease Model. The Journal of Neuroscience, 40(3), 694–709.

Chui, D.-H., Tanahashi, H., Ozawa, K., Ikeda, S., Checler, F., Ueda, O., et al. (1999). Transgenic mice with Alzheimer presenilin 1 mutations show accelerated neurodegeneration without amyloid plaque formation. Nature Medicine, 5(5), 560–564.

Clancy, B., Darlington, R. B., & Finlay, B. L. (2001). Translating developmental time across mammalian species. Neuroscience, 105(1), 7–17.

Clinton, L. K., Billings, L. M., Green, K. N., Caccamo, A., Ngo, J., Oddo, S., et al. (2007). Age-dependent sexual dimorphism in cognition and stress response in the 3xTg-AD mice. Neurobiology of Disease, 28(1), 76–82.

Cuzon, V. C., Yeh, P. W. L., Yanagawa, Y., Obata, K., & Yeh, H. H. (2008). Ethanol Consumption during Early Pregnancy Alters the Disposition of Tangentially Migrating GABAergic Interneurons in the Fetal Cortex. The Journal of Neuroscience, 28(8), 1854–1864.

Davis, K. M., Gagnier, K. R., Moore, T. E., & Todorow, M. (2013). Cognitive aspects of fetal alcohol spectrum disorder. Wiley Interdisciplinary Reviews. Cognitive Science, 4(1), 81–92.

DeKosky, S. T., & Scheff, S.W. (1990). Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Annals of Neurology, 27(5), 457–464.

Delatour, L. C., & Yeh, H. H. (2017). FASD and Brain Development: Perspectives on Where We are and Where We Need to Go. OBM Neurobiology, 1.

Delatour, L. C., Yeh, P. W., & Yeh, H. H. (2019a). Ethanol Exposure In Utero Disrupts Radial Migration and Pyramidal Cell Development in the Somatosensory Cortex. Cerebral Cortex, 29(5), 2125–2139.

Delatour, L. C., Yeh, P. W. L., & Yeh, H. H. (2019b). Prenatal Exposure to Ethanol Alters Synaptic Activity in Layer V/VI Pyramidal Neurons of the Somatosensory Cortex. Cerebral Cortex, 30(3), 1735–1751.

Denny, C. H. (2019). Consumption of Alcohol Beverages and Binge Drinking Among Pregnant Women Aged 18–44 Years – United States, 2015–2017. MMWR. Morbidity and Mortality Weekly Report, 68(16), 365–368.

Downing, C., Balderrama-Durbin, C., Broncucia, H., Gilliam, D., & Johnson, T. E. (2009). Ethanol Teratogenesis in Five Inbred Strains of Mice. Alcoholism: Clinical and Experimental Research, 33(7), 1238–1245.

Edwards, G. A., III., Gamez, N., Escobedo, G., Jr., Calderon, O., & Moreno-Gonzalez, I. (2019). Modifiable Risk Factors for Alzheimer's Disease. Frontiers in Aging Neuroscience, 11.

España, J., Giménez-Llort, L., Valero, J., Miñano, A., Rábano, A., Rodriguez-Alvarez, J.,

LaFerla, F. M., & Saura, C. A. (2010). Intraneuronal β-Amyloid Accumulation in the Amygdala Enhances Fear and Anxiety in Alzheimer's Disease Transgenic Mice. Biological Psychiatry, 67(6), 513–521.

Fowler, S. W., Walker, J. M., Klakotskaia, D., Will, M. J., Serfozo, P., Simonyi, A., et al. (2013). Effects of a metabotropic glutamate receptor 5 positive allosteric modulator, CDPPB, on spatial learning task performance in rodents. Neurobiology of Learning and Memory, 99, 25–31.

Gouras, G. K., Tampellini, D., Takahashi, R. H., & Capetillo-Zarate, E. (2010). Intraneuronal beta-amyloid accumulation and synapse pathology in Alzheimer's disease. Acta Neuropathologica, 119(5), 523–541.

Hansen, K. B., Yuan, H., & Traynelis, S .F. (2007). Structural aspects of AMPA receptor activation, desensitization and deactivation. Current Opinion in Neurobiology, 17(3), 281–288.

Hardy, J., & Selkoe, D. J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science, 297(5580), 353–356.

Head, E., Lott, I. T., Hof, P. R., Bouras, C., Su, J. H., Kim, R., Haier, R., & Cotman, C. W. (2003). Parallel compensatory and pathological events associated with tau pathology

in middle aged individuals with Down syndrome. Journal of Neuropathology and Experimental Neurology, 62(9), 917–926.

Heymann, D., Stern, Y., Cosentino, S., Tatarina-Nulman, O., Dorrejo, J. N., & Gu, Y. (2016). The association between alcohol use and the progression of Alzheimer's disease. Current Alzheimer Research, 13(12), 1356–1362.

Hijazi, S., Heistek, T. S., Scheltens, P., Neumann, U., Shimshek, D. R., Mansvelder, H. D., et al. (2019). Early restoration of parvalbumin interneuron activity prevents memory loss and network hyperexcitability in a mouse model of Alzheimer's disease. Molecular Psychiatry, 25(12), 3380–3398.

Hoffman, J. L., Faccidomo, S., Kim, M., Taylor, S. M., Agoglia, A. E., May, A. M., et al. (2019). Alcohol drinking exacerbates neural and behavioral pathology in the 3xTg-AD mouse model of Alzheimer's disease. International Review of Neurobiology, 148, 169–230.

Huang, W.-J., Zhang, X., & Chen, & W.-W. (2016). Association between alcohol and Alzheimer's disease. Experimental and Therapeutic Medicine 12(3), 1247–1250.

Iacobucci, G. J., & Popescu, G. K. (2018). Kinetic models for activation and modulation of NMDA receptor subtypes. Current Opinion in Physiology, 2, 114–122.

Iulita, M.F., Allard, S., Richter, L., Munter, L.-M., Ducatenzeiler, A., Weise, C., et al. (2014). Intracellular Aβ pathology and early cognitive impairments in a transgenic rat overexpressing human amyloid precursor protein: a multidimensional study. Acta Neuropathologica Communications, 2, 61.

Jacobson, J. L., Akkaya-Hocagil, T., Ryan, L. M., Dodge, N. C., Richardson, G. A., Olson, H. C., et al. (2021). Effects of prenatal alcohol exposure on cognitive and behavioral development: Findings from a hierarchical meta-analysis of data from six prospective longitudinal U.S. cohorts. Alcoholism: Clinical and Experimental Research, 45(10), 2040–2058.

Jafari, Z., Okuma, M., Karem, H., Mehla, J., Kolb, B. E., & Mohajerani, M. H. (2019). Prenatal noise stress aggravates cognitive decline and the onset and progression of beta amyloid pathology in a mouse model of Alzheimer's disease. Neurobiology of Aging, 77, 66–86.

Jones, K. L., & Smith, D. W. (1973). Recognition of the fetal alcohol syndrome in early infancy. Lancet, 302, 999–1001.

Kiss, E., Gorgas, K., Schlicksupp, A., Groß, D., Kins, S., Kirsch, J., et al. (2016). Biphasic Alteration of the Inhibitory Synapse Scaffold Protein Gephyrin in Early and Late Stages of an Alzheimer Disease Model. The American Journal of Pathology, 186(9), 2279–2291.

Knopman, D. S., Amieva, H., Petersen, R. C., Chételat, G., Holtzman, D. M., Hyman, B. T., et al. (2021). Alzheimer disease. Nature Reviews. Disease Primers, 7(1), 33.

Koch, M., Costanzo, S., Fitzpatrick, A. L., Lopez, O. L., DeKosky, S., Kuller, L. H., et al. (2020). Alcohol Consumption, Brain Amyloid-β Deposition, and Brain Structural Integrity Among Older Adults Free of Dementia. Journal of Alzheimer's Disease, 74(2), 509–519.

Koike, M., Tsukada, S., Tsuzuki, K., Kijima, H., & Ozawa, S. (2000). Regulation of Kinetic Properties of GluR2 AMPA Receptor Channels by Alternative Splicing. The Journal of Neuroscience, 20(6), 2166–2174.

Koopmans, G., Blokland, A., van Nieuwenhuijzen, P., & Prickaerts, J. (2003). Assessment of spatial learning abilities of mice in a new circular maze. Physiology & Behavior, 79(4–5), 683–693.

Kurucu, H., Colom-Cadena, M., Davies, C., Wilkins, L., King, D., Rose, J., et al. (2021). Inhibitory synapse loss and accumulation of amyloid beta in inhibitory presynaptic terminals in Alzheimer's disease. European Journal of Neurology, 29(5), 1311–1323.

Lee, S. M., Yeh, P. W. L., & Yeh, H. H. (2022). L-Type Calcium Channels Contribute to Ethanol-Induced Aberrant Tangential Migration of Primordial Cortical GABAergic Interneurons in the Embryonic Medial Prefrontal Cortex. eNeuro, 9(1), ENEURO.0359-21.2021.

Leon, W. C., Canneva, F., Partridge, V., Allard, S., Ferretti, M. T., DeWilde, A., et al. (2010). A Novel Transgenic Rat Model with a Full Alzheimer's-Like Amyloid Pathology Displays Pre-Plaque Intracellular Amyloid-β-Associated Cognitive Impairment. Journal of Alzheimer's Disease, 20(1), 113–126.

Leslie, S. W., Brown, L. M., Dildy, J. E., & Sims, J. S. (1990). Ethanol and neuronal calcium channels. Alcohol, 7(3), 233–236.

Leung, L., Andrews-Zwilling, Y., Yoon, S. Y., Jain, S., Ring, K., Dai, J., et al. (2012). Apolipoprotein E4 causes age- and sex-dependent impairments of hilar GABAergic interneurons and learning and memory deficits in mice. PLoS One, 7(12), e53569.

Li, Y., Sun, H., Chen, Z., Xu, H., Bu, G., & Zheng, H. (2016). Implications of GABAergic Neurotransmission in Alzheimer's Disease. Frontiers in Aging Neuroscience, 8(31).

Li, Y., Zhu, K., Li, N., Wang, X., Xiao, X., Li, L., et al. (2021). Reversible GABAergic dysfunction involved in hippocampal hyperactivity predicts early-stage Alzheimer disease in a mouse model. Alzheimer's Research & Therapy, 13(1), 114.

Lim, J. P., Zou, M. E., Janak, P. H., & Messing, R. O. (2012). Responses to ethanol in C57BL/6 versus C57BL/6 × 129 hybrid mice. Brain and Behavior, 2(1), 22–31.

Lovinger, D. M. (2018). Presynaptic Ethanol Actions: Potential Roles in Ethanol Seeking. Handbook of Experimental Pharmacology, 248, 29–54.

Lunde, E. R., Washburn, S. E., Golding, M. C., Bake, S., Miranda, R. C., & Ramadoss, J. (2016). Alcohol-Induced Developmental Origins of Adult-Onset Diseases. Alcoholism: Clinical and Experimental Research, 40(7), 1403–1414.

Madden, J. T., Thompson, S. M., Magcalas, C. M., Wagner, J. L., Hamilton, D. A., Savage, D. D., et al. (2020). Moderate prenatal alcohol exposure reduces parvalbumin

expressing GABAergic interneurons in the dorsal hippocampus of adult male and female rat offspring. Neuroscience Letters, 718, 134700.

Marguet, F., Friocourt, G., Brosolo, M., Sauvestre, F., Marcorelles, P., Lesueur, C., et al. (2020). Prenatal alcohol exposure is a leading cause of interneuronopathy in humans. Acta Neuropathologica Communications, 8(1), 208.

Martinez-Losa, M., Tracy, T. E., Ma, K., Verret, L., Clemente-Perez, A., Khan, A. S., et al. (2018). Nav1.1-Overexpressing Interneuron Transplants Restore Brain Rhythms and Cognition in a Mouse Model of Alzheimer's Disease. Neuron, 98(1), 75-89.e5.

Mastrangelo, M. A., & Bowers, W. J. (2008). Detailed immunohistochemical characterization of temporal and spatial progression of Alzheimer's disease-related pathologies in male triple-transgenic mice. BMC Neuroscience, 9, 81.

Mattson, S. N., Crocker, N., & Nguyen, T. T. (2011). Fetal Alcohol Spectrum Disorders: Neuropsychological and Behavioral Features. Neuropsychology Review, 21(2), 81–101.

May, P. A., & Gossage, J. P. (2011). Maternal risk factors for fetal alcohol spectrum disorders: not as simple as it might seem. Alcohol Research & Health, 34(1), 15–26.

Milstein, A. D., & Nicoll, R. A. (2008). Regulation of AMPA receptor gating and pharmacology by TARP auxiliary subunits. Trends in Pharmacological Sciences, 29(7), 333–339.

Mitew, S., Kirkcaldie, M. T. K., Dickson, T. C., & Vickers, J. C. (2013). Altered synapses and gliotransmission in Alzheimer's disease and AD model mice. Neurobiology of Aging, 34(10), 2341–2351.

Moore, E. M., & Riley, E. P. (2015). What Happens When Children with Fetal Alcohol Spectrum Disorders Become Adults? Current Developmental Disorders Reports, 2(3), 219–227.

Möykkynen, T., & Korpi, E. R. (2012). Acute Effects of Ethanol on Glutamate Receptors. Basic & Clinical Pharmacology & Toxicology, 111(1), 4–13.

Muñoz, G., Urrutia, J. C., Burgos, C. F., Silva, V., Aguilar, F., Sama, M., et al. (2015). Low concentrations of ethanol protect against synaptotoxicity induced by Aβ in hippocampal neurons. Neurobiology of Aging, 36(2), 845–856.

Murray, A. J., Sauer, J.-F., Riedel, G., McClure, C., Ansel, L., Cheyne, L., et al. (2011). Parvalbumin-positive CA1 interneurons are required for spatial working but not for reference memory. Nature Neuroscience, 14(3), 297–299.

Nahar, L., Delacroix, B. M., & Nam, H. W. (2021). The Role of Parvalbumin Interneurons in Neurotransmitter Balance and Neurological Disease. Frontiers in Psychiatry, 12, 679960.

Naseri, N. N., Wang, H., Guo, J., Sharma, M., & Luo, W. (2019). The complexity of tau in Alzheimer's disease. Neuroscience Letters, 705, 183–194.

Oddo, S., Caccamo, A., Shepherd, J. D., Murphy, M. P., Golde, T. E., Kayed, R., et al. (2003). Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron, 39(3), 409–421.

Ogawa, T., Kuwagata, M., Ruiz, J., & Zhou, F. C. (2005). Differential teratogenic effect of alcohol on embryonic development between C57BL/6 and DBA/2 mice: a new view. Alcoholism: Clinical and Experimental Research, 29(5), 855–863.

Oh, K.-J., Perez, S. E., Lagalwar, S., Vana, L., Binder, L., & Mufson, E. J. (2010). Staging of Alzheimer's pathology in triple transgenic mice: a light and electron microscopic analysis. International Journal of Alzheimer's Disease, 2010, 780102.

Palop, J. J., & Mucke, L. (2016). Network abnormalities and interneuron dysfunction in Alzheimer disease. Nature Reviews. Neuroscience, 17(12), 777–792.

Patterson, C. (2018). World Alzheimer Report 2018. The state of the art of dementia research: New frontiers. Alzheimer's Disease International. Retrieved from https://apo.org.au/sites/default/files/resource-files/2018-09/apo-nid260056.pdf

Peng, B., Yang, Q., Joshi, R. B., Liu, Y., Akbar, M., Song, B.-J., et al. (2020). Role of Alcohol Drinking in Alzheimer's Disease, Parkinson's Disease, and Amyotrophic Lateral Sclerosis. International Journal of Molecular Sciences, 21(7), 2316.

Pietropaolo, S., Feldon, J., & Yee, B. K. (2008). Age-dependent phenotypic characteristics of a triple transgenic mouse model of Alzheimer disease. Behavioral Neuroscience, 122(4), 733–747.

Pimenova, A. A., Raj, T., & Goate, A. M. (2018). Untangling Genetic Risk for Alzheimer's Disease. Biological Psychiatry, 83(4), 300–310.

Prince, S. M., Paulson, A. L., Jeong, N., Zhang, L., Amigues, S., & Singer, A. C. (2021). Alzheimer's pathology causes impaired inhibitory connections and reactivation of spatial codes during spatial navigation. Cell Reports, 35(3), 109008.

Ramirez, D. M., & Kavalali, E. T. (2011). Differential regulation of spontaneous and evoked neurotransmitter release at central synapses. Current Opinion in Neurobiology, 21(2), 275–282.

Rasmussen, C. (2005). Executive functioning and working memory in fetal alcohol spectrum disorder. Alcoholism: Clinical and Experimental Research, 29(8), 1359–1367.

Rasmussen, J., & Langerman, H. (2019). Alzheimer's Disease – Why We Need Early Diagnosis. Degenerative Neurological and Neuromuscular Disease, 9, 123–130.

Roberto, M., Treistman, S. N., Pietrzykowski, A. Z., Weiner, J., Galindo, R., Mameli, M., et al. (2006). Actions of Acute and Chronic Ethanol on Presynaptic Terminals. Alcoholism: Clinical and Experimental Research, 30(2), 222–232.

Roberto, M., & Varodayan, F. (2017). Synaptic Targets: Chronic Alcohol Actions. Neuropharmacology, 122, 85–99.

Sanchez-Mejias, E., Nuñez-Diaz, C., Sanchez-Varo, R., Gomez-Arboledas, A., Garcia-Leon, J. A., Fernandez-Valenzuela, J. J., et al. (2020). Distinct disease-sensitive

GABAergic neurons in the perirhinal cortex of Alzheimer's mice and patients. Brain Pathology, 30(2), 345–363.

Scheff, S. W., Price, D. A., Schmitt, F. A., & Mufson, E. J. (2006). Hippocampal synaptic loss in early Alzheimer's disease and mild cognitive impairment. Neurobiology of Aging, 27(10), 1372–1384.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods, 9(7), 676–682.

Seifan, A., Schelke, M., Obeng-Aduasare, Y., & Isaacson, R. (2015). Early Life Epidemiology of Alzheimer's Disease--A Critical Review. Neuroepidemiology, 45(4), 237–254.

Selkoe, D. J. (2002). Alzheimer's Disease Is a Synaptic Failure. Science, 298(5594), 789–791.

Siggins, G. R., Roberto, M., & Nie, Z. (2005). The tipsy terminal: presynaptic effects of ethanol. Pharmacology & Therapeutics, 107(1), 80–98.

Skorput, A. G., Lee, S. M., Yeh, P. W., & Yeh, H. H. (2019). The NKCC1 antagonist bumetanide mitigates interneuronopathy associated with ethanol exposure in utero. eLife, 8, e48648.

Skorput, A. G. J., Gupta, V. P., Yeh, P. W. L., & Yeh, H. H. (2015). Persistent Interneuronopathy in the Prefrontal Cortex of Young Adult Offspring Exposed to Ethanol In Utero. The Journal of Neuroscience, 35(31), 10977–10988.

Stevens, L. M., & Brown, R. E. (2015). Reference and working memory deficits in the 3xTg-AD mouse between 2 and 15-months of age: A cross-sectional study. Behavioural Brain Research, 278, 496–505.

Stincic, T. L., & Frerking, M. E. (2015). Different AMPA receptor subtypes mediate the distinct kinetic components of a biphasic EPSC in hippocampal interneurons. Frontiers in Synaptic Neuroscience, 7, 7.

Stover, K. R., Campbell, M. A., Van Winssen, C. M., & Brown, R. E. (2015). Early detection of cognitive deficits in the 3xTg-AD mouse model of Alzheimer's disease. Behavioural Brain Research, 289, 29–38.

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126(4), 663–676.

Takahashi, R. H., Milner, T. A., Li, F., Nam, E. E., Edgar, M. A., Yamaguchi, H., et al. (2002). Intraneuronal Alzheimer abeta42 accumulates in Multivesicular Bodies and Is Associated with Synaptic Pathology. The American Journal of Pathology, 161(5), 1869–1879.

Tampellini, D. (2015). Synaptic activity and Alzheimer's disease: A critical update. Frontiers in Neuroscience, 9, 423.

Tong, B.C.-K., Wu, A. J., Li, M., & Cheung, K.-H. (2018). Calcium signaling in Alzheimer's disease & therapies. Biochimica et Biophysica Acta. Molecular Cell Research, 1865(11 Pt B), 1745–1760.

Vivanti, G., Tao, S., Lyall, K., Robins, D. L., & Shea, L. L. (2021). The prevalence and incidence of early-onset dementia among adults with autism spectrum disorder. Autism Research. 10, 2189-2199.

Verret, L., Mann, E. O., Hang, G. B., Barth, A. M. I., Cobos, I., Ho, K., et al. (2012). Inhibitory Interneuron Deficit Links Altered Network Activity and Cognitive Dysfunction in Alzheimer Model. Cell, 149(3), 708–721.

Vetreno, R. P., & Crews, F. T. (2012). Adolescent binge drinking increases expression of the danger signal receptor agonist HMGB1 and Toll-like receptors in the adult prefrontal cortex. Neuroscience, 226, 475–488.

Welikovitch, L. A., Do Carmo, S., Maglóczky, Z., Szocsics, P., Lőke, J., Freund, T., et al. (2018). Evidence of intraneuronal Aβ accumulation preceding tau pathology in the entorhinal cortex. Acta Neuropathologica, 136(6), 901–917.

Wozniak, J. R., Riley, E. P., & Charness, M. E. (2019). Clinical presentation, diagnosis, and management of fetal alcohol spectrum disorder. The Lancet. Neurology, 18(8), 760–770.

Xu, Y., Zhao, M., Han, Y., & Zhang, H. (2020). GABAergic Inhibitory Interneuron Deficits in Alzheimer's Disease: Implications for Treatment. Frontiers in Neuroscience, 14, 660.

Yu, J.-T., Xu, W., Tan, C.-C., Andrieu, S., Suckling, J., Evangelou, E., et al. (2020). Evidence-based prevention of Alzheimer's disease: systematic review and meta-analysis of 243 observational prospective studies and 153 randomised controlled trials. Journal of Neurology, Neurosurgery, and Psychiatry, 91(11), 1201–1209.

Table 3.1. Age, genotype/exposure, and sex of behaviorally tested animals

Age	B6129		3xTg-AD		3xTg-AD + EtOH	
	F	M	F	M	F	M
4 months	10 (3)	9 (3)	10 (3)	10 (3)	7 (3)	6 (3)
6 months	9 (3)	11 (3)	10 (3)	11 (4)	5 (3)	10 (3)

*n (N) where n = number of mice tested; N = number of litters included.

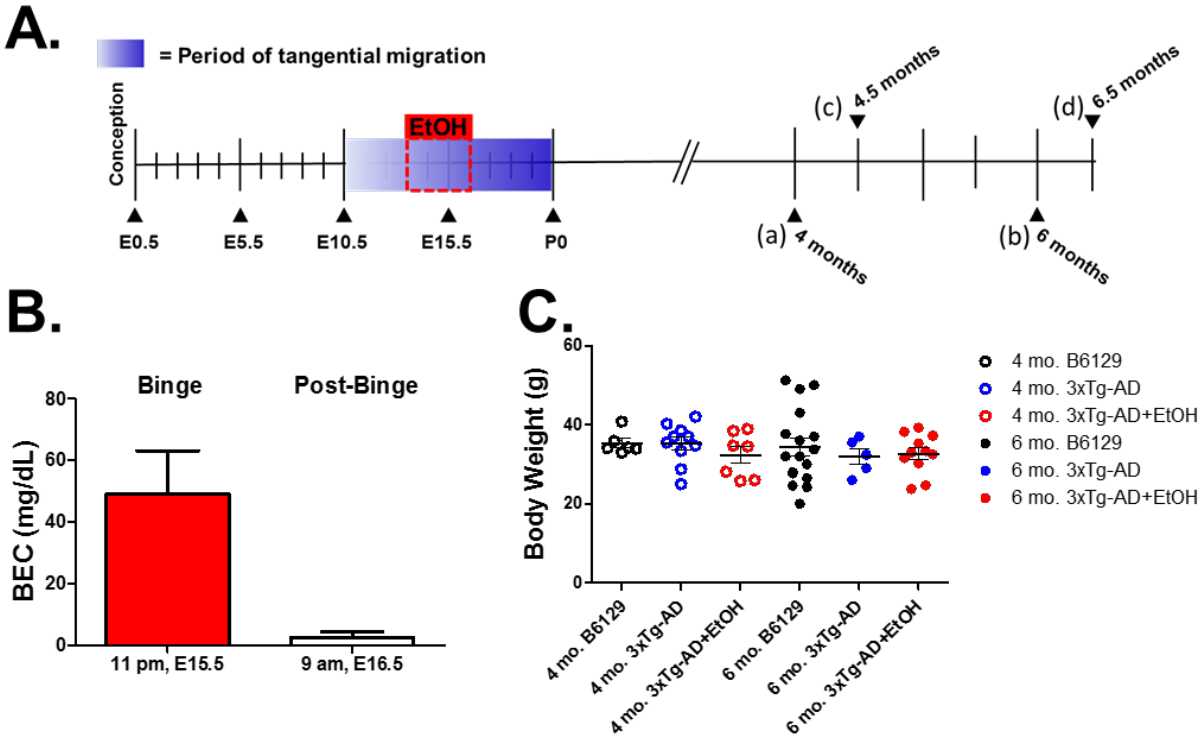


Figure 3.1. Experimental timeline, blood ethanol concentration (BEC) in 3xTg-AD pregnant dams, and group-dependent differences in body weight.

(A) Pregnant 3xTg-AD dams were exposed to ethanol during a period of peak tangential migration of GABAergic interneurons from the medial ganglionic eminence into the developing cortex: embryonic day (E) 13.5–E16.5. Mice were then aged to (a) 4 months or (b) 6 months, at which point they completed the 10-day modified Barnes maze testing paradigm. Subsequently, mice were sacrificed in the 2 weeks following behavioral testing for immunohistochemistry and/or electrophysiology experiments at (c) 4.5 months or (d) 6.5 months. (B) Pregnant 3xTg-AD dams drank liquid food containing ethanol (5% w/w) and yielded a mean BEC of 49.17 mg/dL at 11:00 PM on E15.5 (N = 3 dams) when allowed on E13.5–E16.5, while BECs had dropped to a mean of 2.66 mg/dL at 9:00 AM on E16.5 (N = 2 dams) (C) Body weight did not differ significantly in 4-month-old mice: B6129 (empty black dots, N = 6, n = 6 M, 3 litters); 3xTg-AD (empty blue dots, N = 11, n = 4 F, 7 M, 3 litters); 3xTg-AD + EtOH (empty red dots, N = 7, n = 4 F, 3 M, 2 litters); 6-month-old: B6129 (filled black dots, N = 17, n = 9 F, 8 M, 5 litters); 3xTg-AD (filled blue dots, N = 6, n = 2 F, 4 M, 4 litters); 3xTg-AD + EtOH (filled red dots N = 12, n = 3 F, 9 M, 5 litters). N = total number of mice per group, n = total number of mice by sex: F (female), M (male).

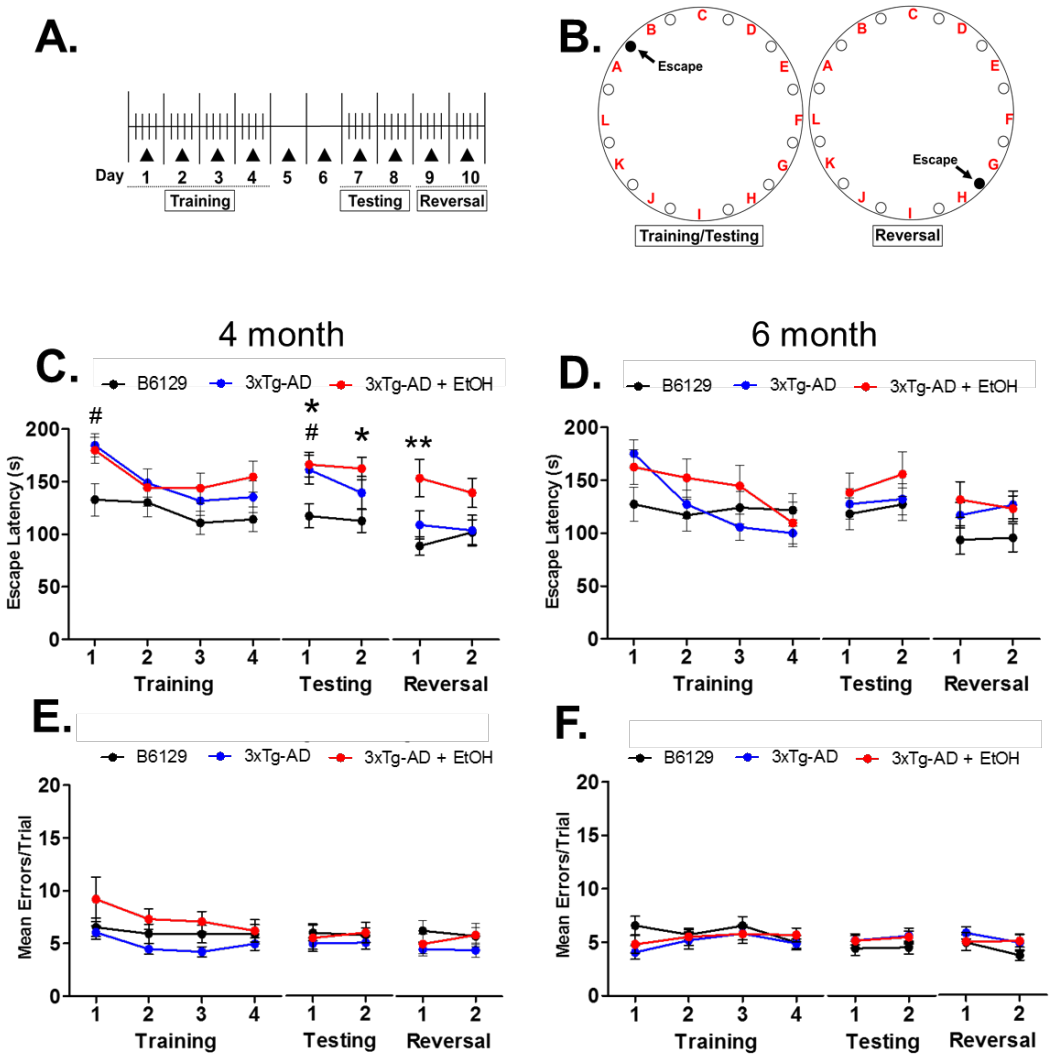
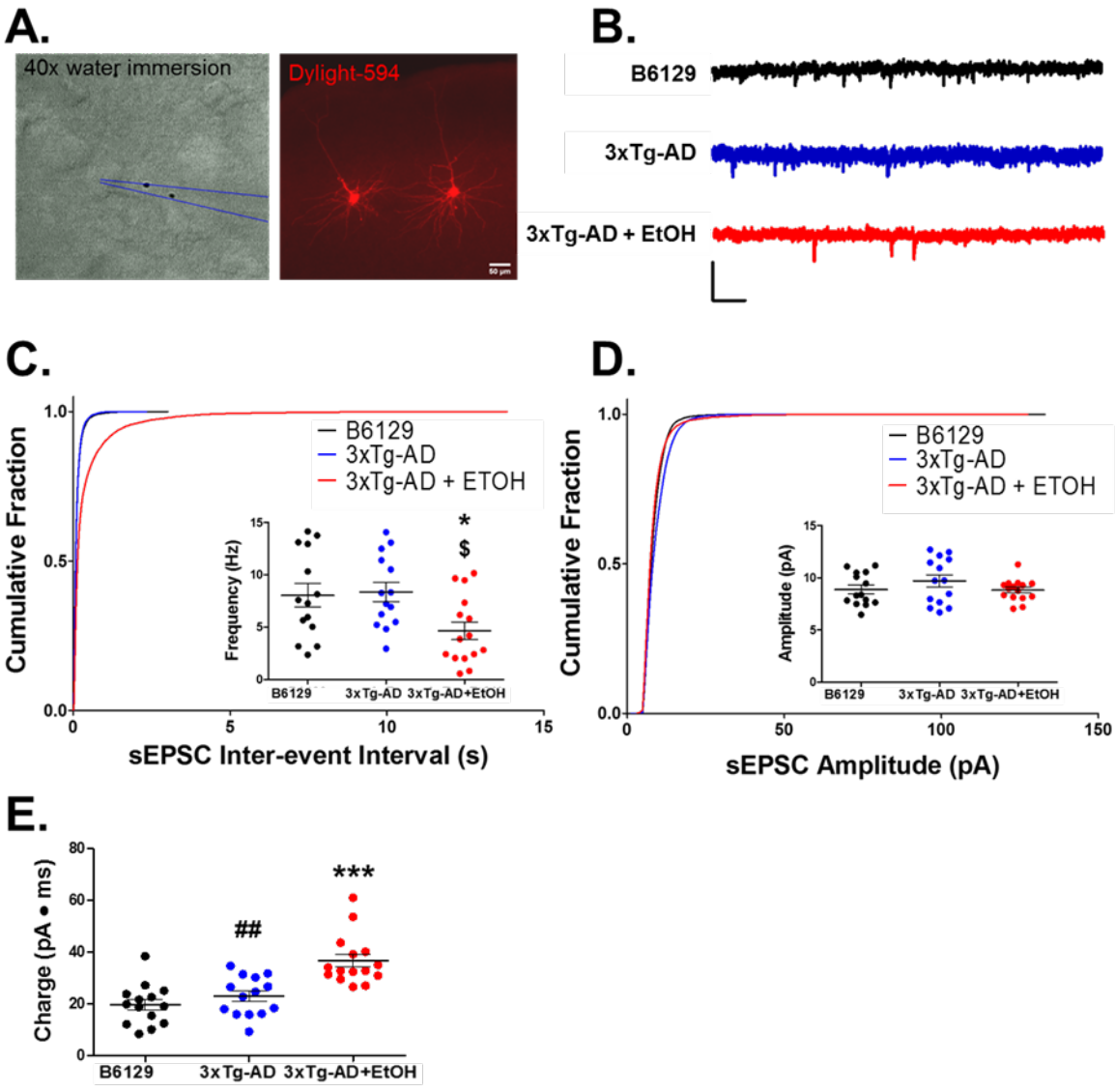


Figure 3.2. Prenatal ethanol exposure results in an earlier onset of spatial memory deficits in 3xTg-AD mice, as well as deficits in reversal learning.

(A, B) 4-month-old and 6-month-old mice completed the 10-day modified Barnes maze testing paradigm, consisting of a 4-day training phase, 2-day testing phase, and 2-day reversal phase (C, D) Mean latency to correctly identify the escape hole (sec, n = 4 trials) for B6129 (black dots connected by black lines), 3xTg-AD (blue dots connected by blue lines), and ethanol-exposed 3xTg-AD mice (red dots connected by red lines) in 4-month-old (C) and 6-month-old mice (D). (E, F) Mean errors per trial (nose pokes not in the escape hole) for B6129 (black dots connected by black lines), 3xTg-AD (blue dots connected by blue lines), and ethanol-exposed 3xTg-AD mice (red dots connected by red lines) in 4-month-old (E) and 6-month-old mice (F). (C) $*$ = $p < 0.05$ B6129 vs. 3xTg-AD + EtOH, $**$ = $p < 0.01$ B6129 vs. 3xTg-AD + EtOH, # = $p < 0.05$ B6129 vs. 3xTg-AD, two-way repeated-measures ANOVA with Bonferroni post hoc tests. For sample sizes by experimental group see Table 1.

Figure 3.3. Prenatal ethanol exposure decreases the frequency and increases the charge of spontaneous excitatory post-synaptic currents (sEPSCs) in the medial prefrontal cortex (mPFC) of 4-month-old 3xTg-AD mice.

(A, left panel) 40× magnification Hoffman Modulated Contrast image of a Layer V/VI pyramidal neuron in the mPFC from a 4-month-old mouse during recording. (A, right panel) Image of a neurobiotin-filled pyramidal neuron after recording; scale bar = 50 μM. (B) Representative traces recorded under whole-cell voltage clamp of sEPSCs recorded at a holding potential −70 mV from Layer V/VI pyramidal neurons in acute mPFC slices from 4-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red) mice; scale bar = 250 ms × 20 pA. (C) Cumulative distribution of sEPSC inter-event intervals (s) recorded from Layer V/VI pyramidal neurons of 4-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red). (inset): Mean sEPSC frequency of Layer V/VI pyramidal neurons of mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (D) Cumulative distribution of sEPSC amplitude intervals (s) recorded in Layer V/VI pyramidal neurons of 4-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red). (inset): Mean sEPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (E) Mean sEPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (C) $*$ = $p < 0.05$, B6129 vs. 3xTg-AD + EtOH, $ = $p < 0.05$ 3xTg-AD vs. 3xTg-AD + EtOH, one-way ANOVA with Bonferroni post hoc tests (E) $***$ = $p < 0.001$, B6129 vs. 3xTg-AD + EtOH, # = $p < 0.05$, one-way ANOVA with Bonferroni post hoc tests. For all measures: B6129 (N = 14 cells, n = 4 F, 5 M, 6 litters); 3xTg-AD (N = 15 cells, n = 5 F, 5 M, 6 litters); 3xTg-AD + ETOH (N = 15 cells, n = 3 F, 7 M, 7 litters). N = total number of cells per group (dots) from n = total number mice by sex: F (female), M (male).


A.
40x water immersion
Dylight-594
B.
B6129
3xTg-AD
3xTg-AD + EtOH
C.
Cumulative Fraction
B6129
3xTg-AD
3xTg-AD + ETOH
Frequency (Hz)
B6129
3xTg-AD
3xTg-AD+EtOH
sEPSC Inter-event Interval (s)
D.
Cumulative Fraction
B6129
3xTg-AD
3xTg-AD + ETOH
Amplitude (pA)
B6129
3xTg-AD
3xTg-AD+EtOH
sEPSC Amplitude (pA)
E.
Charge (pA • ms)
B6129
3xTg-AD
3xTg-AD+EtOH

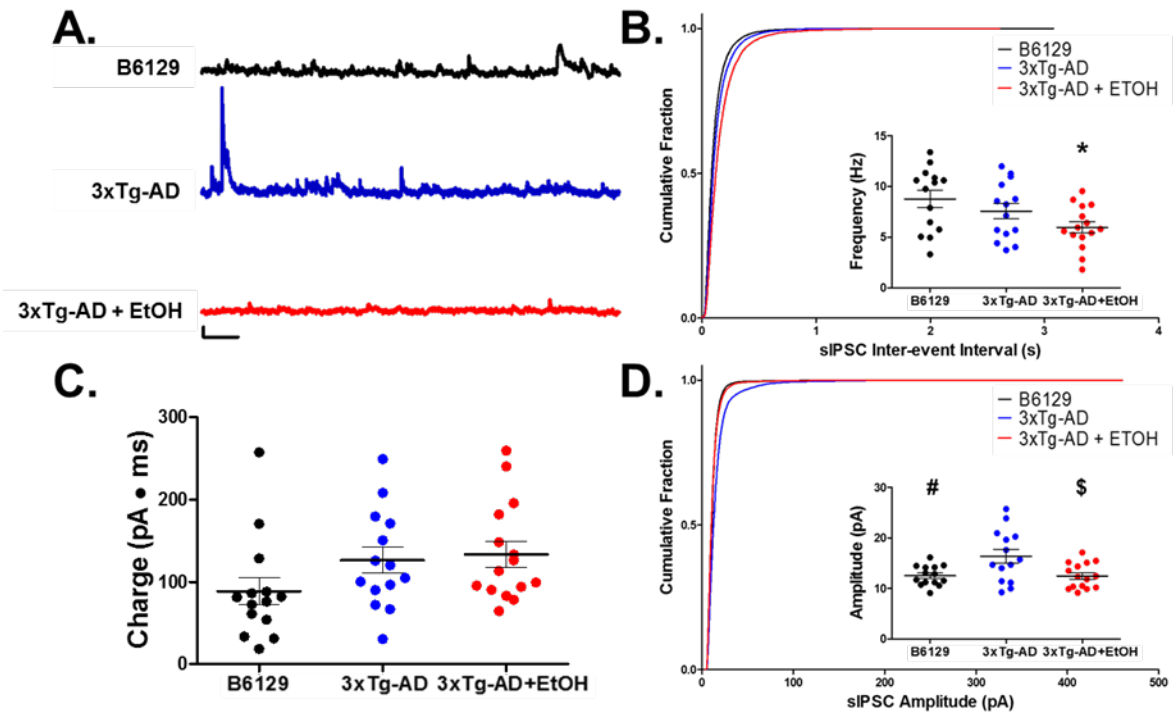


Figure 3.4. Prenatal ethanol exposure decreases the frequency of spontaneous inhibitory post-synaptic currents (sIPSCs) in the medial prefrontal cortex (mPFC) of 4-month-old 3xTg-AD mice.

(A) Representative traces of whole-cell voltage clamp recordings of sIPSCs recorded at a holding potential 0 mV from Layer V/VI pyramidal neurons in acute mPFC slices from 4-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red) mice; scale bar = 250 ms × 20 pA. (B) Cumulative distribution of sIPSC inter-event intervals (s) recorded Layer V/VI pyramidal neurons of 4-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red) mice. (inset): Mean sIPSC frequency of Layer V/VI pyramidal neurons of mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (C) Cumulative distribution of sIPSC amplitude intervals (s) recorded from Layer V/VI pyramidal neurons of 4-month-old B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red) mice. (inset): Mean sIPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH mice (red dots). (D) Mean sIPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (B) $*$ = $p < 0.05$, B6129 vs. 3xTg-AD + EtOH, one-way ANOVA with Bonferroni post hoc tests. (D) # = $p < 0.05$, 3xTg-AD vs. B6129, $ = $p < 0.05$, 3xTg-AD vs. 3xTg-AD + EtOH, one-way ANOVA with Bonferroni post hoc tests. For all measures: B6129 (N = 14 cells, n = 4 F, 5 M, 6 litters), 3xTg-AD (N = 15 cells, n = 5 F, 5 M, 6 litters), and 3xTg-AD + ETOH (N = 15 cells, n = 3 F, 7 M, 7 litters). N = total number of cells per group (dots) from n = total number mice by sex: F (female), M (male).

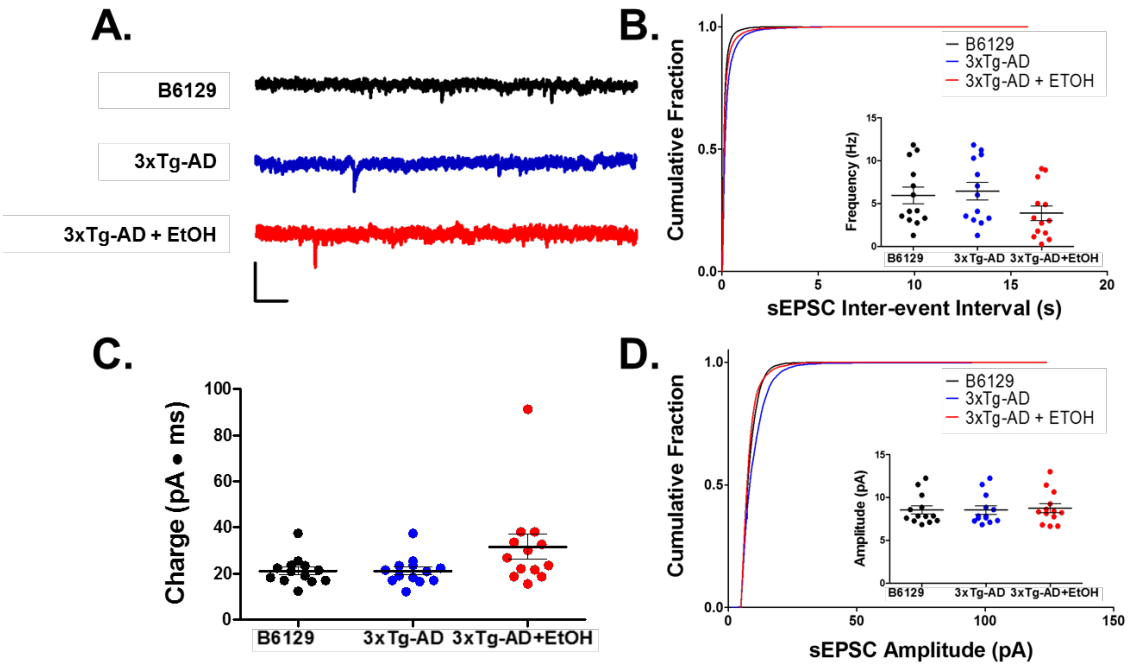


Figure 3.5. Prenatal ethanol exposure does not alter the frequency, amplitude, or charge of spontaneous excitatory post-synaptic currents (sEPSCs) in the medial prefrontal cortex (mPFC) of 6-month-old 3xTg-AD mice.

(A) Representative traces of whole-cell voltage clamp recordings of sEPSCs recorded at a holding potential −70 mV from Layer V/VI pyramidal neurons in acute mPFC slices from 6-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red) mice. (B) Cumulative distribution of sEPSC inter-event intervals (s) recorded from Layer V/VI pyramidal neurons of 6-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red). (inset): Mean sEPSC frequency of Layer V/VI pyramidal neurons of mPFC of 6-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (C) Cumulative distribution of sEPSC amplitude intervals (s) recorded Layer V/VI pyramidal neurons of 6-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH mice (red). (inset): Mean sEPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 6-month-old B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (D) Mean sEPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 6-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. For all measures: B6129 (N = 13 cells, n = 4 F, 4 M, 5 litters), 3xTg-AD (N = 13 cells, n = 4 F, 6 M, 3 litters), and 3xTg-AD + ETOH (N = 14 cells, n = 4 F, 6 M, 5 litters). N = total number of cells per group (dots) from n = total number mice by sex: F (female), M (male).

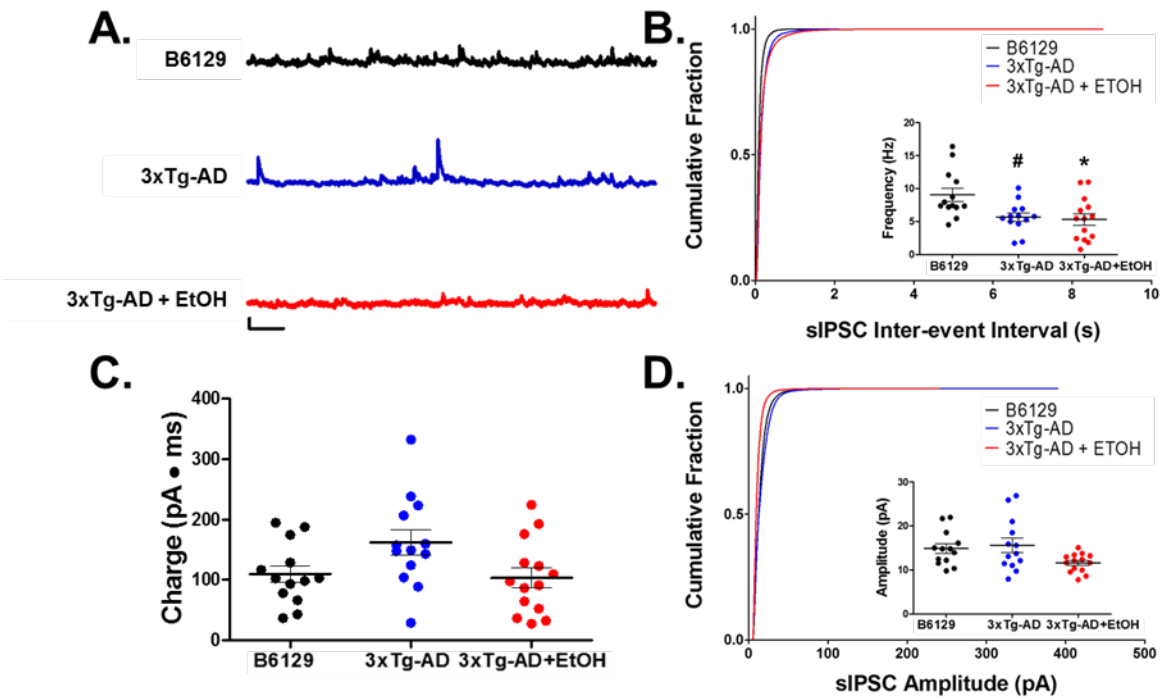


Figure 3.6. Prenatal ethanol exposure decreases the frequency of spontaneous inhibitory post-synaptic currents (sIPSCs) in the medial prefrontal cortex (mPFC) of 6-month-old 3xTg-AD mice.

(A) Representative traces of whole-cell voltage clamp recordings of sIPSCs recorded at a holding potential 0 mV from Layer V/VI pyramidal neurons in acute mPFC slices from 6-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red). (B) Cumulative distribution of sIPSC inter-event intervals (s) recorded from Layer V/VI pyramidal neurons of 6-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH (red) mice, (inset): Mean sIPSC frequency of Layer V/VI pyramidal neurons of mPFC of 6-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (C) Cumulative distribution of sIPSC amplitude intervals (s) recorded Layer V/VI pyramidal neurons of 6-month-old: B6129 (black), 3xTg-AD (blue), and 3xTg-AD + EtOH mice (red). (inset): Mean sIPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 6-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (D) Mean sIPSC amplitude of Layer V/VI pyramidal neurons of mPFC of 6-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH mice (red dots). (B) # = $p < 0.05$, 3xTg-AD vs. B6129, $*$ = $p < 0.05$, B6129 vs. 3xTg-AD + EtOH, one-way ANOVA with Bonferroni post hoc tests. (D) ### = $p < 0.001$, 3xTg-AD vs. B6129, $$$ = $p < 0.001$, 3xTg-AD vs. 3xTg-AD + EtOH, one-way ANOVA with Bonferroni post hoc tests. For all measures: B6129 (N = 13 cells, n = 4 F, 4 M, 5 litters), 3xTg-AD (N = 13 cells, n = 4 F, 6 M, 3 litters), and 3xTg-AD + ETOH (N = 14 cells, n = 4 F, 6 M, 5 litters). N = total number of cells per group (dots) from n = total number mice by sex: F (female), M (male).

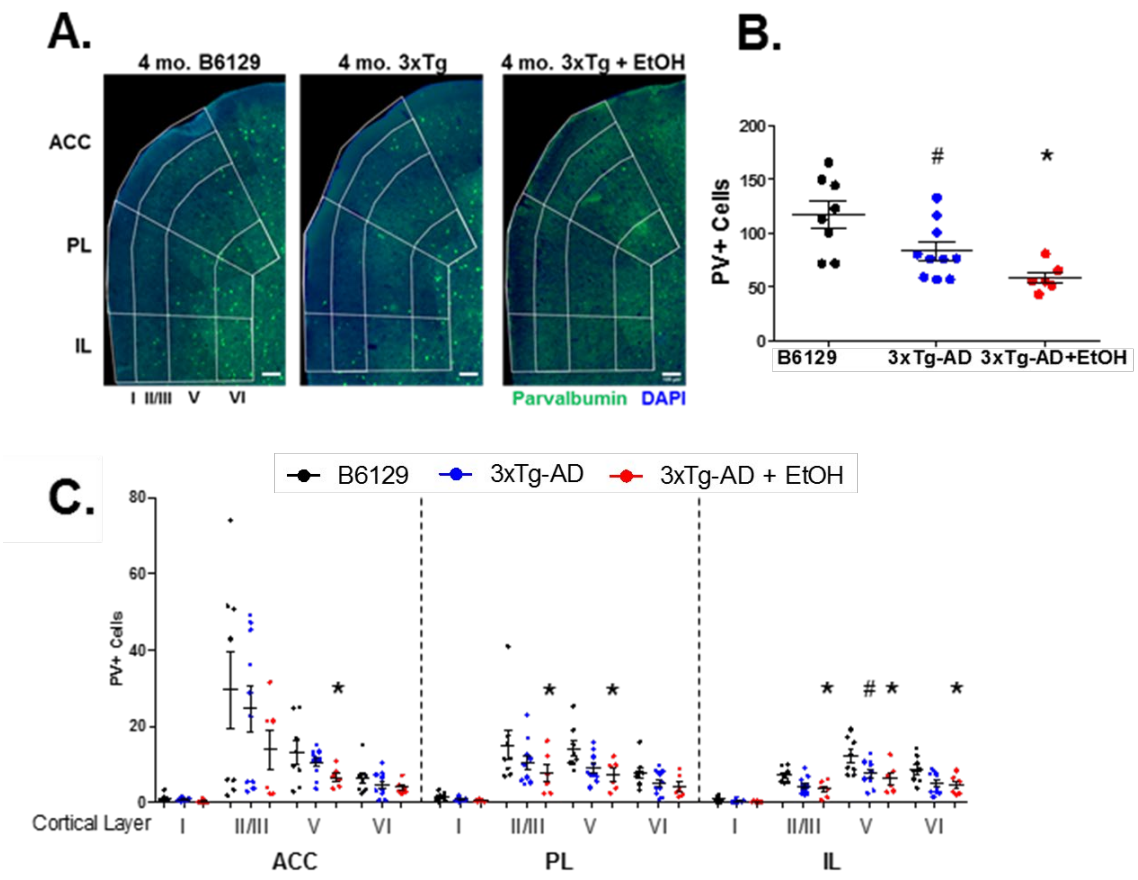


Figure 3.7. Prenatal ethanol exposure results in more widespread diminution of parvalbumin immunoreactive (PV+) GABAergic interneurons in the medial prefrontal cortex (mPFC) of 4-month-old 3xTg-AD mice.

(A) Example PV+ cells (green) in coronal sections of mPFC of 4-month-old: B6129, 3xTg-AD, and 3xTg-AD + EtOH mice with DAPI-counterstain (blue); scale bar = 100 μM. White lines demarcate the cortical layers: I, II/III, V, VI and subregions of the mPFC: anterior cingulate cortex (ACC), prelimbic cortex (PL), infralimbic cortex (IL). (B) Total number of PV+ cells in the mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH mice (red dots). (C) Number of PV+ cells in layers I, II/II, V, and VI of the ACC, PL, and IL regions of the mPFC of 4-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (B) # = $p < 0.05$ B6129 vs. 3xTg-AD, ∗ = $p < 0.05$ B6129 vs. 3xTg-AD + EtOH, one-way ANOVA with Bonferroni post hoc tests. (C) # = $p < 0.05$ B6129 vs. 3xTg-AD, ∗ = $p < 0.05$ B6129 vs. 3xTg-AD + EtOH, two-way ANOVA with Bonferroni post hoc tests. For all measures: B6129 (N = 8 brains, n = 4 F, 4 M, 5 litters), 3xTg-AD (N = 10 brains, n = 4 F, 4 M, 5 litters), and 3xTg-AD + ETOH mice (N = 6 brains, n = 3 F, 3 M, 3 litters). N = total number of brains per group (dots) from n = total number brains by sex: F (female), M (male).

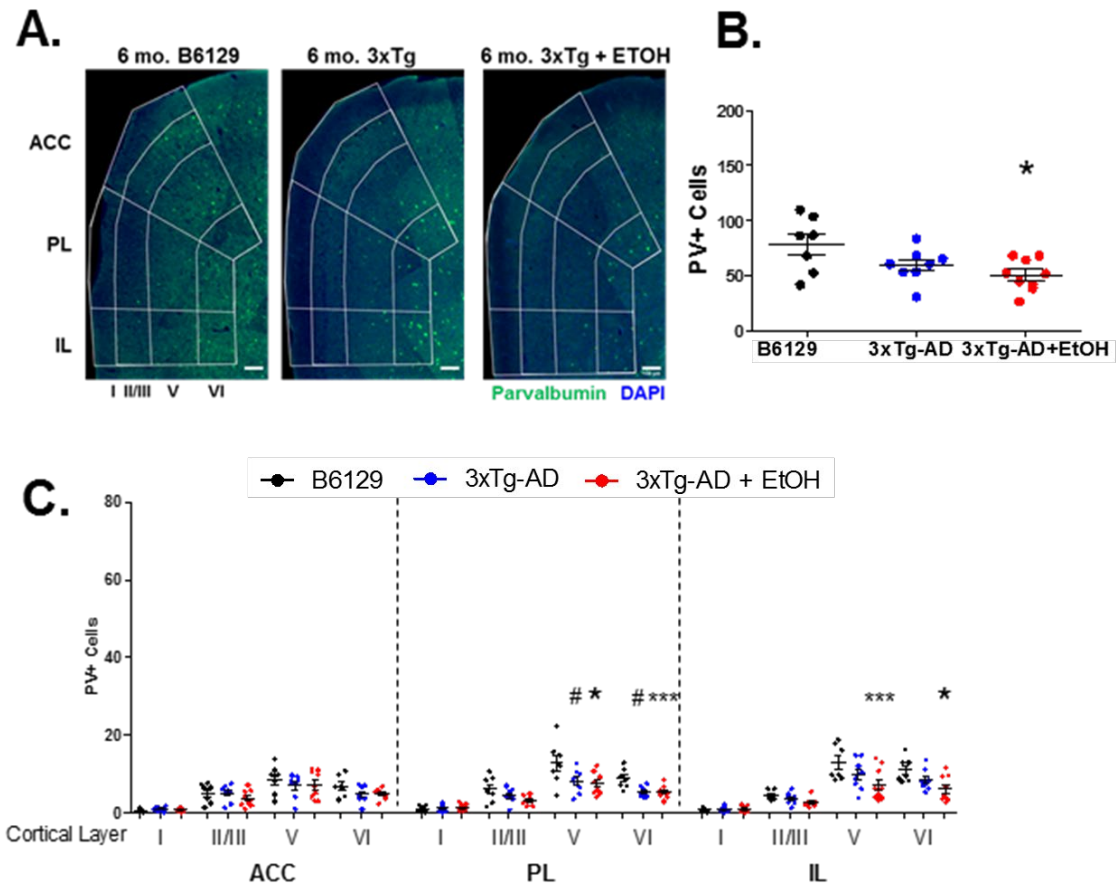


Figure 3.8. Prenatal ethanol exposure results in layer-specific and region-specific diminution of immunoreactive

(PV+) GABAergic interneurons in the medial prefrontal cortex (mPFC) of 6-month-old 3xTg-AD mice. (A) Example PV+ cells (green) in coronal sections of mPFC of 6-month-old: B6129, 3xTg-AD, and 3xTg-AD + EtOH mice with DAPI-counterstain (blue); scale bar = 100 µM. White lines demarcate the cortical layers: I, II/III, V, VI and subregions of the mPFC: anterior cingulate cortex (ACC), prelimbic cortex (PL), infralimbic cortex (IL). (B) Total number of PV+ cells in the mPFC of 6-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH (red dots) mice. (C) Number of PV+ cells in layers I, II/II, V, and VI of the ACC, PL, and IL regions of the mPFC of 6-month-old: B6129 (black dots), 3xTg-AD (blue dots), and 3xTg-AD + EtOH mice (red dots). (B) $*$ = $p < 0.05$ B6129 vs. 3xTg-AD + EtOH, one-way ANOVA with Bonferroni post hoc tests. (C) # = $p < 0.05$ B6129 vs. 3xTg-AD, $*$ = $p < 0.05$, $***$ = $p < 0.001$ B6129 vs. 3xTg-AD + EtOH, two-way ANOVA with Bonferroni post hoc tests. For all measures: B6129 (N = 7 brains, n = 4 F, 3 M, 4 litters), 3xTg-AD (N = 7 brains, n = 3 F, 4 M, 4 litters), and 3xTg-AD + ETOH (N = 7 brains, n = 4 F, 5 M, 4 litters). N = total number of brains per group (dots) from n = total number brains by sex: F (female), M (male).

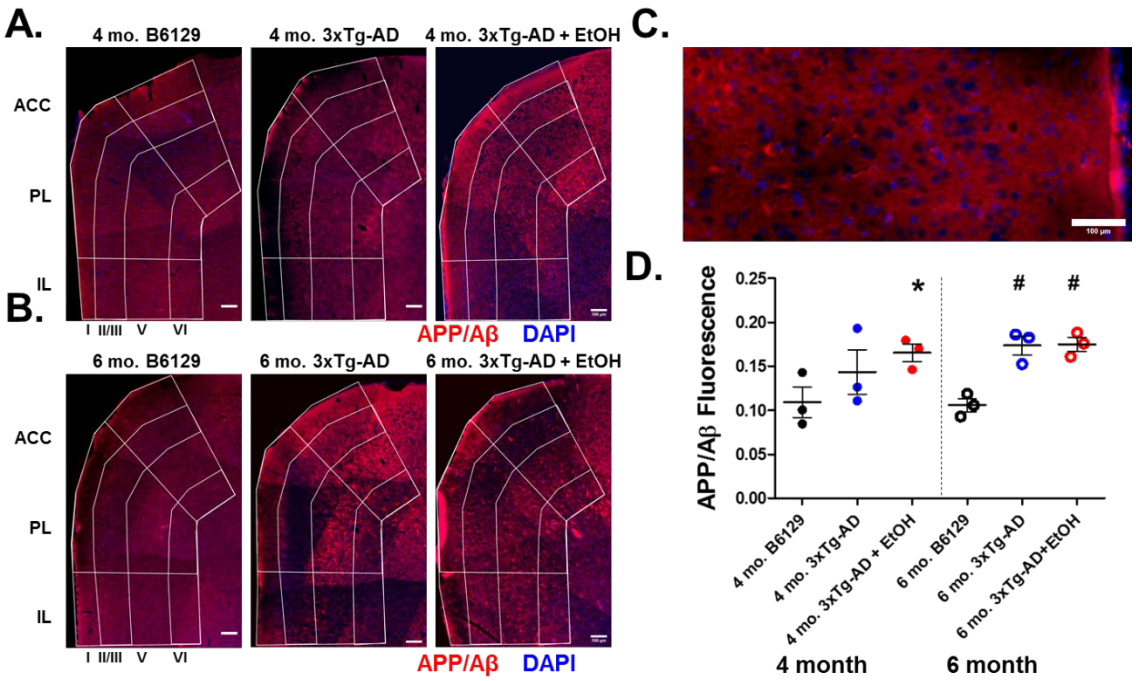
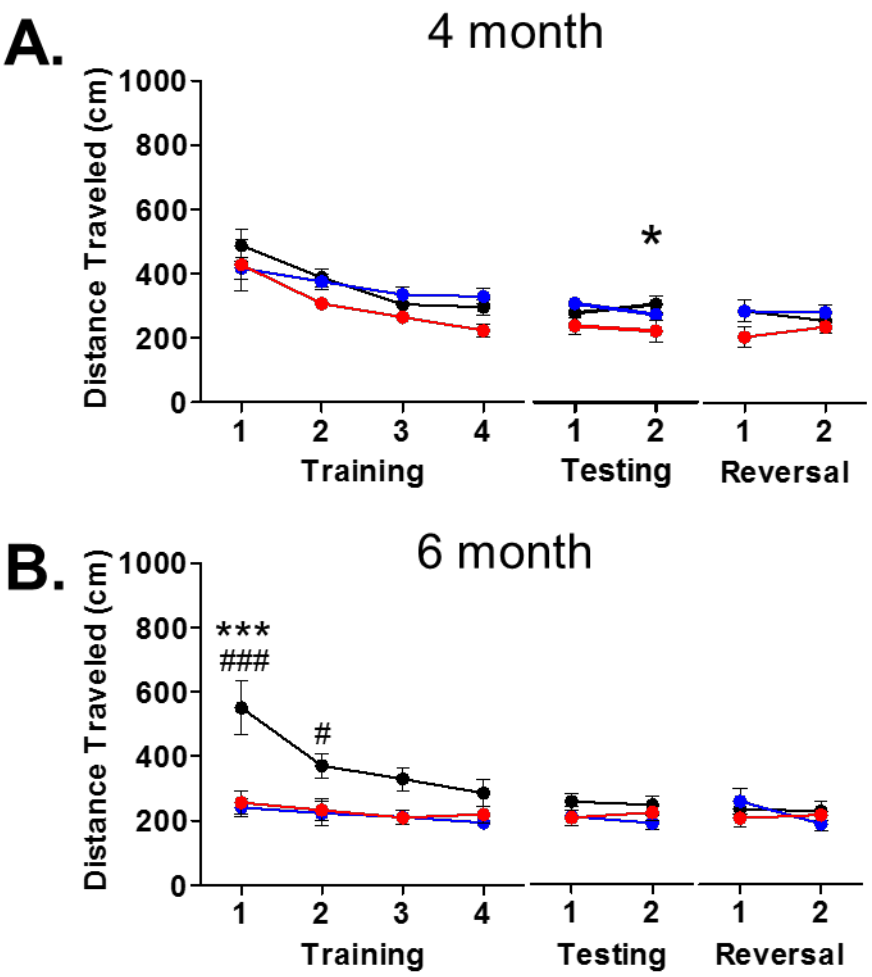
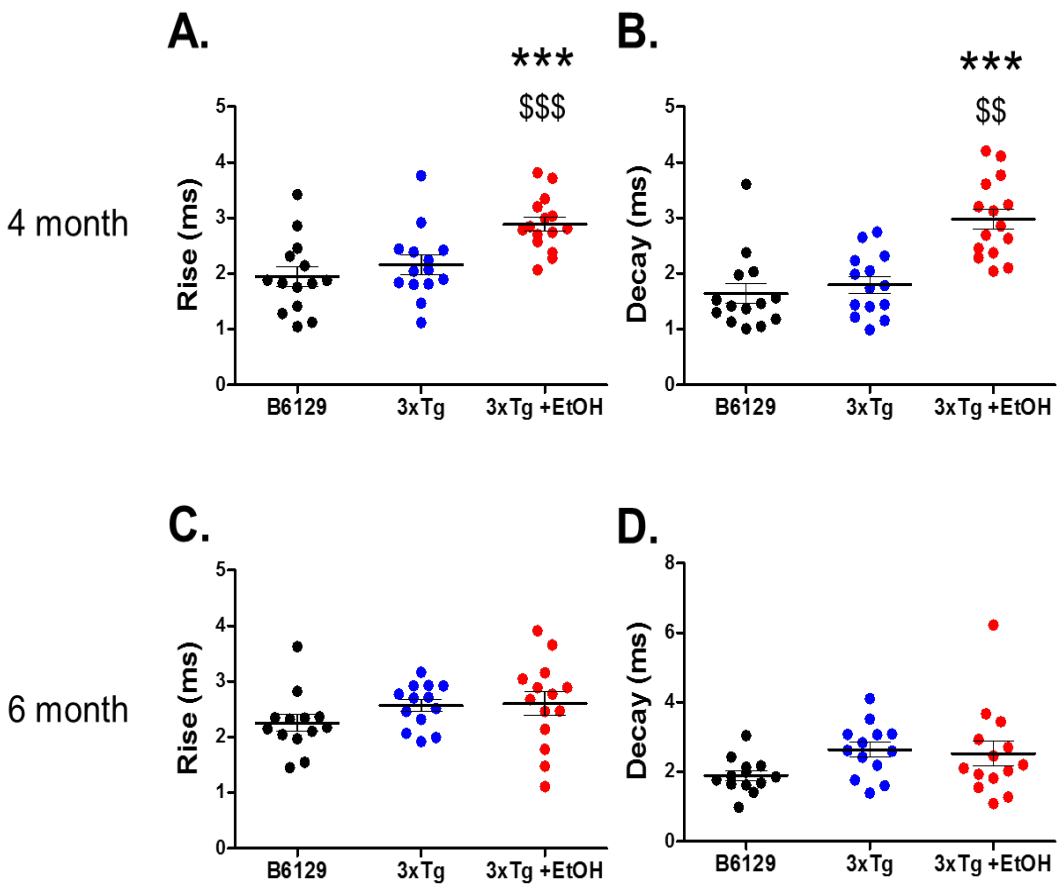


Figure 3.9. Prenatal ethanol exposure increases amyloid precursor protein/beta amyloid (APP/Aβ) immunoreactivity in the medial prefrontal cortex (mPFC) of 4-month-old 3xTg-AD mice.

(A) Representative images of APP/Aβ immunostaining (red) in coronal sections of mPFC of 4-month-old: B6129, 3xTg-AD, and 3xTg-AD + EtOH mice with DAPI-counterstain (blue); scale bar = 100 µM. White lines demarcate the cortical layers: I, II/III, V, VI and subregions of the mPFC: anterior cingulate cortex (ACC), prelimbic cortex (PL), infralimbic cortex (IL). (C) Example diffuse and intraneuronal APP/Aβ immunostaining (red) in Layers I–VI of the PL cortex of a 6-month-old 3xTg-AD + EtOH mPFC. (D) Relative APP/Aβ immunofluorescence intensity in the mPFC of 4-month-old: B6129 (filled black dots), 3xTg-AD (filled blue dots), and 3xTg-AD + EtOH (filled red dots) mice, and 6-month-old: B6129 (empty black dots), 3xTg-AD (empty blue dots), and 3xTg-AD + EtOH (empty red dots) mice. $*$ = $p < 0.05$, 4-month-old B6129 vs. 4-month-old 3xTg-AD + EtOH, two-way ANOVA with Bonferroni post hoc tests, # = $p < 0.05$, 6-month-old B6129 vs. 6-month-old 3xTg-AD and 6-month-old B6129 vs. 6-month-old 3xTg-AD + EtOH. 4-month-old B6129 (N = 3 brains, n = 1 F, 2 M, 3 litters), 4-month-old 3xTg-AD (N = 3 brains, n = 2 F, 1 M, 3 litters), 4-month-old 3xTg-AD + ETOH (N = 3 brains, n = 2 F, 1 M, 2 litters), 6-month-old B6129 (N = 3 brains, n = 2 F, 1 M, 3 litters), 6-month-old 3xTg-AD (N = 3 brains, n = 1 F, 2 M, 3 litters), and 6-month-old 3xTg-AD + ETOH (N = 3 brains, n = 1 F, 2 M, 3 litters). N = total number of brains per group (dots) from n = total number brains by sex: F (female), M (male).



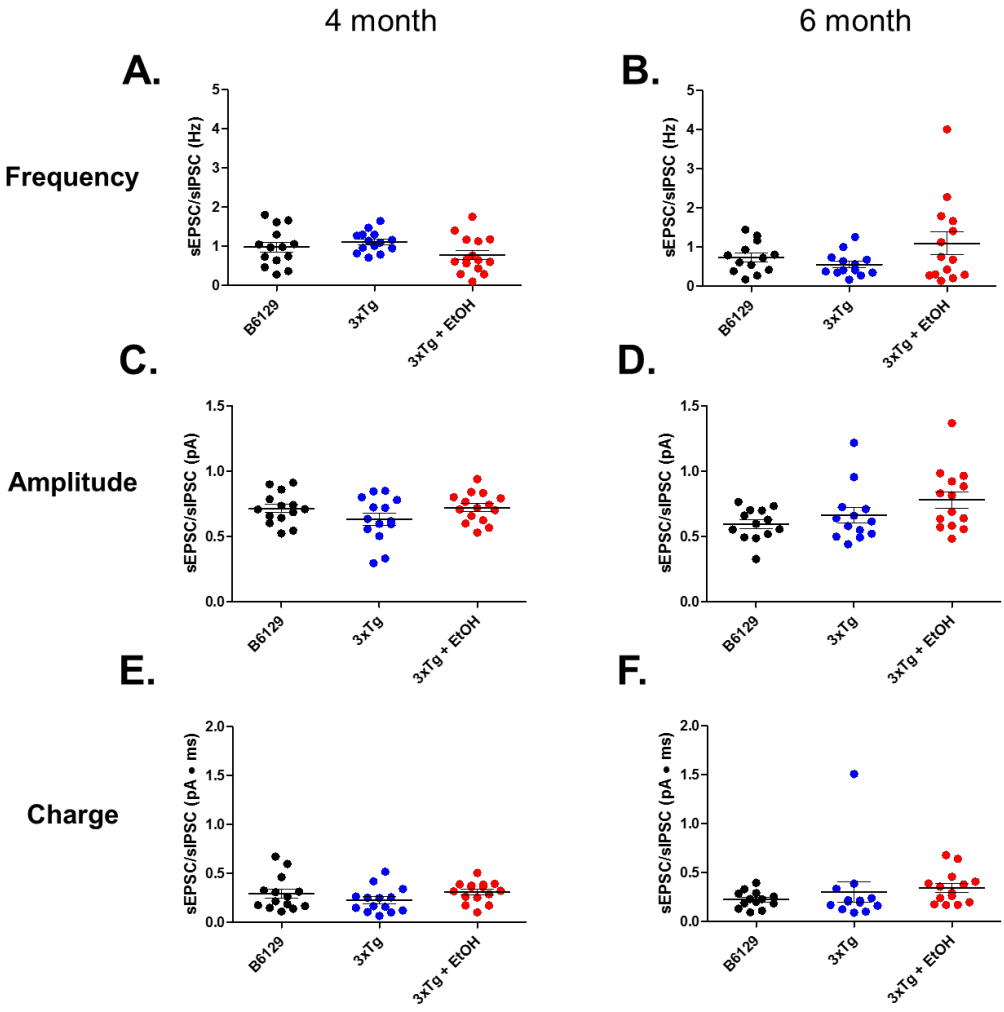
Supplementary Figure 3.1. Total distance traveled (cm) in the modified Barnes Maze by 4-month and 6-month old mice. (A, B) Mean total distance traveled (cm, n= 4 trials) for B6129 (black dots connected by black lines), 3xTg-AD (blue dots connected by blue lines) and ethanol-exposed 3xTg-AD mice (red dots connected by red lines) in 4-month (A) and 6-month old mice (B). (A) * = p< 0.05 B6129 vs. 3xTg-AD+EtOH, two-way repeated measures ANOVA with Bonferroni post-hoc tests (B) *** = p< 0.001 B6129 vs. 3xTg-AD+EtOH, ### = p<0.001 B6129, # = p< 0.05 B6129 vs. 3xTg-AD, Two-way repeated measures ANOVA with Bonferroni post-hoc tests. For sample sizes by experimental group see Table 1.

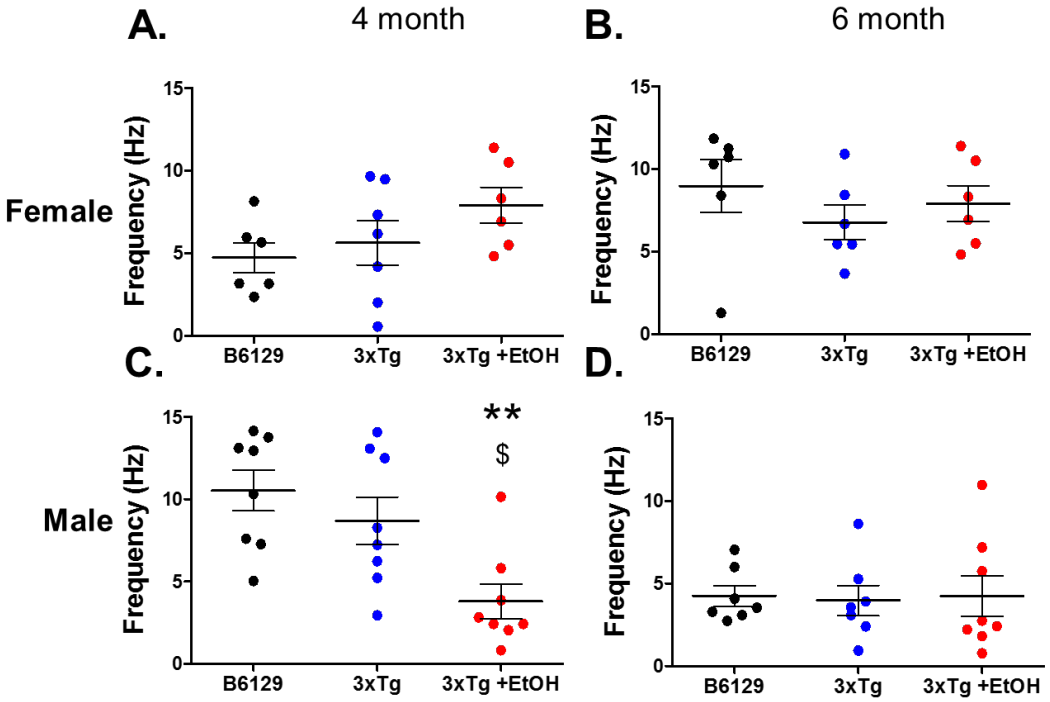


Supplementary Figure 3.2. Prenatal ethanol exposure increases the rise and decay times of spontaneous excitatory post-synaptic currents (sEPSCs) in the medial prefrontal cortex (mPFC) of 4-month old 3xTg-AD mice. (A) Mean sEPSC rise time of Layer V/VI pyramidal neurons of mPFC of 4-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH (red dots) mice. (B) Mean sEPSC decay time of Layer V/VI pyramidal neurons of mPFC of 4-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH (red dots) mice. (C) Mean sEPSC rise time of Layer V/VI pyramidal neurons of mPFC of 6-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH mice (red dots). (D) Mean sEPSC decay time of Layer V/VI pyramidal neurons of mPFC of 6-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH (red dots) mice. (A), (B) *** = p< 0.001, B6129 vs. 3xTg-AD+EtOH, one-way ANOVA with Bonferroni post-hoc tests, $$$ = p< 0.001, 3xTg-AD vs. 3xTg-AD+EtOH, $$ = p< 0.01 3xTg-AD vs. 3xTg-AD +EtOH, one-way ANOVA with Bonferroni post-hoc tests. For all measures: 4-month old: B6129 (N= 14 cells, n= 4 F, 5 M, 6 litters), 3xTg-AD (N= 15 cells, n= 5 F, 5 M, 6 litters) 3xTg-AD+ ETOH (N= 15 cells, n= 3 F, 7 M, 7 litters); 6-month old: B6129 (N= 13 cells, n= 4 F, 4 M, 5 litters), 3xTg-AD (N= 13 cells, n= 4 F, 6 M, 3 litters) 3xTg-AD+ ETOH (N= 14 cells, n= 4 F, 6 M, 5 litters); N = total number of cells per group (dots) from n = total number mice by sex: F (female), M (male).

Supplementary Figure 3.3. Prenatal ethanol exposure does not alter the excitatory: inhibitory (E/I) ratio of the of Layer V/VI pyramidal neurons in the medial prefrontal cortex (mPFC) of 4-month and 6-month old 3xTg-AD mice.

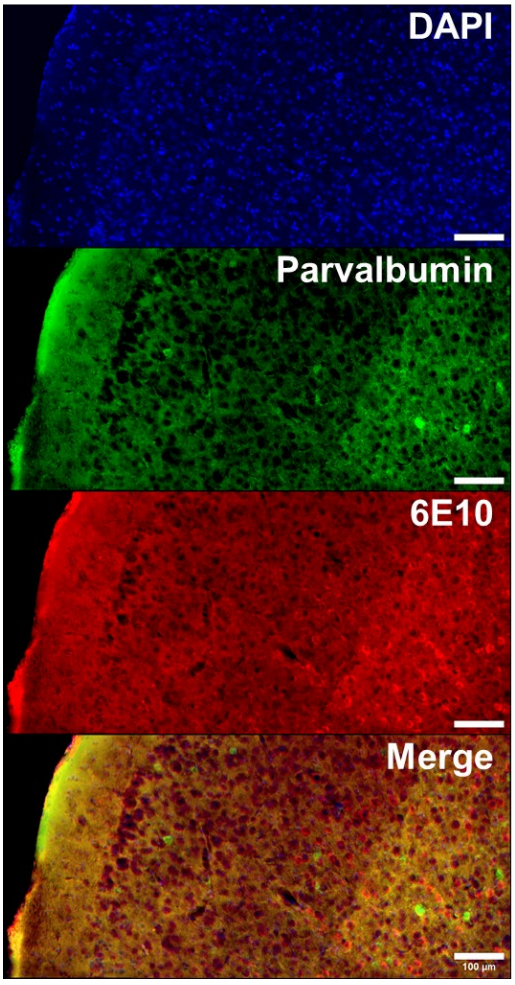
(A) Mean E/I ratio for spontaneous post-synaptic current (sPSC) frequency of 4-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH (red dots) mice. (B) Mean E/I ratio for sPSC frequency of 6-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH mice (red dots). (C) Mean E/I ratio for sPSC amplitude of 4-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH (red dots) mice. (D) Mean E/I ratio for sPSC amplitude of 6-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH mice (red dots). (E) Mean E/I ratio for sPSC charge of 4-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH (red dots) mice. (F) Mean E/I ratio for sPSC charge of 6-month old: B6129 (black dots), 3xTg-AD (blue dots) and 3xTg-AD+EtOH (red dots) mice. For all measures: 4-month old: B6129 (N= 14 cells, n= 4 F, 5 M, 6 litters), 3xTg-AD (N= 15 cells, n= 5 F, 5 M, 6 litters) 3xTg-AD+ ETOH (N= 15 cells, n= 3 F, 7 M, 7 litters); 6-month old: B6129 (N= 13 cells, n= 4 F, 4 M, 5 litters), 3xTg-AD (N= 13 cells, n= 4 F, 6 M, 3 litters) 3xTg-AD+ ETOH (N= 14 cells, n= 4 F, 6 M, 7 litters); N = total number of cells per group (dots) from n = total number mice by sex: F (female), M (male).


4 month
6 month
A.
B.
Frequency
sEPSC/sIPSC (Hz)
C.
D.
Amplitude
sEPSC/sIPSC (pA)
E.
F.
Charge
sEPSC/sIPSC (pA • ms)
B6129
3xTg
3xTg + EtOH



Supplemental Figure 3.4. Summary of age-dependent effects of prenatal ethanol exposure on PV+ GABAergic interneuron number and synaptic function in the mPFC of 4- and 6-month old 3xTg-AD mice.

(A) Mean total number of PV+ cells in the mPFC of 4- and 6-month old 3xTg-AD mice. (B, left panel) Mean sEPSC frequency in mPFC pyramidal neurons of 4- and 6-month old 3xTg-AD mice, * = p< 0.05, 4 mo. B6129 vs. 4 mo. 3xTg-AD+EtOH, $ = p<0.05 4 mo. 3xTg-AD vs. 4 mo. 3xTg-AD+EtOH, one-way ANOVA with Bonferroni post-hoc tests. (B, right panel) Mean sIPSC frequency in mPFC pyramidal neurons of 4- and 6-month old 3xTg-AD mice. * = p< 0.05, 4 mo. B6129 vs. 4 mo. 3xTg-AD+EtOH, # = p<0.05 6 mo. B6129 vs. 6 mo. 3xTg-AD, & = p<0.05 6 mo. B6129 vs. 6 mo. 3xTg-AD+EtOH, one-way ANOVA with Bonferroni post-hoc tests. (C, left panel) Mean sEPSC amplitude in mPFC pyramidal neurons of 4- and 6-month old 3xTg-AD mice.(C, right panel)) Mean sIPSC amplitude in mPFC pyramidal neurons of 4- and 6-month old 3xTg-AD mice. # = p<0.05 4 mo. 3xTg-AD vs. 4 mo. B6129, $ = p<0.05 4 mo. 3xTg-AD vs. 4 mo. 3xTg-AD+EtOH one-way ANOVA with Bonferroni post-hoc tests. (D, left panel) Mean sEPSC charge in mPFC pyramidal neurons of 4- and 6-month old 3xTg-AD mice. *** = p< 0.001, 4 mo. B6129 vs. 4 mo. 3xTg-AD+EtOH, $ = p<0.05 4 mo. 3xTg-AD vs. 4 mo. 3xTg-AD+EtOH, one-way ANOVA with Bonferroni post-hoc tests.(D, right panel) Mean sIPSC charge in mPFC pyramidal neurons of 4- and 6-month old 3xTg-AD mice. For all: 4-month old: B6129 (filled black dots), 3xTg-AD (filled blue dots) and 3xTg-AD+EtOH (filled red dots) mice, and 6-month old: B6129 (empty black dots), 3xTg-AD (empty blue dots) and 3xTg-AD+EtOH (empty red dots) mice.



Supplementary Figure 3.5. Intraneuronal amyloid does not colocalize with PV+GABAergic interneurons in 3xTg-AD mice.

(A) Representative images of parvalbumin (green) and intraneuronal-APP/Aβ (6E10-red) co-immunostaining from a 4-month old 3xTg-AD+EtOH mouse, with DAPI counterstaining (blue) in Layers I-VI of the prelimbic (PL) mPFC. (B) Higher magnification representative image of parvalbumin (green) and intraneuronal-APP/Aβ (6E10-red) co-immunostaining from a 6-month old 3xTg-AD+EtOH mouse, with DAPI counterstaining (blue) in Layers V/VI of the PL mPFC.

CHAPTER 4

Discussion

4.1 Summary

My thesis work explored the effects of prenatal ethanol exposure on the brain during both early development, and aging, examining across the lifespan differences in functional and behavioral responses related to biological sex, neuronal subtype, and postnatal age. In Chapter 2, I determined that prenatal ethanol exposure results in delays in motor development that are more pronounced in male mice and coincide with differences in the development of functional properties, synaptic inputs, and morphological features of striatal neurons. I found that functional and synaptic changes resulting from prenatal ethanol exposure also differ between two populations of striatal neurons: striatal GINs and SPNs, and observe that the effects of prenatal ethanol exposure change dynamically over the first two postnatal weeks, coinciding with the maturation of both striatal neurons and the development of early motor behaviors. In Chapter 3, I found that prenatal ethanol exposure resulted in an earlier onset of cognitive deficits in 3xTg-AD mice, associated with pathological changes to the mPFC, including: GABAergic synaptic deficits, decreased numbers of PV+ GINs, and increased intraneuronal APP/β-amyloid. Together, these data indicate that the effects of prenatal ethanol exposure may result in phenotypes that are: (1) sexually dymorphic (2) neuronal subtype specific (3) dynamic in early development, and (4) persistent in aging animals.

4.2 Mechanistic explanations for sex differences in susceptibility to prenatal ethanol exposure

In Chapter 2, I found sex differences in the susceptibility of female and male mice to the effects of a prenatal ethanol exposure on early motor development, adding to previous work that had identified male vulnerability to prenatal ethanol exposure in both adolescent and adult rodents and individuals with FASD. In my discussion of possible mechanisms underlying these sex differences I addressed the potential contribution of differential function of striatal GINs and SPNs to the observed behavioral differences.

However, male vulnerability to pre and perinatal insults is not specific to CNS targets. Sex differences in responses to toxic environmental exposures are first observed in utero, when male fetuses have an increased risk of fetal heart rate variability, preterm birth, low birth weight, obstetric complications and related morbidities (DiPietro & Voegtline, 2017). Postnatally, males are more likely to be diagnosed with neurodevelopmental disorder; epidemiological studies estimate that neurodevelopmental disorders, including autism, ADHD and intellectual disability are 1.2-4 times more prevalent in children identifying as male (Bölte et al., 2023). A similar pattern is observed in cases of maternal substance use; males have higher rates of neonatal abstinence syndrome, susceptibility to developmental defects following neonatal methadone exposure, and low-birth weight with perinatal nicotine exposure, which have been recapitulated in rodent models (Hou et al., 2004; Jansson et al., 2010; Sikic et al., 2022; Terasaki et al., 2016; Willoughby et al., 2007).

The sex differences I uncovered in motor behavior and the development of striatal neurons could develop acutely during the embryonic ethanol exposure, or begin to be expressed postnatally, due to ethanol induced changes to the postnatal sexual

differentiation of the brain in male animals. Potential embryonic sources of postnatal sex differences in the response of striatal neurons and early motor behaviors to prenatal ethanol exposure include ethanol-induced changes in the embryonic expression of Y-chromosome specific genes, resulting from epigenetic modifications of gene expression such as histone modifications or DNA methylation. Prenatal ethanol exposure has been shown to alter postnatal gene expression via modification of variety of epigenomic pathways, and sex differences observed in recent transcriptomic analyses of the effects of acute ethanol on the embryonic cortex support the potential contribution of epigenomic modification to genes found on sex chromosomes (Chater-Diehl et al., 2017; Kleiber et al., 2014; Salem et al., 2021). Alternatively, the differences I found the later development of male and female animals could also be secondary to their strategy in managing the physiological stress of ethanol exposure in utero (Bosco and Diaz, 2012; Sutherland and Brunwasser, 2018).

However, previous work in the lab failed to identify sex differences in the effects of prenatal ethanol exposure on the embryonic development of the somatosensory cortex, but did identify subtle differences in the effects of prenatal ethanol exposure on the synaptic function of pyramidal neurons of adolescent mice using the same brief binge exposure model (Delatour et al., 2019a, 2019b). Thus, I hypothesize that the sex-dependent differences in the effects of prenatal ethanol exposure on striatal development are more likely to result from ethanol-induced changes during postnatal development.

Masculinization of the developing brain occurs with onset of early androgen expression in late embryonic and early postnatal male mice (McCarthy et al., 2017). The mechanism underlying this transition involves differing mechanisms in male and female mice. In

male mice, this begins with an increase in testosterone production in the developing testes. Testosterone is converted to estradiol by aromatase, resulting in increased levels of estradiol in the developing brain of male mice, ultimately giving rise to masculine traits (McCarthy et al., 2017). In female mice, CNS estrogen levels remain low due to the lack active estrogen production in the ovaries, and the suppression of estrogen by residual maternal alpha-fetoprotein in female mice (Bakker et al., 2006; McCarthy et al., 2017). The masculinization or feminization of the developing brain is set during an early developmental critical period beginning around E16 with male fetal androgen production, and ending at the around the end of the first postnatal week in mice (Konkle and McCarthy, 2011; Weisz and Ward, 1980). Prenatal ethanol exposure has been shown to decrease embryonic production of testosterone in ethanol-exposed male rats; a heavy binge (35% v/v) ethanol exposure E17-20 prevented the surge in testosterone observed in control-fed male rats on E18-19, and was related to a decreased responsiveness of the developing testes to luteinizing hormone (McGivern et al., 1988). In our model, ethanol exposure occurs just prior to the expected timing of the testosterone surge in mice (E13.5-16.5), and a much lower dose (5% v/v) such that it is impossible to predict if a similar suppression occurs.

Regardless, if the effects of prenatal ethanol exposure were purely in inhibiting the masculinization of the brain, by decreasing either the stimulation of testosterone via modification of hypothalamic GnRH production, by directly increasing testosterone production at the level of the testes, or by preventing the conversion to of testosterone to estrogen in the striatum, I would expect phenotypes in male mice to be identical to those in control-fed female mice (Clarkson and Herbison, 2016; Küppers and Beyer, 1998). This is true in some phenotypes at specific postnatal time points, but is not consistent across all phenotypes I assessed in Chapter 2. However, if prenatal ethanol exposure

does turn out to inhibit androgen production in male mice in utero, it could prevent trophic actions of early postnatal androgen expression, contributing to the increased severity of deficits observed in male mice (Tibrewal et al., 2018; Toran-Allerand, 1996)

Differences in the response of female and male mice to prenatal ethanol exposure could also be influenced by sex differences in the number and function of striatal microglia. In other brain regions, sexually dimorphisms have been observed in the number and degree of activation of microglia in female and male mice, with male mice consistently demonstrating increased numbers of active amoeboid microglia in early postnatal development, while this trend reverses by the end of the first postnatal month (Lenz et al., 2013; Schwarz et al., 2012). Microglia have been shown to demonstrate sex-dependent susceptibility to prenatal ethanol exposure in the hippocampus of early postnatal rats, and can facilitate both the development and pruning of GABAergic synaptic afferents from GINs (Favuzzi et al., 2021; Gallo et al., 2022; Ruggiero et al., 2018). Failure of early postnatal microglia to facilitate the formation of GABAergic synapses between striatal neurons in male mice could also contribute to the synaptic deficits I observe male mice at P2. Assessing the effects of prenatal ethanol exposure on striatal development in the context of: microglial depletion, masculinization of female neonates via early postnatal androgen exposure, or a transgenic mouse with an autosomally expressed SRY-gene allowing for the differentiation between chromosomal and gonadal sex determinants, could all contribute to resolving the etiology of sex differences in striatal and motor development following prenatal ethanol exposure (De Vries et al., 2002).

4.3 Sex differences in developing striatal neurons and their response to prenatal ethanol exposure

In addition to sex differences in the effects of prenatal ethanol exposure on striatal GINs and SPNs, Chapter 2, I also identified several sex differences between control-fed male and female animals. For example, male mice developed mature surface righting behavior more rapidly during the first postnatal week (P4-6), coinciding with a developmental increase GIN AP firing rate that preceded a similar increase observed in female mice. Interestingly this change in firing rate occurred immediately, after a detected increase glutamatergic sPSC frequency recorded in GINs from male mice at P2. We also determined sex differences between the AP firing rate of SPN at P14, with SPNs from male mice firing at a higher rate than those of female mice coinciding with a decreased frequency of GABAergic sPSCs recorded from GINs and a trend towards a decreased frequency of GABAergic sPSCs recorded from SPNs.

Our findings add to extensive work by Meitzen and colleagues characterizing sex differences in the function of SPNs of both prepubertal and adult rodents. In prepubertal rats, SPNs of the dorsal striatum of females have a more hyperpolarized AP threshold and higher firing rate relative to male animals, independent of differences in intrinsic membrane properties or synaptic inputs (Dorris et al., 2015). Sex differences in SPN function persist following the onset of puberty and are apparent in multiple striatal subregions and in both direct and indirect pathway SPNs (Willett et al., 2019). Indeed, the of function SPNs is also modified by changing levels of sex hormones during the estrous cycle of female rats (Proaño et al., 2018; Willett et al., 2019). Importantly, exposure to androgens during early neonatal development can alter the sex-associated functional properties of SPN (Cao et al., 2016). SPNs in female adolescent rats can be masculinized after perinatal treatment with estrogen receptor (ER) α or β agonists,

resulting in decreased SPN excitability relative to those of untreated female rats (Cao and Meitzen, 2021). Alternatively, rather than direct effects on SPNs, and striatal GINs, sex differences in the function of striatal neurons in control animals could result from sex differences in basal levels of striatal dopamine (Castner et al., 1993). All in all, our investigation of SPN and GIN function during early development indicates that in addition to sex differences in SPN excitability, and dopaminergic signaling in the striatum of juvenile and adolescent rodents, sex differences occur in the function of both striatal GINs and SPNs, as well as GABAergic synaptic activity recorded from both populations of neurons beginning early striatal development.

How sex differences in GABAergic signaling may contribute to the baseline sexualization of SPN neuron function is not known. However, given that sex differences in excitatory projections to the striatum have been identified in clinical imaging studies, and are not unique to rodents, further investigation into the mechanisms underlying sex differences in the function of GABAergic connections in the striatum may prove informative (Lei et al., 2016).

4.4 Cell-subtype specific susceptibility to PAE

Our findings indicate that prenatal ethanol exposure differentially affects two of the major sub-populations of striatal neurons: GINs and SPNs, despite their proximity in developmental time and location, and shared local and distant afferent connections. In Chapter 2, I postulated that differing effects of prenatal ethanol exposure on the functional and synaptic development of striatal neurons may contribute to cell-type specific effects of prenatal ethanol exposure. However, there are several alternative explanations for these observed differences.

In addition to direct effects of prenatal ethanol exposure on the gene expression, the function of ion channels, and the development of glutamatergic or GABAergic inputs to striatal GINs, that I described in Chapter 2, it is possible that the differences I observed between striatal GINs and SPNs reflect their differing functional roles in the developing striatal network. Investigation of the relationship between the activity of striatal neurons and the development of striatal afferents, suggests that the activity of SPNs in the developing striatum may be required to facilitate the establishment of connections between striatal neurons and other brain regions (Kozorovitskiy et al., 2012). Alternatively, GINs have been shown to make a critical contribution to local network dynamics in the development of other brain regions (Deidda et al., 2015; Modol et al., 2020). These data lead me to hypothesize that the differences I observe in SPNs may reflect the effects of prenatal ethanol exposure on connections to distant brain regions, while alterations to GIN function may reflect an attempt to balance or maintain the activity required to drive local striatal network development. The temporal coincidence of changes to striatal GINs and SPNs following prenatal ethanol exposure, suggests that the effects of this exposure on these two populations of neurons may be closely connected. Although the direction and source of changes in the function of striatal GINs and SPNs differed, when shifts in the effect of prenatal ethanol exposure occurred, they were generally reflected in both cell subtypes. Correct interpretation of the interplay between these differing roles will require further investigation of the development of hyperpolarizing GABA activity in both striatal GINs and SPNs, the potential impact of prenatal ethanol exposure on this developmental shift, and the effects of prenatal ethanol exposure on the embryonic proliferation and postnatal survival of striatal neurons.

Differences in the response of striatal GINs and SPNs to prenatal ethanol exposure could also reflect differences in the maturational trajectory of these two populations of striatal neurons. Although GINs and SPNs share common embryonic birth dates, and timing for the receipt of afferent connections based on their shared location, they have been reported to mature at different rates (Chesselet et al., 2007; Tepper et al., 1998). Previous study of striatal GIN and SPN development beginning in the second postnatal week suggested that the morphology and synaptic inputs of striatal GINs might mature more rapidly than those of SPNs (Chesselet et al., 2007; Tepper et al., 1998). However, our findings in Chapter 2 suggest that, during very early postnatal development (P2-4) striatal GINs may trail behind SPNs in their maturational trajectory, receiving fewer synaptic inputs and demonstrating a greater difference from adult-like firing rates and intrinsic properties than SPNs observed at P28. A delay in maturation in developing GINs relative to SPNs could confer a difference in susceptibility to the effects of prenatal ethanol exposure. Whether or not differences in the maturational state of striatal GINs and SPNs are reflected embryonically, and thus potentially susceptible to the acute effects of prenatal ethanol exposure is not yet known.

Relatedly, the metabolic demands of maintaining the differing level of activity GINs as they acquire their fast-spiking qualities in the striatum during the first two postnatal weeks, may also confer differential susceptibility to the effects of prenatal ethanol exposure. The high firing rate of PV+ GINs has been shown to result in a unique susceptibility to oxidative stress (Behrens et al., 2007). Analysis of genetic pathways altered by acute ethanol exposure in mouse embryos indicate increases in oxidative stress may contribute to postnatal vulnerabilities to the effects of prenatal ethanol exposure (Bhatia et al., 2019; Boschen et al., 2022).

Finally, a crucial unanswered question, is how shifting the timing of our prenatal ethanol exposure might in turn modify susceptibility of cell subtypes within the developing striatum, and across other brain regions. Evidence for the critical importance of embryonic timing to the susceptibility of populations comes from the unique vulnerability of earlier born Layer V pyramidal tract cortical pyramidal neurons, relative to later born Layer 2/3 intratelencephalic pyramidal neurons using our binge ethanol model (E13.5-16.5) (Delatour et al., 2019b, 2019a; Skorput et al., 2015). The findings in Chapter 3, also demonstrated that considerable changes in GIN number are more pronounced deep cortical layers in the mPFC of 3xTg-AD mice exposed prenatally to ethanol during the same embryonic period.

Similar to neurons in the developing cortex, the adult location of striatal neurons is determined by their embryonic birth date (Knowles et al., 2021). A recent study by Ma and colleagues provides evidence for regional specificity of the effects of prenatal ethanol exposure in the striatum; exposing pregnant dams to ethanol (E17-20) resulted in alterations in SPN function biased to the posterior dorsomedial striatum, relative to SPNs recorded from more anterior slices (Roselli et al., 2020). How the earlier timing of our ethanol-exposure paradigm might differentially effect subregions or compartments of the developing striatum remains an open question.

All in all, further investigation into the source of cell-type specific differences in the effects of prenatal ethanol exposure during development are warranted. These could include efforts to investigate the source and strength of excitatory inputs to the developing striatum using viral tracing and optogenetic approaches. Alternatively, assessing the acute effects of ethanol on gene expression in striatal GINs and SPNs

embryonically, or during time periods where differences in early motor behavior are present could lead to novel pharmacological targets for the treatment of FASD.

4.5 Opposing effects of early developmental exposures on cortical vs. striatal synaptic activity

Both common themes and differences emerge when I consider how prenatal ethanol exposure alters synaptic activity in striatal GINs and SPNs in the developing striatum, in mPFC pyramidal neurons of 3xTg-AD mice, and in the context of previously reported effects of our brief binge ethanol exposure paradigm on the synaptic activity of cortical neurons (Delatour et al., 2019b, 2019a; Skorput et al., 2019). Among all brain regions I identified I found presynaptic changes indicated by differences in sPSC frequency. These data suggest a consistent impact of prenatal ethanol exposure on presynaptic structures. Studies of ethanol exposure in adult animals have also highlighted the effects of acute and chronic ethanol exposure on function at pre-synaptic terminals (Lovinger and Roberto, 2013; Roberto et al., 2006). In the case of our studies of the effects of prenatal ethanol exposure on the developing striatum and on the mPFC of aging 3xTg-AD mice, further investigation into the etiology of presynaptic deficits is warranted as the changes in sPSC frequency may be due to alterations in either or both spontaneous vesicle release or action potential-evoked synaptic mechanisms, which would indicate differing effects of the ethanol exposure on the molecular components of the synapse (Ramirez and Kavalali, 2011).

Alternatively, the direction of presynaptic changes following our brief binge prenatal ethanol exposure paradigm diverges depending on both the age and cortical region assessed. Prenatal ethanol exposure has previously been observed to increase excitatory and inhibitory sPSC frequency in the mPFC and somatosensory cortex of

young adult, and adolescent mice respectively (Delatour et al., 2019b, 2019a; Skorput et al., 2015). Alternatively, in Chapters 2 and 3 I showed that prenatal ethanol exposure resulted in both increases and decreases in sPSC frequency varied depending on cell-subtype, animal sex and postnatal age.

Previous reports suggest that opposing activity in the cortex and striatum may occur following developmental exposures and precipitate changes in network excitability. For example, acute exposure to a mu-opioid agonist resulted in increased activity, as measured by the frequency of Ca2+ transients in cortical pyramidal neurons, contrasting with the agonist's effect of significantly decreasing the number of active D2R+ SPNs, and a trend towards a decreased number of active D1+ SPNs (Barry et al., 2022). Alternatively, disruption of auditory sensory inputs or decreased excitatory afferent activity in the developing striatum resulted in a contrasting increase in SPN excitability that persisted into adulthood (Mowery et al., 2017). These data suggest a reciprocal relationship between the activity of cortical and striatal neurons, consistent with evidence that differences in the intrinsic properties of striatal neurons can modify the presynaptic function of corticostriatal projections, and conversely that differences in cortical input can alter the excitability of SPNs during development (Kozorovitskiy et al., 2012).

4.6 Mechanisms underlying age-dependent phenotypes following a prenatal exposure to ethanol: Evidence for critical periods in striatal development

In Chapter 2, I identified sex differences in the responses of striatal GINs to prenatal ethanol exposure that corresponded with either protection from or susceptibility to early motor differences. GINs in other brain regions have been shown to undergo dynamic change across development, but demonstrate particularly dramatic functional differences and contributions to behavior across the lifespan, with their contribution to the closure of

developmental critical periods (Amegandjin et al., 2021; Deidda et al., 2015; Fagiolini et al., 2004; Okaty et al., 2009; Takesian et al., 2018). If striatal GINs facilitate the closure of a developmental critical period for the maturation of the striatal network, or for striatal-mediated behaviors is not yet known. However, recent studies indicate patterns suggestive of critical period plasticity in the striatum, particularly in response to dopaminergic modulation.

Recent work identified a developmental critical period during which the striatal circuit is better able to recover from insult is evident in the differential susceptibility of neonatal, adolescent and adult animals to loss of dopaminergic signaling mediated by dopamine depletion or genetic inhibition of dopamine release. Neonatal mice survive 6-hydroxydopamine-mediated dopamine depletion without the overt locomotor deficits, while similar lesions are detrimental in adult animals by way of decreasing the firing threshold and increasing the excitability of DMS D1R+ SPNs in response to inputs from prelimbic, cingulate and supplementary motor cortical areas (Braz et al., 2015; Galiñanes et al., 2009; Keifman et al., 2022; Kravitz et al., 2010). Alternatively, dopaminergic replacement in Pitx3KO mouse model with genetic loss of neonatal dopamine input only resulted in recovery of striatal neuron excitability and motor deficits when exposure occurred during a juvenile (P18-28) vs. adult period (P90-120), or when KO animals were exposure to supplemental dopamine from birth to P28 (Lieberman et al., 2018).

Further, dopamine depletion resulted in hyperlocomotion in subset of juvenile animals (P21-25), coinciding with a decreased responsiveness to cortical excitation that recover by adulthood (P84-112). Hyperlocomotion in these animals may be due to the delayed development of mature network activity observed in adult animals: clusters of responsive

neurons and non-responsive SPNs vs. maintenance of the non-specific excitability of neonatal SPNs in response to cortical stimulation (Galiñanes et al., 2009). Similarly, an adolescent (P30-37) critical period is observed in the responses of the developing striatum to the dopamine-2 receptor-inhibiting antipsychotic haloperidol and its effects on motor behavior (Soiza-Reilly and Azcurra, 2009).Alternatively, the response of striatal circuit maturation to sensory deprivation and loss of glutamatergic corticostriatal inputs resulting in lasting differences in SPN properties in adulthood, and the facilitation of glutamatergic synapse formation by early postnatal SPN firing, are reminiscent of critical period-like activity patterns (Kozorovitskiy et al., 2012; Mowery et al., 2017).

The influence of GABA on the release of both dopamine and glutamate is well described in adult animals, but further examination of the role of GABA in documented striatal critical periods, and related changes in functional and morphological phenotypes during early development may be altered by prenatal ethanol exposure warrants future study (Lopes et al., 2019; Mitchell, 1980; Ouyang et al., 2007).

4.7 Age-dependence of behavioral phenotypes in mouse models of neurodevelopmental disorders

In Chapter 2, I observed an age-dependence of striatal phenotypes resulting from prenatal ethanol exposure. Evidence of age-dependent phenotypes in the behavioral consequences of genetic mutations and neonatal exposures have been observed in several animal models of neurodevelopmental disorders (Kim et al., 2022; Peixoto et al., 2019, 2016; Petroni et al., 2022). Further support for the hypothesis that prenatal ethanol exposure results in neurodevelopmental differences in ongoing changes in normal network development come from the clinical supervision of individuals with diagnosed neurodevelopmental disorders throughout childhood and adolescence. Longitudinal

surveillance of children beginning in childhood suggests that symptoms of neurodevelopmental disorders may change during development, with increases and decreases in severity observed in some individuals but not others (Jacobson et al., 2021; Rivard et al., 2023). Given the rapid changes I observe in the impact of prenatal ethanol exposure on the developing striatum, I hypothesize that the neurons within the striatal microcircuit may continue to demonstrate shifts in phenotypes in their response to prenatal ethanol exposure in adolescence, adulthood and further in the aged states. These data highlight the need to follow and reassess the needs of individuals with FASD across their childhood development.

4.8 Evidence for early network dysfunction as a risk factor for neurodegenerative disease later in life: susceptibility of GINs

Exploration of potential mechanisms underlying the effects of prenatal ethanol exposure on synaptic activity in the developing striatum in Chapter 2, and the mPFC 3xTg-AD mice in Chapter 3, raises several related questions including: why increases in GABAergic firing are both specific to and ostensibly protective in female mice exposed prenatally to ethanol, and why GABAergic synaptic activity is uniquely vulnerable to the effects of ethanol and aging in the mPFC. Evidence from both past work in the lab and, other disease models points to two possible mechanisms: (1) the direct and specific effects of acute ethanol exposure on developing GINs during gestation, which I explored in Chapter 2 and a preceding section this discussion, and (2) the distinct susceptibility of GINs to CNS network activity imbalance resulting from prenatal ethanol exposure.

The vulnerability of GABAergic interneurons to deficits resulting from embryonic exposures, genetic mutations related to neurodevelopmental disorders and neurodegenerative diseases is well-documented. Indeed, the disruption of GIN

migration, number and function collectively termed: “interneuronopathy,” is a common phenotype among neurodevelopmental disorders including ASD, DS and attention deficit hyperactivity disorder (ADHD) and FASD, as well as epilepsy (Cuzon et al., 2008; Giorgi et al., 2020; Kato and Dobyns, 2005; Marguet et al., 2020; Reiner et al., 2016; Skorput et al., 2019, 2015; Yang et al., 2021; Zorrilla de San Martin et al., 2018). Relatedly, as described in Chapter 3, each of these disorders is also associated with increased risk of developing AD (Ballard et al., 2016; Becker et al., 2021; Fluegge and Fluegge, 2018; Giorgi et al., 2020; Lott and Head, 2019; Vivanti et al., 2021). The common occurrence of disrupted GABAergic function within developing CNS networks across neurodevelopmental disorders, and its association with increased AD risk supports the need to examine the role of GIN dysfunction and loss in the relationship between neurodevelopmental disorders and AD risk. Evidence from several brain regions, and disorders indicate that GINs are uniquely able to respond to and correct network imbalances (Klausberger et al., 2003). The mechanisms underlying these responses to E/I imbalance vary but include: altering the postnatal survival of GINs to correct ectopic GIN proliferation in neonatal animals, and adjusting GIN AP firing rate, modifying excitatory plasticity at glutamatergic synapses, and facilitating GABAergic synapse formation in responses to differences in excitatory inputs, in adult animals (Chan et al., 2022; Gao and Penzes, 2015; Klausberger et al., 2003; Magno et al., 2021; Pouille and Scanziani, 2001).

Alternatively, modifying network activity in the mPFC of neonatal mice, or specifically targeting the activity of mPFC GINs in adolescence results in lasting changes in mPFC E/I balance, and reversal learning deficits in adult animals (Bitzenhofer et al., 2021; Caballero et al., 2020; Canetta et al., 2022) Additionally, recent work suggests that mPFC PV+ GINs are subject to an adolescent critical period associated with appropriate

development of social behaviors (Amegandjin et al., 2021). Past work in the lab indicates that a prenatal ethanol exposure can result in a persistent E/I imbalance in young adult mice, that is associated with differences in reversal learning behavior (Skorput et al., 2015). Collectively these data suggest a potential explanation for the earlier onset of spatial memory and reversal learning deficits I observed in 3xTg-AD mice following a prenatal ethanol exposure in Chapter 3, in which early postnatal dysfunction in mPFC GINs after prenatal ethanol exposure, may result in a lasting network imbalance that leaves GINs either more vulnerable to or less able to respond to changes in excitability related to the progression of AD pathology.

In addition, there is considerable evidence for age-dependent effects on both glutamatergic and GABAerigc synaptic activity in several brain regions in the absence of an additional stressors (Morrison and Baxter, 2012; Petralia et al., 2014; Rozycka and Liguz-Lecznar, 2017). It is not unsurprising that I see differing effects of prenatal ethanol exposure on synaptic activity in the mPFC in 3xTg-AD mice, when compared to young adult mice exposed-prenatally to ethanol, but without a transgenic background conferring increased risk for AD-like phenotypes. However, these data raise the question of whether synaptic deficits in young adult 3xTg-AD mice would parallel those observed in control (B6129) mice, and whether prenatal ethanol exposure might result in synaptic differences, even in younger 3xTg-AD mice.

4.9 Role of E/I imbalance in executive function deficits in FASD

Past work in the lab identified an E/I imbalance in the mPFC of young adult mice after prenatal ethanol exposure using the same brief binge ethanol exposure paradigm, during the same embryonic time period (Skorput et al., 2015). In Chapter 3 I identified

deficits in both spatial memory and reversal learning that occurred in the absence of E/I imbalance sPSC recordings in 3xTg-AD mice exposed-prenatally to ethanol. One explanation for this observation is the difference in our experimental approach. E/I imbalance was identified in young adult animals using experiments assessing the amplitude of electrically-evoked action potential mediated post-synaptic currents (Skorput et al., 2015). Consistent with this hypothesis, assessment of E/I balance from sPSC recordings also failed to identify an E/I imbalance in the neonatal somatosensory cortex following a brief binge ethanol exposure, however measurement of optogenetically-evoked GABAergic and glutamatergic synaptic currents resolved an imbalance in adolescent somatosensory cortex (Delatour et al., 2019a). Action-potential mediated and spontaneous synaptic events rely on different voltage gated calcium channels and neurotransmitter pools (Ramirez and Kavalali, 2011). It is possible that spontaneous vesicle release is intact and able to compensate for differences in action-potential mediated changes in E/I balance in 3xTg-AD mice following prenatal ethanol exposure.

Alternatively, despite the lack of apparent E/I balance I observed in recordings of putative GABAergic and glutamatergic spontaneous currents, an imbalance in excitation and inhibition may still be present, resulting from differences in net activity change resulting from GABA action on the GABA-A receptor. While the shift from depolarizing to hyperpolarizing action of GABA has been observed at P10-20 in the developing mPFC, past work from the lab suggests that prenatal ethanol exposure can modify the depolarizing action of GABA in the embryonic brain, suggesting that ethanol-exposure could also influence the timing of the depolarizing to hyperpolarizing GABA shift in development (Skorput et al., 2019). Ectopic expression of depolarizing GABA may also contribute to network excitability observed in AD models (Capsoni et al., 2022; Chen et

al., 2017). Future experiments assessing spontaneous- and action potential-mediated currents, and the reversal potential of GABA in 3xTG-AD mice exposed prenatally to ethanol will further clarify this point.

4.10 Experimental limitations

Perhaps the most obvious limitation to this research relates to the challenge of using a mouse model to make inferences about a human disorder. This is particularly apparent in interpreting how developmental changes I observed may relate to age-dependent developmental differences in individuals with an FASD diagnosis. Based on neuroanatomical comparisons, the first week of the early postnatal time period I assessed is considered to best represent third trimester of human fetal growth. However, when considering the onset of early motor behaviors, early postnatal mice share more similarities with the early childhood development of human children. Similarly, while I observe a clear impact of prenatal ethanol exposure on AD-relevant phenotypes in 3xTg-AD mice, these are an imperfect model of human AD and it is thus challenging to predict how observed differences may translate to human populations.

Additionally, while I identified a strong temporal association between in the differences in the development of striatal GINs in male mice, and the male specific differences I observed in early motor behaviors following prenatal exposure further investigation is required to confirm the importance of striatal GINs to the development of early motor behaviors. Future experiments investigating whether modifying the excitability of striatal GINs, their excitatory inputs, or their ability to release GABA in early development, results in early motor deficits will either prove or disprove this hypothesis.

Other notable gaps in our experimental design include our inability to consider the potentially differing effects of prenatal ethanol exposure on direct and indirect pathway

SPNs, striosomal and matrix SPNs, and both striatal GINs and SPNs across the four striatal quadrants. Given the well documented functional differences between direct and indirect pathway SPNs, and associations between embryonic birth date and postnatal localization of striatal neurons, it will be necessary for future study to clarify the consistency or specificity of the effect of prenatal ethanol exposure on striatal neurons of different subpopulations, and residing in compartments and regions (Alloway et al., 2017; Kelly et al., 2018; Marin et al., 2000; Matsushima and Graybiel, 2020; Monteiro et al., 2022; Newman et al., 2015).

Also, as mentioned in previous sections this work employs a brief binge exposure to ethanol during a period when both SPNs and GINs are proliferating and migrating to form the developing striatum. How a binge exposure during a different embryonic time period, a shorter or longer exposure time, or a more or less severe BEC change might alter the response of cells within the developing striatal circuit remains unanswered. It is also challenging to predict how well this model may recapitulate gestational exposures that most commonly precede FASD diagnoses.

4.11 Conclusions: Implications for the etiology, clinical assessment and management of FASD

My purpose in the experiments outlined in this thesis was to identify differences in the effects of prenatal ethanol exposures on the longitudinal effects of prenatal ethanol exposure spanning the lifespan. Below I outline the potential implications of our findings related to the effects of prenatal ethanol exposure on both early development and aging, for individuals with FASD and their caregivers.

Sex differences

Our findings in Chapter 2 highlight the specific vulnerability of male mice to differences in the development of striatal neurons and early motor behaviors following prenatal ethanol exposure. These data are consistent with observations from both clinical and rodents studies, which suggest that males and females may be differentially susceptible to the effects of prenatal exposure. Our data adds the caveat that sex-dependent differences the effects of prenatal ethanol exposure may be present even very early in development, and thus should inform approaches to identifying and diagnosis FASD in early childhood.

Cell-type dependence

In Chapter 2, I also observe unique effects of prenatal ethanol exposure on striatal GINs and SPNs varying based on both the postnatal age and the sex of the animal assessed, The synaptic changes I observed in striatal neurons also stand in contrast with the consistent increases in glutamatergic and GABAergic synaptic inputs previously observed in pyramidal neurons prefrontal and somatosensory cortex in neonatal and young adult animals after prenatal ethanol exposure using the same brief binge paradigm (Delatour et al., 2019a, 2019b; Skorput et al., 2015). These data suggest that future development of pharmacological interventions in FASD will need to consider that variation in glutamatergic and GABAergic signaling depending on the cell-type and brain region assessed. Our findings in Chapter 3 further imply that neurons within the same region may also be differentially affected by prenatal ethanol exposure in aging vs. young adults. Specifically, these data indicate that targeted pharmacological intervention targeting specific subpopulations of neurons may be required to adequately address the clinical symptoms of FASD.

Age-dependence

In Chapters 2 and 3, I identified age-dependent differences in the responses of striatal and mPFC neurons to prenatal ethanol exposure that may occur rapidly in early development, or result in long-lasting effects on neuronal activity and behavior in aging animals. The age-dependence of the effects of prenatal ethanol exposure suggests that clinical management of FASD may be improved by continued supervision, and reassessment of treatment approaches across childhood development, and highlights the need for increased research into the experience of individuals with FASD in the aging process.

4.10 References

Alloway, K.D., Smith, J.B., Mowery, T.M., Watson, G.D.R., 2017. Sensory Processing in the Dorsolateral Striatum: The Contribution of Thalamostriatal Pathways. Front Syst Neurosci 11, 53. https://doi.org/10.3389/fnsys.2017.00053

Amegandjin, C.A., Choudhury, M., Jadhav, V., Carriço, J.N., Quintal, A., Berryer, M., Snapyan, M., Chattopadhyaya, B., Saghatelyan, A., Di Cristo, G., 2021. Sensitive period for rescuing parvalbumin interneurons connectivity and social behavior deficits caused by TSC1 loss. Nat Commun 12, 3653. https://doi.org/10.1038/s41467-021-23939-7

Bakker, J., De Mees, C., Douhard, Q., Balthazart, J., Gabant, P., Szpirer, J., Szpirer, C., 2006. Alpha-fetoprotein protects the developing female mouse brain from masculinization and defeminization by estrogens. Nat Neurosci 9, 220–226. https://doi.org/10.1038/nn1624

Ballard, C., Mobley, W., Hardy, J., Williams, G., Corbett, A., 2016. Dementia in Down's syndrome. The Lancet Neurology 15, 622–636. https://doi.org/10.1016/S1474-4422(16)00063-6

Barry, J., Oikonomou, K.D., Peng, A., Yu, D., Yang, C., Golshani, P., Evans, C.J., Levine, M.S., Cepeda, C., 2022. Dissociable effects of oxycodone on behavior, calcium transient activity, and excitability of dorsolateral striatal neurons. Frontiers in Neural Circuits 16.

Becker, S., Sharma, M.J., Callahan, B.L., 2021. ADHD and Neurodegenerative Disease Risk: A Critical Examination of the Evidence. Front Aging Neurosci 13, 826213. https://doi.org/10.3389/fnagi.2021.826213

Behrens, M.M., Ali, S.S., Dao, D.N., Lucero, J., Shekhtman, G., Quick, K.L., Dugan, L.L., 2007. Ketamine-Induced Loss of Phenotype of Fast-Spiking Interneurons Is Mediated by NADPH-Oxidase. Science 318, 1645–1647. https://doi.org/10.1126/science.1148045

Bhatia, S., Drake, D.M., Miller, L., Wells, P.G., 2019. Oxidative stress and DNA damage in the mechanism of fetal alcohol spectrum disorders. Birth Defects Research 111, 714–748. https://doi.org/10.1002/bdr2.1509

Bitzenhofer, S.H., Pöpplau, J.A., Chini, M., Marquardt, A., Hanganu-Opatz, I.L., 2021. A transient developmental increase in prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice. Neuron. https://doi.org/10.1016/j.neuron.2021.02.011

Boschen, K.E., Steensen, M.C., Simon, J.M., Parnell, S.E., 2022. Short-term transcriptomic changes in the mouse neural tube induced by an acute alcohol exposure. Alcohol S0741-8329(22)00088-X. https://doi.org/10.1016/j.alcohol.2022.09.001

Bosco, C., Diaz, E., 2012. Placental Hypoxia and Foetal Development Versus Alcohol Exposure in Pregnancy. Alcohol and Alcoholism 47, 109–117. https://doi.org/10.1093/alcalc/agr166

Braz, B.Y., Galiñanes, G.L., Taravini, I.R., Belforte, J.E., Murer, M.G., 2015. Altered Corticostriatal Connectivity and Exploration/Exploitation Imbalance Emerge as

Intermediate Phenotypes for a Neonatal Dopamine Dysfunction. Neuropsychopharmacology 40, 2576–2587. https://doi.org/10.1038/npp.2015.104

Caballero, A., Flores-Barrera, E., Thomases, D.R., Tseng, K.Y., 2020. Downregulation of parvalbumin expression in the prefrontal cortex during adolescence causes enduring prefrontal disinhibition in adulthood. Neuropsychopharmacology 45, 1527–1535. https://doi.org/10.1038/s41386-020-0709-9

Canetta, S.E., Holt, E.S., Benoit, L.J., Teboul, E., Sahyoun, G.M., Ogden, R.T., Harris, A.Z., Kellendonk, C., 2022. Mature parvalbumin interneuron function in prefrontal cortex requires activity during a postnatal sensitive period. eLife 11, e80324. https://doi.org/10.7554/eLife.80324

Cao, J., Dorris, D., Meitzen, J., 2016. Neonatal Masculinization Blocks Increased Excitatory Synaptic Input in Female Rat Nucleus Accumbens Core. Endocrinology 157. https://doi.org/10.1210/en.2016-1160

Cao, J., Meitzen, J., 2021. Perinatal activation of ERα and ERβ but not GPER-1 masculinizes female rat caudate-putamen medium spiny neuron electrophysiological properties. Journal of Neurophysiology 125, 2322–2338. https://doi.org/10.1152/jn.00063.2021

Capsoni, S., Arisi, I., Malerba, F., D'Onofrio, M., Cattaneo, A., Cherubini, E., 2022. Targeting the Cation-Chloride Co-Transporter NKCC1 to Re-Establish GABAergic Inhibition and an Appropriate Excitatory/Inhibitory Balance in Selective Neuronal Circuits: A Novel Approach for the Treatment of Alzheimer's Disease. Brain Sci 12, 783. https://doi.org/10.3390/brainsci12060783

Castner, S.A., Xiao, L., Becker, J.B., 1993. Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. Brain Res 610, 127–134. https://doi.org/10.1016/0006-8993(93)91225-h

Chan, E.S., Ge, Y., So, Y.W., Bai, Y.-F., Liu, L., Wang, Y.T., 2022. Allosteric potentiation of GABAA receptor single-channel conductance by netrin-1 during neuronal-excitation-induced inhibitory synaptic homeostasis. Cell Reports 41. https://doi.org/10.1016/j.celrep.2022.111584

Chater-Diehl, E.J., Laufer, B.I., Singh, S.M., 2017. Changes to histone modifications following prenatal alcohol exposure: An emerging picture. Alcohol 60, 41–52. https://doi.org/10.1016/j.alcohol.2017.01.005

Chen, M., Wang, J., Jiang, J., Zheng, X., Justice, N.J., Wang, K., Ran, X., Li, Y., Huo, Q., Zhang, J., Li, H., Lu, N., Wang, Y., Zheng, H., Long, C., Yang, L., 2017. APP modulates KCC2 expression and function in hippocampal GABAergic inhibition. eLife 6, e20142. https://doi.org/10.7554/eLife.20142

Chesselet, M.-F., Plotkin, J.L., Wu, N., Levine, M.S., 2007. Development of striatal fast-spiking GABAergic interneurons, in: Tepper, J.M., Abercrombie, E.D., Bolam, J.P. (Eds.), Progress in Brain Research, Gaba and the Basal Ganglia. Elsevier, pp. 261–272. https://doi.org/10.1016/S0079-6123(06)60015-0

Clarkson, J., Herbison, A.E., 2016. Hypothalamic control of the male neonatal testosterone surge. Philosophical Transactions of the Royal Society B: Biological Sciences 371, 20150115. https://doi.org/10.1098/rstb.2015.0115

Cuzon, V.C., Yeh, P.W.L., Yanagawa, Y., Obata, K., Yeh, H.H., 2008. Ethanol Consumption during Early Pregnancy Alters the Disposition of Tangentially Migrating GABAergic Interneurons in the Fetal Cortex. J. Neurosci. 28, 1854–1864. https://doi.org/10.1523/JNEUROSCI.5110-07.2008

De Vries, G.J., Rissman, E.F., Simerly, R.B., Yang, L.-Y., Scordalakes, E.M., Auger, C.J., Swain, A., Lovell-Badge, R., Burgoyne, P.S., Arnold, A.P., 2002. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. J Neurosci 22, 9005–9014. https://doi.org/10.1523/JNEUROSCI.22-20-09005.2002

Deidda, G., Allegra, M., Cerri, C., Naskar, S., Bony, G., Zunino, G., Bozzi, Y., Caleo, M., Cancedda, L., 2015. Early depolarizing GABA controls critical period plasticity in the rat visual cortex. Nat Neurosci 18, 87–96. https://doi.org/10.1038/nn.3890

Delatour, L.C., Yeh, P.W., Yeh, H.H., 2019a. Ethanol Exposure In Utero Disrupts Radial Migration and Pyramidal Cell Development in the Somatosensory Cortex. Cereb Cortex 29, 2125–2139. https://doi.org/10.1093/cercor/bhy094

Delatour, L.C., Yeh, P.W.L., Yeh, H.H., 2019b. Prenatal Exposure to Ethanol Alters Synaptic Activity in Layer V/VI Pyramidal Neurons of the Somatosensory Cortex. Cereb. Cortex. https://doi.org/10.1093/cercor/bhz199

Dorris, D.M., Cao, J., Willett, J.A., Hauser, C.A., Meitzen, J., 2015. Intrinsic excitability varies by sex in prepubertal striatal medium spiny neurons. J Neurophysiol 113, 720–729. https://doi.org/10.1152/jn.00687.2014

Fagiolini, M., Fritschy, J.-M., Löw, K., Möhler, H., Rudolph, U., Hensch, T.K., 2004. Specific GABAA circuits for visual cortical plasticity. Science 303, 1681–1683. https://doi.org/10.1126/science.1091032

Favuzzi, E., Huang, S., Saldi, G.A., Binan, L., Ibrahim, L.A., Fernández-Otero, M., Cao, Y., Zeine, A., Sefah, A., Zheng, K., Xu, Q., Khlestova, E., Farhi, S.L., Bonneau, R., Datta, S.R., Stevens, B., Fishell, G., 2021. GABA-receptive microglia selectively sculpt developing inhibitory circuits. Cell. https://doi.org/10.1016/j.cell.2021.06.018

Fluegge, Keith, Fluegge, Kyle, 2018. Antecedent ADHD, dementia, and metabolic dysregulation: A U.S. based cohort analysis. Neurochemistry International 112, 255–258. https://doi.org/10.1016/j.neuint.2017.08.005

Galiñanes, G.L., Taravini, I.R.E., Murer, M.G., 2009. Dopamine-Dependent Periadolescent Maturation of Corticostriatal Functional Connectivity in Mouse. J. Neurosci. 29, 2496–2509. https://doi.org/10.1523/JNEUROSCI.4421-08.2009

Gallo, N.B., Berisha, A., Van Aelst, L., 2022. Microglia regulate chandelier cell axo-axonic synaptogenesis. Proceedings of the National Academy of Sciences 119, e2114476119. https://doi.org/10.1073/pnas.2114476119

Gao, R., Penzes, P., 2015. Common Mechanisms of Excitatory and Inhibitory Imbalance in Schizophrenia and Autism Spectrum Disorders. Curr Mol Med 15, 146–167.

Giorgi, F.S., Saccaro, L.F., Busceti, C.L., Biagioni, F., Fornai, F., 2020. Epilepsy and Alzheimer's Disease: Potential mechanisms for an association. Brain Research Bulletin 160, 107–120. https://doi.org/10.1016/j.brainresbull.2020.04.009

Jacobson, J.L., Akkaya-Hocagil, T., Ryan, L.M., Dodge, N.C., Richardson, G.A., Olson, H.C., Coles, C.D., Day, N.L., Cook, R.J., Jacobson, S.W., 2021. Effects of prenatal alcohol exposure on cognitive and behavioral development: Findings from a hierarchical meta-analysis of data from six prospective longitudinal U.S. cohorts. Alcohol Clin Exp Res 45, 2040–2058. https://doi.org/10.1111/acer.14686

Kato, M., Dobyns, W.B., 2005. X-Linked Lissencephaly With Abnormal Genitalia as a Tangential Migration Disorder Causing Intractable Epilepsy: Proposal for a New Term, "Interneuronopathy." J Child Neurol 20, 392–397. https://doi.org/10.1177/08830738050200042001

Keifman, E., Coll, C., Tubert, C., Paz, R.M., Belforte, J.E., Murer, M.G., Braz, B.Y., 2022. Preserved motility after neonatal dopaminergic lesion relates to hyperexcitability of direct pathway medium spiny neurons. J. Neurosci. https://doi.org/10.1523/JNEUROSCI.1992-21.2022

Kelly, S.M., Raudales, R., He, M., Lee, J.H., Kim, Y., Gibb, L.G., Wu, P., Matho, K., Osten, P., Graybiel, A.M., Huang, Z.J., 2018. Radial Glial Lineage Progression and Differential Intermediate Progenitor Amplification Underlie Striatal Compartments and Circuit Organization. Neuron 99, 345-361.e4. https://doi.org/10.1016/j.neuron.2018.06.021

Kim, S., Oh, H., Choi, S.H., Yoo, Y.-E., Noh, Y.W., Cho, Y., Im, G.H., Lee, C., Oh, Y., Yang, E., Kim, G., Chung, W.-S., Kim, H., Kang, H., Bae, Y., Kim, S.-G., Kim, E., 2022. Postnatal age-differential ASD-like transcriptomic, synaptic, and behavioral deficits in Myt1l-mutant mice. Cell Reports 40, 111398. https://doi.org/10.1016/j.celrep.2022.111398

Klausberger, T., Magill, P.J., Márton, L.F., Roberts, J.D.B., Cobden, P.M., Buzsáki, G., Somogyi, P., 2003. Brain-state- and cell-type-specific firing of hippocampal interneurons in vivo. Nature 421, 844–848. https://doi.org/10.1038/nature01374

Kleiber, M.L., Diehl, E.J., Laufer, B.I., Mantha, K., Chokroborty-Hoque, A., Alberry, B., Singh, S.M., 2014. Long-term genomic and epigenomic dysregulation as a consequence of prenatal alcohol exposure: a model for fetal alcohol spectrum disorders. Frontiers in Genetics 5.

Knowles, R., Dehorter, N., Ellender, T., 2021. From Progenitors to Progeny: Shaping Striatal Circuit Development and Function. J. Neurosci. 41, 9483–9502. https://doi.org/10.1523/JNEUROSCI.0620-21.2021

Konkle, A.T.M., McCarthy, M.M., 2011. Developmental time course of estradiol, testosterone, and dihydrotestosterone levels in discrete regions of male and female rat brain. Endocrinology 152, 223–235. https://doi.org/10.1210/en.2010-0607

Kozorovitskiy, Y., Saunders, A., Johnson, C.A., Lowell, B.B., Sabatini, B.L., 2012. Recurrent network activity drives striatal synaptogenesis. Nature 485, 646–650. https://doi.org/10.1038/nature11052

Kravitz, A.V., Freeze, B.S., Parker, P.R.L., Kay, K., Thwin, M.T., Deisseroth, K., Kreitzer, A.C., 2010. Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. Nature 466, 622–626. https://doi.org/10.1038/nature09159

Küppers, E., Beyer, C., 1998. Expression of aromatase in the embryonic and postnatal mouse striatum. Molecular Brain Research 63, 184–188. https://doi.org/10.1016/S0169-328X(98)00279-4

Lei, X., Han, Z., Chen, C., Bai, L., Xue, G., Dong, Q., 2016. Sex Differences in Fiber Connection between the Striatum and Subcortical and Cortical Regions. Frontiers in Computational Neuroscience 10.

Lenz, K.M., Nugent, B.M., Haliyur, R., McCarthy, M.M., 2013. Microglia Are Essential to Masculinization of Brain and Behavior. J. Neurosci. 33, 2761–2772. https://doi.org/10.1523/JNEUROSCI.1268-12.2013

Lieberman, O.J., McGuirt, A.F., Mosharov, E.V., Pigulevskiy, I., Hobson, B.D., Choi, S., Frier, M.D., Santini, E., Borgkvist, A., Sulzer, D., 2018. Dopamine Triggers the Maturation of Striatal Spiny Projection Neuron Excitability during a Critical Period. Neuron 99, 540-554.e4. https://doi.org/10.1016/j.neuron.2018.06.044

Lopes, E.F., Roberts, B.M., Siddorn, R.E., Clements, M.A., Cragg, S.J., 2019. Inhibition of Nigrostriatal Dopamine Release by Striatal GABAA and GABAB Receptors. J. Neurosci. 39, 1058–1065. https://doi.org/10.1523/JNEUROSCI.2028-18.2018

Lott, I.T., Head, E., 2019. Dementia in Down syndrome: unique insights for Alzheimer disease research. Nat Rev Neurol 15, 135–147. https://doi.org/10.1038/s41582-018-0132-6

Lovinger, D.M., Roberto, M., 2013. Synaptic Effects Induced by Alcohol. Curr Top Behav Neurosci 13, 31–86. https://doi.org/10.1007/7854_2011_143

Magno, L., Asgarian, Z., Pendolino, V., Velona, T., Mackintosh, A., Lee, F., Stryjewska, A., Zimmer, C., Guillemot, F., Farrant, M., Clark, B., Kessaris, N., 2021. Transient developmental imbalance of cortical interneuron subtypes presages long-term changes in behavior. Cell Reports 35, 109249. https://doi.org/10.1016/j.celrep.2021.109249

Marguet, F., Friocourt, G., Brosolo, M., Sauvestre, F., Marcorelles, P., Lesueur, C., Marret, S., Gonzalez, B.J., Laquerrière, A., 2020. Prenatal alcohol exposure is a leading cause of interneuronopathy in humans. Acta Neuropathologica Communications 8, 208. https://doi.org/10.1186/s40478-020-01089-z

Marin, O., Anderson, S.A., Rubenstein, J.L., 2000. Origin and molecular specification of striatal interneurons. J. Neurosci. 20, 6063–6076.

Matsushima, A., Graybiel, A.M., 2020. Combinatorial Developmental Controls on Striatonigral Circuits. Cell Reports 31, 107778. https://doi.org/10.1016/j.celrep.2020.107778

McCarthy, M.M., Nugent, B.M., Lenz, K.M., 2017. Neuroimmunology and neuroepigenetics in the establishment of sex differences in the brain. Nat Rev Neurosci 18, 471–484. https://doi.org/10.1038/nrn.2017.61

McGivern, R.F., Raum, W.J., Salido, E., Redei, E., 1988. Lack of Prenatal Testosterone Surge in Fetal Rats Exposed to Alcohol: Alterations in Testicular Morphology and Physiology. Alcohol: Clinical and Experimental Research 12, 243–247. https://doi.org/10.1111/j.1530-0277.1988.tb00188.x

Mitchell, R., 1980. A novel GABA receptor modulates stimulus-induced glutamate release from cortico-striatal terminals. European Journal of Pharmacology 67, 119–122. https://doi.org/10.1016/0014-2999(80)90018-7

Modol, L., Bollmann, Y., Tressard, T., Baude, A., Che, A., Duan, Z.R.S., Babij, R., De Marco García, N.V., Cossart, R., 2020. Assemblies of Perisomatic GABAergic Neurons in the Developing Barrel Cortex. Neuron 105, 93-105.e4. https://doi.org/10.1016/j.neuron.2019.10.007

Monteiro, C.E., Da Silva, E., Sodré, R., Costa, F., Trindade, A.S., Bunn, P., Costa e Silva, G., Di Masi, F., Dantas, E., 2022. The Effect of Physical Activity on Motor Skills of Children with Autism Spectrum Disorder: A Meta-Analysis. International Journal of Environmental Research and Public Health 19, 14081. https://doi.org/10.3390/ijerph192114081

Morrison, J.H., Baxter, M.G., 2012. The Aging Cortical Synapse: Hallmarks and Implications for Cognitive Decline. Nat Rev Neurosci 13, 240–250. https://doi.org/10.1038/nrn3200

Mowery, T.M., Penikis, K.B., Young, S.K., Ferrer, C.E., Kotak, V.C., Sanes, D.H., 2017. The Sensory Striatum Is Permanently Impaired by Transient Developmental Deprivation. Cell Reports 19, 2462–2468. https://doi.org/10.1016/j.celrep.2017.05.083

Newman, H., Liu, F.-C., Graybiel, A.M., 2015. Dynamic ordering of early generated striatal cells destined to form the striosomal compartment of the striatum. J. Comp. Neurol. 523, 943–962. https://doi.org/10.1002/cne.23725

Okaty, B.W., Miller, M.N., Sugino, K., Hempel, C.M., Nelson, S.B., 2009. Transcriptional and Electrophysiological Maturation of Neocortical Fast-Spiking GABAergic Interneurons. J. Neurosci. 29, 7040–7052. https://doi.org/10.1523/JNEUROSCI.0105-09.2009

Ouyang, C., Guo, L., Lu, Q., Xu, X., Wang, H., 2007. Enhanced activity of GABA receptors inhibits glutamate release induced by focal cerebral ischemia in rat striatum. Neuroscience Letters 420, 174–178. https://doi.org/10.1016/j.neulet.2007.05.004

Peixoto, R.T., Chantranupong, L., Hakim, R., Levasseur, J., Wang, W., Merchant, T., Gorman, K., Budnik, B., Sabatini, B.L., 2019. Abnormal Striatal Development Underlies the Early Onset of Behavioral Deficits in Shank3B-/- Mice. Cell Rep 29, 2016-2027.e4. https://doi.org/10.1016/j.celrep.2019.10.021

Peixoto, R.T., Wang, W., Croney, D.M., Kozorovitskiy, Y., Sabatini, B.L., 2016. Early hyperactivity and precocious maturation of corticostriatal circuits in Shank3B −/− mice. Nature Neuroscience 19, 716–724. https://doi.org/10.1038/nn.4260

Petralia, R.S., Mattson, M.P., Yao, P.J., 2014. Communication breakdown: The impact of ageing on synapse structure. Ageing Research Reviews 14, 31–42. https://doi.org/10.1016/j.arr.2014.01.003

Petroni, V., Subashi, E., Premoli, M., Memo, M., Lemaire, V., Pietropaolo, S., 2022. Long-term behavioral effects of prenatal stress in the Fmr1-knock-out mouse model for fragile X syndrome. Front Cell Neurosci 16, 917183. https://doi.org/10.3389/fncel.2022.917183

Pouille, F., Scanziani, M., 2001. Enforcement of Temporal Fidelity in Pyramidal Cells by Somatic Feed-Forward Inhibition. Science 293, 1159–1163. https://doi.org/10.1126/science.1060342

Proaño, S.B., Morris, H.J., Kunz, L.M., Dorris, D.M., Meitzen, J., 2018. Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core. J Neurophysiol 120, 1356–1373. https://doi.org/10.1152/jn.00263.2018

Ramirez, D.M., Kavalali, E.T., 2011. Differential regulation of spontaneous and evoked neurotransmitter release at central synapses. Current Opinion in Neurobiology, Synaptic function and regulation 21, 275–282. https://doi.org/10.1016/j.conb.2011.01.007

Reiner, O., Karzbrun, E., Kshirsagar, A., Kaibuchi, K., 2016. Regulation of neuronal migration, an emerging topic in autism spectrum disorders. Journal of Neurochemistry 136, 440–456. https://doi.org/10.1111/jnc.13403

Rivard, M., Mestari, Z., Coulombe, P., Morin, D., Mello, C., Morin, M., 2023. Developmental and behavioral groupings can predict changes in adaptive behavior over time in young children with neurodevelopmental disorders. Research in Developmental Disabilities 132, 104390. https://doi.org/10.1016/j.ridd.2022.104390

Roberto, M., Treistman, S.N., Pietrzykowski, A.Z., Weiner, J., Galindo, R., Mameli, M., Valenzuela, F., Zhu, P.J., Lovinger, D., Zhang, T.A., Hendricson, A.H., Morrisett, R., Siggins, G.R., 2006. Actions of Acute and Chronic Ethanol on Presynaptic Terminals. Alcohol Clin Exp Res 30, 222–232. https://doi.org/10.1111/j.1530-0277.2006.00030.x

Roselli, V., Guo, C., Huang, D., Wen, D., Zona, D., Liang, T., Ma, Y.-Y., 2020. Prenatal alcohol exposure reduces posterior dorsomedial striatum excitability and motivation in a sex- and age-dependent fashion. Neuropharmacology 180, 108310. https://doi.org/10.1016/j.neuropharm.2020.108310

Rozycka, A., Liguz-Lecznar, M., 2017. The space where aging acts: focus on the GABAergic synapse. Aging Cell 16, 634–643. https://doi.org/10.1111/acel.12605

Ruggiero, M.J., Boschen, K.E., Roth, T.L., Klintsova, A.Y., 2018. Sex Differences in Early Postnatal Microglial Colonization of the Developing Rat Hippocampus Following a Single-Day Alcohol Exposure. J Neuroimmune Pharmacol 13, 189–203. https://doi.org/10.1007/s11481-017-9774-1

Salem, N.A., Mahnke, A.H., Konganti, K., Hillhouse, A.E., Miranda, R.C., 2021. Cell-type and fetal-sex-specific targets of prenatal alcohol exposure in developing mouse cerebral cortex. iScience 24, 102439. https://doi.org/10.1016/j.isci.2021.102439

Schwarz, J.M., Sholar, P.W., Bilbo, S.D., 2012. Sex differences in microglial colonization of the developing rat brain. Journal of Neurochemistry 120, 948–963. https://doi.org/10.1111/j.1471-4159.2011.07630.x

Skorput, A., Gupta, V.P., Yeh, P.W.L., Yeh, H.H., 2015. Persistent Interneuronopathy in the Prefrontal Cortex of Young Adult Offspring Exposed to Ethanol In Utero. J. Neurosci. 35, 10977–10988. https://doi.org/10.1523/JNEUROSCI.1462-15.2015

Skorput, A., Lee, S.M., Yeh, P.W., Yeh, H.H., 2019. The NKCC1 antagonist bumetanide mitigates interneuronopathy associated with ethanol exposure in utero. eLife 8, e48648. https://doi.org/10.7554/eLife.48648

Soiza-Reilly, M., Azcurra, J.M., 2009. Developmental striatal critical period of activity-dependent plasticity is also a window of susceptibility for haloperidol induced adult motor alterations. Neurotoxicol Teratol 31, 191–197. https://doi.org/10.1016/j.ntt.2009.03.001

Sutherland, S., Brunwasser, S.M., 2018. Sex Differences in Vulnerability to Prenatal Stress: A Review of the Recent Literature. Curr Psychiatry Rep 20, 102. https://doi.org/10.1007/s11920-018-0961-4

Takesian, A.E., Bogart, L.J., Lichtman, J.W., Hensch, T.K., 2018. Inhibitory circuit gating of auditory critical-period plasticity. Nat Neurosci 21, 218–227. https://doi.org/10.1038/s41593-017-0064-2

Tepper, J.M., Sharpe, N.A., Koós, T.Z., Trent, F., 1998. Postnatal development of the rat neostriatum: electrophysiological, light- and electron-microscopic studies. Dev. Neurosci. 20, 125–145. https://doi.org/10.1159/000017308

Tibrewal, M., Cheng, B., Dohare, P., Hu, F., Mehdizadeh, R., Wang, P., Zheng, D., Ungvari, Z., Ballabh, P., 2018. Disruption of Interneuron Neurogenesis in Premature Newborns and Reversal with Estrogen Treatment. J. Neurosci. 38, 1100–1113. https://doi.org/10.1523/JNEUROSCI.1875-17.2017

Toran-Allerand, C.D., 1996. Mechanisms of estrogen action during neural development: Mediation by interactions with the neurotrophins and their receptors? The Journal of Steroid Biochemistry and Molecular Biology, International Symposium of The Journal of Steroid Biochemistry and Molecular Biology 56, 169–178. https://doi.org/10.1016/0960-0760(95)00234-0

Vivanti, G., Tao, S., Lyall, K., Robins, D.L., Shea, L.L., 2021. The prevalence and incidence of early-onset dementia among adults with autism spectrum disorder. Autism Res. https://doi.org/10.1002/aur.2590

Weisz, J., Ward, I.L., 1980. Plasma Testosterone and Progesterone Titers of Pregnant Rats, Their Male and Female Fetuses, and Neonatal Offspring. Endocrinology 106, 306–316. https://doi.org/10.1210/endo-106-1-306

Willett, J.A., Cao, J., Dorris, D.M., Johnson, A.G., Ginnari, L.A., Meitzen, J., 2019. Electrophysiological Properties of Medium Spiny Neuron Subtypes in the Caudate-Putamen of Prepubertal Male and Female Drd1a-tdTomato Line 6 BAC Transgenic Mice. eNeuro 6. https://doi.org/10.1523/ENEURO.0016-19.2019

Yang, J., Yang, X., Tang, K., 2021. Interneuron development and dysfunction. The FEBS Journal n/a. https://doi.org/10.1111/febs.15872

Zorrilla de San Martin, J., Delabar, J.-M., Bacci, A., Potier, M.-C., 2018. GABAergic over-inhibition, a promising hypothesis for cognitive deficits in Down syndrome. Free Radical Biology and Medicine, Down Syndrome: From Development to Adult Life to Alzheimer Disease 114, 33–39. https://doi.org/10.1016/j.freeradbiomed.2017.10.