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Development of GABAergic Inputs to Adult Born Dentate Granule Neurons in the Mouse Hippocampus

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Abstract

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New neurons are continuously produced in the subgranular zone of the dentate gyrus of the hippocampus throughout life. These newborn dentate granule cells (DGCs) undergo a stereotyped process of morphological and functional maturation during the first few weeks after differentiation that recapitulates some aspects of postnatal neuronal development. The inhibitory neurotransmitter GABA is critical for survival, morphological development, and functional maturation of adult-born DGCs (abDGCs). While the importance of GABAergic synaptogenesis to abDGCs survival and development has been well characterized, little is known about the precise pattern of connectivity between abDGCs and local interneurons, the main source of GABA inputs onto abDGCs. This process of maturation and functional integration of abDGCs can be influenced by an array of intrinsic and extrinsic factors ranging from disease to experience.

In this dissertation, I will discuss the development of GABAergic inputs to developing abDGCs and assess whether this process is differentially regulated in a model of a neurodevelopmental disorder as well as by experience. In Chapter 2, I discuss the role of the gene disrupted in Fragile X syndrome *Fmr1* in the functional maturation of abDGCs. Fragile X mental retardation protein (FMRP) has been shown to play a role in adult neurogenesis and behaviors that rely on this process, however it is not known whether the functional synaptic maturation and integration of abDGCs into hippocampal circuits is affected in *Fmr1* knockout (KO) mice. These studies systematically characterized the functional development of abDGCs during the first four weeks after differentiation and demonstrate that the maturation of GABAergic synaptic inputs to these

neurons is not grossly affected by the loss of FMRP. In Chapter 3, I characterize the development of inputs from two morphologically and chemically distinct populations of interneurons in the dentate gyrus. The dentate gyrus contains a diverse population of interneurons, but the present study focused on somatostatin (SST) expressing and parvalbumin (PV) expressing interneurons because these are among the most well-studied interneurons in the dentate gyrus, they can be identified by distinct chemical markers allowing us to genetically label them, and their axons project to distinct regions of the dentate where they innervate separate cellular compartments of mature DGCs. PV interneurons are the most well-characterized inputs to abDGCs, however they have not been studied beyond the first week of abDGC development. I also determined the effects of voluntary wheel running of mice on functional connectivity between abDGCs and these specific populations of interneurons. I show that the time course of development of inputs from PV and SST interneurons is likely explained by the organization of interneuron projections within the dentate. Young abDGCs first receive synaptic input from PV interneurons in the granule cell layer, as these cells mature and extend increasingly complex dendrites into the molecular layer where they receive synaptic inputs from SST interneurons. I also show that voluntary wheel running increases input from both PV and SST interneurons, but with different time courses. Understanding the process of integration of abDGCs into the existing hippocampal circuitry will contribute to our understanding of the role of adult neurogenesis in information processing and disease.

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List of Abbreviations

abDGCs: adult born dentate granule cell

aIPSC: asynchronous inhibitory postsynaptic current

aNSCs: adult neural stem cells

BrdU: 5-bromo-2'-deoxyuridine

CCK: cholecystokinin

ChR2: channelrhodpsin-2

DGC: dentate granule cell

DGCs: dentate granule cells

Dpi: days post injection

DREADDs: designer receptor exclusively activated by designer drugs

Fmr1: Fragile X mental retardation 1

FMRP: Fragile X mental retardation protein

FXS: Fragile X syndrome

GABA: gamma-aminobutyric acid

GAD: glutamic acid decarboxylase

GCL: granule cell layer

GP2: gamma-retroviral gag and pol

HEK: human embryonic kidney

HICAP: hilar commissural associational path

HIPP: hilar perforant pathway associated

LTP: long term potentiation

mIPSC: miniature inhibitory postsynaptic current

MMLV: Moloney Murine Leukemia Virus

MOPP: molecular layer perforant pathway

NKCC1: sodium-potassium-chloride cotransporter 1

nNOS: neuronal nitric oxide synthase

NPY: neuropeptide Y

PV: parvalbumin

SST: somatostatin

RFP: red fluorescent protein

SGZ: subgranular zone

sIPSC: spontaneous inhibitory postsynaptic current

UTR: untranslated region

VIP: vasoactive intestinal polypeptide

Table of Contents

Abstract ……..……..2

Acknowledgement ……..……...4

List of abbreviations ……...……...5

List of figures ……...……...8

Chapter 1: Introduction: Adult hippocampal neurogenesis ……..……..9

Chapter 2: On the development of GABAergic synapses in adult born dentate granule neurons in the Fragile X mouse model …….……..…..……..29

Chapter 3: On the input-specific development of GABAergic synapses in adult born dentate granule neurons ……...……..51

Chapter 4: Discussion and Concluding Remarks ……..……..82

References ……..……..92

List of Tables and Figures

Chapter 1:

Figure 1.1 Summary of interneuron connectivity to abDGCs.

Figure 1.2 Figure 1.2 Adult neurogenesis is altered in Fmr1 KO mice

Chapter 2:

Figure 2.1 Frequency of sIPSCs and mIPSCs is not altered in abDGCs in Fmr1 KO

Figure 2.2 mIPSC amplitude is unaffected by loss of FMRP, but mIPSC decay is slower in abDGCs in Fmr1 KO

Figure 2.3 E_{GABA} in 21 dpi abDGCs in Fmr1 KO and WT

Figure 2.4 Morphology of 21 dpi abDGCs in Fmr1 KO and WT

Chapter 3:

Figure 3.1 Expression of ChR2-EYFP

Figure 3.2 Input output curve of IPSC amplitudes in SST-ChR2 and PV-ChR2 mice

Figure 3.3 Maturation of SST inputs to abDGCs

Figure 3.4 Maturation of PV inputs to abDGCs

Figure 3.5 Differential synaptogenesis of SST and PV inputs to abDGCs

Figure 3.6 Quantal IPSCs evoked by SST and PV interneurons

Figure 3.7 Running changes SST synaptogenesis onto abDGCs

Figure 3.8 Running changes PV synaptogenesis onto abDGCs

Chapter 4:

Figure 4.1 Adult neurogenesis is altered in Fmr1 KO mice, but GABAergic signaling in abDGCs is mostly unaffected by loss of FMRP.

Figure 4.2 PV and SST inputs to abDGCs

Chapter 1.

Introduction:

Adult hippocampal neurogenesis

Adult neurogenesis

The process of generating functional neurons, called neurogenesis, was originally described by Ramon y Cajal and was thought to occur exclusively in the embryonic and perinatal brain. The first evidence to the contrary was found using tritiated thymidine and autoradiography to label dividing cells in the adult rat brain. These studies found neurons and neuroblasts that had undergone mitosis following injection of tritiated thymidine, indicating that new cells were being produced in the adult brain (Altman 1962, Altman and Das 1965). Over a decade later, tritiated thymidine injections combined with electron microscopy confirmed that there were indeed newly generated neurons in the adult rat brain (Kaplan and Hinds 1977). These studies identified the new born neurons as granule cells in the dentate gyrus and olfactory bulb (Kaplan and Hinds 1977). Later studies confirmed the proliferation of neurons in the adult brain by colocalizing BrdU (5-bromo-2'-deoxyuridine) labeling with staining for the neuronal makers such as NeuN, NR1, and MAP2 and confirming the absence of glial markers such as GFAP and S100B (Reynolds and Weiss 1992). The first evidence that adult born dentate granule cells (abDGCs) formed synaptic connections used retrograde labeling combined with BrdU to show that abDGCs form synaptic connections with CA3 neurons (Markakis and Gage 1999). Furthermore, synaptophysin staining surrounding BrdU labeled abDGCs and the presence of dendritic spines suggested the presence of presynaptic connections to 4-week-old abDGCs (Markakis and Gage 1999, van Praag et al. 2002).

Adult neurogenesis in humans

Adult neurogenesis was first described in rodents but has since been demonstrated to be an evolutionarily conserved process that occurs across many species. The first evidence of adult hippocampal neurogenesis in humans utilized postmortem tissue from cancer patients who received intravenous BrdU for diagnostic purposes. Eriksson and colleagues identified BrdU-

positive cells that were co-labeled with the neuronal markers NeuN, calbindin, and NSE (neuron specific enolase) in the adult dentate gyrus (Eriksson et al. 1998). More recently, Spalding and colleagues used environmental ^{14}C exposure from above ground nuclear bomb testing to determine the rate of neurogenesis in the adult human hippocampus (Spalding et al. 2013). Any cells that underwent mitosis prior to the nuclear bomb test would not contain ^{14}C, while those that divided after would. There is a uniform elevation of ^{14}C levels in the genomic DNA of isolated hippocampal neurons from individuals born prior to the onset of nuclear bomb tests, even in the oldest individual in the study who was 42 years old at the onset of the tests, indicating that new neurons were produced in these individuals in adulthood (Spalding et al. 2013). These studies confirm the presence of adult hippocampal neurogenesis in humans, although it may occur at a lower rate that in rodents. About 700 new neurons are added to the adult human hippocampus each day, compared to the roughly 9,000 produced each day in the adult rodent hippocampus (Cameron and McKay 2001, Spalding et al. 2013). However, most of these cells do not survive the first 21 days post-mitosis in the adult rodent hippocampus (Dayer et al. 2003).

Further characterization of postmortem human tissue for evidence of adult hippocampal neurogenesis using histological markers for neural progenitors and immature neurons has proven more controversial. Boldrini and colleagues recently reported the presence of quiescent neural progenitors, intermediate neural progenitors, and neuroblasts (post-mitotic cells that will eventually become immature neurons) in the dentate gyrus of adults ages 14-79 (Boldrini et al. 2018). Conversely, Sorrells and colleagues reported that intermediate neural progenitors were present in human embryonic brains and infant brains up to 1 year of age; however, proliferating cells were rarely observed in the hippocampus of a 7-year-old, and not seen at all in older subjects (Sorrells et al. 2018). They also reported that the number of immature neurons declined sharply with age and were not observed beyond 13 years of age (Sorrells et al. 2018). However, these

studies relied on antibody labeling to identify neurochemical markers of mitosis and immature neurons in postmortem human tissue. One caveat to both of these studies is that the postmortem delay prior to was quite long, up to 48 hours in the study by Sorrels and colleagues and 26 hours in the study by Boldrini and colleagues. Sorrells and colleagues concluded that immature neurons were not present in the adult human brain due to a lack of doublecortin and polysialylated neuronal cell adhesion molecule (DCX+/PSA-NCAM+) labeled cells in individuals older than 13. However, a previous study reported a loss of DCX immunoreactivity with an 8 hour postmortem delay prior to fixation (Boekhoorn et al. 2006). Thus, the absence of DCX staining does not necessarily indicate an absence of immature neurons. Furthermore, these histological markers of adult hippocampal neurogenesis have been well validated in rodents, but the expression of the same markers of proliferating and immature neurons in the adult human hippocampus has not been well characterized. It is possible that there are variations in the rate at which newborn neurons mature across species. Indeed, abDGCs in rats mature much faster than those in mice, but take significantly longer to mature in nonhuman primates (Snyder et al. 2009, Kohler et al. 2011). Therefore, further validation of the use of histological markers will be necessary to fully understand adult neurogenesis in the human hippocampus. While these recent studies have surely sparked debate in the field and reinvigorated investigation into human adult hippocampal neurogenesis, the use of ^{14}C and BrdU labeling to identify cells that have undergone mitosis offers much more compelling evidence of adult neurogenesis in humans.

Role of adult-born dentate granule cells in information processing

Not only are new neurons produced throughout life, these newly generated cells have a critical role in information processing in the hippocampus. The hippocampus is important for learning, memory, mood, and anxiety. The dentate gyrus is the gatekeeper of the hippocampus, it receives excitatory input from the entorhinal cortex and passes information on to the CA3 hippocampal

subfield. Computational studies proposed a role of the dentate in pattern separation, meaning that it transforms similar inputs into distinct outputs (Becker 2005). Biologically, this process would be supported by modulating the firing rate depending on the input or by recruitment of distinct populations of neurons for each input. This is supported by the fact that the dentate has 5-10 times more neurons than its input, the entorhinal cortex (Amaral et al. 2007). The dentate also has sparse coding, whereby a single event is encoded by activation of a small number of neurons (Deng et al. 2010). Indeed, sparse coding is facilitated by feedback and feedforward inhibition from the network of local interneurons (Houser 2007, Booker and Vida 2018). This sparse activity of the dentate is still able to pass on information due to the fact that individual mossy fibers can reliably activate downstream CA3 pyramidal cells (Henze et al. 2002). AbDGCs confer an additional source of plasticity and computing power to this region. While the newborn neurons will mature to become nearly indistinguishable from developmentally born DGCs, there is evidence that these cells are preferentially recruited during specific learning and behavioral tasks and that they play a unique role in information processing, specifically pattern separation, that cannot be replicated by mature DGCs (Ramirez-Amaya et al. 2006, Tashiro et al. 2007, Clelland et al. 2009, Sahay et al. 2011, Nakashiba et al. 2012).

For example, ablation of adult neurogenesis using x-irradiation leads to impaired performance on the pattern separation variation of the radial arm maze and a non-navigable touch screen task (Clelland et al. 2009). Specifically, ablation of adult neurogenesis leads to impaired discrimination of low separation pairs of stimuli, but not high separation pairs (Clelland et al. 2009). Conversely, increasing hippocampal neurogenesis by genetic inhibition of programmed cell death improves pattern separation performance (Sahay et al. 2011). Inhibition of mature DGCs by genetic expression of tetanus toxin while leaving synaptic transmission of abDGCs intact does not interfere with pattern separation behavior (Nakashiba et al. 2012). Together these studies

demonstrate a distinct role for abDGCs in learning that cannot be replicated by mature or developmentally born DGCs.

Newborn neurons in the dentate gyrus are preferentially recruited by the hippocampal circuitry during specific behavioral tasks and are reactivated by subsequent exposure to the same stimuli. Staining for immediate early gene expression combined with BrdU labeling to birthdate abDGCs has been used to determine patterns of neuronal recruitment. For instance, following exploration of a novel environment, the immediate early gene Arc was expressed in more 5-month-old abDGCs than developmentally born DGCs (Ramirez-Amaya et al. 2006). Another study found that abDGCs that are more than 4-weeks-old are more likely to be recruited during training on the Morris water maze task and are re-activated during a probe trial at 10 weeks (Kee et al. 2007). Environmental enrichment recruits 2-3 week old abDGCs, and these cells are preferentially recruited during re-exposure to the same environment when they are 6-weeks old (Tashiro et al. 2007).

Adult hippocampal neurogenesis has also been linked to mood and anxiety. Exposure to contextual fear conditioning increases expression of the immediate early gene cFos in 2-week-old abDGCs (Kirby et al. 2011). In addition, unpredictable chronic mild stress reduces proliferation and survival of abDGCs (Tanti et al. 2013), while ablation of adult hippocampal neurogenesis by focal x-irradiation or genetic ablation impairs contextual fear conditioning (Saxe et al. 2006). In addition, chronic antidepressant treatment increases proliferation (Malberg et al. 2000). In order to understand how abDGCs contribute to hippocampal circuits, we must first know what makes these newly generated neurons unique from the surrounding mature cells.

Development of adult-born neurons

The neurogenic niche in the rodent hippocampus lies in the subgranular zone (SGZ) of the dentate gyrus. Radial glia-like neural stem cells (NSCs) reside in the SGZ and extend an apical process to the molecular layer. These adult NSCs (aNSCs) can transition from quiescence to active division giving rise to cells that will either become neurons or astrocytes. At this point the fate of these cells has been determined and the rapidly dividing neural progenitor cells (NPCs) produce the neuroblasts which will eventually differentiate into neurons (Toni and Schinder 2015). These neurons will then integrate into the existing hippocampal circuit over the first several weeks post-mitosis.

Morphological maturation of abDGCs

Morphological maturation of abDGCs parallels the maturation of developmentally born neurons (Esposito et al. 2005, Ge et al. 2006). abDGCs migrate into the granule cell layer (GCL) during the second week post-differentiation and begin extending dendrites towards the molecular layer (Zhao et al. 2006). Dendritic spines begin to form around 16 days, consistent with the fact that glutamatergic synaptic transmission is rarely observed prior to 14 days post mitosis (Ge et al. 2006, Zhao et al. 2006). Dendritic morphology resembles that of mature DGCs as early as 21 days after differentiation, when distal dendrites reach the outer molecular layer and significant arborization is observed (Zhao et al. 2006); however, the dendritic arbor continues to develop over the first 4 months (van Praag et al. 2002). The axons, or mossy fibers, of newborn DGCs project to the hilus as early as 7 days and they extend to CA3 around 10 days (Zhao et al. 2006). Mossy fibers from abDGCs form synapses with interneurons and mossy cells in the hilus and form functional glutamatergic synapses onto CA3 cells by 17 days (Zhao et al. 2006). Optogenetic activation of 4-week-old and 7-week-old abDGCs reliably evoked EPSCs and IPSCs in CA3 pyramidal cells that were blocked by the AMPAR antagonist NBQX, indicating direct mossy fiber

excitation and feedforward inhibition through interneurons (Temprana et al. 2015). In the same study activation of 4-week-old abDGCs evoked a small inhibitory response in neighboring mature DGCs and activation of 7-week-old abDGCs led to a larger IPSC in mature DGCs (Temprana et al. 2015). Therefore, abDGCs form functional synaptic connections with CA3 pyramidal neurons and provide disynaptic inhibitory feedback onto mature DGCs (Temprana et al. 2015).

Functional maturation of abDGCs

Newborn DGCs have electrical properties characteristic of immature neurons such as high input resistance, increased excitability, and reduced strength of GABAergic inhibition (Schmidt-Hieber et al. 2004, Esposito et al. 2005, Ge et al. 2006, Marin-Burgin and Schinder 2012). Early studies used the characteristically high input resistance of newborn neurons to characterize the functional properties of abDGCs (Schmidt-Hieber et al. 2004). The development of techniques to label abDGCs with genetically encoded fluorescent proteins has allowed for more detailed characterization of the functional characteristics of newborn neurons in the adult hippocampus (van Praag et al. 2002, Overstreet et al. 2004). Labeling abDGCs using viruses or transgenic mice eliminates the need for fixation and staining that are required for BrdU labeling allowing. Moreover, viral delivery or induction of gene expression have been adapted to birthdate neurons. One of the most common methods of labeling newborn neurons is by injecting a mutated oncoretrovirus, the Moloney murine leukemia virus (MMLV), directly into the SGZ by stereotaxic injection. Retroviral infection leads to incorporation of the retroviral genome into the host genome. However, MMLV cannot insert its genome into the nucleus so it can only transduce host cells that are undergoing mitosis, when the nuclear envelope breaks down (Lewis and Emerman 1994). This allows for efficient and selective transduction of rapidly dividing neural progenitors in the neurogenic region (Tashiro et al. 2006). Using retroviral-mediated expression of fluorescent

proteins in NPCs and subsequently in abDGCs combined with electrophysiology has allowed for functional characterization of the maturation and integration of newborn neurons (van Praag et al. 2002, Tashiro et al. 2006). Since the fluorescent protein encoded by the retrovirus is only incorporated into dividing cells, any fluorescent cells underwent mitosis at the time of injection and the number of days post injection (dpi) is correlated with the age of the abDGC allowing the method to accurately birthdate neurons. It is possible that labeled neural stem cells may undergo mitosis several days after viral injection, however Zhao and colleagues showed that only 3.5% of fluorescent cells also expressed the mitotic marker Ki67 at 7 dpi and that this fraction is further reduced to 0.9 % at 10 dpi (Zhao et al. 2006). Therefore, most of the labeled cells are born shortly after viral injection. Retroviral labeling offers more precise approximation of the age of cells that other methods. Genetic labeling of abDGCs using inducible expression of a fluorescent marker in nestin-creERT2 or ascl1-creERT2 mice will label all cells born after the time of induction of cre expression (Lagace et al. 2007, Kim et al. 2011) and in POMC-GFP mice labeled cells are 3-30 days post-mitosis (Overstreet et al. 2004).

AbDGCs undergo a stereotyped pattern of functional synaptic development that recapitulates perinatal neuronal maturation, and eventually become nearly indistinguishable from developmentally born DGCs (Laplagne et al. 2006, Laplagne et al. 2007). AbDGCs are initially activated by tonic GABA, and the first synaptic inputs to developing abDGCs are GABAergic (Ge et al. 2006). Evoked GABA-mediated postsynaptic currents are first detected at 7 dpi, and robust spontaneous GABA-mediated postsynaptic currents are observed by 14 dpi (Ge et al. 2006). Esposito and colleagues were unable to detect evoked GABA-mediated postsynaptic currents at 7 dpi (Esposito et al. 2005). Early GABAergic inputs are excitatory due to a depolarized GABA reversal potential (E_{GABA}). This is due to elevated expression of the juvenile chloride cotransporter NKCC1 in abDGCs during the first 2-3 weeks of development, which results in a relatively high

intracellular chloride concentration in young neurons (Overstreet Wadiche et al. 2005, Ge et al. 2006). Evoked glutamate-mediated postsynaptic currents are first detected at 14 dpi (Ge et al. 2006). Newly generated neurons have NMDAR-only silent synapses and undergo a period of enhanced synaptic plasticity due to a reduced threshold for induction and an increased amplitude of LTP during development (Ge et al. 2007, Chancey et al. 2013). Interestingly, LTP can be induced with intact GABA signaling in 4-week-old abDGCs, but can only be induced in the presence of the $GABA_AR$ antagonist bicuculine in 8-week-old abDGCs (Ge et al. 2007). In fact, the presence of abDGCs is required for the induction of LTP in the dentate gyrus with intact inhibition (Saxe et al. 2006). The immature properties of these newly generated neurons are thought to confer a distinct role in information processing by allowing abDGCs to be preferentially recruited by network activity and undergo plasticity that neighboring mature DGCs cannot (Ge et al. 2007, Dieni et al. 2012).

The role of GABA in adult hippocampal neurogenesis

GABA inputs to abDGCs are important for survival, dendritic development, and subsequent formation and unsilencing of glutamatergic synapses. In adult neural stem cells and abDGCs, GABA is initially depolarizing (Tozuka et al. 2005, Ge et al. 2006, Chancey et al. 2013), similar to what occurs in early postnatal development in the cortex and hippocampus (He et al. 2014, Tyzio et al. 2014). The depolarizing response to GABA is due to a depolarized reversal potential for $GABA_A$ receptors (E_{GABA}), which is determined by the chloride equilibrium potential. The juvenile chloride transporter NKCC1 transports chloride into the cell, leading to a relatively elevated intracellular chloride concentration in abDGCs (Ge et al. 2006). By four weeks after differentiation, an increase in the expression of the mature chloride cotransporter KCC2, which extrudes chloride, relative to expression of NKCC1 leads to a reduction of the intracellular chloride concentration and a mature, hyperpolarized E_{GABA} (Ge et al. 2006).

Early depolarizing GABA facilitates the trophic, or development-promoting, effects of GABA in newborn neurons (Owens and Kriegstein 2002, Overstreet-Wadiche et al. 2006). Optogenetic activation of parvalbumin (PV) expressing interneurons *in vivo* reduces the number of newly generated neurons, while inhibition of PV interneurons increases the number of new neurons, indicating that GABA regulates proliferation by maintaining the quiescence of aNSCs (Song et al. 2012). However, GABA also promotes survival and differentiation of abDGCs. Blocking GABA signaling by systemic injection of the $GABA_A$ receptor ($GABA_AR$) antagonists picrotoxin or pentylenetetrazole prevents neural progenitors from exiting the cell cycle, while the $GABA_AR$ agonists phenobarbital or pentobarbital increases the number of newly generated neurons (Tozuka et al. 2005). Eliminating depolarizing GABA by shRNA-mediated knockdown of NKCC1 in abDGCs reduces survival and arrests dendritic development (Jagasia et al. 2009). *In vivo* administration of diazepam to enhance the response of γ2 subunit-containing $GABA_ARs$ to GABA maintains quiescence of aNSCs, while conditional deletion of γ2 from aNSCs increases proliferation (Song et al. 2012). *In vivo* optogenetic activation of GABAergic inputs to 1-4 dpi abDGCs increases survival, indicating opposing roles for GABA in regulating the proliferation and survival aNSCs and aNPCs (Song et al. 2013). These studies demonstrate opposing roles for GABA in promoting quiescence of aNSCs and promoting proliferation and survival of aNPCs and abDGCs.

GABA also plays a role in morphological maturation of abDGCs. In young abDGCs, optogenetic activation of GABAergic inputs from 1-4 dpi promotes dendritic development (Song et al. 2013). Disruption of tonic GABA signaling by deletion of the $GABA_AR$ α4 subunit impairs dendritic development in 2-week-old abDGCs, while deletion of the α2 subunit disrupted dendritic development in 4-week-old abDGCs (Duveau et al. 2011). The trophic effects of GABA are

thought to be mediated by tonic GABA currents (Song et al. 2012, Song et al. 2013), however disruption of tonic GABA currents by deletion of the $GABA_AR$ α4 subunit did not affect survival or proliferation, indicating that the early trophic effects of GABA might be at least partially mediated by phasic GABA. GABA inputs are also required for experience-dependent dendritic remodeling in abDGCs (Alvarez et al. 2016). Inhibition of parvalbumin (PV) interneurons with DREADDs prevented increased dendritic complexity in abDGCs following exposure to environmental enrichment, and activation of PV interneurons alone lead to increased dendritic complexity (Alvarez et al. 2016). Together, these studies indicate that GABA promotes the morphological maturation of newborn neurons in the adult hippocampus.

GABA also plays a pivotal role in synaptic development in abDGCs. Blocking depolarizing GABA by shRNA-mediated knockdown of NKCC1 disrupts dendritic development and delays the formation of GABA and glutamate-mediated synapses in abDGCs, even at time points after GABA would be expected to be hyperpolarizing (Ge et al. 2006). At glutamatergic synapses, depolarizing GABA is necessary for NMDAR activation and subsequent incorporation of AMPARs at previously silent synapses as well as experience-dependent synapse unsilencing in abDGCs (Chancey et al. 2013). The NKCC1 inhibitor bumetanide prevented increased AMPAR incorporation in abDGCs in slices and prevented synapse unsilencing *in vivo* following exposure to an enriched environment (Chancey et al. 2013). Together these studies demonstrate a critical role for GABA inputs in the functional maturation of abDGCs, however the specific interneurons providing GABAergic inputs to developing abDGCs remain largely unknown.

Interneurons in the dentate gyrus and adult hippocampal neurogenesis

The dentate gyrus contains a diverse population of interneurons. Early morphological characterization of dentate interneurons identified at least 21 different cell types in the hilus alone (Amaral 1978). These neurons have since been classified by chemical identity, morphology, and specificity of post-synaptic target domain (Freund and Buzsáki 1996, Houser 2007, Booker and Vida 2018). While the pattern of development of connections between abDGCs and interneurons is not exactly known, a few studies have identified presynaptic interneurons at single time points during maturation (**Figure 1.1**). Retrograde transynaptic tracing using modified rabies virus combined with retroviral birthdating show that somatostatin (SST), parvalbumin (PV), and cholecystokinin (CCK) expressing interneurons synapse onto abDGCs at 3-5 weeks (Li et al. 2013, Bergami et al. 2015). Paired recordings and glutamate uncaging have identified molecular layer perforant pathway (MOPP) (Li et al. 2013) and Ivy/neurogliaform cells (Markwardt et al. 2011) as presynaptic inputs to abDGCs. However, these studies did not quantify the number of each type of interneuron or compare how these inputs change during maturation.

Much more is known about the connectivity of dentate interneurons with mature DGCs. PV interneurons in the dentate are basket cells with their somata located at the base of the GCL and axons projecting within the GCL to form perisomatic synapses onto mature DGCs (Houser 2007, Savanthrapadian et al. 2014). PV interneurons also form axo-axonic synapses onto DGCs (Soriano and Frotscher 1989, Booker and Vida 2018). PV interneurons are the most well-studied

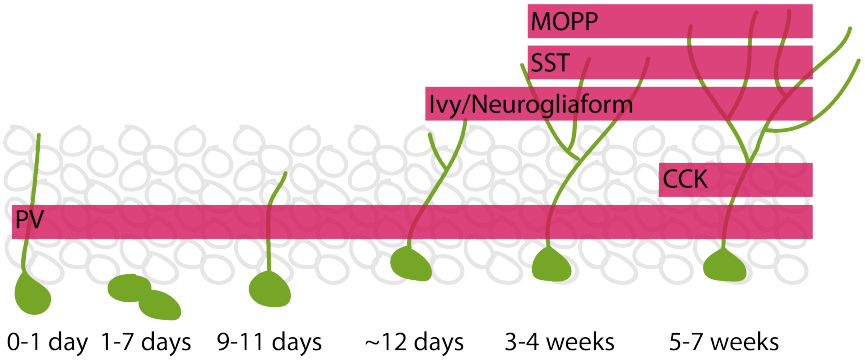

Figure 1.1 Summary of interneuron connectivity to abDGCs
Optogenetic activation of specific interneurons, paired recordings, and retrograde transynaptic tracing have been used to identify presynaptic inputs to abDGCs at individual time points.

source of GABA input to abDGCs. Tonic GABA from PV interneurons maintains quiescence of aNSCs (Song et al. 2012), while *in vivo* activation of PV interneurons using DREAADs or optogenetics increases the survival and promotes dendritic growth of abDGCs (Song et al. 2013, Alvarez et al. 2016).

SST interneurons in the dentate are primarily classified as HIPP (hilar perforant pathway associated) cells with somata in the hilus and axons projecting through the GCL to form synapses onto the dendrites of mature DGCs in the molecular layer (Houser 2007, Savanthrapadian et al. 2014). Much less is known about SST inputs to abDGCs. Song and colleagues did report that they were unable to detect SST inputs to 4 dpi abDGCs using optogenetic activation (Song et al. 2013).

PV and SST interneurons will be the focus of Chapter 3 but it is important to note that these are not the only known or potential interneuron inputs to abDGCs. Cholecystokinin (CCK) interneurons have also been identified as presynaptic inputs to abDGCs (Bergami et al. 2015). CCK interneurons in the dentate have been classified as both basket cells and HICAP (hilar commissural/associational pathway) cells. The somata of hilar commissural associational path (HICAP) interneurons reside on the border of the hilus and the GCL and axons project to the inner molecular layer, so it is possible that CCK-expressing basket cells and HICAP cells are actually a single overlapping population of dentate interneurons (Hefft and Jonas 2005, Houser 2007, Booker and Vida 2018). Paired recordings from POMC-GFP labeled abDGCs identified Ivy/neurogliaform cells as presynaptic partners to abDGCs that are approximately 2 weeks old (Markwardt et al. 2011). Ivy/ neurogliaform cells are found in both the molecular layer and the hilus and form synapses onto the proximal dendrites of mature DGCs and abDGCs (Armstrong et al. 2011, Markwardt et al. 2011). These interneurons release the neuromodulator neuropeptide

Y (NPY) which regulates proliferation of aNSCs *in vitro* and *in vivo* (Howell et al. 2003, Howell et al. 2007). The somata and axons of MOPP cells are found in the outer molecular layer where they synapse onto the distal dendrites of mature DGCs and abDGCs (Halasy and Somogyi 1993, Li et al. 2013, Booker and Vida 2018). MOPP cells represent a heterogenous population of dentate interneurons, some of which are Ivy/neurogliaform cells (Armstrong et al. 2011). The somata of vasoactive intestinal peptide-expressing (VIP) interneurons are found in the GCL with axons projecting to the GCL and inner molecular layer, but connectivity with mature or abDGCs has not been described (Sik et al. 1997). However, previous studies have indicated a role for VIP expressing interneurons in adult neurogenesis as the VIP receptors VPAC1 and VPAC2 have been shown to regulate fate specification and symmetric division of aNSCs (Zaben et al. 2009).

It is possible that inputs from specific interneuron populations differentially regulate abDGC maturation. Indeed, Song and colleagues have demonstrated this type of input specificity in regulation of aNSC quiescence (Song et al. 2012). *In vivo* optogenetic activation of PV interneurons maintains quiescence of aNSCs while inhibition promotes activation and division of aNSCs, but similar manipulation of SST or VIP interneurons had no effect on aNSC quiescence (Song et al. 2012). Survival of abDGCs was also promoted specifically by *in vivo* activation of PV but not SST interneurons (Song et al. 2013). While it is clear from these studies that the role of GABA in adult neurogenesis may be input-specific, studies characterizing this input-specific regulation of adult neurogenesis have thus far focused on the earliest stages of neurogenesis (proliferation and survival) and have not assessed the role of specific inputs in maturation of abDGCs.

Understanding the temporal sequence of interneuron innervation of abDGCs by specific interneurons may help us better understand how specific populations of interneurons regulate the

different stages of abDGC maturation. The organization of the axons from dentate interneurons may determine the temporal sequence of innervation of abDGCs. As the neurites of newborn neurons extend through the GCL and into the molecular layer they encounter an increasingly diverse population of interneurons (Dieni et al. 2012). Determining the time course of development of inputs from specific population of interneurons and abDGCs will shed light on how different interneuron subtypes contribute to the various roles in GABA signaling in developing abDGCs. It is possible that inputs from specific interneurons contribute differentially to the role of GABA signaling in maturation and later on as a regulator of neural timing and synchronization. In Chapter 3, I will describe experiments I performed to dissect the time course of development of inputs from two distinct populations of interneurons, SST+ HIPP cells and PV+ basket cells, onto abDGCs in the first four weeks after differentiation.

Regulation of adult hippocampal neurogenesis

The proliferation, differentiation, survival, morphological maturation, and functional integration of adult born neurons is a dynamic process that can be regulated by a number of different intrinsic and extrinsic factors. For example, genetic and physiological conditions such as epilepsy, neuropsychiatric disorders, and aging alter adult hippocampal neurogenesis. Adult neurogenesis is also regulated by experiences such as stress, running, and environmental enrichment (van Praag et al. 1999, Toda et al. 2018).

Adult neurogenesis and disease

The importance of abDGCs to hippocampal function and memory has raised the possibility that alterations in neural stem cell proliferation as well as maturation and integration of abDGCs contribute to the pathology of neuropsychiatric and neurodevelopmental disorders. Indeed, knockdown of the schizophrenia-linked gene *Disc1* in abDGCs leads to aberrant migration, dendritic morphology, and axonal targeting in abDGCs as well as behavioral deficits (Zhou et al. 2013). Animal models of autism spectrum disorders (ASDs) also have deficits in adult neurogenesis. CNTNAP2 KO mice and Shank3 mutant mice both have a reduced number of immature neurons and a reduced number of radial-glia-like neural progenitors in the adult dentate gyrus (Cope et al. 2016). The gene responsible for Rett syndrome, MeCP2, has been shown to regulate quiescence of NSCs, morphological maturation, and the transition to expressing NeuN, a marker of mature neurons (Smrt et al. 2007, Gao et al. 2015, Chen et al. 2017). Alterations in adult hippocampal neurogenesis have also been described in an animal model of Fragile X syndrome (FXS) (**Figure 1.2**). Loss of FMRP increased the rate of proliferation of adult NPCs and altered their fate specification in adult *Fmr1* KO mice (Luo et al. 2010). In addition, conditional deletion of FMRP from aNSCs leads to deficits in behavioral tasks that rely on adult hippocampal neurogenesis (Luo et al. 2010). I will discuss the role of FMRP in adult neurogenesis in more detail in Chapter 2, where I will describe studies that characterized the functional synaptic maturation and integration of abDGCs into

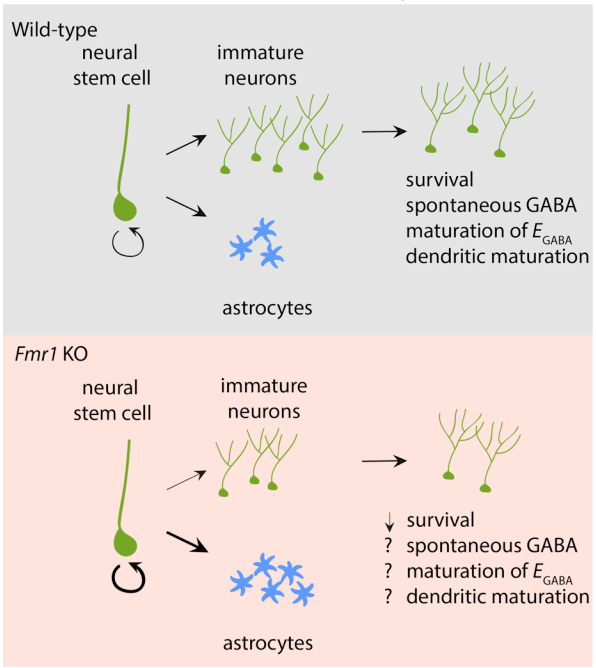

Figure 1.2 Adult neurogenesis is altered in *Fmr1* KO mice
Loss of FMRP leads to an increased rate of proliferation of adult NPCs, altered fate specification, and reduced survival of abDGCs in adult *Fmr1* KO mice. Whether loss of FMRP leads to changes in GABAergic signaling in abDGCs is not known.

hippocampal circuits during the first four weeks after differentiation in *Fmr1* KO mice.

Effect of experience on adult hippocampal neurogenesis

Experience can alter adult hippocampal neurogenesis in a number of ways. The first evidence of experience-dependent changes in adult hippocampal neurogenesis found that housing mice in an enriched environment (EE) increases the number of new neurons in the adult mouse brain (Kempermann et al. 1997). This increase in neurogenesis correlates with improved spatial learning as assessed by the Morris water maze task (Kempermann et al. 1997). However, EE is a complex combination of experiences including larger housing, increased social and novel inanimate object stimulation, and exercise (van Praag et al. 1999). Later studies found that voluntary wheel running in the absence of other enrichment similarly increases the proliferation and survival of abDGCs, improves acquisition on the Morris water maze task, and increases LTP in the dentate gyrus (van Praag et al. 1999, van Praag et al. 1999). Although running has a clear effect on adult hippocampal neurogenesis, other forms of exercise such as swimming which is required for completion of the Morris water maze task do not alter adult hippocampal neurogenesis (van Praag et al. 1999). In addition, running increases dendritic length and branching in 7 dpi abDGCs (Sah et al. 2017). These studies demonstrate a role for running in the early stages of adult hippocampal neurogenesis, which lead to improved performance on behavioral tasks. However, how experience affects the functional maturation and integration of abDGCs into the hippocampal network is just beginning to be explored.

Retrograde transynaptic tracing has been used to assess experience-dependent changes in abDGC connectivity (Deshpande et al. 2013, Bergami et al. 2015, Vivar et al. 2016, Sah et al. 2017). These studies have found that running increases the total number of labeled abDGCs and presynaptic cells in 1-week-old and 5-week-old abDGCs, but decreases connectivity between

local hippocampal neurons and 5-week-old abDGCs (Vivar et al. 2016, Sah et al. 2017). However, another study found that running increases the overall connectivity ratio of presynaptic cells to postsynaptic abDGCs that were 7 weeks old (Deshpande et al. 2013). Bergami and colleagues also found that EE increases local and long range connectivity to 6 week-old abDGCs, but running specifically increases connectivity with cells in the molecular layer of the dentate gyrus and cells in the entorhinal cortex, medial septum and nucleus diagonal band of Broca (Bergami et al. 2015). Conversely, Vivar and colleagues found that running decreases connectivity between local hippocampal neurons and 5-week-old abDGCs (Vivar et al. 2016). While these studies indicate an effect of running on abDGC connectivity, the nature of this change is unclear and the methods used to date do not fully assess functional connectivity. I will further discuss the effects of running on adult hippocampal neurogenesis in Chapter 3, where I will describe studies aimed at determining the effect of running on integration of abDGCs into the existing hippocampal circuit during the first four weeks after differentiation.

In this dissertation, I will discuss the development of GABAergic inputs to developing abDGCs and how this process is differentially regulated by disease and experience. In Chapter 2, I will present data on the effect of disruption of the gene that causes Fragile X syndrome, *Fmr1,* on the functional maturation of abDGCs. The protein product of the *Fmr1* gene, FMRP, has been shown to play a role in adult neurogenesis and behaviors that rely on this process, however it is not known whether the functional synaptic maturation and integration of abDGCs into hippocampal circuits is affected in *Fmr1* KO mice. I have systematically characterized the functional development of abDGCs during the first four weeks after differentiation and demonstrate that the maturation of GABAergic synaptic inputs to these neurons is not grossly affected by the loss of FMRP. In Chapter 3, I will demonstrate the input-specific development of GABAergic synapses onto abDGCs and the effects of experience on this process. I systematically characterized the

development of inputs from two morphologically and chemically distinct populations of interneurons in the dentate gyrus, somatostatin (SST) and parvalbumin (PV) expressing interneurons and determine the effects of running on functional connectivity between abDGCs and these specific populations of interneurons. I demonstrate that the temporal sequence of inhibitory synaptogenesis is likely explained by the organization of interneuron projections within the dentate. I also show that running significantly alters input from both PV and SST interneurons, but with different time courses. Understanding the process of integration of abDGCs into the existing hippocampal circuitry contributes to our understanding of the role of adult neurogenesis in information processing and disease.

Chapter 2.

On the development of GABAergic synapses in adult born dentate granule neurons in the Fragile X mouse model

Abstract:

Fragile X syndrome is the most common form of inherited mental retardation and the most common known single gene cause of autism. Loss of Fragile X mental retardation protein (FMRP) in mice (*Fmr1* KO) leads to altered synaptic and circuit maturation in the hippocampus that are correlated with alterations in hippocampal-dependent behaviors. Previous studies have demonstrated that loss of FMRP increased the rate of proliferation of progenitor cells and altered their fate specification in adult *Fmr1* KO mice. While these studies clearly demonstrate a role for FMRP in adult neurogenesis in the hippocampus, it is not known whether the functional synaptic maturation and integration of adult born dentate granule cells (abDGCs) into hippocampal circuits is affected in *Fmr1* KO mice. Here I used retroviral labeling to birthdate abDGCs in *Fmr1* KO mice which allowed me to perform targeted patch clamp recording to measure the development of synaptic inputs to these neurons at precise time points after differentiation. The frequency and amplitude of spontaneous GABAergic events increased over the first three weeks after differentiation, however this normal development of GABAergic synapses was not altered in *Fmr1* KO mice. Furthermore, the relatively depolarized GABA reversal potential (E_{GABA}) in immature abDGCs was unaffected by loss of FMRP as was the development of dendritic arbor of the adult generated neurons. These studies systematically characterized the functional development of abDGCs during the first four weeks after differentiation and demonstrate that the maturation of GABAergic synaptic inputs to these neurons is not grossly affected by the loss of FMRP.

Introduction

Fragile X Syndrome

Fragile X syndrome (FXS) is the most common known genetic cause of autism and intellectual disability. Individuals with FXS often have developmental delays, hyperactivity, attention deficits, anxiety, and sensory hypersensitivity. FXS is caused by the expansion of the CGG repeat in the 5' UTR of the *Fmr1* gene, which leads to hypermethylation, transcriptional silencing, and loss of expression of the protein product, Fragile X mental retardation protein (FMRP) (Willemsen et al. 2011). FMRP is an RNA-binding protein that regulates a large number of messenger RNAs, many of which encode synaptic proteins. Dysregulated expression of synaptic proteins is thought to perturb synapse maturation and plasticity, however the specific mechanisms underlying synaptic and cognitive deficits in FXS remain unclear. In many neuronal types FMRP and related proteins play a role in neuronal development and synaptic function. There are alterations in the development of synapses particularly during early cortical development in *Fmr1* KO mice (Nimchinsky et al. 2001, Cruz-Martin et al. 2010, Harlow et al. 2010). In several instances, these morphological and functional changes normalize later in development (Bureau et al. 2008); however, phenotypes associated with these circuits persist in adult *Fmr1* KO mice (Arnett et al. 2014, He et al. 2017).

Adult neurogenesis is altered in Fragile X Syndrome

There are a number of studies demonstrating that FMRP plays an important role in adult neural stem cells in the neurogenic niche in the subgranular zone (SGZ) of the hippocampus (Li and Zhao 2014). As discussed in Chapter 1, the importance of abDGCs to hippocampal function, memory processes, and potentially to several neuropsychiatric disorders (Goncalves et al. 2016) has raised the possibility that alterations in neural stem cell proliferation and maturation and integration of abDGCs contribute to the pathology of FXS. Proliferation of adult neural progenitors

is enhanced in mice lacking FMRP (*Fmr1* KO) (Luo et al. 2010) (but also see (Eadie et al. 2009)) and fate specification is altered with fewer neural progenitors differentiating into neurons (Luo et al. 2010). There is also an overall reduction in the number DGCs in older adult *Fmr1* KO mice, which is likely due to reduced survival of adult-born neurons (Lazarov et al. 2012). Furthermore, knockdown of FMRP specifically in adult neural stem cells results in cell autonomous effects on proliferation, fate specification, and hippocampal-dependent behaviors (Guo et al. 2011). Interestingly, difficult hippocampal-dependent behaviors that require intact adult hippocampal neurogenesis are impaired in the *Fmr1* KO. *Fmr1* KO mice have deficits in trace fear conditioning (Zhao et al. 2005). This phenotype can be recapitulated by specific deletion of FMRP in adult neural stem cells and can be rescued by re-expression of FMRP only in adult neural stem cells in a global *Fmr1* KO (Guo et al. 2011). Despite these known disruptions in neurogenesis in *Fmr1* KO mice, whether FMRP loss also affects how abDGCs mature and integrate into the existing hippocampal circuitry after differentiation is unclear.

Evidence of altered GABA signaling in Fragile X Syndrome

Previous studies have found that the development of a mature, hyperpolarized E_{GABA} is delayed in the somatosensory cortex and hippocampus of *Fmr1* KO mice during postnatal development due to aberrant expression of the juvenile chloride cotransporter NKCC1 (He et al. 2014, Tyzio et al. 2014). In addition, expression of some $GABA_A$ receptor subunits is reduced in the cortex, hippocampus, or forebrain of *Fmr1* KO mice (El Idrissi et al. 2005, D'Hulst et al. 2006, Gantois et al. 2006, Adusei et al. 2010). In some cases, these alterations in expression occur only early in development (Adusei et al. 2010). Expression of the glutamate decarboxylase enzyme (GAD) responsible for converting glutamate to GABA is increased in the hippocampus but decreased in the amygdala of *Fmr1* KO mice (El Idrissi et al. 2005, Olmos-Serrano et al. 2010). Functionally, both the frequency and amplitude of miniature and spontaneous IPSCs were reduced in the

amygdala of *Fmr1* KO mice (Olmos-Serrano et al. 2010). However, a similar alteration was not observed in the subiculum of older mice (Curia et al. 2009). Since GABA plays a critical role in the maturation of abDGCs and GABA signaling is dysregulated in FXS, it is possible that GABAergic inputs may be altered in abDGCs in *Fmr1* KO mice.

Evidence of altered neuronal morphology in Fragile X Syndrome

Neuronal morphology is often used to assess maturation of abDGCs, and there is evidence that FMRP regulates neuronal morphology in other brain regions. Some groups have reported reduced dendritic spine density or immature spine morphology in *Fmr1* KO mice, however there is no consensus on the effect of loss of FMRP on dendritic spines (He and Portera-Cailliau 2013). Total neurite length and complexity were reduced in cultured cells derived from aNSCs lacking FMRP and dendritic complexity was reduced in 56 dpi abDGCs in *Fmr1* KO mice (Guo et al. 2011). In 4-week-old abDGCs, loss of FMRP exacerbates the effects of loss of its autosomal paralog Fxr2, leading to a further reduction in surface GluA1 expression and dendritic growth compared to loss of Fxr2 alone (Guo et al. 2015).

FMRP clearly plays a role in postnatal neuron development and function, but the precise role of FMRP in functional maturation of abDGCs has not been characterized. As described in Chapter 1, abDGCs undergo a stereotyped process of morphological and functional maturation that recapitulates postnatal development in the first few weeks after differentiation (Esposito et al. 2005, Ge et al. 2006). Given the critical role of GABA signaling in early post-mitotic maturation of abDGCs (Ge et al. 2006), and also the known alterations in GABA signaling during early postnatal development (He et al. 2014, Nomura et al. 2017) and in juvenile *Fmr1* KO mice (Paluszkiewicz et al. 2011, Martin et al. 2014, Zhang et al. 2017), one possibility is that the time course of development of GABAergic inputs onto abDGCs is altered in *Fmr1* KO mice, affecting their

integration into hippocampal circuitry. To test this hypothesis, I used retroviral labeling to birthdate abDGCs in *Fmr1* KO mice, allowing me to perform targeted patch clamp recording to measure the development of synaptic inputs to these neurons at precise time points after differentiation. The frequency and amplitude of spontaneous GABAergic events increased over the first three weeks after differentiation in recordings from control mice. Comparisons with recordings from *Fmr1* KO mice revealed no difference in the size or frequency of events demonstrating that this normal process of synaptic development is not affected by loss of FMRP. Furthermore, the relatively depolarized GABA reversal potential (E_{GABA}) in immature abDGCs was unaffected in *Fmr1* KO mice, nor was the development of dendritic arbor of abDGCs. These studies systematically characterized the functional development of abDGCs during the first four weeks after differentiation and demonstrate that the maturation of GABAergic synaptic inputs to these neurons and their morphological development is not grossly affected by the loss of FMRP. The work presented in this Chapter has been published (Remmers and Contractor 2018).

Materials and Methods

Animals

All procedures related to the care and treatment of animals were performed in accordance with Northwestern University IACUC. *Fmr1* KO mice (C57Bl/6) were maintained by breeding heterozygous females with WT or KO males. All experiments were performed blind to genotype in age-matched male littermates ($Fmr1^{-/y}$). *Fmr1* is an X-linked gene and therefore the use of male mice is relevant to the human disorder. Tail biopsies were used to perform *post hoc* genotyping of all mice used in the study.

Retroviral birthdating

A replication incompetent retrovirus based on MMLV (Moloney Murine Leukemia Virus) expressing RFP was prepared as described (Tashiro et al. 2006, Tashiro et al. 2015). Briefly, HEK-293 cells stably expressing GP2 were co-transfected with RFP and VSVG using Lipofectamine 2000. The media was collected from transfected cells 3 and 6 days post-transfection, filtered and centrifuged at 25,000 rpm to precipitate the virus. 8-10 week old *Fmr1* WT or KO males were anesthetized using ketamine/xylazine and 1 µl of virus was injected bilaterally into the subgranular zone of the dentate gyrus at a rate of ~0.3 µl/min.

Slice preparation and electrophysiology

250 µm coronal slices were prepared at 14, 21, and 28 (± 1) days post injection. Slices were prepared using a Leica Vibratome in ice-cold high-sucrose artificial CSF (ACSF) containing the following (in mM): 85 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 0.5 $CaCl_2$, and 4 $MgCl_2$, equilibrated with 95% O_2 and 5% CO_2 and including 10 µM DL-APV and 100 µM kynurenate. Slices were heated to 28°C in the same sucrose-ACSF, then the sucrose solution was gradually exchanged for recovery ACSF containing the following (in mM): 125 NaCl, 2.4 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 1 $CaCl_2$, 2 $MgCl_2$, 0.01 DL-APV, and 0.1 kynurenic acid.

After a 60 min recovery, individual slices were transferred to a recording chamber and continuously perfused with oxygenated ACSF containing 2 mM $CaCl_2$ and 1 mM $MgCl_2$ at an elevated temperature of 32°C. The dentate gyrus was visually identified, and targeted recordings were made from RFP-expressing dentate granule cells. Recording electrodes were manufactured from borosilicate glass pipettes and had tip resistances of 4–6 MΩ when filled with internal recording solution. For whole-cell recordings, internal recording solution contained the following (in mM): 95 CsF, 25 CsCl, 10 Cs-HEPES, 10 Cs-EGTA, 2 NaCl, 2 Mg-ATP, 10 QX-314, 5 TEA-Cl, and 5 4-AP, pH adjusted to 7.3 with CsOH. Data were collected and analyzed using pClamp

10 software (Molecular Devices). Neurons were voltage-clamped at -70 mV to record sIPSCs and mIPSCs, which were isolated by inclusion of D-APV (50 μM), CNQX (10 μM), and TTX (1 μM) for mIPSCs. MiniAnalysis (Synaptosoft) was used to analyze sIPSCs and mIPSCs. For perforated patch recordings, the pipette solution contained the following (in mM): 150 KCl and 100 HEPES, pH adjusted to 7.2 with Tris-OH. The pipette tip was filled with gramicidin-free KCl solution and then backfilled with solution containing gramicidin (100 μg/ml). GABAergic currents were evoked using a picospritzer to deliver a 50 ms puff of 10 μM GABA in the presence of 50 μM D-APV and 10 μM CNQX. Responses were recorded at holding potentials between -100 and 0 mV. The GABA reversal potential was calculated as the x-axis intercept of the best-fit line of the current-voltage plot.

Two-photon laser scanning microscopy

Labeled abDGCs were patched in the whole cell configuration as described above with the structural dye Alexa Fluor 488 (50 μM) included in the internal solution. Dye was allowed to perfuse through the cell for ~20 min prior to image acquisition. Fluorescent images were acquired with picosecond pulsed excitation at 790 nM. Images of the soma and dendrites were acquired with 0.19-μm^2 pixels with 10 μs pixel dwell time with 1.0 μm focal steps. Neuron Studio was used to create 3-D reconstructions of the dendrites and morphological analysis was performed in NeuronStudio (Wearne et al. 2005) and ImageJ.

Data analysis

Data analysis was performed using Microsoft Excel and OriginPro 2017 software. mIPSC and sIPSCs were analysed using MiniAnalysis (Synaptosoft). Decay kinetics of mIPSC events were measured as the time to decay from 90 to 37% of the peak amplitude on the falling phase of the

response. Comparisons were made with a Mann-Whitney *U* test, unless otherwise indicated. Differences were considered significant when $p < 0.05$. Data are shown as mean ± SEM.

Results

Development of spontaneous GABA currents in abDGCs in Fmr1 KO mice

To identify and birthdate newborn DGCs, I injected a modified retrovirus expressing RFP into the subgranular zone of 8-10 week old *Fmr1* KO mice and WT littermates (Tashiro et al. 2015). Retroviral injection clearly labeled neurons located in the SGZ and GCL of the dentate gyrus (**Figure 2.1 A**). Evoked IPSCs have been detected in abDGCs as early as 7 days post-injection (dpi) (Ge et al. 2006) and in our recordings the earliest time point at which I consistently observed spontaneous inhibitory events was at 14 dpi. Based on this, I performed targeted patch-clamp recordings from RFP-expressing cells at 14, 21, and 28 dpi. I first measured the frequency of spontaneous IPSCs (sIPSCs) and miniature IPSCs (mIPSCs) at these time points. sIPSC frequency increased over time as abDGCs matured in both genotypes (**Figure 2.1 B**). Despite the known delays in maturation of neurons in other cortical regions in *Fmr1* KO mice, I found that there was no difference in the sIPSC frequency at any post-differentiation age of abDGC tested spanning this early period of development of these neurons (14 dpi WT: 0.028 ± 0.004 Hz; 14 dpi KO: 0.032 ± 0.004 Hz, n = 6/3 (cells, animals respectively), p = 0.49 Mann-Whitney *U*; 21 dpi WT: 0.480 ± 0.074 Hz, n=14/7, 21 dpi KO: 0.468 ± 0.070 Hz, n = 15/9, p = 0.95 Mann-Whitney *U*; 28 dpi WT: 1.16 ± 0.031 Hz, n=12/4 KO: 0.90 ± 0.086 Hz, 11/ 6, p = 0.70 Mann-Whitney *U*) (**Figure 2.1 B, D-F**). In addition, I measured action potential-independent spontaneous inhibitory events (mIPSCs) in abDGCs, which can be a good indicator of the number of inhibitory connections, or the release probability of those synapses. Again, I did not find a difference in the frequency of these events in comparisons between recordings in WT and *Fmr1* KO mice at any of the ages

tested (**Figure 2.1 C, G-I**). A comparison of the average mIPSC frequencies in each recording over time demonstrated an equivalent increase across this developmental period for abDGCs in both genotypes, indicating an increase in the number of synaptic connections or an increased release probability, or both. (**Figure 2.1 C**). Together, the lack of a difference in frequency of sIPSCs or mIPSCs during maturation of abDGCs indicates that there is no difference in the number of inhibitory synaptic connections in these neurons in the *Fmr1* KO mice during the first 4 weeks after differentiation.

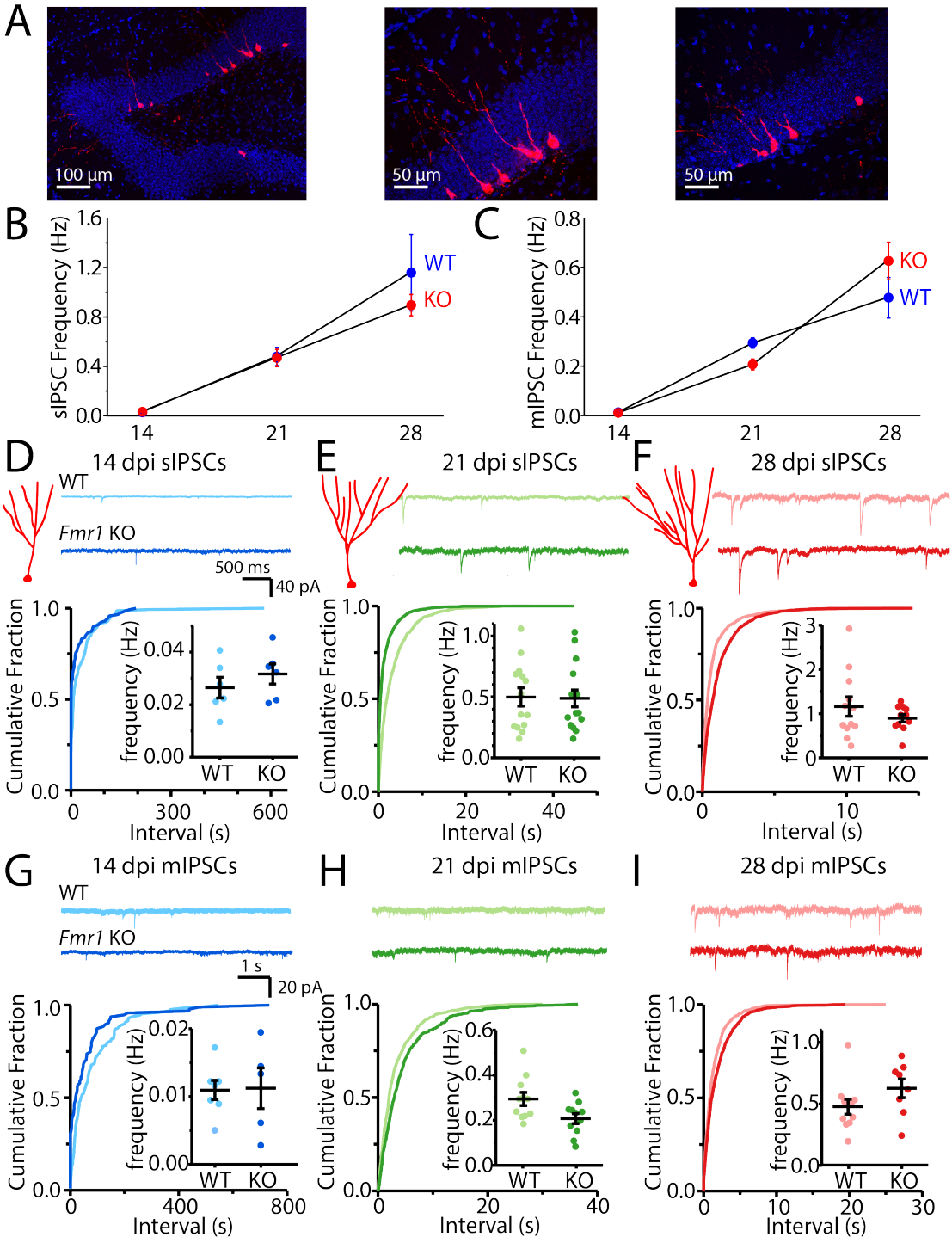
A
100 µm
50 µm
50 µm
B
sIPSC Frequency (Hz)
WT
KO
C
mIPSC Frequency (Hz)
KO
WT
D
14 dpi sIPSCs
WT
Fmr1 KO
500 ms
40 pA
E
21 dpi sIPSCs
F
28 dpi sIPSCs
Cumulative Fraction
frequency (Hz)
Interval (s)
G
14 dpi mIPSCs
1 s
20 pA
H
21 dpi mIPSCs
I
28 dpi mIPSCs

Figure 2.1 Frequency of sIPSCs and mIPSCs is not altered in abDGCs in *Fmr1* KO
A) Representative images of 21 dpi abDGCs virally labeled with RFP. Calibration: 100 μm for low magnification image (left), 50 μm for high magnification (right). B) Average sIPSC and C) mIPSC frequencies across all time points measured in *Fmr1* WT (blue) and *Fmr1* KO mice (red) D) Schematic of dendritic morphology of abDGCs and representative traces (top panel), cumulative distribution of inter-event-interval and average frequency of each recorded neuron (inset) of sIPSCs (bottom panel) at 14 dpi, and E) 21 dpi, and F) 28 dpi. Calibration: 40 pA, 500 ms. G-H) Representative traces, cumulative distribution of inter-event-intervals and average frequency of mIPSCs in each recorded abDGC (inset) at 14, 21, and 28 dpi, respectively. Calibration: 20 pA, 1 s.

The amplitude of spontaneous events, particularly mIPSCs, is an indicator of the strength of individual synapses and is expected to increase as abDGCs undergo maturation (Ge et al. 2006). Comparison of the mIPSC amplitudes at each of the days post-differentiation spanning this period again did not reveal any difference between recordings in each of the genotypes (WT 14 dpi: 10.4 ± 0.827 pA, n= 7/3; KO 14 dpi: 11.7 ± 1.81 pA, n =,5 / 5 , p = 0.64, Mann-Whitney *U*; WT 21 dpi: 15.9 ± 1.45 pA, n = 12/5, KO 21 dpi: 15.6 ±1.78 pA, n = 12/ 5, p = 1.00, Mann-Whitney *U*; WT 28 dpi: 15.4 ± 1.04 pA, n =11/4, KO 28 dpi: 16.6 ± 1.13 pA, n= 8/ 3 , p = 0.49, Mann-Whitney *U*) (**Figure 2.2 A-C**). Consistent with this lack of a genotype-dependent change in synaptic strength during the maturation of abDGCs in *Fmr1* KO mice, there were no differences in the amplitude of sIPSCs (**Figure 2.2 E**). In recordings of both mIPSCs and sIPSCs there was an equivalent increase of the average amplitude of events in older neurons in both genotypes (**Figure 2.2 D & E**). The increased amplitude of mIPSCs between 14 and 21 dpi indicates that the strength of individual synapses is increasing as abDGCs mature. Interestingly, at 14 dpi the average amplitude for both mIPSCs and sIPSCs was similar, suggesting that at this time point presynaptic inhibitory neurons make a single synaptic connection per axon. However, the amplitudes diverged at 21 dpi when sIPSC amplitudes were larger than those of mIPSCs, indicating that single presynaptic axons make multiple contacts onto abDGCs in these older neurons (**Figure 2.2 D & E**).

Measuring the decay of mIPSCs, I found that there was a significant increase in the decay time course as abDGCs matured in both genotypes that could be indicative of a change in the subunit composition of $GABA_A$Rs (Overstreet Wadiche et al. 2005). At the youngest time point measured, mIPSC decay was not significantly different in *Fmr1* KO (WT 14dpi: 2.39 ±0.612 ms, n= 7/3 KO 14 dpi: 2.15 ± 0.517 ms, n = 5/4 p = 1.00, Mann-Whitney *U*) (**Figure 2.2 F**). However, in older abDGCs the decay of mIPSCs in *Fmr1* KO neurons was slower (WT 21 dpi: 7.45 ± 0.722 ms, n = 12/5; KO: 11.1 ± 0.987 ms, n=12/ 5, p = 0.008, Mann-Whitney *U*; WT 28 dpi: 9.36 ± 0.492 ms, n = 11/4 KO 28 dpi: 10.8 ± 0.410 ms, n = 8/3 cells, p=0.041, Mann-Whitney *U*) (**Figure 2.2 G & H**). In addition, sIPSC decay was not significantly different in *Fmr1* KO (WT 14 dpi: 1.66 ± 0.275 ms, n= 6/3 KO 14 dpi: 1.57 ± 0.127 ms, n = 6/3 p = 0.7, Mann-Whitney *U*; WT 21 dpi: 12.6 ± 1.19 ms, n = 14/8; KO: 16.0 ± 1.39 ms, n=15/9, p = 0.08, Mann-Whitney *U*; WT 28 dpi: 14.2 ± 1.32 ms, n = 12/4 KO 28 dpi: 13.3 ± 0.80 ms, n = 11/6 cells, p=0.651, Mann-Whitney *U*).

Previous studies have found that the decay kinetics of sIPSCs are slower in abDGCs than in mature granule cells because of the incorporation of α1 subunit into postsynaptic $GABA_A$ receptors as neurons mature (Overstreet Wadiche et al. 2005). I tested whether the increased decay of the mIPSCs in abDGCs in *Fmr1* KO mice might reflect a lower α1 subunit incorporation by measuring the effect of zolpidem, an α1-specific positive allosteric modulator, on the decay kinetics of mIPSCs. mIPSCs were recorded at 21 dpi, when decay was significantly slower in *Fmr1* KO, and the decay was measured before and after the addition of 0.5 μM zolpidem (**Figure 2.2 I**). I found that zolpidem lengthened mIPSC decay in 21 dpi abDGCs in both *Fmr1* KO and WT to the same degree, suggesting that α1 subunit content is not a major determinant of the differences in decay observed in the *Fmr1* KO mice (decay zolpidem/decay control WT: 1.40 ± 0.11, n= 17/7 KO: 1.25 ± 0.05, n = 15/7, p=0.87, Mann-Whitney *U*).

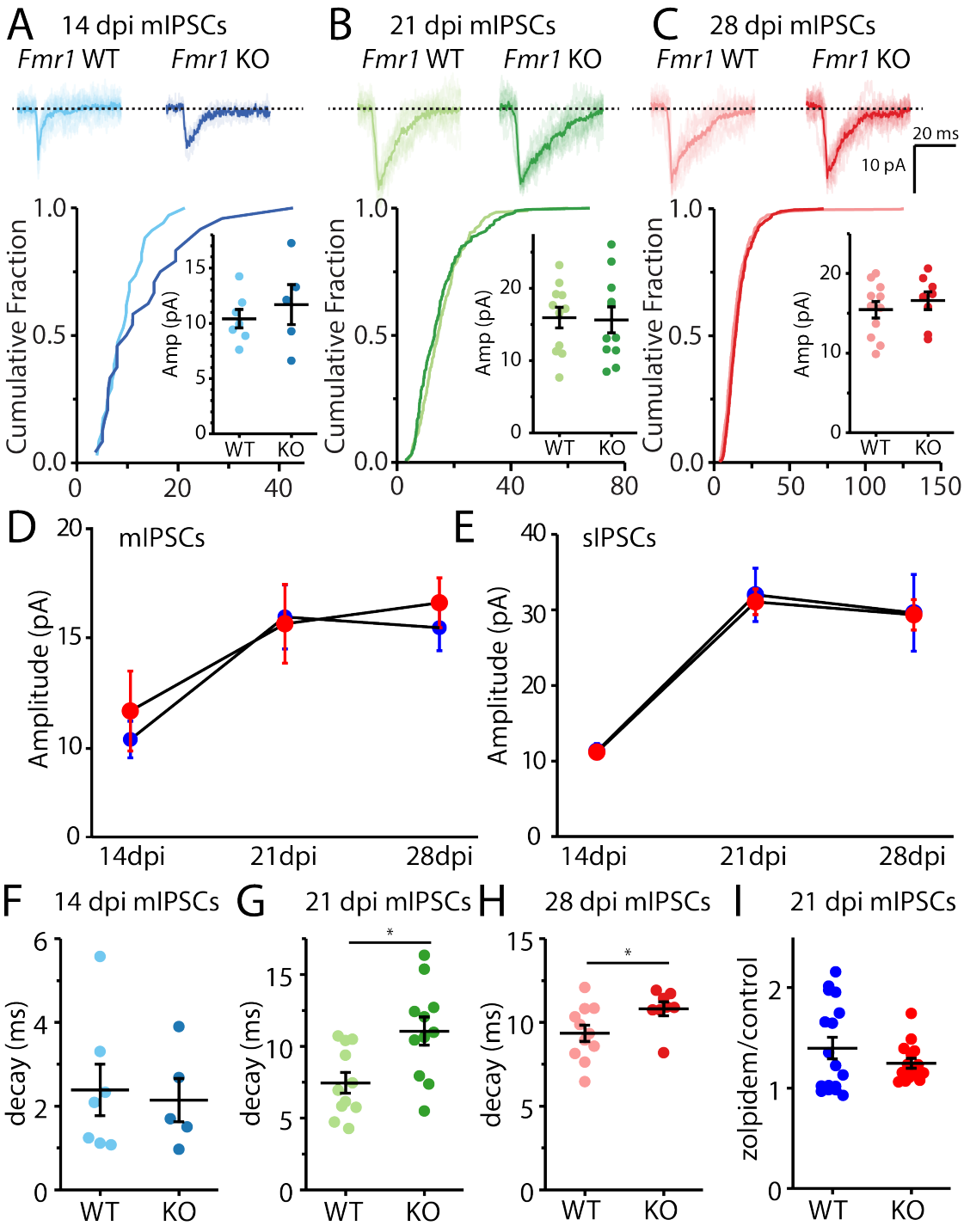

Figure 2.2 mIPSC amplitude is unaffected by loss of FMRP, but mIPSC decay is slower in abDGCs in *Fmr1* KO

A) Representative traces of individual and averaged mIPSC events (top) and cumulative distribution and average amplitudes mIPSCs in each recording measured at 14 dpi, or B) 21 dpi and C) 28 dpi abDGCs. Calibration: 10 pA, 20 ms. D) Average mIPSC amplitudes and E) sIPSC amplitude across all time points measured in *Fmr1* WT (blue) and KO (red). F) Average decay of mIPSCs in 14 dpi, G) 21 dpi, and H) 28 dpi abDGCs I) Average mIPSC decay with zolpidem normalized to decay pre-zolpidem (* $p < 0.05$, Mann-Whitney *U*)

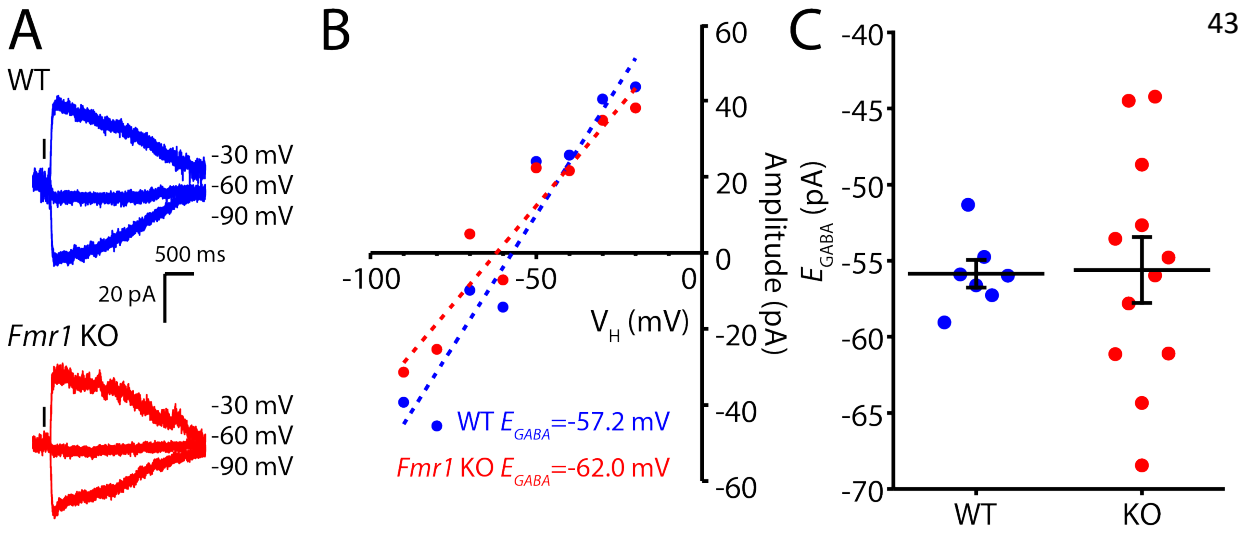

Figure 2.3 E_{GABA} in 21 dpi abDGCs in *Fmr1* KO and WT
A) Representative traces of GABA responses from perforated-patch recordings in 21 dpi abDGCs in *Fmr1* WT (top, blue) and *Fmr1* KO (bottom, red) measured at three holding potentials (-30mV, -60mV & -90mV). The response to a 50 ms puff of 10 μM GABA was measured at several holding potentials in the presence of 50 μM D-APV and 10 μM CNQX. B) Representative current-voltage curves constructed from responses in two cells (blue WT, red KO). E_{GABA} was calculated as the x-axis intercept of the best-fit line of the current-voltage plot. Calibration is 20 pA, 500 ms. C) Grouped E_{GABA} data from all recordings.

Maturation of the GABA reversal potential (E_{GABA}) in abDGCs

In abDGCs, E_{GABA} has been shown to be depolarized during early maturation, similar to what has been observed in developing neurons in other regions of the brain in perinatal mice (Ge et al. 2006, Chancey et al. 2013). The chloride equilibrium potential in large part determines the reversal potential for $GABA_A$ receptors (E_{GABA}) and therefore affects the strength of inhibitory transmission. E_{GABA} reaches its mature value by 4 weeks after differentiation in abDGCs (Ge et al. 2006). Prior analysis of *Fmr1* KO mice has demonstrated that E_{GABA} maturation is delayed in the developing cortex and hippocampus of *Fmr1* KO mice (He et al. 2014, Tyzio et al. 2014). Therefore, I measured E_{GABA} using perforated patch recording from abDGCs 21 days after differentiation. In WT abDGCs the reversal potential was still depolarized from the mature value at this age (WT E_{GABA}: -55.9 ± 0.91 mV, n=7/3). However, this value did not differ from that recorded in abDGCs in *Fmr1* KO animals (KO E_{GABA}: -55.6 ± 2.17 mV, n = 12/6, p = 0.967, Mann-

Whitney *U*) (**Figure 2.3 A-C**). Therefore, while the reversal potential for GABA is still relatively immature and depolarized in 21 dpi abDGCs there is no effect of the loss of FMRP on E_{GABA}, as has been reported in other developing neurons.

Development of dendrites of abDGCs in Fmr1 KO mice

Dendritic morphology of abDGCs resembles that of mature DGCs as early as 21 dpi, when distal dendrites reach the outer molecular layer and elaborate arborization is observed (Zhao et al. 2006). Dendritic spines begin to form around 16 dpi, consistent with the fact that glutamatergic signaling is rarely observed prior to 14 dpi (Ge et al. 2006, Zhao et al. 2006). I thus sought to determine if loss of FMRP would lead to alterations in dendritic morphology in 21 dpi abDGCs during this period of rapid functional maturation. Retrovirus-labeled abDGCs were filled with a morphological dye and imaged using a two-photon microscope (**Figure 2.4 A**). Quantification of their dendritic complexity at 21 dpi, as assessed by Sholl analysis did not uncover any significant difference between the genotypes (2-way ANOVA for unbalanced design, p = 0.999 for genotype x radius interaction) (**Figure 2.4 B**). In addition, measurement of total dendritic length did not reveal any difference in 21 dpi abDGCs in *Fmr1* KO (WT: 701 ± 94.2 μm, n = 8/5, KO: 729 ± 57.5

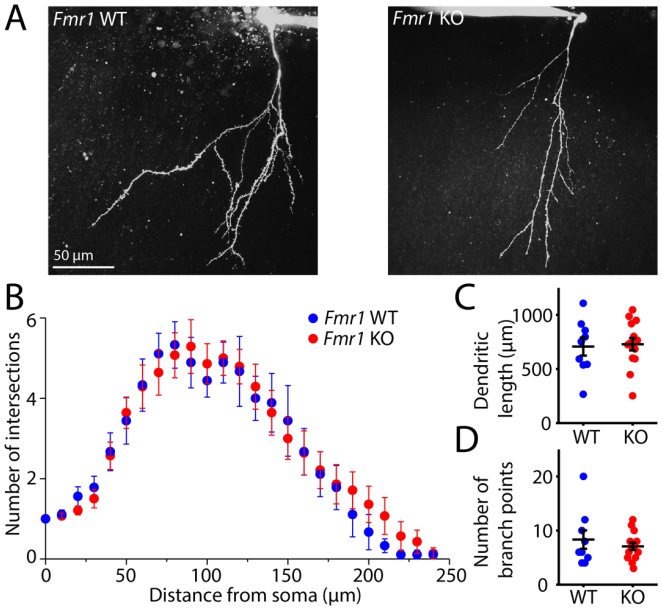

Figure 2.4 Morphology of 21 dpi abDGCs in *Fmr1* KO and WT
A) Representative 2-photon images of 21 dpi abDGCs filled with Alexa-488 in slices from *Fmr1* WT (right) and *Fmr1* KO (left) mice. Calibration: 50 μm. B) Sholl analysis of dendritic complexity of 21 dpi abDGCs in Fmr1 WT (blue) and Fmr1 KO mice (red) (B) Average total dendritic length and (D) Total number of branch points of 21 dpi abDGCs in *Fmr1* WT (blue) and *Fmr1* KO (red) mice.

μm n = 15/8, p = 0.781, Mann-Whitney *U*) and there was no difference in the number of dendritic branch points in *Fmr1* KO at 21 dpi (WT: 8.63 ± 1.92, n = 9/5, KO: 7.20 ± 0.66, n =14/8, p=0.917, Mann-Whitney *U*) (**Figure 2.4 C & D**).

Discussion

In this study I set out to systematically describe the development of abDGCs in the Fragile X mouse model, focusing on the formation of GABAergic inputs to these neurons during the first four weeks after differentiation. There are multiple studies that have found that loss of FMRP can delay neuronal development in the Fragile X brain, therefore a similar delay in development of abDGCs could have an impact on how these neurons become connected to hippocampal circuits, and how they contribute to circuit function. Studying GABA synapse development is particularly relevant as these are the first synapses to form onto abDGCs and GABA also has a trophic effect on abDGCs (Ge et al. 2006, Song et al. 2013, Alvarez et al. 2016). Surprisingly, our data indicate that the development of inhibitory signaling onto abDGCs during the first four weeks is mostly unaffected by the loss of FMRP in the *Fmr1* KO mice. Given the range of impairments in GABA signaling that have been described in the hippocampus of *Fmr1* KO mice including altered expression of GABA receptor subunits and GAD 65/67, and the alterations in E_{GABA} in *Fmr1* KO mice (Braat and Kooy 2015), it is surprising that GABA signaling appears to be unaltered in developing abDGCs during this early period of their maturation.

Critical period development and chloride homeostasis in Fragile X

At the cellular level, Fragile X is a complex disorder because loss of FMRP leads to the dysregulation of expression of many neuronal proteins (Tang et al. 2015). The *Fmr1* KO mouse model has been particularly useful in describing these complex alterations in neuronal development (Contractor et al. 2015). An important aspect that has emerged from some of these

studies is the alteration in synaptic development of neurons during early critical periods in the cortex (Cruz-Martin et al. 2010, Harlow et al. 2010, Nomura et al. 2017). While these delays in both excitatory and inhibitory neuron maturation have been well documented, up until now it has not been established whether the development of abDGCs shows similar alterations in maturation to the developmentally generated neurons.

Among the known effects in developing neurons is the delay in the maturation of the GABA reversal potential in both the somatosensory cortex (He et al. 2014) and the hippocampus (Tyzio et al. 2014). In abDGCs E_{GABA} has been demonstrated to be depolarized during early maturation in the weeks following differentiation in a manner similar to developing neurons in other regions of the brain in perinatal mice (Tozuka et al. 2005, Ge et al. 2006). This occurs because of the relatively elevated intracellular chloride concentrations in abDGCs, which is established by the juvenile chloride transporter NKCC1 (Ge et al. 2006). Disrupting chloride homeostasis in abDGCs leads to a profound alteration in the formation of synapses and in the maturation of the dendritic arbor (Ge et al. 2006). As there is growing evidence of altered E_{GABA} in developing neurons, I also measured E_{GABA} in abDGCs 21 days after differentiation when this measure is still maturing and not at its adult value (Ge et al. 2006). I confirmed that in 21 dpi abDGCs in both WT and *Fmr1* KO mice the measured E_{GABA} is still relatively depolarized, but the values of E_{GABA} were not significantly different between the genotypes. Therefore, this crucial measure that regulates the strength of GABA signaling, and has a major impact on neuronal development, is not affected in abDGCs in *Fmr1* KO mice.

GABA and Fragile X

The sequence of the formation of inputs and neurotransmitter signaling onto abDGCs broadly reflects that of developing neurons in other brain regions, including the early establishment of

tonic and phasic GABAergic signaling followed by the establishment of excitatory connections (Esposito et al. 2005, Ge et al. 2007). GABA has an established role in brain development affecting proliferation (LoTurco et al. 1995, Haydar et al. 2000), migration, and maturation of progenitors and neurons (Represa and Ben-Ari 2005, Wang and Kriegstein 2009). After postnatal development there are multiple known defects in GABA signaling associated with Fragile X, including age and region specific changes in $GABA_A$ receptor subunit expression (Braat and Kooy 2015), changes in tonic and phasic GABA currents onto excitatory neurons (Centonze et al. 2008, Curia et al. 2009, Olmos-Serrano et al. 2010, Zhang et al. 2017) as well as defects in interneurons themselves (Gibson et al. 2008, Nomura et al. 2017). Expression of several $GABA_A$ receptor subunits is reduced in the cortex, hippocampus, or forebrain of *Fmr1* KO mice (El Idrissi et al. 2005, D'Hulst et al. 2006, Gantois et al. 2006, Adusei et al. 2010). Expression of the glutamate decarboxylase enzyme (GAD) responsible for converting glutamate to GABA is increased in the hippocampus, but decreased in the amygdala of *Fmr1* KO mice (El Idrissi et al. 2005, Olmos-Serrano et al. 2010). Functionally, both the frequency and amplitude of miniature and spontaneous IPSCs are reduced in the amygdala of juvenile *Fmr1* KO mice (Olmos-Serrano et al. 2010); however, a similar alteration is not observed in the subiculum of older mice (Curia et al. 2009). In some cases, these alterations in GABAergic signaling occur only early in postnatal development (Adusei et al. 2010, Nomura et al. 2017). Thus, loss of FMRP clearly affects GABA signaling in the postnatal mouse brain, but whether this is also the case for abDGCs was not known. By mapping spontaneous events by recording both sIPSCs and mIPSCs at three time points after differentiation of abDGCs I were able to establish how inhibitory synapses form onto these neurons. I found that in both genotypes the frequency of IPSCs increases over time as would be expected if new synapses were being formed over the post differentiation period I analyzed. Interestingly, the amplitude of both the sIPSC and mIPSC events increased between 14 and 21 dpi. The increase in the mean quantal size at this developmental time point suggests

that individual synapses become stronger. At the earliest time points, the amplitude of both mIPSC and sIPSCs is equivalent suggesting that presynaptic axons of the inhibitory cells make a single contact whereas by 21 dpi the mean amplitude of sIPSCs is double the mean amplitude of quantal events, suggesting that the sIPSCs represent the release at multiple synapses. While again I did not observe any genotype differences in these parameters, I did observe consistent lengthening of the decay kinetics of mIPSCs in *Fmr1* KO mice which were significant in recordings from both 21 dpi and 28 dpi neurons. Prior work that compared the decay kinetics of inhibitory events in abDGCs and mature granule neurons found that the sIPSCs in abDGCs are slower because of the subunit composition of postsynaptic $GABA_A$ receptors (Overstreet Wadiche et al. 2005). This study found that the zolpidem sensitivity of inhibitory events was increased in mature neurons suggesting that the α1 subunit is increasingly incorporated into neurons as they mature (Overstreet Wadiche et al. 2005). Given this, I considered the possibility that a reduction in the incorporation of the α1 subunit in abDGCs in *Fmr1* KO mice could underlie the prolonged decay of mIPSCs in *Fmr1* KO. However, I did not observe a significant difference in the effect of zolpidem on mIPSC decay in 21 dpi abDGCs between *Fmr1* KO and WT, indicating that a reduction in α1 expression is unlikely to underlie the changes in mIPSC decay. It is possible that alterations in expression of other GABA receptor subunits or even differences in the location of GABAergic synapses on developing neurons may underlie the observed change in mIPSC decays recorded by a somatic electrode. Interestingly, while prior work has reported that abDGCs lack expression of the $GABA_A$ α1 subunit (Overstreet Wadiche et al. 2005), I found zolpidem had a significant effect on most mIPSCs in the 21 dpi birth-dated neurons in both genotypes. This may reflect differences in the populations of neurons that were analyzed in the previous study since the neurons labeled in the POMC-GFP mice are ~3-30 days post-mitosis. ~~which recorded from POMC-GFP labelled neurons which are a more heterogeneous aged group~~ (Overstreet et al. 2004).

In addition to the analysis of synaptic function, I also measured the dendritic arbor to quantify both the complexity as well as the total dendritic length in 21 dpi abDGCs. A prior study has reported that selective deletion of FMRP in adult neural stem cells isolated from the dentate gyrus, as well as *in situ*, caused a reduction in both the dendritic complexity and total dendritic length when measured in neurons 56 days after differentiation (Guo et al. 2011). Dendritic length and complexity are also reduced in mice with knockout of the FMRP paralog FXR2P and double knockout of FXR2P and FMRP induced an additive effect on dendrites of abDGCs (Guo et al. 2015). In our experiments I patched and filled abDGCs with morphological dyes in live slices and imaged the dendritic arbor at 21 dpi. In these younger neurons I did not observe any genotype related differences in the total dendritic length or complexity in *Fmr1* KO mice. It is possible that this difference in our results and those of the earlier study is due to a dendritic phenotype that emerges later on in the development of FMRP lacking abDGCs. There have also been reports of region specific or developmental age specific alterations in dendritic spine density or immature spine morphology in *Fmr1* KO mice, although there is no consensus on the effect of loss of FMRP on dendritic spines (reviewed here: (He and Portera-Cailliau 2013)). While I did not examine this measure in these immature abDGCs prior work has reported normal dendritic spines in the mature dentate gyrus in *Fmr1* KO (Grossman et al. 2010).

In summary, I performed a systematic analysis of the functional properties of GABAergic synapses in abDGCs during the first four weeks after differentiation in *Fmr1* KO mice. Previous studies have demonstrated that cell proliferation and fate specification of adult neural stem cells is altered by FMRP loss. Our data suggest that neurons that develop from these stem cells do not have major alterations in the maturation of their GABAergic synaptic inputs and dendrites during the first four weeks of their development.

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Chapter 3.

On the input-specific development of GABAergic synapses in adult born dentate granule neurons

Abstract:

The inhibitory neurotransmitter GABA is critical for survival, dendritic development, and glutamatergic synaptogenesis in abDGCs. While the process of GABAergic synaptogenesis in abDGCs has been studied by several groups, little is known about the precise pattern of connectivity between abDGCs and local interneuron types. In the present study I focused on two morphologically and chemically distinct populations of dentate interneurons: PV-expressing basket cells and SST-expressing HIPP cells. PV interneurons are the most well-studied source of GABA input to abDGCs, but little is known about the development of inputs from SST interneurons onto abDGCs. Previous studies have proposed that GABAergic regulation of adult neural stem cell (aNSC) quiescence, proliferation, survival, and early morphological development of abDGCs is specifically mediated by tonic and phasic GABA inputs from PV interneurons. However abDGCs receive input from multiple other interneuron classes whose function and timing of input remain unknown. This raises the possibility that inputs from specific interneuron populations differentially regulate abDGC maturation. I have characterized the development of inputs from SST+ HIPP cells and PV+ basket cells onto abDGCs in the first four weeks after differentiation using retroviral birthdating combined with optogenetic activation of interneurons.

I found that the process of synaptogenesis is likely explained by the organization of interneuron projections within the dentate. AbDGCs receive input from PV-expressing basket cells in the GCL in the first week after differentiation and in the second week, when neurites have begun to extend through the GCL and into the molecular layer, they receive input from SST-expressing HIPP cells. I also found that the number of synapses from both PV and SST interneurons onto abDGCs increases as the cells mature. I also assessed the effects of running on GABAergic synaptogenesis. Previous studies have demonstrated that voluntary wheel running in mice increases adult hippocampal neurogenesis and improves performance on tasks that rely on this process. Retrograde tracing studies have reported no change or decreased connectivity between

local interneurons and abDGCs following voluntary wheel running. Interestingly, I find that voluntary wheel running increases the maximal response of abDGCs to optogenetic activation of both PV and SST interneurons. Together these studies further our understanding of the role of interneurons in adult hippocampal neurogenesis.

Introduction

As discussed in the previous chapters, GABA inputs to abDGCs play a critical role in survival, dendritic development, and subsequent formation and unsilencing of glutamatergic synapses (Tozuka et al. 2005, Ge et al. 2006, Chancey et al. 2013). However, the specific populations of neurons that provide GABAergic inputs onto developing abDGCs and precisely how each of these are changing with time, have not been well characterized. Given the location of PV and SST interneurons within the dentate and known information about the location of synapses from these cells onto mature abDGCs, it is likely that these cell types form synapses onto abDGCs at different stages of maturation. PV neurons are likely to form synapses close to the soma whereas SST neurons form synapses on dendritic regions including distal dendrites (Houser 2007, Booker and Vida 2018). Furthermore, running increases both neurogenesis and pattern separation performance, but how exactly this occurs is unclear. While this could simply be due to an increased number of abDGCs, it seems likely that alterations in the functional connectivity and rate of maturation of abDGCs also occurs. In this Chapter, I will present work aimed at determining the time course of development of GABAergic inputs from PV and SST interneurons onto abDGCs during the first four weeks of development, and I will determine how the integration of abDGCs into the GABAergic network might be altered by voluntary wheel running.

Interneurons in the dentate gyrus

The dentate gyrus contains a diverse population of interneurons which can be classified by chemical identity, morphology, and location within the dentate (Houser 2007, Booker and Vida 2018). While the exact pattern of development of connectivity between abDGCs and interneurons has not been well described, a few studies have identified presynaptic interneurons at single time points during maturation. Retrograde transynaptic tracing using modified rabies virus shows that SST, PV, and CCK expressing interneurons synapse onto 3-5-week-old abDGCs (Li et al. 2013, Bergami et al. 2015). In addition, glutamate uncaging studies have identified MOPP cells as presynaptic inputs to 3-week-old abDGCs (Li et al. 2013) and paired recordings of from POMC-GFP labeled abDGCs identified Ivy/neurogliaform cells as presynaptic inputs to abDGCs that are approximately 2-weeks-old (Markwardt et al. 2011). However, these studies did not quantify the number of each type of interneuron or compare how these inputs change during maturation. The present study focused on two morphologically and chemically distinct types of dentate interneurons: PV-expressing basket cells and SST-expressing HIPP cells.

In the dentate gyrus, PV-expressing interneurons are basket cells in the GCL and synapse onto the soma and axon initial segment of mature DGCs (Houser 2007). PV interneurons are the most well-characterized source of GABA inputs to abDGCs. GABA release from PV interneurons maintains quiescence of aNSCs while i*n vivo* optogenetic activation of PV interneurons from 1-4 dpi increases the survival and promotes dendritic growth of abDGCs (Song et al. 2012, Song et al. 2013). *In vivo* activation of PV interneurons with DREAADs also promotes dendritic development at 9-11 dpi (Alvarez et al. 2016). SST interneurons in the dentate are primarily classified as HIPP cells with somata in the hilus and axons projecting through the GCL to form synapses onto the dendrites of mature DGCs in the molecular layer (Houser 2007, Savanthrapadian et al. 2014). Much less is known about SST inputs to abDGCs, although Song and colleagues did report that they were unable to detect SST inputs to 4 dpi abDGCs using

optogenetic activation (Song et al. 2013).

Experience regulates adult hippocampal neurogenesis

The proliferation, differentiation, survival, morphological maturation, and functional integration of adult born neurons is a dynamic process that is regulated by a number of different intrinsic and extrinsic factors (van Praag et al. 1999, Toda et al. 2018). In the previous chapter I described the effects of a genetic mutation on the functional maturation of abDGCs. As described in Chapter 1, experiences such as voluntary wheel running, environmental enrichment, and stress can also alter adult hippocampal neurogenesis. Here, I will focus on the role of voluntary wheel running in the maturation and functional integration of abDGCs. In a series of seminal studies, van Praag and colleagues found that voluntary wheel running increases the proliferation and survival of abDGCs, that these newborn cells were indeed neurons, and that this upregulated hippocampal neurogenesis had functional consequences (van Praag et al. 1999, van Praag et al. 1999, van Praag et al. 2002). Voluntary wheel running improves acquisition on the Morris water maze task and leads to increased potentiation following LTP induction in the dentate gyrus (van Praag et al. 1999, van Praag et al. 1999). Voluntary wheel running also leads to improved acquisition and reversal learning on a touchscreen pattern separation task when the stimuli are spatially similar (Creer et al. 2010). In addition, running improves novel object recognition of similar but not distinct objects with a long delay (24h) between stimulus presentations (Bolz et al. 2015). Together, these studies clearly demonstrate an effect of running on adult hippocampal neurogenesis and neurogenesis-dependent behaviors.

However, whether these effects of running are due to an increase in the number of abDGCs, or if they are accompanied by changes in functional connectivity of abDGCs remains to be determined. A few studies have assessed changes in the organization of afferents to abDGCs

following running and environmental enrichment. Retrograde transynaptic tracing using modified rabies virus combined with retroviral birthdating has been employed to assess connectivity of abDGCs. These tracing studies found that running increased the overall connectivity ratio of presynaptic cells to 7-week-old postsynaptic abDGCs (Deshpande et al. 2013). Running also increases the total number of labeled abDGCs and presynaptic cells in 1 and 5-week-old abDGCs, but decreased connectivity between local hippocampal neurons and 5-week-old abDGCs (Vivar et al. 2016, Sah et al. 2017). However, in a similar study, Bergami and colleagues also found that running had no effect on the connectivity of 3-week-old abDGCs, but increased connectivity with cells in the molecular layer of the dentate gyrus, entorhinal cortex, medial septum, and nucleus of the diagonal band of Broca (Bergami et al. 2015).

While these studies indicate an effect of voluntary running on local and long-range connectivity to abDGCs, the nature of this change is unclear, and the methods used to do not assess changes in functional connectivity such as the number of synapses or synaptic strength. Changes in the number of cells providing presynaptic inputs to abDGCs as measured by transynaptic tracing is only one possible way in which the integration of abDGCs to the existing hippocampal circuit might be altered due to experience. It is equally likely that the strength of individual synapses or the number of synapses between the same population of cells would also be susceptible to experience-dependent remodeling. One recent study found that mIPSC frequency and amplitude in 5-week-old abDGCs are not affected by running (Vivar et al. 2016). Most of these studies assessing the functional alterations caused by running have focused on 5-7-week-old abDGCs. However, changes in GABA inputs to abDGCs seem likely to have a larger impact on the development of abDGCs during early development, when GABA has been shown to be important for the survival, dendritic development, and subsequent formation and unsilencing of glutamatergic synapses (Tozuka et al. 2005, Chancey et al. 2013, Song et al. 2013, Alvarez et al.

2016). Thus, further investigation of the effects of running on functional connectivity of abDGCs is necessary to fully understand how running leads to behavioral changes.

GABAergic inputs from the diverse population of interneurons in the dentate gyrus are critical regulators of adult hippocampal neurogenesis. There is evidence that distinct populations of dentate interneurons make specific contributions to adult neurogenesis. PV interneurons regulate the maintenance of quiescence of aNSCs and promote survival and morphological maturation of newly generated neurons (Song et al. 2012, Song et al. 2013). It is likely that other specific interneuron populations differentially contribute to abDGC maturation, but the precise temporal pattern of innervation of abDGCs by local interneurons in unknown. In this Chapter, I will describe experiments using optogenetic activation of two morphologically and chemically distinct populations of interneurons, PV and SST, to assess the temporal pattern of development of GABAergic synapses from these specific populations of interneurons onto abDGCs during the first four weeks of maturation. Furthermore, while running is known to promote adult neurogenesis, the functional consequences of running on the development of GABAergic synapses onto abDGCs is not known. I will demonstrate that voluntary wheel running alters the functional connectivity of SST and PV interneurons abDGCs. These studies are the first to systematically characterize the time course of development of GABAergic inputs from PV and SST interneurons onto abDGCs during the first four weeks of development and how voluntary wheel running might alter this process.

Materials and Methods

Animals

All procedures related to the care and treatment of animals were performed in accordance with Northwestern University IACUC. PV-cre and SST-cre mice crossed with Ai32 ChR2/EYFP mice

(PV-ChR2, SST-ChR2). Both lines were maintained on a C57Bl/6 background and were maintained by breeding females heterozygous for *Cre* recombinase and homozygous for the Ai32 allele with homozygous Ai32 males. Tail biopsies were used to perform genotyping of all mice used in the study.

Retroviral birthdating

A replication incompetent retrovirus based on MMLV (Moloney Murine Leukemia Virus) expressing RFP was prepared as described (Tashiro et al. 2006, Tashiro et al. 2015). Briefly, HEK-293 cells stably expressing GP2 were co-transfected with RFP and VSVG using Lipofectamine 2000. The media was collected from transfected cells 3 and 6 days post-transfection, filtered and centrifuged at 25,000 rpm to precipitate the virus. 6-8 week old PV-ChR2 and SST-ChR2 mice were anesthetized using ketamine/xylazine and 1 µl of virus was injected bilaterally into the subgranular zone of the dentate gyrus at a rate of ~0.3 µl/min.

Slice preparation and electrophysiology

250 µm coronal slices were prepared at 7, 14, 21, and 28 (± 1) days post injection. Slices were prepared using a Leica Vibratome in ice-cold high-sucrose artificial CSF (ACSF) containing the following (in mM): 85 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 0.5 $CaCl_2$, and 4 $MgCl_2$, equilibrated with 95% O_2 and 5% CO_2 and including 10 µM DL-APV and 100 µM kynurenate. Slices were heated to 28°C in the same sucrose-ACSF, then the sucrose solution was gradually exchanged for recovery ACSF containing the following (in mM): 125 NaCl, 2.4 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 1 $CaCl_2$, 2 $MgCl_2$, 0.01 DL-APV, and 0.1 kynurenic acid.

After a 60 min recovery, individual slices were transferred to a recording chamber and continuously perfused with oxygenated ACSF containing 2 mM $CaCl_2$ and 1 mM $MgCl_2$ at an

elevated temperature of 32°C. The dentate gyrus was visually identified and targeted recordings were made from RFP-expressing dentate granule cells. Recording electrodes were manufactured from borosilicate glass pipettes and had tip resistances of 4–6 MΩ when filled with internal recording solution. For whole-cell recordings, internal recording solution contained the following (in mM): 95 CsF, 25 CsCl, 10 Cs-HEPES, 10 Cs-EGTA, 2 NaCl, 2 Mg-ATP, 10 QX-314, 5 TEA-Cl, and 5 4-AP, pH adjusted to 7.3 with CsOH. For asynchronous release, slices were perfused with oxygenated ACSF containing 6mM $SrCl_2$, 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$. Data were collected and analyzed using pClamp 10 software (Molecular Devices). Neurons were voltage-clamped at -70 mV to record IPSCs, which were isolated by inclusion of D-APV (50 μM), CNQX (10 μM), and TTX (1 μM) for mIPSCs. MiniAnalysis (Synaptosoft) was used to analyze aIPSCs. Channelrhodopsin-expressing interneurons were activated using a 5 ms, 500 mV pulse of LED light to the entire visual field. Input-output curves indicate that these conditions consistently evoked the maximal IPSC amplitude in postsynaptic mature and birthdated DGCs and that the response plateaus at higher stimulation intensities **(Figure 3.2 A, B)**. Mature, unlabeled cells were recorded from the outer third of the GCL since most abDGCs have been shown to migrate to the inner third of the GCL and only 10% migrate to the outer third of the GCL (Kempermann et al. 2003).

Data analysis

Data analysis was performed using Microsoft Excel and OriginPro 2017 software. mIPSC and sIPSCs were analyzed using MiniAnalysis (Synaptosoft). Decay kinetics of compound IPSC events were measured by fitting a single or double exponential to decay from 90 to 10% of the peak amplitude on the falling phase of the response. Decay kinetics of aIPSC events were measured as the time to decay from 90 to 37% of the peak amplitude on the falling phase of the

response. Comparisons were made with a Mann-Whitney U test, unless otherwise indicated. Differences were considered significant when $p < 0.05$. Data are shown as mean $\pm$ SEM.

Results

Development of SST inputs to abDGCs

To identify and birthdate newborn DGCs, I injected a modified retrovirus expressing RFP into the subgranular zone of 6-8 week old PV-ChR2 or SST-ChR2 mice (**Figure 3.1 A, B**) (Tashiro et al. 2015). Evoked IPSCs have been detected in abDGCs as early as 7 dpi (Ge et al. 2006). Based upon this and the known role of GABA in early maturation of abDGCs, I performed targeted patch-clamp recordings from RFP-expressing cells at 7, 14, 21, and 28 dpi. I used full-field LED illumination to deliver a 5 ms light pulse at an intensity that routinely elicited a maximal response in postsynaptic DGCs (**Figure 3.2 A, B**). In SST-ChR2 mice, no postsynaptic response was detected in any of the cells I recorded at 7 dpi (n = 13/2 cells, animals respectively), but the same stimulation elicited a compound IPSC in 92% (n = 7/1) of 14. dpi abDGCs and 100% of cells recorded at later timepoints (**Figure 3.3 A, B**). The amplitude of the maximal compound

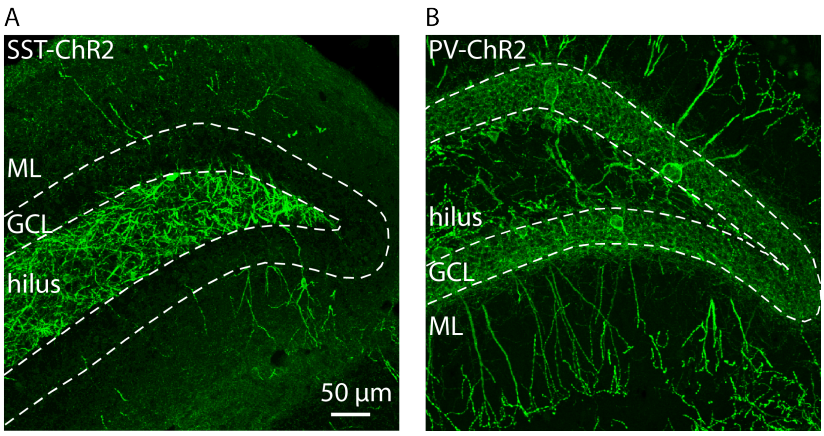

Figure 3.1 Expression of ChR2-EYFP
Representative confocal images of ChR2-EYFP in SST-cre **(A)** and PV-cre **(B)** mice.

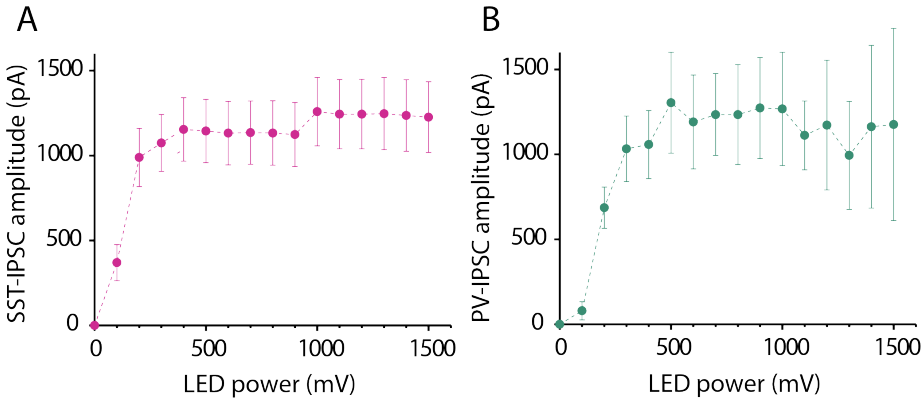

Figure 3.2 Input output curve of IPSC amplitudes in SST-ChR2 and PV-ChR2 mice
The amplitude of the IPSC response recorded from mature, unlabeled DGCs in SST-ChR2 **(A)** and PV-ChR2 **(B)** mice is plotted against the power input to the LED light source.

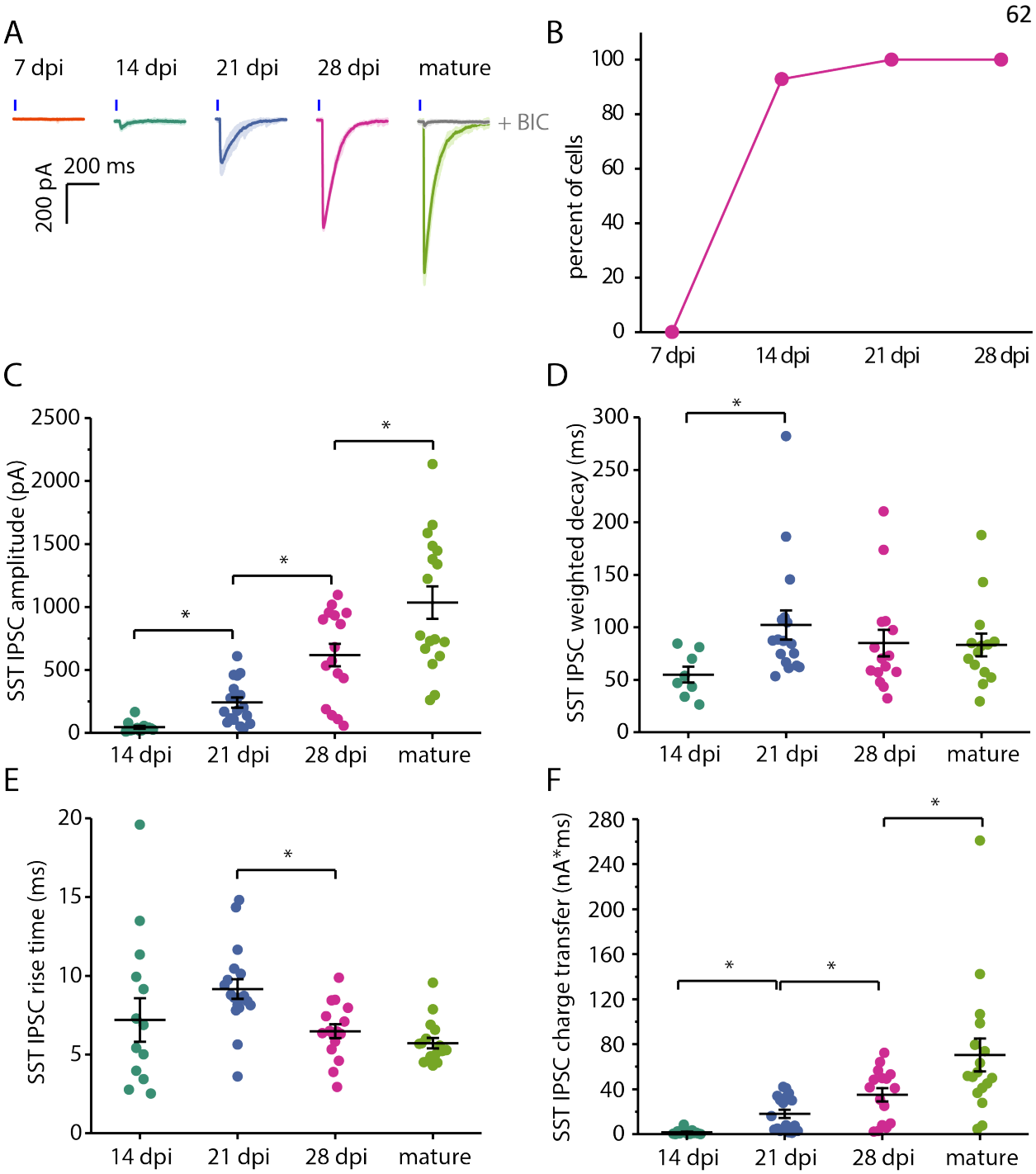

Figure 3.3 Maturation of SST inputs to abDGCs
A) Representative traces show the maximal compound IPSC evoked by ChR2-expressing SST interneurons. Calibration is 200 pA, 200 ms. Blue line indicates 5 ms light pulse. **B)** Shows the percent of abDGCs in which a light-evoked response was observed. Gray trace in mature cell shows response after addition of 10 μM bicuculine. **C)** Amplitude of light-evoked IPSCs in SST-ChR2 mice **D)** Weighted decay of light-evoked IPSCs in SST-ChR2 mice **E)** Rise time of light-evoked IPSCs in SST-ChR2 mice **F)** Charge transfer of light-evoked IPSCs in SST-ChR2 mice. *p<0.05, Mann-Whitney *U*.

IPSC in SST-ChR2 mice increased as the abDGCs matured (**Figure 3.3 C**), indicating an increase in SST input with age. The weighted decay of compound IPSCs in SST-ChR2 mice is faster at 14 dpi than at 21 dpi (14 dpi: 59.4 ± 7.6 ms, n = 14/5, 21 dpi: 102.2 ± 13.9 ms, n = 18/5, $p<0.01$, Mann-Whitney *U*) (**Figure 3.3 D**) which may reflect a change in the location of SST synapses onto abDGCs since dendritic inputs would be expected to have a slower decay than perisomatic inputs. In addition, the rise time of the maximal compound IPSCs is reduced between 21 and 28 dpi (21 dpi: 9.2 ± 0.6 ms, n = 18/5, 28 dpi: 6.5 ± 0.4 ms, n = 17/14, p=0.001, Mann-Whitney *U*) (**Figure 3.3 E**). Rise time may similarly be affected by the location of SST inputs relative to the soma. Finally, charge transfer of the maximal IPSC increased as abDGCs matured, likely reflecting the observed increase in amplitude with age (**Figure 3.3 F)**.

Development of PV inputs to abDGCs

I also recorded the maximal IPSC response in abDGCs following optogenetic activation of PV interneurons. Phasic GABA responses have been observed as early as 4 dpi in response to an 8 Hz optogenetic stimulation of PV interneurons and 7 dpi using an extracellular electrical stimulation electrode placed in the molecular layer (Esposito et al. 2005, Ge et al. 2006, Song et al. 2012). In PV-ChR2 mice, optogenetic activation of PV interneurons with a single pulse of light elicited an IPSC in 66.67% of 7 dpi abDGCs (n = 6/3), and 100% of cells at later timepoints (**Figure 3.4 A, B**). The amplitude of the compound IPSC increased as abDGCs matured, indicating an increase in PV inputs during the first four weeks of development of abDGCs (**Figure 3.4 C).** The weighted decay of compound IPSCs in PV-ChR2 mice decreases between 21 and 28 dpi (21 dpi: 74.5 ± 4.9 ms, n = 13/6, 28 dpi: 55.6 ± 7.5 ms n = 14/3, p = 0.049, Mann-Whitney *U*) (**Figure 3.4 D)**. The rise time of the maximal compound IPSC in PV-ChR2 mice increased between 7 and 14 dpi (7 dpi: 9.5 ± 1.1 ms, n = 6/2, 14 dpi: 17.1 ± 2.6 ms, n = 12/3, p = 0.004, Mann-Whitney *U*) and then decreased at 21 dpi (11.7 ± 1.1 ms, n = 13/6, p = 0.021, Mann-Whitney

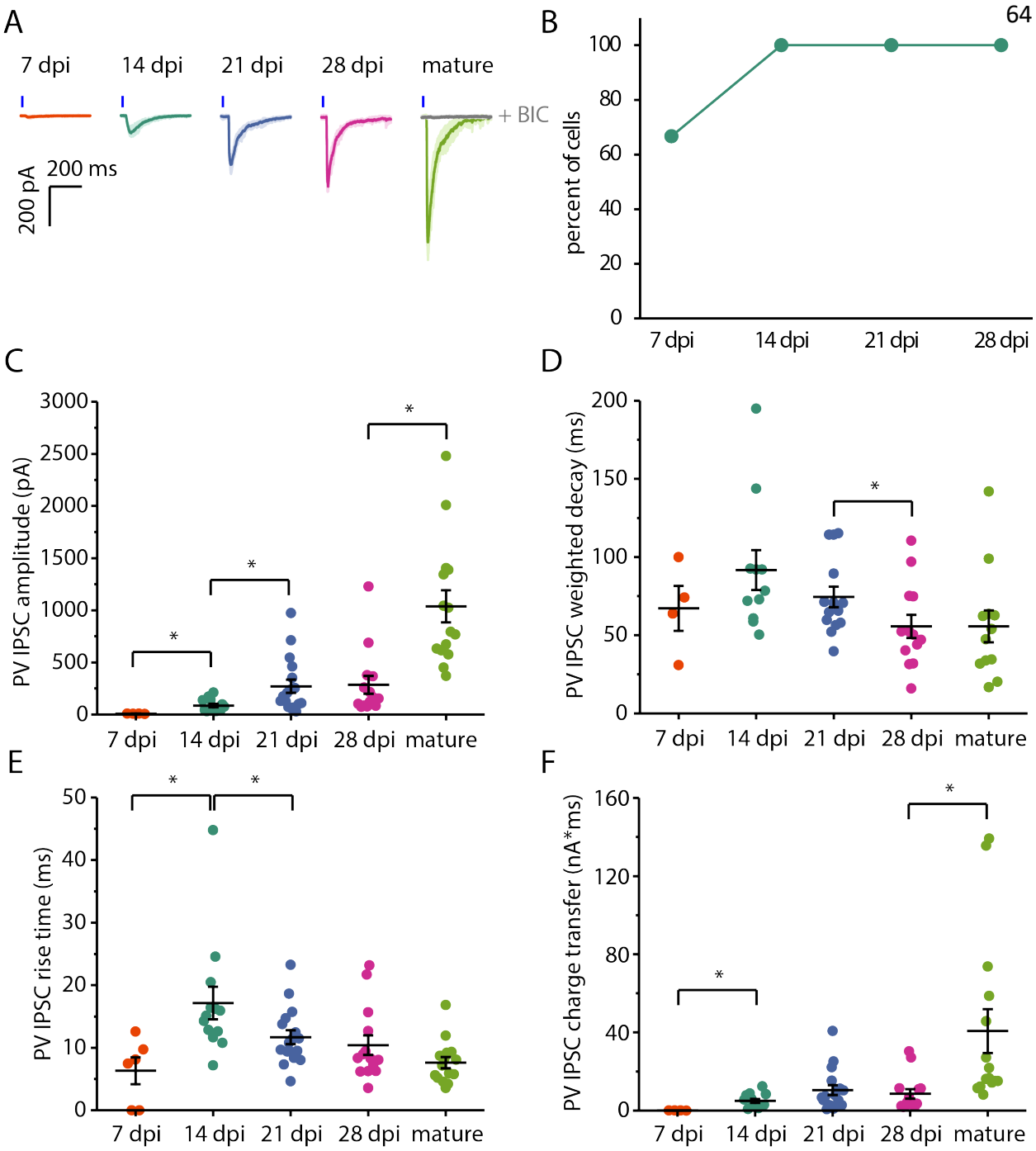

Figure 3.4 Maturation of PV inputs to abDGCs
A) Representative traces show the maximal compound IPSC evoked by ChR2-expressing PV interneurons. Calibration is 200 pA, 200 ms. Blue line indicates 5 ms light pulse. Gray trace in mature cell shows response after addition of 10 μM bicuculine. **B)** Shows the percent of abDGCs in which a light-evoked response was observed in PV-ChR2 mice **C)** Amplitude of light-evoked IPSCs in PV-ChR2 mice **D)** Weighted decay of light-evoked IPSCs in PV-ChR2 mice **E)** Rise time of light-evoked IPSCs in PV-ChR2 mice **F)** Charge transfer of light-evoked IPSCs in PV-ChR2 mice. *p<0.05, Mann-Whitney *U*.

U) (**Figure 3.4 E**). These developmental changes in the kinetics of IPSCs are likely due to changes in the $GABA_AR$ subunit composition (Kapur and Macdonald 1999, Overstreet Wadiche et al. 2005) . The increased rise time from 14 to 21 dpi could be due to an increase in the abundance of dendritic inputs, since dendrites are still developing at these time points. Charge transfer of the maximal compound IPSC in PV-ChR2 mice increased as cells matured, specifically between 7 and 14 dpi (7 dpi: 81.5 ± 17.3, n = 6/2, 14 dpi: $4.96 \times 10^3 \pm 9.82 \times 10^2$, n = 12/3, p = 0.004) and 28 dpi and mature, unlabeled DGCs ($8.6 \times 10^3 \pm 2.5 \times 10^3$, n = 14/3, mature: $4.1 \times 10^4 \pm 1.1 \times 10^1$, n = 15/6, p<0.001) (**Figure 3.4 F**). These changes correlate with the developmental increase in amplitude of compound IPSCs and are likely due to the changes in amplitude.

Differential innervation of abDGCs by PV and SST interneurons

I also compared the development of inputs from SST and PV interneurons. I found that PV inputs were present earlier in maturation than SST inputs. While activation of SST interneurons failed to evoke a response in 7 dpi abDGCs and only evoked an IPSC in 92.86% (13 of 14) of 14 dpi abDGCs, activation of PV interneurons evoked an IPSC in 66.67% of cells (4 out of 6) at 7 dpi and 100% of cells (13 out of 13) at 14 dpi (**Figure 3.5 A**). This is consistent with a previous study that found that optogenetic activation of SST interneurons did not evoke an IPSC in 4 dpi abDGCs, but activation of PV interneurons did elicit an IPSC (Song et al. 2013). Moreover, the amplitude of the maximal compound IPSC was larger in PV-ChR2 mice than in SST-ChR2 mice at 14 dpi (PV: 85.6 ± 16.6 pA, SST: 44.6 ± 11.2 pA, p = 0.2) (**Figure 3.5 B**). But at 28 dpi the maximal compound IPSC amplitude was larger in SST-ChR2 mice than in PV-ChR2 mice (PV: 285.9 ± 85.5 pA, SST: 618.9 ± 89.1, p = 0.01), indicating that developing abDGCs receive more input from PV interneurons early in development, and increasing input from SST interneurons later in development. The weighted tau of the maximal compound IPSC was slower in PV-ChR2 than in

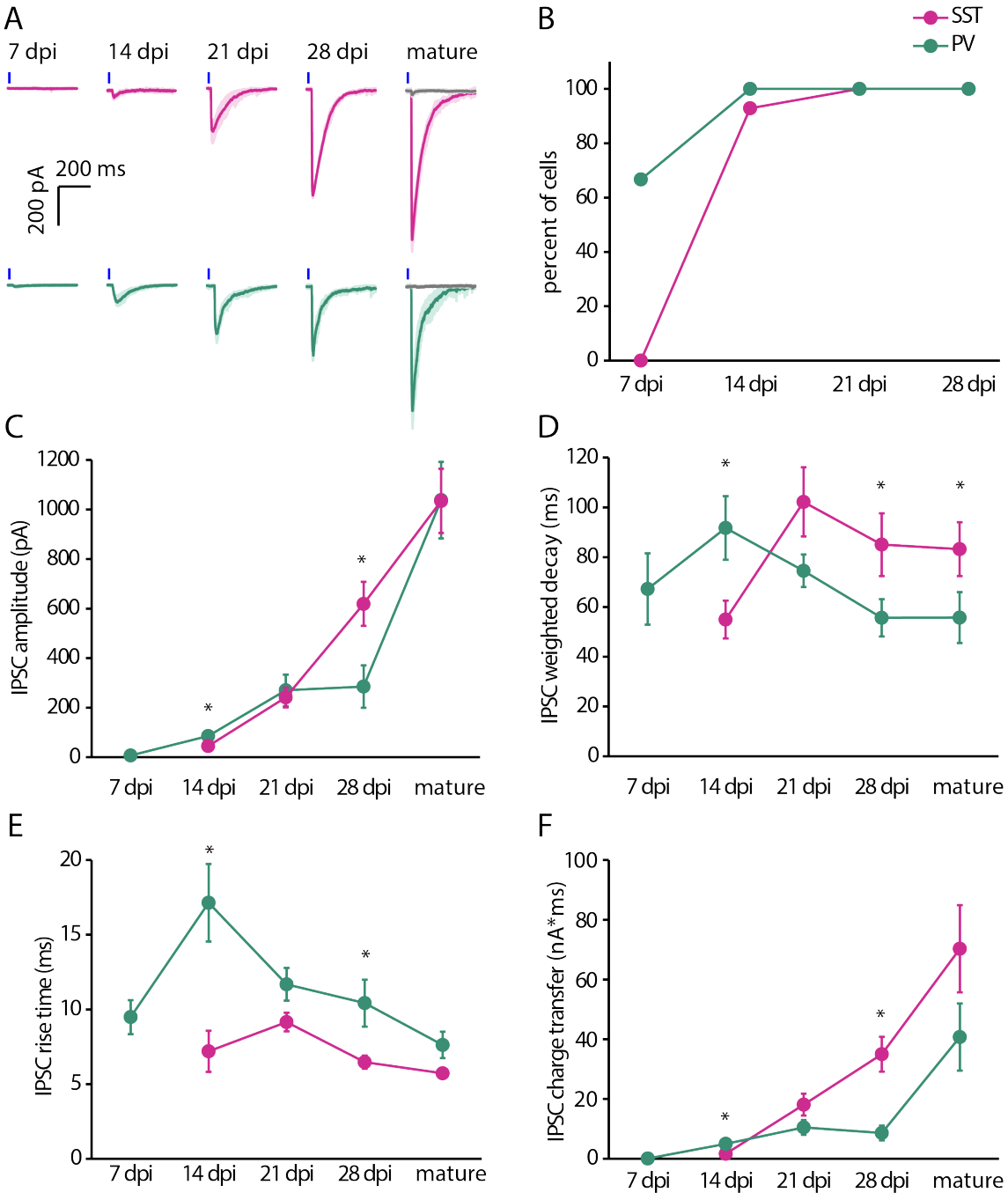

Figure 3.5 Differential synaptogenesis of SST and PV inputs to abDGCs
A) Representative traces show the maximal compound IPSC evoked by ChR2-expressing SST and PV interneurons. Blue line indicates 5 ms light pulse. Gray trace in mature cell shows response after addition of 10 μM bicuculine. **B)** Shows the percent of abDGCs in which a light-evoked response was observed in SST-ChR2 and PV-ChR2 mice **C)** Amplitude of light-evoked IPSCs in SST-ChR2 and PV-ChR2 mice **D)** Weighted decay of light-evoked IPSCs in SST-ChR2 and PV-ChR2 mice **E)** Rise time of light-evoked IPSCs in SST-ChR2 and PV-ChR2 mice **F)** Charge transfer of light-evoked IPSCs in SST-ChR2 and PV-ChR2 mice. *p<0.05, Mann-Whitney *U*.

SST-ChR2 mice at 14 dpi (PV: 91.7 ± 12.8 ms, SST: 54.9 ± 7.6 ms, p = 0.02, Mann-Whitney *U*) but was slower in 28 dpi and mature DGCs in SST-ChR2 mice (28 dpi PV: 54.2 ± 6.7 ms, 28 dpi SST: 85.0 ± 12.6 ms, p = 0.038, mature PV: 55.7 ± 10.2 ms, mature SST: 83.2 ± 10.8 ms, p = 0.04, Mann-Whitney *U)* (**Figure 3.5 C**). The slower decay of IPSCs in SST-ChR2 mice at 28 dpi and in mature DGCs is consistent with previous findings that dendritic synapses have slower kinetics and contain different $GABA_AR$ subunits than perisomatic(Pearce 1993, Soltesz et al. 1995, Brooks-Kayal et al. 2001). SST+ HIPP cells project to the molecular layer where they form synapses with dendrites of mature DGCs, while the axons of PV+ basket cells remain in the GCL and form perisomatic synapses (Houser 2007, Laplagne et al. 2007, Savanthrapadian et al. 2014, Booker and Vida 2018). In addition, the rise time of the compound IPSC was slower in PV-ChR2 than in SST-ChR2 mice at 14 and 28 dpi (14 dpi PV: 17.1 ± 2.6 ms, 14 dpi SST 7.2 ± 1.4 ms, p=0.001, 28 dpi PV: 10.4 ± 1.6 ms, 28 dpi SST: 6.5 ± 0.4 ms, p=0.04, Mann-Whitney *U*) (**Figure 3.5 D**). I also measured the charge transfer of the compound IPSCs and found that the charge transfer was larger in PV-ChR2 mice at 14 dpi (PV: $4.96 \times 10^3 \pm 9.82 \times 10^2$, SST: $1.6 \times 10^3 \pm 6.1 \times 10^2$, p=0.003, Mann-Whitney *U*) and larger in SST-ChR2 mice at 28 dpi (PV: $8.6 \times 10^3 \pm 2.5 \times 10^3$, SST: $3.5 \times 10^4 \pm 5.8 \times 10^3$, p=0.003) (**Figure 3.5 E**).

Quantal IPSCs evoked by SST and PV interneurons

I also analyzed evoked asynchronous IPSCs (aIPSCs) by adding strontium chloride to the extracellular solution and reducing the calcium concentration. Strontium reduces the amplitude of the evoked IPSC by desynchronizing GABA release and allowing the analysis of synaptic events up to one second after stimulation (Goda and Stevens 1994). These aIPSCs are desynchonized release of quantal events originating at individual synapses from the optogenetically stimulated PV or SST interneurons. I did not observe a significant change in amplitude or decay of aIPSCs in either SST-ChR2 or PV-ChR2 mice at any of the timepoints measured, indicating that the

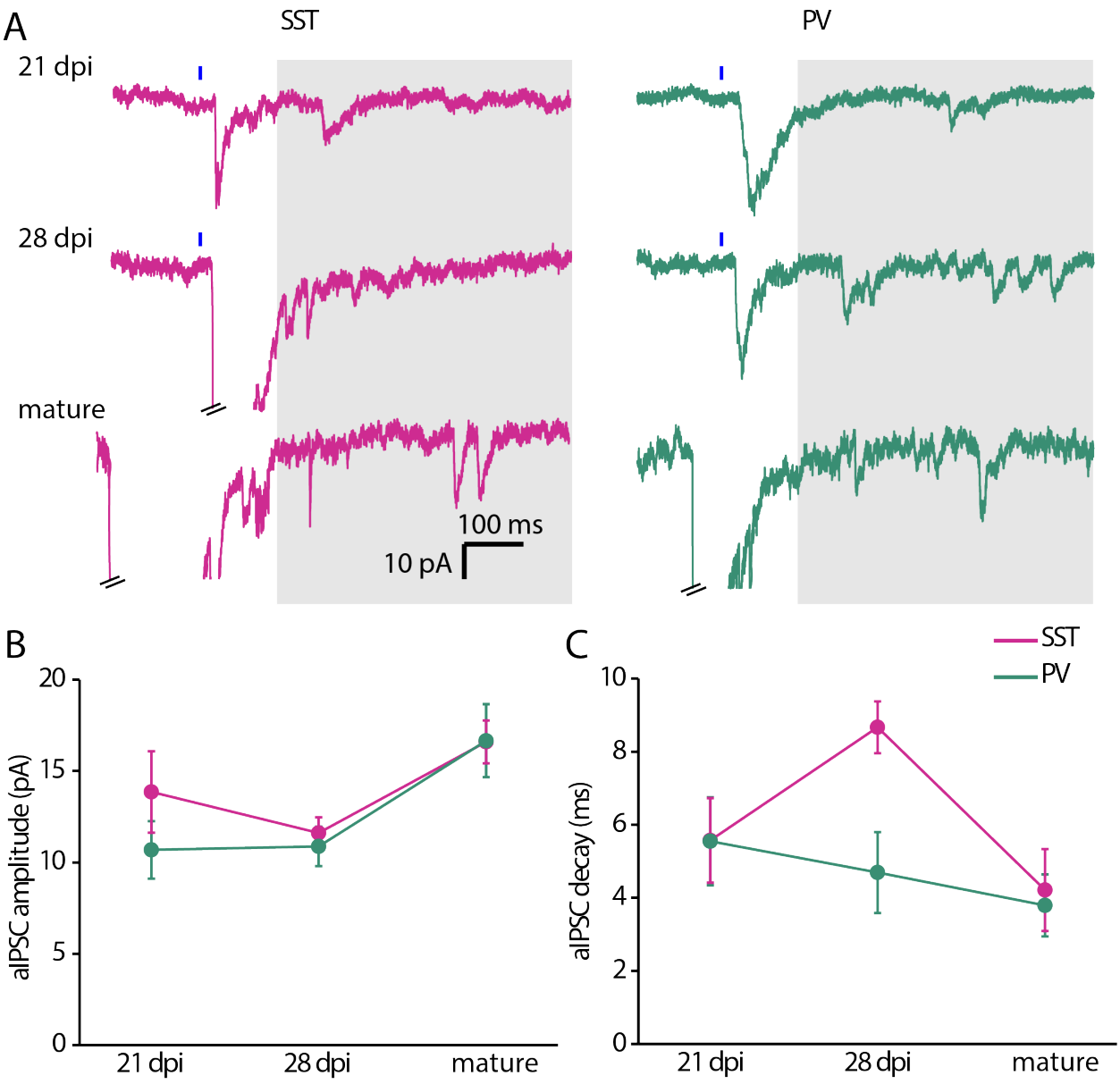

Figure 3.6 Quantal IPSCs evoked by SST and PV interneurons
A) Representative traces show asynchronous IPSCs evoked by ChR2-expressing SST and PV interneurons in 6 mM $SrCl_2$, 2 mM $MgCl_2$, 0.5 mM$CaCl_2$. Blue line indicates 5 ms light pulse. Shaded region indicates 500 ms analyzed following return to baseline of the synchronous IPSC. **B)** Amplitude of aIPSCs in abDGCs in SST-ChR2 and PV-ChR2 mice **C)** Decay of aIPSCs in abDGCs in SST-ChR2 and PV-ChR2 mice. *p<0.05, Mann-Whitney *U.*

strength of individual synapses is not changing during this time (Figure 3.4 A-E). Although the size of aIPSCs in abDGCs is constant, the increase in amplitude of compound IPSCs across these same time points indicates that the number of synaptic inputs from both SST and PV interneurons is increasing as abDGCs mature. Furthermore, the similar size of individual aIPSCs in 28 dpi abDGCs together with the significantly larger amplitude of compound IPSCs in abDGCs in SST-ChR2 mice compared to PV-ChR2 mice at the same time point indicates that SST interneurons form more synapses with 28 dpi abDGCs than PV interneurons.

Effect of running on PV and SST innervation of abDGCs

Voluntary wheel running alters adult hippocampal neurogenesis and improves performance on tasks that rely on neurogenesis (van Praag et al. 1999, van Praag et al. 1999, van Praag et al. 2002, Creer et al. 2010, Bolz et al. 2015). There is evidence that running may increase connectivity of abDGCs to the existing circuitry, but these studies have only found significant changes in the connectivity of abDGCs with cortical regions at older time points (5-7 weeks post injection) (Deshpande et al. 2013, Bergami et al. 2015). In addition, prior studies have used retrograde transynaptic tracing to measure the number of cells forming presynaptic connections to abDGCs. However, this is only one measure of connectivity and does not account for changes in the number or strength of synapses between the same population of cells. Therefore, I asked whether voluntary wheel running altered the development of PV and SST inputs to abDGCs during the first four weeks of maturation, when GABA is critical for neuronal development. SST-ChR2 and PV-ChR2 were single housed with a running wheel beginning 3 weeks prior to surgeries and retroviral injection to label neurons when mice were 6-8 weeks old. I then performed targeted patch-clamp recordings from RFP-expressing cells at 7, 14, 21, and 28 dpi (**Figures 3.7 & 3.8**). Mice ran an average of 7.4 km/day, consistent with what has been previously reported in C57Bl/6 mice (Garrett et al. 2012). By recording the maximal compound optogenetically activated

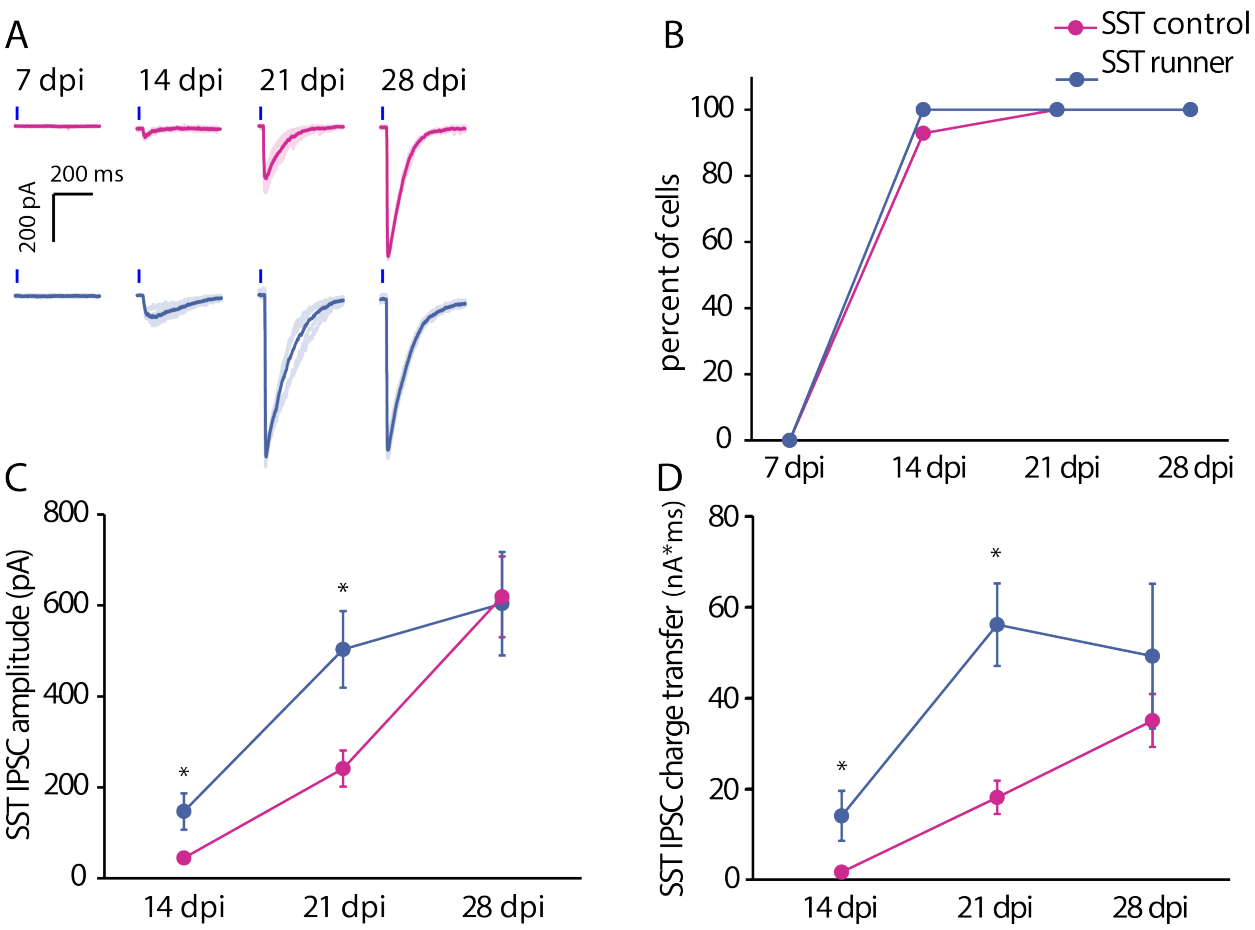

Figure 3.7 Running alters SST synaptogenesis onto abDGCs
A) Representative traces show the maximal compound IPSC evoked by ChR2-expressing SST interneurons in runners and controls. Calibration is 200 pA, 200 ms. Blue line indicates 5 ms light pulse. **B)** Shows the percent of abDGCs in which a light-evoked response was observed in SST-ChR2 runners and controls. **C)** Amplitude of light-evoked IPSCs in SST-ChR2 runners and controls. **D)** Charge transfer of light-evoked IPSCs in SST-ChR2 runners and controls. *p<0.05, Mann-Whitney *U.*

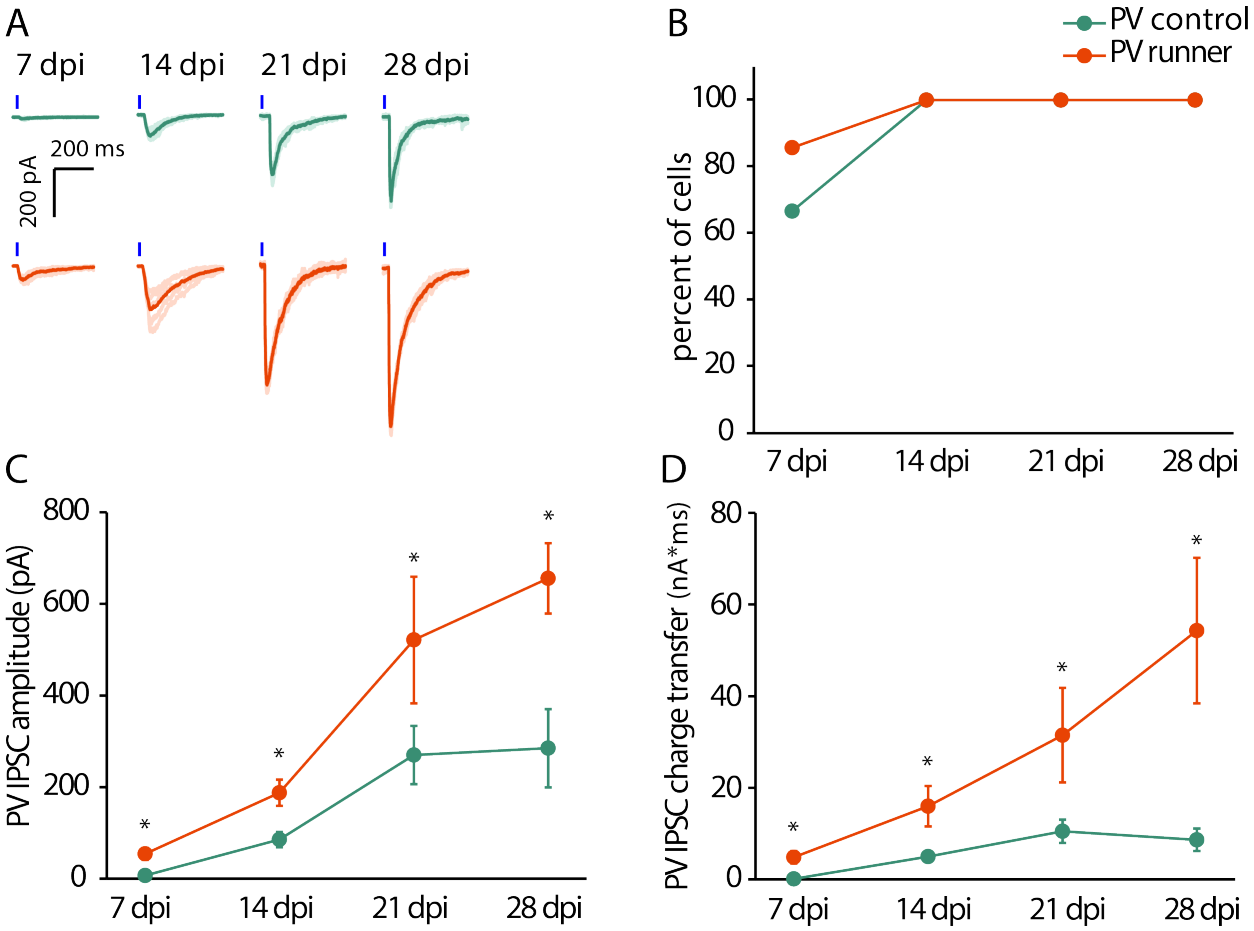

Figure 3.8 Running alters PV synaptogenesis onto abDGCs
A) Representative traces show the maximal compound IPSC evoked by ChR2-expressing PV interneurons in runners and controls. Calibration is 200 pA, 200 ms. Blue line indicates 5 ms light pulse. **B)** Shows the percent of abDGCs in which a light-evoked response was observed in PV-ChR2 runners and controls. **C)** Amplitude of light-evoked IPSCs in PV-ChR2 runners and controls **D)** Charge transfer of light-evoked IPSCs in PV-ChR2 runners and controls. *p<0.05, Mann-Whitney *U.*

IPSC as described above, I found that voluntary wheel running altered the development of SST inputs to abDGCs. While optogenetic activation of SST interneurons still did not evoke an IPSC in any abDGCs at 7 dpi (n = 13/1), the same stimulus evoked an IPSC in 100% of 14 dpi abDGCs (n = 13/2) compared to 92.9% of 14 dpi (13 from 14) abDGCs in controls (**Figure 3.7 B**). In addition, running altered the development of IPSC amplitude in SST-ChR2 mice. At 14 and 21 dpi, SST-ChR2 mice housed with a running wheel had a larger IPSC amplitude and charge transfer than controls (SST runner 14 dpi 146.9 ± 40.0 pA, n = 13/2, SST control 14 dpi 44.6 ± 11.2 pA, n = 14/5 p = 0.01, SST runner 21 dpi: 503.3 ± 84.4 pA, n = 13,2 SST control 21 dpi: 241.2 ± 39.9 pA, n = 18/5, p=0.01, Mann-Whitney *U*) (**Figure 3.7 C & D**). In PV-ChR2 mice, 85.7% of cells at 7 dpi responded to optogenetic activation of PV interneurons in runners, compared to 66.67% in controls (7 dpi PV runner: n = 7/2, control n = 6/3) (**Figure 3.8 B**). Running also increased the amplitude and charge transfer of compound IPSCs in abDGCs at all time points measured in PV-ChR2 mice (PV runner 7 dpi: 54.4 ± 13.1 pA, n = 7/2, PV control 7 dpi: 7.2 ± 0.8 pA, n = 6/2, p = 0.014, PV runner 14 dpi: 187.8 ± 28.5 pA, n = 16/2, PV control 14 dpi: 85.6 ± 16.6 pA, n = 12/3, p=0.007, PV runner 21 dpi: 521.3 ± 138.4 pA, n = 9/1, PV control 21 dpi: 270.2 ± 63.6 pA, p=0.04, n = 13/6, PV runner 28 dpi: 655.9 ± 76.9 pA, n = 4/1, PV control 28 dpi: 285.0 ± 85.5 pA, n = 14/3, p=0.02, Mann-Whitney *U*) (**Figure 3.8 C & D**). These findings indicate that running alters connectivity of SST interneurons to abDGCs in younger abDGCs but increases the strength of PV inputs across all timepoints measured, although additional experiments will be necessary to confirm these conclusions.

Discussion

In this study I characterized the development of synaptic inputs from the two primary populations of interneurons in the dentate gyrus (PV and SST) onto developing abDGCs and determined how these were modified by voluntary wheel running. Given the critical role of GABA in adult

neurogenesis, it is important to understand the contribution of different presynaptic cell types to this process. I found that the amplitude of ChR2-evoked compound IPSCs in both SST-ChR2 and PV-ChR2 mice increased in abDGCs across the first four weeks of maturation and was still larger in mature, unlabeled DGCs indicating that the overall GABAergic input increased as abDGCs mature. PV+ basket cells form synapses onto abDGCs prior to SST+ HIPP cells at 7 dpi, and the maximal contribution of PV interneurons to GABAergic input is larger at 14 dpi. Interestingly at 28 dpi the maximal input from SST interneurons is larger than that from the PV cells. I found that the number of synaptic inputs from both SST and PV interneurons increased as abDGCs mature, but the contribution of SST surpasses that from PV interneurons at 28 dpi. Given these different temporal sequences and patterns of synaptogenesis, and the known differences in the compartmentalized input they provide, it is possible that these two types of interneurons play distinct roles in the maturation of abDGCs.

Voluntary wheel running alters adult hippocampal neurogenesis and improves performance on behavioral tasks that rely on neurogenesis (van Praag et al. 1999, Creer et al. 2010, Bolz et al. 2015). Prior studies demonstrated that wheel running increased the number of presynaptic partners forming connections onto abDGCs, but these changes in connectivity were mostly accounted for by long range connections from outside of the hippocampus and increased connectivity with older abDGCs (Deshpande et al. 2013, Bergami et al. 2015). Furthermore, these studies did not assess the number or strength of synapses between these cells (Deshpande et al. 2013, Bergami et al. 2015). I used optical stimulation of all inputs from two identified classes of interneuron combined with retroviral birthdating to address how voluntary wheel running affects the development of PV and SST inputs to abDGCs during the first four weeks of maturation, when GABA is critical for development. Preliminary data indicates that voluntary wheel running increases the maximal input from both PV and SST interneurons. Running increased the

probability of 14 dpi abDGCs being connected to SST interneurons and increased the maximal IPSC amplitude in 14 dpi and 21 dpi abDGCs. Analysis of PV-ChR2 mice demonstrated that wheel running increased the compound IPSC amplitude at all timepoints that were analyzed after differentiation. The specific effects of running on synaptogenesis may indicate different roles of PV and SST interneurons in mediating the effects of running on adult neurogenesis. SST inputs are not altered by running after 21 dpi, so perhaps SST inputs facilitate morphological changes due to running prior to 28 dpi. The lack of an effect of running on SST inputs at 28 dpi also coincides with the development of a mature hyperpolarized E_{GABA} and the beginning of the critical period of enhanced LTP in abDGCs (Ge et al. 2006, Ge et al. 2007). Running also increases LTP in the dentate gyrus, presumably through the addition of abDGCs (van Praag et al. 1999), so perhaps the lack of effects of running on SST-mediated inhibition at later time points allows for increased plasticity.

Maturation of GABAergic synapses onto abDGCs

The first synaptic inputs to abDGCs are from local GABAergic interneurons in the dentate (Ge et al. 2006). These GABA inputs are important for survival, dendritic development, and glutamatergic synaptogenesis and synapse unsilencing (Ge et al. 2006, Chancey et al. 2013). Phasic GABA responses have been observed in abDGCs as early as 4 dpi using a train of optogenetic activation of presynaptic PV interneurons (Song et al. 2013) and 7 dpi using electrical stimulation (Ge et al. 2006). PV basket cells provide perisomatic inputs to mature DGCs in the GCL, while SST interneurons in the dentate are primarily HIPP cells that project axons to the molecular layer where they synapse onto the dendrites of mature DGCs (Houser 2007, Savanthrapadian et al. 2014, Booker and Vida 2018). Therefore, it is likely that the temporal sequence of innervation from these distinct populations of interneurons is determined in part by the laminar organization of interneuron axons in the dentate. At the earliest timepoints, when dendrites have not extended

beyond the GCL, abDGCs receive perisomatic input from PV interneurons with axons in the GCL. As the abDGCs mature and extend their dendrites into the molecular layer, they encounter the axons of SST interneurons. This input-specific development may due to the organization of dentate interneurons, or interneurons may target specific cellular domains of the postsynaptic cell. Comparing connectivity of DGCs based on their location within the GCL could help determine how this process works. For example, PV interneurons may form more synaptic connections with DGCs residing in the inner GCL than DGCs in the outer GCL if connectivity is simply determined by the spatial relationship of DGC dendrites to the axons of local interneurons. It is also possible that axons seek out specific cellular domains of the postsynaptic cell with a degree of specificity that allows for similar innervation of DGCs regardless of their location within the GCL.

IPSCs in abDGCs have characteristically slow kinetics compared to mature DGCs (Overstreet Wadiche et al. 2005). Esposito and colleagues describe two distinct types of GABA-mediated PSC in abDGCs: fast IPSCs that originate from somatic inputs, and slow IPSCs originating from dendritic inputs (Esposito et al. 2005). Indeed, stimulation of GABAergic inputs from the GCL elicits IPSCs with faster kinetics than IPSCs resulting from molecular layer stimulation (Laplagne et al. 2007). This is consistent with previously described fast somatic IPSCs and slow dendritic IPSCs in developmentally born DGCs (Soltesz et al. 1995) and CA1 pyramidal neurons (Pearce 1993, Banks et al. 1998). This distribution of IPSC kinetics is likely due to inputs from different populations of interneurons targeting different cellular compartments and different $GABA_AR$. subunit composition between these synaptic sites (Pearce 1993, Soltesz et al. 1995, Banks et al. 1998). I find that the decay of compound IPSCs in SST-ChR2 mice is slower than in PV-ChR2 mice in 28 dpi and mature DGCs, consistent with the idea that SST interneurons are forming synapses onto the distal dendrites while PV interneurons are providing perisomatic inputs and that these synapses may have different $GABA_AR$ subunit composition. However, the kinetics of

IPSCs at 14 dpi do not necessarily line up with the idea that perisomatic inputs have faster kinetics while dendritic inputs have slower kinetics. $GABA_AR$ subunit expression has been shown to change during postnatal development of DGCs and during abDGCs maturation, so there may also be developmental changes that contribute to IPSC decay (Kapur and Macdonald 1999, Overstreet Wadiche et al. 2005). Future studies could use pharmacology to determine the $GABA_AR$ subunit composition at synapses from PV and SST interneurons onto abDGCs and how these change over time. Banks and colleagues showed that fast somatic IPSCs, but not slow dendritic IPSCs were mediated by α4-containing $GABA_ARs$ due to the lack of sensitivity to furosemide in CA1 pyramidal cells (Banks et al. 1998). In addition, the $GABA_ARs$ undergo a developmental shift from α4, γ2 containing $GABA_ARs$ with a high sensitivity to furosemide, moderate sensitivity to zinc, and low sensitivity to diazepam in early postnatal development to α1, γ2 containing $GABA_ARs$ that are sensitive to zolpidem and diazepam and insensitive to furosemide and zinc (Kapur and Macdonald 1999). POMC-labeled abDGCs also have a reduced sensitivity to the $GABA_AR$ α1-specific positive allosteric modulator zolpidem compared to mature DGCs (Overstreet Wadiche et al. 2005). PV and SST synapses could have similar increases in α1 incorporation across development, or perhaps this developmental change occurs at a specific subset of synapses. It is also possible that perisomatic inputs from PV interneurons occur at synapses with furosemide-sensitive $GABA_ARs$ containing the α4 subunit and that SST dendritic inputs from SST interneurons don't have the α4 subunit and are insensitive to furosemide. In addition, the use of ChR2 to stimulate presynaptic interneurons may interfere with the interpretation of IPSC kinetics. Since I used repeated maximal stimulation of presynaptic cells, it is possible that GABA spillover could activate extrasynaptically localized receptors contributing to the kinetics of the response and complicating the interpretation of these results. Additional experiments analyzing the kinetics of IPSCs using sub-saturating stimulation will be necessary for these data to be interpreted.

Interneurons in the dentate gyrus

I found that synaptogenesis of PV inputs to abDGCs occurs prior to SST inputs, consistent with the idea that the process of synaptogenesis coincides with morphological maturation of abDGCs. As the neurites of newborn neurons extend through the GCL and into the molecular layer they encounter the axons of different populations of dentate interneurons. However, it is important to consider that not all PV interneurons are basket cells with axons in the GCL. A subset of PV interneurons in the dentate are axoaxonic cells (Soriano and Frotscher 1989, Booker and Vida 2018), although this population of interneurons is not well characterized, it is possible that these two groups of PV interneurons could have different roles in the maturation of abDGCs and may have a distinct pattern of synaptogenesis. A recent study also identified an additional population of SST-expressing interneurons in the hilus with axons forming perisomatic inputs onto basket cells in the GCL (Yuan et al. 2017). Interestingly, they found that focal activation of ChR2-expressing SST interneurons near the soma of mature DGCs evoked an IPSC with slow kinetics. This is likely due to activation of axons of SST+ HIPP cells passing through the GCL that synapse onto the distal dendrites of DGCs in the molecular layer, but does not exclude the possibility of a perisomatic input.

Furthermore, while most SST and PV interneurons that target mature DGCs reside in the dentate, there is the possibility that some of the axon terminals being activated in the current study project from cells outside of the dentate. A recent study found that approximately 15-20% of all PV and SST cells that form synapses onto developmentally born DGCs reside in other hippocampal regions, specifically CA1 and CA3 (Szabo et al. 2017).

Given the distinct patterns of synaptogenesis of PV and SST inputs to abDGCs, it is possible that these inputs regulate different stages of abDGC maturation. Indeed, Song and colleagues have demonstrated this type of input specificity in regulation of aNSC quiescence (Song et al. 2012). *In vivo* optogenetic activation of PV interneurons maintained quiescence of aNSCs while silencing of PV interneurons promoted increased proliferation, but similar manipulation of SST or VIP interneurons had no effect on aNSC quiescence (Song et al. 2012). Survival of abDGCs was also promoted specifically by *in vivo* activation of PV but not SST interneurons (Song et al. 2013). It will be interesting to determine the specific role of inputs from PV and SST interneurons in the maturation of abDGCs.

Running alters the development GABAergic inputs to developing abDGCs

Adult neurogenesis is a highly regulated process that can be regulated by factors such as genes, age, and environment. Voluntary wheel running in mice increases both proliferation and survival of abDGCs, leading to an overall increase in the number of newborn neurons in the dentate (van Praag et al. 1999). Voluntary wheel running also improves performance on a pattern separation task, indicating a functional change in the hippocampal circuitry following running (Creer et al. 2010, Bolz et al. 2015). These behavioral effects of running may be due to an increased number of newborn neurons, but can also reflect additional alterations in the maturation and integration of abDGCs in mice that have run or been exposed to other environmental enrichment. One indication that the maturation of abDGCs may be accelerated by running is the increased dendritic length and branching in 7 dpi abDGCs following voluntary wheel running (Sah et al. 2017). Previous studies have assessed the effects of running on abDGC connectivity using retrograde transynaptic tracing to assess changes in the organization of afferents to abDGCs following running and environmental enrichment. While initial studies found that running increased connectivity of 7-week-old abDGCs, a later study found that this was likely due to changes in long-

range connectivity between the entorhinal cortex, medial septum and nucleus diagonal band of Broca and abDGCs (Deshpande et al. 2013, Bergami et al. 2015). On the other hand, Vivar and colleagues found that running decreased connectivity between local hippocampal neurons and 5-week-old abDGCs and did not result in changes in mIPSC frequency or amplitude (Vivar et al. 2016). To our knowledge, the current study is the first comprehensive analysis of the effects of running on functional connectivity of abDGCs with specific interneuron populations during the early development of abDGCs. Although previous studies found little to no effect of running on local connectivity, there are several possible explanations for our findings. Retrograde tracing only measures the number of presynaptic cells and does not account for changes in synaptic strength or number synapses between the same population of cells. In addition, previous studies using retrograde transynaptic tracing mostly assessed connectivity in 5-7-week-old abDGCs (Deshpande et al. 2013, Bergami et al. 2015). It is possible that running increases the rate of maturation and functional integration of abDGCs, but they eventually reach the same mature state, so these studies may reflect ages when the effects of running on connectivity are no longer present. In SST-ChR2 mice, the effects of running are only observed at 14 and 21 dpi and thus if there was a corresponding change in the number of presynaptic cells, it would not have been seen in these studies. Furthermore, the increase in new abDGCs following running was most profound in the dorsal dentate and is not observed in the ventral dentate (Vivar et al. 2016). The present study was performed entirely in the dorsal dentate, whereas the prior tracing studies were not restricted to this region. Therefore, small changes in the connectivity ratio between abDGCs and local interneurons might be obscured by a lack of alterations in the ventral hippocampus.

Running has been shown to have an effect on GABA signaling throughout the hippocampus. Changes in $GABA_A$ receptor subunit expression and the GABA synthesizing enzyme GAD 67 have been reported in mice housed with a running wheel (Hill et al. 2010, Schoenfeld et al. 2013).

Thus, it is possible that alteration in GABA signaling and interneuron activity are driving the observed changes in synaptogenesis onto abDGCs. However, spontaneous GABA signaling to mature DGCs was unaffected by voluntary wheel running (Vivar et al. 2016). Future experiments assessing the effects of running on GABAergic inputs to mature DGCs will be necessary to address this question.

In summary, the present studies are the first to systematically characterize the temporal sequence of development of inputs from SST and PV interneurons onto abDGCs during the first month after differentiation and to determine the effects of voluntary wheel running on functional connectivity between abDGCs and specific populations of interneurons. I found that the process of synaptogenesis is likely explained by the organization of interneuron projections within the dentate. PV interneurons in the GCL form synapses onto young abDGCs. Then as abDGCs mature and extend increasingly complex dendrites into the molecular layer, they receive synaptic inputs from SST interneurons. I also found that the number of synapses from both SST and PV interneurons increased as abDGCs mature, and that SST interneurons have more synapses onto 28 dpi abDGCs than PV interneurons. In addition, I found that voluntary wheel running increases input from both PV and SST interneurons. Together these studies further our understanding of the role of interneurons in adult hippocampal neurogenesis.

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Chapter 4.

Discussion and Concluding Remarks

Discussion

In this work I analyzed details of the development of abDGCs focusing on their functional maturation. I have presented evidence that the development of GABAergic inputs onto abDGCs during the first four weeks is mostly unaffected by the loss of FMRP in the *Fmr1* KO mice (**Figure 4.1**). Given the range of impairments in GABA signaling that have been described in the hippocampus and the known impairments in adult neurogenesis and neurogenesis-dependent behaviors in *Fmr1* KO mice, it is surprising that GABA signaling appears to be unaltered in developing abDGCs during this early period of maturation and integration. I have also presented evidence that GABAergic synapses onto abDGCs develop in an input-specific manner. PV interneurons in the granule cell layer form synapses onto young abDGCs. Then as abDGCs mature and extend increasingly complex dendrites into the molecular layer, they receive synaptic inputs from SST interneurons. I also show that voluntary wheel running regulates the input from both PV and SST interneurons, but with different time courses. Understanding the process of integration of abDGCs into the existing hippocampal circuitry will contribute to our understanding of the role of adult neurogenesis in information processing and disease.

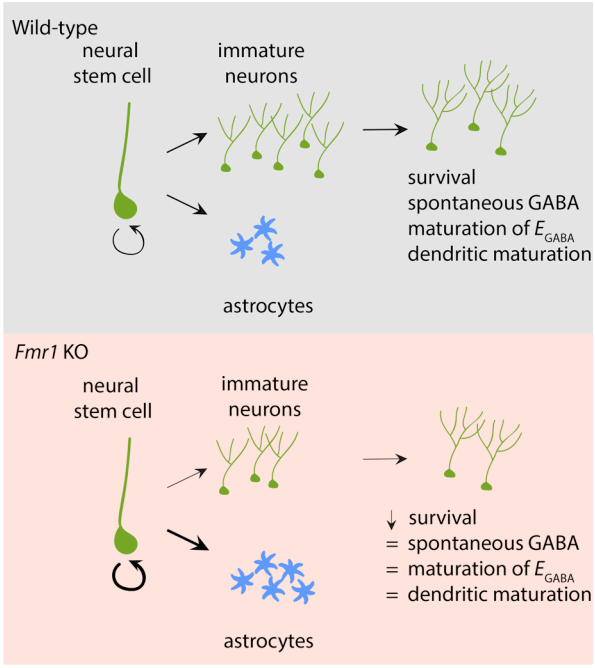

Figure 4.1 Adult neurogenesis is altered in *Fmr1* KO mice, but GABAergic signaling in abDGCs is mostly unaffected by loss of FMRP
Loss of FMRP leads to an increased rate of proliferation of adult NPCs, altered fate specification, and reduced survival of abDGCs in adult *Fmr1* KO mice. The development of GABAergic inputs onto abDGCs during the first four weeks is mostly unaffected by the loss of FMRP in the *Fmr1* KO mice.

Interneurons and adult hippocampal neurogenesis

I have demonstrated that the formation of GABAergic synapses in developing abDGCs occurs in an input specific manner. In the present work, I have dissected the temporal sequence of innervation of abDGCs by two chemically and morphologically distinct populations of interneurons. A remaining question is whether this specificity is simply due to the spatial organization of interneurons in the dentate, or if it is due to specificity of the postsynaptic target domain. I have characterized the development of inputs from PV interneurons onto developing abDGCs and these inputs have been shown to play a role in early development of abDGCs (Song et al. 2012, Song et al. 2013, Alvarez et al. 2016). It will be interesting to determine whether CCK-expressing basket cells, which are morphologically similar but chemically distinct, have a similar pattern of synaptogenesis onto abDGCs and what role they play in the process of abDGC maturation. Although VIP-expressing interneurons have not been well-characterized in the dentate, VIP interneurons in the CA3 region of the hippocampus are basket cells that provide perisomatic inhibition onto pyramidal cells (Booker and Vida 2018). Song and colleagues found that *in vivo* optogenetic manipulation of PV, but not VIP or SST, interneurons regulated quiescence of aNSCs (Song et al. 2012). Similar

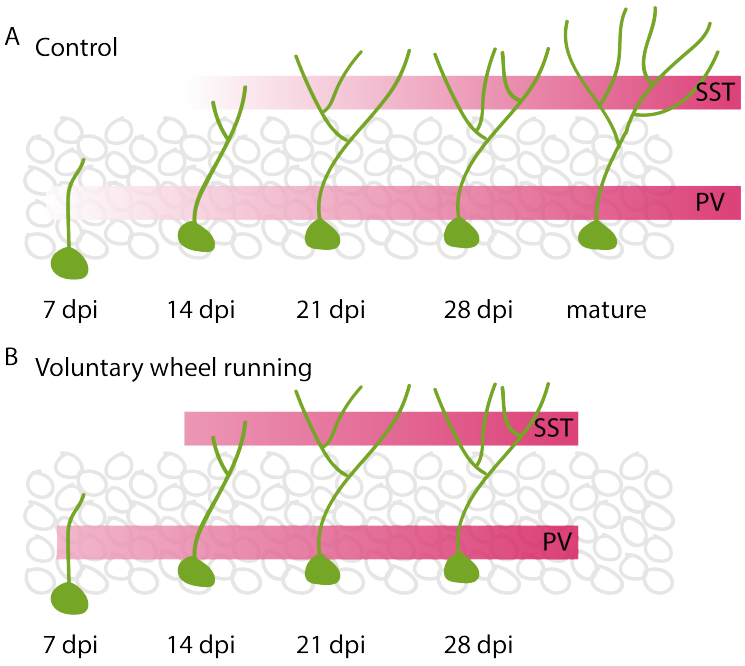

Figure 4.2 PV and SST inputs to abDGCs.
A) Inputs from PV interneurons are detected at 7 dpi, while inputs from SST interneurons are not detected until 14 dpi. The amplitude of the compound IPSC from PV and SST inputs increases as abDGCs mature, likely due to an increase in the number of synapses. **B)** Voluntary wheel running increased the maximal response amplitude of the compound IPSC from PV interneurons at all time point measured, while the SST-evoked response was only

activation of PV, but not SST, interneurons at a later timepoint promotes survival of abDGCs (Song et al. 2013). It is not surprising that SST interneurons do not affect quiescence or survival since these studies were in abDGCs that are less than 7 dpi, when my data indicates that there is no synaptic input from SST interneurons. However, the lack of an effect due to activation of VIP interneurons would indicate that there are additional factors, besides the spatial organization of interneurons, that contribute to the input-specific regulation of adult hippocampal neurogenesis. There are also populations of interneurons that have distinct morphological and chemical identities, but that synapse on to the same domain of the postsynaptic cell. MOPP, Ivy/neurogliaform, and SST/HIPP cells all target the dendrites of mature DGCs in the molecular layer. Again, if the spatial organization of axon terminals in relation to the developing abDGCs determines the development of synaptic inputs, these cells would be expected to have similar temporal patterns of synaptic development. While it is possible that inputs from morphologically similar cells would have similar roles in regulating adult hippocampal neurogenesis, there is evidence that this might not be the case (Song et al. 2012). The present studies have focused on the development of inputs from local interneurons in the dentate gyrus, but it is important to consider that abDGCs receive inputs from other hippocampal regions as well as extra-hippocampal brain regions that might also regulate abDGC maturation. For example, approximately 15-20% of all PV and SST cells that form synapses onto developmentally born DGCs reside in other hippocampal regions, specifically CA1 and CA3 (Szabo et al. 2017).

In addition to the critical role of GABA in regulating adult hippocampal neurogenesis, local interneurons also contribute to this process through release of neuropeptides. Ivy/Neurogliaform cells release the neuromodulator neuropeptide Y (NPY) which regulates proliferation of aNSCs *in vitro* and *in vivo* (Howell et al. 2003, Howell et al. 2007). The VIP receptor VPAC1 promotes a neurogenic fate while VPAC2 receptors promote survival of neural precursors *in vitro* (Zaben et

al. 2009). Deletion of VPAC2 in mice (*VIPR2* KO) leads to reduced survival of abDGCs and a reduced number of neural precursors *in vivo* (Zaben et al. 2009). These studies point to a non-GABA-mediated role of interneurons in regulating adult hippocampal neurogenesis, which will be important to consider when dissecting the role of these interneuron populations.

Activity-dependent regulation of maturation of abDGCs

While this dissertation has focused on the role of GABA in adult hippocampal neurogenesis, other neurotransmitters, neuromodulators, and neuropeptides also play a role in this process. For instance, abDGCs receive glutamatergic inputs from mossy cells in the dentate gyrus as early as 14 dpi and glutamatergic inputs from the entorhinal cortex are established between 3-5 weeks (Ge et al. 2006, Deshpande et al. 2013, Bergami et al. 2015). These inputs play a role in adult hippocampal neurogenesis since glutamate signaling through GluN1-containing NMDARs has been shown to promote survival of abDGCs. Retrovirus-mediated deletion of GluN1 reduces survival of 2-3-week-old abDGCs compared to neighboring WT abDGCs (Tashiro et al. 2006). Furthermore, treatment with the NMDAR antagonist CPP increases survival of GluN1 KO abDGCs, indicating NMDAR-dependent competition for survival of abDGCs (Tashiro et al. 2006). Since glutamatergic synapses are not present prior to 14 dpi it is unlikely that glutamate mediates abDGC survival prior to this time, but it does leave open the question of whether GABA and glutamate work in concert to promote abDGC survival or if there is a critical period during which these inputs regulate adult hippocampal neurogenesis. Interestingly, this period of NMDAR-dependent competitive survival of abDGCs coincides with the development of a hyperpolarized E_{GABA}, raising the possibility that survival is mediated by excitation and that glutamate may take over the role of promoting activity-dependent survival. Further studies investigating the specific role of glutamatergic and GABAergic inputs on abDGC survival at earlier and later timepoints will

be necessary to determine the interplay between these two neurotransmitters in regulating abDGC survival.

Adult hippocampal neurogenesis is also directly and indirectly regulated by dopaminergic input from the ventral tegmental area, acetylcholine (ACh) from the medial septum, and serotonin from the raphe nuclei (Malberg et al. 2000, Kulkarni et al. 2002, Frazier et al. 2003, Kaneko et al. 2006, Mu et al. 2011, Takamura et al. 2014). Dopamine promotes survival and modulates synaptic plasticity in abDGCs. Systemic administration of the dopamine D1-like receptor agonist (±)-SKF38393 increased survival of abDGCs (Takamura et al. 2014). In addition, dopamine suppressed medial perforant path inputs onto newborn and mature DGCs and reduced the potentiation of EPSCs after LTP induction exclusively in abDGCs (Mu et al. 2011). This is likely due to a development shift from expression of D1 dopamine receptor in abDGCs to D2 receptors in mature DGCs (Mu et al. 2011), suggesting that DA may act directly on abDGCs. Cholinergic inputs from the medial septum innervate the hilus and regulate the activity of hilar interneurons, which may in turn regulate the activity of DGCs and aNPCs. Increasing cholinergic signaling by systemic injection an acetylcholinesterase inhibitor increases the survival of abDGCs (Kaneko et al. 2006). Bath application of the muscarinic acetylcholine receptor activator carbachol increased spontaneous GABA-mediated PSCs in nestin-GFP labeled NPCs, presumably by activating hilar interneurons as this effect was blocked by the $GABA_AR$ antagonist bicuculine (Tozuka et al. 2005). Nicotinic acetylcholine receptors may also regulate inputs to abDGCs since bath application of the nicotinic acetylcholine receptor agonist methyllycaconitine enhances evoked IPSC amplitude in developmentally born DGCs (Frazier et al. 2003). These studies demonstrate an indirect role for ACh in adult neurogenesis through its regulation of GABAergic interneurons. However, retrograde tracing studies have identified direct inputs to abDGCs from cholinergic neurons in the

medial septum (Deshpande et al. 2013, Bergami et al. 2015) so there may be an additional role for cholinergic neurons in adult hippocampal neurogenesis through direct inputs onto abDGCs.

The dentate also receives serotonergic inputs from the raphe nuclei. The first indication that serotonin (5-HT) might play a role in adult hippocampal neurogenesis came from the observation that 5-HT-selective SSRIs such as fluoxetine increased proliferation (Malberg et al. 2000). Deletion of tryptophan hydroxylase (TPH, an enzyme required 5-HT synthesis) in mice prevents the effects of running on adult hippocampal neurogenesis (Klempin et al. 2013), indicating that 5-HT may specifically modulate environmental effects on adult neurogenesis. It remains unknown whether 5-HT modulates neurogenesis through direct action on abDGCs or modulates the activity of cells projecting to the neurogenic niche. There is also evidence that norepinephrine regulates adult hippocampal neurogenesis, since treatment with the noradrenaline reuptake inhibitor reboxetine leads to increased neurogenesis (Malberg et al. 2000). Kulkarni and colleagues systemically injected the selective noradrenergic neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromo-benzylamine hydrochloride (DSP-4), to deplete norepinephrine and found that this depletion led to reduced proliferation of aNPCs (Kulkarni et al. 2002). Together, these studies demonstrate the complexity of signaling in the dentate gyrus. While the present work has focused on inputs from local GABAergic interneurons as a key regulator of adult hippocampal neurogenesis, it is important to keep in mind that there are many other signaling pathways working in concert. These actions may occur through direct regulation of abDGCs or through regulation of GABAergic interneurons.

Experience-dependent changes in abDGC connectivity

Voluntary wheel running in mice leads to improved performance on a pattern separation task, indicating a functional change in the hippocampal circuitry following running (Creer et al. 2010,

Bolz et al. 2015). These behavioral effects of running may be due to an increased number of newborn neurons, but running also leads to additional alterations in the maturation and integration of abDGCs. However, the effects of running on abDGC maturation and not well understood. One indication that maturation of abDGCs may be accelerated by running is the increased dendritic length and branching in 7 dpi abDGCs following voluntary wheel running (Sah et al. 2017). Voluntary wheel running also increased the number of cells forming synapses onto abDGCs (Deshpande et al. 2013, Bergami et al. 2015). I have shown that running increases the GABAergic innervation of abDGCs from specific populations of interneurons. Previous studies have indicated a role for PV interneurons in mediating experience-dependent changes in abDGC morphology following brief exposure to an enriched environment (Alvarez et al. 2016). It is possible that increased input following voluntary wheel running also alters the functional maturation and integration of abDGCs and GABA signaling may similarly be required for running-induced changes in abDGC morphology. Future studies using *in vivo* inhibition of PV and SST interneurons with currently available tools such as optogenetics or DREADDs could determine whether these cells types facilitate running-induced changes in abDGC proliferation, survival, or maturation.

In addition, it is likely that other environmental factors such as learning, enrichment, or stress alter the functional connectivity of abDGCs. For example, exposure to an enriched environment leads to preferential recruitment of abDGCs during re-expose to the same environment (Tashiro et al. 2007). Retrograde transynaptic tracing studies found that environmental enrichment also leads to changes in the number of presynaptic cells projecting to 6-week-old abDGCs (Bergami et al. 2015). Moreover, silencing of PV interneurons prevents increased dendritic complexity following a brief exposure to environmental enrichment in 11 dpi abDGCs (Alvarez et al. 2016). Future studies could similarly determine whether environmental enrichment leads to changes to

GABAergic signaling in abDGCs and if inputs from specific interneuron populations are differentially altered by experiences. In addition, further *in vivo* studies can determine how other types of interneurons contribute to experience-dependent changes in adult hippocampal neurogenesis.

Correlation between altered GABA signaling and neurogenesis in disease

The importance of abDGCs to hippocampal function and memory has raised the possibility that alterations in neural stem cell proliferation and maturation and integration of abDGCs contribute to the pathophysiology of neuropsychiatric and neurodevelopmental disorders. Indeed, adult hippocampal neurogenesis is altered in animal models of autism spectrum disorders, schizophrenia, Alzheimer's disease, and epilepsy. Interestingly, these alterations in adult hippocampal neurogenesis are often accompanied by interneuron loss or dysfunction (Masiulis et al. 2011). Given the critical role of GABA in adult neurogenesis, it is likely that disruptions to the local interneuron network in the dentate gyrus might lead to impaired adult neurogenesis. I have presented work addressing this possibility in a disease model and found that although there is evidence of impaired GABA signaling and adult hippocampal neurogenesis in the *Fmr1* KO mouse model of FXS, the development of GABAergic inputs to abDGCs is mostly unaffected by the loss of FMRP. Since most studies of GABA signaling in the *Fmr1* KO mice have focused on other brain regions or used protein and mRNA analysis of larger regions that may include the dentate (i.e. forebrain, hippocampus), how exactly loss of *FMRP* affects local GABA signaling in the dentate gyrus is not known (El Idrissi et al. 2005, D'Hulst et al. 2006, Gantois et al. 2006, Adusei et al. 2010). Therefore, it is possible that GABA signaling in the dentate gyrus is unaffected by the loss of FMRP. Although loss of FMRP leads to a delayed maturation of E_{GABA} in postnatal development, perhaps this is in part mediated by changes in the neural circuitry that normalize as the mice mature. Similar changes may not be present in abDGCs since they are integrating into

an already mature neural circuit. Future studies characterizing GABAergic signaling to developmentally born DGCs in the dentate gyrus of *Fmr1* KO during early postnatal development and in adults could determine whether GABAergic signaling in the dentate is simply unaffected by loss of FMRP or if there is a developmental change in GABAergic signaling in *Fmr1* KO mice.

This lack of an alteration in GABA signaling in *Fmr1* KO mice does not exclude the possibility that perturbations in GABAergic signaling might facilitate disruptions in adult hippocampal neurogenesis in other disorders. For example, mice expressing the Alzheimer's disease risk factor ApoE4 have impaired adult neurogenesis as well as a loss of hippocampal interneurons and impaired GABAergic signaling in abDGCs (Li et al. 2009). There is evidence of altered hippocampal GABA signaling in an animal model of Rett syndrome (Chao et al. 2010, El-Khoury et al. 2014). The gene responsible for Rett syndrome, *MeCP2*, has been shown to regulate NSC quiescence and maturation of abDGCs (Smrt et al. 2007, Gao et al. 2015, Chen et al. 2017). Furthermore, knockout of the autism-associated gene *CNTNAP2* in mice leads to alterations in adult neurogenesis and a specific loss of hippocampal PV interneurons (Penagarikano et al. 2011, Cope et al. 2016). Knockdown of the schizophrenia-linked gene *Disc1* in abDGCs leads to aberrant migration, dendritic morphology, and axonal targeting in abDGCs as well as behavioral deficits in mice (Zhou et al. 2013). Expression of mutated *Disc1* in mice leads to loss of PV interneurons (Shen et al. 2008, Nakai et al. 2014). In the pilocarpine-induced model of temporal lobe epilepsy, seizures induce increased proliferation, altered migration leading to the presence of ectopic hilar cells, and abnormal axonal connectivity to CA3 and the molecular layer (Parent et al. 1997, Parent 2007). This aberrant adult neurogenesis correlates with a selective loss of SST interneurons in mouse models of epilepsy (Sloviter 1987). In addition, the surviving SST interneurons have larger somata, longer dendrites, sprout axons in the molecular layer and have an increased the probability of having monosynaptic connections with DGCs (Zhang et al. 2009).

Therefore, there are multiple disease models in which both abDGCs are affected and that GABA signaling is also altered.

Since perturbations in adult hippocampal neurogenesis in disease models are often accompanied by alterations in GABA signaling, a better understanding of the role of GABA in adult neurogenesis could expand our understanding of the mechanisms of a range of human diseases. Furthermore, since in some cases the perturbations in GABA signaling are cell-type specific, it is possible that specific populations of interneurons might differentially contribute to disease pathology. A better understanding of the specific role of interneuron subtypes in regulating adult neurogenesis might shed light on the mechanisms of altered neurogenesis in these disorders.

Concluding remarks

In summary, I have presented work demonstrating that the functional maturation of abDGCs in the first four weeks after differentiation is largely unaffected by loss of FMRP. I have also demonstrated that the development of GABAergic synapses onto abDGCs and the effects of running on this process are input-specific. This work expands our understanding of the role of interneurons and GABA signaling in adult hippocampal neurogenesis.

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