The effects of methamphetamine exposure during brain development on the cholinergic system and cognition in mice

Jessica A. Siegel

Department of Behavioral Neuroscience

Oregon Health & Science University

3181 SW Sam Jackson Park Road

Portland, Oregon 97239

May, 2011

School of Medicine

Oregon Health & Science University

Certificate of Approval

This is to certify that the PhD Dissertation of

Jessica A. Siegel

"The effects of methamphetamine exposure during brain development on the cholinergic system and cognition in mice"

Has been approved

_____
Dissertation Advisor

_____
Committee Member

_____
Committee Member

_____
Committee Member

_____
Committee Member

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

5-choice serial reaction time task	5-SRTT
Acetylcholine	ACh
Acetylcholinesterase	AChE
Amphetamine	AMPH
Analysis of variance	ANOVA
Apolipoprotein E	ApoE
Basal forebrain	BF
Bone morphogenetic protein	BMP
Choline acetyltransferase	ChAT
Choline transporter	CHT
Conditioned stimulus	CS
Dopamine transporter	DAT
Dopamine	DA
Gestational day	GD
Glutamic acid decarboxylase	GAD
Horizontal limb nucleus of the diagonal band	HDB
Medial septum	MS
Methamphetamine	MA
Muscarinic acetylcholine receptor	mAChR
Nerve growth factor	NGF
Nicotinic acetylcholine receptor	nAChR
Norepinephrine transporter	NET
Norepinephrine	NE
Nucleus basalis of Meynert	NB
Parvalbumin	PVA
Phosphate-activated glutaminase	PAG
Phosphate-buffered saline	PBS
Phosphate-buffered saline with Triton X	PBT
Phosphate-buffered saline with Tween-20	PT
Postnatal day	PND
Pre-pulse inhibition	PPI
Saline	SA
Serotonin transporter	SERT
Serotonin	5-HT
Unconditioned stimulus	US
Ventral tegmental area	VTA
Vertical limb nucleus of the diagonal band	VDB
Vesicular acetylcholine transporter	VAChT

ACKNOWLEDGEMENTS

I would like to thank many people for their help and support. First, I would like to thank my mentor, Dr. Jacob Raber, for his mentorship and dedication to my projects and pursuits, scientific and otherwise. Dr. Raber has opened my mind to a wide range of scientific methods and questions and the knowledge I have gained from his mentorship will undoubtedly serve me in my future. Dr. Raber is always willing to examine data and ideas from multiple angles and has always helped me interpret my findings in a positive light. I thank him for keeping me positive and for encouraging me throughout my projects over the past five years. I would also like to thank my dissertation committee for their advice, support, and contribution to this document: Dr. Gregory Mark, Dr. Steven Matsumoto, Dr. Aaron Janowsky, Dr. Andrey Ryabinin, Dr. Jacob Raber, and Dr. Charles Meshul. Dr. Gregory Mark (AKA the "Cholinergic God") deserves a special thank you for his willingness to talk with me about the cholinergic system and to listen to my concerns over the past years.

I want to thank the faculty, students, and staff of the Department of Behavioral Neuroscience. In particular, thank you to Dr. Matt Lattal for his mentorship during my rotation and second year project. I would also like to thank Dr. Amy Eshleman for teaching me how to perform transporter uptake assays. Also, thank you to Dr. Suzanne Mitchell who has not only been a fantastic resource for all department-related questions but has been a fantastic friend over the years. I thank the members of the Raber lab past and present for their help with experiments and interpretation of data. Specifically I must thank Dr. Ted Benice, Laura Villasana, and Dr. Brian Piper for their advice and input on

my work. Also, thank you to Catherine Dayger, Mike Craytor, Josh Smith, Timothy Pfankuch, Tony Bader, Peter Van Meer, and Cadence True for their help with breeding, injecting, and testing my mice.

Finally, I must thank my friends and family. Thank you to James Stafford and Lauren Dobbs for their humor, energy, support, and friendship over the past five years. I cannot image what graduate school would have been like without them and I believe that The Band will live on forever! Thank you to JSLM +A for their love and support not just throughout graduate school but throughout my *entire* life. I thank all of my family for their support. In particular, thank you Aunt Ruth, Uncle Robert and Aunt Joan, and Grandma Shirley and Grandpa Bert for supporting me through college. I thank Bubba and Tata for spoiling me and loving me to the fullest extent possible. I am blessed to have grown up with such wonderful grandparents and a grandmother who constantly insists on feeding me! I would like to extend a special thank you to my Mom and Dad and Jared, three of my best friends in the world. Without the guidance, advice, and love from my parents, I would not have believed in myself enough to pursue a PhD. Words cannot begin to express how grateful I am to them. Based on my work over the past five years, I must also thank my Mom for not taking methamphetamine while she was pregnant with me! Last, but certainly not least, I want to thank Ted, my partner and best friend. I thank him not only for mentoring me through graduate school and for reading this dissertation multiple times, but I also thank him for his unwavering dedication, love, and support. This dissertation would not have been possible without him.

CHAPTER 1: GENERAL INTRODUCTION

1. Introduction to methamphetamine

Methamphetamine (MA) is a highly addictive central nervous system psychomotor stimulant. In America, MA became popular in the 1940s when it was administered to World War II soldiers to promote wakefulness and fight fatigue, effects that are common among all psychomotor stimulants (Gilman, Rall, Nies, & Taylor, 1993; Gonzales, Mooney, & Rawson, 2010). Production and use of MA for non-medical purposes began to rise in the 1960s and in response to this increase MA was eventually classified as a Schedule II drug by the United States Drug Enforcement Administration (Gettig, Grady, & Nowosadzka, 2006; Gonzales *et al.*, 2010; NIDA, 2010).

MA has a high abuse potential compared to other psychomotor stimulants, likely due in part to its long half-life, its high lipid solubility making transfer across the blood-brain barrier relatively easy, and the fact that MA is metabolized into another active psychomotor stimulant, amphetamine (AMPH), prior to excretion (Barr *et al.*, 2006; Cruickshank & Dyer, 2009; Good, Solt, Acuna, Rotmensch, & Kim, 2010). The prevalence of MA abuse is also likely due in part to the wide availability of the precursors, such as pseudoephedrine, and its ease of manufacture (Gonzales *et al.*, 2010; Sulzer, Sonders, Poulsen, & Galli, 2005). In 2005 the Combat Methamphetamine Epidemic Act was passed, placing federal regulations on the sale of pseudoephedrine (DEA, 2006; Gonzales *et al.*, 2010). Despite a reduction in "mom and pop" meth labs, the use and abuse of MA continues to be a problem.

Usage statistics: Recent data suggest an overall decline in MA use in the United States in recent years (NSDUH, 2007), however not all epidemiological data show this decrease. Emergency department visits for MA-related issues, for example, increased over 50% from 1995 to 2002 (NIDA, 2006). Similarly, the number of individuals admitted to drug abuse treatment centers for MA addiction increased substantially from 1992 to 2004 (DASIS, 2008; NIDA, 2006). MA use has historically been localized to the rural western portion of the United States (DASIS, 2008; NSDUH, 2007). In more recent years, however, use of the drug has spread across the country (NIDA, 2006).

Actions on monoamine neurotransmitters: The addictive nature of MA is in large part due to its effects on the central monoamine neurotransmitters, specifically dopamine (DA), serotonin (5-HT), and norepinephrine (NE) (Katzung, 2007; Sulzer *et al.*, 2005). MA is a lipophilic weak base that crosses the neuronal plasma membrane and acts as a substrate for the vesicular monoamine transporter. Once transported into the vesicle, MA binds free protons and alters the acidic pH of the vesicles, causing monoamine release into the cytosol (Sulzer *et al.*, 2005). MA also acts at the plasma membrane transporters. According to Sulzer *et al.* (2005), it is still debatable whether MA acts to block the uptake of monoamines from the synaptic cleft or if MA itself is a substrate for the transporters and induces reverse-transport of monoamines into the synaptic cleft. Regardless of the precise mechanism, the effect of MA is an increase in non-vesicular release of DA, 5-HT, and NE and the prevention of their reuptake from the synaptic cleft (Katzung, 2007; Sulzer *et al.*, 2005). It is generally thought that MA's affinity is greatest for the DA transporter (DAT) > NE transporter (NET) > 5-HT transporter (SERT).

However, some studies suggest that MA is more potent at the NET than the DAT and causes more NE than DA release. Nonetheless, the affinity for the DAT and NET, and the release of DA and NE, are much greater than that for the SERT and release of 5-HT (Han & Gu, 2006; Rothman *et al.*, 2001).

2. The long-term effects of methamphetamine in the adult brain

Much research has examined the effects of MA on the adult brain. Although not the focus of this dissertation, the following is a brief review of the long-term effects of MA in adults as these are relevant to MA effects during development.

Effects on the dopamine and serotonin systems: MA has long-term toxic effects on the adult brain, as evidenced by long-lasting changes in the monoaminergic neurotransmitter systems. For example, MA users who are abstinent anywhere between 3 months and 5 years show reduced levels of DAT in the striatum and SERT in the thalamus, caudate, putamen, and amygdala compared to non- users (Johanson *et al.*, 2006; Sekine *et al.*, 2006; Volkow, Chang, Wang, Fowler, Franceschi *et al.*, 2001). In adult rodents, SERT and DAT levels in the nucleus accumbens are reduced 2 weeks following MA exposure (Broening, Pu, & Vorhees, 1997; Guilarte, Nihei, McGlothan, & Howard, 2003). Immunoreactivity for tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of DA, is reduced 60 days following MA exposure in adult rats in the nucleus accumbens, frontal cortex, and ventral tegmental area (VTA) (Trulson, Cannon, Faegg, & Raese, 1987). Other neuroadaptations in adult rats following MA exposure include reductions in DA and 5-HT concentrations in the nucleus accumbens (Wallace, Gudelsky, & Vorhees,

1999), the number of 5-HT_2 receptors in the hippocampus, and DA D_1 and D_2 receptors in the nucleus accumbens and the caudate-putamen (McCabe, Hanson, Dawson, Wamsley, & Gibb, 1987). Although beyond the scope of this dissertation, the long-term changes in the DA and 5-HT system contribute to the powerful addictiveness of MA.

Effects on the acetylcholine system: Although it is widely accepted that the monoamines are MA's main biological target, MA also affects other systems, likely through indirect mechanisms. One such system that is altered by MA is the central acetylcholine (ACh) system, or the cholinergic system. Research into the effects of MA on the cholinergic system is limited compared to that for the monoamines. Nonetheless, studies show that heavy MA use in adults impairs various aspects of the cholinergic system (see **section 5**). *One of the goals of this dissertation is to further delineate the long-term effects of MA exposure on the ACh system (**Chapters 2 and 3**).*

Effects on cognition: MA-induced cognitive impairments may be due in part to MA's actions on the monoamine systems, as well as on the ACh system. Increased levels of released monoamines results in decreased sleep and appetite, and increased arousal, heart rate, blood pressure, euphoria, locomotor activity, and with high enough doses, psychotic episodes (Katzung, 2007). Long-term MA use is associated with a variety of cognitive and behavioral impairments in adults. The major cognitive impairments occur in the domains of learning, memory, and executive function (for a review, see (Cruickshank & Dyer, 2009; Scott *et al.*, 2007)). For example, adults who used MA for an average of 10.5 years show deficits on a word-recall memory task (Thompson *et al.*, 2004) while use for

an average of 30 months results in impaired memory and greater impulsivity on a delayed-discounting task (Hoffman *et al.*, 2006). However, not all studies find such pronounced cognitive impairments in MA abusers, albeit cognitive function is consistently lower than that of non-users (Johanson *et al.*, 2006).

While the effects of MA exposure on the adult brain are well documented, much less is understood about the effects of MA exposure on the developing brain. From studies in adults, however, it is clear that MA use can have long-lasting neurotoxic effects and can impair cognition.

3. Studying methamphetamine exposure during brain development

Our understanding of the long-term effects of MA exposure during brain development remains limited. The section below describes why *in utero* MA exposure is a public health concern and the ways in which this can be modeled in rodents.

Usage demographics: The number of children exposed to MA *in utero* increased over the past two decades due to the increased prevalence of MA use amongst pregnant women (Terplan, Smith, Kozloski, & Pollack, 2009). MA use in the 1980's and 90's was generally confined to Caucasian men driving trucks, working construction, or performing other physically demanding jobs. However, the demographic profile of MA use has widened to other populations, including women (Gonzales *et al.*, 2010). MA use among women is abnormally high compared to other drugs of abuse. According to the National Survey on Drug Use and Health, women make up 45% of individuals admitted to drug

abuse treatment centers for MA abuse in the United States. In contrast, women make up only 26% of individuals admitted to drug abuse treatment centers for alcohol or marijuana abuse (DASIS, 2008; NSDUH, 2007). Furthermore, MA users tend to be of child-bearing age. In 2008, 23.7% of emergency department visits involving MA-related issues were made by patients between the ages of 18 and 24 while 34.7% of visits were made by patients between the ages of 25 and 34 (DAWN, 2010). In 2009, the average age of initiating MA use was 19.3 years (NSDUH, 2009). Finally, MA use increases the likelihood of engaging in risky sexual behavior, thereby increasing the chance of a female MA user becoming pregnant (Zule, Costenbader, Meyer, & Wechsberg, 2007).

Children exposed to MA *in utero* have long-term cognitive problems (see **section 7**), yet the neurobiological mechanism underlying these problems are unknown. *It is difficult to study the effects of MA exposure in humans, in part because mothers who use MA are also likely to use other drugs during pregnancy (see **section 7**; (L. M. Smith et al., 2006; L. M. Smith et al., 2011)). Thus animal models of developmental MA exposure are a pertinent and attractive alternative.*

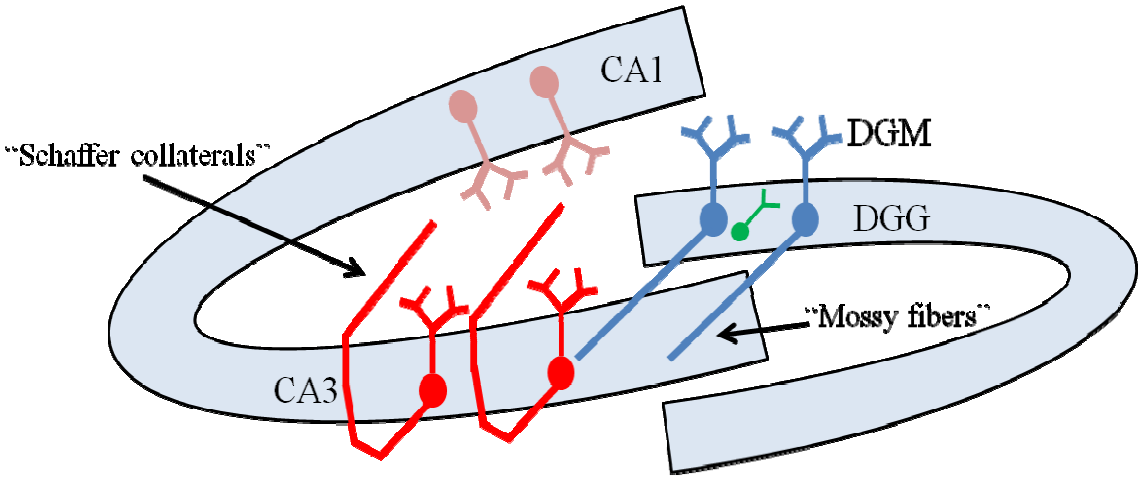
Modeling methamphetamine exposure during brain development in rodents: One way to model human MA exposure *in utero* is to expose animals to MA during gestation by injecting the pregnant mother with MA. An alternative animal model of human *in utero* MA exposure is to expose rodents to MA during the first three weeks of life. Some stages of rodent hippocampal development occur during the first three postnatal weeks, modeling human hippocampal development during the third trimester (Clancy, Finlay, Darlington, & Anand, 2007; Clancy, Kersh *et al.*, 2007; Winzer-Serhan, 2008). The

hippocampus is an area of the brain that is important for many aspects of cognitive function (see **section 6**).

One advantage of exposing pups to MA is that it removes the effects of altered maternal care due to drug administration and withdrawal. Early life parental care can affect cognition later in life and postnatal exposure to MA controls for this possibility (Bredy, Lee, Meaney, & Brown, 2004; Rice, Sandman, Lenjavi, & Baram, 2008). Furthermore, postnatal exposure controls for potential variations in the dose of MA that each pup receives *in utero* depending on the size of the litter, providing a clean way to assess the effects of MA exposure during hippocampal development in rodents.

As discussed in detail below, MA exposure during the first three postnatal weeks in rodents is intended to model MA exposure during the third trimester in humans. However, humans are likely to be exposed to MA during the first or first and second trimesters in addition to the third trimester, or only during the first and second trimesters, as some women discontinue MA use by the third trimester. Therefore it is important to examine the effects of MA exposure at various points of brain development in animal models. Studies in rodents show greater cognitive effects of MA exposure during the postnatal period versus during gestation. MA exposure during both gestation and postnatal development impairs righting reflexes and sensorimotor function, but cognitive impairments are dependent only on postnatal MA exposure (Hruba, Schutova, Pometlova, Rokyta, & Slamberova, 2009; Hruba, Schutova, Slamberova, Pometlova, & Rokyta, 2008; Schutova, Hruba, Pometlova, Deykun, & Slamberova, 2008; Schutova, Hruba, Pometlova, & Slamberova, 2009; Slamberova, Pometlova, & Charousova, 2006; Slamberova, Pometlova, & Rokyta, 2007).

Anatomy of the hippocampus: The hippocampus is divided into the dentate gyrus and the hippocampus proper, which includes the CA1 and CA3 regions (Amaral & Witter, 1989). The primary cells of the dentate gyrus are the granule cells, which are located in the granule layer of the dentate gyrus. Granule cell dendrites extend into the molecular layer of the dentate gyrus while the axons (so called "mossy fibers") make excitatory glutamatergic synapses with pyramidal neurons in the CA3 (I. L. Crawford & Connor, 1973; Frotscher, 1991). The dentate gyrus granule layer also contains basket cells that inhibit the activity of the granule cells (Amaral, Scharfman, & Lavenex, 2007). The pyramidal cell is the prominent cell type within the CA1 and CA3. The CA3 pyramidal neurons send glutamatergic projections (the so called "Schaffer collaterals") to the CA1 (Figure 1) (Storm-Mathisen, 1977).



ıins granule
ʻer while the
s"). Basket
ake
ıls). DGG =

Development of the hippocampus: Birth of the dentate gyrus granule cells occurs as early as gestational day (GD) 21 and continues until postnatal day (PND) 21 in rats (Altman & Bayer, 1990; Bayer, 1980). Approximately 85% of granule cells are generated after birth in rats (Bayer, Altman, Russo, & Zhang, 1993) and dendritic growth of the granule cells continues as late as PND 40 (Rihn & Claiborne, 1990). In humans, the dentate gyrus granule cells are generated beginning around the twelfth week of gestation and continue possibly beyond birth. The majority of the granule neurons migrate from week 19 and beyond in humans (Bayer *et al.*, 1993) and neurogenesis of granule cells continues throughout life (Eriksson *et al.*, 1998). Pyramidal cells of the CA1 and CA3 regions generate between GD 16-20 in rodents, which corresponds to weeks 7-15 of human gestation (Figure 2) (Bayer *et al.*, 1993). *As the cognitive effects of MA exposure are more severe following postnatal exposure and a significant portion of hippocampal development occurs during the postnatal period, a postnatal exposure model is used in all experiments described in this dissertation (see also (Acevedo, de Esch, & Raber, 2007; C. A. Crawford, Williams, Newman, McDougall, & Vorhees, 2003; Grace, Schaefer, Gudelsky, Williams, & Vorhees, 2010; Vorhees et al., 2000; Vorhees, Skelton, & Williams, 2007; Williams, Blankenmeyer et al., 2003; Williams, Vorhees, Boon, Saber, & Cain, 2002)).*

Most studies using the postnatal exposure model examine the effects of MA in adult animals. However, the effects of MA exposure during brain development may differ when assessed in the adolescent rather than the adult and adolescent rodents have more translational relevance to younger human populations exposed to MA *in utero*. Thus one

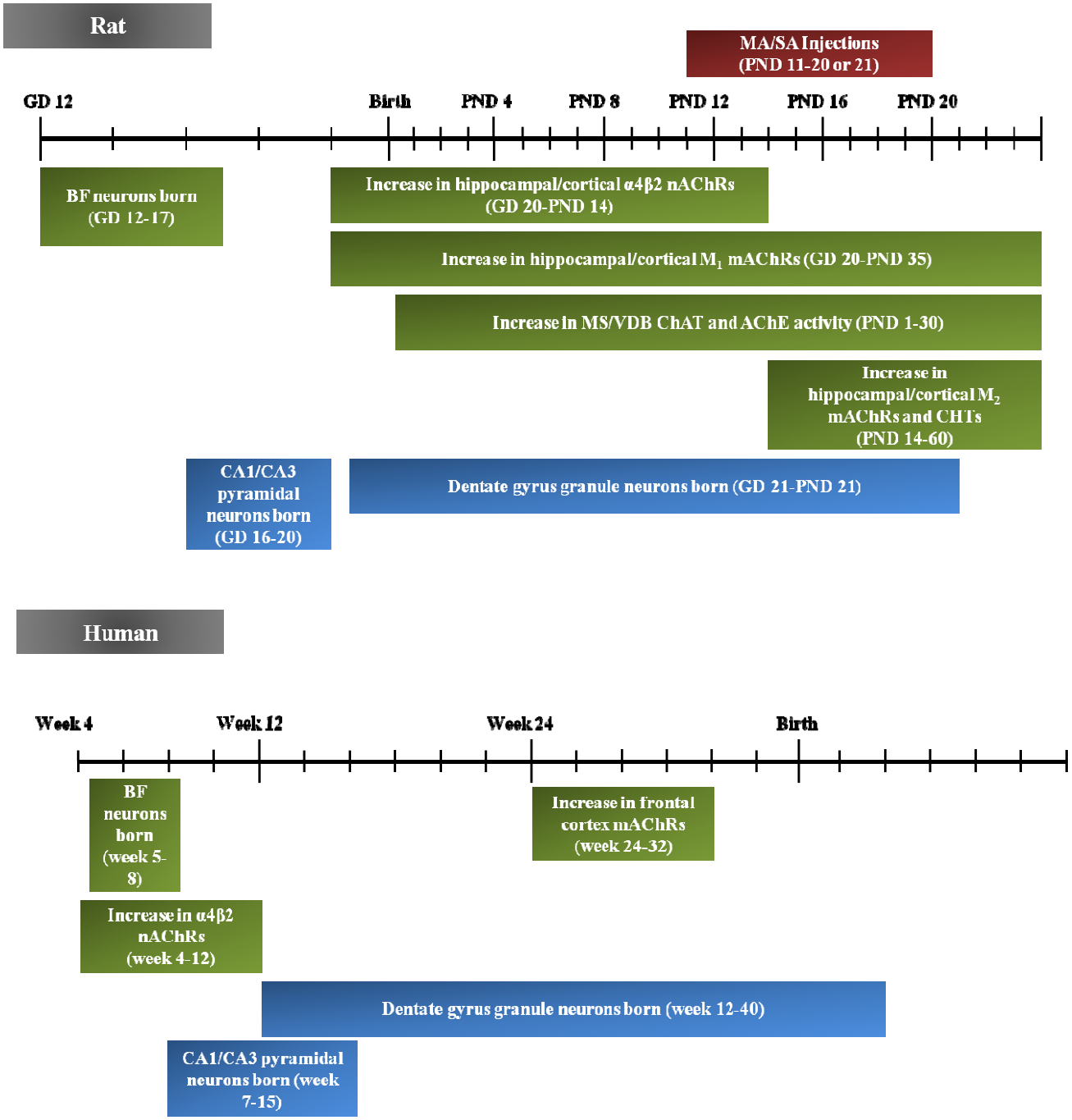


Figure 2. Timeline of hippocampal and basal forebrain cholinergic development. The pyramidal neurons of the CA1/CA3 are born from gestational day (GD) 16-20 in rats and week 7-15 (1st and 2nd trimesters) in humans. The dentate gyrus granule neurons are born from GD 21 to postnatal day (PND) 21 in rats and from week 12-40 (2nd and 3rd trimesters) in humans. Not depicted in this figure, the granule cells show neurogenesis throughout life in both humans and rodents after this initial development of the dentate gyrus. The basal forebrain (BF) cholinergic neurons are born from GD 12-17 in rats, while the α4β2 nicotinic acetylcholine receptors (nAChRs) increase from GD 20 to PND 14 and the muscarinic acetylcholine receptors (mAChRs) increase until PND 35 or 60. Choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) activity increases after birth until PND 30 in rats. Choline transporter (CHT) expression increases between PND 14-16 in rats. In humans, BF cholinergic neurons are born between weeks 5-8 (1st trimester). α4β2 nAChRs increase between weeks 4-12 (1st trimester) while the mAChRs increase between weeks 24-32 (3rd trimester). Methamphetamine (MA) or saline (SA) exposure in this dissertation was from PND 11-20 or 21.

important aspect of the animal model is *when* the effects are measured. *One goal of this dissertation is to assay the long-term effects of MA exposure during brain development on the adolescent brain and behavior (**Chapters 2 and 4**).*

4. The central cholinergic system

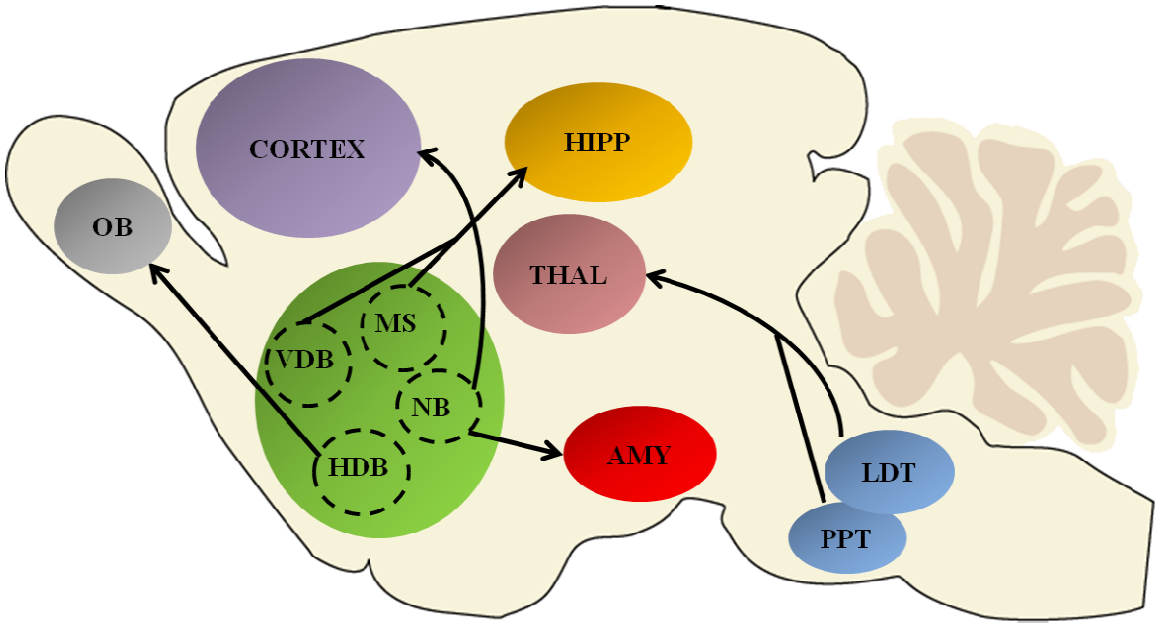
The cholinergic system plays a unique and important role in cognition (see **section 6**) and thus the effects of MA on cholinergic system development may be directly related to MA-induced cognitive impairments later in life. The section below describes the anatomy, circuitry, and development of the basal forebrain (BF) cholinergic system. The effects of MA on the cholinergic system are discussed in **section 5**.

Cholinergic anatomy and circuitry: ACh was first identified as a central neurotransmitter in the 1930s (Halbach & Dermietzel, 2002; Mesulam, 2004). In addition to the BF cholinergic system, cholinergic nuclei are located within the pedunculopontine tegmental nucleus and the laterodorsal tegmental nucleus (Feldman, Meyer, & Quenzer, 1997; Halbach & Dermietzel, 2002; Mesulam, Mufson, Wainer, & Levey, 1983). These neurons send ascending cholinergic projections primarily to the thalamus (Mesulam *et al.*, 1983). For the purpose of this dissertation, the rest of the discussion will focus only on the BF cholinergic system.

The BF contains four cholinergic nuclei: the medial septum (MS), vertical limb nucleus of the diagonal band (VDB), horizontal limb nucleus of the diagonal band (HDB), and the nucleus basalis of Meynert (NB) (Feldman *et al.*, 1997; Halbach & Dermietzel, 2002; Mesulam *et al.*, 1983). The MS and VDB neurons are the major source

of cholinergic innervation in the hippocampus (Gaykema, Luiten, Nyakas, & Traber, 1990; Linke, Schwegler, & Boldyreva, 1994). Studies using acetylcholinesterase (AChE; the enzyme that breaks down ACh) and choline acetyltransferase (ChAT; the enzyme that synthesizes ACh) as markers for cholinergic axons show extensive and dense cholinergic innervation in all regions of the hippocampus, including the CA1, CA3, and dentate gyrus (Houser, Crawford, Barber, Salvaterra, & Vaughn, 1983; Schwegler, Boldyreva, Pyrlik-Gohlmann *et al.*, 1996; Storm-Mathisen, 1977). The cholinergic neurons form excitatory synapses with pyramidal cells, granule cells, and GABAergic interneurons in the hippocampus (Freund & Buzsaki, 1996; Frotscher, 1991; Storm-Mathisen, 1977). Severing the axons from the MS/VDB to the hippocampus dramatically reduces hippocampal levels of ChAT and AChE (Storm-Mathisen, 1977). The cholinergic neurons in the HDB project to the olfactory bulb, while the cholinergic neurons in the NB project mainly to the amygdala and cortex (Figure 3) (Gaykema *et al.*, 1990; Halbach & Dermietzel, 2002; Houser *et al.*, 1983; Mesulam *et al.*, 1983; Zaborszky, Carlsen, Brashear, & Heimer, 1986). The cholinergic NB neurons innervate glutamatergic neurons and GABAergic interneurons in the cortex (Mesulam, 2004).

Non-cholinergic anatomy and circuitry: The BF also contains non-cholinergic neurons that are GABAergic or glutamatergic and play an important role in BF signaling and function (Gritti, Mainville, & Jones, 1993, 1994; Gritti, Manns, Mainville, & Jones, 2003; Henny & Jones, 2006; Manns, Mainville, & Jones, 2001; Mesulam, 2004). GABAergic MS and VDB neurons project along with cholinergic neurons to the hippocampus (Freund & Antal, 1988; Linke *et al.*, 1994). Linke *et al.* (1994) finds that



f
s

10% of MS and 15% VDB neurons that project to the hippocampus in mice are positive for parvalbumin (PVA), a calcium-binding protein that is expressed in many GABAergic cells and is commonly used as a GABAergic marker in the mammalian brain (Baimbridge, Celio, & Rogers, 1992; Freund, 1989; Kiss, Patel, Baimbridge, & Freund, 1990; Linke *et al.*, 1994; Schwegler, Boldyreva, Pyrlik-Gohlmann *et al.*, 1996). The GABAergic projection neurons from the MS/VDB synapse onto GABAergic interneurons in the hippocampus, serving to disinhibit the hippocampal pyramidal and granule cells (Freund & Antal, 1988; Frotscher, 1991). GABAergic BF neurons also project to the cortex and synapse onto inhibitory cortical interneurons, disinhibiting the principle cortical neurons (Freund & Meskenaite, 1992).

The glutamatergic BF neurons were recently discovered and express mRNA for the vesicular glutamate transporter 1 and 2 (Danik *et al.*, 2005; Fremeau *et al.*, 2001; Sotty *et al.*, 2003). Twenty-five to thirty percent of MS/VDB hippocampal projection neurons are glutamatergic and they project to the CA1, CA3, and dentate gyrus (Colom, Castaneda, Reyna, Hernandez, & Garrido-Sanabria, 2005; Sotty *et al.*, 2003). Huh *et al.* (2010) show that glutamatergic MS/VDB neurons make synaptic connections with CA3 pyramidal neurons (Huh, Goutagny, & Williams, 2010). However, not all of the summed pyramidal responses following MS/VDB stimulation are excitatory, suggesting that glutamatergic MS/VDB neurons may also excite inhibitory hippocampal interneurons. To date no study has directly examined whether glutamatergic MS/VDB neurons make synaptic connections with hippocampal interneurons, although these interneurons do express metabotropic glutamate receptors (Boscia, Ferraguti, Moroni, Annunziato, & Pellegrini-Giampietro, 2008; Huh *et al.*, 2010). Glutamatergic MS/VDB neurons also project to the cortex. Manns *et al.* (2001) find that approximately 80% of projection neurons from the MS/VDB to the entorhinal cortex are positive for phosphate-activated glutaminase (PAG; the enzyme that synthesizes glutamate) and that a large proportion of cholinergic and GABAergic neurons projecting to the entorhinal cortex also express PAG (Manns *et al.*, 2001). Cholinergic and GABAergic neurons can express the vesicular glutamate transporter (Danik *et al.*, 2005; Halbach & Dermietzel, 2002; Sotty *et al.*, 2003), but the majority of MS/VDB/HDB glutamatergic neurons do not express ChAT or glutamic acid decarboxylase (GAD; the enzyme that synthesizes GABA) (Colom *et al.*, 2005). A small percent (< 1%) of MS/VDB/HDB neurons express ChAT, GAD, and

glutamate, suggesting that some neurons within the BF can synthesize all three neurotransmitters (Colom *et al.*, 2005).

In addition to projecting externally, neurons within the BF can synapse onto each other and internally regulate signaling. Synapses between glutamatergic neurons within the MS/VDB, for example, can lead to their synchronous firing (Manseau, Danik, & Williams, 2005). Also, both cholinergic and GABAergic MS/VDB neurons form functional synapses with glutamatergic MS/VDB neurons (Manseau *et al.*, 2005) while MS/VDB glutamatergic and GABAergic neurons express ACh receptors, suggesting that these neurons receive cholinergic projections from local and/or extrinsic cholinergic cells (Manseau *et al.*, 2005). In summary, in addition to the cholinergic BF neurons, the GABAergic neurons play an integral part in brain function as these neurons project to the hippocampus and cortex and regulate signaling within these areas. The glutamatergic BF neurons, which have received considerably less attention, also seem to play an important role in BF neurotransmission and regulation of cholinergic signaling within the brain.

External control of the basal forebrain: The BF receives reciprocal projections from the hippocampus and cortex. The hippocampus sends excitatory projections to GABAergic neurons in the lateral septum, which inhibit cholinergic neurons in the MS (Leranth & Frotscher, 1989). GABAergic CA1/CA3 neurons synapse onto MS/VDB GABAergic neurons and to a lesser extent onto cholinergic neurons (Toth, Borhegyi, & Freund, 1993). Various cortical regions also project to the BF (Alonso & Kohler, 1984; Woolf, 1991) and most of these projections are glutamatergic and synapse almost exclusively onto BF GABAergic neurons (Hajszan, Alreja, & Leranth, 2004; Mesulam, 2004;

Zaborszky, Gaykema, Swanson, & Cullinan, 1997). The BF also receives glutamatergic projections from the olfactory bulb, amygdala, thalamus, and medial hypothalamus (Carnes, Fuller, & Price, 1990).

Retrograde labeling studies show that the BF receives tyrosine hydroxylase-positive innervation from the VTA and locus coeruleus and 5-HT-positive innervation from the raphe nucleus (Gaykema & Zaborszky, 1996; B. E. Jones & Cuello, 1989; Semba, Reiner, McGeer, & Fibiger, 1988; Woolf, 1991), providing a potential indirect mechanism for MA effects on the BF. The expression of receptors in the BF matches the neurochemical signature of the innervation it receives. The MS/VDB expresses glutamate, $GABA_A$, 5-HT_1 and 5-HT_2, DA D_1, D_2 and D_5, and noradrenergic α_1 receptors (Berlanga, Simpson, & Alcantara, 2005; Zilles, Werner, Qu, Schleicher, & Gross, 1991). Very similar patterns of receptor expression are found in the NB (Zilles *et al.*, 1991). Thus the BF is influenced by many brain regions, including its target regions and the VTA, locus coeruleus, and raphe nucleus, which are directly affected by MA.

Cholinergic neurochemistry: ACh is synthesized from acetyl coenzyme A (acetyl CoA) and choline by ChAT, which is found primarily in the cytoplasm of the nerve terminal (Feldman *et al.*, 1997; Halbach & Dermietzel, 2002; Oda, 1999). Acetyl CoA is derived from pyruvate in the mitochondria while choline is derived from either dietary sources, ACh hydrolysis (see below), or is produced in the liver and transported across the blood brain barrier. Once synthesized within the neuronal cytoplasm, ACh is transported into synaptic vesicles via the vesicular ACh transporter (VAChT), an antiporter that couples the movement of ACh into the vesicle with the removal of a proton (Feldman *et al.*, 1997;

Usdin, Eiden, Bonner, & Erickson, 1995). After being released into the synaptic cleft, ACh is hydrolyzed into choline and acetic acid primarily by extracellular AChE, which is located either in the synaptic cleft or bound to the pre-synaptic membrane (Cummings, 2000; Halbach & Dermietzel, 2002). Choline is taken back into the pre-synaptic terminal via the low-affinity choline transporter (CHT) or the high-affinity Na^+/Cl^--dependent CHT (Figure 4) (Ferguson & Blakely, 2004; Halbach & Dermietzel, 2002; Lockman & Allen, 2002; Sarter & Parikh, 2005). The low-affinity CHT is ubiquitously distributed in the brain and primarily supplies choline for the synthesis of phospholipids. The high-affinity CHT is localized to cholinergic terminals and supplies choline for the synthesis of ACh (Ferguson & Blakely, 2004; Lockman & Allen, 2002). The transport of choline via the high-affinity CHT is the rate limiting step in neuronal ACh synthesis and positively correlates with the activity of cholinergic neurons (Ferguson & Blakely, 2004; Lockman & Allen, 2002; Takashina *et al.*, 2008). Thus the high-affinity CHT is essential for ACh production, release, and transmission. Reference to CHTs from here on will refer specifically to the high-affinity Na^+/Cl^--dependent CHT.

There are two classes of ACh receptors: nicotinic ACh receptors (nAChRs) and muscarinic ACh receptors (mAChRs) (Cummings, 2000; Feldman *et al.*, 1997; Halbach & Dermietzel, 2002). The nAChRs are ligand-gated ion channels comprised of five membrane-spanning subunits in a 2α3β subunit or 5α7 subunit stoichiometry (Halbach & Dermietzel, 2002; Paterson & Nordberg, 2000). In the central nervous system, the majority of nAChRs constitute combinations of α2-α6 and β2 and β4, or homo-oligomeric α7 nAChRs (Paterson & Nordberg, 2000). Two molecules of ACh must bind to α subunits in order to open the ion channel which depolarizes the neuron (the nAChRs

are permeable to K^+, Na^+, and Ca^{+2}). The nAChRs are located on both pre- and post-synaptic membranes (Halbach & Dermietzel, 2002; Paterson & Nordberg, 2000).

Compared to the mAChRs, the nAChRs show low levels of expression in the brain. Ligand binding studies and *in vivo* imaging studies in humans reveal that nAChRs are expressed primarily in the cortex, thalamus, hippocampus, striatum, cerebellum, and BF (Paterson & Nordberg, 2000). The α7 nAChRs show highest expression levels in the hippocampus (Paterson & Nordberg, 2000).

The mAChRs are metabotropic G protein-coupled receptors. There are five mAChRs subtypes: M_1, M_3, and M_5 mAChRs (M_1-type) and M_2 and M_4 mAChRs (M_2-type). The M_1-type mAChRs are coupled to the G_q protein, which activates phospholipase C, leading to calcium release from intracellular stores and increased neuronal excitation. The M_2-type mAChRs are coupled to the G_i protein, which inhibits adenylate cyclase activity and reduces cyclic AMP, thereby reducing neuronal excitation. Thus, the M_1-type mAChRs are considered excitatory while the M_2-type mAChRs are inhibitory. The M_2-type receptors are often located on the pre-synaptic terminals of cholinergic neurons and provide negative feedback on ACh release (Figure 4).

As mentioned above, the mAChRs are more widely distributed throughout the mammalian brain compared to the nAChRs. The M_1 mAChRs are most commonly expressed in the cortex, hippocampus, amygdala, striatum, and olfactory bulb while the M_2 mAChRs are located in areas of the brain containing cholinergic neurons such as the BF and the pontomesencephalic nuclei, but are also found in the thalamus, hippocampus, cortex, brainstem, striatum, and cerebellum. The levels of M_3 mAChRs are lower in the brain than M_1 or M_2 mAChRs and are found primarily in the cortex, olfactory bulb,

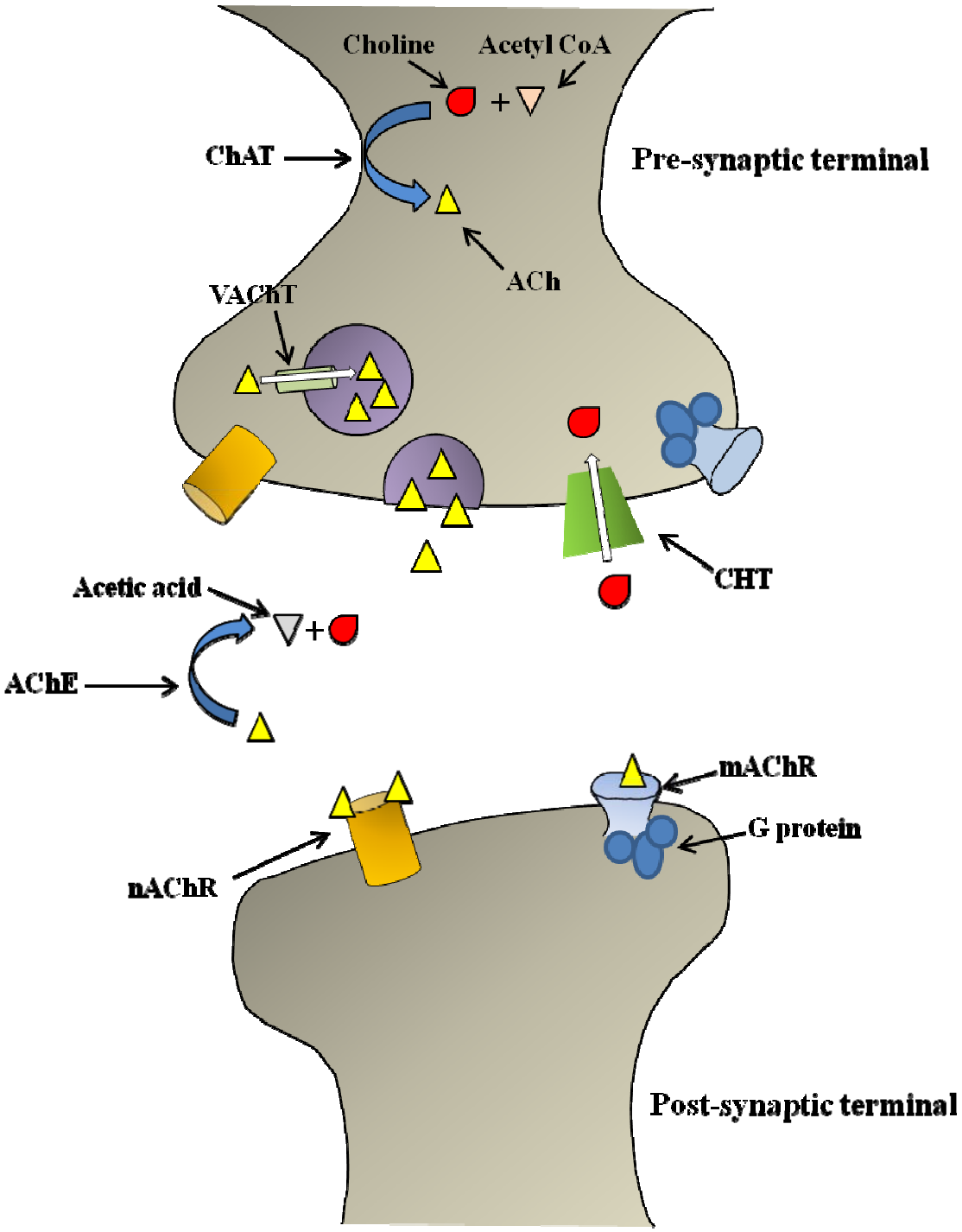


Figure 4. Diagram of a cholinergic synapse. Acetylcholine (ACh) is synthesized by choline acetyltransferase (ChAT) from choline and acetyl coenzyme A (CoA) in the pre-synaptic terminal. ACh is packaged into pre-synaptic vesicles via the vesicular ACh transporter (VAChT). Once ACh is released into the synaptic cleft, it binds to either nicotinic ACh (nAChR) or muscarinic ACh (mAChR) receptors. These receptors can function as either post-synaptic or pre-synaptic receptors. ACh is degraded within the synaptic cleft by acetylcholinesterase (AChE). AChE hydrolyzes ACh into choline and acetic acid. Choline is taken back into the pre-synaptic neuron via the high affinity Na^+/Cl^--dependent choline transporter (CHT), where it is then used for the synthesis of ACh. Diagram adapted from (Abreu-Villaca, Filgueiras, & Manhaes, 2010).

striatum, thalamus, and brain stem. The distribution of the M_4 mAChRs is similar to that of the M_1 mAChRs, except levels are higher in the striatum. Finally the M_5 mAChRs show very low levels in most areas of the mammalian brain (Aubert, Cecyre, Gauthier, & Quirion, 1992; Halbach & Dermietzel, 2002; Schwab, Bruckner, Rothe, Castellano, & Oliverio, 1992). Of note, both the hippocampus and cortex, which receive BF projections and are important for cognitive function (see **section 6**), express high levels of mAChRs and nAChRs.

Development of the cholinergic basal forebrain: BF ACh neurons migrate prenatally but continue to develop mature phenotypes well into postnatal development. In C57BL/6J mice, mitotically-active cells in the germinal zone of the lateral ventricle are present from GD 14-17. These cells divide in the germinal zone and then migrate medially and ventrally to the MS, their final destination, with peak generation occurring on GD 15 (Schambra, Sulik, Petrusz, & Lauder, 1989). The cholinergic cells of the VDB and HDB are born during the same period and migrate medially from the germinal zone of the lateral ventricle (Schambra *et al.*, 1989). A similar pattern occurs in the MS/VDB/HDB of rats, but development begins earlier on GD 12 (Brady, Phelps, & Vaughn, 1989; Semba & Fibiger, 1988). Cholinergic neurons of the NB, which generate between GD 12-16, originate in the germinal zone of the caudatopallial angle and migrate ventrally to the NB (Figure 2) (Brady *et al.*, 1989). The time of peak generation of the NB neurons is GD 16 in mice (Schambra *et al.*, 1989) and GD 13 in rats (Brady *et al.*, 1989).

ChAT activity in rodents is very low in the MS and VDB during gestation. However, activity levels increase substantially after birth, peaking around PND 30 in rats

(Figure 2). A similar developmental pattern occurs for AChE activity in the BF (Thal, Gilbertson, Armstrong, & Gage, 1992). CHT mRNA levels in the mouse septum show steady increases from GD 14, when they are barely detectible, to PND 30 (the last time point measured) (Berse *et al.*, 2005). Protein levels for CHT in the mouse septum parallel this pattern, with higher levels during postnatal development compared to embryonic development (Berse *et al.*, 2005). *Taken together, cholinergic BF cells divide and migrate well before birth in rodents, but mature neurochemistry does not appear until several weeks after birth. Thus postnatal MA exposure might affect the neurochemistry but not the birthdates and migration of BF cells (**Chapter 2**).*

Development of the non-cholinergic basal forebrain: Postnatal MA treatment could also affect the cholinergic BF by changing the development of non-cholinergic BF neurons. Various studies have argued that BF GABAergic development occurs prior to cholinergic development and may contribute in part to the development of the cholinergic phenotype (Bender, Plaschke, Naumann, Wahle, & Frotscher, 1996; Kenigsberg, Hong, & Theoret, 1998). Immunoreactivity for GABA within the BF is first observed on GD 13 in rats, but it is unclear whether it stems from endogenous GABAergic neurons or from fibers innervating the region. By GD 17, GABAergic neurons within the BF are clearly seen (Lauder, Han, Henderson, Verdoorn, & Towle, 1986). *In situ* hybridization shows that mRNA expression for the β subunits of the $GABA_A$ receptor is low at birth and continues to increase until PND 21 in the BF, CA1, CA3, and dentate gyrus, while mRNA levels for the α1 subunit reach maximal levels on PND 14 in the VDB/HDB of rats (Zhang, Sato, Araki, & Tohyama, 1992; Zhang, Sato, & Tohyama, 1991). GAD mRNA is

detected in the MS on GD 17 and shows high levels by GD 20, reaching adult patterns by PND 22 in rats (Bender *et al.*, 1996). To the best of my knowledge, no studies have examined the developmental profile of BF glutamatergic neurons.

Development of the cholinergic system in target regions: Many ACh markers in the BF's target regions develop postnatally in rodents, making them susceptible to postnatal MA exposure. In the hippocampus, AChE and ChAT activity levels are nearly undetectable during gestation and show large increases from birth until PND 30 in rats (Thal *et al.*, 1992). AChE-positive fibers in the dentate gyrus are barely detectable at PND 2 but by PND 11 the projections appear to be mature, suggesting that development of the cholinergic fibers in the dentate gyrus may occur faster than in other regions of the rat hippocampus (Makuch *et al.*, 2001). Kiss & Patel (1992) find very few AChE-positive fibers in the cortex on PND 4 in rats, but the levels increase steadily until PND 28 when the intensity of AChE staining matches that seen in adults (Kiss & Patel, 1992). Similarly, peak AChE and ChAT activity levels are reached in the frontal cortex of rats on approximately PND 30 (Thal *et al.*, 1992; Zahalka, Seidler, Lappi, Yanai, & Slotkin, 1993). The VAChTs develop prior to birth in the hippocampus and cortex in rats, but the CHTs show a late developmental profile, increasing substantially from PND 14-60 (Figure 2) (Aubert, Cecyre, Gauthier, & Quirion, 1996). *Thus the rodent hippocampal and cortical cholinergic projections do not reach neurochemical maturity until approximately 3 weeks after birth, and MA exposure during these first postnatal weeks may have a detrimental effect on this development (**Chapters 2 and 3**).*

The developmental profile of the ACh receptors has been documented in both rodents and humans. The ACh receptor subtypes show differential developmental timelines, with the nAChRs developing prenatally and the mAChRs developing postnatally in rodents. The α7 nAChRs appear in the hippocampus as early as GD 12 in rats (Abreu-Villaca *et al.*, 2010). While the α4β2 nAChRs are present well before birth in rodents, levels in the cortex and hippocampus do increase between PND 1-14 (Abreu-Villaca *et al.*, 2010; Aubert *et al.*, 1996). Similar early development of nAChRs has been shown in humans, with mRNA for various subunits (α3-5 and α7 and β2-4) seen in fetuses as early as week 5 of gestation in the medulla, pons, cerebellum, and forebrain. Cortical mRNA levels are detected beginning at gestational week 6 (Hellstrom-Lindahl, Gorbounova, Seiger, Mousavi, & Nordberg, 1998). Binding studies in humans substantiate these findings, with α4β2 nAChR binding increasing from gestational week 4-12 (Hellstrom-Lindahl *et al.*, 1998). Thus the nAChRs show an early developmental profile in both rodents and humans. In contrast, M_1 mAChRs levels are very low prior to birth and show large increases after birth and up to PND 21 and 35 in the rat hippocampus and cortex, respectively (Aubert *et al.*, 1996). The M_2 mAChRs show large increases in receptor binding sites between PND 20-40 in cortical regions and maximal levels on PND 21 in the hippocampus of the rat (Aubert *et al.*, 1996). A similar late pattern of mAChR development is observed in humans. Binding of mAChRs in the frontal cortex is undetectable at gestational week 14 in humans. The mAChRs appear around weeks 16-18 and represent approximately 24% of the receptor density at birth in the frontal cortex. Between weeks 24-32 there is a dramatic increase in mAChR binding sites, with over 60% of the total receptors found at birth developing during the third

trimester (Figure 2) (Ravikumar & Sastry, 1985). Receptor binding in areas other than the frontal cortex was not measured in this study and may reflect a different developmental pattern. Nonetheless, this study demonstrates the late development of the mAChRs in humans that matches that seen in rats (Ravikumar & Sastry, 1985).

Extracellular factors produced in target regions of the BF can regulate the development of the cholinergic phenotype. Once again, any disruption or alteration of these target-derived factors during development, such as that potentially caused by MA, could alter cholinergic development and have long-term effects on the cholinergic system. The best characterized target-derived factor is nerve growth factor (NGF), which provides trophic support to cholinergic neurons in the BF by activating one of two receptors: the low-affinity neurotrophin receptor p75NTR or the high-affinity TrkA receptor (Abreu-Villaca *et al.*, 2010). NGF is produced in target regions of the BF (e.g. the hippocampus and cortex) where it binds to the NGF receptors on the terminals of cholinergic BF neurons. NGF is transported back to the cell body where it, and its activated receptors, can induce intracellular signal transduction cascades and transcription factors that promote cholinergic cell activity and survival (Counts & Mufson, 2005; Wainer *et al.*, 1993). Cell culture and *in vivo* studies definitively demonstrate that NGF exposure enhances various aspects of cholinergic development and function in the BF, hippocampus, and cortex (Hartikka & Hefti, 1988; Li *et al.*, 1995; Madziar, Lopez-Coviella, Zemelko, & Berse, 2005; Mobley *et al.*, 1986; Tian, Sun, & Suszkiw, 1996). NGF levels in the cortex and hippocampus increase after birth in rats and peak around PND 21 before declining to adult levels, preceding an increase in ChAT activity in these regions by several days (Conner & Varon, 1997; Large *et al.*, 1986). There is also an

increase in p75NTR-immunoreactive fibers in the hippocampus after birth until approximately PND 14, when levels and patterns look similar to those seen in adult rats (Conner & Varon, 1997). Thus it is suspected that NGF supports the differentiation and maturation of developing cholinergic fibers as they reach their target regions (Large *et al.*, 1986).

5. The effects of methamphetamine exposure during brain development on the cholinergic system

Very little is known about the long-term effects of developmental MA exposure on the cholinergic system. The majority of the literature on MA and ACh focuses on the adult brain and will be briefly described prior to discussing the effects of MA exposure during brain development.

Effects on the adult cholinergic system: The data presented below suggest that MA has effects on the cholinergic system at least in adult humans and rodents. Heavy MA use reduces the activity of ChAT in various areas of the human brain, including the caudate and the hippocampus (Kish *et al.*, 1999; Siegal *et al.*, 2004). Heavy MA use in adult humans is also associated with a 48% increase in concentrations of the VAChT in the caudate (Siegal *et al.*, 2004). High doses of MA acutely increase ACh levels in the VTA (Dobbs & Mark, 2008), interpeduncular nucleus (Hussain, Taraschenko, & Glick, 2008), and the striatum (Taguchi *et al.*, 1998) in adult rodents and reduce the total number of cholinergic cells in the dorsal striatum of adult rats (Zhu, Xu, & Angulo, 2006). Additionally, MA alters both types of ACh receptors: the nAChRs up-regulate in cell

culture following MA incubation (Garcia-Rates, Camarasa, Escubedo, & Pubill, 2007) while the mAChRs down-regulate in the striatum, cortex, and hippocampus following MA exposure in adult rats (McCabe, Gibb, Wamsley, & Hanson, 1987).

Effects of exposure during brain development on the adolescent cholinergic system: *The effects of developmental MA exposure on the adolescent cholinergic system remain to be examined.* Most studies that have examined the effects of MA exposure on the adolescent brain focus on the DA/NE/5-HT systems. In the only study to examine a neurotransmitter system other than DA/NE/5-HT in the pre-adolescent or adolescent brain, Acevedo *et al.* (2008) show that MA exposure on PND 11 immediately increases histamine levels in the brains of C57BL/6J mice (Acevedo, Pfankuch, van Meer, & Raber, 2008).

Effects of exposure during brain development on the adult cholinergic system: The long-term effects of MA exposure during brain development have been examined in adult rodents. Once again, most of these studies focus on the monoaminergic systems and not the cholinergic system. The only studies to examine the long-term effect of MA exposure during brain development on the cholinergic system show reduced AChE-positive fibers in the cortex and dentate gyrus in adult gerbils exposed to a single toxic dose of MA on PND 14 (Busche, Bagorda, Lehmann, Neddens, & Teuchert-Noodt, 2006; Lehmann, Hundsdorfer, Hartmann, & Teuchert-Noodt, 2004). *As the cholinergic system plays a unique and important role in cognition (see **section 6**), it is important to further understand how MA exposure during brain development affects the cholinergic system (**Chapters 2 and 3**).*

Mechanism of action on the cholinergic system: The mechanism by which MA affects the ACh system is unknown. Cholinergic neurons in the BF express receptors for DA, 5-HT, and NE and receive input from DA, NE, and 5-HT neurons within the brainstem (Berlanga *et al.*, 2005; Smiley, Subramanian, & Mesulam, 1999; Zilles *et al.*, 1991). Thus MA-induced increases in DA/NE/5-HT may influence the cholinergic BF neurons. A more detailed discussion about the potential mechanisms by which MA might influence the cholinergic system is presented in the general discussion (**Chapter 6**).

6. The cholinergic system and cognition

The central cholinergic system plays a unique and important role in cognitive function, especially attention (for a review, see (Sarter & Bruno, 2004)) and learning and memory (for a review, see (McKinney & Jacksonville, 2005)). The section below describes the evidence for the ACh system's unique role in cognition. The importance of the hippocampus and cortex, both of which receive cholinergic projections from the BF, are also introduced.

Acetylcholine and cognition in adults: Cortical ACh release increases during performance of attention-demanding tasks (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002; Dalley *et al.*, 2001; Himmelheber, Sarter, & Bruno, 2000; Passetti, Dalley, O'Connell, Everitt, & Robbins, 2000) and hippocampal ACh release increases during memory retrieval in contextual fear conditioning tests in rats (Nail-Boucherie, Dourmap, Jaffard, & Costentin, 2000). Lesions of the BF in rats impair target detection (Risbrough, Bontempi, &

Menzaghi, 2002) and response accuracy (Muir, Everitt, & Robbins, 1994) in the 5-choice serial reaction time task (5-SRTT), an attention test for rodents (Robbins, 2002). AChE inhibitors improve attentional performance on the 5-SRTT in BF-lesioned rats and enhance object location memory in mice (Muir *et al.*, 1994; Murai, Okuda, Tanaka, & Ohta, 2007). Similar impairments in attention following BF lesions are seen in non-human primates (Voytko *et al.*, 1994). Specific lesions of the cholinergic BF neurons in rats impair spatial learning and memory in the water maze, a test commonly used to measure hippocampus-dependent learning and memory ((Frick, Kim, & Baxter, 2004; Lin, LeBlanc, Deacon, & Isacson, 1998; Morris, Garrud, Rawlins, & O'Keefe, 1982) but see (Galani *et al.*, 2002)).

Genetic and pharmacological studies targeting the ACh receptors provide much evidence supporting a role for the cholinergic system in cognition. Studies in both rodents and primates demonstrate the importance of nAChRs in cognitive function (for a review, see (Paterson & Nordberg, 2000)). Briefly, nicotine, which is a potent nAChR agonist, improves learning and memory in a spatial discrimination task in rats with septum lesions (Decker, Majchrzak, & Anderson, 1992) and improves attention in rodents (J. M. Phillips, McAlonan, Robb, & Brown, 2000; Young *et al.*, 2004). Mice lacking the α7 nAChR show impairments in the 5-SRTT and olfactory working memory span performance (Young *et al.*, 2007). In humans, acute nicotine administration decreases reaction time and increases target detection accuracy and memory recognition (Froeliger, Gilbert, & McClernon, 2009).

Blockade of mAChRs in rodents impairs spatial learning in the water maze (Fontana, Inouye, & Johnson, 1994), passive avoidance retention memory (Fontana *et al.*,

1994), object location recognition memory (Murai *et al.*, 2007), memory in a delayed non-matching to position task (van Hest, Stroet, van Haaren, & Feenstra, 1990), spatial discrimination learning (Steckler & Holsboer, 2001), contextual and cued fear memory (Feiro & Gould, 2005; Rogers & Kesner, 2004), sensorimotor gating in the pre-pulse inhibition (PPI) test (C. K. Jones & Shannon, 2000), and attention in the 5-SRTT (Mirza & Stolerman, 2000; Ruotsalainen, Miettinen, MacDonald, Koivisto, & Sirvio, 2000). mAChR blockade also impairs object recognition memory in adult rats, an effect that is reversed by AChE inhibitors and nicotine (Sambeth, Riedel, Smits, & Blokland, 2007). Scopolamine, a general mAChR antagonist, impairs object recognition and the number of words recalled in a working memory task while AChE inhibitors improve reaction time on a working memory task in humans (Thiel, 2003). Taken together, this literature demonstrates the vital importance of the cholinergic system in a variety of cognitive tasks in multiple species.

The developing acetylcholine system and cognition: Cholinergic disruption during brain development induces cognitive impairments later in life (for a review, see (Berger-Sweeney, 2003)). Irreversible cholinergic BF lesions on PND 7 impair memory for social olfactory information (Ricceri *et al.*, 2004) and spatial learning in the water maze (Pappas, Payne, Fortin, & Sherren, 2005) in male rats in adulthood. Cholinergic BF lesions on PND 1 or 3 impair acquisition of a passive avoidance task in adolescent female rats and impair novel location recognition memory in adult male and female rats (Ricceri, Hohmann, & Berger-Sweeney, 2002). Irreversible lesions of the NB on PND 1 reduce spatial learning in the water maze in male mice at 8 weeks of age (Arters, Hohmann,

Mills, Olaghere, & Berger-Sweeney, 1998). In contrast, pre- and postnatal choline supplementation improves working memory in the radial arm maze in adult rats (Meck, Smith, & Williams, 1989). *The proper development of the cholinergic system is important for cognitive function later in life. Any disruption in cholinergic function during brain development, such as that potentially caused by MA, is likely to result in a variety of cognitive deficits.*

The role of the hippocampus and cortex in cognition: The BF cholinergic cells project to the hippocampus and cortex, two areas of the brain that are intimately involved in cognitive function. Lesions of the hippocampus in rodents cause deficits in working memory in the T-maze, spatial learning and memory in the water maze, and impair acquisition of passive avoidance (Deacon, Bannerman, Kirby, Croucher, & Rawlins, 2002; Gerlai, McNamara, Williams, & Phillips, 2002; Morris *et al.*, 1982). Hippocampal lesions in rats also impair contextual, but not cued, fear conditioning memory (Otto & Poon, 2006; R. G. Phillips & LeDoux, 1992) and impair novel location recognition and context memory, but not novel object recognition memory (Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002; Save, Poucet, Foreman, & Buhot, 1992). The novel location recognition test is a spatial memory test, whereas the novel object recognition test does not have a spatial component. Thus the hippocampus is considered to be important for contextual and spatial learning and memory. However, it should be noted that humans with hippocampal brain damage show impaired object recognition memory, an effect not seen in rodents (Reed & Squire, 1997).

Cortical lesions do not impair spatial learning and memory in the water maze or radial arm maze (Bussey, Muir, & Aggleton, 1999; Morris *et al.*, 1982), but do impair attention in the 5-SRTT and passive avoidance memory (Muir, Everitt, & Robbins, 1996). The effects of cortical lesions on novel location and novel object recognition memory depend on the exact location of the lesion. For example, lesions specific to the posterior parietal cortex impair both novel location and novel object recognition (Save *et al.*, 1992) while lesions of the cingulate cortex impair novel location, but not novel object, recognition memory in rats (Ennaceur, Neave, & Aggleton, 1997). Lesions of the perirhinal and postrhinal cortex impair novel object recognition memory (Bussey *et al.*, 1999) while lesions of the medial prefrontal cortex fail to induce impairments in either novel location or novel object recognition memory (Ennaceur *et al.*, 1997). Humans with lesions of the left ventrolateral frontal cortex and right superior medial frontal cortex show impairments in "cognitive control" or inhibition of responses in a modified stroop task (Alexander, Stuss, Picton, Shallice, & Gillingham, 2007). Taken together, the hippocampus and the cortex are important for various cognitive functions. *Disruption of the development of either of these regions that might occur with MA exposure is expected to result in impaired cognitive performance later in life. The work described in this dissertation examines how MA exposure during hippocampal development affects cognitive performance on a variety of tasks, including novel location and novel object recognition memory, in adolescence and adulthood (**Chapters 4 and 5**).*

7. The effects of methamphetamine exposure during brain development on cognition

Effects in infants and children: Although the precise mechanism by which MA causes impairments is not understood, it is clear that MA exposure during brain development results in a variety of long-term abnormalities and impairments. Assessing the effects of MA exposure in humans is difficult since mothers who use MA are also likely to use alcohol, nicotine, and marijuana during pregnancy (L. M. Smith *et al.*, 2006; L. M. Smith *et al.*, 2011). While many studies try to control for the effects of poly drug exposure, this is an inherent limitation of studying the effects of MA exposure during brain development in humans.

To the best of my knowledge, no studies have examined the long-term effects of *in utero* MA exposure on adult behavior or cognition. Thus, the discussion below focuses only on the effects in infants and children. Exposure to MA *in utero* increases the risk of being born small for gestational age (L. Smith *et al.*, 2003; L. M. Smith *et al.*, 2006), increases the risk of birth defects (Forrester & Merz, 2007), and decreases birth weight, length, and head circumference (Little, Snell, & Gilstrap, 1988). Maternal MA use during pregnancy is also associated with higher incident of preterm birth, complications associated with maternal hypertension, and placental abruption (Good *et al.*, 2010). Infants exposed to MA or MA and AMPH during pregnancy show reduced visual recognition in the Fagan Test of Infant Intelligence (Struthers & Hansen, 1992). Heavy MA use at any point during pregnancy is also associated with lower arousal, increased lethargy, and physiological stress during the first 5 days of life. MA exposure during the third trimester is specifically associated with poor movement quality (Paz *et al.*, 2009; L. M. Smith *et al.*, 2008).

The effects of MA exposure during brain development persist into childhood and adolescence. Heavy MA use at any point during pregnancy is associated with poor motor ability, as measured by grasping, at 1 year of age. However this impairment is gone by 3 years (L. M. Smith *et al.*, 2011). MA exposure results in impaired visual motor integration, attention, and verbal memory in 3-16 year old children, and these impairments are associated with reduced hippocampal, putamen, and globus pallidus volumes (Chang *et al.*, 2004). MA exposure during any point of pregnancy also impairs visual motor integration in 3-4 year old children (Chang *et al.*, 2009). MA exposed children show impairments in hippocampus-dependent spatial memory at 7-9 years of age, but no effects are found on other cognitive tasks, including intelligence quotient and visual-spatial working memory (Piper *et al.*, 2011). MA-exposed children are also more likely to be behind in school, diagnosed with Attention Deficit Hyperactivity Disorder, and to show pronounced problems in executive function as assessed by parental ratings on the Behavioral Rating Inventory of Executive Function questionnaire compared to unexposed children (Piper *et al.*, 2011). Finally, a study from Sweden followed children exposed to AMPH and/or MA to the age of 14. This series of studies finds that MA/AMPH exposure is associated with reductions in intelligence quotient, impairments in school performance due to delays in math and language development, and impairments in physical ability (for a review, see (Lester & Lagasse, 2010)). Despite difficulties controlling for poly drug exposure, these studies demonstrate that MA exposure during brain development leads to a variety of cognitive deficits in infants and children.

Children exposed to both MA and alcohol *in utero* show reduced striatal volumes that are correlated with cognitive deficits measured by the full-scale intelligence quotient

compared to children exposed only to alcohol (Sowell *et al.*, 2010). MA/alcohol-exposed children also show more diffuse brain activation during a word recall task compared to children exposed only to alcohol, suggesting a compensatory enhancement in brain activation in MA exposed children to support impaired recall on a word memory task and a weaker verbal memory network (Lu *et al.*, 2009).

Although there are consistent effects of MA exposure during brain development on cognition, there are individual differences in the degree of these impairments. For example, despite overall group differences between MA-exposed and unexposed children, some MA-exposed children are rated on the Behavior Rating Inventory of Executive Function and perform in a test of spatial memory similar to unexposed children (Piper *et al.*, 2011). Individual differences in the degree of cognitive impairment after MA exposure suggest that other factors, such as genetic factors, may modulate the susceptibility to develop cognitive impairment after *in utero* MA exposure. However, these potential genetic contributions have not yet been examined. *As the genetic contribution to MA-induced effects is a relatively unexplored field, one aim of this dissertation is to examine how genetics might affect MA-induced cognitive impairments following exposure during brain development (see* ***Chapters 3 and 5*** *for more details).*

Effects in adolescent rodents: Adolescence in rodents occurs between PND 30-50 (Spear, 2000). This time period models some of the brain, behavioral, and hormonal changes that occur in humans during adolescence (Payne & Hales, 2004; Spear, 2000). Very few studies have examined the long-term effects of MA exposure during adolescence and the findings described below include studies that measure behavior or cognition in pre-

adolescent and adolescent animals. Rats exposed to MA during the entirety of gestation show impairments in righting reflexes from PND 1-12, motor coordination on the rotarod on PND 23, and decreases in pain withdrawal thresholds on PND 30 (J. Y. Chen *et al.*, 2010; Hruba *et al.*, 2008; Slamberova *et al.*, 2006). Furthermore, rats exposed to MA during gestation show reductions in activity levels on PND 14 (Acuff-Smith, Schilling, Fisher, & Vorhees, 1996) and PND 30 (Weissman & Caldecott-Hazard, 1993). Exposure to MA during the first 3 weeks of life through nursing from an exposed mother also impairs righting reflexes on PND 12 while exposure during the entirety of gestation and the first 3 weeks of life impairs motor coordination on the rotarod on PND 23 (Hruba *et al.*, 2008).

To the best of my knowledge, only two studies have examined adolescent cognition following MA exposure during brain development. Male rats exposed to MA from either GD 7-12 or GD 13-18 show impairments in retention of passive avoidance memory on PND 20, an effect not observed in female rats (Acuff-Smith *et al.*, 1996). In a study by Vorhees *et al.* (2007), rats exposed to MA (5 mg/kg four times daily) from PND 11-20 show impairments in spatial learning and memory in the water maze on PND 30 and 40 (Vorhees *et al.*, 2007). *To date, no studies have examined cognitive function in adolescent mice exposed to MA during brain development or behavior on tests other than passive avoidance and water maze. However, the studies described above suggest that behavior and cognition are affected in adolescence and warrant further investigation.*

Effects in adult rodents: A greater body of literature has focused on the long-term effects of MA exposure during brain development in adulthood, and many of the effects mirror

what is found in humans following MA exposure *in utero*. For example, exposure to MA during the first 3 weeks of life through nursing impairs spatial learning in the water maze in adult male rats (Hruba *et al.*, 2009). Rats exposed to MA (40 mg/kg once daily or 5, 10, or 15 mg/kg four times daily) from PND 11-20 also show reductions in spatial learning and memory in the water maze (Grace, Schaefer, Graham *et al.*, 2010; Skelton, Williams, Schaefer, & Vorhees, 2007; Vorhees *et al.*, 2000; Vorhees *et al.*, 2007; Williams, Morford, Wood, Wallace *et al.*, 2003; Williams *et al.*, 2002) and the Barnes maze (Williams, Blankenmeyer *et al.*, 2003) in adulthood. The impairments in spatial learning and memory following postnatal MA exposure are seen as late as PND 360 in rats, suggesting that the effects of MA are long lasting (Vorhees *et al.*, 2007). These spatial learning and memory impairments in rodents are associated with MA-induced hippocampal impairments, evidenced by reductions in microtubule-associated protein-2 levels in the hippocampus (Acevedo *et al.*, 2008) and reduced neurogenesis in the dentate gyrus (Schaefers, Teuchert-Noodt, Bagorda, & Brummelte, 2009) in adult rodents exposed to MA during hippocampal development.

Exposure to a much lower dose of MA (5 mg/kg once daily) from PND 11-20 in C57BL/6J mice also impairs adult learning and memory in the water maze as well as sensorimotor gating in the PPI test, novel location recognition memory, and novel object recognition memory (Acevedo *et al.*, 2007). *Most developmental MA research has used rats and very high doses of MA, and the behavioral testing has generally been limited to a few cognitive tests. Thus further work is warranted to determine if lower doses of MA can induce impairments in a variety of behavioral and cognitive domains in mice* ***(Chapters 4 and 5).***

8. Summary and dissertation goals

Infants and children exposed to MA during brain development show physical, brain, and cognitive abnormalities. However, the neurobiological mechanisms underlying these abnormalities are unknown, and there are currently no approved treatments for MA-exposed children. The work presented in this dissertation aims to increase our understanding of the effects of MA exposure during brain development. My overarching hypothesis is that MA exposure during brain development will alter the cholinergic system and impair cognition in adolescent and adult male and female mice.

MA exposure detrimentally affects many neurotransmitter systems, including ACh. The ACh system plays a unique and important role in cognitive function and thus potential MA-induced alterations in the ACh system may contribute to the cognitive deficits in exposed children and animals. Many important aspects of the cholinergic system develop late in rodents and humans and overlap with the late development of the hippocampal granule cells. The effects of MA exposure on the developing ACh system, however, are poorly understood. Furthermore, animal models of MA exposure during brain development have typically focused on the long-term effects of exposure in adulthood. In terms of translating results to human populations, it is important to understand how MA exposure during brain development affects the brain and behavior in adolescence, as potential treatments or interventions for MA-induced impairments in humans will likely occur during adolescence as opposed to adulthood.

The goal of this dissertation is to investigate the effects of MA exposure during brain development on the BF cholinergic system and cognition in both adolescent and adult mice in a series of studies asking the following general questions:

1. What are the effects of MA exposure during brain development on the adolescent BF cholinergic system? By what mechanism might MA alter the BF cholinergic system? In order to begin to address these questions, the current work examines the effects of MA exposure during brain development on cholinergic and GABAergic BF cells and cholinergic projections and receptors in the hippocampus and cortex in adolescent mice. Based on the literature suggesting that developmental MA exposure decreases cholinergic axons in adult male gerbils, I hypothesize that MA exposure during brain development will decrease the density of cholinergic cells in the BF, the area occupied by cholinergic axons in the hippocampus and cortex, and the number of mAChRs in the hippocampus and cortex in male and female adolescent mice.

2. What are the effects of MA exposure during brain development on the adult BF cholinergic system and how are they similar to, or different from, the effects seen in adolescence? To address these questions, the effects of MA exposure during brain development on cholinergic projections and receptors in the hippocampus and cortex are examined in adult mice. Similar to my hypothesis for adolescent mice, I predict that MA exposure during brain development will decrease the area occupied by cholinergic axons and the number of mAChRs in the hippocampus and cortex in male and female adult mice.

3. What are the effects of MA exposure during brain development on adolescent behavior and cognition? This work assays the effects of MA exposure during brain development

on a variety of behavioral and cognitive tasks in adolescent mice. Based on previous studies in rats showing impairments in spatial learning and memory and passive avoidance memory in adolescence following developmental MA exposure, I hypothesize that MA exposure during brain development will impair cognitive function in various cognitive tasks in male and female adolescent mice.

4. What are the effects of MA exposure during brain development on adult behavior and cognition and how might the effects differ between adolescents and adults? Previous studies have investigated the long-term cognitive effects of MA exposure during brain development in adult wild type mice. How might these results differ from those in mice expressing different human genes? In order to address these questions, the current work examines the effects of MA exposure during brain development on a variety of behavioral and cognitive tasks in adult mice expressing human apolipoprotein (apoE) E3 or E4. Previous data show impairments in cognition in adult wild type mice exposed to MA during brain development, and these impairments are more severe in adult female than male mice. Thus I hypothesize that mice expressing both apoE3 and apoE4 will show cognitive impairments in adulthood following developmental MA exposure, and that the adult female mice will show greater impairments compared to the male mice and apoE4 mice will show greater MA-induced impairments compared to apoE3 mice.

CHAPTER 2: LONG-TERM EFFECTS OF METHAMPHETAMINE EXPOSURE DURING BRAIN DEVELOPMENT ON THE CHOLINERGIC SYSTEM IN ADOLESCENT MICE

1. Introduction

Children and rodents exposed to MA during brain development show cognitive impairments on various tasks (Acevedo *et al.*, 2007; Chang *et al.*, 2009; Lester & Lagasse, 2010; Piper *et al.*, 2011; Vorhees *et al.*, 2000), some of which are dependent on the hippocampus (Morris *et al.*, 1982; Save *et al.*, 1992). Hippocampal granule cell birth and development occur during the first three postnatal weeks in rodents, modeling this stage of human hippocampal development during the third trimester (Altman & Bayer, 1990; Bayer, 1980; Bayer *et al.*, 1993; Clancy, Finlay *et al.*, 2007; Clancy, Kersh *et al.*, 2007; Winzer-Serhan, 2008). MA exposure in humans and rodents disrupts hippocampal function: MA-exposed children have smaller hippocampal volumes compared to unexposed children (Chang *et al.,* 2004) while postnatal MA exposure in rodents results in altered expression of NGF (Skelton *et al.,* 2007), decreased dendritic spine density (Williams, Brown, & Vorhees, 2004), and decreased microtubule-associated protein-2 levels (Acevedo *et al.,* 2008) in the hippocampus. Thus postnatal MA exposure targets hippocampal function as does MA exposure *in utero* in humans.

The biological mechanisms underlying MA-induced cognitive impairments are not well understood. The BF ACh system plays a unique and important role in cognition (Muir *et al.*, 1994; Sarter & Bruno, 2004; Voytko *et al.*, 1994) and MA-induced cognitive impairments may be associated with disruption of the ACh system. The BF cholinergic

neurons project to the hippocampus and cortex, two areas of the brain that are important for many cognitive tasks (Halbach & Dermietzel, 2002; Morris *et al.*, 1982; Save *et al.*, 1992). Furthermore, postnatal MA exposure may target important aspects of cholinergic development that occur after birth in rodents (Aubert *et al.*, 1996; Berse *et al.*, 2005; Kiss & Patel, 1992; Thal *et al.*, 1992; Zahalka *et al.*, 1993), modeling the effects of MA exposure on late stages of cholinergic development in humans (Ravikumar & Sastry, 1985). The effects of MA on cholinergic neurons in the BF and projections to the hippocampus and cortex, however, have not been examined.

In order to determine the effects of MA exposure on the BF cholinergic system, the current work measured ChAT expression in the BF, hippocampus, and cortex, as ChAT is commonly used as marker for cholinergic cells and processes (Oda, 1999). mAChR binding was also examined in the hippocampus and cortex, as the mAChRs are present in high concentrations in these brain regions (Aubert *et al.*, 1992; Halbach & Dermietzel, 2002; Paterson & Nordberg, 2000). The BF GABAergic neurons were also measured. PVA is good marker of GABAergic BF cells. PVA-positive cells in the BF do not express ChAT (Gritti *et al.*, 1993; Kiss *et al.*, 1990) and nearly all PVA-positive BF cells also stain positive for GABAergic markers (Freund, 1989; Gritti *et al.*, 2003). Other calcium-binding proteins, such as calretinin and calbindin-D28K, are also expressed in GABAergic cells within the brain (Baimbridge *et al.*, 1992). However, calretinin-positive cells in the BF are distinct from cells expressing PVA or ChAT and they do not project to the hippocampus or cortex. Furthermore, while less than 10% of calretinin-positive BF cells co-localize with GAD, over 80% co-localize with PAG, suggesting that calretinin is a good marker of glutamatergic BF cells in rats (Gritti *et al.*, 2003; Kiss, Magloczky,

Somogyi, & Freund, 1997). Calbindin-positive cells have also been found in the BF, and these neurons are distinct from those expressing PVA and ChAT. Calbindin-positive cells do not project to the cortex and a very small percentage (< 5%) co-localize with GAD (Gritti *et al.*, 2003; M. L. Smith, Hale, & Booze, 1994). Other studies suggest that calbindin is not present in the MS/VDB/HDB (Freund, 1989). Thus of the calcium-binding proteins, PVA is the best marker of GABAergic BF cells and was used in this study for this purpose.

Animal models of MA exposure during brain development have typically focused on the long-term effects of MA in adulthood whereas relatively little research has focused on the effects in adolescence. Rats exposed to MA during brain development show impairments in the DA, NE, and 5-HT systems during pre-adolescence and adolescence (Cabrera, Levy, Li, van de Kar, & Battaglia, 1993; Grace, Schaefer, Gudelsky *et al.*, 2010; Schaefer *et al.*, 2008; Weissman & Caldecott-Hazard, 1993), but the effects of MA on the adolescent ACh system are unknown. Thus the BF ACh system was examined in adolescent mice following MA exposure during hippocampal development. It was hypothesized that MA exposure during brain development would decrease the density of BF cholinergic neurons, the area occupied by cholinergic axons in the hippocampus and cortex, and the mAChRs in the hippocampus and cortex in male and female mice.

2. Methods

Animals: Three- to 5-month-old male and female C57BL/6J mice were bred in our colony using breeding cages containing one male and two female mice. Female mice were singly housed from the first sign of pregnancy, when they gained a noticeable

amount of weight. Lab chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO) and water were given *ad libitum*. On PND 21, pups were weaned and group housed with five mice per cage according to sex. Litters were provided soft foods during the injection period and two weeks after weaning to maintain stable weight gain. The mice were kept on a 12 hr light/dark schedule (lights on at 06:00). All procedures conformed to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University.

Injections: (*d*)-MA hydrochloride (5 mg/kg), obtained from the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse drug supply program, was diluted with 0.9% sodium chloride (saline (SA)) to the appropriate concentration. This dose of MA was selected based on previous findings that it causes long-term cognitive impairments in adult mice exposed during postnatal hippocampal development (Acevedo *et al.*, 2007). Male and female pups from a total of 18 separate litters were weighed and given a single intra-peritoneal injection of MA or SA daily at 10:00 from PND 11-20 (injection volume of 0.1 mL). A within-litter injection design was used to balance the number of MA and SA injections within a litter and across sexes. This design has been used in prior postnatal MA investigations (Acevedo *et al.*, 2007; C. A. Crawford *et al.*, 2003; Grace, Schaefer, Gudelsky *et al.*, 2010; Schaefer *et al.*, 2008; Vorhees *et al.*, 2000; Vorhees *et al.*, 2007; Williams, Blankenmeyer *et al.*, 2003; Williams *et al.*, 2002).

Choline acetyltransferase immunohistochemistry: On PND 30, mice were deeply anesthetized with a cocktail containing 100 mg/kg ketamine, 10 mg/kg xylazine, and 2 mg/kg acepromazine and were transcardially perfused with phosphate-buffered SA (PBS) followed by 4% paraformaldehyde (pH = 7.4). Brains were removed and post-fixed overnight in 4% paraformaldehyde at 4° C, transferred to 30% sucrose solution, and were embedded in cryoprotectant and stored at -80° C until sectioning. Serial coronal sections (50 μm) were collected onto Superfrost microscope slides (Fisher Scientific, Pittsburgh, PA) through the entire MS/VDB/HDB (MA: n = 10, SA: n = 10), NB (MA: n = 11, SA: n = 11) or hippocampus/cortex (MA: n = 10, SA: n = 9) with a 200 μm inter-section distance, guided by the mouse brain atlas (Franklin & Paxinos, 1997). For the MS/VDB/HDB region, a total of 6 sections were collected per mouse (+1.18 to 0.00 mm Bregma). For the NB, a total of 4 sections were collected per mouse (-0.25 to -1.00 mm Bregma). For the hippocampus/cortex, a total of 12 sections were collected per mouse (-1.00 mm to -4.00 mm Bregma). Sections were stained for immunoreactivity of ChAT, a marker of cholinergic neurons and processes (Oda, 1999). Following an antigen retrieval step according to the manufacturer's instructions (H-3000; Vector Laboratories, Burlingame, CA), sections were incubated for 2 hrs at room temperature in a blocking solution (5% normal donkey serum in PBS containing 0.2% Triton X-100 and 0.2% bovine serum albumen (PBT)), followed by incubation overnight at 4° C in goat-anti-ChAT primary antibody (1:400; Millipore, Billerica, MA). Following 4 washes with PBT (10 min each) and 1 wash with PBS (15 min), sections were incubated for 2 hrs at room temperature in donkey-anti-goat IgG antibody (1:50) conjugated to Texas Red for fluorescent visualization (Jackson Immunoresearch, West Grove, PA). Following 4

washes with PBT (10 min each) and 1 wash with PBS (15 min), the sections were covered with anti-fade solution containing a DAPI counterstain (Vectashield, Vector Laboratories) and were coverslipped (Fisher Scientific). Slides were stored in the dark at 4° C until they were imaged.

Parvalbumin and NeuN immunohistochemistry: All procedures for the PVA and NeuN immunohistochemistry were identical to those for ChAT, except that PVA and NeuN imaging were performed only on sections from the MS/VDB/HDB. The same PND 30 mice used for the ChAT-positive cell counts in the MS/VDB/HDVB were used for the current studies (MA: $n = 10$, SA: $n = 10$) and the sections were directly adjacent to those used for the ChAT-positive cell counts in the MS/VDB/HDB. Sections were stained for fluorescent visualization of PVA, a marker of GABAergic neurons in the basal forebrain (Freund, 1989; Gritti *et al.*, 2003), and NeuN, a marker of all neurons (Mullen, Buck, & Smith, 1992). Following an antigen retrieval step according to the manufacturer's instructions (H-3000; Vector Laboratories), sections were incubated for 2 hrs at room temperature in a blocking solution (5% normal donkey serum in PBS containing 0.05% Tween-20 (PT)), followed by incubation overnight at 4° C with guinea pig-anti-PVA (1:300; Chemicon International, Billerica, MA) and mouse-anti-NeuN (1:400; Millipore, Billerica, MA) primary antibodies. Following 4 washes with PT (10 min each) and 1 wash with PBS (15 min), sections were incubated with donkey-anti- guinea pig and donkey-anti-mouse IgG antibody (1:50 for each) conjugated to fluorescein isothiocyanate (PVA) or Texas Red (NeuN) for fluorescent visualization (Jackson Immunoresearch) for 2 hrs at room temperature. Following 4 washes with PT (10 min each) and 1 wash with

PBS (15 min), the sections were covered with anti-fade solution containing a DAPI counterstain (Vectashield, Vector Laboratories) and were coverslipped (Fisher Scientific). Slides were stored in the dark at 4° C until they were imaged.

Confocal microscopy and unbiased stereology analysis: The density of ChAT-positive cells in the MS/VDB/HDB and NB was calculated by counting the total number of cells found within the region, using an unbiased rare event stereological procedure. Because there are so few ChAT-positive cells in the BF, each cell was counted within the defined region in 6 separate sections for the MS/VDB/HDB and 4 separate sections for the NB using the 60X objective lens (N.A. = 1.42, W.D. = 0.15, F.N. = 26.5) of an Olympus spinning disc confocal microscope (IX81, Olympus Imaging Corp., Center Valley, PA) equipped with Slidebook software (Intelligent Imaging Solutions). The density of ChAT-positive cells within each section was calculated by dividing the number of counted cells by the sample volume. The average density was calculated by averaging the sample densities for each mouse.

The density of PVA-positive and NeuN-positive cells in the MS/VDB/HDB was estimated using the nonbiased stereological physical volume fractionator technique (West, Slomianka, & Gundersen, 1991) using the 60X objective lens of the confocal microscope equipped with Slidebook software. Six separate sections spanning the MS/VDB/HDB were imaged for each mouse using 15 separate dissectors for each section. Each dissector (50 x 50 μm) consisted of a stack of 10 images with a 2 μm inter-image distance. PVA-positive cells and NeuN-positive cells were counted when they appeared within the dissector in one image of the 10 image stack but not in the preceding

image. Cells were counted only when the staining appeared with a distinctly labeled DAPI stained nucleus. Thus a total of approximately 90 individual dissectors were counted to obtain an unbiased and accurate average density of PVA-positive and NeuN-positive cells per cubic centimeter within the MS/VDB/HDB of each mouse. The density of PVA-positive and NeuN-positive cells within each dissector was averaged for each mouse across all sections.

Densitometry analysis: Densitometry analysis was used to quantify the area occupied by ChAT-immunoreactive fibers innervating the hippocampus and cortex. Immunofluorescence was imaged in stacks of 10 images with a 1 μm inter-image range in selected sub-regions of the hippocampus (CA1, CA3, stratum radiatum of the CA1 and CA3, and the granule and molecular layer of the dentate gyrus) and cortex (motor, primary and secondary sensory, entorhinal and lateral entorhinal, and piriform cortex) using the 60X objective lens of a confocal microscope equipped with Slidebook software. Stacks of images were vertically collapsed into a single image using a summation algorithm. An intensity threshold was chosen for each group of brains stained together, containing mice from each treatment group, based on the average intensities of immunoreactivity. The total number of pixels with ChAT immunofluorescence intensity above the threshold was determined within each image and converted to area. Twelve sections per mouse were used.

Muscarinic receptor binding: Mice were killed by cervical dislocation on PND 30 and receptor saturation binding experiments were performed using hippocampal (MA: $n = 20$,

SA: $n = 20$) and cortical (MA: $n = 13$, SA: $n = 14$) membrane preparations and radioligands specific for M_1 ([^{3}H] Pirenzepine) or M_2 ([^{3}H] AF-DX-384) mAChRs (Siegel, Benice, Van Meer, Park, & Raber, 2011; Vaucher *et al.*, 2002; Watson, Roeske, & Yamamura, 1986). Bilateral hippocampi and cortices were dissected and freshly frozen. Tissue preparations were homogenized in 1 mL of ice-cold buffer (140 mmol NaCl, 0.2 mmol KCl, 10 mmol $MgCl_2$, 0.5 mmol EDTA, 50 mmol Tris, pH = 7.5), centrifuged at 16,000 g for 25 min, washed in ice-cold buffer, and re-centrifuged. The washing procedure was repeated four times and the membrane pellet stored at -80° C until use. Tissue pellets were re-suspended in ice-cold binding buffer (10 mmol NaH_2PO_4, 10 mmol Na_2HPO_4, 1 mmol $MgCl_2$, pH = 7.4) and protein concentrations measured (BCA protein assay kit, Pierce, Rockford IL).

Tissue samples (50 µL) were incubated for 1 hr in triplicate (hippocampus) or duplicate (cortex) in a total volume of 200 µL, containing binding buffer (50 µL), radioligand (50 µL), and 2.5 mmol atropine to measure nonspecific binding or water (50 µL). Single point saturation binding was performed for hippocampal preparations due to the limited amount of tissue (98 nM [^{3}H] pirenzepine and 85 nM [^{3}H] AF-DX-384). For the cortical binding, eight different increasing concentrations of [^{3}H] pirenzepine (330 pM - 46 nM) or [^{3}H] AF-DX-384 (252 pM - 46 nM) were used. Some brains were used for both hippocampal and cortical binding studies. Following incubation, 5 mL of ice-cold binding buffer was added to stop the reaction and tissues were bound to filter paper (Beckman, Fullerton, CA) using a cell harvester (Brandel, Gaithersburg, MD). Filters were placed in vials and 5 mL of scintillation fluid (Optiphase 2, Perkin Elmer, Waltham, MA) was added before counting radioactivity using a beta counter (LS 6000SC, Beta

Counter, Beckman). The maximal number of binding sites (B_{max}) and the equilibrium dissociation constant (K_d, for cortical binding only) were determined according to the Hill equation (Whiteaker, Jimenez, McIntosh, Collins, & Marks, 2000) using nonlinear regression analysis and Graphpad Prism 4.0 software (Graphpad, San Diego, CA).

Statistical analysis: All statistical analyses were performed using SPSS software (Chicago, IL). Two-way analysis of variance (ANOVA) was used to assess the effects of treatment and sex on the density of ChAT-positive cells in the MS/VDB/HDB and NB, the density of PVA-positive and NeuN-positive cells in the MS/VDB/HDB, and the B_{max} and K_d of the mAChRs in the hippocampus and cortex. A linear mixed model with unstructured covariance as an optimal correlation was used to assess potential treatment and sex differences in the proportion of the percent of ChAT-positive, PVA-positive, and other (ChAT- and PVA-negative) cells out of total NeuN-positive neurons in the MS/VDB/HDB after accounting for the structural independency of the data (% PVA + % ChAT + % other = 100%). A Bayesian Information Criteria (BIC) was used to determine an optimal covariance structure. A repeated measure ANOVA was used to assess the effects of treatment and sex on the area occupied by ChAT immunoreactivity in all regions of the hippocampus and cortex. Lower-bound estimates of epsilon were used for all repeated measure ANOVAs. Data from four mice were removed from the cortical M_1 mAChR binding study due to technical errors. Data within the text are reported as mean ± S.E.M. Only significant interactions are reported. All statistical tests were conducted with a two-tailed significance alpha level of 0.05.

3. Results

Density of basal forebrain neurons: Due to a lack of clear anatomical boundaries between the MS, VDB, and HDB, the MS/VDB/HDB was considered as one region and examined separately from the NB. The volumes of the MS/VDB/HDB and the NB did not differ between MA- and SA-exposed or male and female mice. Representative images of these areas are shown in Figure 5a.

For the density of ChAT-positive cells in the MS/VDB/HDB, there was an interaction between treatment and sex (F (1, 16) = 6.53, p = 0.02). Thus the effects of treatment were explored in male and female mice separately. There was no difference between MA- and SA-exposed males in the density of ChAT-positive cells. However, female MA-exposed mice had a higher density of ChAT-positive cells than female SA-exposed mice in the MS/VDB/HDB (main effect of treatment; F (1, 8) = 5.80, p = 0.04; Figure 5b and 5c). There was no effect of treatment on the density of ChAT-positive cells in the NB (Figure 5d). Male mice, however, had a higher density of ChAT-positive cells in the NB compared to female mice (main effect of sex; F (1, 18) = 8.15, p = 0.01).

MA exposure during brain development did not alter the density of PVA-positive (Figure 6a and 6c) or NeuN-positive (Figure 6b and 6d) cells in the MS/VDB/HDB. There was no difference between the treatment groups or sexes in the proportion of the percent of ChAT- positive (cholinergic), PVA-positive (GABAergic), or other neurons in the MS/VDB/HDB (Table 1). However, among the female mice, MA exposure significantly increased the percent of ChAT-positive cells compared to SA exposure (t (1, 20) = -2.17, p = 0.04; Table 1). Negative controls with secondary antibody only revealed no fluorescent visualization, indicating that all of the antibodies used were specific.

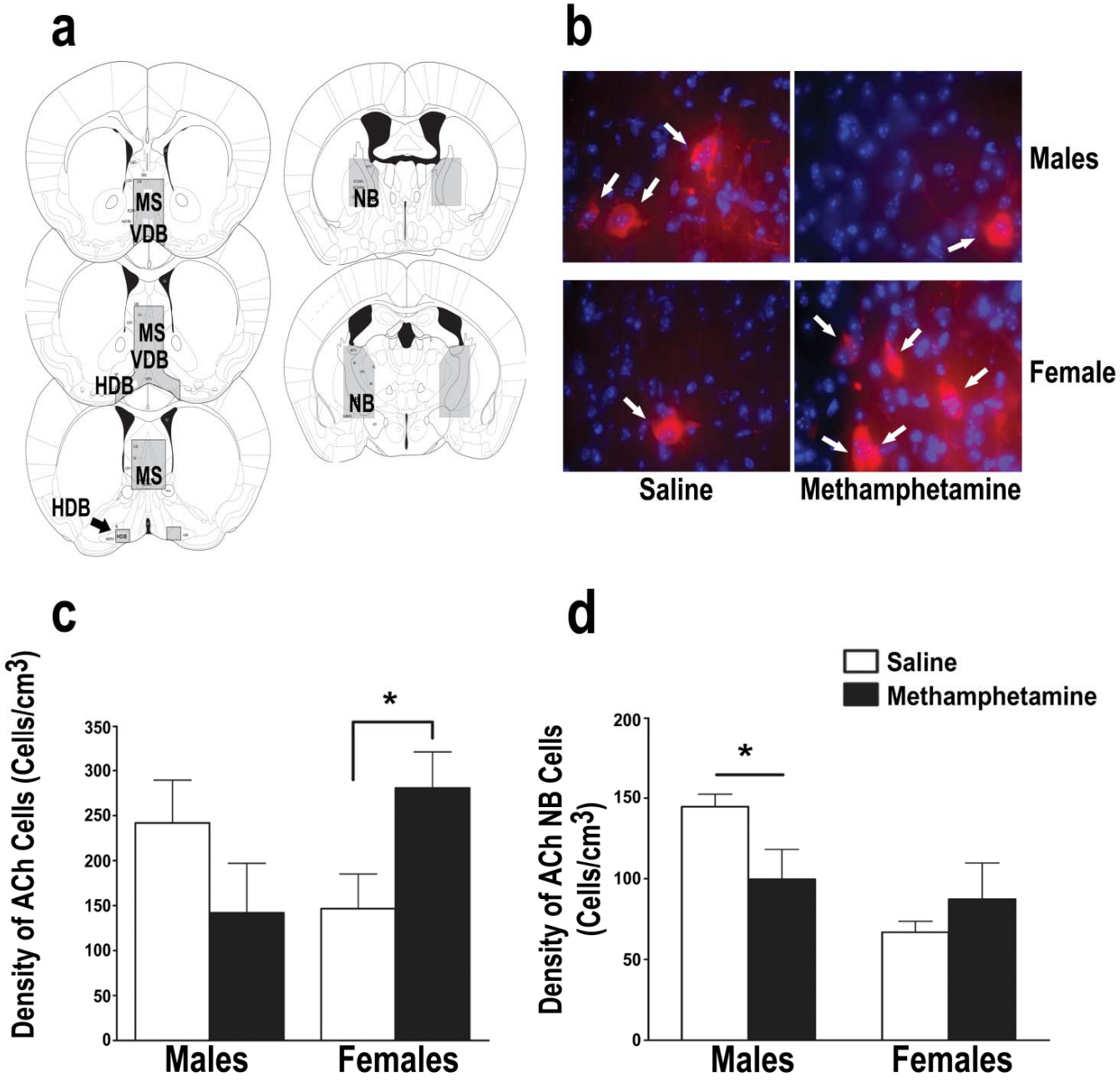


Figure 5. Density of cholinergic cells in the basal forebrain of adolescent mice. (a) Representative images of the area defined as the medial septum /vertical/horizontal limb nucleus of the diagonal band and the nucleus basalis in which cells were counted. (b) Representative images of cholinergic cells (choline acetyltransferase-positive; white arrows) in the medial septum/vertical/horizontal limb nucleus of the diagonal band. Cell nuclei are marked with blue Dapi stain. (c) Methamphetamine exposure in female mice increased the density of cholinergic cells (choline acetyltransferase-positive) in the medial septum/vertical/ horizontal limb nucleus of the diagonal band compared to female mice exposed to saline. This effect was not observed in male mice. (d) Methamphetamine exposure did not alter the density of cholinergic cells (choline acetyltransferase-positive) in the nucleus basalis. Male mice had a higher density of cholinergic cells in the nucleus basalis compared to female mice. *$p < 0.05$, data expressed as mean ± S.E.M. MS = medial septum, VDB = vertical limb nucleus of the diagonal band, HDB = horizontal limb nucleus of the diagonal band, NB = nucleus basalis, ACh = acetylcholine.

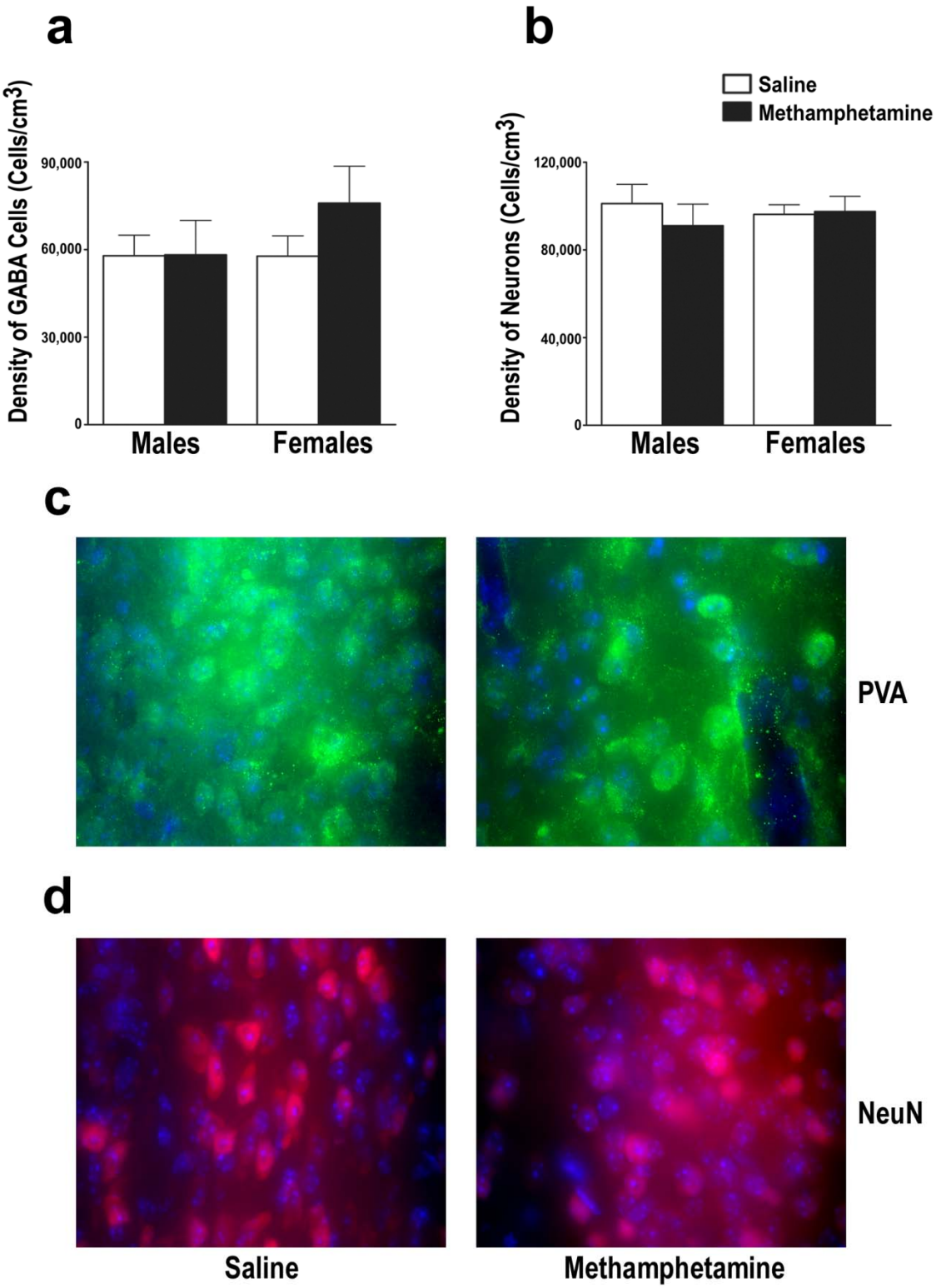


Figure 6. Density of cells in the medial septum/vertical/horizontal limb nucleus of the diagonal band of adolescent mice. (a) Methamphetamine exposure did not alter the density of GABAergic cells (parvalbumin-positive) or (b) the density of total neurons (NeuN-positive) in the medial septum/vertical/ horizontal limb nucleus of the diagonal band. (c) Representative images of GABAergic cells (parvalbumin-positive) in the medial septum/vertical/horizontal limb nucleus of the diagonal band. Cell nuclei are marked with blue Dapi stain. (d) Representative images of neurons (NeuN-positive) in the medial septum/vertical/horizontal limb nucleus of the diagonal band. Cell nuclei are marked with blue Dapi stain. PVA = parvalbumin.

Table 1

The percent of cholinergic, GABAergic, and other neurons in the medial septum/vertical/horizontal limb nucleus of the diagonal band of adolescent mice.

Treatment	Sex	Cholinergic	GABAergic	Other
Saline	Males	0.25 ± 0.05	58.12 ± 7.61	41.64 ± 7.64
Methamphetamine	Males	0.14 ± 0.05	61.48 ± 7.61	38.38 ± 7.64
Saline	Females	0.15 ± 0.05	59.54 ± 7.61	40.31 ± 7.64
Methamphetamine	Females	0.29 ± 0.05*	75.86 ± 7.61	23.85 ± 7.64

*$p < 0.05$, methamphetamine female mice higher than saline female mice for cholinergic cells.
Data expressed as mean percent ± estimated S.E.M.

Area occupied by cholinergic fibers in the hippocampus and cortex: Representative images of the areas in the hippocampus that were imaged and the ChAT-positive fibers in the hippocampus are shown in Figures 7a and 7b, respectively. In the hippocampus there was a treatment x sex interaction (F (1, 15) = 7.53, p = 0.02). The effects of treatment on the area occupied by ChAT immunoreactivity in all areas of the hippocampus collapsed together were explored in each sex separately as there was no interaction between treatment, sex, and region. In the male mice, there was no effect of treatment on the area occupied by ChAT-positive fibers. In contrast, female MA-exposed mice had a higher area occupied by ChAT-positive fibers in all regions of the hippocampus compared to female SA-exposed mice (main effect of treatment; F (1, 7) = 6.99, p = 0.03; Figure 7c). Unlike the hippocampus, there was no effect of treatment or sex on the area occupied by ChAT-positive fibers in the cortex (Figure 7d).

Muscarinic acetylcholine receptors in the hippocampus and cortex: In the hippocampus, MA-exposed mice had a higher number (B_{max}) of M_1 mAChRs compared to SA-exposed

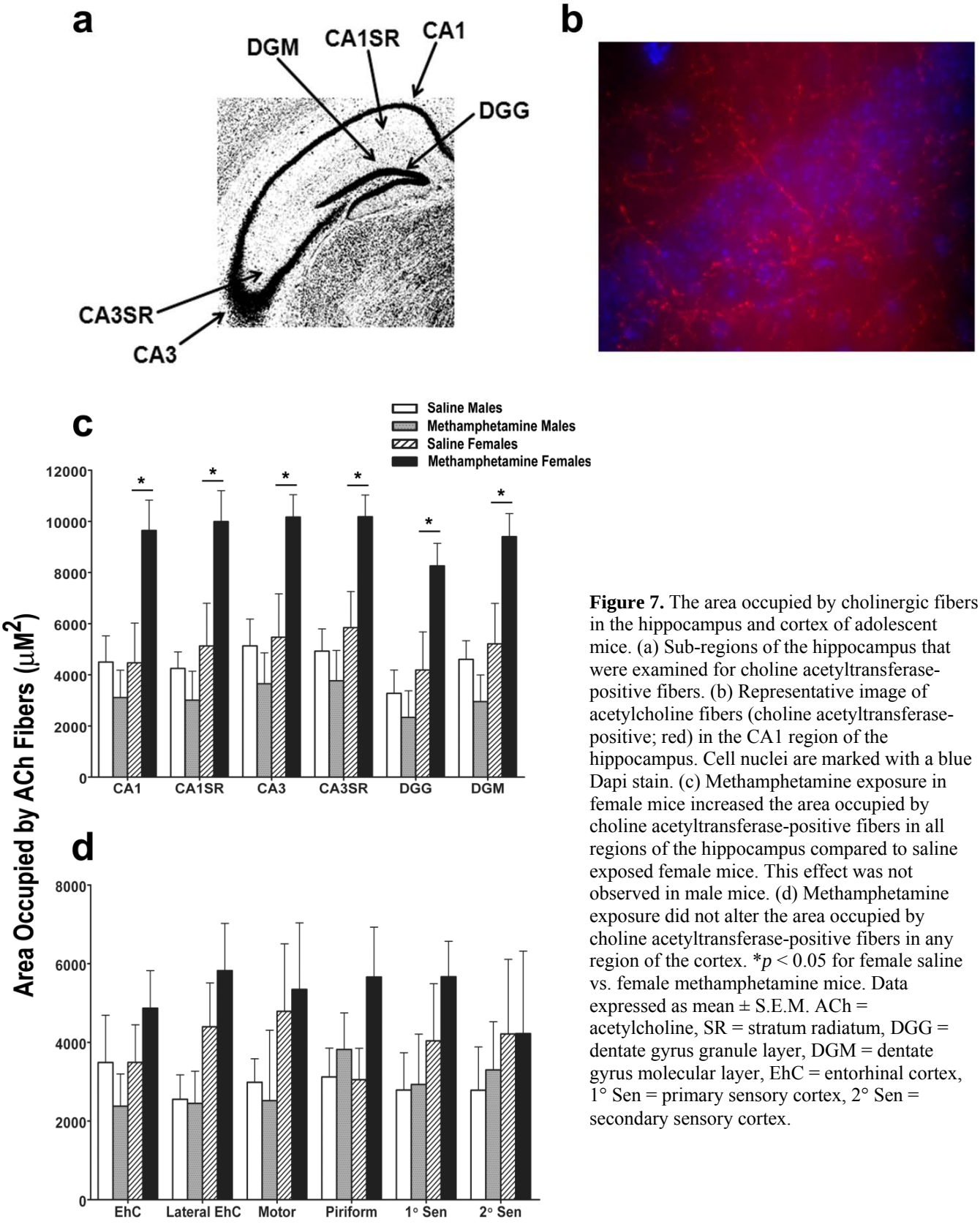


Figure 7. The area occupied by cholinergic fibers in the hippocampus and cortex of adolescent mice. (a) Sub-regions of the hippocampus that were examined for choline acetyltransferase-positive fibers. (b) Representative image of acetylcholine fibers (choline acetyltransferase-positive; red) in the CA1 region of the hippocampus. Cell nuclei are marked with a blue Dapi stain. (c) Methamphetamine exposure in female mice increased the area occupied by choline acetyltransferase-positive fibers in all regions of the hippocampus compared to saline exposed female mice. This effect was not observed in male mice. (d) Methamphetamine exposure did not alter the area occupied by choline acetyltransferase-positive fibers in any region of the cortex. $*p < 0.05$ for female saline vs. female methamphetamine mice. Data expressed as mean ± S.E.M. ACh = acetylcholine, SR = stratum radiatum, DGG = dentate gyrus granule layer, DGM = dentate gyrus molecular layer, EhC = entorhinal cortex, 1° Sen = primary sensory cortex, 2° Sen = secondary sensory cortex.

mice (main effect of treatment; F (1, 15) = 4.63, p = 0.04; Figure 8a). Female mice had a higher number of M_1 mAChRs compared to male mice (main effect of sex; F (1, 16) = 7.45, p = 0.02). There was no effect of treatment or sex on the number of M_2 mAChRs in the hippocampus (Figure 8b). In the cortex, there was no effect of treatment or sex on the number or dissociation constant (K_d) of either the M_1 or M_2 mAChRs (Table 2).

4. Discussion

The findings from this study show for the first time that MA exposure during brain development causes long-term alterations in the adolescent BF cholinergic system. In contrast to the original hypothesis, MA exposure increased the density of cholinergic cells in the MS/VDB/HDB and the area occupied by cholinergic fibers in the hippocampus of female, but not male, adolescent mice. MA exposure also increased the number of M_1 mAChRs in the hippocampus of both male and female adolescent mice. In contrast, there was no effect of MA on the density of GABAergic cells or total neuronal density in the MS/VDB/HDB or the density of cholinergic cells in the NB. The area occupied by cholinergic fibers in the cortex, the number of M_2 mAChRs in the hippocampus, and the number and dissociation constant of M_1 and M_2 mAChRs in the cortex were also not affected by MA.

The results show a sex-dependent effect of MA on the cholinergic system; female adolescent mice had increased cholinergic cell density in the MS/VDB/HDB and ChAT-positive fibers in the hippocampus following MA exposure, whereas these effects were not seen in males. However, both male and female mice showed an increase in M_1 mAChR binding in the hippocampus. The mechanism underlying these sex-dependent

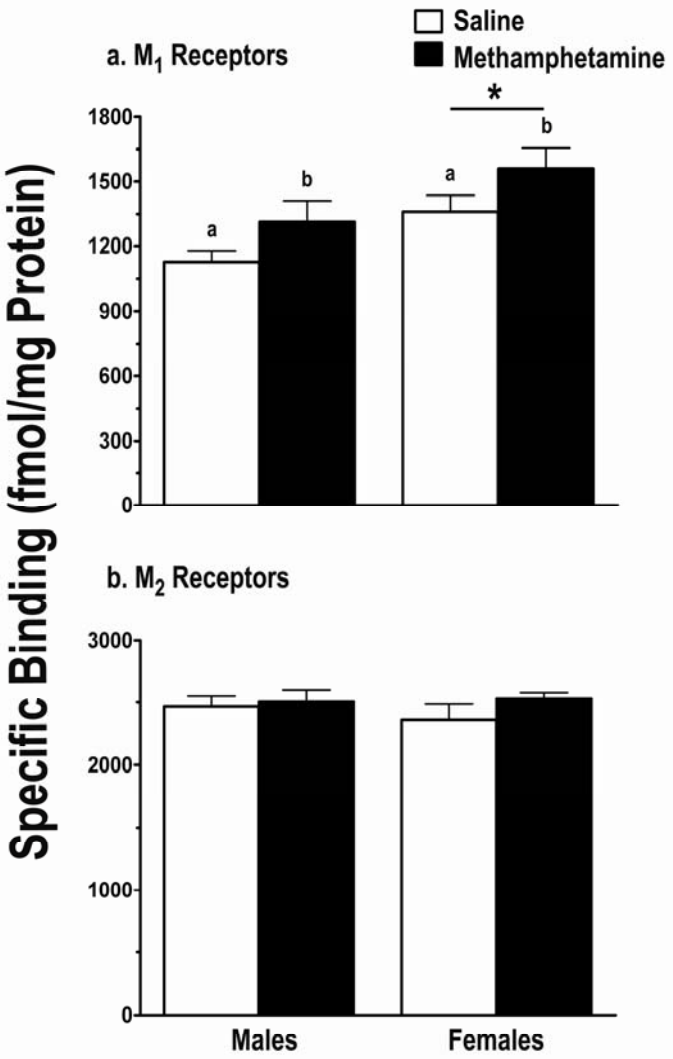


Figure 8. Number of M_1 and M_2 muscarinic acetylcholine receptors in the hippocampus of adolescent mice. (a) Methamphetamine-exposed mice[b] had a higher number (B_{max}) of M_1 muscarinic acetylcholine receptors compared to saline-exposed mice[a]. Female mice also had a higher number of M_1 muscarinic receptors compared to male mice. (b) There was no difference between methamphetamine- and saline-exposed mice or male and female mice in the number of M_2 muscarinic acetylcholine receptors in the hippocampus. Data expressed as mean ± S.E.M. [a]Saline-exposed mice significantly lower than [b]methamphetamine-exposed mice, $p < 0.05$. *$p < 0.05$ female mice higher than male mice.

Table 2

The number and dissociation constant of cortical muscarinic acetylcholine receptors in adolescent mice.

		M_1 muscarinic receptors		M_2 muscarinic receptors	
Treatment	Sex	B_{max}	K_d	B_{max}	K_d
Saline	Males	1,028.0 ± 131.8	5,954.0 ± 582.4	2,047.7 ± 96.0	10,927.6 ± 2,021.5
Methamphetamine	Males	1,045.2 ± 56.2	6,527.6 ± 543.5	1,835.0 ± 106.8	8,348.6 ± 747.1
Saline	Females	1,302.0 ± 96.9	8,621.0 ± 1,053.7	1,819.1 ± 145.1	10,434.3 ± 749.3
Methamphetamine	Females	1,045.1 ± 167.1	7,527.7 ± 1,532.9	2,051.4 ± 62.2	10,827.0 ± 1,498.2

B_{max} = number of receptors (fmol/mg protein), K_d = receptor dissociation constant (pM).
Data expressed as mean ± S.E.M.

effects is unknown. Female mice show higher concentrations of MA in the cortex 120 minutes following single injection of MA (5 mg/kg) on PND 11 compared to male mice. Female mice also show a trend toward higher MA concentrations in the cortex and hippocampus on PND 15 and 20 compared to male mice one hour post MA injections from PND 11-20, suggesting that female mice metabolize MA in the brain at a slower rate than male mice (Acevedo *et al.*, 2008). The slower metabolism of MA in female mice may increase MA-induced neurotoxicity on the ACh system. Alternatively, the sex-dependent effects of MA on the cholinergic system could be due in part to sex differences in the timeline of cholinergic development. ChAT activity levels in the MS mature earlier in female rats, while male rats show increases in ChAT activity after PND 18 (Loy & Sheldon, 1987). Thus PND 11-20 may target a sensitive period of ChAT development in female but not male mice. A more detailed discussion of the potential mechanisms underlying the sex-dependent effects of MA on the cholinergic system is provided in **Chapter 6**.

Increased mAChRs in both male and female adolescent mice suggest that MA has effects in male mice, but they are more limited compared to those in female mice. MA exposure decreased the density of cholinergic cells in the male mice as opposed to the increase seen in female mice, although these changes did not reach statistical significance. However, this finding parallels a decrease seen in AChE-positive fibers in the hippocampus of adult male gerbils following postnatal MA exposure (Busche *et al.*, 2006). MA-induced decreases in cholinergic cells may result in a compensatory up-regulation of mAChRs in the hippocampus in male mice. Female mice also showed a

MA-induced increase in hippocampal mAChRs, suggesting that different mechanisms may underlie this effect in males and females.

The precise mechanism by which MA affects the cholinergic system is not yet understood. The time period of MA exposure (PND 11-20) overlaps with important aspects of cholinergic BF development in rodents, including developmental increases in ChAT and AChE activity, CHT levels, and mAChR levels in the BF, hippocampus, and cortex (Aubert *et al.*, 1996; Berse *et al.*, 2005; Kiss & Patel, 1992; Thal *et al.*, 1992; Zahalka *et al.*, 1993). MA-induced increases in cholinergic cell density in the MS/VDB/HDB of female mice could arise by MA-induced enhancement of cholinergic differentiation of proliferating precursor cells, by increased cholinergic cell survival, or by enhancement of the cholinergic phenotype within differentiated cholinergic cells. It is unlikely that MA exposure from PND 11-20 affects differentiation of proliferating precursor cells, as the BF cholinergic neurons divide and migrate before birth (Brady *et al.*, 1989; Schambra *et al.*, 1989). MA exposure also does not promote overall increases in new neurons, as there was no increase in total neuronal density, suggesting no effects on cell survival. MA exposure may have increased cholinergic phenotype expression within cholinergic neurons from basal levels, or induced a switch from another phenotype to the cholinergic phenotype. The BF contains GABAergic and glutamatergic neurons in addition to the cholinergic neurons (Gritti *et al.*, 2003). There was no change in GABAergic cells, and if anything, MA exposure in the female mice increased the density of GABAergic cells, suggesting that MA did not induce a phenotype change from GABAergic to cholinergic. Although there was no significant decrease in percent of non-cholinergic or GABAergic (presumably glutamatergic) “other” neurons, MA-exposed

female mice did show a lower percent of "other" neurons and an increase in cholinergic neurons compared to SA-exposed female mice. A very small reduction in the percent of "other" neurons could contribute to a significant change in the percent of cholinergic cells, as the percentage of the cholinergic cells was quite low compared to the other phenotypes. Thus while a significant reduction in "other" neurons was not observed, there could have been a small MA-induced change in phenotype toward a detectable increase in the cholinergic phenotype. Further research is needed to quantify the density of glutamatergic neurons in addition to cholinergic and GABAergic neurons to determine MA-induced changes in this neuronal population.

Many transcription and extracellular factors contribute to differentiation of cholinergic and/or GABAergic phenotypes and could potentially be affected by MA exposure (for a more detailed discussion, see **Chapter 6**). For example, bone morphogenetic protein (BMP) and the LIM homeobox family of transcription factors support the development and/or maintenance of cholinergic BF cells and can enhance cholinergic cell differentiation (Bachy & Retaux, 2006; Fragkouli *et al.*, 2005; Lopez-Coviella, Berse, Krauss, Thies, & Blusztajn, 2000; Manabe *et al.*, 2007; Manabe *et al.*, 2005; Mori *et al.*, 2004; Schnitzler *et al.*, 2010). To the best of my knowledge the effects of MA or other psychomotor stimulants on these factors have not been examined.

Another way that MA may increase cholinergic function is by increasing levels of the neurotrophic factor NGF in the hippocampus. NGF promotes cholinergic cell activity and survival (Counts & Mufson, 2005; Wainer *et al.*, 1993; Yuhara *et al.*, 2003). NGF can increase ChAT activity and the number of ChAT-positive neurons in basal forebrain culture from PND 10-14 rats (Yuhara *et al.*, 2003) while postnatal exposure to NGF

increases ChAT mRNA levels in the septum of rats on PND 21 (Tian *et al.*, 1996), suggesting that this trophic factor continues to promote cholinergic development after birth. MA exposure from PND 11-20 increases hippocampal NGF levels on PND 20 in rats (Skelton *et al.*, 2007). These effects, however, are seen in both male and female rats. In mice the effects of MA on NGF may be more prominent in females and may result in long-term increases in the expression of ChAT and the density of cholinergic cells in adolescent female mice.

MA may increase ChAT and mAChR levels via activation of BF cholinergic cells. MA increases DA, NE, and 5-HT release from brainstem nuclei and the BF neurons express receptors for DA, 5-HT, and NE (Berlanga *et al.*, 2005; Smiley *et al.*, 1999; Zilles *et al.*, 1991). ChAT and mAChR levels may be directly linked to increases in cAMP and other intracellular signaling mechanisms that would occur following DA binding to D_1 or D_5 receptors in the BF following MA exposure (Zilles *et al.*, 1991). However, findings from Arnold *et al.* (2001) suggest that the AMPH-induced increase in cortical ACh release from the NB is not dependent upon the D_1 or D_2 DA receptors, as this increase occurs in the presence of D_1 and D_2 receptor antagonists in the NB (Arnold, Fadel, Sarter, & Bruno, 2001). Thus it is also feasible that the effects of MA on the BF are independent of DA receptor stimulation. Alternatively, MA exposure may decrease activation of BF cholinergic cells and subsequent ACh release, resulting in a compensatory up-regulation of ChAT and mAChRs (see **Chapter 6** for a more thorough discussion). No studies have assessed the long-term effects of MA exposure during brain development on ACh release into the hippocampus and much more work is needed to delineate the ways in which MA affects the cholinergic system. However, the findings

suggest that the effects of MA on the cholinergic system are very pronounced, especially in female mice, and that they may contribute to MA-induced impairments in other aspects of brain function and cognition.

There was no effect of MA exposure on the density of NB cholinergic neurons. In parallel with this finding, there was also no increase in the area occupied by cholinergic fibers in any region of the cortex examined. As the NB is the primary source of cholinergic innervation in the cortex (Mesulam *et al.*, 1983), these findings are consistent with the region-specific effects of MA exposure in the BF. Similarly there were no changes in cortical mAChRs, again suggesting specific effects of MA on the MS/VDB/HDB and the corresponding projections to the hippocampus. These findings imply that the effects of MA in the MS/VDB/HDB are linked to those in the hippocampus, and that these MA-induced alterations might contribute to MA-induced cognitive impairments (Acevedo *et al.*, 2007).

In summary, the findings presented in this chapter show for the first time that MA exposure during brain development increases BF cholinergic cell density and the area occupied by cholinergic fibers in the hippocampus of adolescent female mice. MA exposure also increases the number of M_1 mAChRs in the hippocampus of adolescent male and female mice. These cholinergic changes may be related to MA-induced cognitive impairments that are observed following MA exposure from PND 11-20 in adolescent and adult rodents (Acevedo *et al.*, 2007; Vorhees *et al.*, 2000; Vorhees *et al.*, 2007; Williams *et al.*, 2002).

CHAPTER 3: LONG-TERM EFFECTS OF METHAMPHETAMINE EXPOSURE DURING BRAIN DEVELOPMENT ON THE CHOLINERGIC SYSTEM IN ADULT MICE

1. Introduction

MA affects various neurotransmitters in the adult brain, including DA and 5-HT (Sulzer *et al.*, 2005). Although less well understood, MA can also affect ACh in the adult brain (Dobbs & Mark, 2008; Garcia-Rates *et al.*, 2007; Hussain *et al.*, 2008; Kish *et al.*, 1999; McCabe, Gibb *et al.*, 1987; Siegal *et al.*, 2004; Taguchi *et al.*, 1998; Zhu *et al.*, 2006). The data presented in **Chapter 2** suggest that MA exposure during brain development affects the adolescent cholinergic system, and that these effects are more severe in adolescent female than male mice. In the only studies to examine the long-term effects of MA exposure during brain development on the adult cholinergic system, it was found that male gerbils exposed to a single toxic dose of MA on PND 14 show reduced AChE-positive fibers in the cortex and dentate gyrus in adulthood (Busche *et al.*, 2006; Lehmann *et al.*, 2004). These findings in adult rodents warrant further investigation as the hippocampus and cortex are two areas of the brain that are important for cognition (Alexander *et al.*, 2007; Deacon *et al.*, 2002; Morris *et al.*, 1982; Save *et al.*, 1992) and the effects of MA exposure on the adult cholinergic system are relatively understudied.

Despite consistent effects of MA exposure on brain function and cognition in humans, there are individual differences in the degree of MA-induced impairments (Piper *et al.*, 2011). Individual differences in the degree of impairment following MA exposure suggest that other factors, such as genetic factors, may modulate the susceptibility to

brain impairments following *in utero* MA exposure. Given its role in neuronal repair after injury, apoE may be one factor influencing the degree of brain damage resulting from *in utero* MA exposure (Arendt *et al.*, 1997; Mahley, 1988). ApoE plays a role in lipid transport and metabolism in the brain and exists as 3 major isoforms in humans; apoE2, apoE3, and apoE4 (Mahley, 1988). ApoE modulates responses to central nervous system injury (Buttini *et al.,* 1999; Lomnitski *et al.,* 1999) and might also modulate susceptibility to brain impairments following exposure to MA during brain development.

The cholinergic system is modulated by apoE. Mice lacking apoE show lower mAChR binding in the cortex and hippocampus compared to wild type mice (Siegel *et al.*, 2011) and apoE4 is associated with lower NB neuronal activity (Salehi, Dubelaar, Mulder, & Swaab, 1998) and ChAT activity than apoE2 and apoE3 (Allen *et al.*, 1997; Lai *et al.*, 2006; Poirier *et al.*, 1995). The interaction between apoE and MA's affects on the cholinergic system, however, has not been examined. In this study, the effect of MA exposure during brain development on mAChR binding in adulthood was assessed in mice expressing human apoE3 or apoE4. Furthermore, in order to determine if the effects of MA exposure on cholinergic fibers in the adolescent hippocampus (**Chapter 2**) persist into adulthood, the effects of MA exposure on ACh fibers in the hippocampus and cortex were also examined in adult wild type mice. It was hypothesized that MA exposure during brain development would decrease the area occupied by the cholinergic axons and the mAChRs in the hippocampus and cortex, and that the effects of MA on the mAChRs would be more severe in the apoE4 mice compared to the apoE3 mice.

2. Methods

Animals: For the mAChR binding study, human *APOE* targeted replacement mice expressing human apoE3 or apoE4 under control of the mouse apoE promoter, on the C57BL/6J background (Knouff *et al.*, 1999; Sullivan *et al.*, 1997), were provided by Dr. Patrick Sullivan for breeding. Mice were housed and bred in our colony using homozygous mating. Breeding cages contained one male and two female mice and female mice were singly housed from the first sign of pregnancy, when they gained a noticeable amount of weight. Pups were weaned and group housed with five mice per cage according to sex on PND 22. For the cholinergic fiber experiments, 3-5 month-old male and female C57BL/6J mice were bred in our colony using breeding cages containing one male and two female mice. Female mice were singly housed from the first sign of pregnancy, when they gained a noticeable amount of weight. On PND 21, pups were weaned and group housed with five mice per cage according to sex. For both experiments, lab chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO) and water were given *ad libitum*. Litters were provided soft foods during the injection period and 2 weeks after weaning to maintain stable weight gain. The mice were kept on a 12 hr light/dark schedule (lights on at 06:00). All procedures conformed to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University.

Injections: (*d*)-MA hydrocholoride (5 mg/kg), obtained from the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse drug supply program, was diluted with 0.9% sodium chloride (SA) to the appropriate

concentration. For the mAChR binding study, a total of 18 litters were used; 10 apoE3 litters and 8 apoE4 litters. Male and female pups were weighed and given a single intra-peritoneal injection of MA or SA daily at 10:00 from PND 11-21 (injection volume of 0.1 mL). For the cholinergic fiber study, male and female pups from 5 separate litters were given a single intra-peritoneal injection of MA or SA daily at 10:00 from PND 11-20 (injection volume of 0.1 mL). For both studies, a within-litter injection design was used to balance the number of MA and SA injections within a litter and across sexes.

Muscarinic receptor binding: Receptor saturation binding experiments were performed on hippocampal (n = 48) and cortical (n = 39) membrane preparations in adult apoE3 and apoE4 mice following behavioral testing (see **Chapter 5**) using radioligands specific for M_1 ([^{3}H] pirenzepine) or M_2 ([^{3}H] AF-DX-384) mAChRs (Siegel *et al.*, 2011; Vaucher *et al.*, 2002; Watson *et al.*, 1986). Following the last day of behavioral testing, bilateral hippocampi and cortices were dissected and freshly frozen. Tissue preparation and the binding procedure were performed in an identical fashion to those described in the methods section of **Chapter 2**.

Choline acetyltransferase immunohistochemistry: On PND 90 mice were deeply anesthetized and their brains were removed and fixed overnight at 4° C. All immunohistochemistry tissue processing and staining procedures were identical to those described in the methods section of **Chapter 2**, except serial coronal sections (50 μm) were collected onto Superfrost microscope slides (Fisher Scientific) through only the

hippocampus/cortex (MA: $n = 9$, SA: $n = 9$) with a 200 μm inter-section distance. A total of 12 sections were collected per mouse (-1.00 mm to -4.00 mm Bregma).

Densitometry analysis: Densitometry analysis was used to quantify the area occupied by ChAT-immunoreactive fibers innervating the hippocampus and cortex. The procedure was identical to that described in the methods section of **Chapter 2**.

Statistical analysis: All statistical analyses were performed using SPSS software (Chicago, IL). A three-way ANOVA was used to assess the effects of treatment, genotype, and sex on the B_{max} and K_d of the mAChRs in the hippocampus and cortex. A repeated measure ANOVA was used to assess the effects of treatment and sex on the area occupied by ChAT immunoreactivity in all regions of the hippocampus and cortex. Lower-bound estimates of epsilon were used for all repeated measure ANOVAs. Only significant interactions are reported. All statistical tests were conducted with a two-tailed significance alpha level of 0.05.

3. Results

Muscarinic receptor binding: In the hippocampus, there was no main effect of sex or genotype on the number (B_{max}) of M_1 mAChRs. However, MA-treated mice had more M_1 mAChRs (higher B_{max}) than SA-treated mice (main effect of treatment; F (1, 16) = 11.24, $p < 0.01$; Figure 9). There was no main effect of treatment, sex, or genotype on the number of M_2 mAChRs in the hippocampus, although there was a trend for apoE4 mice to have more M_2 mAChRs than apoE3 mice (F (1, 16) = 4.32, $p = 0.054$). As the mice

used for the binding studies were of different ages when they finished behavioral testing (see **Chapter 5**), the binding data using age as a covariate was also examined. When age was included as a covariate, the results for hippocampal binding remained unchanged. There was no main effect of treatment, sex, or genotype on the number or dissociation constant (K_d) of either M_1 or M_2 mAChRs in the cortex (Table 3). When age was included in the analyses as a covariate, the results for cortical binding remained unchanged.

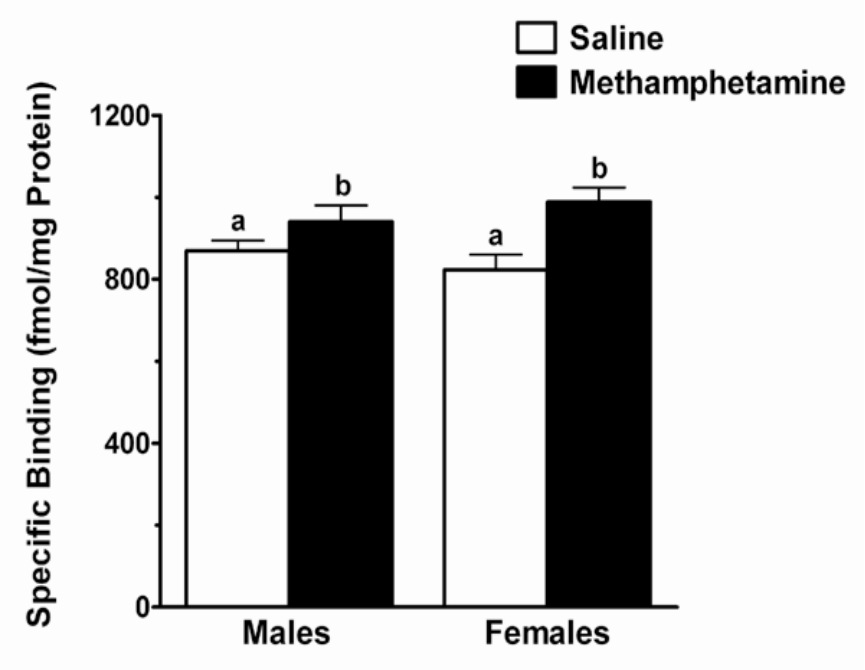


Figure 9. Number of M_1 muscarinic acetylcholine receptors in the hippocampus of adult mice collapsed across *APOE* genotype. Methamphetamine-exposed mice[b] had increased number (B_{max}) of M_1 muscarinic receptors in the hippocampus compared to saline-exposed mice[a]. Data are expressed as mean ± S.E.M. [a]Saline-exposed mice significantly lower than [b]methamphetamine-exposed mice, $p < 0.05$.

Area occupied by cholinergic fibers in the hippocampus and cortex: There was no effect of treatment or sex on the area occupied by ChAT-positive fibers in any region of the hippocampus or cortex in adult C57BL/6J mice (Table 4). Negative controls with secondary antibody only revealed no fluorescent visualization, indicating that the antibodies used were specific.

Table 3

Hippocampal and cortical M_1 and M_2 muscarinic acetylcholine receptors in adult mice.

	Males				Females			
	ApoE3		ApoE4		ApoE3		ApoE4	
Receptor	SA	MA	SA	MA	SA	MA	SA	MA
M_2 Hipp								
B_{max}	1162.2 ± 84	1262.4 ± 113	1405.53 ± 154	1350.7 ± 38	1224.9 ± 86	1454.9 ± 114	1390.1 ± 90	1549.0 ± 85
M_1 Cortex								
B_{max}	851.6 ± 67	882.1 ± 26	790.1 ± 115	830.9 ± 74	743.3 ± 94	887.7 ± 46	926.3 ± 64	931.5 ± 82
K_d	6606.0 ± 523	6958.8 ± 1754	6022.2 ± 568	10,363.4 ± 2904	5793.3 ± 513	6094.6 ± 743	7449.5 ± 1337	7231.0 ± 1311
M_2 Cortex								
B_{max}	1565.5 ± 225	1257.3 ± 29	1176.1 ± 74	1445.6 ± 258	1205.8 ± 90	1297.7 ± 162	1400.5 ± 88	1710.7 ± 135
K_d	12670.8 ± 5243	4993.3 ± 638	6226.8 ± 374	12322.6 ± 4449	5802.8 ± 1373	6979.2 ± 1399	8495.5 ± 1394	12976.8 ± 3964

B_{max} = number of receptors (fmol/mg protein), K_d = receptor dissociation constant (pM).
MA = methamphetamine, SA = saline, Hipp = hippocampus.

Data expressed as mean ± S.E.M.

Table 4

The area occupied by cholinergic fibers in the hippocampus and cortex of adult mice.

		Hippocampus					
Treatment	Sex	CA1	CA1SR	CA3	CA3SR	DGG	DGM
Saline	Males	2,624.8 ± 1,502.2	2,026.0 ± 798.0	2,780.2 ± 1,287.5	2,409.3 ± 905.3	2,292.4 ± 1,025.5	3,074.0 ± 1,213.9
Methamphetamine	Males	1,951.2 ± 544.8	2,184.6 ± 290.5	2,513.1 ± 516.7	2,521.4 ± 454.2	2,521.2 ± 489.8	3,861.7 ± 403.1
Saline	Females	3,454.1 ± 1,524.7	3,360.1 ± 1,496.9	4,594.2 ± 1,675.6	4,139.3 ± 1,557.1	3,802.9 ± 1,328.5	4,067.6 ± 1,428.6
Methamphetamine	Females	3,301.0 ± 587.1	2,942.0 ± 592.0	3,833.2 ± 715.5	3,464.2 ± 708.2	2,763.7 ± 755.8	3,689.8 ± 536.8

		Cortex					
Treatment	Sex	EhC	Lateral EhC	Motor	Piriform	1° Sensory	2° Sensory
Saline	Males	2,253.7 ± 1,069.1	3,196.7 ± 1,476.4	289.7 ± 286.8	3,377.1 ± 1,395.5	790.4 ± 286.6	261.9 ± 247.2
Methamphetamine	Males	4,781.4 ± 1,963.6	5,814.0 ± 1,839.6	1,946.8 ± 599.6	4,971.8 ± 1,367.8	2,141.9 ± 748.8	4,718.3 ± 2,367.4
Saline	Females	3,561.8 ± 1,297.3	3,599.2 ± 1,441.9	1,426.6 ± 747.3	4,404.9 ± 1,248.8	1,889.7 ± 1,140.4	2,635.4 ± 1,556.7
Methamphetamine	Females	3,709.0 ± 684.3	4,876.1 ± 742.5	1,744.1 ± 695.8	5,638.3 ± 670.1	2,255.9 ± 904.9	1,784.2 ± 547.1

Data expressed as mean area occupied by choline acetyltransferase immunoreactivity (μM^2) ± S.E.M.

SR = stratum radiatum, DGG = dentate gyrus granule layer, DGM = dentate gyrus molecular layer, EhC = entorhinal cortex.

4. Discussion

The results presented in this chapter show that MA exposure during brain development increased the number of M_1 mAChRs in the hippocampus of adult mice. Similar to what was found in adolescent mice (**Chapter 2**), this effect was observed in both males and females. In contrast, MA exposure did not affect the cortical mAChRs or the area occupied by ChAT-positive fibers innervating the hippocampus or cortex in male or female adult mice. These results differed from the original hypothesis that MA would decrease these cholinergic markers. Furthermore, no differences in the effects of MA were found between the apoE3 and apoE4 mice, suggesting *APOE* genotype does not modulate the effects of MA on the mAChR system.

Both adolescent and adult mice exposed to MA show increased M_1 mAChRs in the hippocampus but not in the cortex. Although the mAChR binding study was performed in adult mice expressing human apoE3 or apoE4, there was no mediating effect of *APOE* genotype, suggesting that similar effects would be observed in wild type mice. Taken together with the findings from **Chapter 2**, the effect of MA exposure during brain development on the mAChR system persists into adolescence and adulthood, suggesting this effect is long-lasting and may contribute to MA-induced cognitive impairments in adolescent and adult rodents (Acevedo *et al.*, 2007; Vorhees *et al.*, 2000; Vorhees *et al.*, 2007; Williams *et al.*, 2002). Despite the lack of an effect of apoE genotype, future studies examining the effects of MA treatment during brain development on the mAChRs in adulthood in wild type mice are warranted.

The lack of apoE effects suggests that apoE does not influence MA's effects on the mAChR system. However, it is important to keep in mind that only one aspect of the

cholinergic system was investigated in this study and that apoE isoform may modulate the effects of MA on other aspects of the cholinergic or other neurotransmitter systems. Relatively few studies have examined how apoE might interact with the effects of drugs of abuse, and those that have focus mainly on alcohol. For example, apoE4 is associated with increased cognitive impairments in alcoholic patients with Wernicke-Korsakoff syndrome (Muramatsu *et al.*, 1997) and with smaller hippocampal volumes in female alcoholics (Bleich *et al.*, 2003). ApoE3 is associated with a history of alcohol withdrawal seizures compared with apoE2 (Wilhelm *et al.*, 2007), and alcoholic patients carrying one ε4 allele show better visual memory than patients carrying one ε2 allele (Bartres-Faz *et al.*, 2002). Finally, mice lacking apoE show enhanced ethanol-conditioned place preference compared to wild type mice (Bechtholt, Smith, Raber, & Cunningham, 2004), suggesting that apoE modulates the rewarding effects of ethanol in a mouse model. The current findings contribute important information to the field of MA exposure and suggest that apoE genotype may not be a factor influencing differences in brain function following exposure to MA *in utero* (Piper *et al.*, 2011).

The effects of MA exposure during brain development on the cholinergic system differed between adolescent and adult female mice. While MA exposure increased the area occupied by cholinergic fibers in the hippocampus in adolescent female mice (**Chapter 2**), this effect was not observed in adult female mice. Changes in hormone levels with age may account for the differential effects observed between adolescents and adults. Estrogens are generally considered to be beneficial for cholinergic function (for a review, see (Gibbs, 2010); see **Chapter 6**) and thus it may be the case that during adolescence, the beneficial effects of estrogens are not present. By adulthood, in contrast,

estrogens may promote recovery from MA-induced changes in the cholinergic projections. There were no cholinergic differences following MA between adolescent and adult male mice, suggesting that recovery with age occurs only within the females.

There are sex differences in the effects of MA on the DA system that might relate to sex differences in the effects on the ACh system. For example, adult male mice show more severe MA-induced reductions in striatal DA that persists for two weeks following exposure compared to adult female mice (D. B. Miller, Ali, O'Callaghan, & Laws, 1998; L. Yu & Liao, 2000b). Male rats exposed to MA from PND 11-20 show greater reductions in protein kinase A activity in the striatum compared to female rats in adulthood (C. A. Crawford *et al.*, 2003). In the case of the DA system, it seems that males are more susceptible to the effects of MA. In contrast, the current data suggest that males are relatively protected from the effects of MA on the ACh system. However, in corroboration with the current findings, sex hormones seem to play a role in the recovery from MA-induced DA effects in females. For example, ovariectomized female mice supplemented with estrogen show greater recovery of striatal DA levels two weeks after MA exposure compared to female mice devoid of estrogens (L. Yu & Liao, 2000a). Thus it may also be the case that circulating sex hormones help promote recovery from MA-induced cholinergic impairments as well, potentially explaining why the adolescent female mice show greater cholinergic alterations than the adult female mice. The interaction between sex hormones, MA exposure, and cholinergic function has not been examined and further work is required to better understand these relationships.

The density of cholinergic BF neurons was not examined in adult mice. In adolescent mice, the increase in cholinergic fibers in the hippocampus was accompanied

by an increase in the density of cholinergic neurons in the MS/VDB/HDB (**Chapter 2**). The lack of an effect of MA on the cholinergic fibers in the hippocampus and cortex in adult mice suggests that there might not be a change in the density of cholinergic neurons, although these effects may be independent of each other. Future studies should examine the long-term effects of MA exposure during hippocampal development on the density of cholinergic cells in adult mice.

In summary, the data from this chapter show that MA exposure during brain development has long-lasting effects on the mAChR system in the hippocampus. The lack of an effect of MA on the area occupied by cholinergic fibers in the hippocampus suggests that this system recovers with age in female mice.

CHAPTER 4: LONG-TERM EFFECTS OF METHAMPHETAMINE EXPOSURE DURING BRAIN DEVELOPMENT ON COGNITIVE FUNCTION IN ADOLESCENT MICE

Adapted from:

Siegel, J.A., Park, B.S., & Raber, J. (2011). Long-term effects of neonatal methamphetamine exposure on cognitive function in adolescent mice. *Behavioural Brain Research, 219 (1),* 159-164.

1. Introduction

Compared to other drugs of abuse, MA use among pregnant women has increased over the past decade (Terplan *et al.*, 2009). MA-exposed children show cognitive impairments and have smaller hippocampal volumes compared to unexposed children (Chang *et al.*, 2009; Chang *et al.*, 2004; Lester & Lagasse, 2010; Piper *et al.*, 2011). Animal studies examining the effects of MA exposure during brain development are consistent with the human studies. For example, mice exposed to MA during brain development show impaired hippocampus-dependent novel location recognition and novel object recognition memory, and reduced sensorimotor gating in a PPI test in adulthood (Acevedo *et al.*, 2007).

Animal models of MA exposure during hippocampal development have typically focused on the long-term effects of MA in adulthood and relatively little research has focused on the effects of MA exposure on cognition in adolescence. In the only two studies to examine adolescent cognition, it was found that rats exposed to MA during

gestation have impaired retention of passive avoidance memory on PND 20 (Acuff-Smith *et al.*, 1996) while rats exposed to MA (5 mg/kg four times daily) during postnatal hippocampal development have impaired spatial learning and memory in the water maze at both PND 30 and 40 (Vorhees *et al.*, 2007). However, cognitive performance on tests other than passive avoidance and the water maze has not been assessed and no studies have been performed in mice. Furthermore, the findings from **Chapter 2** show that the cholinergic system, which plays an important role in cognitive function (Muir *et al.*, 1994; Sarter & Bruno, 2004; Voytko *et al.*, 1994), is altered by developmental MA exposure in adolescent mice. Therefore, the current work examined the effects of MA exposure during brain development on performance on a variety of behavioral and cognitive tests in adolescent male and female mice.

Because postnatal MA exposure has been shown to alter measures of anxiety in rats (Grace, Schaefer, Graham *et al.*, 2010) and increased anxiety can influence performance on cognitive tasks, the open field and elevated zero maze were used to measure anxiety-like behaviors in adolescent mice exposed to MA. Previous studies in adult wild type mice exposed to MA from PND 11-20 show impairments in novel location and novel object recognition memory, as well as PPI (Acevedo *et al.*, 2007). Thus performance on these tests was also examined. Finally, psychomotor stimulants can affect conditioned fear memory in adult rodents (Tsuchiya, Inoue, & Koyama, 1996). However, the effects of developmental MA exposure on conditioned fear have not been examined. Therefore, contextual and cued fear conditioning was also performed in adolescent mice exposed to MA or SA during postnatal brain development. It was hypothesized that MA exposure during brain development would impair performance in

the novel location and novel object recognition test, PPI, and contextual and cued fear conditioning in male and female adolescent mice.

2. Methods

Animals: Three- to 5-month-old male and female C57BL/6J mice were bred in our colony using breeding cages containing one male and two female mice. Female mice were singly housed from the first sign of pregnancy, when they gained a noticeable amount of weight. Lab chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO) and water were given *ad libitum*. On PND 21, pups were weaned and group housed with five mice per cage according to sex. The litters were provided soft foods during the injection period and two weeks after weaning to maintain stable weight gain. The mice were kept on a 12 hr light/dark schedule (lights on at 06:00). Behavioral testing took place during the light cycle. All procedures conformed to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University.

Injections: (*d*)-MA hydrocholoride (5 mg/kg), obtained from the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse drug supply program, was diluted with 0.9% sodium chloride (SA) to the appropriate concentration. Male and female pups from a total of 10 separate litters were weighed and given a single intra-peritoneal injection of MA or SA daily at 10:00 from PND 11-20

(injection volume of 0.1mL). A within-litter injection design was used to balance the number of MA and SA injections within a litter and across sexes.

Behavioral testing: Beginning on PND 28, mice (MA: $n = 24$, SA: $n = 27$) were individually housed. Behavioral testing began on PND 30. All mice were tested consecutively using the following sequence of tests: open field (PND 30), elevated zero maze (PND 31), novel location and novel object recognition (PND 32-34), PPI (PND 44), and contextual and cued fear conditioning (PND 45-46). There was a one-week interval between novel location and novel object recognition and the PPI tests. This order of testing was used to begin with the least stressful assessment and end with those thought to be most stressful.

Open field: Open field activity was assessed for 10 min in brightly lit enclosures (40.6 cm x 40.6 cm square) equipped with an array of infrared photocells for measuring horizontal movements and quantified automatically by a computer (Hamilton-Kinder, Poway, CA, USA). Distance moved and percent time spent in the center of the enclosure was measured. Percent time spent in the periphery versus center of the enclosure was used as a measure of anxiety-like behavior (Clement, Calatayud, & Belzung, 2002).

Elevated zero maze: In addition to the open field, anxiety-like behavior was also assessed in the elevated zero maze. The elevated zero maze (Hamilton-Kinder) consisted of a circular arena with two enclosed areas and two open areas of equal size. Mice were placed in the closed part of the maze and allowed to explore for 10 min. A video tracking

system (Ethovision XT, Noldus, Leesburg, VA) was used to analyze distance moved and the percent time spent in the open areas of the maze. Percent time spent in the closed versus open areas was used as a measure of anxiety-like behavior (Clement *et al.*, 2002).

Novel location novel object recognition: Mice were individually habituated to a lit open arena with clear Plexiglas walls (40.6 cm x 40.6 cm; Hamilton-Kinder) for 5 min on three consecutive days. On the fourth day, the mice were trained in five consecutive 10 min trials. The first three 10 min trials were familiarization trials with three objects placed within the arena (one in each of three corners). For the novel location recognition test (fourth trial, 10 min), one object was relocated to a novel location in the arena. The same object was moved for all mice. For the novel object recognition test (fifth trial, 10 min), one object was replaced with a novel object. The same object was replaced for all mice. There was a four min inter-trial interval between all trials. The objects were cleaned during the inter-trial interval to remove olfactory cues. The time spent exploring each object during all trials was analyzed using a multiple body point video tracking system that can track the nose-point of a mouse, as described ((Benice & Raber, 2008); Ethovision XT, Noldus). Exploration of an object was defined when the nose of the mouse was in a zone containing the object and the center point of the mouse was not. The zone was approximately 3 cm surrounding the object (Benice & Raber, 2008). Subsequently, the percent time the animal explored a particular object out of the total time exploring all three objects was calculated. The difference between the percent time spent exploring the object in the novel location (trial four) and the percent time spent exploring the same object in its original location (trial three) was calculated to measure

hippocampus-dependent novel location recognition. The percent time exploring the novel object in trial five was calculated to measure hippocampus-independent novel object recognition.

Pre-pulse inhibition: Mice were placed in an enclosure within a startle monitor sound-attenuated chamber and startle response amplitudes were measured with a force transducer (Hamilton-Kinder). Following a 5 min acclimation period, mice were exposed to 3, 40 ms acoustic stimuli (110 db). The testing phase consisted of 20 ms pre-pulses (70-80 db) followed by 50 ms delays and 40 ms acoustic stimuli (110 db). The same pattern of acoustic stimuli and testing with pre-pulses then occurred with a 120 db stimulus. Random inter-trial intervals were used between trials (15-30 sec). PPI was calculated using the following formula: % response = 100 x ((S-PS)/S), where S was the mean startle amplitude without a pre-pulse and PS was the mean startle response following a pre-pulse. Thus, a 100% response in the PPI test indicates complete inhibition of the startle response during pre-pulse trials.

Fear conditioning: Contextual and cued fear conditioning tests were performed as previously described (Villasana, Rosenberg, & Raber, 2009). Briefly, in contextual fear conditioning, the mice learn to associate the environment (fear conditioning chamber, conditioned stimulus (CS)) with a mild foot shock (unconditioned stimulus (US)), whereas in cued fear conditioning, the mice learn to associate a cue (tone (CS)) with a mild foot shock (US). During training the mice were placed in a fear conditioning chamber and allowed to explore for 2 min before the delivery of a 30 sec tone which was

immediately followed by a 2 sec foot shock. Two minutes later a second CS-US pair was delivered. Freezing was assessed for the first 2 min of the training session. Testing 24 hours later consisted of two phases: contextual testing where the mouse was placed in the same training context in the absence of the US, and cued testing where the mouse was placed in a different context in the absence of the US but in the presence of the tone. Re-exposure to either the context or tone results in freezing behavior (Villasana *et al.*, 2009). For contextual fear conditioning, mice were placed in the same training context for 5 min and freezing was analyzed for the first 3 min. For cued fear conditioning, mice were placed in a new context (containing a different odor, floor texture, walls, and shape) where they were allowed to explore for 3 min before being re-exposed to the tone for a duration of 3 min. Freezing was analyzed for the entire 6 min of the cued fear conditioning test (Ethovision XT, Noldus).

Statistical analysis: All statistical analyses were performed using SPSS software (Chicago, IL). As the pups were not individually tagged during the injection period, repeated measures ANOVA with litter as the experimental unit was used to assess the amount of weight gained each day during the injection period. Lower-bound estimates of epsilon were used for the repeated measure ANOVA. Two-way ANOVAs were used to assess effects of treatment and sex on performance on the behavioral and cognitive tests. Six mice were removed from the novel location and novel object recognition analysis due to influential outlier scores (greater than two standard deviations from the mean) or failure to explore the objects during any of the trials (less than a total of 2 seconds of

exploration over the 5 trials). Only significant interactions are reported. All statistical tests were conducted with a two-tailed significance alpha level of 0.05.

3. Results

Weight gain: MA- and SA-injected pups were weighed each day during the injection period to monitor weight gain. Repeated measures ANOVA showed an effect of postnatal day on the amount of weight gained from the previous day (main effect of day; F (1, 29) = 5.20, p = 0.03). *Post hoc* tests showed that all of the mice tended to gain more weight at the beginning of the injection period and the amount of weight gained from the previous day declined toward the end of the injection period. The repeated measures ANOVA also showed an effect of treatment on weight gained from the previous day (main effect of treatment; F (1, 29) = 6.82, p = 0.01). MA-exposed mice gained on average less weight each day than SA-exposed mice, regardless of sex (F (1, 31) = 7.35, p = 0.01; Figure 10). There was no effect of sex on weight gained from the previous day during the injection period.

Exploration and measures of anxiety in the open field and elevated zero maze: Distance moved and the percent time spent in the center of the arena were determined in the open field test. There was no effect of MA treatment on distance moved or percent time in the center of the arena. Female mice moved a greater distance than male mice, irrespective of treatment (main effect of sex; F (1, 47) = 5.08, p = 0.03; Table 5). There was no effect of sex on percent time in the center of the arena. Next, distance moved and the percent of time in the open anxiety-provoking and closed areas of the elevated zero maze were

measured. Similar to the open field, there was no effect of MA treatment on distance moved or percent time in the open areas of the maze. Female mice moved a greater distance compared to male mice, irrespective of treatment (main effect of sex; F (1, 47) =10.88, $p < 0.01$; Table 5). There was no effect of sex on percent time in the open areas of the maze.

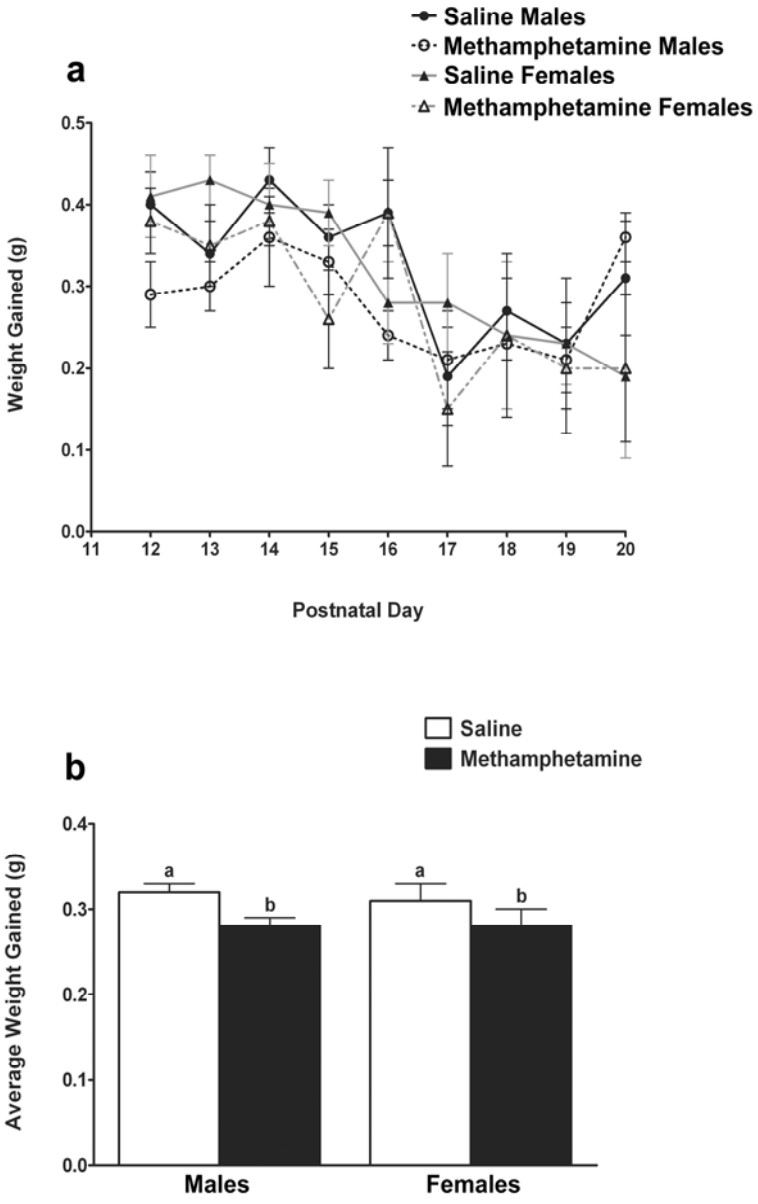


Figure 10. Weight gained during injections of methamphetamine or saline. (a) Weight gained each day from the previous day across the injection period. (b) Weight gained each day from the previous day averaged across all days of the injection period. Regardless of sex, methamphetamine-exposed pups gained less weight each day from the previous day during the injection period than saline-exposed pups. Data expressed as mean ± S.E.M. [a]Saline-exposed mice significantly higher than [b]methamphetamine-exposed mice, $p < 0.05$.

Table 5

Explorative and anxiety-like behavior in the open field and elevated zero maze in adolescent mice.

Treatment	Sex	Test	Distance moved (cm)	Percent time in center/open areas
Saline	Males	Open field	4660.49 ± 254.95	10.69 ± 1.44
Methamphetamine	Males	Open field	4247.40 ± 289.68	9.29 ± 1.78
Saline	Females	Open field	5163.67 ± 314.43[a]	11.12 ± 1.68
Methamphetamine	Females	Open field	4936.13 ± 180.81[a]	12.83 ± 1.45
Saline	Males	Zero maze	1735.52 ± 64.08	17.23 ± 1.47
Methamphetamine	Males	Zero maze	1666.08 ± 91.50	14.69 ± 1.63
Saline	Females	Zero maze	2033.54 ± 89.42[a]	16.65 ± 1.38
Methamphetamine	Females	Zero maze	1892.94 ± 73.71[a]	20.75 ± 2.34

Data expressed as mean ± S.E.M.

[a]Measures higher in females than males for respective test.

Novel location novel object recognition: There was no effect of MA treatment or sex on the total amount of time spent exploring the objects over the five trials in the novel location novel object recognition test (Figure 11a). There was also no effect of MA treatment or sex on the average distance moved in the novel location novel object test (data not shown). The percent time spent exploring an object in a novel versus familiar location was used as a measure of novel location recognition (Benice & Raber, 2008). MA-exposed mice showed reduced novel location recognition compared to SA-exposed mice (main effect of treatment; F (1, 41) = 4.26, p = 0.045; Figure 11b). There was no effect of sex on novel location recognition. The percent time spent exploring a novel object was used as a measure of novel object recognition (Benice & Raber, 2008). MA-exposed mice showed reduced novel object recognition compared to SA-exposed mice

(main effect of treatment; F (1, 41) = 8.09, $p < 0.01$; Figure 11c). There was no effect of sex on novel object recognition.

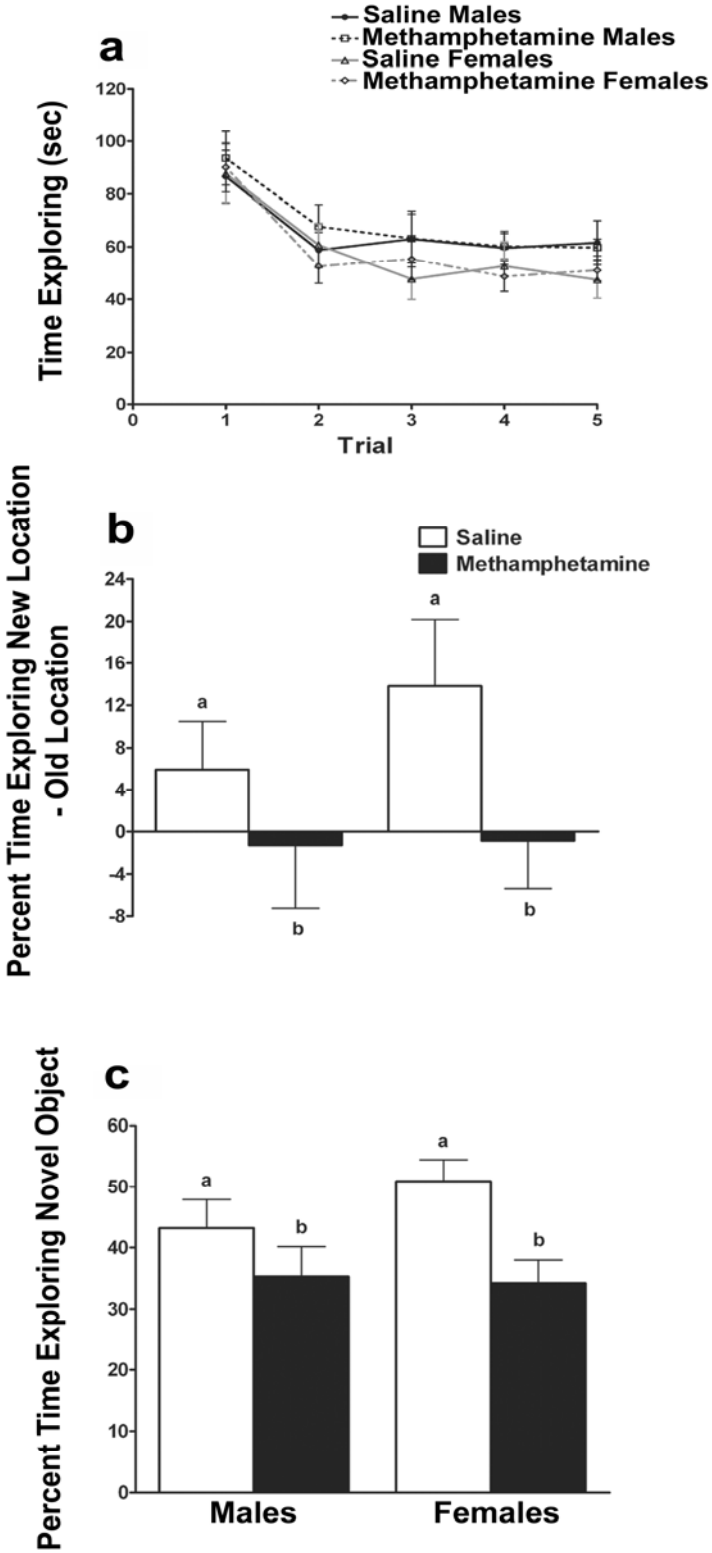


Figure 11. Novel location novel object recognition memory in adolescent mice. (a) There was no difference between the groups in the total amount of time spent exploring the 3 objects in the arena for each trial of the test. (b) Methamphetamine-exposed mice[b] spent less time exploring the object in the new location versus the old location compared to saline-exposed mice[a]. (c) Methamphetamine-exposed mice[b] also spent less time exploring the novel object compared to saline-exposed mice[a]. Data expressed as mean ± S.E.M. [a]Saline-exposed mice significantly higher than [b]methamphetamine-exposed mice, $p < 0.05$.

Sensorimotor gating in the pre-pulse inhibition test: A 100% response in the PPI test indicates complete inhibition of the startle response during pre-pulse trials. There was no effect of MA treatment or sex on the baseline acoustic startle response or PPI with the 110 db stimulus (data not shown). For the 120 db stimulus trials, there was no effect of MA treatment or sex on the baseline acoustic startle response. Male mice showed increased PPI compared to female mice for the 120 db stimulus (main effect of sex; F (1, 47) = 10.78, $p < 0.01$; Table 6), but there was no difference between the MA and SA-exposed mice in PPI during the 120 db stimulus.

Table 6

Baseline acoustic startle response and pre-pulse inhibition in adolescent mice[a].

Treatment	Sex	Baseline startle (N)	Pre-pulse inhibition
Saline	Males	2.86 ± 0.22	59.63 ± 3.67[b]
Methamphetamine	Males	2.68 ± 0.25	52.47 ± 4.65[b]
Saline	Females	3.01 ± 0.25	36.25 ± 4.97
Methamphetamine	Females	2.84 ± 0.25	45.46 ± 5.10

Data expressed as mean ± S.E.M.
[a]Only 120 db trials are shown as there were no sex differences in the 110 db trials.
[b]Males higher than females.

Contextual and cued fear conditioning: The first two minutes of the training (prior to tone-US presentation) were analyzed to exclude potential locomotor or anxiety-like effects contributing to freezing behavior (baseline freezing behavior). There was no difference between the treatment groups or sexes in baseline percent freezing during the training phase (Table 7). The first three minutes of the contextual fear conditioning test

were analyzed for group differences in percent time freezing. There were no differences between the treatment groups or sexes in the percent time freezing during the contextual fear conditioning test (Table 7). For the cued fear conditioning test, the first three minutes (prior to tone onset) and last three minutes (during tone presentation) were analyzed separately. Similar to the contextual fear conditioning results, there was no difference between the treatment groups or sexes in the percent time freezing during either phase of the cued fear conditioning test (Table 7). When the pre- and post-tone freezing behavior was analyzed using a repeated measure ANOVA, there were also no effects of treatment or sex (data not shown).

Table 7

Percent freezing in contextual and cued fear conditioning in adolescent mice.

Treatment	Sex	Baseline	Contextual test	Pre-tone cued test	During tone cued test
Saline	Males	13.44 ± 2.50	39.98 ± 4.57	19.83 ± 4.11	61.22 ± 4.07
Methamphetamine	Males	10.56 ± 2.41	41.65 ± 4.52	17.41 ± 2.69	57.72 ± 4.78
Saline	Females	10.17 ± 2.34	33.60 ± 4.74	12.00 ± 1.88	58.67 ± 4.51
Methamphetamine	Females	10.19 ± 2.37	36.48 ± 4.32	21.28 ± 3.14	66.86 ± 4.18

Data are expressed as mean percentages ± S.E.M.

4. Discussion

The results presented in this chapter show that MA exposure during hippocampal development reduces postnatal weight gain and impairs novel location recognition and novel object recognition memory in adolescent male and female mice. This is the first study to examine the effects of postnatal MA exposure on cognitive function in multiple tests during adolescence in mice. The results align with the original hypothesis that MA

exposure would impair cognitive performance, although no effects were found on the PPI and fear conditioning tests.

The effects of MA exposure on cognition during adolescence are similar to those previously found in adult C57BL/6J mice, but the mediating effects of sex differed in the two age groups. Similar to what was found in adolescence, adult MA-exposed male and female mice are impaired in the novel object recognition task (Acevedo *et al.*, 2007). However, both male and female mice showed novel location recognition impairments during adolescence, whereas only female mice show novel location recognition impairments in adulthood (Acevedo *et al.*, 2007), suggesting recovery of this cognitive deficit in the males with age. The increased susceptibility in adult female mice might relate to the slower rate of MA metabolism in female than male mice (see the discussion section of **Chapter 2**; (Acevedo *et al.*, 2008)). In addition, male sex hormones may be protective against MA-induced cognitive impairments. Indeed, androgens are protective against other brain challenges, such as β-amyloid toxicity (Pike, 2001) and apoE4 expression (Raber, Bongers, LeFevour, Buttini, & Mucke, 2002), and in the context of age-related cognitive decline (Benice & Raber, 2009b). However, the protective effects of androgens may be insufficient during adolescence when mice are going through puberty. Increases in gonadotropin-releasing hormone receptor expression, which is coupled with increased responsiveness to follicle-stimulating hormone and luteinizing hormone, occur earlier in female than male rats (Becu-Villalobos, Gonzalez Iglesias, Diaz-Torga, Hockl, & Libertun, 1997). Thus, the slower hormonal development in male rodents may relate to delayed maturation of cognitive function and delayed neuroprotection of gonad hormones in the males. By adulthood, in contrast, androgens

might be protective due to their higher levels and longer presence since the MA insult, thus ameliorating MA-induced impairments in novel location recognition observed in only adult females.

MA exposure did not impair performance on the PPI test in adolescent mice. Studies in adult C57BL/6J mice exposed to MA from PND 11-20 show PPI impairments (Acevedo *et al.*, 2007). These data suggest that the effects of neonatal MA exposure on PPI performance are age-dependent. There were also no effects of developmental MA exposure in adolescent mice on contextual or cued fear conditioning, which are considered to be dependent on the hippocampus and amygdala, respectively (Otto & Poon, 2006; R. G. Phillips & LeDoux, 1992; Stafford & Lattal, 2009). Previous reports have shown that psychomotor stimulants can affect fear conditioning in adulthood. A low pre-test dose of cocaine enhances both contextual and cued fear conditioning and a high dose impairs this conditioning when mice are tested in the absence of the drug (Wood, Fay, Sage, & Anagnostaras, 2007). Pre-treatment with MA also enhances freezing in a contextual fear conditioning paradigm in adult rats (Tsuchiya *et al.*, 1996). However, in the current investigation there were no effects of developmental MA exposure on contextual or cued fear conditioning. Thus, fear conditioning behavior may be less sensitive to the effects of MA exposure during hippocampal development due to the involvement of other brain regions such as the amygdala.

The mice in this study were tested on all the behavioral tests. Testing began on PND 30, which is considered to be toward the beginning of adolescence in rodents (Spear, 2000). However, the mice were at different stages of adolescence as they progressed through each test, with the final day of testing occurring on PND 46. PND 42

is often considered the end of adolescence in rats, and the end of adolescence in mice a few days later (Spear, 2000). However, more detailed analyses of the biological changes that occur during adolescence suggest that the end may be closer to PND 50 in rodents (Spear, 2000). Thus, although the mice were tested at different ages for each behavioral test, all of the tests occurred during what can be considered adolescence. There is the potential concern of carryover effects with repeated testing in the same animal and the effect of behavioral testing order must be taken into account when considering these results. The testing battery was designed to begin with the test considered to be least stressful and end with the test considered to be most stressful. While all the mice received the same order of testing, experience in one test could have altered performance on subsequent tests (McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001). Therefore, masking of potential treatment and sex effects in the cognitive tests toward the end of the battery cannot be ruled out. However, previous studies have shown effects of developmental MA exposure on cognitive performance in multiple tests using a similar testing battery and order of testing, suggesting that it is unlikely that consecutive testing masked any effects of MA in the current study (Acevedo *et al.*, 2007).

In addition to long-term effects of MA during brain development on cognition in adolescence, MA has acute effects on cognition in the adolescent brain. MA use during adolescence impairs executive function in the Stroop interference task, fine motor speed on the Grooved Pegboard task, and spatial organization in the WAIS/WISC-IV matrices test when the participants are tested during adolescence (G. King, Alicata, Cloak, & Chang, 2010). The effects of acute or long-term (chronic) drug exposure may also differ in the adolescent versus adult brain. Indeed, these results and results from previous

studies demonstrate age-dependent interactions between MA exposure and sex. More studies are warranted to examine the effects of drugs of abuse other than MA on adolescent brain function as they may drastically differ from those in adults.

In conclusion, the findings presented in this chapter show that exposure to MA during a time period equivalent to the third trimester of human fetal gestation impairs novel location recognition and novel object recognition memory in both male and female mice during adolescence. Future studies are warranted to better understand the mechanisms underlying the effects of developmental MA exposure on brain function in adolescence.

CHAPTER 5: LONG-TERM EFFECTS OF METHAMPHETAMINE EXPOSURE DURING BRAIN DEVELOPMENT ON COGNITIVE FUNCTION IN ADULT MICE

Adapted from:

Siegel, J.A., Craytor, M.J., & Raber, J. (2010). Long-term effects of methamphetamine exposure on cognitive function and muscarinic acetylcholine receptor levels in mice. *Behavioural Pharmacology, 21 (7),* 602-614.

1. Introduction

Exposure to MA during brain development causes physiological and cognitive impairments in infants that persist into adolescence (Chang *et al.*, 2009; Chang *et al.*, 2004; Piper *et al.*, 2011; L. M. Smith *et al.*, 2008; Struthers & Hansen, 1992). As discussed in **Chapters 1 and 4**, animal studies examining the long-term cognitive effects of MA exposure during brain development in adulthood mirror the findings in humans. The data presented in **Chapter 4** additionally show that cognitive function during adolescence is also impaired by MA exposure during postnatal brain development.

Despite consistent effects of MA exposure on cognition, some MA-exposed children are rated similarly by their parents on the Behavior Rating Inventory of Executive Function questionnaire and perform as well on a spatial memory task as unexposed children (Piper *et al.*, 2011). ApoE may be one factor influencing the degree of brain damage resulting from *in utero* MA exposure (Arendt *et al.*, 1997; Mahley, 1988). Since apoE modulates responses to central nervous system injury (Buttini *et al.,* 1999; Lomnitski *et al.,* 1999), it might also modulate susceptibility to cognitive

impairments following exposure to MA during brain development. Compared to apoE3, apoE4 is associated with an increased risk of developing Alzheimer's disease (Saunders *et al.,* 1993) and cognitive impairments following environmental challenges (Brichtova & Kozak, 2008; F. C. Crawford *et al.*, 2002; Guangda, Bangshun, Xiujian, & Yangzhong, 1999; Nathoo, Chetty, van Dellen, & Barnett, 2003). Children carrying the ε4 allele show impaired cognition compared to children without the ε4 allele (Acevedo, Piper, Craytor, Benice, & Raber, 2010). Mice lacking apoE show impaired acquisition of a test of attention (Siegel *et al.*, 2011) and mice expressing apoE4 in neurons (Raber *et al.,* 1998) or astrocytes (van Meer, Acevedo, & Raber, 2007) show impaired spatial learning and memory.

Little research has examined the effects of apoE in the context of drugs of abuse, and those that have focus mainly on alcohol (see discussion section of **Chapter 3**). These studies show that apoE modulates the way in which alcohol affects cognition. However, the effects of apoE on MA-induced cognitive impairments have not yet been examined. As previous studies have shown that MA exposure during brain development alters cognitive function in adult wild type mice (Acevedo *et al.,* 2007), the goal of the current study was to determine the potential ability of apoE to modulate the effects of MA on cognition by assessing the long-term effects of MA exposure during brain development on adult cognitive function in mice expressing human apoE isoforms.

As previous data show impaired novel location and novel object recognition memory in wild type adolescent and adult mice exposed to MA during brain development (**Chapter 4**; (Acevedo *et al.*, 2007)), these tests were also examined in the current study. Adult wild type mice show impaired PPI following MA exposure (Acevedo *et al.*, 2007),

and thus PPI was examined. Altered sensorimotor function can affect performance in cognitive tasks that require movement and therefore it is important to examine the potential effects of MA on sensorimotor function, which was done in the current study using the rotarod test. Finally, children exposed to MA *in utero* are more likely to have a diagnosis of Attention Hyperactivity Deficit Disorder compared to unexposed children (Piper *et al.*, 2011). Therefore, 5-SRTT performance was assessed in adult mice in order to determine any long-lasting effects of developmental MA exposure on attention. Performance on these cognitive tasks were also of interests as they all involve cholinergic signaling, which is altered by MA exposure during brain development in adulthood (**Chapter 3**; (Arnold *et al.*, 2002; C. K. Jones & Shannon, 2000; Muir *et al.*, 1994; Murai *et al.*, 2007; Sambeth *et al.*, 2007)). It was hypothesized that MA exposure during brain development would impair cognitive performance in the novel location and novel object recognition test, PPI, and the 5-SRTT. Furthermore, it was predicted that these effects would be more severe in female mice and mice expressing apoE4 compared to male mice and mice expressing apoE3, respectively.

2. Methods

Animals: Human *APOE* targeted replacement mice expressing human apoE3 or apoE4 under control of the mouse apoE promoter, on the C57BL/6J background (Knouff *et al.*, 1999; Sullivan *et al.*, 1997), were provided by Dr. Patrick Sullivan for breeding. Mice were housed and bred in our colony using homozygous mating. Breeding cages contained one male and two female mice and female mice were singly housed from the first sign of pregnancy, when they gained a noticeable amount of weight. Pups were weaned and

group housed with five mice per cage according to sex on PND 22. Lab chow (PicoLab Rodent Diet 20, #5053; PMI Nutrition International, St. Louis, MO) and water were given *ad libitum*. Litters were provided soft foods during the injection period and 2 weeks after weaning to maintain stable weight gain. The mice were kept on a 12 hr light/dark schedule (lights on at 06:00). Behavioral testing took place during the light cycle. All procedures conformed to the standards of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of the Oregon Health and Science University.

Injections: (*d*)-MA hydrocholoride (5 mg/kg), obtained from the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse drug supply program, was diluted with 0.9% sodium chloride (SA) to the appropriate concentration. A total of 18 litters were used for the current study; 10 apoE3 litters and 8 apoE4 litters. Male and female pups were weighed and given a single intra-peritoneal injection of MA or SA daily at 10:00 from PND 11-21 (injection volume of 0.1 mL). A within-litter injection design was used to balance the number of MA and SA injections within a litter and across sexes.

Weight gain and maternal care: Due to time and resource limitations, 11 of the 18 litters were chosen at random to be included in the weight gain and maternal care measures. The pups (n = 59 pups; 5 apoE3 and 6 apoE4 litters) were weighed each day to measure weight gain over the injection period. These pups were individually labeled and video recorded from PND 8-21. One hour video recordings were obtained at hours 1, 4, and 12

post injection time (10:00). Maternal nursing and grooming were scored every 4 minutes within each hour. For individual pups, one point was given if nursing or grooming was observed (Bredy *et al.*, 2004) and the percent of maternal nursing and grooming out of the total possible observations was calculated and averaged across all days for each post injection hour.

Behavioral testing: Litters born within a 2-week period were combined into cohorts for behavioral testing beginning on PND 90. Mice (MA: $n = 44$, SA: $n = 40$) were singly housed 48 hrs prior to testing and all mice were tested consecutively using the following sequence of tests: novel location novel object recognition, rotarod, PPI, and the 5-SRTT.

Novel location novel object recognition: The testing procedure for the novel location novel object recognition test was identical to that described in the methods section of **Chapter 4**. The difference between the percent time spent exploring the object in the novel location (trial 4) and the percent time spent exploring the same object in its original location (trial 3) was calculated to measure novel location recognition. The percent time exploring the novel object in trial five was calculated to measure novel object recognition. The percent time exploring the novel object as compared to the two familiar objects in trial 5 was also calculated as a measure of novel object recognition.

Rotarod: Sensorimotor function was assessed using a rotarod. The rod had a diameter of 7 cm and was placed 64 cm above the floor of a chamber (Hamilton-Kinder). The rod

initially rotated at 5 rpm and accelerated by 5 rpm every 15 sec. Mice were tested in 3 consecutive trials for 3 days. The latency to fall was measured.

Pre-pulse inhibition: The procedure for the PPI test was identical to that described in the methods section of **Chapter 4**, except that the mice were tested only on the 120 db trials.

5-Choice serial reaction time task: Operant learning and attention were assessed in the 5-SRTT. First, mice (n = 82) were food restricted to 80% of their free-feeding weight and tested in a 2-bottle preference test for sucrose solution as previously described (Siegel *et al.*, 2011). Mice were tested for 20 min once a day in operant testing chambers ((Robbins, 2002); Med Associates., St. Albans VT, USA). Sucrose solution (5%, 20 μL) was used as the reward.

5-SRTT training and testing was performed as previously described (Siegel *et al.*, 2011), with minor modifications. For training of the mice to retrieve a reward from the magazine (magazine training), mice were placed into the chambers with the house light off. After 15 sec, the magazine light illuminated and the reward was dispensed. Upon consumption, the magazine light was turned off and a 15 sec inter-trial interval elapsed. For training of the mice to poke their nose in a hole to receive a reward (nosepoke training), all 5 holes in the chamber were illuminated and a nosepoke into any hole resulted in reward. The magazine light remained illuminated until consumption. Following consumption and a 2 sec inter-trial interval, all of the holes were re-illuminated. For magazine and nosepoke training, mice were required to perform 40 responses for 2 consecutive days before advancing to the next level. Mice that failed to

learn nosepoke training (more than 15 days without advancing) were returned to magazine training.

Level testing followed nosepoke training. For level testing, 1 of the 5 holes was illuminated and a nosepoke into the lit hole was rewarded. An incorrect nosepoke or failure to respond during the stimulus duration of the hole illumination (omission) resulted in a 5 sec timeout (the house light was illuminated and the mice received no rewards). Mice progressed through levels 1, 2, 3, 4, 5, and 6 (20, 10, 8, 4, 2, and 1 sec stimulus duration, respectively). Mice were required to perform at least 10 correct nosepoke responses at a maximum latency of half the stimulus duration for 2 consecutive days to advance to the next level. The number of days to pass a training level and the level at which a mouse failed to learn the task (more than 15 days without advancing) were measured. Mice that failed to learn a level were taken back to nosepoke training. Subsequent failure on any level resulted in removal from testing (dropout). At level 6, attention was measured by calculating the percent of correct responses and omissions before and during treatment with scopolamine, a general mAChR antagonist. Once asymptotic performance was reached (percent correct responses with 90-110% of average percent correct from previous 5 days), the mice were given a single intra-peritoneal injection of saline or scopolamine (1.4, 0.8, or 0.2 mg/kg) each day prior to testing. All mice began with 3 consecutive days of saline injections, followed by 3 consecutive days of scopolamine 1.4 mg/kg, 3 days of saline, 3 days of scopolamine 0.8 mg/kg, 3 days of saline, and finally 3 days of scopolamine 0.2 mg/kg. If asymptotic performance was not reached after 55 days on level 6, mice with fewer than 15 total responses for more than 60% of the days or 115 days on level 6, whichever occurred first, were removed from

testing (dropouts). The doses of scopolamine were based on previous reports showing scopolamine-induced impairments in cognition in rodents (Pattij *et al.*, 2007; J. M. Phillips *et al.*, 2000; Shannon & Eberle, 2006).

Statistical analysis: All statistical analyses were performed using SPSS software (Chicago, IL). Three-way ANOVAs were used to assess the effects of treatment, genotype, and sex on weight gain and performance in the behavioral tests. Repeated measures ANOVA was used to assess maternal care and the effects of scopolamine administration on performance in the 5-SRTT (repeated factors: post-injection hour and scopolamine dose). Lower-bound estimates of epsilon were used for all repeated measures ANOVA tests. Four mice were removed from the novel location recognition analysis due to influential outlier scores (greater than two standard deviations from the mean). One cohort of mice (representing all genotypes, sexes, and treatments, $n = 16$) was removed from the novel object recognition analysis due to technical equipment problems during this phase of testing. The Kaplan-Meier method was used to assess the median training level at which mice were unable to learn the 5-SRTT (dropout level). The Fisher's Exact Test was conducted to assess dropout rates at each level of training in the 5-SRTT. Only significant interactions are reported. Data within the text are reported as mean ± S.E.M. All statistical tests were conducted with a two-tailed significance alpha level of .05.

3. Results

Weight gain and maternal care: MA- and SA-injected pups were weighed each day during the injection period to monitor weight gain. MA-treated mice gained less weight than SA-treated mice (main effect of treatment; F (1, 51) = 4.37, p = 0.04; Figure 12). In addition, apoE3 mice (1.90 ± 0.15) gained less weight than apoE4 mice (3.05 ± 0.10; main effect of genotype; F (1, 51) = 43.03, $p < 0.01$). There was no effect of sex on weight gain during the injection period.

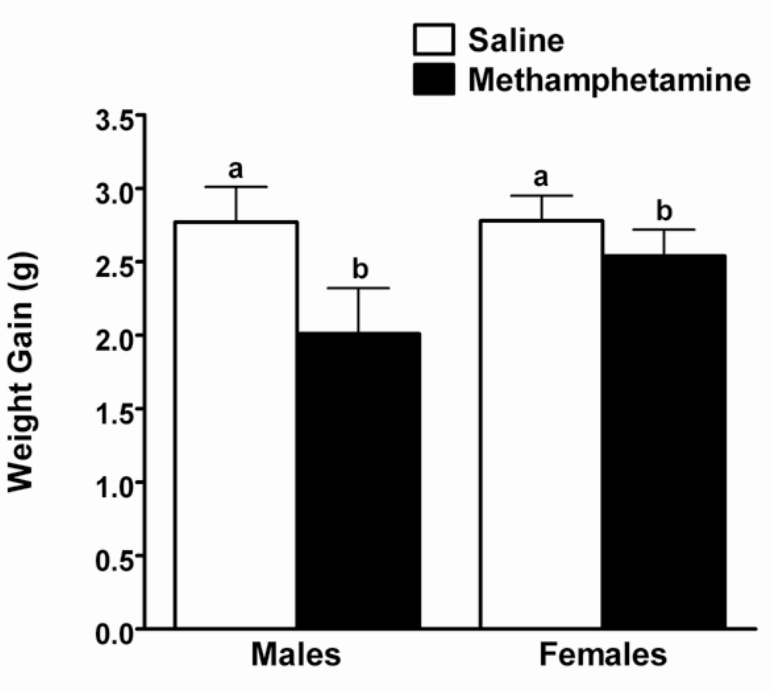


Figure 12. Weight gained during injections of methamphetamine or saline in mice collapsed across *APOE* genotype. There was a main effect of treatment on weight gain, with saline-exposed mice[a] gaining more weight over the injection period compared to methamphetamine-exposed mice[b]. Data expressed as mean ± S.E.M. [a]Saline-exposed mice significantly higher than [b]methamphetamine-exposed mice, $p < 0.05$.

As maternal care can influence behavior of the offspring in rodents (Bredy *et al.,* 2004), maternal feeding and grooming was measured to assess potential differences in the maternal care that MA- and SA-exposed pups received during injections. During the baseline period (PND 8-10), there was no main effect of treatment, sex, or genotype on maternal feeding. Similarly, during the injection period (PND 11-21), there was no main effect of treatment, sex, or genotype on maternal feeding. However, there was a genotype

x post-injection hour interaction (F (1, 51) = 5.16, p = 0.027). ApoE3 mice received more maternal feeding than apoE4 mice at both 1 and 4 hours post injection (Table 8 describes significant effects for all outcome measures). There was no difference in maternal feeding between apoE3 and apoE4 mice at 12 hours post injection (Figure 13).

During the baseline period, there was no effect of treatment, sex, or genotype on maternal grooming. However, there was a genotype x post-injection hour interaction (F (1, 51) = 4.48, p = 0.039). ApoE3 mice (3.73 ± 1.28) received more maternal grooming than apoE4 mice (0.82 ± 0.26) at 4 hours post injection time prior to the start of injections (PND 8-10). There was no difference in baseline maternal grooming between apoE3 and apoE4 mice at 1 hour and 12 hours post injection time. There was no main effect of treatment, sex, or genotype on the percent of maternal grooming observations during the injection period (PND 11-21).

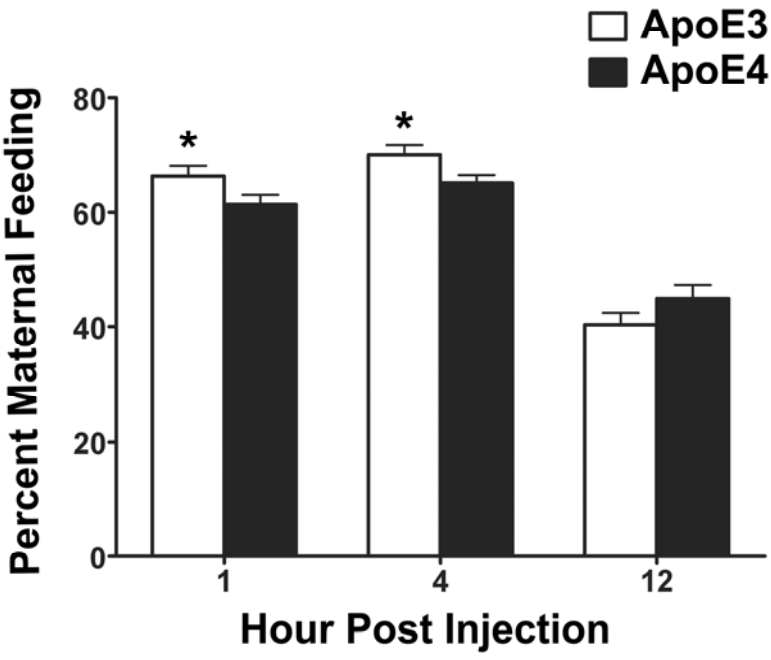


Figure 13. Maternal feeding during the injection period collapsed across treatment and sex. ApoE3 pups received a greater percent of maternal feeding compared to apoE4 pups 1 hour and 4 hours post injection time from postnatal day 11-21. There was no difference in the percent of maternal feeding 12 hours post injection time. $^{*}p <$ 0.05 apoE3 mice higher than apoE4 mice. Data expressed as mean ± S.E.M.

Novel location novel object recognition: There was no main effect of treatment, sex, or genotype on the total amount of time spent exploring the objects over the 5 trials (Figure 14a). There was a treatment x sex interaction for novel location recognition (F (1, 73) = 7.04, p = .01; Table 9). MA-treated female mice spent less time exploring the object in the new versus the old location than SA-treated female mice. Furthermore, paired t-tests collapsed across genotype for each group showed that MA-treated female mice did not show a difference in the percent time exploring the object in the new versus the old location (Figure 14b), indicating impaired novel location recognition. There was no main effect of treatment, sex, or genotype on the percent time spent exploring the novel object over the 10 minute trial (Table 9). Previous studies suggest that the majority of object exploration occurs in the first few minutes of the novel object recognition test (Dix & Aggleton, 1999; Mumby *et al.*, 2002). Furthermore, the total time spent exploring the objects within each minute over the 10 minute trial decreased in the current study (repeated measures ANOVA; F (9, 540) = 5.85, $p < 0.01$), suggesting that most of the exploration of the novel object occurred during the first part of the trial. Therefore, novel object recognition in the first 5 minutes of the 10 minute trial was examined. Over the first 5 minutes of the trial, MA-exposed mice spent less time exploring the novel object than SA-exposed mice (main effect of treatment; F (1, 60) = 4.48, p = 0.038; Table 9). Furthermore, an ANOVA conducted with planned *a priori* contrasts between each of the objects, collapsed across genotype and sex, showed that SA-exposed mice spent more time exploring the novel object over the other two objects while MA-exposed mice did not (Figure 14c). There was no effect of sex or genotype on novel object recognition.

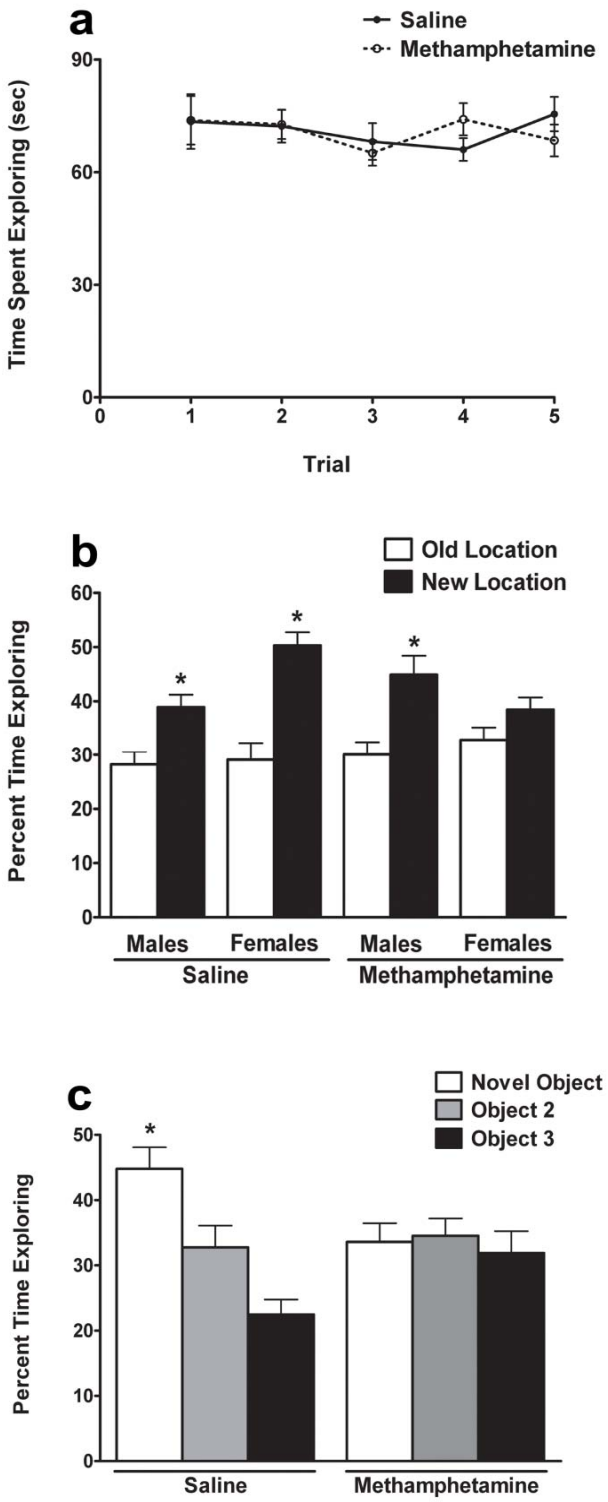


Figure 14. Novel location novel object recognition memory in adult mice. (a) There was no effect of methamphetamine treatment, sex, or genotype on the total amount of time spent exploring the objects over the 5 testing trials. Data are collapsed across sex and apolipoprotein E genotype. (b) All groups except the methamphetamine-treated female mice explored the object more in the new location than the old location. Data are collapsed across apolipoprotein E genotype. *$p < 0.05$ percent time spent exploring the object in the new location was greater than the percent time spent exploring the object in the old location. (c) Saline-exposed mice explored the novel object more than the other two familiar objects while methamphetamine-exposed mice did not show this exploration preference for the novel object. Data are collapsed across sex and apolipoprotein E genotype. *$p < 0.05$ percent time spent exploring the novel object was greater than percent time exploring the other two familiar objects. Data are expressed as mean ± S.E.M.

Rotarod: As motor coordination can affect performance on cognitive tests, sensorimotor function was measured using the latency to fall in the rotarod test (Soderling *et al.,* 2003).

There was no main effect of treatment, sex, or genotype on sensorimotor function in the rotarod test (Table 9).

Pre-pulse inhibition: Sensorimotor gating was assessed using PPI. A 100% response in this test indicates complete inhibition of the startle response during pre-pulse trials. There was no main effect of treatment or sex on the baseline acoustic startle response or PPI. However, compared to apoE4 mice, apoE3 mice showed an increased baseline acoustic startle response to the 120 db stimulus (main effect of genotype; F (1, 76) = 6.05, p = .02; Table 9) and increased PPI (main effect of genotype; F (1, 76) = 7.38, p < .01; Table 9).

Attention behavior in the 5-choice serial reaction time task: All mice showed preference for a 5% sucrose solution over a 10% and 20% sucrose solution in a 2-bottle preference test (F (2, 156) = 462.40, p < 0.001; data not shown). Therefore, the 5% sucrose solution was used as the reward in the 5-SRTT. There was no main effect of treatment or genotype on the median dropout training level in the 5-SRTT (Kaplan-Meier analysis; p > 0.45 for both treatment and genotype, log-rank test). There was also no main effect of treatment or genotype on the dropout rates at each individual level of testing. The median dropout training level was lower among female than male mice (female median level; level 1: male median level; level 6: p < 0.01, log-rank test; Figure 15a). There was also a difference in dropout rates between male and female mice at level 1 (p < 0.001, Fisher's Exact Test). Female mice (53%) dropped out more than male mice (11%).

For the mice that remained in the 5-SRTT through level 6, there was no main effect of treatment, sex, or genotype on percent correct responses during level 6 testing

prior to scopolamine injections. There was no main effect of treatment or sex on percent omissions during level 6 testing. However, apoE3 mice showed more omissions than apoE4 mice (main effect of genotype; F (1, 37) = 5.75, p = 0.02; Table 9).

Scopolamine-induced attention impairments: The effects of scopolamine on attention were assessed during level 6 of the 5-SRTT among the mice that did not dropout. There was no difference in the percent correct responses or omissions during the 3 saline administration periods (F (1, 16) = 1.06, p = 0.32 and F (1, 16) = 3.64, p = 0.074, respectively). Thus, percent correct responses and omissions were averaged for all saline injection days for comparisons with performance during scopolamine injections. There was an effect of scopolamine dose on the percent correct responses. For all mice, the percent correct responses were lower during both the 1.4 mg/kg and 0.8 mg/kg scopolamine injections compared to saline injections (F (1, 16) = 8.22, p = 0.01; Figure 15b). There was no main effect of treatment, sex, or genotype on percent correct responses. However, there was a treatment x sex x genotype interaction (F (1, 16) = 4.82, p = 0.043). Thus, the effects of sex and treatment were examined in each genotype. In apoE4 mice, there was no effect of sex or treatment on percent correct responses during the saline and scopolamine injections. In the apoE3 mice, there was a treatment x sex interaction (F (1, 6) = 9.23, p = 0.02). When this interaction was explored further, there was no effect of treatment in female apoE3 mice. There was a significant effect of treatment in the male mice. However, the group sizes at this point in the 5-SRTT were low (apoE3 SA male: n = 1; apoE3 MA male: n = 3), rendering this significant effect of treatment as un-interpretable.

Scopolamine treatment also affected percent omissions. For all mice, the percent omissions were higher during scopolamine 1.4 mg/kg treatment than saline, scopolamine 0.8 mg/kg, and scopolamine 0.2 mg/kg treatment (F (1, 16) = 14.29, $p < 0.01$; Figure 15c). There was no main effect of treatment on percent omissions following the scopolamine or saline injections. There were more omissions in male mice (75.8 ± 3.1) than female mice (61.0 ± 4.3; F (1, 16) = 16.41, $p < 0.01$) and apoE3 mice (72.9 ± 3.6) than apoE4 mice (66.2 ± 4.3; F (1, 16) = 9.20, $p < 0.01$).

4. Discussion

The data presented in this chapter show that adult mice expressing human apoE3 or apoE4 have long-term cognitive impairments in novel location recognition and novel object recognition memory following MA exposure during brain development. Similar to findings in wild type mice (Acevedo *et al.*, 2007) and to what was predicted, both male and female MA-exposed mice showed deficits in novel object recognition while only female MA-exposed mice showed deficits in novel location recognition, suggesting an increased susceptibility of female mice to the effects of postnatal MA on performance of a hippocampus-dependent test (Save *et al.*, 1992). In contrast to the original hypothesis, this effect was independent of apoE isoform.

The results are similar to those found in adolescent wild type mice, where MA exposure during brain development impaired both novel location and novel object recognition memory in male and female mice (**Chapter 4**). Similar to adolescence, MA impaired novel object recognition memory in adulthood. However, MA exposure impaired novel location recognition memory only in the female mice in adulthood,

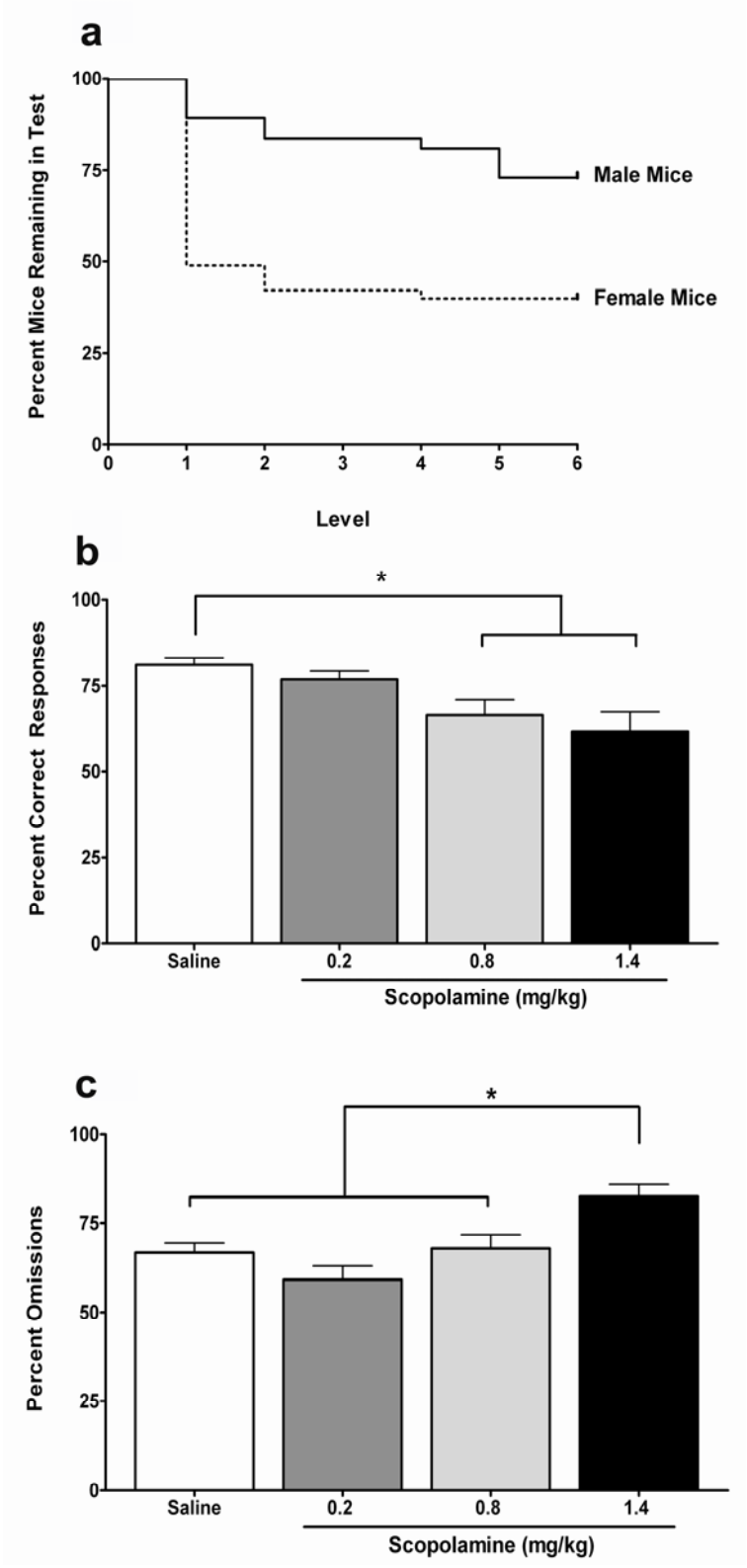


Figure 15. Dropout rates and performance during scopolamine injections on the 5-choice serial reaction time task in adult mice. (a) Female mice dropped out of the 5-choice serial reaction time task at a lower median level (level 1) than male mice (level 6). Data are collapsed across treatment and apolipoprotein E genotype. (b) Percent of correct responses during level 6 testing were reduced by scopolamine 1.4 mg/kg and 0.8 mg/kg injections compared to saline injections in all mice. (c) Percent of omissions during level 6 testing were increased by scopolamine 1.4 mg/kg injections compared to saline, scopolamine 0.8 mg/kg, and scopolamine 0.2 mg/kg injections in all mice. $*p < 0.05$ compared to indicated treatments. Data are expressed as mean ± S.E.M.

Table 8

Summary of significant effects of treatment, sex, and *APOE* genotype in adult mice.

Factor	Test	Measure	Effect
MA exposure	Weight gain	Body weight gain	Decreased in MA
(MA vs. SA)	Novel location recognition	Novel location recognition	Decreased in MA-female
	Novel object recognition (5 min)	Novel object recognition	Decreased in MA
	5-SRTT	Percent correct responses during scopolamine injections	Increased in MA-apoE3
Sex	5-SRTT	Median dropout training level	Increased in male
(Males vs. females)	5-SRTT	Dropout rates on level 1	Decreased in male
	5-SRTT	Percent correct responses during scopolamine injections	Decreased in male apoE3
	5-SRTT	Percent omissions during scopolamine injections	Increased in male
APOE genotype	Maternal care	Feeding (hours 1 and 4)	Decreased in apoE4
(ApoE4 vs. apoE3)	Maternal care	Grooming (hour 4)	Decreased in apoE4
	Weight gain	Body weight gain	Increased in apoE4
	PPI	Baseline acoustic startle to 120 db stimulus	Decreased in apoE4
	PPI	PPI to 120 db stimulus	Decreased in apoE4
	5-SRTT	Percent omissions on level 6	Decreased in apoE4
	5-SRTT	Percent omissions during scopolamine injections	Decreased in apoE4

MA = methamphetamine, SA = saline, ApoE = apolipoprotein E, 5-SRTT = 5-choice serial reaction time task, PPI = pre-pulse inhibition.
All effects are mice of the same treatment, genotype, or sex compared to group listed.

Table 9

Performance on the behavioral tests in adult mice.

		Males				Females			
		ApoE3		ApoE4		ApoE3		ApoE4	
Test	Measure	SA	MA	SA	MA	SA	MA	SA	MA
Novel location	Novel location rec.	14.9 ± 4.2	16.8 ± 7.0	7.6 ± 4.2	13.4 ± 4.2	21.1 ± 8.3	5.4 ± 3.9	21.2 ± 3.9	5.8 ± 5.3
Novel object	Novel object rec. 10 min	42.1 ± 2.7	38.3 ± 4.0	49.7 ± 3.8	35.0 ± 6.2	30.55 ± 4.9	34.4 ± 6.5	41.6 ± 6.1	33.78 ± 5.11
Novel object	Novel object rec. 5 min	46.3 ± 4.8	39.7 ± 5.6	53.9 ± 6.4	34.0 ± 7.7	34.0 ± 5.6	30.7 ± 5.4	40.9 ± 5.8	32.44 ± 4.82
Rotarod	Latency to fall (s)	60 ± 2.9	53.7 ± 2.4	56.7 ± 2.5	54.2 ± 1.0	58.9 ± 2.4	61.2 ± 2.0	57.1 ± 2.8	56.3 ± 1.7
PPI	Baseline startle (N)*	2.1 ± 0.5	2.7 ± 0.5	1.5 ± 0.3	1.8 ± 0.4	1.8 ± 0.3	1.8 ± 0.3	1.1 ± 0.4	1.5 ± 0.4
	PPI*	67.9 ± 6.0	67.9 ± 7.2	57.3 ± 5.4	57.9 ± 8.5	68.9 ± 6.2	61.9 ± 4.4	30.9 ± 20.3	44.8 ± 8.4
5-SRTT	Level 6 % omissions*	71.1 ± 1.8	70.4 ± 3.0	62.5 ± 3.9	65.9 ± 2.2	64.3 ± 4.4	69.1 ± 5.1	65.4 ± 8.1	50.4 ± 5.2

MA = methamphetamine, SA = saline, ApoE = apolipoprotein E, PPI = pre-pulse inhibition, 5-SRTT = 5-choice serial reaction time task, Rec = recognition.

All Measures shown as group means ± S.E.M.

* $p < .05$ main effect of genotype, apoE3 higher than apoE4 mice.

suggesting a recovery of impairment in the male mice with age. As discussed in **Chapters 2-4**, the mechanism underlying the sex difference in MA's affects are currently unknown, although slower metabolism of MA in the brain and the protective effects of androgens may contribute. Androgen levels have not reached maturity by PND 30 (Selmanoff, Goldman, & Ginsburg, 1977), suggesting that the adolescent male mice are not protected from MA-induced impairments and show novel location recognition memory impairments while the adult male mice are protected and do not show these impairments. MA-induced hippocampal alterations may be less severe in the male mice by adulthood, thus ameliorating novel location recognition impairments. In contrast, other brain areas important for novel object recognition may be equally affected in the male and female mice, thus impairing performance on this task in both sexes in adulthood. A more thorough discussion of sex differences is presented in **Chapter 6**.

Novel object recognition memory was consistently impaired in both male and female mice during adolescence (**Chapter 4**) and adulthood. The mechanism underlying the enhanced sensitivity of performance on this task following MA exposure is not yet understood. Potential alterations in other neurotransmitter systems in male and female mice following MA exposure might relate to impairments in novel object recognition memory. Alternatively, altered hippocampal projections to the perirhinal cortex following MA exposure may relate to novel object recognition impairments. Lesions of the perirhinal cortex impair novel object recognition memory in rodents and non-human primates (for a review, see (Brown & Aggleton, 2001)). The perirhinal cortex may be equally altered in male and female mice following MA exposure. Future studies are

needed to assess this possibility and MA's affects on the perirhinal cortex. A more detailed discussion of this issue is presented in **Chapter 6**.

There was no effect of postnatal MA exposure on PPI or attention as assessed in the 5-SRTT. The long-term effects of postnatal MA exposure on brain function may not be sufficiently severe to cause impairments in PPI and 5-SRTT performance, but such impairments, at least in the 5-SRTT, might be revealed at earlier time points following MA exposure. In contrast to the current findings, previous studies in wild type mice show impairments in PPI following postnatal MA exposure (Acevedo *et al.,* 2007). Differences in PPI impairments suggest a protective effect of human apoE against the effects of postnatal MA exposure on sensorimotor gating. This difference in PPI impairments between wild type and apoE3 or apoE4 mice may be a result of apoE functionality. ApoE is involved in the restoration of neuronal function and neurite outgrowth following brain injury (for a review, see; (Raber, 2004)). Astrocytes synthesize apoE and secrete an apoE-cholesterol complex that circulates to injured regions within the brain where the cholesterol is used for membrane and synapse formation (Poirier, 1994). Mouse apoE and human apoE may differ in trafficking and promotion of neurite outgrowth and species differences in ability of apoE to promote neurite outgrowth may account for differential brain repair and cognitive function following MA exposure during brain development.

One concern with the postnatal MA exposure model is that the mother will not care for MA-exposed pups to the same degree as SA-exposed pups, thus causing long-term effects on behavior. This possibility was investigated by measuring maternal feeding and grooming throughout the injection period. There were no differences in feeding or grooming received in the MA- and SA-exposed pups, but there were significant

differences in maternal care between apoE3 and apoE4 mice. These data suggest that the maternal care experiment was sensitive enough to detect group differences and that altered maternal care cannot account for the long-term cognitive effects of MA exposure.

The lack of apoE effects in this chapter and in **Chapter 3** suggests that apoE does not influence MA's effects on the brain or behavior. Relatively few studies have examined how apoE might interact with the effects of drugs of abuse, and those that have focus mainly on alcohol (Bartres-Faz *et al.*, 2002; Bechtholt *et al.*, 2004; Bleich *et al.*, 2003; Muramatsu *et al.*, 1997; Wilhelm *et al.*, 2007). Thus, the current findings contribute important information to the field of MA exposure and suggest that apoE genotype may not be a factor influencing differences in cognitive impairments observed in children following exposure to MA *in utero*.

Homozygous breeding was used for the mice in this study and the mAChR binding experiment in **Chapter 3**. Homozygous breeding can lead to genetic mutations that can alter brain function and behavior. However, new breeders are used every couple of years in our colony with the goal of preventing any potential mutations from manifesting. As there was no mediating effect of genotype on any of the effects of MA, mutations due to homozygous breeding are likely not a concern for the current findings. One potential limitation of this study is that more genotypes were not included in the testing. Mice expressing human apoE2, as well as apoE knockout mice and wild type mice, were not included. Aside from practical limitations with space and resources, wild type mice were not included because previous studies have examined the effects of MA exposure during hippocampal development on adult cognitive and brain function in wild type mice (Acevedo *et al.*, 2007; Acevedo *et al.*, 2008). As the apoE2 isoform is the least

common in humans (Wilson *et al.,* 1994), potential differences in MA-induced effects between mice expressing apoE3 and apoE4 isoforms was determined. While there were no effects of apoE genotype, future studies comparing the effects of MA treatment during brain development on cognition in adulthood in apoE and wild type mice are warranted.

In summary, the results presented in this chapter show that exposure to MA during a time period equivalent to the third trimester of human fetal gestation impaired novel location recognition memory in adult female mice and impaired novel object recognition memory in both adult male and female mice, replicating previous findings in adult wild type mice (Acevedo *et al.*, 2007).

CHAPTER 6: GENERAL DISCUSSION

1. Summary of experimental results

The overarching goal of this dissertation was to elucidate the long-term effects of MA exposure during brain development on the cholinergic system and cognition in adolescent and adult mice. The main findings are that MA exposure during brain development altered the cholinergic system in both adolescent and adult mice in a sex-dependent manner: cholinergic cell density in the MS/VDB/HDB and area occupied by cholinergic fibers in the hippocampus were increased in adolescent MA-exposed female mice and M_1 mAChRs were increased in the hippocampus of adolescent and adult MA-exposed male and female mice. MA exposure also impaired cognitive function in a sex-dependent manner: hippocampus-dependent novel location recognition memory was impaired in adolescent MA-exposed male and female mice but only in adult MA-exposed female mice, while novel object recognition memory was impaired in adolescent and adult MA-exposed male and female mice.

The original hypotheses of this dissertation were that MA exposure during brain development would decrease the density of cholinergic cells in the BF, the area occupied by cholinergic axons in the hippocampus and cortex, and the number of mAChRs in male and female mice. Furthermore, I hypothesized that developmental MA exposure would impair cognitive function in male and female mice, and that these impairments would be more severe in adult female than male mice and more sever in apoE4 than apoE3 mice. In contrast to my original predictions, MA exposure increased the cholinergic markers measured in these studies and affected apoE3 and apoE4 mice to the same degree.

However, in parallel with my original predictions, MA exposure impaired cognitive function in both adolescent and adult male and female mice, and the impairments in novel location recognition were more severe in adult female than male mice.

The findings from this dissertation are significant because they demonstrate for the first time an effect of MA exposure during brain development on the adolescent cholinergic system and that some of these effects persist into adulthood. MA exposure also impaired cognitive function in adult mice, replicating previous studies in rodents (Acevedo *et al.*, 2007; Vorhees *et al.*, 2000; Williams, Morford, Wood, Wallace *et al.*, 2003; Williams *et al.*, 2002). This dissertation adds to this literature by showing that MA exposure from PND 11-20 impairs cognitive function in adolescent mice. Taken together, these results have important implications for human MA exposure and suggest that the cholinergic system may be disrupted in children and adults exposed to MA *in utero*. A better understanding of how MA exposure during brain development alters the cholinergic system advances our understanding of MA's actions in the brain, as relatively little research has focused on this and the mechanism by which MA might induce cognitive impairments. Furthermore, as other psychomotor stimulant drugs such as cocaine and AMPH have similar actions in the brain, it may be the case that exposure to these drugs during brain development also alters the cholinergic system in a manner similar to that shown in this dissertation, and this may contribute to cognitive impairments seen in children exposed to these drugs *in utero*.

In the sections below, the potential mechanisms by which MA may alter cholinergic function and the relationship between the MA-induced cholinergic changes and cognitive impairments are discussed. The significance of the sex differences in MA's

effects are also explored prior to a discussion of model and technical considerations. Within each section, future studies that will contribute to the current findings are presented.

2. Hypothesized mechanisms of methamphetamine's effect on the cholinergic system

Effects on extracellular and transcription factors: The mechanism by which MA enhanced ChAT and mAChR expression in this dissertation is not known (**Chapters 2 and 3**). MA exposure could increase trophic factors that support the differentiation of cholinergic cells or enhance the expression of cholinergic markers. For example, MA exposure could have altered BMP signaling, thus causing long-term changes in the cholinergic system. BMP-4 increases CHT and VAChT mRNA levels in culture from GD 14 mouse septum (Berse *et al.*, 2005) and BMP-9 increases ACh levels, ChAT immunofluorescence, ChAT and VAChT mRNA levels, and the expression and release of NGF in MS cell cultures, but has no effect on GAD immunofluorescence (Lopez-Coviella *et al.*, 2000; Schnitzler *et al.*, 2010). BMP-9 application to mouse embryos on GD 14 increases ACh levels on GD 18, but this effect is not seen in mice exposed later in gestation or on PND 1-3 (Lopez-Coviella *et al.*, 2000). Furthermore, lesions of the DA midbrain neurons reduce mRNA levels for various BMP subtypes in the cortex and hippocampus in adult rats, suggesting that long-term MA-induced alterations in DA function may alter BMP signaling (H. L. Chen *et al.*, 2003). However, the receptors required for BMP-9 are present only during early gestation and decline with age in mice (Lopez-Coviella *et al.*, 2006). MA may alter BMP receptor expression during postnatal development, thus inducing BMP-9 signaling.

The LIM homeobox family of transcription factors supports the differentiation of cholinergic BF cells. Potential MA-induced alterations in these transcription factors could then greatly affect cholinergic BF neurons later in life. Mouse embryonic stem cell or neuroblastoma culture lacking the gene encoding Lhx7 (also known as L3/Lhx8) show reduced ChAT and VAChT levels, an effect that is reversed by the subsequent over-expression of Lhx7 (Manabe *et al.*, 2007; Manabe *et al.*, 2005). Interestingly, these cells also show increased GABAergic markers, suggesting that Lhx7 enhances cholinergic differentiation and also suppresses GABAergic differentiation in culture (Manabe *et al.*, 2005). *In vivo* studies show that Lhx7 is required for cholinergic cell differentiation, as mice lacking the gene encoding Lhx7 show dramatic decreases in the number of ChAT-positive cells in the BF in adulthood, but no changes in GABAergic cells (Fragkouli *et al.*, 2005; Mori *et al.*, 2004). These mice also show decreased AChE staining in the BF, cortex, and hippocampus in adulthood (Fragkouli *et al.*, 2005). These data suggest that the LIM homeobox transcription factors are required for the differentiation of the cholinergic phenotype in the BF and that Lhx7 could function to direct the phenotype of post-mitotic neurons toward cholinergic characteristics (Fragkouli *et al.*, 2005). To the best of my knowledge no studies have examined the effects of MA or other psychomotor stimulants on LIM homeobox transcription factors and the mechanism by which MA might alter these transcription factors is unknown. However, one could hypothesize that MA increases the activity of the LIM homeobox transcription factors, increasing cholinergic phenotype expression in post-mitotic BF cells. Under normal circumstances these transcription factors act during gestation, but MA exposure could alter this processes, thus resulting in a long-term increase in ChAT and mAChR expression levels.

A more likely way in which MA exposure might alter the BF cholinergic system is by increasing levels of NGF in the hippocampus. In cultures of mouse septum, NGF increases ChAT activity, ACh production (Madziar *et al.*, 2005), the number of ChAT-positive cells (Hartikka & Hefti, 1988), and CHT mRNA levels (Berse *et al.*, 2005). *In vivo* experiments also show that NGF promotes cholinergic development. Rats injected with NGF into the ventricles on PND 2, 4, 6 and 8 show increased ChAT activity in the septum, NB, cortex, and hippocampus on PND 12 (Mobley *et al.*, 1986). Injections of NGF into the ventricles on PND 4 or PND 18 increases both ChAT and VAChT mRNA levels in the septum of rats on PND 7 and PND 21, respectively (Tian *et al.*, 1996). Finally, blockade of NGF reduces ChAT mRNA in the septum of rats on PND 11 (Li *et al.*, 1995). GABAergic BF neurons do not express NGF receptors (Hartikka & Hefti, 1988) and NGF has no effect on GAD activity in the septum, hippocampus, or cortex (Mobley *et al.*, 1986). These results suggest that NGF's trophic effects are specific to cholinergic neurons and that NGF influences cholinergic phenotype expression during postnatal development. MA exposure from PND 11-20 increases NGF levels in the rat hippocampus on PND 20 (Skelton *et al.*, 2007), suggesting that in the female mice in the current experiments, MA may have increased NGF in the hippocampus, altering normal developmental patterns of NGF expression and the development of the BF cholinergic system.

Microglia activation and oxidative stress: MA-induced activation of microglia is another plausible mechanism by which MA may alter cholinergic function. Activated microglia can produce cytokines, chemokines, and reactive oxygen species (Ni *et al.*, 2007).

Although beyond the scope of this dissertation, reactive oxygen species are needed in a delicate balance for proper neuronal function and disruption of the appropriate balance may be detrimental to brain function. In terms of cholinergic function, microglia stimulation increases ChAT activity and the percent of ChAT-positive cells in cortical neuron cultures from GD 15 rats (Ni *et al.*, 2007). The cytokine interferon-γ enhances ACh synthesis, ChAT mRNA levels, and cholinergic cell number in BF cell culture (Jonakait, Wei, Sheng, Hart, & Ni, 1994) and interleukin-1 increases AChE mRNA levels in the cortex of adult rats (Li *et al.*, 2000). Thus microglia activation and the subsequent effects enhance the cholinergic system, and anything that activates microglia may alter cholinergic phenotype expression.

MA exposure in adult rodents increases microglia activation and thus oxidative stress (Escubedo, Chipana, Perez-Sanchez, Camarasa, & Pubill, 2005; Fantegrossi *et al.*, 2008; Goncalves *et al.*, 2010; Guilarte *et al.*, 2003; LaVoie, Card, & Hastings, 2004; Thrash, Karuppagounder, Uthayathas, Suppiramaniam, & Dhanasekaran, 2010). MA increases oxidative stress also by releasing DA into the cytoplasm where DA is rapidly oxidized to form reactive oxygen species (Krasnova & Cadet, 2009). MA elevates mRNA levels for interleukin-1β in adult rats (Yamaguchi *et al.*, 1991). Furthermore, MA increases reactive oxygen species in cultured human neuroblastoma cells (Wu *et al.*, 2007) and abstinent adult MA abusers show increased microglia activation (Sekine *et al.*, 2008). Treatment with anti-oxidants protects against MA-induced depletion of monoaminergic axons (for a review, see (Krasnova & Cadet, 2009)) and MA neurotoxicity on the DA and 5-HT systems is reduced in adult mice lacking interleukin-6 (Ladenheim *et al.*, 2000). As microglia activation and increased cytokines and oxidative

stress play a role in cholinergic phenotype expression, MA exposure during brain development may act to enhance aspects of the cholinergic system via activation of microglia and increased oxidative stress.

Increased cholinergic activity via interactions with other neurotransmitter systems: MA exposure during brain development may increase cholinergic enzymes and receptors via activation of other neurotransmitters. For example, the cholinergic system is modulated by the histaminergic system (for a review, see (Bacciottini, Passani, Mannaioni, & Blandina, 2001)) and histamine release from the tuberomammillary nucleus to the MS increases ACh release from the MS to the hippocampus and anti-histamine drugs decrease cholinergic transmission (Bacciottini *et al.,* 2001). MA exposure during brain development increases brain histamine levels in neonates and this increase might mediate MA-induced cognitive impairments in adulthood, as blockade of histamine activity during MA exposure blocks the effects of MA on cognition (Acevedo *et al.*, 2007; Acevedo *et al.*, 2008). Furthermore, the tuberomammillary histamine neurons project directly to the hippocampus, where histamine can regulate hippocampal function in conjunction with cholinergic regulation of the hippocampus (Bacciottini *et al.*, 2001). Histamine also modulates the effects of ACh released from the NB to the cortex and amygdala and is implicated in cognitive function (Bacciottini *et al.*, 2001). Thus, the potential effects of MA on the histamine system could have led to downstream effects on the BF cholinergic system and contributed to the MA-induced cognitive impairments observed in this dissertation.

MA also has long-term effects on the DA, NE, and 5-HT systems following exposure during brain development that may mediate its effects on the cholinergic system (Cabrera *et al.*, 1993; C. A. Crawford *et al.*, 2003; Grace, Schaefer, Gudelsky *et al.*, 2010; Lehmann & Lehmann, 2007; Schaefer *et al.*, 2008; Weissman & Caldecott-Hazard, 1993). ChAT and mAChR levels may be altered by increases in cAMP and other intracellular signaling molecules that would occur following DA binding to D_1/D_5 receptors on BF cholinergic neurons following MA exposure (Figure 16a). Alternatively, the VTA contains DA neurons that project to the nucleus accumbens where MA acutely increases DA levels (Bardo, 1998; Halbach & Dermietzel, 2002). DA release from the VTA can inhibit subsequent GABA release from the nucleus accumbens into the BF by activating inhibitory DA D_2 receptors on GABAergic nucleus accumbens neurons (Casamenti, Deffenu, Abbamondi, & Pepeu, 1986; H. Moore, Fadel, Sarter, & Bruno, 1999). In addition, increased synaptic levels of 5-HT, which can be caused by MA, reduce GAD mRNA levels in the nucleus accumbens in adult male rats (Mijnster, Galis-de Graaf, & Voorn, 1998), potentially decreasing GABA synthesis and release into the BF. An acute reduction in GABA inhibition with MA would disinhibit the cholinergic BF neurons, perhaps leading to long-term increases in the cholinergic system (Figure 16a).

Decreased cholinergic activity via interactions with other neurotransmitter systems: MA exposure during brain development may decrease cholinergic BF cellular activity via activation of other neurotransmitters, thus resulting in a compensatory up-regulation of ChAT expression levels and the mAChRs. Indeed, BF cholinergic lesions increase M_1 mAChR levels in the hippocampus (Rossner, Schliebs, Hartig, & Bigl, 1995). However,

reductions in ACh release are associated with decreases in ChAT mRNA and protein levels (Jamal *et al.*, 2007), suggesting that ChAT expression levels do not show compensatory up-regulations under certain conditions. Nonetheless this possibility remains to be determined in the context of postnatal MA exposure.

There are a variety of mechanisms by which MA may cause a decrease in ACh synthesis and release. MA exposure from PND 11-20 has been shown to reduce striatal DA D_2 receptor levels in adult rats (C. A. Crawford *et al.*, 2003), potentially disinhibiting the activity of the accumbens GABAergic neurons and GABA release into the BF. Another hypothesized mechanism by which MA may decrease BF cholinergic cell activity is through the BF GABAergic neurons. Acute MA-induced increases in DA in the BF may excite GABAergic BF activity, thus inhibiting the cholinergic neurons and causing long-term decreases in ACh release and compensatory up-regulations in ChAT and mAChRs (Figure 16b). Lesions of the VTA can increase the levels of ACh released into the hippocampus (Robinson, Malthe-Sorenssen, Wood, & Commissiong, 1979), suggesting that under normal conditions DA from the VTA can inhibit cholinergic neurotransmission potentially by activating GABAergic BF cells. Finally, potential long-term reductions in DA function in the VTA following exposure to MA during brain development may reduce excitation of the BF cholinergic cells, leading to long-term reductions in ACh synthesis and release. MA exposure during gestation reduces tyrosine-hydroxylase mRNA levels in the VTA on PND 7 and 14 in female rats, suggesting a down-regulation in dopaminergic function (Gomes-da-Silva *et al.*, 2002). Thus MA-induced down-regulation of the DA system may in turn result in a loss of cholinergic BF cell excitation and a compensatory up-regulation of ChAT and mAChRs.

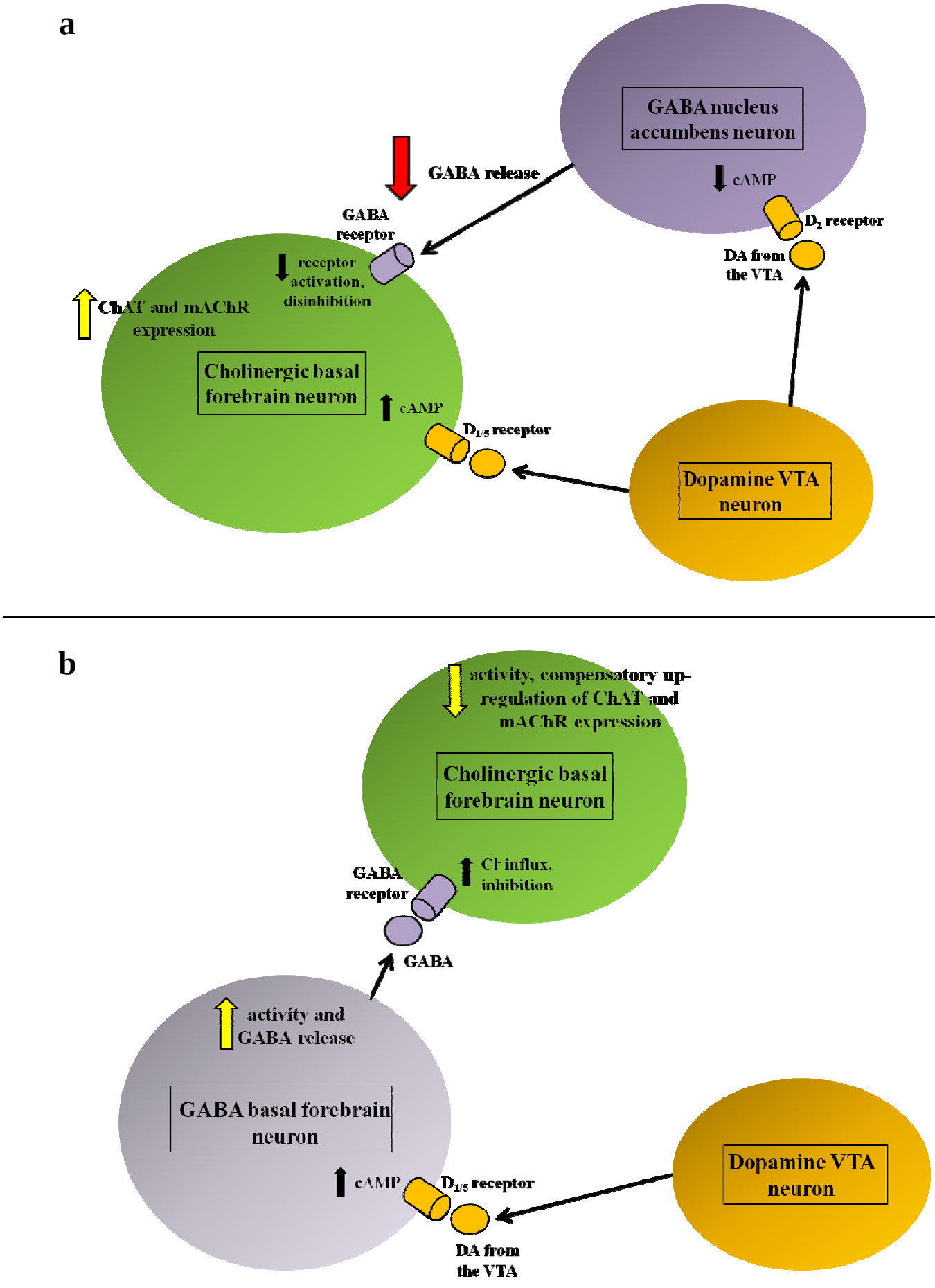

a
GABA nucleus accumbens neuron
GABA release
cAMP
GABA receptor
D2 receptor
DA from the VTA
receptor activation, disinhibition
ChAT and mAChR expression
Cholinergic basal forebrain neuron
cAMP
D1/5 receptor
Dopamine VTA neuron
b
activity, compensatory up-regulation of ChAT and mAChR expression
Cholinergic basal forebrain neuron
GABA receptor
Cl- influx, inhibition
GABA
activity and GABA release
GABA basal forebrain neuron
cAMP
D1/5 receptor
DA from the VTA
Dopamine VTA neuron

Figure 16. Mechanisms by which methamphetamine might alter cholinergic function in the basal forebrain. (a) Methamphetamine-induced increases in dopamine (DA) from the ventral tegmental area (VTA) may activate DA D_2 receptors, thus decreasing cyclic AMP (cAMP) and reducing activation of GABAergic neurons in the nucleus accumbens. Subsequently, the release of GABA to the basal forebrain cholinergic neurons would be reduced, disinhibiting the cholinergic neurons and increasing cellular activity. DA from the VTA could also activate DA $D_{1/5}$ receptors on the basal forebrain cholinergic neurons, increasing cAMP and cellular activity. The result of this increase in cellular activity could be increased choline acetyltransferase (ChAT) and muscarinic acetylcholine receptor (mAChR) levels. (b) Methamphetamine-induced increases in DA from the VTA may activate DA $D_{1/5}$ receptors on basal forebrain GABAergic cells, increasing cAMP and cellular activity. Subsequently, the release of GABA to the cholinergic basal forebrain neurons would be increased, inhibiting the cholinergic cell by increasing chloride (Cl^-) influx. This may result in decreased cell activity and a compensatory up-regulation of ChAT and the mAChRs.

Future studies: Many more studies are needed to elucidate the precise mechanism underlying MA's affects on the cholinergic system. First, I propose that BMP and LIM homeobox transcription factors, NGF, and activated microglia and oxidative stress are measured in the BF, hippocampus and cortex of adolescent and adult mice exposed to MA from PND 11-20. Furthermore, these measures can be taken during the injection period in order to determine the acute versus long-term effects of MA. In order to determine the potential role of these factors in the long-term cholinergic changes following MA exposure, each of these factors can be blocked during concurrent MA or SA injections from PND 11-20 followed by cholinergic measurements in adolescents and adults. Blocking potential MA-induced changes in the above mentioned factors would allow for a direct quantification of their contribution to MA's affects on the cholinergic system and cognition later in life. I hypothesize that blocking MA-induced increases in NGF and microglia activation will block MA's long-term effects on the BF cholinergic system.

Another study I propose is to examine the acute and long-term effects of MA exposure on DA, 5-HT, and NE release into the BF in order to elucidate the ways in which early MA exposure might alter the developing cholinergic BF system. Potential

changes in extracellular GABA and glutamate should also be measured in the BF using microdialysis following DA/NE/5-HT increases in order to determine if changes in cholinergic cells are related to changes in GABA and/or glutamate release. MA-induced increases in BF DA/5-HT/NE levels can also be blocked during MA exposure to determine the contribution of these effects on the MA-induced cholinergic changes later in life. Coupled with additional cholinergic measurements (see **section 5**), these experiments would help determine if MA exposure is exciting cholinergic cells, thus resulting in the observed increases in ChAT and mAChRs, or if MA exposure is inhibiting cholinergic cells, thus resulting in a compensatory up-regulation in ChAT levels and mAChRs. I predict that MA increases DA levels in the BF, thus resulting in increased cholinergic cell activity and persistent increases in ChAT and the mAChRs. Furthermore, I hypothesize that blocking MA-induced increases in DA in the BF will block MA's long-term effects on the BF cholinergic system.

Finally, in order to determine more precisely how MA alters the expression of cholinergic markers, I propose that future studies measure glutamate neuronal density in addition to GABA and cholinergic neuronal density in MA- and SA-exposed mice in adolescence and adulthood. This study would help elucidate if MA is inducing a phenotype change from glutamatergic or GABAergic to cholinergic, thus increasing cholinergic markers later in life. On the other hand, this study might show no alterations in glutamatergic or GABAergic cells, which would suggest that MA is somehow increasing cholinergic cell survival during development or enhancing cholinergic marker expression within cholinergic neurons while not altering other neuronal populations. Based on the lack of an affect of MA on GABAergic and total neuronal density in this

dissertation (**Chapter 2**), I hypothesize that MA exposure will not alter the density of glutamatergic neurons, and consistent with the current findings, that MA exposure will also have no effect on the density of GABAergic neurons. I theorize that in this dissertation MA increased cholinergic marker expression within differentiated cholinergic cells, thus resulting in increased ChAT and mAChR expression levels.

3. Cholinergic involvement in methamphetamine-induced cognitive impairments

Enhanced cholinergic function is generally considered to be positive for cognitive function (see **section 6** of **Chapter 1** for a detailed discussion: (Cummings, 2000, 2003)). Thus the increases in cholinergic cell density, area occupied by cholinergic fibers, and mAChRs in this dissertation initially seem at odds with the impaired cognitive function following MA exposure. However, increased ChAT expression mAChR binding levels may be coupled with decreases in other functional measures of the cholinergic system (see **section 5**) and might not necessarily reflect increased cholinergic function. Alternatively, MA-induced increases in cholinergic function may disrupt the balance of cholinergic signaling that is essential for cognitive function. These issues are explored in the sections below.

Cholinergic involvement in cognitive tasks: The MA-induced alterations in ChAT and mAChRs (**Chapters 2 and 3**) may contribute to the MA-induced cognitive impairments in adolescent and adult mice (**Chapters 4 and 5**). Novel location recognition is impaired by mAChR antagonism (Murai *et al.,* 2007). Furthermore, performance on this test is hippocampus-dependent: hippocampal lesions impair novel location recognition (Save *et*

al., 1992) and hippocampal neurons show increased activity during cognitive tasks that involve a spatial component, including novel location recognition ((Lenck-Santini, Rivard, Muller, & Poucet, 2005); for a review, see (Brown & Aggleton, 2001)). Thus altered cholinergic signaling within the hippocampus following MA exposure may be related to impaired novel location recognition memory. With the exception of the adult male mice, all MA-exposed mice showed novel location recognition memory impairments. Since the MA-exposed adult male mice showed the same cholinergic changes (increased M_1 mAChRs) as the MA-exposed adult female mice, these results suggest that the female mice may be more sensitive to the cognitive effects of altered cholinergic function than male mice in adulthood (see **section 4** for a discussion of sex differences).

Novel object recognition is also disrupted by systemic mAChR antagonism (Sambeth *et al.*, 2007). However, novel object recognition is typically considered a hippocampus-independent task (Save *et al.*, 1992). Lesions of the perirhinal cortex, which has reciprocal connections with the CA1 (Furtak, Wei, Agster, & Burwell, 2007), impairs novel object recognition memory (Bussey *et al.*, 1999; Ennaceur, Neave, & Aggleton, 1996). Furthermore, neurons within the perirhinal cortex show decreased activity following re-exposed to a familiar object (for a review, see (Brown & Aggleton, 2001)). Thus alterations in the hippocampal cholinergic system following MA exposure may affect CA1 output to the perirhinal cortex, thus disrupting performance on this task. Although no changes in cortical mAChRs or area occupied by ChAT fibers in various cortical regions were found in this dissertation (**Chapters 2 and 3**), potential MA-

induced alterations in the perirhinal cortex or altered cortical ACh release may relate to novel object recognition impairments in MA-exposed mice (**Chapters 4 and 5**).

The 5-SRTT and PPI tests are also dependent upon cholinergic transmission (see **section 6** of **Chapter 1**). However, MA did not affect 5-SRTT or PPI performance. These findings question the correlation between MA-induced cholinergic and cognitive impairments in this dissertation and warrant future studies examining cholinergic and cognitive measures within the same animal (see below). However, the correlation between MA-induced cholinergic and cognitive impairments cannot be ruled out based on my data. For example, MA-induced deficits in 5-SRTT may recover by adulthood but be present during adolescence when MA-induced cholinergic effects are more severe. In addition, the lack of mAChR and cholinergic fiber changes in the cortex may leave attention behavior intact in adulthood following MA exposure. PPI behavior involves cholinergic signaling, but is also dependent upon intricate interactions between subcoritcal and limbic structures and multiple neurotransmitter systems (for a review, see (Swerdlow & Geyer, 1998)). Thus brain areas and neurotransmitter systems left unaffected by MA may have left PPI undisrupted despite changes in the cholinergic system.

<u>Cholinergic involvement in methamphetamine-induced cognitive impairments in children:</u> Alterations in cholinergic function may also be related to some of the cognitive impairments observed in children following *in utero* MA exposure (see **section 7** of **Chapter 1**). Briefly, MA exposure *in utero* results in impaired attention, verbal and spatial memory, and executive function, and increased likelihood of a diagnosis of

Attention Deficit Hyperactivity Disorder (Chang *et al.*, 2004; Piper *et al.*, 2011). As the cholinergic system is known to play an important role in attention and memory (see **section 6** of **Chapter 1**), potential MA-induced alterations in the cholinergic system may be related to the cognitive impairments in these children.

Detrimental cognitive effects of increased cholinergic function: Potential MA-induced increases in cholinergic function may disrupt hippocampal signaling and thus cognition by disrupting cholinergic MS/VDB regulation of the hippocampal theta rhythm. Hippocampal theta rhythm is an oscillatory EEG pattern with a frequency of 4-12 Hz that can be recorded from the hippocampus of various mammals, including humans (O'Keefe & Burgess, 1999). Theta rhythm represents synchronized pyramidal cell activity and is prominent during voluntary locomotion and attention to spatial information within the environment. Hippocampal theta rhythm is also important for encoding and retrieval of learned information (Buzsaki, 2005; Hasselmo, Bodelon, & Wyble, 2002). For example, rabbits trained in eye-blink conditioning, a form of associative learning, take half as many trials to learn the task when trials occur during hippocampal theta (Seager, Johnson, Chabot, Asaka, & Berry, 2002). In addition, there is greater theta activity in the hippocampus and surrounding cortices during goal-directed navigation in a virtual reality water maze task in humans, and theta activity is correlated with navigation performance (Cornwell, Johnson, Holroyd, Carver, & Grillon, 2008).

The hippocampal theta rhythm is modulated by input from the MS/VDB. Scopolamine increases the peak frequency (Givens & Olton, 1995) while lesions of the cholinergic MS cells decrease the area under the curve (power) of hippocampal theta in

adult rats (Lee, Chrobak, Sik, Wiley, & Buzsaki, 1994). Systemic activation of the α7 nAChRs enhances hippocampal theta activity (Siok, Rogers, Kocsis, & Hajos, 2006). More recent studies suggest that the cholinergic MS/VDB cells serve to potentiate and regulate the theta rhythm rather than generate it, as ablation of the cholinergic input to the hippocampus disrupts, but does not abolish, the hippocampal theta rhythm (C. King, Recce, & O'Keefe, 1998). MA-induced increases in cholinergic BF activity may then serve to disrupt the theta rhythm. High concentrations of carbachol, a general mAChR agonist, can induce gamma EEG in the hippocampus, which has a much higher frequency (40-100 Hz) than theta (Fellous & Sejnowski, 2000). Furthermore, high doses of the AChE inhibitor physostigmine, which increase ACh levels, suppress theta activity at the higher frequencies (5.7 – 11.9 Hz) and shift the peak of the theta to a lower frequency (Podol'skii, Vorob'ev, & Belova, 2001). Taken together, these data suggest that an increase in ACh release into the hippocampus can distort hippocampal theta wave activity in a fashion that may disrupt cognition.

MA-induced increases in VTA DA release into the BF may also contribute to altered hippocampal theta. Bursting activity of MS/VDB neurons at the theta frequency can be induced by stimulation of the VTA and blockade of DA D_1 and D_5 receptors in the MS/VDB attenuates the theta burst activity of these neurons (Fitch, Sahr, Eastwood, Zhou, & Yang, 2006). Injections of DA into the MS/VDB of rats increases the amplitude and power of hippocampal theta activity (Miura, Ito, & Kadokawa, 1987). Furthermore, MA exposure in rabbits shifts the hippocampal theta toward higher frequencies (J. Yamamoto, 1997). The high levels of DA in the BF that occur following systemic MA

exposure could then function to alter MS/VDB cholinergic cell activity, altering the theta rhythm in the hippocampus and disrupting cognition.

Future studies: Future studies are required to confirm the cholinergic contribution to MA-induced cognitive impairments. The BF cholinergic system should be measured in mice following behavioral testing in order to establish correlations between MA-induced cholinergic and cognitive changes in the same animal. Furthermore, I propose that cholinergic treatments should be used to attempt to rescue the MA-induced cognitive impairments in adolescence and adulthood. For example, adolescent and adult mice exposed to MA or SA from PND 11-20 could receive intraventricular injections of a mAChR agonist, such as oxotremorine, or vehicle each day prior to behavioral testing. The results from this study would provide evidence for the involvement of the cholinergic system in MA-induced cognitive impairments. In addition, the results from a study such as this might yield information about potential treatment options for MA-induced cognitive impairments in children. I hypothesize that MA will induce similar cholinergic and cognitive impairments within the same animals as those observed in different groups of animals in this dissertation, and that treating the cholinergic changes will reverse MA-induced cognitive impairments.

In order to better understand the mechanism underlying MA's affects on novel object recognition memory, I propose that a future study investigate the long-term effects of MA exposure on the perirhinal cortex. Responding of individual neurons within the perirhinal cortex should be measured following exposure to MA, as studies show that neurons within this region respond less to subsequent presentation of objects that have

previously been encountered (for a review, see (Brown & Aggleton, 2001)). Measuring potential alterations in perirhinal cortex neuronal activity during performance of a novel object recognition test following MA exposure would provide information as to whether this brain region is involved in the novel object recognition impairments found in both male and female mice at both adolescent and adult ages (**Chapters 4 and 5**). I hypothesize that the perirhinal cortex is altered by MA exposure and that perirhinal neurons will not show reduced activity upon repeated stimuli presentation, explaining why novel object recognition memory is impaired by MA exposure during brain development.

Future studies are also required to better elucidate whether ACh-induced theta rhythm changes contribute to MA-induced cognitive impairments. To the best of my knowledge, no studies have examined the effects of MA exposure during brain development on hippocampal theta and how these changes might contribute to MA-induced cognitive impairments. Potential changes in hippocampal theta rhythm following MA exposure from PND 11-20 should be measured in adolescent and adult mice. I hypothesize that MA will alter hippocampal theta via its actions on the BF cholinergic system, thus altering hippocampal signaling and novel location and novel object recognition memory.

4. Sex differences in the effects of methamphetamine exposure

The results from this dissertation show a sex-dependent effect of MA on the cholinergic system and cognition. The mechanism underlying the sex differences in MA's affects is unknown, although as discussed in **Chapter 2**, female mice metabolize MA at a slower

rate than male mice following acute exposure on PND 11 (Acevedo *et al.*, 2008), potentially increasing MA-induced neurotoxicity and causing greater long-term impairments. The discussion below explores possible explanations for the sex differences in MA's actions on the cholinergic system and cognition and the potential involvement of sex hormones in these effects.

Sex differences in the cholinergic system: The different effects of MA on the cholinergic system between the sexes (**Chapters 2 and 3**) could be due sex differences in BF development. ChAT activity levels in the MS mature earlier in female rats during postnatal development (Loy & Sheldon, 1987), and thus MA exposure from PND 11-20 could target a sensitive period of ChAT development in female mice and miss this period in male mice. There are also sex differences in the mature cholinergic system. Adult female rats show greater decreases in hippocampal ACh levels following a cholinergic insult (Hortnagl *et al.,* 1993), lower levels of ACh release in the hippocampus over a 24 hour period (Masuda, Mitsushima, Funabashi, & Kimura, 2005), and lower stress-induced ACh release in the hippocampus compared to males (Mitsushima, Masuda, & Kimura, 2003). The decreased ACh levels in the hippocampus of female rodents under basal and stress/injury conditions could make this system more sensitive to insult compared to males, potentially explaining why there are greater MA-induced changes in the BF cholinergic system in female mice.

The effects of sex hormones on the central nervous system can be either organizational or activational. Organizational effects happen in response to sex hormones during development and are long-lasting. Activational effects happen on the developed

nervous system during adulthood and induce changes in the brain and/or behavior (M. C. Moore, 1991). In rodents, masculinization of the brain and behavior is dependent upon the organizational effects of estradiol in the brain during development (Schwarz & McCarthy, 2008). Female rat pups exposed to high levels of estradiol from PND 1-7 show increased ChAT activity in the VDB compared to untreated females in adulthood, and the levels are similar to those of adult males. Blockade of the conversion of testosterone to estrogen decreases adult VDB ChAT levels in adult male rats down to the level of adult female rats (Luine, Renner, & McEwen, 1986). Neonatal treatment with testosterone or estradiol increases hippocampal ACh release in adult female rats to match the levels of adult males (Mitsushima, Takase, Funabashi, & Kimura, 2009). These findings demonstrate that sex hormones play an organizational role in the development of the BF cholinergic system. The sex differences in MA's cholinergic effects may then be due in part to an interaction between MA and sex hormones during development.

The cholinergic effects of MA differed with age in the female mice but remained the same between adolescent and adult male mice. Thus the activational effects of estradiol may contribute to the partial recovery of MA-induced cholinergic alterations in the female mice. Sex hormones have an activational effect on the cholinergic system: there is an estrogen response element on the ChAT gene where estrogen receptor α can translocate to the nucleus and enhance transcription of ChAT (M. M. Miller *et al.*, 1999). Ovariectomy reduces ChAT activity in the cortex and hippocampus, the number of ChAT-positive cells in the MS, and ACh release in the hippocampus in adult rats, and these effects are reversed by estradiol treatment (Mitsushima *et al.*, 2009; Ping, Trieu, Wlodek, & Barrett, 2008; H. Yamamoto *et al.*, 2007). Thus the adult female mice may

recover from MA-induced cholinergic insults due to mature hormone levels. In contrast, the adolescent female mice may not be producing adult levels of circulating estrogens on PND 30 (Drickamer, 1984) and thus MA could have induced cholinergic alterations in the MS/VDB/HDB and hippocampus. Testosterone also has activational affects on the cholinergic system: gonadectomy reduces cholinergic cell density in the MS and cholinergic fiber innervation in the hippocampus of male rats, an effect that is reversed by exogenous testosterone administration (Nakamura, Fujita, & Kawata, 2002). Since adolescent mice do not have mature levels of androgens, these findings suggest that the activational effects of androgens may not account for the more limited effects of MA on the cholinergic system in the adolescent male mice.

Sex differences in cognition: Sex hormones play a role in cognitive function and thus they may influence the long-term effects of MA on cognition (**Chapters 4 and 5**). Under normal conditions male rodents typically show better spatial memory compared to female rodents (Veng, Granholm, & Rose, 2003). Neonatal exposure to testosterone improves spatial memory performance in adult female rats to the level of males while neonatal castration impairs spatial memory in adult males (Isgor & Sengelaub, 2003). Much literature has also demonstrated the activational effects of sex hormones on cognition. While a thorough review of this literature is beyond the scope of this dissertation, it is generally thought that estradiol enhances cognition in female animals (Ping *et al.*, 2008; Vaucher *et al.*, 2002) and androgens enhance cognition in male rodents (Benice & Raber, 2009a; Sandstrom, Kim, & Wasserman, 2006). MA exposure during brain development may alter sex hormone levels during development or later in life, thus affecting cognitive

function. Unpublished findings from our lab show that MA exposure from PND 11-20 does not alter menstrual cycling in adult wild type female mice. However, estrogen levels may still be altered, and to the best of my knowledge, the effects of postnatal MA exposure on androgen and estrogen levels in rodents have not yet been examined.

Future studies: In order to determine the potential contribution of sex hormones to the differential effects of MA on the cholinergic system and cognition in male and female mice, future studies should examine the long-term effects of MA exposure from PND 11-20 in the context of gonadectomy and hormone replacement. To look at the organizational effects of hormones, gonadectomy of male and female mice should occur at birth and a group of male mice could be "de-masculinized" by the administration of an androgen that cannot be converted to estrogen and a group of female mice could be "masculinized" by the administration of estradiol during development. If sex hormones during development contribute to the effects of MA on the cholinergic system and cognition, I would expect that estrogen treated female mice exposed to MA would show the same cholinergic and cognitive changes as the MA-exposed male mice in this dissertation. Conversely, I predict that the estrogen-deprived male mice exposed to MA would show the same cholinergic and cognitive changes as the MA-exposed female mice in this dissertation. To address the potential activational effects of sex hormones, the mice should be gonadectomized after MA/SA injections. If the activational effects of sex hormones are protective against MA's affects, I expect that male and female mice will show similar MA-induced cholinergic and cognitive impairments. In addition, I predict that there will be no recovery of cholinergic function in the adult female mice.

MA may alter circulating levels of hormones in adulthood. A simple study to investigate this possibility would be to expose mice to MA or SA from PND 11-20 and measure serum sex hormone levels in adolescence and adulthood. No changes in estrous cycling are expected based on previous unpublished findings, but changes in hormone levels may be present and contribute to the recovery from MA's effects during development, especially in the female mice. Understanding if there are long-term changes in hormone levels that persist into adulthood would enhance our understanding of MA's affects in the brain and potentially the mechanism underlying the robust sex differences in MA-induced changes in brain function and behavior.

5. Model and technical considerations

Prenatal versus gestational methamphetamine exposure: Postnatal MA exposure was intended to target both hippocampal and cholinergic development and model MA exposure during the third trimester in humans. Humans will likely be exposed to MA during other time points of pregnancy as many women report using MA throughout pregnancy (L. Smith *et al.*, 2003). While this is one potential limitation of the postnatal model, studies in rodents show greater cognitive and behavioral effects of MA exposure during the postnatal period versus during gestation (see **section 3** of **Chapter 1)**. Thus for the current body of work, postnatal MA exposure was used to produce the most robust behavioral outcome.

Exposing fetuses to MA by injecting pregnant mothers has its own relative strengths and weaknesses. The strengths include that this more directly models *in utero* MA exposure in a human whose mother takes MA during pregnancy. It also removes any

potential confounding issues of injecting the pups, potentially causing injection-induced stress that can alter brain function and cognition later in life. In order to address this issue in the postnatal exposure model, control mice are exposed to injections of SA, theoretically eliminating any effects of the injections themselves. Corticosterone and other stress markers were not measured in this dissertation, but the adolescent mice showed no MA-induced effects on anxiety or locomotor activity, suggesting no alterations in the behavioral expression of anxiety or stress. However, the stress of the injections contributing to altered cognition later in life cannot be ruled out.

One limitation of the gestational exposure model is that MA exposure or withdrawal in the mother can alter maternal care. Indeed, early life parental care can greatly affect cognition later in life (Bredy *et al.*, 2004; Rice *et al.*, 2008). Exposing the pups to MA after birth removes this confound, but also introduces the potential confound of differences in maternal care between the SA- and MA-exposed pups. In order to address this concern, maternal licking and grooming was measured in the study presented in **Chapter 5**. No differences in maternal care were found between the SA- and MA-exposed pups during the injection period, suggesting that altered maternal care between the treatment groups cannot account for group differences in cognition later in life.

A final limitation of the gestational model of MA exposure is that all pups within a single litter are exposed to the same treatment. Thus a within-litter design cannot be used and only one animal from each litter can be examined to remove potential litter effects. Postnatal exposure to MA gets around this issue since a within-litter injection design can be used (half the litter exposed to MA, half exposed to SA). However, studies find that litter effects on the outcome measures can still be large and inflate the

possibility of a Type I error in statistical analyses (Holson & Pearce, 1992). One way to address this issue is to use litter instead of individual pups as the experimental unit in the statistical analyses. In this dissertation, the results from the cognitive studies were re-analyzed with litter as the experimental unit to address this issue, and the effects of MA were little changed. Thus it seems that in the current studies, litter influences are not a great concern.

Effects of methamphetamine on body weight: MA caused a small but significant reduction in weight gain over the injection period in both male and female mice in the studies presented in **Chapters 4 and 5**. Potential malnutrition in the MA-exposed mice may have contributed to the cholinergic and cognitive effects observed in this dissertation. However, studies in rats exposed to MA from PND 11-20 show that reduced weight persists until PND 42 but weight differences are not present in adulthood (Vorhees *et al.*, 2000). Another study in rats shows significant weight differences between MA- and SA-exposed pups from PND 11-20, but no differences by PND 35 (Williams, Blankenmeyer *et al.*, 2003). It should be noted that the doses of MA used in these studies are much higher and the resulting weight differences between treatment groups much more severe than what was observed in the current work.

In an effort to address the issue of under-nutrition in postnatal psychomotor stimulant exposure, Williams *et al.* (2003) include a group of pups with a large litter size in a study examining the long-term effects of 3,4 methylenedioxy-methamphetamine (MDMA; ecstasy) from PND 11-20. Larger liter size (approximately 16 pups) results in weights that are equivalent to the MDMA-exposed pups, allowing the researchers to

examine the effects of reduced weight gain on cognition later in life (Williams, Morford, Wood, Rock *et al.*, 2003). The MDMA-exposed rats show cognitive impairments in adulthood that are not present in the large litter rats, corroborating other findings that under-nutrition during development does not alter cognition later in life (Strupp & Levitsky, 1995; Williams, Morford, Wood, Rock *et al.*, 2003). These findings in rats argue that reduced weight gain during postnatal development cannot account for the cognitive effects of MA later in life.

Maternal feeding was also measured in the experiment presented in **Chapter 5**. This experiment was sensitive enough to detect genotype differences in maternal feeding (apoE3 pups received more feeding that apoE4 pups), yet the cognitive differences between the apoE3 and apoE4 mice in adulthood were very subtle and did not interact with MA's affects. There were no differences in maternal feeding between MA- and SA-exposed pups. In addition, MA-exposed mice were not impaired on a test of conditioned fear memory or an operant test of attention. These findings suggest that MA-induced reductions in weight gain during the injection period do not contribute to MA-induced cognitive impairments in adulthood.

Dose of methamphetamine: Only one dose of MA (5 mg/kg, once daily) was used in this dissertation. This dose was based on previous studies showing brain and cognitive effects of postnatal MA exposure in mice (Acevedo *et al.*, 2007; Acevedo *et al.*, 2008). Previous studies in rats use much higher doses of MA from PND 11-20 to induce brain and behavioral changes later in life (C. A. Crawford *et al.*, 2003; Grace, Schaefer, Graham *et al.*, 2010; Grace, Schaefer, Gudelsky *et al.*, 2010; Schaefer *et al.*, 2008; Skelton *et al.*,

2007; Vorhees, Ahrens, Acuff-Smith, Schilling, & Fisher, 1994; Vorhees *et al.*, 2000; Vorhees *et al.*, 2009; Vorhees *et al.*, 2007; Williams, Blankenmeyer *et al.*, 2003; Williams *et al.*, 2004; Williams, Morford, Wood, Wallace *et al.*, 2003; Williams *et al.*, 2002). Vorhees *et al.* use this larger dose of MA in an attempt to mimic doses used by humans. Studies suggest that the average daily dose of MA in adult users is about 1.6 g, with a range between 0.25-10.0 g (McCann *et al.*, 1998; Volkow, Chang, Wang, Fowler, Leonido-Yee *et al.*, 2001). Women who use MA during pregnancy report using approximately 0.3 g per day (Chang *et al.*, 2009). Extrapolating human self-administered doses of MA to rodents is a challenge considering the pharmacokinetic differences between species. For example, the half-life of MA in humans is 10-12 hours while in rats is approximately 1 hour (Brien, Kitney, Peachey, & Rogers, 1978; Cho, Melega, Kuczenski, & Segal, 2001; Cook *et al.*, 1993; Mendelson *et al.*, 2006; Riviere, Gentry, & Owens, 2000).

Doses of MA that are self-administered in humans (average 1.6 g per day) are sufficient to cause long-term reductions in striatal DAT levels (McCann *et al.*, 1998; Volkow, Chang, Wang, Fowler, Leonido-Yee *et al.*, 2001). In non-human primates, 2-4 mg/kg per day is sufficient to induce long-term reductions in striatal DAT (Harvey, Lacan, Tanious, & Melega, 2000; Villemagne *et al.*, 1998). These doses are considerably lower than those required for striatal neurotoxicity in rodents. For example, in adult mice, 10 mg/kg of MA is not enough to decrease striatal DAT levels, but 20 mg/kg reduces striatal DAT levels to 50% while 40 mg/kg reduces levels to approximately 25% of control values (J. Yu, Cadet, & Angulo, 2002). In rats, 16 mg/kg is sufficient to reduce striatal DAT levels (Eisch, Gaffney, Weihmuller, O'Dell, & Marshall, 1992). These data

show that proportionally larger doses of MA are required to induce brain deficits in rodents compared to primates.

Another challenge with dosing is extrapolating relevant doses of MA in adults to young animals. Studies on the pharmacology of MA in neonatal rodents are limited, although experiments in rats show that neonates are less sensitive to the effects of MA than adults. Exposure to MA from PND 7-10 or 17-20 reduces striatal DA levels to a lesser degree than MA exposure in adults (Lucot, Wagner, Schuster, & Seiden, 1982) and MA exposure on PND 20 does not reduce tyrosine hydroxylase-positive fibers in the caudate putamen whereas severe reductions are found following exposure on PND 60 and 80 in rats (Pu & Vorhees, 1993). These age-related differences in the effects of MA are attributed to a developmental increase in the number of DATs from PND 1-40 in rats, limiting the ability of MA to induce DA release and DA toxicity prior to PND 40 (Kirksey & Slotkin, 1979). Thus comparing MA dosing regimens between neonatal and adult rodents also has its limitations. The low dose of MA used in the current work was chosen in part because Voorhees *et al.* show high mortality rates with 40 mg/kg MA from PND 11-20 in rats (Vorhees *et al.*, 2000). Exposing postnatal C57BL/6J mice to 40, 20, or 10 mg/kg MA results in 100% mortality (Acevedo *et al.*, 2007). Thus the current dose of 5 mg/kg was used to minimize MA-induced deaths but still cause long-term changes in the brain and behavior. The fact that alterations were found with a relative low dose of MA in the current studies suggests that a higher dose that may be more physiologically relevant to human MA exposure may cause even more severe alterations and impairments.

Parvalbumin as a marker for GABAergic cells: PVA was used as a surrogate marker of GABAergic cells (**Chapter 2**), although it likely did not label all GABAergic neurons in the BF. Pilot studies for the work presented in **Chapter 2** attempted to use antibodies against GAD and NeuN to examine the density of GABAergic neurons following MA exposure, but the co-labeling was difficult to image and it was not possible to distinguish GABAergic neurons due to diffuse staining patterns. PVA is good marker of GABAergic BF cells (see the introduction to **Chapter 2**; (Baimbridge *et al.*, 1992; Freund, 1989; Kiss *et al.*, 1990; Linke *et al.*, 1994; Schwegler, Boldyreva, Linke *et al.*, 1996; Schwegler, Boldyreva, Pyrlik-Gohlmann *et al.*, 1996)). However, it will be important in the future to use direct markers of GABA cells to further delineate the effects of MA exposure on GABA neurons in the BF.

Additional cholinergic markers: As previously mentioned, MA may alter ACh synthesis and release, resulting in a compensatory changes in ChAT expression levels and mAChRs (**Chapters 2 and 3**). In order to begin to address the effects of MA on ACh synthesis, pilot studies were performed to measure the rate of choline uptake by the CHTs in the hippocampus and cortex as the transport of choline into the pre-synaptic neuron via the CHT is the rate limiting step in ACh synthesis (Ferguson & Blakely, 2004; Lockman & Allen, 2002; Takashina *et al.*, 2008). However, an interest in examining CHT function only arose following the ChAT studies. Functional uptake assays can only be performed in fresh tissue, and as all of the tissues used in this study were frozen prior to the interest in examining the CHTs, functional transporter assays were not possible.

It is also important to measure both ChAT expression and activity levels in order to get a clear idea about the effects of MA on ChAT. Experimental manipulations that reduce ACh transmission or cellular electrical activity are typically associated with a decrease in ChAT activity (Ishii, Miwa, Nishio, & Yagasaki, 1990; Jackson, Lecar, Brenneman, Fitzgerald, & Nelson, 1982; Lapchak, Jenden, & Hefti, 1991; Rossner *et al.*, 1995), although some find no changes in ChAT activity or ChAT levels in the BF with electrical activity manipulations (Agoston, Komoly, & Palkovits, 1994; Lund *et al.*, 1978). Furthermore, ChAT is present is excessively high concentrations compared to ACh precursor molecules and it is not the rate limiting step in ACh production (Blusztajn & Wurtman, 1983), suggesting that the observed increases in ChAT expression levels are not likely caused by decreases in ACh transmission. Finally, AChE is often used as a marker of cholinergic processes and activity assays for AChE would provide additional information about how much ACh is being released and degraded in the synaptic cleft.

In terms of ACh receptors, the mAChRs were the focus of this dissertation since they play an important role in cognition and show high levels in the hippocampus and cortex (see **Chapter 1**). However, the M_4 mAChRs and nAChRs also play an important role in brain function and cognition and are expressed in both the hippocampus and cortex (Halbach & Dermietzel, 2002; Paterson & Nordberg, 2000). MA can displace ligand binding to α7 and α4β2 nAChRs and increase α7 and α4β2 nAChR number (B_{max}) in cell culture (Garcia-Rates *et al.*, 2007), suggesting an interesting interaction between MA and the nAChRs that might relate to MA-induced cognitive impairments. Finally, the increased number of hippocampal M_1 mAChRs following MA exposure may be associated with altered ability of the receptors to form binding complexes with G-proteins

and induce intracellular signaling cascades. Understanding how MA alters G-protein coupling, as well as the M_4 mAChRs and nAChRs, will contribute significantly to this body of work and potentially help explain why there was a MA-induced increase in hippocampal M_1 mAChRs.

Future studies: Future studies should include a no injection control group that would serve to delineate the potential long-term effects of the injection on the cholinergic system and cognition later in life. Future studies should use GAD or another direct GABAergic marker to determine the effects of MA on the BF GABA system. I predict that the results from this study will be similar to those found from the current studies, as PVA is a good marker of GABAergic BF cells. However, any potential contradictions in the results will be important in order to better determine the relationship with cholinergic BF changes following MA exposure.

Another important avenue for future research will be to explore the effects of MA on cholinergic markers other than ChAT levels and the mAChRs. More specifically, ACh release, ChAT activity, CHT function, AChE levels, nAChRs, M_4 mAChRs, and mAChR G-protein coupling should be measured to further elucidate the long-term effects of MA on the cholinergic system. As mentioned earlier, one possibility is that MA impairs cholinergic transmission, and that the observed increases in ChAT and mAChRs are a compensatory response to other MA-induced cholinergic insults. By studying other aspects of the cholinergic system, this possibility would be elucidated. Another important reason to explore the effects of MA on these additional cholinergic measures is that they might help direct the development of treatments for MA-induced cognitive impairments

in children. I predict that MA exposure *will not* reduce ACh signaling but rather will increase ChAT activity, CHT function, and ACh release, resulting in an up-regulation of ChAT expression and mAChR levels.

6. Final conclusions

In conclusion, the data from this dissertation demonstrate long-term effect of MA exposure during brain development on the adolescent and adult cholinergic system and cognition in mice. The results from this dissertation suggest that MA exposure *in utero* may alter the cholinergic system in children, especially in girls, and this may directly affect cognitive function later in life. As the findings from these mouse studies may have significant translational relevance to the condition of human MA exposure *in utero*, further investigations into the long-term effects of MA exposure during brain development on the cholinergic system and cognition are warranted.

REFERENCES

Abreu-Villaca, Y., Filgueiras, C. C., & Manhaes, A. C. (2010). Developmental aspects of the cholinergic system. *Behavioural Brain Research*.

Acevedo, S. F., de Esch, I. J., & Raber, J. (2007). Sex- and histamine-dependent long-term cognitive effects of methamphetamine exposure. *Neuropsychopharmacology, 32*(3), 665-672.

Acevedo, S. F., Pfankuch, T., van Meer, P., & Raber, J. (2008). Role of histamine in short- and long-term effects of methamphetamine on the developing mouse brain. *J Neurochem, 107*(4), 976-986.

Acevedo, S. F., Piper, B. J., Craytor, M. J., Benice, T. S., & Raber, J. (2010). Apolipoprotein E4 and sex affect neurobehavioral performance in primary school children. *Pediatr Res, 67*(3), 293-299.

Acuff-Smith, K. D., Schilling, M. A., Fisher, J. E., & Vorhees, C. V. (1996). Stage-specific effects of prenatal d-methamphetamine exposure on behavioral and eye development in rats. *Neurotoxicol Teratol, 18*(2), 199-215.

Agoston, D. V., Komoly, S., & Palkovits, M. (1994). Selective up-regulation of neuropeptide synthesis by blocking the neuronal activity: galanin expression in septohippocampal neurons. *Experimental Neurology, 126*(2), 247-255.

Alexander, M. P., Stuss, D. T., Picton, T., Shallice, T., & Gillingham, S. (2007). Regional frontal injuries cause distinct impairments in cognitive control. *Neurology, 68*(18), 1515-1523.

Allen, S. J., MacGowan, S. H., Tyler, S., Wilcock, G. K., Robertson, A. G., Holden, P. H., Smith, S. K., & Dawbarn, D. (1997). Reduced cholinergic function in normal and Alzheimer's disease brain is associated with apolipoprotein E4 genotype. *Neurosci Lett, 239*(1), 33-36.

Alonso, A., & Kohler, C. (1984). A study of the reciprocal connections between the septum and the entorhinal area using anterograde and retrograde axonal transport methods in the rat brain. *J Comp Neurol, 225*(3), 327-343.

Altman, J., & Bayer, S. A. (1990). Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. *J Comp Neurol, 301*(3), 365-381.

Amaral, D. G., Scharfman, H. E., & Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res, 163*, 3-22.

Amaral, D. G., & Witter, M. P. (1989). The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience, 31*(3), 571-591.

Arendt, T., Schindler, C., Bruckner, M. K., Eschrich, K., Bigl, V., Zedlick, D., & Marcova, L. (1997). Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein epsilon 4 allele. *Journal of Neuroscience, 17*(2), 516-529.

Arnold, H. M., Burk, J. A., Hodgson, E. M., Sarter, M., & Bruno, J. P. (2002). Differential cortical acetylcholine release in rats performing a sustained attention

task versus behavioral control tasks that do not explicitly tax attention. *Neuroscience, 114*(2), 451-460.
Arnold, H. M., Fadel, J., Sarter, M., & Bruno, J. P. (2001). Amphetamine-stimulated cortical acetylcholine release: role of the basal forebrain. *Brain Res, 894*(1), 74-87.
Arters, J., Hohmann, C. F., Mills, J., Olaghere, O., & Berger-Sweeney, J. (1998). Sexually dimorphic responses to neonatal basal forebrain lesions in mice: I. Behavior and neurochemistry. *Journal of Neurobiology, 37*(4), 582-594.
Aubert, I., Cecyre, D., Gauthier, S., & Quirion, R. (1992). Characterization and autoradiographic distribution of [3H]AF-DX 384 binding to putative muscarinic M2 receptors in the rat brain. *Eur J Pharmacol, 217*(2-3), 173-184.
Aubert, I., Cecyre, D., Gauthier, S., & Quirion, R. (1996). Comparative ontogenic profile of cholinergic markers, including nicotinic and muscarinic receptors, in the rat brain. *J Comp Neurol, 369*(1), 31-55.
Bacciottini, L., Passani, M. B., Mannaioni, P. F., & Blandina, P. (2001). Interactions between histaminergic and cholinergic systems in learning and memory. *Behavioural Brain Research, 124*(2), 183-194.
Bachy, I., & Retaux, S. (2006). GABAergic specification in the basal forebrain is controlled by the LIM-hd factor Lhx7. *Dev Biol, 291*(2), 218-226.
Baimbridge, K. G., Celio, M. R., & Rogers, J. H. (1992). Calcium-binding proteins in the nervous system. *Trends Neurosci, 15*(8), 303-308.
Bardo, M. T. (1998). Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. *Crit Rev Neurobiol, 12*(1-2), 37-67.
Barr, A. M., Panenka, W. J., MacEwan, G. W., Thornton, A. E., Lang, D. J., Honer, W. G., & Lecomte, T. (2006). The need for speed: an update on methamphetamine addiction. *J Psychiatry Neurosci, 31*(5), 301-313.
Bartres-Faz, D., Clemente, I. C., Monras, M., Munoz, M., Lopez-Alomar, A., Valveny, N., Moral, P., Gual, A., Sanchez-Turet, M., Guardia, J., & Junque, C. (2002). Relation of Apo E and ACE genes to cognitive performance in chronic alcoholic patients. *Addict Biol, 7*(2), 227-233.
Bayer, S. A. (1980). Development of the hippocampal region in the rat. I. Neurogenesis examined with 3H-thymidine autoradiography. *J Comp Neurol, 190*(1), 87-114.
Bayer, S. A., Altman, J., Russo, R. J., & Zhang, X. (1993). Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology, 14*(1), 83-144.
Bechtholt, A. J., Smith, R., Raber, J., & Cunningham, C. L. (2004). Enhanced ethanol-, but not cocaine-induced, conditioned place preference in Apoe(-/-) mice. *Pharmacol Biochem Behav, 77*(4), 783-792.
Becu-Villalobos, D., Gonzalez Iglesias, A., Diaz-Torga, G., Hockl, P., & Libertun, C. (1997). Brain sexual differentiation and gonadotropins secretion in the rat. *Cell Mol Neurobiol, 17*(6), 699-715.
Bender, R., Plaschke, M., Naumann, T., Wahle, P., & Frotscher, M. (1996). Development of cholinergic and GABAergic neurons in the rat medial septum: different onset of choline acetyltransferase and glutamate decarboxylase mRNA expression. *J Comp Neurol, 372*(2), 204-214.

Benice, T. S., & Raber, J. (2008). Object recognition analysis in mice using nose-point digital video tracking. *J Neurosci Methods, 168*(2), 422-430.
Benice, T. S., & Raber, J. (2009a). Dihydrotestosterone modulates spatial working-memory performance in male mice. *J Neurochem, 110*(3), 902-911.
Benice, T. S., & Raber, J. (2009b). Testosterone and dihydrotestosterone differentially improve cognition in aged female mice. *Learn Mem, 16*(8), 479-485.
Berger-Sweeney, J. (2003). The cholinergic basal forebrain system during development and its influence on cognitive processes: important questions and potential answers. *Neurosci Biobehav Rev, 27*(4), 401-411.
Berlanga, M. L., Simpson, T. K., & Alcantara, A. A. (2005). Dopamine D5 receptor localization on cholinergic neurons of the rat forebrain and diencephalon: a potential neuroanatomical substrate involved in mediating dopaminergic influences on acetylcholine release. *J Comp Neurol, 492*(1), 34-49.
Berse, B., Szczecinska, W., Lopez-Coviella, I., Madziar, B., Zemelko, V., Kaminski, R., Kozar, K., Lips, K. S., Pfeil, U., & Blusztajn, J. K. (2005). Expression of high affinity choline transporter during mouse development in vivo and its upregulation by NGF and BMP-4 in vitro. *Brain Res Dev Brain Res, 157*(2), 132-140.
Bleich, S., Wilhelm, J., Graesel, E., Degner, D., Sperling, W., Rossner, V., Javaheripour, K., & Kornhuber, J. (2003). Apolipoprotein E epsilon 4 is associated with hippocampal volume reduction in females with alcoholism. *J Neural Transm, 110*(4), 401-411.
Blusztajn, J. K., & Wurtman, R. J. (1983). Choline and cholinergic neurons. *Science, 221*(4611), 614-620.
Boscia, F., Ferraguti, F., Moroni, F., Annunziato, L., & Pellegrini-Giampietro, D. E. (2008). mGlu1alpha receptors are co-expressed with CB1 receptors in a subset of interneurons in the CA1 region of organotypic hippocampal slice cultures and adult rat brain. *Neuropharmacology, 55*(4), 428-439.
Brady, D. R., Phelps, P. E., & Vaughn, J. E. (1989). Neurogenesis of basal forebrain cholinergic neurons in rat. *Brain Res Dev Brain Res, 47*(1), 81-92.
Bredy, T. W., Lee, A. W., Meaney, M. J., & Brown, R. E. (2004). Effect of neonatal handling and paternal care on offspring cognitive development in the monogamous California mouse (Peromyscus californicus). *Horm Behav, 46*(1), 30-38.
Brichtova, E., & Kozak, L. (2008). Apolipoprotein E genotype and traumatic brain injury in children--association with neurological outcome. *Childs Nerv Syst, 24*(3), 349-356.
Brien, J. F., Kitney, J. C., Peachey, J. E., & Rogers, B. J. (1978). Methamphetamine-induced behavioural effects and brain concentrations of methamphetamine and its metabolite amphetamine in mice. *Res Commun Chem Pathol Pharmacol, 22*(2), 313-328.
Broening, H. W., Pu, C., & Vorhees, C. V. (1997). Methamphetamine selectively damages dopaminergic innervation to the nucleus accumbens core while sparing the shell. *Synapse, 27*(2), 153-160.
Brown, M. W., & Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat Rev Neurosci, 2*(1), 51-61.

Busche, A., Bagorda, A., Lehmann, K., Neddens, J., & Teuchert-Noodt, G. (2006). The maturation of the acetylcholine system in the dentate gyrus of gerbils (Meriones unguiculatus) is affected by epigenetic factors. *J Neural Transm, 113*(2), 113-124.

Bussey, T. J., Muir, J. L., & Aggleton, J. P. (1999). Functionally dissociating aspects of event memory: the effects of combined perirhinal and postrhinal cortex lesions on object and place memory in the rat. *Journal of Neuroscience, 19*(1), 495-502.

Buttini, M., Orth, M., Bellosta, S., Akeefe, H., Pitas, R. E., Wyss-Coray, T., Mucke, L., & Mahley, R. W. (1999). Expression of human apolipoprotein E3 or E4 in the brains of Apoe-/- mice: isoform-specific effects on neurodegeneration. *Journal of Neuroscience, 19*(12), 4867-4880.

Buzsaki, G. (2005). Theta rhythm of navigation: link between path integration and landmark navigation, episodic and semantic memory. *Hippocampus, 15*(7), 827-840.

Cabrera, T. M., Levy, A. D., Li, Q., van de Kar, L. D., & Battaglia, G. (1993). Prenatal methamphetamine attenuates serotonin mediated renin secretion in male and female rat progeny: evidence for selective long-term dysfunction of serotonin pathways in brain. *Synapse, 15*(3), 198-208.

Carnes, K. M., Fuller, T. A., & Price, J. L. (1990). Sources of presumptive glutamatergic/aspartatergic afferents to the magnocellular basal forebrain in the rat. *J Comp Neurol, 302*(4), 824-852.

Casamenti, F., Deffenu, G., Abbamondi, A. L., & Pepeu, G. (1986). Changes in cortical acetylcholine output induced by modulation of the nucleus basalis. *Brain Res Bull, 16*(5), 689-695.

Chang, L., Cloak, C., Jiang, C. S., Farnham, S., Tokeshi, B., Buchthal, S., Hedemark, B., Smith, L. M., & Ernst, T. (2009). Altered neurometabolites and motor integration in children exposed to methamphetamine in utero. *Neuroimage, 48*(2), 391-397.

Chang, L., Smith, L. M., LoPresti, C., Yonekura, M. L., Kuo, J., Walot, I., & Ernst, T. (2004). Smaller subcortical volumes and cognitive deficits in children with prenatal methamphetamine exposure. *Psychiatry Res, 132*(2), 95-106.

Chen, H. L., Lein, P. J., Wang, J. Y., Gash, D., Hoffer, B. J., & Chiang, Y. H. (2003). Expression of bone morphogenetic proteins in the brain during normal aging and in 6-hydroxydopamine-lesioned animals. *Brain Res, 994*(1), 81-90.

Chen, J. Y., Yeh, G. C., Tao, P. L., Kuo, C. T., Chen, K. B., & Wen, Y. R. (2010). Prenatal exposure to methamphetamine alters the mechanical withdrawal threshold and tonic hyperalgesia in the offspring. *Neurotoxicology, 31*(5), 432-438.

Cho, A. K., Melega, W. P., Kuczenski, R., & Segal, D. S. (2001). Relevance of pharmacokinetic parameters in animal models of methamphetamine abuse. *Synapse, 39*(2), 161-166.

Clancy, B., Finlay, B. L., Darlington, R. B., & Anand, K. J. (2007). Extrapolating brain development from experimental species to humans. *Neurotoxicology, 28*(5), 931-937.

Clancy, B., Kersh, B., Hyde, J., Darlington, R. B., Anand, K. J., & Finlay, B. L. (2007). Web-Based Method For Translating Neurodevelopment From Laboratory Species To Humans. *Neuroinformatics, 5*(1), 79-94.

Clement, Y., Calatayud, F., & Belzung, C. (2002). Genetic basis of anxiety-like behaviour: a critical review. *Brain Res Bull, 57*(1), 57-71.
Colom, L. V., Castaneda, M. T., Reyna, T., Hernandez, S., & Garrido-Sanabria, E. (2005). Characterization of medial septal glutamatergic neurons and their projection to the hippocampus. *Synapse, 58*(3), 151-164.
Conner, J. M., & Varon, S. (1997). Developmental profile of NGF immunoreactivity in the rat brain: a possible role of NGF in the establishment of cholinergic terminal fields in the hippocampus and cortex. *Brain Res Dev Brain Res, 101*(1-2), 67-79.
Cook, C. E., Jeffcoat, A. R., Hill, J. M., Pugh, D. E., Patetta, P. K., Sadler, B. M., White, W. R., & Perez-Reyes, M. (1993). Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. *Drug Metab Dispos, 21*(4), 717-723.
Cornwell, B. R., Johnson, L. L., Holroyd, T., Carver, F. W., & Grillon, C. (2008). Human hippocampal and parahippocampal theta during goal-directed spatial navigation predicts performance on a virtual Morris water maze. *Journal of Neuroscience, 28*(23), 5983-5990.
Counts, S. E., & Mufson, E. J. (2005). The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. *J Neuropathol Exp Neurol, 64*(4), 263-272.
Crawford, C. A., Williams, M. T., Newman, E. R., McDougall, S. A., & Vorhees, C. V. (2003). Methamphetamine exposure during the preweanling period causes prolonged changes in dorsal striatal protein kinase A activity, dopamine D2-like binding sites, and dopamine content. *Synapse, 48*(3), 131-137.
Crawford, F. C., Vanderploeg, R. D., Freeman, M. J., Singh, S., Waisman, M., Michaels, L., Abdullah, L., Warden, D., Lipsky, R., Salazar, A., & Mullan, M. J. (2002). APOE genotype influences acquisition and recall following traumatic brain injury. *Neurology, 58*(7), 1115-1118.
Crawford, I. L., & Connor, J. D. (1973). Localization and release of glutamic acid in relation to the hippocampal mossy fibre pathway. *Nature, 244*(5416), 442-443.
Cruickshank, C. C., & Dyer, K. R. (2009). A review of the clinical pharmacology of methamphetamine. *Addiction, 104*(7), 1085-1099.
Cummings, J. L. (2000). Cholinesterase inhibitors: A new class of psychotropic compounds. *Am J Psychiatry, 157*(1), 4-15.
Cummings, J. L. (2003). Use of cholinesterase inhibitors in clinical practice: evidence-based recommendations. *Am J Geriatr Psychiatry, 11*(2), 131-145.
Dalley, J. W., McGaughy, J., O'Connell, M. T., Cardinal, R. N., Levita, L., & Robbins, T. W. (2001). Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. *Journal of Neuroscience, 21*(13), 4908-4914.
Danik, M., Cassoly, E., Manseau, F., Sotty, F., Mouginot, D., & Williams, S. (2005). Frequent coexpression of the vesicular glutamate transporter 1 and 2 genes, as well as coexpression with genes for choline acetyltransferase or glutamic acid decarboxylase in neurons of rat brain. *J Neurosci Res, 81*(4), 506-521.
DASIS. (2008). The Drug and Alcohol Services Information System Report: Primary Methamphetamine/Amphetamine Admissions to Substance Abuse Treatment 1-4.

DAWN. (2010, *The Drug Abuse Warning Network Report: Emergency Department Visits Involving Methamphetamine* from http://www.oas.samhsa.gov/2k10/DAWN017/Meth.htm

DEA. (2006). General information regarding "The Combat Methamphetamine Epidemic Act of 2005". 1-8.

Deacon, R. M., Bannerman, D. M., Kirby, B. P., Croucher, A., & Rawlins, J. N. (2002). Effects of cytotoxic hippocampal lesions in mice on a cognitive test battery. *Behavioural Brain Research, 133*(1), 57-68.

Decker, M. W., Majchrzak, M. J., & Anderson, D. J. (1992). Effects of nicotine on spatial memory deficits in rats with septal lesions. *Brain Res, 572*(1-2), 281-285.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behavioural Brain Research, 99*(2), 191-200.

Dobbs, L. K., & Mark, G. P. (2008). Comparison of systemic and local methamphetamine treatment on acetylcholine and dopamine levels in the ventral tegmental area in the mouse. *Neuroscience, 156*(3), 700-711.

Drickamer, L. C. (1984). Acceleration of puberty in female mice by a urinary chemosignal from pregnant or lactating females: timing and duration of stimulation. *Dev Psychobiol, 17*(5), 451-455.

Eisch, A. J., Gaffney, M., Weihmuller, F. B., O'Dell, S. J., & Marshall, J. F. (1992). Striatal subregions are differentially vulnerable to the neurotoxic effects of methamphetamine. *Brain Res, 598*(1-2), 321-326.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1996). Neurotoxic lesions of the perirhinal cortex do not mimic the behavioural effects of fornix transection in the rat. *Behavioural Brain Research, 80*(1-2), 9-25.

Ennaceur, A., Neave, N., & Aggleton, J. P. (1997). Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Exp Brain Res, 113*(3), 509-519.

Eriksson, P. S., Perfilieva, E., Bjork-Eriksson, T., Alborn, A. M., Nordborg, C., Peterson, D. A., & Gage, F. H. (1998). Neurogenesis in the adult human hippocampus. *Nat Med, 4*(11), 1313-1317.

Escubedo, E., Chipana, C., Perez-Sanchez, M., Camarasa, J., & Pubill, D. (2005). Methyllycaconitine prevents methamphetamine-induced effects in mouse striatum: involvement of alpha7 nicotinic receptors. *J Pharmacol Exp Ther, 315*(2), 658-667.

Fantegrossi, W. E., Ciullo, J. R., Wakabayashi, K. T., De La Garza, R., 2nd, Traynor, J. R., & Woods, J. H. (2008). A comparison of the physiological, behavioral, neurochemical and microglial effects of methamphetamine and 3,4-methylenedioxymethamphetamine in the mouse. *Neuroscience, 151*(2), 533-543.

Feiro, O., & Gould, T. J. (2005). The interactive effects of nicotinic and muscarinic cholinergic receptor inhibition on fear conditioning in young and aged C57BL/6 mice. *Pharmacol Biochem Behav, 80*(2), 251-262.

Feldman, R. S., Meyer, J. S., & Quenzer, L. F. (1997). *Principles of Neuropsychopharmacology*. Sunderland: Sinauer Associates, Inc.

Fellous, J. M., & Sejnowski, T. J. (2000). Cholinergic induction of oscillations in the hippocampal slice in the slow (0.5-2 Hz), theta (5-12 Hz), and gamma (35-70 Hz) bands. *Hippocampus, 10*(2), 187-197.

Ferguson, S. M., & Blakely, R. D. (2004). The choline transporter resurfaces: new roles for synaptic vesicles? *Mol Interv, 4*(1), 22-37.

Fitch, T. E., Sahr, R. N., Eastwood, B. J., Zhou, F. C., & Yang, C. R. (2006). Dopamine D1/5 receptor modulation of firing rate and bidirectional theta burst firing in medial septal/vertical limb of diagonal band neurons in vivo. *J Neurophysiol, 95*(5), 2808-2820.

Fontana, D. J., Inouye, G. T., & Johnson, R. M. (1994). Linopirdine (DuP 996) improves performance in several tests of learning and memory by modulation of cholinergic neurotransmission. *Pharmacol Biochem Behav, 49*(4), 1075-1082.

Forrester, M. B., & Merz, R. D. (2007). Risk of selected birth defects with prenatal illicit drug use, Hawaii, 1986-2002. *J Toxicol Environ Health A, 70*(1), 7-18.

Fragkouli, A., Hearn, C., Errington, M., Cooke, S., Grigoriou, M., Bliss, T., Stylianopoulou, F., & Pachnis, V. (2005). Loss of forebrain cholinergic neurons and impairment in spatial learning and memory in LHX7-deficient mice. *Eur J Neurosci, 21*(11), 2923-2938.

Franklin, K., & Paxinos, G. (1997). *The mouse brain in stereotaxic coordinates*. San Diego: Academic Press.

Fremeau, R. T., Jr., Troyer, M. D., Pahner, I., Nygaard, G. O., Tran, C. H., Reimer, R. J., Bellocchio, E. E., Fortin, D., Storm-Mathisen, J., & Edwards, R. H. (2001). The expression of vesicular glutamate transporters defines two classes of excitatory synapse. *Neuron, 31*(2), 247-260.

Freund, T. F. (1989). GABAergic septohippocampal neurons contain parvalbumin. *Brain Res, 478*(2), 375-381.

Freund, T. F., & Antal, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature, 336*(6195), 170-173.

Freund, T. F., & Buzsaki, G. (1996). Interneurons of the hippocampus. *Hippocampus, 6*(4), 347-470.

Freund, T. F., & Meskenaite, V. (1992). gamma-Aminobutyric acid-containing basal forebrain neurons innervate inhibitory interneurons in the neocortex. *Proceedings of the National Academy of Sciences of the United States of America, 89*(2), 738-742.

Frick, K. M., Kim, J. J., & Baxter, M. G. (2004). Effects of complete immunotoxin lesions of the cholinergic basal forebrain on fear conditioning and spatial learning. *Hippocampus, 14*(2), 244-254.

Froeliger, B., Gilbert, D. G., & McClernon, F. J. (2009). Effects of nicotine on novelty detection and memory recognition performance: double-blind, placebo-controlled studies of smokers and nonsmokers. *Psychopharmacology (Berl), 205*(4), 625-633.

Frotscher, M. (1991). Target cell specificity of synaptic connections in the hippocampus. *Hippocampus, 1*(2), 123-130.

Furtak, S. C., Wei, S. M., Agster, K. L., & Burwell, R. D. (2007). Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus, 17*(9), 709-722.

Galani, R., Lehmann, O., Bolmont, T., Aloy, E., Bertrand, F., Lazarus, C., Jeltsch, H., & Cassel, J. C. (2002). Selective immunolesions of CH4 cholinergic neurons do not disrupt spatial memory in rats. *Physiol Behav, 76*(1), 75-90.

Garcia-Rates, S., Camarasa, J., Escubedo, E., & Pubill, D. (2007). Methamphetamine and 3,4-methylenedioxymethamphetamine interact with central nicotinic receptors and induce their up-regulation. *Toxicol Appl Pharmacol, 223*(3), 195-205.

Gaykema, R. P., Luiten, P. G., Nyakas, C., & Traber, J. (1990). Cortical projection patterns of the medial septum-diagonal band complex. *J Comp Neurol, 293*(1), 103-124.

Gaykema, R. P., & Zaborszky, L. (1996). Direct catecholaminergic-cholinergic interactions in the basal forebrain. II. Substantia nigra-ventral tegmental area projections to cholinergic neurons. *J Comp Neurol, 374*(4), 555-577.

Gerlai, R. T., McNamara, A., Williams, S., & Phillips, H. S. (2002). Hippocampal dysfunction and behavioral deficit in the water maze in mice: an unresolved issue? *Brain Res Bull, 57*(1), 3-9.

Gettig, J. P., Grady, S. E., & Nowosadzka, I. (2006). Methamphetamine: putting the brakes on speed. *J Sch Nurs, 22*(2), 66-73.

Gibbs, R. B. (2010). Estrogen therapy and cognition: a review of the cholinergic hypothesis. *Endocr Rev, 31*(2), 224-253.

Gilman, A. G., Rall, T. W., Nies, A. S., & Taylor, P. (Eds.). (1993). *Goodman and Gilman's the pharmacological basis of therapeutics* (Eighth ed.). New York: McGraw-Hill, INC.

Givens, B., & Olton, D. S. (1995). Bidirectional modulation of scopolamine-induced working memory impairments by muscarinic activation of the medial septal area. *Neurobiol Learn Mem, 63*(3), 269-276.

Gomes-da-Silva, J., Perez-Rosado, A., de Miguel, R., Fernandez-Ruiz, J., Silva, M. C., & Tavares, M. A. (2002). Prenatal exposure to methamphetamine in the rat: ontogeny of tyrosine hydroxylase mRNA expression in mesencephalic dopaminergic neurons. *Ann N Y Acad Sci, 965*, 68-77.

Goncalves, J., Baptista, S., Martins, T., Milhazes, N., Borges, F., Ribeiro, C. F., Malva, J. O., & Silva, A. P. (2010). Methamphetamine-induced neuroinflammation and neuronal dysfunction in the mice hippocampus: preventive effect of indomethacin. *Eur J Neurosci, 31*(2), 315-326.

Gonzales, R., Mooney, L., & Rawson, R. A. (2010). The methamphetamine problem in the United States. *Annu Rev Public Health, 31*, 385-398.

Good, M. M., Solt, I., Acuna, J. G., Rotmensch, S., & Kim, M. J. (2010). Methamphetamine use during pregnancy: maternal and neonatal implications. *Obstet Gynecol, 116*(2 Pt 1), 330-334.

Grace, C. E., Schaefer, T. L., Graham, D. L., Skelton, M. R., Williams, M. T., & Vorhees, C. V. (2010). Effects of inhibiting neonatal methamphetamine-induced corticosterone release in rats by adrenal autotransplantation on later learning, memory, and plasma corticosterone levels. *Int J Dev Neurosci, 28*(4), 331-342.

Grace, C. E., Schaefer, T. L., Gudelsky, G. A., Williams, M. T., & Vorhees, C. V. (2010). Neonatal methamphetamine-induced corticosterone release in rats is inhibited by adrenal autotransplantation without altering the effect of the drug on hippocampal serotonin. *Neurotoxicol Teratol, 32*(3), 356-361.

Gritti, I., Mainville, L., & Jones, B. E. (1993). Codistribution of GABA- with acetylcholine-synthesizing neurons in the basal forebrain of the rat. *J Comp Neurol, 329*(4), 438-457.
Gritti, I., Mainville, L., & Jones, B. E. (1994). Projections of GABAergic and cholinergic basal forebrain and GABAergic preoptic-anterior hypothalamic neurons to the posterior lateral hypothalamus of the rat. *J Comp Neurol, 339*(2), 251-268.
Gritti, I., Manns, I. D., Mainville, L., & Jones, B. E. (2003). Parvalbumin, calbindin, or calretinin in cortically projecting and GABAergic, cholinergic, or glutamatergic basal forebrain neurons of the rat. *J Comp Neurol, 458*(1), 11-31.
Guangda, X., Bangshun, X., Xiujian, L., & Yangzhong, H. (1999). Apovarepsilon(4) allele increases the risk for exercise-induced silent myocardial ischemia in non-insulin-dependent diabetes mellitus. *Atherosclerosis, 147*(2), 293-296.
Guilarte, T. R., Nihei, M. K., McGlothan, J. L., & Howard, A. S. (2003). Methamphetamine-induced deficits of brain monoaminergic neuronal markers: distal axotomy or neuronal plasticity. *Neuroscience, 122*(2), 499-513.
Hajszan, T., Alreja, M., & Leranth, C. (2004). Intrinsic vesicular glutamate transporter 2-immunoreactive input to septohippocampal parvalbumin-containing neurons: novel glutamatergic local circuit cells. *Hippocampus, 14*(4), 499-509.
Halbach, O., & Dermietzel, H. (2002). *Neurotransmitters and Neuromodulators: Handbook of Receptors and Biological Effects* (Vol. 1). Weinheim: Wiley-VCH.
Han, D. D., & Gu, H. H. (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol, 6*, 6.
Hartikka, J., & Hefti, F. (1988). Development of septal cholinergic neurons in culture: plating density and glial cells modulate effects of NGF on survival, fiber growth, and expression of transmitter-specific enzymes. *Journal of Neuroscience, 8*(8), 2967-2985.
Harvey, D. C., Lacan, G., Tanious, S. P., & Melega, W. P. (2000). Recovery from methamphetamine induced long-term nigrostriatal dopaminergic deficits without substantia nigra cell loss. *Brain Res, 871*(2), 259-270.
Hasselmo, M. E., Bodelon, C., & Wyble, B. P. (2002). A proposed function for hippocampal theta rhythm: separate phases of encoding and retrieval enhance reversal of prior learning. *Neural Comput, 14*(4), 793-817.
Hellstrom-Lindahl, E., Gorbounova, O., Seiger, A., Mousavi, M., & Nordberg, A. (1998). Regional distribution of nicotinic receptors during prenatal development of human brain and spinal cord. *Brain Res Dev Brain Res, 108*(1-2), 147-160.
Henny, P., & Jones, B. E. (2006). Innervation of orexin/hypocretin neurons by GABAergic, glutamatergic or cholinergic basal forebrain terminals evidenced by immunostaining for presynaptic vesicular transporter and postsynaptic scaffolding proteins. *J Comp Neurol, 499*(4), 645-661.
Himmelheber, A. M., Sarter, M., & Bruno, J. P. (2000). Increases in cortical acetylcholine release during sustained attention performance in rats. *Brain Res Cogn Brain Res, 9*(3), 313-325.
Hoffman, W. F., Moore, M., Templin, R., McFarland, B., Hitzemann, R. J., & Mitchell, S. H. (2006). Neuropsychological function and delay discounting in methamphetamine-dependent individuals. *Psychopharmacology (Berl), 188*(2), 162-170.

Holson, R. R., & Pearce, B. (1992). Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. *Neurotoxicol Teratol, 14*(3), 221-228.

Hortnagl, H., Hansen, L., Kindel, G., Schneider, B., el Tamer, A., & Hanin, I. (1993). Sex differences and estrous cycle-variations in the AF64A-induced cholinergic deficit in the rat hippocampus. *Brain Res Bull, 31*(1-2), 129-134.

Houser, C. R., Crawford, G. D., Barber, R. P., Salvaterra, P. M., & Vaughn, J. E. (1983). Organization and morphological characteristics of cholinergic neurons: an immunocytochemical study with a monoclonal antibody to choline acetyltransferase. *Brain Res, 266*(1), 97-119.

Hruba, L., Schutova, B., Pometlova, M., Rokyta, R., & Slamberova, R. (2009). Effect of methamphetamine exposure and cross-fostering on cognitive function in adult male rats. *Behavioural Brain Research, 208*(1), 63-71.

Hruba, L., Schutova, B., Slamberova, R., Pometlova, M., & Rokyta, R. (2008). Effect of methamphetamine exposure and cross-fostering on sensorimotor development of male and female rat pups. *Dev Psychobiol*.

Huh, C. Y., Goutagny, R., & Williams, S. (2010). Glutamatergic neurons of the mouse medial septum and diagonal band of Broca synaptically drive hippocampal pyramidal cells: relevance for hippocampal theta rhythm. *Journal of Neuroscience, 30*(47), 15951-15961.

Hussain, R. J., Taraschenko, O. D., & Glick, S. D. (2008). Effects of nicotine, methamphetamine and cocaine on extracellular levels of acetylcholine in the interpeduncular nucleus of rats. *Neurosci Lett, 440*(3), 270-274.

Isgor, C., & Sengelaub, D. R. (2003). Effects of neonatal gonadal steroids on adult CA3 pyramidal neuron dendritic morphology and spatial memory in rats. *Journal of Neurobiology, 55*(2), 179-190.

Ishii, T., Miwa, T., Nishio, H., & Yagasaki, O. (1990). The biochemical and ultrastructural examinations in central cholinergic damage of the rat induced by the intraperitoneal administration of AF64A. *Jpn J Pharmacol, 54*(4), 415-423.

Jackson, M. B., Lecar, H., Brenneman, D. E., Fitzgerald, S., & Nelson, P. G. (1982). Electrical development in spinal cord cell culture. *Journal of Neuroscience, 2*(8), 1052-1061.

Jamal, M., Ameno, K., Ameno, S., Morishita, J., Wang, W., Kumihashi, M., Ikuo, U., Miki, T., & Ijiri, I. (2007). Changes in cholinergic function in the frontal cortex and hippocampus of rat exposed to ethanol and acetaldehyde. *Neuroscience, 144*(1), 232-238.

Johanson, C. E., Frey, K. A., Lundahl, L. H., Keenan, P., Lockhart, N., Roll, J., Galloway, G. P., Koeppe, R. A., Kilbourn, M. R., Robbins, T., & Schuster, C. R. (2006). Cognitive function and nigrostriatal markers in abstinent methamphetamine abusers. *Psychopharmacology (Berl), 185*(3), 327-338.

Jonakait, G. M., Wei, R., Sheng, Z. L., Hart, R. P., & Ni, L. (1994). Interferon-gamma promotes cholinergic differentiation of embryonic septal nuclei and adjacent basal forebrain. *Neuron, 12*(5), 1149-1159.

Jones, B. E., & Cuello, A. C. (1989). Afferents to the basal forebrain cholinergic cell area from pontomesencephalic--catecholamine, serotonin, and acetylcholine--neurons. *Neuroscience, 31*(1), 37-61.

Jones, C. K., & Shannon, H. E. (2000). Muscarinic cholinergic modulation of prepulse inhibition of the acoustic startle reflex. *J Pharmacol Exp Ther, 294*(3), 1017-1023.

Katzung, B. G. (2007). *Basic and Clinical Pharmacology* (10th ed.). New York: McGraw-Hill Companies, Inc.

Kenigsberg, R. L., Hong, Y., & Theoret, Y. (1998). Cholinergic cell expression in the developing rat medial septal nucleus in vitro is differentially controlled by GABAA and GABAB receptors. *Brain Res, 805*(1-2), 123-130.

King, C., Recce, M., & O'Keefe, J. (1998). The rhythmicity of cells of the medial septum/diagonal band of Broca in the awake freely moving rat: relationships with behaviour and hippocampal theta. *Eur J Neurosci, 10*(2), 464-477.

King, G., Alicata, D., Cloak, C., & Chang, L. (2010). Neuropsychological deficits in adolescent methamphetamine abusers. *Psychopharmacology (Berl), 212*(2), 243-249.

Kirksey, D. F., & Slotkin, T. A. (1979). Concomitant development of [3H]-dopamine and [3H]-5-hydroxytryptamine uptake systems in rat brain regions. *Br J Pharmacol, 67*(3), 387-391.

Kish, S. J., Kalasinsky, K. S., Furukawa, Y., Guttman, M., Ang, L., Li, L., Adams, V., Reiber, G., Anthony, R. A., Anderson, W., Smialek, J., & DiStefano, L. (1999). Brain choline acetyltransferase activity in chronic, human users of cocaine, methamphetamine, and heroin. *Mol Psychiatry, 4*(1), 26-32.

Kiss, J., Magloczky, Z., Somogyi, J., & Freund, T. F. (1997). Distribution of calretinin-containing neurons relative to other neurochemically identified cell types in the medial septum of the rat. *Neuroscience, 78*(2), 399-410.

Kiss, J., & Patel, A. J. (1992). Development of the cholinergic fibres innervating the cerebral cortex of the rat. *Int J Dev Neurosci, 10*(2), 153-170.

Kiss, J., Patel, A. J., Baimbridge, K. G., & Freund, T. F. (1990). Topographical localization of neurons containing parvalbumin and choline acetyltransferase in the medial septum-diagonal band region of the rat. *Neuroscience, 36*(1), 61-72.

Knouff, C., Hinsdale, M. E., Mezdour, H., Altenburg, M. K., Watanabe, M., Quarfordt, S. H., Sullivan, P. M., & Maeda, N. (1999). Apo E structure determines VLDL clearance and atherosclerosis risk in mice. *J Clin Invest, 103*(11), 1579-1586.

Krasnova, I. N., & Cadet, J. L. (2009). Methamphetamine toxicity and messengers of death. *Brain Res Rev, 60*(2), 379-407.

Ladenheim, B., Krasnova, I. N., Deng, X., Oyler, J. M., Polettini, A., Moran, T. H., Huestis, M. A., & Cadet, J. L. (2000). Methamphetamine-induced neurotoxicity is attenuated in transgenic mice with a null mutation for interleukin-6. *Mol Pharmacol, 58*(6), 1247-1256.

Lai, M. K., Tsang, S. W., Garcia-Alloza, M., Minger, S. L., Nicoll, J. A., Esiri, M. M., Wong, P. T., Chen, C. P., Ramirez, M. J., & Francis, P. T. (2006). Selective effects of the APOE epsilon4 allele on presynaptic cholinergic markers in the neocortex of Alzheimer's disease. *Neurobiol Dis, 22*(3), 555-561.

Lapchak, P. A., Jenden, D. J., & Hefti, F. (1991). Compensatory elevation of acetylcholine synthesis in vivo by cholinergic neurons surviving partial lesions of the septohippocampal pathway. *Journal of Neuroscience, 11*(9), 2821-2828.

Large, T. H., Bodary, S. C., Clegg, D. O., Weskamp, G., Otten, U., & Reichardt, L. F. (1986). Nerve growth factor gene expression in the developing rat brain. *Science, 234*(4774), 352-355.

Lauder, J. M., Han, V. K., Henderson, P., Verdoorn, T., & Towle, A. C. (1986). Prenatal ontogeny of the GABAergic system in the rat brain: an immunocytochemical study. *Neuroscience, 19*(2), 465-493.

LaVoie, M. J., Card, J. P., & Hastings, T. G. (2004). Microglial activation precedes dopamine terminal pathology in methamphetamine-induced neurotoxicity. *Experimental Neurology, 187*(1), 47-57.

Lee, M. G., Chrobak, J. J., Sik, A., Wiley, R. G., & Buzsaki, G. (1994). Hippocampal theta activity following selective lesion of the septal cholinergic system. *Neuroscience, 62*(4), 1033-1047.

Lehmann, K., Hundsdorfer, B., Hartmann, T., & Teuchert-Noodt, G. (2004). The acetylcholine fiber density of the neocortex is altered by isolated rearing and early methamphetamine intoxication in rodents. *Experimental Neurology, 189*(1), 131-140.

Lehmann, K., & Lehmann, D. (2007). Transmitter balances in the olfactory cortex: adaptations to early methamphetamine trauma and rearing environment. *Brain Res, 1141*, 37-47.

Lenck-Santini, P. P., Rivard, B., Muller, R. U., & Poucet, B. (2005). Study of CA1 place cell activity and exploratory behavior following spatial and nonspatial changes in the environment. *Hippocampus, 15*(3), 356-369.

Leranth, C., & Frotscher, M. (1989). Organization of the septal region in the rat brain: cholinergic-GABAergic interconnections and the termination of hippocampo-septal fibers. *J Comp Neurol, 289*(2), 304-314.

Lester, B. M., & Lagasse, L. L. (2010). Children of addicted women. *J Addict Dis, 29*(2), 259-276.

Li, Y., Holtzman, D. M., Kromer, L. F., Kaplan, D. R., Chua-Couzens, J., Clary, D. O., Knusel, B., & Mobley, W. C. (1995). Regulation of TrkA and ChAT expression in developing rat basal forebrain: evidence that both exogenous and endogenous NGF regulate differentiation of cholinergic neurons. *Journal of Neuroscience, 15*(4), 2888-2905.

Li, Y., Liu, L., Kang, J., Sheng, J. G., Barger, S. W., Mrak, R. E., & Griffin, W. S. (2000). Neuronal-glial interactions mediated by interleukin-1 enhance neuronal acetylcholinesterase activity and mRNA expression. *Journal of Neuroscience, 20*(1), 149-155.

Lin, L., LeBlanc, C. J., Deacon, T. W., & Isacson, O. (1998). Chronic cognitive deficits and amyloid precursor protein elevation after selective immunotoxin lesions of the basal forebrain cholinergic system. *Neuroreport, 9*(3), 547-552.

Linke, R., Schwegler, H., & Boldyreva, M. (1994). Cholinergic and GABAergic septo-hippocampal projection neurons in mice: a retrograde tracing study combined with double immunocytochemistry for choline acetyltransferase and parvalbumin. *Brain Res, 653*(1-2), 73-80.

Little, B. B., Snell, L. M., & Gilstrap, L. C., 3rd. (1988). Methamphetamine abuse during pregnancy: outcome and fetal effects. *Obstet Gynecol, 72*(4), 541-544.

Lockman, P. R., & Allen, D. D. (2002). The transport of choline. *Drug Dev Ind Pharm, 28*(7), 749-771.

Lomnitski, L., Chapman, S., Hochman, A., Kohen, R., Shohami, E., Chen, Y., Trembovler, V., & Michaelson, D. M. (1999). Antioxidant mechanisms in apolipoprotein E deficient mice prior to and following closed head injury. *Biochim Biophys Acta, 1453*(3), 359-368.

Lopez-Coviella, I., Berse, B., Krauss, R., Thies, R. S., & Blusztajn, J. K. (2000). Induction and maintenance of the neuronal cholinergic phenotype in the central nervous system by BMP-9. *Science, 289*(5477), 313-316.

Lopez-Coviella, I., Mellott, T. M., Kovacheva, V. P., Berse, B., Slack, B. E., Zemelko, V., Schnitzler, A., & Blusztajn, J. K. (2006). Developmental pattern of expression of BMP receptors and Smads and activation of Smad1 and Smad5 by BMP9 in mouse basal forebrain. *Brain Res, 1088*(1), 49-56.

Loy, R., & Sheldon, R. A. (1987). Sexually dimorphic development of cholinergic enzymes in the rat septohippocampal system. *Brain Res, 431*(1), 156-160.

Lu, L. H., Johnson, A., O'Hare, E. D., Bookheimer, S. Y., Smith, L. M., O'Connor, M. J., & Sowell, E. R. (2009). Effects of prenatal methamphetamine exposure on verbal memory revealed with functional magnetic resonance imaging. *J Dev Behav Pediatr, 30*(3), 185-192.

Lucot, J. B., Wagner, G. C., Schuster, C. R., & Seiden, L. S. (1982). Decreased sensitivity of rat pups to long-lasting dopamine and serotonin depletions produced by methylamphetamine. *Brain Res, 247*(1), 181-183.

Luine, V. N., Renner, K. J., & McEwen, B. S. (1986). Sex-dependent differences in estrogen regulation of choline acetyltransferase are altered by neonatal treatments. *Endocrinology, 119*(2), 874-878.

Lund, D. D., Knuepfer, M. M., Brody, M. J., Bhatnagar, R. K., Schmid, P. G., & Roskoski, R., Jr. (1978). Comparison of tyrosine hydroxylase and choline acetyltransferase activity in response to sympathetic nervous system activation. *Brain Res, 156*(1), 192-197.

Madziar, B., Lopez-Coviella, I., Zemelko, V., & Berse, B. (2005). Regulation of cholinergic gene expression by nerve growth factor depends on the phosphatidylinositol-3'-kinase pathway. *J Neurochem, 92*(4), 767-779.

Mahley, R. W. (1988). Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science, 240*(4852), 622-630.

Makuch, R., Baratta, J., Karaelias, L. D., Lauterborn, J. C., Gall, C. M., Yu, J., & Robertson, R. T. (2001). Arrival of afferents and the differentiation of target neurons: studies of developing cholinergic projections to the dentate gyrus. *Neuroscience, 104*(1), 81-91.

Manabe, T., Tatsumi, K., Inoue, M., Makinodan, M., Yamauchi, T., Makinodan, E., Yokoyama, S., Sakumura, R., & Wanaka, A. (2007). L3/Lhx8 is a pivotal factor for cholinergic differentiation of murine embryonic stem cells. *Cell Death Differ, 14*(6), 1080-1085.

Manabe, T., Tatsumi, K., Inoue, M., Matsuyoshi, H., Makinodan, M., Yokoyama, S., & Wanaka, A. (2005). L3/Lhx8 is involved in the determination of cholinergic or GABAergic cell fate. *J Neurochem, 94*(3), 723-730.

Manns, I. D., Mainville, L., & Jones, B. E. (2001). Evidence for glutamate, in addition to acetylcholine and GABA, neurotransmitter synthesis in basal forebrain neurons projecting to the entorhinal cortex. *Neuroscience, 107*(2), 249-263.

Manseau, F., Danik, M., & Williams, S. (2005). A functional glutamatergic neurone network in the medial septum and diagonal band area. *J Physiol, 566*(Pt 3), 865-884.

Masuda, J., Mitsushima, D., Funabashi, T., & Kimura, F. (2005). Sex and housing conditions affect the 24-h acetylcholine release profile in the hippocampus in rats. *Neuroscience, 132*(2), 537-542.

McCabe, R. T., Gibb, J. W., Wamsley, J. K., & Hanson, G. R. (1987). Autoradiographic analysis of muscarinic cholinergic and serotonergic receptor alterations following methamphetamine treatment. *Brain Res Bull, 19*(5), 551-557.

McCabe, R. T., Hanson, G. R., Dawson, T. M., Wamsley, J. K., & Gibb, J. W. (1987). Methamphetamine-induced reduction in D1 and D2 dopamine receptors as evidenced by autoradiography: comparison with tyrosine hydroxylase activity. *Neuroscience, 23*(1), 253-261.

McCann, U. D., Wong, D. F., Yokoi, F., Villemagne, V., Dannals, R. F., & Ricaurte, G. A. (1998). Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11C]WIN-35,428. *Journal of Neuroscience, 18*(20), 8417-8422.

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). The use of behavioral test batteries: effects of training history. *Physiol Behav, 73*(5), 705-717.

McKinney, M., & Jacksonville, M. C. (2005). Brain cholinergic vulnerability: relevance to behavior and disease. *Biochem Pharmacol, 70*(8), 1115-1124.

Meck, W. H., Smith, R. A., & Williams, C. L. (1989). Organizational changes in cholinergic activity and enhanced visuospatial memory as a function of choline administered prenatally or postnatally or both. *Behav Neurosci, 103*(6), 1234-1241.

Mendelson, J., Uemura, N., Harris, D., Nath, R. P., Fernandez, E., Jacob, P., 3rd, Everhart, E. T., & Jones, R. T. (2006). Human pharmacology of the methamphetamine stereoisomers. *Clin Pharmacol Ther, 80*(4), 403-420.

Mesulam, M. M. (2004). The cholinergic innervation of the human cerebral cortex. *Prog Brain Res, 145*, 67-78.

Mesulam, M. M., Mufson, E. J., Wainer, B. H., & Levey, A. I. (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience, 10*(4), 1185-1201.

Mijnster, M. J., Galis-de Graaf, Y., & Voorn, P. (1998). Serotonergic regulation of neuropeptide and glutamic acid decarboxylase mRNA levels in the rat striatum and globus pallidus: studies with fluoxetine and DOI. *Brain Res Mol Brain Res, 54*(1), 64-73.

Miller, D. B., Ali, S. F., O'Callaghan, J. P., & Laws, S. C. (1998). The impact of gender and estrogen on striatal dopaminergic neurotoxicity. *Ann N Y Acad Sci, 844*, 153-165.

Miller, M. M., Hyder, S. M., Assayag, R., Panarella, S. R., Tousignant, P., & Franklin, K. B. (1999). Estrogen modulates spontaneous alternation and the cholinergic phenotype in the basal forebrain. *Neuroscience, 91*(3), 1143-1153.

Mirza, N. R., & Stolerman, I. P. (2000). The role of nicotinic and muscarinic acetylcholine receptors in attention. *Psychopharmacology (Berl), 148*(3), 243-250.

Mitsushima, D., Masuda, J., & Kimura, F. (2003). Sex differences in the stress-induced release of acetylcholine in the hippocampus and corticosterone from the adrenal cortex in rats. *Neuroendocrinology, 78*(4), 234-240.

Mitsushima, D., Takase, K., Funabashi, T., & Kimura, F. (2009). Gonadal steroids maintain 24 h acetylcholine release in the hippocampus: organizational and activational effects in behaving rats. *Journal of Neuroscience, 29*(12), 3808-3815.

Miura, Y., Ito, T., & Kadokawa, T. (1987). Effects of intraseptally injected dopamine and noradrenaline on hippocampal synchronized theta wave activity in rats. *Jpn J Pharmacol, 44*(4), 471-479.

Mobley, W. C., Rutkowski, J. L., Tennekoon, G. I., Gemski, J., Buchanan, K., & Johnston, M. V. (1986). Nerve growth factor increases choline acetyltransferase activity in developing basal forebrain neurons. *Brain Res, 387*(1), 53-62.

Moore, H., Fadel, J., Sarter, M., & Bruno, J. P. (1999). Role of accumbens and cortical dopamine receptors in the regulation of cortical acetylcholine release. *Neuroscience, 88*(3), 811-822.

Moore, M. C. (1991). Application of organization-activation theory to alternative male reproductive strategies: a review. *Horm Behav, 25*(2), 154-179.

Mori, T., Yuxing, Z., Takaki, H., Takeuchi, M., Iseki, K., Hagino, S., Kitanaka, J., Takemura, M., Misawa, H., Ikawa, M., Okabe, M., & Wanaka, A. (2004). The LIM homeobox gene, L3/Lhx8, is necessary for proper development of basal forebrain cholinergic neurons. *Eur J Neurosci, 19*(12), 3129-3141.

Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature, 297*(5868), 681-683.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1994). AMPA-induced excitotoxic lesions of the basal forebrain: a significant role for the cortical cholinergic system in attentional function. *Journal of Neuroscience, 14*(4), 2313-2326.

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1996). The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cereb Cortex, 6*(3), 470-481.

Mullen, R. J., Buck, C. R., & Smith, A. M. (1992). NeuN, a neuronal specific nuclear protein in vertebrates. *Development, 116*(1), 201-211.

Mumby, D. G., Gaskin, S., Glenn, M. J., Schramek, T. E., & Lehmann, H. (2002). Hippocampal damage and exploratory preferences in rats: memory for objects, places, and contexts. *Learn Mem, 9*(2), 49-57.

Murai, T., Okuda, S., Tanaka, T., & Ohta, H. (2007). Characteristics of object location memory in mice: Behavioral and pharmacological studies. *Physiol Behav, 90*(1), 116-124.

Muramatsu, T., Kato, M., Matsui, T., Yoshimasu, H., Yoshino, A., Matsushita, S., Higuchi, S., & Kashima, H. (1997). Apolipoprotein E epsilon 4 allele distribution

in Wernicke-Korsakoff syndrome with or without global intellectual deficits. *J Neural Transm, 104*(8-9), 913-920.
Nail-Boucherie, K., Dourmap, N., Jaffard, R., & Costentin, J. (2000). Contextual fear conditioning is associated with an increase of acetylcholine release in the hippocampus of rat. *Brain Res Cogn Brain Res, 9*(2), 193-197.
Nakamura, N., Fujita, H., & Kawata, M. (2002). Effects of gonadectomy on immunoreactivity for choline acetyltransferase in the cortex, hippocampus, and basal forebrain of adult male rats. *Neuroscience, 109*(3), 473-485.
Nathoo, N., Chetty, R., van Dellen, J. R., & Barnett, G. H. (2003). Genetic vulnerability following traumatic brain injury: the role of apolipoprotein E. *Mol Pathol, 56*(3), 132-136.
Ni, L., Acevedo, G., Muralidharan, B., Padala, N., To, J., & Jonakait, G. M. (2007). Toll-like receptor ligands and CD154 stimulate microglia to produce a factor(s) that promotes excess cholinergic differentiation in the developing rat basal forebrain: implications for neurodevelopmental disorders. *Pediatr Res, 61*(1), 15-20.
NIDA. (2006). Research Report Series : Methamphetamine Abuse and Addiction. 1-8.
NIDA. (2010). InfoFacts: Methamphetamine. 1-4.
NSDUH. (2007). The National Survey on Drug Use and Health Report: Methamphetamine Use. 1-4.
NSDUH. (2009). Results from the 2009 National Survey on Drug Use and Health: Volume I. Summary of National Findings. 1-104.
O'Keefe, J., & Burgess, N. (1999). Theta activity, virtual navigation and the human hippocampus. *Trends Cogn Sci, 3*(11), 403-406.
Oda, Y. (1999). Choline acetyltransferase: the structure, distribution and pathologic changes in the central nervous system. *Pathol Int, 49*(11), 921-937.
Otto, T., & Poon, P. (2006). Dorsal hippocampal contributions to unimodal contextual conditioning. *Journal of Neuroscience, 26*(24), 6603-6609.
Pappas, B. A., Payne, K. B., Fortin, T., & Sherren, N. (2005). Neonatal lesion of forebrain cholinergic neurons: further characterization of behavioral effects and permanency. *Neuroscience, 133*(2), 485-492.
Passetti, F., Dalley, J. W., O'Connell, M. T., Everitt, B. J., & Robbins, T. W. (2000). Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. *Eur J Neurosci, 12*(8), 3051-3058.
Paterson, D., & Nordberg, A. (2000). Neuronal nicotinic receptors in the human brain. *Prog Neurobiol, 61*(1), 75-111.
Pattij, T., Janssen, M. C., Loos, M., Smit, A. B., Schoffelmeer, A. N., & van Gaalen, M. M. (2007). Strain specificity and cholinergic modulation of visuospatial attention in three inbred mouse strains. *Genes Brain Behav, 6*(6), 579-587.
Payne, A. H., & Hales, D. B. (2004). Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones. *Endocr Rev, 25*(6), 947-970.
Paz, M. S., Smith, L. M., LaGasse, L. L., Derauf, C., Grant, P., Shah, R., Arria, A., Huestis, M., Haning, W., Strauss, A., Della Grotta, S., Liu, J., & Lester, B. M. (2009). Maternal depression and neurobehavior in newborns prenatally exposed to methamphetamine. *Neurotoxicol Teratol, 31*(3), 177-182.

Phillips, J. M., McAlonan, K., Robb, W. G., & Brown, V. J. (2000). Cholinergic neurotransmission influences covert orientation of visuospatial attention in the rat. *Psychopharmacology (Berl), 150*(1), 112-116.

Phillips, R. G., & LeDoux, J. E. (1992). Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. *Behav Neurosci, 106*(2), 274-285.

Pike, C. J. (2001). Testosterone attenuates beta-amyloid toxicity in cultured hippocampal neurons. *Brain Res, 919*(1), 160-165.

Ping, S. E., Trieu, J., Wlodek, M. E., & Barrett, G. L. (2008). Effects of estrogen on basal forebrain cholinergic neurons and spatial learning. *J Neurosci Res, 86*(7), 1588-1598.

Piper, B. J., Acevedo, S. F., Kolchugina, G. K., Butler, R. W., Corbett, S. M., Honeycutt, E. B., Craytor, M. J., & Raber, J. (2011). Abnormalities in parentally rated executive function in methamphetamine/polysubstance exposed children. *Pharmacol Biochem Behav, 98*, 432-439.

Podol'skii, I. Y., Vorob'ev, V. V., & Belova, N. A. (2001). Long-term changes in hippocampus and neocortex EEG spectra in response to pharmacological treatments affecting the cholinergic system. *Neurosci Behav Physiol, 31*(6), 589-595.

Poirier, J. (1994). Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. *Trends Neurosci, 17*(12), 525-530.

Poirier, J., Delisle, M. C., Quirion, R., Aubert, I., Farlow, M., Lahiri, D., Hui, S., Bertrand, P., Nalbantoglu, J., Gilfix, B. M., & Gauthier, S. (1995). Apolipoprotein E4 allele as a predictor of cholinergic deficits and treatment outcome in Alzheimer disease. *Proc Natl Acad Sci U S A., 92*(26), 12260-12264.

Pu, C., & Vorhees, C. V. (1993). Developmental dissociation of methamphetamine-induced depletion of dopaminergic terminals and astrocyte reaction in rat striatum. *Brain Res Dev Brain Res, 72*(2), 325-328.

Raber, J. (2004). Androgens, apoE, and Alzheimer's disease. *Sci Aging Knowledge Environ, 2004*(11), re2.

Raber, J., Bongers, G., LeFevour, A., Buttini, M., & Mucke, L. (2002). Androgens protect against apolipoprotein E4-induced cognitive deficits. *Journal of Neuroscience, 22*(12), 5204-5209.

Raber, J., Wong, D., Buttini, M., Orth, M., Bellosta, S., Pitas, R. E., Mahley, R. W., & Mucke, L. (1998). Isoform-specific effects of human apolipoprotein E on brain function revealed in ApoE knockout mice: increased susceptibility of females. *Proc Natl Acad Sci U S A., 95*(18), 10914-10919.

Ravikumar, B. V., & Sastry, P. S. (1985). Muscarinic cholinergic receptors in human foetal brain: characterization and ontogeny of [3H]quinuclidinyl benzilate binding sites in frontal cortex. *J Neurochem, 44*(1), 240-246.

Reed, J. M., & Squire, L. R. (1997). Impaired recognition memory in patients with lesions limited to the hippocampal formation. *Behav Neurosci, 111*(4), 667-675.

Ricceri, L., Hohmann, C., & Berger-Sweeney, J. (2002). Early neonatal 192 IgG saporin induces learning impairments and disrupts cortical morphogenesis in rats. *Brain Res, 954*(2), 160-172.

Ricceri, L., Minghetti, L., Moles, A., Popoli, P., Confaloni, A., De Simone, R., Piscopo, P., Scattoni, M. L., di Luca, M., & Calamandrei, G. (2004). Cognitive and neurological deficits induced by early and prolonged basal forebrain cholinergic hypofunction in rats. *Experimental Neurology, 189*(1), 162-172.

Rice, C. J., Sandman, C. A., Lenjavi, M. R., & Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology, 149*(10), 4892-4900.

Rihn, L. L., & Claiborne, B. J. (1990). Dendritic growth and regression in rat dentate granule cells during late postnatal development. *Brain Res Dev Brain Res, 54*(1), 115-124.

Risbrough, V., Bontempi, B., & Menzaghi, F. (2002). Selective immunolesioning of the basal forebrain cholinergic neurons in rats: effect on attention using the 5-choice serial reaction time task. *Psychopharmacology (Berl), 164*(1), 71-81.

Riviere, G. J., Gentry, W. B., & Owens, S. M. (2000). Disposition of methamphetamine and its metabolite amphetamine in brain and other tissues in rats after intravenous administration. *J Pharmacol Exp Ther, 292*(3), 1042-1047.

Robbins, T. W. (2002). The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl), 163*(3-4), 362-380.

Robinson, S. E., Malthe-Sorenssen, D., Wood, P. L., & Commissiong, J. (1979). Dopaminergic control of the septal-hippocampal cholinergic pathway. *J Pharmacol Exp Ther, 208*(3), 476-479.

Rogers, J. L., & Kesner, R. P. (2004). Cholinergic modulation of the hippocampus during encoding and retrieval of tone/shock-induced fear conditioning. *Learn Mem, 11*(1), 102-107.

Rossner, S., Schliebs, R., Hartig, W., & Bigl, V. (1995). 192IGG-saporin-induced selective lesion of cholinergic basal forebrain system: neurochemical effects on cholinergic neurotransmission in rat cerebral cortex and hippocampus. *Brain Res Bull, 38*(4), 371-381.

Rothman, R. B., Baumann, M. H., Dersch, C. M., Romero, D. V., Rice, K. C., Carroll, F. I., & Partilla, J. S. (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse, 39*(1), 32-41.

Ruotsalainen, S., Miettinen, R., MacDonald, E., Koivisto, E., & Sirvio, J. (2000). Blockade of muscarinic, rather than nicotinic, receptors impairs attention, but does not interact with serotonin depletion. *Psychopharmacology (Berl), 148*(2), 111-123.

Salehi, A., Dubelaar, E. J., Mulder, M., & Swaab, D. F. (1998). Aggravated decrease in the activity of nucleus basalis neurons in Alzheimer's disease is apolipoprotein E-type dependent. *Proceedings of the National Academy of Sciences of the United States of America, 95*(19), 11445-11449.

Sambeth, A., Riedel, W. J., Smits, L. T., & Blokland, A. (2007). Cholinergic drugs affect novel object recognition in rats: relation with hippocampal EEG? *Eur J Pharmacol, 572*(2-3), 151-159.

Sandstrom, N. J., Kim, J. H., & Wasserman, M. A. (2006). Testosterone modulates performance on a spatial working memory task in male rats. *Horm Behav, 50*(1), 18-26.

Sarter, M., & Bruno, J. P. (2004). Developmental origins of the age-related decline in cortical cholinergic function and associated cognitive abilities. *Neurobiol Aging, 25*(9), 1127-1139.
Sarter, M., & Parikh, V. (2005). Choline transporters, cholinergic transmission and cognition. *Nat Rev Neurosci, 6*(1), 48-56.
Saunders, A. M., Strittmatter, W. J., Schmechel, D., George-Hyslop, P. H., Pericak-Vance, M. A., Joo, S. H., Rosi, B. L., Gusella, J. F., Crapper-MacLachlan, D. R., Alberts, M. J., & et al. (1993). Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology, 43*(8), 1467-1472.
Save, E., Poucet, B., Foreman, N., & Buhot, M. C. (1992). Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. *Behav Neurosci, 106*(3), 447-456.
Schaefer, T. L., Skelton, M. R., Herring, N. R., Gudelsky, G. A., Vorhees, C. V., & Williams, M. T. (2008). Short- and long-term effects of (+)-methamphetamine and (+/-)-3,4-methylenedioxymethamphetamine on monoamine and corticosterone levels in the neonatal rat following multiple days of treatment. *J Neurochem, 104*(6), 1674-1685.
Schaefers, A. T., Teuchert-Noodt, G., Bagorda, F., & Brummelte, S. (2009). Effect of postnatal methamphetamine trauma and adolescent methylphenidate treatment on adult hippocampal neurogenesis in gerbils. *Eur J Pharmacol, 616*(1-3), 86-90.
Schambra, U. B., Sulik, K. K., Petrusz, P., & Lauder, J. M. (1989). Ontogeny of cholinergic neurons in the mouse forebrain. *J Comp Neurol, 288*(1), 101-122.
Schnitzler, A. C., Mellott, T. J., Lopez-Coviella, I., Tallini, Y. N., Kotlikoff, M. I., Follettie, M. T., & Blusztajn, J. K. (2010). BMP9 (bone morphogenetic protein 9) induces NGF as an autocrine/paracrine cholinergic trophic factor in developing basal forebrain neurons. *Journal of Neuroscience, 30*(24), 8221-8228.
Schutova, B., Hruba, L., Pometlova, M., Deykun, K., & Slamberova, R. (2008). Impact of methamphetamine administered prenatally and in adulthood on cognitive functions of male rats tested in Morris water maze. *Prague Med Rep, 109*(1), 62-70.
Schutova, B., Hruba, L., Pometlova, M., & Slamberova, R. (2009). Impact of prenatal and acute methamphetamine exposure on behaviour of adult male rats. *Prague Med Rep, 110*(1), 67-78.
Schwab, C., Bruckner, G., Rothe, T., Castellano, C., & Oliverio, A. (1992). Autoradiography of muscarinic cholinergic receptors in cortical and subcortical brain regions of C57BL/6 and DBA/2 mice. *Neurochem Res, 17*(11), 1057-1062.
Schwarz, J. M., & McCarthy, M. M. (2008). Cellular mechanisms of estradiol-mediated masculinization of the brain. *J Steroid Biochem Mol Biol, 109*(3-5), 300-306.
Schwegler, H., Boldyreva, M., Linke, R., Wu, J., Zilles, K., & Crusio, W. E. (1996). Genetic variation in the morphology of the septo-hippocampal cholinergic and GABAergic systems in mice: II. Morpho-behavioral correlations. *Hippocampus, 6*(5), 535-545.
Schwegler, H., Boldyreva, M., Pyrlik-Gohlmann, M., Linke, R., Wu, J., & Zilles, K. (1996). Genetic variation in the morphology of the septo-hippocampal cholinergic

and GABAergic system in mice. I. Cholinergic and GABAergic markers. *Hippocampus, 6*(2), 136-148.

Scott, J. C., Woods, S. P., Matt, G. E., Meyer, R. A., Heaton, R. K., Atkinson, J. H., & Grant, I. (2007). Neurocognitive effects of methamphetamine: a critical review and meta-analysis. *Neuropsychol Rev, 17*(3), 275-297.

Seager, M. A., Johnson, L. D., Chabot, E. S., Asaka, Y., & Berry, S. D. (2002). Oscillatory brain states and learning: Impact of hippocampal theta-contingent training. *Proceedings of the National Academy of Sciences of the United States of America, 99*(3), 1616-1620.

Sekine, Y., Ouchi, Y., Sugihara, G., Takei, N., Yoshikawa, E., Nakamura, K., Iwata, Y., Tsuchiya, K. J., Suda, S., Suzuki, K., Kawai, M., Takebayashi, K., Yamamoto, S., Matsuzaki, H., Ueki, T., Mori, N., Gold, M. S., & Cadet, J. L. (2008). Methamphetamine causes microglial activation in the brains of human abusers. *Journal of Neuroscience, 28*(22), 5756-5761.

Sekine, Y., Ouchi, Y., Takei, N., Yoshikawa, E., Nakamura, K., Futatsubashi, M., Okada, H., Minabe, Y., Suzuki, K., Iwata, Y., Tsuchiya, K. J., Tsukada, H., Iyo, M., & Mori, N. (2006). Brain serotonin transporter density and aggression in abstinent methamphetamine abusers. *Arch Gen Psychiatry, 63*(1), 90-100.

Selmanoff, M. K., Goldman, B. D., & Ginsburg, B. E. (1977). Developmental changes in serum luteinizing hormone, follicle stimulating hormone and androgen levels in males of two inbred mouse strains. *Endocrinology, 100*(1), 122-127.

Semba, K., & Fibiger, H. C. (1988). Time of origin of cholinergic neurons in the rat basal forebrain. *J Comp Neurol, 269*(1), 87-95.

Semba, K., Reiner, P. B., McGeer, E. G., & Fibiger, H. C. (1988). Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. *J Comp Neurol, 267*(3), 433-453.

Shannon, H. E., & Eberle, E. L. (2006). Effects of biasing the location of stimulus presentation, and the muscarinic cholinergic receptor antagonist scopolamine, on performance of a 5-choice serial reaction time attention task in rats. *Behav Pharmacol, 17*(1), 71-85.

Siegal, D., Erickson, J., Varoqui, H., Ang, L., Kalasinsky, K. S., Peretti, F. J., Aiken, S. S., Wickham, D. J., & Kish, S. J. (2004). Brain vesicular acetylcholine transporter in human users of drugs of abuse. *Synapse, 52*(4), 223-232.

Siegel, J. A., Benice, T. S., Van Meer, P., Park, B. S., & Raber, J. (2011). Acetylcholine receptor and behavioral deficits in mice lacking apolipoprotein E. *Neurobiol Aging, 32*, 75-84.

Siok, C. J., Rogers, J. A., Kocsis, B., & Hajos, M. (2006). Activation of alpha7 acetylcholine receptors augments stimulation-induced hippocampal theta oscillation. *Eur J Neurosci, 23*(2), 570-574.

Skelton, M. R., Williams, M. T., Schaefer, T. L., & Vorhees, C. V. (2007). Neonatal (+)-methamphetamine increases brain derived neurotrophic factor, but not nerve growth factor, during treatment and results in long-term spatial learning deficits. *Psychoneuroendocrinology, 32*(6), 734-745.

Slamberova, R., Pometlova, M., & Charousova, P. (2006). Postnatal development of rat pups is altered by prenatal methamphetamine exposure. *Prog Neuropsychopharmacol Biol Psychiatry, 30*(1), 82-88.

Slamberova, R., Pometlova, M., & Rokyta, R. (2007). Effect of methamphetamine exposure during prenatal and preweaning periods lasts for generations in rats. *Dev Psychobiol, 49*(3), 312-322.

Smiley, J. F., Subramanian, M., & Mesulam, M. M. (1999). Monoaminergic-cholinergic interactions in the primate basal forebrain. *Neuroscience, 93*(3), 817-829.

Smith, L., Yonekura, M. L., Wallace, T., Berman, N., Kuo, J., & Berkowitz, C. (2003). Effects of prenatal methamphetamine exposure on fetal growth and drug withdrawal symptoms in infants born at term. *J Dev Behav Pediatr, 24*(1), 17-23.

Smith, L. M., LaGasse, L. L., Derauf, C., Grant, P., Shah, R., Arria, A., Huestis, M., Haning, W., Strauss, A., Della Grotta, S., Liu, J., & Lester, B. M. (2006). The infant development, environment, and lifestyle study: effects of prenatal methamphetamine exposure, polydrug exposure, and poverty on intrauterine growth. *Pediatrics, 118*(3), 1149-1156.

Smith, L. M., Lagasse, L. L., Derauf, C., Grant, P., Shah, R., Arria, A., Huestis, M., Haning, W., Strauss, A., Grotta, S. D., Fallone, M., Liu, J., & Lester, B. M. (2008). Prenatal methamphetamine use and neonatal neurobehavioral outcome. *Neurotoxicol Teratol, 30*(1), 20-28.

Smith, L. M., Lagasse, L. L., Derauf, C., Newman, E., Shah, R., Haning, W., Arria, A., Huestis, M., Strauss, A., Della Grotta, S., Dansereau, L. M., Lin, H., & Lester, B. M. (2011). Motor and cognitive outcomes through three years of age in children exposed to prenatal methamphetamine. *Neurotoxicol Teratol, 33*(1), 176-184.

Smith, M. L., Hale, B. D., & Booze, R. M. (1994). Calbindin-D28k immunoreactivity within the cholinergic and GABAergic projection neurons of the basal forebrain. *Experimental Neurology, 130*(2), 230-236.

Soderling, S. H., Langeberg, L. K., Soderling, J. A., Davee, S. M., Simerly, R., Raber, J., & Scott, J. D. (2003). Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. *Proceedings of the National Academy of Sciences of the United States of America, 100*(4), 1723-1728.

Sotty, F., Danik, M., Manseau, F., Laplante, F., Quirion, R., & Williams, S. (2003). Distinct electrophysiological properties of glutamatergic, cholinergic and GABAergic rat septohippocampal neurons: novel implications for hippocampal rhythmicity. *J Physiol, 551*(Pt 3), 927-943.

Sowell, E. R., Leow, A. D., Bookheimer, S. Y., Smith, L. M., O'Connor, M. J., Kan, E., Rosso, C., Houston, S., Dinov, I. D., & Thompson, P. M. (2010). Differentiating Prenatal Exposure to Methamphetamine and Alcohol versus Alcohol and Not Methamphetamine using Tensor-Based Brain Morphometry and Discriminant Analysis. *Journal of Neuroscience, 30*(11), 3876-3885.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev, 24*(4), 417-463.

Stafford, J. M., & Lattal, K. M. (2009). Direct comparisons of the size and persistence of anisomycin-induced consolidation and reconsolidation deficits. *Learn Mem, 16*(8), 494-503.

Steckler, T., & Holsboer, F. (2001). Interaction between the cholinergic system and CRH in the modulation of spatial discrimination learning in mice. *Brain Res, 906*(1-2), 46-59.

Storm-Mathisen, J. (1977). Localization of transmitter candidates in the brain: the hippocampal formation as a model. *Prog Neurobiol, 8*(2), 119-181.

Strupp, B. J., & Levitsky, D. A. (1995). Enduring cognitive effects of early malnutrition: a theoretical reappraisal. *J Nutr, 125*(8 Suppl), 2221S-2232S.

Struthers, J. M., & Hansen, R. L. (1992). Visual recognition memory in drug-exposed infants. *J Dev Behav Pediatr, 13*(2), 108-111.

Sullivan, P. M., Mezdour, H., Aratani, Y., Knouff, C., Najib, J., Reddick, R. L., Quarfordt, S. H., & Maeda, N. (1997). Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis. *J Biol Chem, 272*(29), 17972-17980.

Sulzer, D., Sonders, M. S., Poulsen, N. W., & Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol, 75*(6), 406-433.

Swerdlow, N. R., & Geyer, M. A. (1998). Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophr Bull, 24*(2), 285-301.

Taguchi, K., Atobe, J., Kato, M., Chuma, T., Chikuma, T., Shigenaga, T., & Miyatake, T. (1998). The effect of methamphetamine on the release of acetylcholine in the rat striatum. *Eur J Pharmacol, 360*(2-3), 131-137.

Takashina, K., Bessho, T., Mori, R., Kawai, K., Eguchi, J., & Saito, K. (2008). MKC-231, a choline uptake enhancer: (3) Mode of action of MKC-231 in the enhancement of high-affinity choline uptake. *J Neural Transm, 115*(7), 1037-1046.

Terplan, M., Smith, E. J., Kozloski, M. J., & Pollack, H. A. (2009). Methamphetamine use among pregnant women. *Obstet Gynecol, 113*(6), 1285-1291.

Thal, L. J., Gilbertson, E., Armstrong, D. M., & Gage, F. H. (1992). Development of the basal forebrain cholinergic system: phenotype expression prior to target innervation. *Neurobiol Aging, 13*(1), 67-72.

Thiel, C. M. (2003). Cholinergic modulation of learning and memory in the human brain as detected with functional neuroimaging. *Neurobiol Learn Mem, 80*(3), 234-244.

Thompson, P. M., Hayashi, K. M., Simon, S. L., Geaga, J. A., Hong, M. S., Sui, Y., Lee, J. Y., Toga, A. W., Ling, W., & London, E. D. (2004). Structural abnormalities in the brains of human subjects who use methamphetamine. *Journal of Neuroscience, 24*(26), 6028-6036.

Thrash, B., Karuppagounder, S. S., Uthayathas, S., Suppiramaniam, V., & Dhanasekaran, M. (2010). Neurotoxic effects of methamphetamine. *Neurochem Res, 35*(1), 171-179.

Tian, X., Sun, X., & Suszkiw, J. B. (1996). Developmental age-dependent upregulation of choline acetyltransferase and vesicular acetylcholine transporter mRNA expression in neonatal rat septum by nerve growth factor. *Neurosci Lett, 209*(2), 134-136.

Toth, K., Borhegyi, Z., & Freund, T. F. (1993). Postsynaptic targets of GABAergic hippocampal neurons in the medial septum-diagonal band of broca complex. *Journal of Neuroscience, 13*(9), 3712-3724.

Trulson, M. E., Cannon, M. S., Faegg, T. S., & Raese, J. D. (1987). Tyrosine hydroxylase immunochemistry and quantitative light microscopic studies of the mesolimbic dopamine system in rat brain: effects of chronic methamphetamine administration. *Brain Res Bull, 18*(2), 269-277.

Tsuchiya, K., Inoue, T., & Koyama, T. (1996). Effect of repeated methamphetamine pretreatment on freezing behavior induced by conditioned fear stress. *Pharmacol Biochem Behav, 54*(4), 687-691.

Usdin, T. B., Eiden, L. E., Bonner, T. I., & Erickson, J. D. (1995). Molecular biology of the vesicular ACh transporter. *Trends Neurosci, 18*(5), 218-224.

van Hest, A., Stroet, J., van Haaren, F., & Feenstra, M. (1990). Scopolamine differentially disrupts the behavior of male and female Wistar rats in a delayed nonmatching to position procedure. *Pharmacol Biochem Behav, 35*(4), 903-909.

van Meer, P., Acevedo, S., & Raber, J. (2007). Impairments in spatial memory retention of GFAP-apoE4 female mice. *Behavioural Brain Research, 176*(2), 372-375.

Vaucher, E., Reymond, I., Najaffe, R., Kar, S., Quirion, R., Miller, M. M., & Franklin, K. B. (2002). Estrogen effects on object memory and cholinergic receptors in young and old female mice. *Neurobiol Aging, 23*(1), 87-95.

Veng, L. M., Granholm, A. C., & Rose, G. M. (2003). Age-related sex differences in spatial learning and basal forebrain cholinergic neurons in F344 rats. *Physiol Behav, 80*(1), 27-36.

Villasana, L., Rosenberg, J., & Raber, J. (2009). Sex-dependent effects of (56)Fe irradiation on contextual fear conditioning in C57BL/6J mice. *Hippocampus, 20*(1), 19-23.

Villemagne, V., Yuan, J., Wong, D. F., Dannals, R. F., Hatzidimitriou, G., Mathews, W. B., Ravert, H. T., Musachio, J., McCann, U. D., & Ricaurte, G. A. (1998). Brain dopamine neurotoxicity in baboons treated with doses of methamphetamine comparable to those recreationally abused by humans: evidence from [11C]WIN-35,428 positron emission tomography studies and direct in vitro determinations. *Journal of Neuroscience, 18*(1), 419-427.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Franceschi, D., Sedler, M., Gatley, S. J., Miller, E., Hitzemann, R., Ding, Y. S., & Logan, J. (2001). Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. *Journal of Neuroscience, 21*(23), 9414-9418.

Volkow, N. D., Chang, L., Wang, G. J., Fowler, J. S., Leonido-Yee, M., Franceschi, D., Sedler, M. J., Gatley, S. J., Hitzemann, R., Ding, Y. S., Logan, J., Wong, C., & Miller, E. N. (2001). Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am J Psychiatry, 158*(3), 377-382.

Vorhees, C. V., Ahrens, K. G., Acuff-Smith, K. D., Schilling, M. A., & Fisher, J. E. (1994). Methamphetamine exposure during early postnatal development in rats: I. Acoustic startle augmentation and spatial learning deficits. *Psychopharmacology (Berl), 114*(3), 392-401.

Vorhees, C. V., Inman-Wood, S. L., Morford, L. L., Broening, H. W., Fukumura, M., & Moran, M. S. (2000). Adult learning deficits after neonatal exposure to D-methamphetamine: selective effects on spatial navigation and memory. *Journal of Neuroscience, 20*(12), 4732-4739.

Vorhees, C. V., Skelton, M. R., Grace, C. E., Schaefer, T. L., Graham, D. L., Braun, A. A., & Williams, M. T. (2009). Effects of (+)-methamphetamine on path integration and spatial learning, but not locomotor activity or acoustic startle, align with the stress hyporesponsive period in rats. *Int J Dev Neurosci, 27*(3), 289-298.

Vorhees, C. V., Skelton, M. R., & Williams, M. T. (2007). Age-dependent effects of neonatal methamphetamine exposure on spatial learning. *Behav Pharmacol, 18*(5-6), 549-562.

Voytko, M. L., Olton, D. S., Richardson, R. T., Gorman, L. K., Tobin, J. R., & Price, D. L. (1994). Basal forebrain lesions in monkeys disrupt attention but not learning and memory. *Journal of Neuroscience, 14*(1), 167-186.

Wainer, B. H., Steininger, T. L., Roback, J. D., Burke-Watson, M. A., Mufson, E. J., & Kordower, J. (1993). Ascending cholinergic pathways: functional organization and implications for disease models. *Prog Brain Res, 98*, 9-30.

Wallace, T. L., Gudelsky, G. A., & Vorhees, C. V. (1999). Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behavior, and stimulated dopamine release in the rat. *Journal of Neuroscience, 19*(20), 9141-9148.

Watson, M., Roeske, W. R., & Yamamura, H. I. (1986). [3H]pirenzepine and (-)-[3H]quinuclidinyl benzilate binding to rat cerebral cortical and cardiac muscarinic cholinergic sites. II. Characterization and regulation of antagonist binding to putative muscarinic subtypes. *J Pharmacol Exp Ther, 237*(2), 419-427.

Weissman, A. D., & Caldecott-Hazard, S. (1993). In utero methamphetamine effects: I. Behavior and monoamine uptake sites in adult offspring. *Synapse, 13*(3), 241-250.

West, M. J., Slomianka, L., & Gundersen, H. J. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec, 231*(4), 482-497.

Whiteaker, P., Jimenez, M., McIntosh, J. M., Collins, A. C., & Marks, M. J. (2000). Identification of a novel nicotinic binding site in mouse brain using [(125)I]-epibatidine. *Br J Pharmacol, 131*(4), 729-739.

Wilhelm, J., von Ahsen, N., Hillemacher, T., Bayerlein, K., Frieling, H., Kornhuber, J., & Bleich, S. (2007). Apolipoprotein E gene polymorphism and previous alcohol withdrawal seizures. *J Psychiatr Res, 41*(10), 871-875.

Williams, M. T., Blankenmeyer, T. L., Schaefer, T. L., Brown, C. A., Gudelsky, G. A., & Vorhees, C. V. (2003). Long-term effects of neonatal methamphetamine exposure in rats on spatial learning in the Barnes maze and on cliff avoidance, corticosterone release, and neurotoxicity in adulthood. *Brain Res Dev Brain Res, 147*(1-2), 163-175.

Williams, M. T., Brown, R. W., & Vorhees, C. V. (2004). Neonatal methamphetamine administration induces region-specific long-term neuronal morphological changes in the rat hippocampus, nucleus accumbens and parietal cortex. *Eur J Neurosci, 19*(12), 3165-3170.

Williams, M. T., Morford, L. L., Wood, S. L., Rock, S. L., McCrea, A. E., Fukumura, M., Wallace, T. L., Broening, H. W., Moran, M. S., & Vorhees, C. V. (2003). Developmental 3,4-methylenedioxymethamphetamine (MDMA) impairs sequential and spatial but not cued learning independent of growth, litter effects or injection stress. *Brain Res, 968*(1), 89-101.

Williams, M. T., Morford, L. L., Wood, S. L., Wallace, T. L., Fukumura, M., Broening, H. W., & Vorhees, C. V. (2003). Developmental D-methamphetamine treatment selectively induces spatial navigation impairments in reference memory in the Morris water maze while sparing working memory. *Synapse, 48*(3), 138-148.

Williams, M. T., Vorhees, C. V., Boon, F., Saber, A. J., & Cain, D. P. (2002). Methamphetamine exposure from postnatal day 11 to 20 causes impairments in both behavioral strategies and spatial learning in adult rats. *Brain Res, 958*(2), 312-321.

Wilson, P. W., Myers, R. H., Larson, M. G., Ordovas, J. M., Wolf, P. A., & Schaefer, E. J. (1994). Apolipoprotein E alleles, dyslipidemia, and coronary heart disease. The Framingham Offspring Study. *Jama, 272*(21), 1666-1671.

Winzer-Serhan, U. H. (2008). Long-term consequences of maternal smoking and developmental chronic nicotine exposure. *Front Biosci, 13*, 636-649.

Wood, S. C., Fay, J., Sage, J. R., & Anagnostaras, S. G. (2007). Cocaine and Pavlovian fear conditioning: dose-effect analysis. *Behavioural Brain Research, 176*(2), 244-250.

Woolf, N. J. (1991). Cholinergic systems in mammalian brain and spinal cord. *Prog Neurobiol, 37*(6), 475-524.

Wu, C. W., Ping, Y. H., Yen, J. C., Chang, C. Y., Wang, S. F., Yeh, C. L., Chi, C. W., & Lee, H. C. (2007). Enhanced oxidative stress and aberrant mitochondrial biogenesis in human neuroblastoma SH-SY5Y cells during methamphetamine induced apoptosis. *Toxicol Appl Pharmacol, 220*(3), 243-251.

Yamaguchi, T., Kuraishi, Y., Minami, M., Nakai, S., Hirai, Y., & Satoh, M. (1991). Methamphetamine-induced expression of interleukin-1 beta mRNA in the rat hypothalamus. *Neurosci Lett, 128*(1), 90-92.

Yamamoto, H., Kitawaki, J., Kikuchi, N., Okubo, T., Iwasa, K., Kawata, M., & Honjo, H. (2007). Effects of estrogens on cholinergic neurons in the rat basal nucleus. *J Steroid Biochem Mol Biol, 107*(1-2), 70-79.

Yamamoto, J. (1997). Cortical and hippocampal EEG power spectra in animal models of schizophrenia produced with methamphetamine, cocaine, and phencyclidine. *Psychopharmacology (Berl), 131*(4), 379-387.

Young, J. W., Crawford, N., Kelly, J. S., Kerr, L. E., Marston, H. M., Spratt, C., Finlayson, K., & Sharkey, J. (2007). Impaired attention is central to the cognitive deficits observed in alpha 7 deficient mice. *Eur Neuropsychopharmacol, 17*(2), 145-155.

Young, J. W., Finlayson, K., Spratt, C., Marston, H. M., Crawford, N., Kelly, J. S., & Sharkey, J. (2004). Nicotine improves sustained attention in mice: evidence for involvement of the alpha7 nicotinic acetylcholine receptor. *Neuropsychopharmacology, 29*(5), 891-900.

Yu, J., Cadet, J. L., & Angulo, J. A. (2002). Neurokinin-1 (NK-1) receptor antagonists abrogate methamphetamine-induced striatal dopaminergic neurotoxicity in the murine brain. *J Neurochem, 83*(3), 613-622.

Yu, L., & Liao, P. C. (2000a). Estrogen and progesterone distinctively modulate methamphetamine-induced dopamine and serotonin depletions in C57BL/6J mice. *J Neural Transm, 107*(10), 1139-1147.

Yu, L., & Liao, P. C. (2000b). Sexual differences and estrous cycle in methamphetamine-induced dopamine and serotonin depletions in the striatum of mice. *J Neural Transm, 107*(4), 419-427.

Yuhara, A., Ishii, K., Nishio, C., Abiru, Y., Yamada, M., Nawa, H., Hatanaka, H., & Takei, N. (2003). PACAP and NGF cooperatively enhance choline acetyltransferase activity in postnatal basal forebrain neurons by complementary induction of its different mRNA species. *Biochem Biophys Res Commun, 301*(2), 344-349.

Zaborszky, L., Carlsen, J., Brashear, H. R., & Heimer, L. (1986). Cholinergic and GABAergic afferents to the olfactory bulb in the rat with special emphasis on the projection neurons in the nucleus of the horizontal limb of the diagonal band. *J Comp Neurol, 243*(4), 488-509.

Zaborszky, L., Gaykema, R. P., Swanson, D. J., & Cullinan, W. E. (1997). Cortical input to the basal forebrain. *Neuroscience, 79*(4), 1051-1078.

Zahalka, E. A., Seidler, F. J., Lappi, S. E., Yanai, J., & Slotkin, T. A. (1993). Differential development of cholinergic nerve terminal markers in rat brain regions: implications for nerve terminal density, impulse activity and specific gene expression. *Brain Res, 601*(1-2), 221-229.

Zhang, J. H., Sato, M., Araki, T., & Tohyama, M. (1992). Postnatal ontogenesis of neurons containing GABAA alpha 1 subunit mRNA in the rat forebrain. *Brain Res Mol Brain Res, 16*(3-4), 193-203.

Zhang, J. H., Sato, M., & Tohyama, M. (1991). Different postnatal development profiles of neurons containing distinct GABAA receptor beta subunit mRNAs (beta 1, beta 2, and beta 3) in the rat forebrain. *J Comp Neurol, 308*(4), 586-613.

Zhu, J. P., Xu, W., & Angulo, J. A. (2006). Methamphetamine-induced cell death: selective vulnerability in neuronal subpopulations of the striatum in mice. *Neuroscience, 140*(2), 607-622.

Zilles, K., Werner, L., Qu, M., Schleicher, A., & Gross, G. (1991). Quantitative autoradiography of 11 different transmitter binding sites in the basal forebrain region of the rat--evidence of heterogeneity in distribution patterns. *Neuroscience, 42*(2), 473-481.

Zule, W. A., Costenbader, E. C., Meyer, W. J., Jr., & Wechsberg, W. M. (2007). Methamphetamine use and risky sexual behaviors during heterosexual encounters. *Sex Transm Dis, 34*(9), 689-694.