Abstract

Title of Thesis: **The role of fast spiking parvalbumin interneurons in prefrontal mediated cognition.**

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In a constantly changing environment, the ability to shift from one learned behavioral strategy to another more adaptive strategy is imperative. Research suggests there may be common underlying causes for the similar cognitive etiologies observed in many psychiatric disorders. One of these causes appear to be alterations in cortical GABAergic tone in the prefrontal cortices, particularly in the Orbital Frontal Cortex (OFC) which is known for its role in reversal learning and the Medial Prefrontal Cortex (mPFC) which mediates a form of behavioral flexibility. We tested a mouse model of defective frontal lobe inhibitory GABAergic anatomy on cognitive tasks, including a mouse reversal/set-shift test and fear-conditioning paradigm. We used several lines of mice: a mouse lacking the urokinase plasminogen activator receptor (*uPAR*) gene with a decreased GABA interneuron phenotype, a hepatocyte growth factor/scatter factor (HGF/SF) overexpressing mouse (*Gfap-HGF*), and a cross between the $uPAR^{-/-}$ and the *Gfap-HGF* mice, in which the interneuron deficit appears to be corrected. We have also developed a mouse serial reversal task in which we can record *in vivo* single unit activity in awake

behaving animals, to evaluate murine OFC function during reversal learning. Further, we have studied the role developmental alterations to cortical GABAergic tone play in reversal learning. Using a transgenic animal model to produce a specific frontal cortical GABAergic deficit in adult mice, we have assessed reversal learning through behavioral and *in vivo* psychological techniques, using single cell and local field potential recordings. By studying genetically altered mice, our research illuminated a common neural substrate between mouse circuitry and behavior and human cortical function in psychiatric disease states. We have shown that mice have functional and dissociable prefrontal cortical structures that match rat, primate and human data. We have shown that GABAergic deficits specific to PV^{+} interneurons impact prefrontal mediated cognition and that OFC and mPFC cortices are differentially sensitive to growth factor alterations. We further showed that high frequency oscillations are reduced in *Plaur* mice performing a serial reversal task, and that murine OFC plays a critical role in mediating behavioral flexibility in a first, but not subsequent reversals.

The role of fast-spiking parvalbumin interneurons in prefrontal mediated cognition

By

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Chapter 1

An Introduction to the function of the Prefrontal Cortex

1.1 General Hypothesis

Orbitofrontal cortex (OFC) has been shown to be an important neural substrate for mediating complex cognitive tasks such as reversal learning and has been shown to be similar to primate in terms of connectivity. Many human psychiatric disorders share common cognitive deficits in their phenomenology. Of these, deficits in behavioral flexibility are often noted, especially with regard to reversal learning. Interestingly, while disorders such as Autism Spectrum Disorder, epilepsy and Schizophrenia present a wide range of symptoms, they share some common core cognitive deficits which overlap with prefrontal-mediated cognition. They also share developmental deficits regarding alterations in GABA. Indeed, perturbations in the development of interneurons have been linked to all of the aforementioned disorders. These developmental alterations notably affect the fast-spiking parvalbumin expressing (FS PV^{+}) population of interneurons. It has been shown that this population of interneurons, due to their connectivity, firing rate and position within the neural structures are key cells in the generation of high frequency oscillations. High frequency oscillations, in turn, are generated in normal control humans and experimental animals during learning tasks, whereas humans with disorders such as schizophrenia have deficits in generating these kind of oscillations. Understanding the role of these cells in the OFC during learning will provide a more thorough understanding of the cognitive symptoms that must be overcome to fully treat humans who manifest similar cognitive deficits. *I hypothesize*

that murine OFC functions similarly to that in rodent and primate. Further, I hypothesize that fast-spiking PV^+ interneurons play an integral role in assisting OFC to guide animal behavior in the face of changing contingencies.

1.2 Overview of Dissertation Work

The orbitofrontal cortex (OFC) is an important neuroanatomical region for encoding outcome expectancies to cues by signaling the value of relevant cues and making associations between them. The OFC performs these functions through its reciprocal connections with the thalamus, amygdala and anterior cingulate cortex to directly modulate behavior. The OFC region is thus ideally suited to be the structure that directly mediates some elements of behavioral flexibility. This thesis will directly address the role the OFC plays in mediating a measure of behavioral flexibility: reversal learning. This thesis will also identify underlying perturbations to OFC that have particular psychiatric relevance, focusing on changes in the balance of GABAergic tone within OFC.

Research has shown important cognitive relevance for PV+ fast-spiking interneurons (Sohal et al 2009, Berke 2009, Nakazawa et al 2011) Neuroanatomical deficits regarding PV+ interneurons have been implicated as a potential source of, or contributing factor towards, many of the cognitive deficits observed in human psychiatric diseases, such as Schizophrenia, Tourette syndrome and frontal lobe epilepsy (Hill 2004, Verte et al 2005, Campbell et al, 2008, Sebe & Baraban 2010, Waltz & Gold, 2007, Weiler et al, 2009) This thesis addresses the need for a specific prefrontal-cognitive task in mice by developing a mouse variant of the Wisconsin card sorting task and validating it through

specific neurotoxic lesions to OFC and mPFC areas. This thesis further investigates cognitive deficits observed in animals which lack up to 70% of their PV+ interneurons in adulthood. While important cognitive deficits were uncovered, and were demonstrably rescued by the post-natal endogenous supplementation of HGF/SF, an understanding of the underlying neural circuitry was still lacking. Using our mouse model, we were able to record *in vivo* from mouse OFC in both control and mutant animals, giving us a rare glimpse at the real physiological changes that may underlie the anatomical deficits which manifest as cognitive deficits in humans with psychiatric disorders. Chapter 2 will investigate the behavioral task and NMDA lesions illuminating cognitive specificity of the task. Chapter 3 will cover the cognitive deficits observed in animals with decreased PV+ interneurons, as well as attempts to rescue this deficit postnatally. Chapter 4 will demonstrate the functional network changes that occur in a cortex with decreased PV+ population, potentially offering a glimpse of human disease states in a mouse model. The final chapter will summarize these findings and synthesize them into a more cohesive understanding of prefrontal cortical function and illuminate the role for fast-spiking PV+ expressing interneurons in regulating network dynamics and function.

1.3 Prefrontal Cortical Function

Cognitive rigidity or lack of flexibility is a common behavioral hallmark of many developmental disorders, including autism spectrum disorder (ASD), Tourette syndrome, Rett syndrome, and schizophrenia, as well as neurodegenerative disorders such as Parkinsonôs, Alzheôimerôs disease (Josiassen, Curry et al. 1983; Huntington

Gauntlett-Gilbert, Roberts et al. 1999; Pantelis, Barber et al. 1999; Baddeley, Baddeley et al. 2001; Hill 2004; Verte, Geurts et al. 2005). Patients that suffer frontal lobe deficiencies can easily learn and follow individual rules, but have great difficulty modifying their responses to new rules. For example, schizophrenic patients do not adapt normally to changes in their environments, especially in social and emotional contexts, and they exhibit an inability to modify responses in formal testing situations (Pantelis, Barber et al. 1999; Bowie and Harvey 2006). Performance deficits are observed on the Wisconsin Card Sorting test (WCST), in which the subject must sort a series of cards dependent upon changing rules, such as suit and color. These patients can learn simple rules for sorting the cards, but they are unable to change established behavior once the relevant category changes. In addition, schizophrenia patients are impaired in learning simple reversal tasks, in which the cues signaling correct and incorrect responses are switched (Waltz & Gold, 2007, Weiler et al, 2009, Leeson et al, 2009)

The components of the WCST and reversal learning employed in patient studies have been modified and adapted for research animal models. In agreement with the patient data, lesion studies in the primate and rat demonstrated that disruption in prefrontal areas (dorsal lateral prefrontal cortex) reduces the ability to shift between attentional sets (Dias, Robbins et al. 1996; Dias, Robbins et al. 1996; Birrell and Brown 2000). Similar conclusions about structural and functional analogies have been drawn about the parallel orbital frontal cortical (OFC) regions in primates and rats (McAlonan and Brown 2003; Schoenbaum and Roesch 2005).

Transgenic mice have provided a wealth of information on how individual genes regulate ontogeny and maintenance of the mammalian nervous system. Yet, linking animal responses to human behavior has been extremely challenging, invoking discussion on parallels of anatomy and behavioral testing and interpretation of the data (Gould and Gottesman 2006; Nestler and Hyman 2010). Whether the rodent has a prefrontal cortex has been questioned (Preuss 1995; Uylings, Groenewegen et al. 2003), with the consensus that analogous anatomy and function are present in rat and primate (Kolb 1984; Brown and Bowman 2002; Kolb and Robbins 2003; Groenewegen and Uylings 2010). The main questions pertained to comparable cortico-cortical connections as well as cortico-thalamic connections, cytoarchitecture and presence of appropriate neurotransmitter systems. Rodent OFC is anatomically comparable to primate OFC, with reciprocal corticocortical and corticothalamic projections as well as the presence of projections to anterior cingulate areas. Indeed, while the relative size and proportion of such connections are different, the presence of similar networks strongly implies a comparably similar role in rodent. Though research into primates has demonstrated a more complex level of organization of these connections and generally includes a larger number of slightly defined neuroanatomical regions(Preuss and Goldman-Rakic 1989; Preuss 2000), the rat appears to have a comparable size and complexity.

Importantly, originally disputed connections between Mediodorsal thalamic nuclei and the rodent PFC have been shown to exist, including projections to nearby structures such as motor and somatosensory regions as observed in primate (Uylings, Groenewegen et al. 2003; Van De Werd, Rajkowska et al. 2010). Importantly, strong reciprocal connections

from prefrontal regions to thalamic inputs are observed in primate prefrontal cortex, area 25. In rat, the same strong reciprocal connections are observed in the lateral OFC(Dermon and Barbas 1994), again supporting the idea that rodent PFC is comparable to primate. However, these data contrast with a retrograde tracing study that found few reciprocal connections between rat medial PFC and areas of the thalamus, suggesting that while certain regions of the rodent PFC are involved in primate-like networks of anatomical connectivity, other regions are not (Conde, Audinat et al. 1990; Conde, Maire-Lepoivre et al. 1995). Similar cytoarchitecture and chemoarchitecture is described for the C57Bl/6J strain of the mouse (Van De Werd, Rajkowska et al. 2010). Nonhuman primate research has shed light on many neuroanatomical regions which share similar anatomy and behavioral relevance to humans, the same can be said for rodent research. For a strong comparison, understanding the presence and projections of dopamine into the rodent PFC is essential, as this has been shown to play an important role in guiding primate PFC mediated cognition. While in primate regions which receive dopaminergic input all project back to important origins of the dopamine signals, notably the Ventral Tegmental Area (VTA), the case in the not the same in rodent. Rat cortex has a less diversified reciprocal connection network, with only the medial PFC and ACC projecting back to VTA, while entorhinal and other rostral cortical areas may receive some dopamine but have no reciprocal projections (Carr and Sesack 2000; Carr and Sesack 2000). Lesion data in rodents has implicated this region is not only anatomically comparable, but is responsible for mediating similar cognitive functions. OFC lesioned rats and monkey have difficulty reversing responses on go/no-go tasks. This similarity in

behavior opens the door for tasks/analysis involving transgenic mice, a powerful tool to investigate genes related to human disorders.

1.4 Role of OFC

Normal animal behavior depends upon many factors, including valid sensory information, affective states and normal processing of information. The OFC has been implicated in animals to guide responses by mediating the meaning of cues from the environment (Thorpe 1983, Otto & Eichenbaum 1992, Schoenbaum 1995, Schoenbaum 1995) . The OFC is known to regulate response inhibition in some cases. Studies have shown alterations to OFC through inactivation or lesion to cause preservative or impulse responding to cues (Clarke et al, 2005, Berlin et al, 2004, Schoenbaum et al, 2003). This behavior is a hallmark of addiction, where humans relapse back to former drug use behaviors, or show likelihood of relapsing when presented with familiar drug associated cues, such as needles, cocaine lines and environments (Kalivas et al 2006, Kalivas et al 2007). Studies have shown that OFC does not act alone in cue association. Instead, OFC is part of a complex network where basolateral amygdala (ABL) and OFC interact initially to form associations but where OFC functions to encode predicted outcomes of cues (Stalnaker et al, 2007, Schoenbaum et al 2000). Indeed, OFC in non human primate appears to be responsible for keeping online associations between past choices, alternative choices and predicted outcomes, as well as associations between these possibilities. (Preuss, Gondal et al. 1995)

Evidence linking OFC activity to outcome prediction comes from recording studies which show anticipatory firing to cues when animals have learned to associate those cues with rewards (Schoenbaum, Chiba et al. ; Schoenbaum, Setlow et al.). Recordings from awake behaving rats on an odor discrimination task demonstrated the neural activity in OFC increased after cue presentation and during a delay, signaling the eventual reward, or outcome related to that cue (Schoenbaum et al, 1995a, Schoenbaum et al, 1995b, Saddoris et al, 2005, Ichihara-Takeda & Funhashi 2007, Roesch et al, 2007). Given that OFC activity shows cue related outcome-expectancies, it is not surprising that lesion studies from humans, non-human primates and rats show preservative reversal impairments (Clarke et al, 2005, Berlin et al, 2004, Schoenbaum et al, 2003). Perseverative errors typically occur when an animal is required to modify its behavior when a task has changes, such that a previous behavioral response to a stimuli is no longer the correct response that will yield the rewarded outcome. OFC lesioned animals are able to acquire a discrimination initially, but when the task is changed, such that the subject must now choose the previously incorrect cue, as in a reversal task, then the lesioned subjects require significantly more trials than normal subjects to complete the task (Schneider et al, 2007, Schoenbaum et al, 2002).

Further evidence of the role of OFC in guiding behavior comes from the firing of cue-selective neurons, which initially are not selective for a particular stimuli, but after training, selectively alter their firing to a reward-predicting cue in an odor discrimination task. This cue-selective firing is eliminated during a reversal trial (Schoenbaum et al, 1999). The inflexible behavior in OFC lesioned animals may be due to the inability of the

OFC to use teaching signals to update cue valence, thereby altering behavioral responding to cues. This inflexibility can be seen in the anticipatory firing of OFC neurons related to expected outcomes. If OFC is not properly functioning, these teaching signals are not generated and proper responding to new cues, or inhibition of responding to other cues does not occur.

Proper development of inhibitory neurons is crucial for accurate modulation of neural circuits. As a result, alterations can lead to a variety of neurological deficits. As reviewed above, the orbitofrontal cortex (OFC) is an important neuroanatomical region for encoding outcome expectancies to cues by signaling the value of relevant cues and making associations between them. The OFC performs these functions through its reciprocal connections with the thalamus, amygdala and anterior cingulate cortex to directly modulate behavior. The OFC region is thus ideally suited to be the structure which directly mediated some elements of behavioral flexibility, and interneurons are likely a critical internal part of that regulatory process.

1.5 The role of GABAergic Interneurons in reversal learning

Given that OFC is implicated in cognitive inflexibility through lesion, drug studies and psychiatric disorders (Clarke et al, 2005, Berlin et al, 2004, Schoenbaum et al, 2003) and these studies in rats are validated through their similarities to human case studies, it is of interest to further understand the underlying mechanisms in decision making capabilities in OFC. Monkeys with OFC lesions take more trials to complete reversal tasks (Murray et al, 2007). OFC in monkey has been studied while manipulating several

neurotransmitter systems including dopaminergic and serotonergic systems (Ichihara-Takeda & Funahashi 2007, Liu et al, 2007). These results indicate that alterations to OFC generate the cognitive inflexibility seen through lesion studies with the serotonin changes, but not dopamine (Clarke et al, 2005, Liu et al, 2005). However, in a study regarding reversal learning and aging in rats, reversal learning was found to be mediated in a dopamine dependent manner in aged, but not young, rats (Mizoguchi, Shoji et al. 2010; Mizoguchi, Shoji et al. 2011).

In addition to dopamine, the inhibitory neurotransmitter GABA (-amino butyric acid) has been implicated in schizophrenia (Benes, McSparren et al. 1991; Lewis, Hashimoto et al. 2005; Torrey, Barci et al. 2005) along with several other developmental psychiatric disorders such as frontal lobe epilepsy, ASD and Tourette syndrome (Hill 2004, Verte et al 2005, Campbell et al, 2008, Sebe & Baraban 2010). GABA also modulates excitatory neurotransmission in the cerebral cortex (Ben-Ari 2007, Lydiard 2003) Disruption of the GABAergic interneuron population during development results in improper circuit formation and seizures in humans and mice (Levit, 2004, Benes & Berretta 2001, Cardin et al, 2009, Belforte et al, 2010) The ontogeny of GABAergic interneurons is mediated by various factors including the Met receptor tyrosine kinase (gene: *Met*), its ligand hepatocyte growth factor/scatter factor (HGF/SF; gene: *Hgf)* and the associated molecule urokinase plasminogen activator receptor, uPAR (also known as Plaur; gene: *Plaur*) (Bae, Bissonette et al. ; Powell, Mars et al. 2001; Powell, Campbell et al. 2003; Levitt, Eagleson et al. 2004; Bae, Bissonette et al. 2009; Martins, Shahrohk et al. 2011). Met binds HGF/SF, dimerizes and autophosphorylates, initiating many potential downstream

signaling pathways. However, in order to bind HGF/SF, HGF/SF needs to be activated. Pro-HGF/SF is secreted by astrocyte, sometimes with binding proteins like uPA, in the cortex and pro-HGF/SF is activated in several manners, one of which is through interaction with the uPAR. uPAR activates pro-HGF/SF into HGF/SF, which facilitates binding to the Met receptor. All three genes have been associated with ASD and schizophrenia (Campbell, Sutcliffe et al. 2006; Campbell, Li et al. 2008; Burdick, DeRosse et al. 2010). Mice with the targeted loss of functional *Met* in developing GABAergic interneurons display normal discrimination acquisition but impaired reversal learning ((Bissonette, Bae et al. ; Martins, Shahrohk et al. 2011). The reversal learning deficits are attributed to loss of parvalbumin expressing (PV+) interneurons in the frontal cortical areas. Studying the development of GABAergic interneurons in the cortex *via* HGF/SF and Met, has shown that increases in HGF/SF levels increase the number of interneurons in OFC, while loss of Met results in fewer interneurons (Powell et al, 2001, Martins et al, 2007).

One animal of particular interest is the *Plaur* knockout mouse which has up to a 50% decrease in GABAergic interneurons in frontal and parietal regions **(**Powell et al, 2003). This animal performs normally on basic sensory and motor tests, has normal amygdala and hippocampal function, but displays abnormal reversal and set-shift behavior (Powell et al, 2003, Chapter 3, Chapter 4). The reversal and set-shift behavior deficits correlate well with the anatomy, which shows near normal cell counts for both amygdala and hippocampus in adult **(**Eagleson et al, 2005, Bae et al 2007). Though prefrontal function depends upon several classes of neurotransmitter systems, several studies have implicated

GABA in proper OFC function. Infusion of muscimol ($GABA_A$ receptor antagonist) into OFC impaired performance on a reversal task in rats (Fuchs et al, 2004, Kim & Ragozzino, 2005), while in non-human primates muscimol infusion into ABL limits neuronal firing to cue selectivity by blocking devaluation **(**Wellman et al, 2005). These data match human studies, where decreases of PV+ neurons in prefrontal cortex demonstrate working memory deficits **(**Lewis & Moghaddam, 2006, Hashimoto et al, 2003).

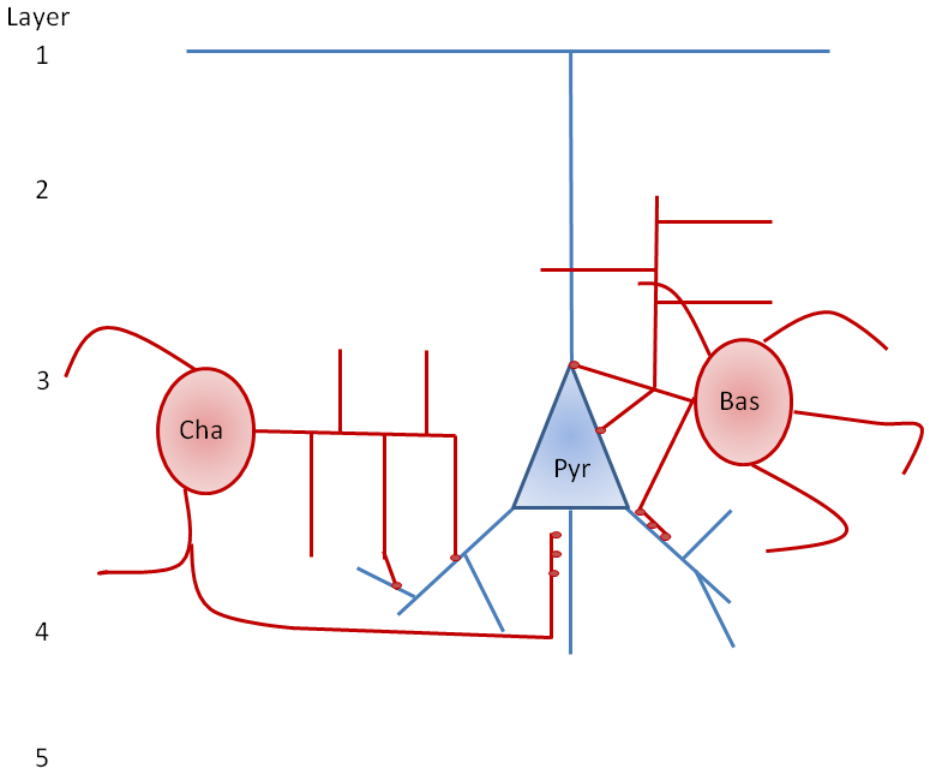
Additionally, long-term deficits in the GABAergic system in rodent and primate forebrain can lead to behavioral and cognitive symptoms commonly associated with mental retardation, mood disorders, schizophrenia, Tourette Syndrome , and ASD (Keverne, 1999; Benes and Berretta, 2001; Andres, 2002; Noebels, 2003; Gross and Hen, 2004; Levitt et al., 2004; Polleux and Lauder, 2004; Kalanithi et al., 2005; Steriade, 2005; DiCicco-Bloom et al., 2006; Leckman et al., 2006; Cristo, 2007).

PV^+ interneurons make up the largest individual subpopulation of GABAergic interneurons in the cerebral cortex and are distinguished by several characteristics including 1) repetitive firing rate 2) action potentials with a short duration due to a short after-hyperpolorization, 3) negative resting potential and 4) a low input resistance (Kubota et al., 1994; Kubota and Kawaguchi, 1994) and are quite different from their regular-spiking Calretinin (CR) or Somatostatin (SST) GABAergic partners (Kubota and Kawaguchi 1994, Blatow et al, 2005, Wonders and Anderson, 2006) These cells are fast-spiking not only due to synaptic input but also due to the presence of electrical

coupling through gap-junctions which have been hypothesized to be the reason for the high synchronization of PV^+ interneurons in the cortex. and are found in two general classifications, chandelier and basket cells (Fig 1.1) of which are axo-axonal and axo-somatic in nature, respectively. Chandelier cells, so named because of their shape, generally synapse onto axon initial segments using short vertical boutons known as cartridges, while basket cells generally synapse onto the soma and proximal dendrites of pyramidal neurons, appearing to encase them in a 'basket'. Interestingly, every portion of excitatory pyramidal neurons in the cortex (and in subcortical structures) are associated with GABAergic innervation of at least one subtype of interneuron.

Figure 1.1 Basic cell types of the cortex.

Representation of fast spiking PV^+ interneurons in the cerebral cortex. Cell in blue represents a pyramidal neuron (Pyr). Cells in red represent PV^+ expressing interneurons, notably Chandelier cells (Cha) and Basket cells (Bas). Chandelier cells typically synapse on axon terminals while basket cells synapse on proximal dendrites, soma and axon hillock of pyramidal neurons. Adapted from Lewis, Hashimoto and Volk (2005)



Other groups of GABAergic interneurons are described as axo-dendritic and disinhibitory synapses which synapse primarily on the dendrites of cortical pyramidal neurons. However, GABAergic interneurons of this group also have very wide axonal arborization, and have been known to synapse also on non-pyramidal cells, including the previously mentioned PV^+ interneurons and other cortical non-pyramidal neurons. This includes calretinin positive neurons which have been found to synapse on both the soma and proximal dendrites of PV^+ interneurons (Kubota et al, 1994). Of great importance is the excitatory input to GABAergic interneurons. Within the cortex, fast spiking interneurons tend to receive a lot of input from mid-layers of the cortex while slower spiking GABAergic interneurons tend to receive input from lower cortical layers. Fast spiking PV^+ interneurons are again of particular interest, as they receive the majority of the extra-cortical excitatory inputs, namely from the thalamus whereas regular spiking and other GABAergic interneurons receive very little or no thalamic input (Gibson et al, 1999). PV+ interneurons also co localize with dopamine receptors, specifically D1 receptors (Muly et al 1998, Davidoff and Benes, 1998). This contrasts with other GABAergic interneuron subtypes in the cortex which co localize with both D1 and D2 receptors. Thus, GABAergic interneurons not only regulate excitatory pyramidal neurons in the cortex, but potentially also regulate themselves.

The combination of shape, connectivity, firing rate and ability to inhibit activity via release of GABA leave fast-spiking PV+ interneurons in a potentially important position (Fig. 1.2). Information traveling down distal dendrites can quickly and efficiently be silenced or altered by activity of PV+ interneurons on proximal dendrites. Alternatively,

depolarized axon terminals may find the time of their depolarization shortened or ablated by fast-spiking PV+ interneurons. Ultimately, PV+ interneurons may play an even greater role in silencing a pyramidal cell's activity, as a great many synapses are found on the soma of pyramidal cells.

1.6 Neural activity in the OFC

While lesion, genetic and pharmacological interventions allow us to probe the function of OFC, understanding how OFC processes information in real time is important to fully grasp how it guides behavior. OFC studies in rat demonstrate OFC neurons can mimic activity patterns of dopaminergic neurons, representing reward prediction (Takahashi, 2009). Though OFC appears integral in representing the outcome of particular cues, it also plays a role in inhibiting responses to non-associated cues (Burke et al, 2009). These data demonstrate another important role for OFC, not simply as an outcome representor but suggest OFC helps guide responding to new associations with old cues (Murray 2007, Furuyashiki et al 2008).

Recordings from awake behaving rats on an odor discrimination task demonstrated the neural activity in OFC increased after cue presentation and during a delay, signaling the eventual reward, or outcome related to that cue (Saddoris et al, 2005, Ichihara-Takeda & Funahashi, 2007, Roesch et al, 2007). Given that OFC activity shows cue related outcome-expectancies, it is not surprising that lesion studies from humans, non-human primates and rats show preservative reversal impairments (Clarke et al 2005, Berlin et al,

2004, Schoenbaum et al, 2003). OFC lesioned animals are able to acquire a discrimination initially, but when the task is changed such that the subject must now choose the previously incorrect cue, as in a reversal task, then the lesioned subjects require significantly more trials than normal subjects to complete the task (Schnider et al, 2007, Schoenbaum et al, 2002).

Further evidence of the role of OFC in guiding behavior comes from the firing of cue-selective neurons, which respond afterwards to a reward predicting cue in an odor discrimination task though the cue selective firing is eliminated during a reversal trial (Schoenbaum et al, 1999). Inflexible behavior observed in OFC lesioned animals may be due to the inability of the OFC to use teaching signals to update cue valence, thereby altering behavioral responding to cues.

While single unit recording is an effective method for understanding how ensembles of neurons in neuroanatomical areas, recording local field potentials (LFPs) are a broader measure of cortical networks (Chrobak & Buszaki, 1996). The LFP represents the total sum of a local network's changing voltage as well as incoming input from outside the local network, especially a local network's dendritic activity (Legatt et al, 1980, Kreiman et al 2006, Liu and Newsome 2006, Berens et al 2008). LFPs, therefore, allow researchers to record large populations of activity and can correlate neural activity with behaviors. In humans performing cognitively taxing working memory tasks and reversal of cue associations, high frequency oscillations are generally observed (Palva et al 2009, Williams & Boksa, 2010). Indeed, deficits in high frequency oscillations may be a

predictor of future cognitive impairments (Missonnier et al, 2010). As we know fast-spiking PV^+ cells may be responsible for generating these oscillations (DeFelipe 1999, Fukuda and Kosaka 2000, Fukuda 2006, Jones 2010) we can hypothesize that they play an important role in many human psychiatric disorders.

Recording *in vivo* in mice is a new and burgeoning field. Indeed, recordings from multiple sites of the mouse brain have demonstrated both the feasibility of recording in mice, the similarities with rat and thus, opened the doors for transgenic experimentation (Dzirasa et al, 2010, Burkhardt, 2009, Nguyen et al, 2009). In mice, awake recordings in the PV-deltaGlurR-A mice, in which the AMPA receptor mediating innervations of PV+ cells is diminished, demonstrated that the hippocampal PV^+ cells were integral in the coordination of pyramidal neurons to generate theta rhythms (Racz et al, 2009). Gamma oscillations can also be suppressed or driven using optogenetic techniques *in vivo*, which impact local excitatory neurotransmission (Sohal et al, 2009). Sigurdsson et al, 2010 used a mouse model of a chromosomal micro deletion, the Df(16)A+/- mouse, to demonstrate deficits in coherence between mouse PFC and hippocampus. Major deficits in terms of coherence between hippocampus and PFC were noted as they corresponded to behavioral performance on a T maze task. Similarly, Popescu et al 2009 demonstrated gamma frequency coherence between the striatum and amygdala as rats learned auditory cues and associated them with outcomes. Studies like these provide a growing framework to understand complex network interactions in the brain.

1.7 Behavioral Testing of Reversal learning

Over the past two decades, behavioral studies in the mouse have demonstrated that although significant strain differences are present, the laboratory mouse appears capable of performing many, if not all, of the cognitive tasks tested in rat and non-human primate (Owen, Logue et al. 1997; Paylor and Crawley 1997; Rossi-Arnaud and Ammassari-Teule 1998). Most recently, reversal learning and attentional set-shifting paradigms have been reported in numerous mouse models of human neuropsychiatric disorders.

Reversal learning in mice has been evaluated by modifying methods initially designed for the rat (see (Floresco and Jentsch 2011) for a current review of the rat literature), including spatial learning with mazes: Morris water maze, T-maze (Bannerman, Deacon et al. 2003) and eight-arm maze (El-Ghundi, O'Dowd et al. 2003) ; with a two-choice digging task; and with operant learning equipment, including the go/no-go (Schoenbaum, Setlow et al. 2003; Kruzich and Grandy 2004) and delayed non-match-to-position task (Krueger, Howell et al. 2006) or visual discrimination paradigms (Bussey, Muir et al. 1997; Chudasama and Robbins 2003; Brigman, Bussey et al. 2005). The two-choice digging task and the touchscreen visual discrimination paradigm have been most popular, especially when assessing both reversal and attentional set-shifting abilities. Both tasks rely on stimulus-reward learning, with the reward being a morsel of food for the food-deprived subject. Reversal learning involves the OFC, dorsal striatum, and amygdala, while set-shifting requires intact medial wall structures (anterior cingulate, prelimbic and infralimbic cortex), amygdala and dorsomedial striatum (Bussey, Everitt et al. 1997; Bussey, Muir et al. 1997; Birrell and Brown 2000; McAlonan and Brown 2003;

Schoenbaum, Setlow et al. 2003; Kim and Ragozzino 2005; Ragozzino 2007; Stalnaker, Franz et al. 2007; Tait and Brown 2007; Tait and Brown 2008). Therefore, the evaluation of reversal learning and set-shifting within the same framework can be informative about multiple areas in the frontostriatal circuitry.

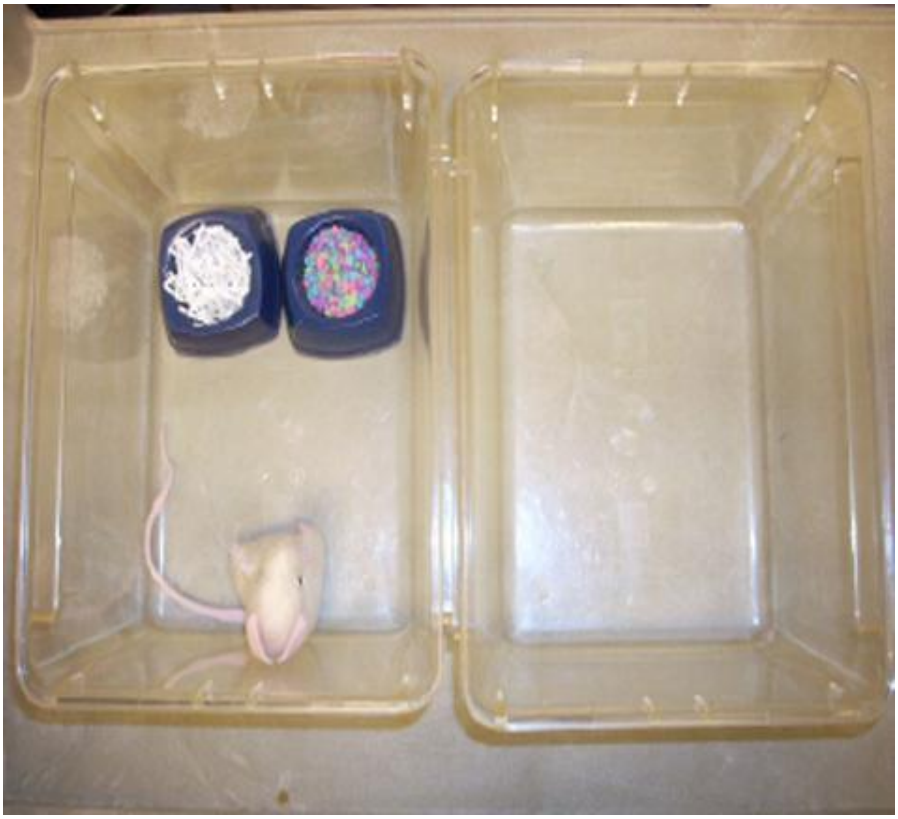
For reversal learning, the mouse must learn to discriminate between two cues. In the touchscreen task, the subject is trained to select between two images, and correct choices are rewarded (Bussey, Everitt et al. 1997; Brigman, Bussey et al. 2005). Once the mouse has reached criterion, usually 85% correct choices, the cues are reversed, such that the previously rewarded image is incorrect, and the previously incorrect image is now rewarded. Preservative errors, those that are contextually inappropriate or an unintentional repetition of the response, as defined by (Crider 1997), are used as a measure to cognitive inflexibility. Unlike maze tasks, the touchscreen requires little movement and can be used to evaluate mice with motor deficits (Morton, Skillings et al. 2006). Data from multiple mouse strains, genetic mutants and pharmacological manipulations are forming a basis to validate the test as animal model of prefrontal cognition.

Common mouse strains have known behavioral differences due to their unique genetic alleles and modifiers. The majority of cognitive testing is performed on the C57Bl/6J (B6) line or congenic mice which have been backcrossed to the B6 background for at least 10 generations. The choice of genetic background can greatly influence behavioral outcome and is critical when comparing across studies.

An alternative to the touchscreen or maze tasks is the two cup digging task based on naturalistic foraging. Two cups contain scented digging medium and a food reward (see Fig.1.2). The rule is set by one of the dimensions, odor or digging medium, and the rodent chooses the cup with the correct odor (or digging medium). Throughout the discrimination trials, the correct cue (i.e. odor) is randomly paired with the other irrelevant dimension (digging medium). For the reversal task, the correct cue (i.e. odor 1) is incorrect, and the previously incorrect cue (i.e. odor 2) is rewarded. Originally designed for rats, lesions in rat OFC lead to impaired performance (McAlonan and Brown 2003). Several successful adaptations show robust performance in mice (Colacicco, Welzl et al. 2002; Garner, Thogerson et al. 2006), with lesions to mouse OFC specifically altering reversal learning. Like the touchscreen task, the two cup digging task has been used in rodents to study the genetic and neurotransmitter alterations in research regarding cognitive elements observed in human psychiatric disorders.

Figure 1.2 Two cup digging task setup.

Depiction of the basic behavioral setup. The mouse is placed in the empty holding chamber adjacent the testing chamber. During a trial, the mouse is placed into a testing chamber with two bowls, each containing different samples of odorized media. A baited bowl is randomized in position (Left or Right). Once the mouse has located the food reward and eaten it, he is placed in the holding chamber and the media bowls changed.



1.8 Behavioral Testing of Attentional set-shifting

Multiple human neuropsychiatric conditions report poor performance on the WCST (Grant and Berg 1948), which requires the subject to alter the response strategy and use previously irrelevant information to solve the new set of problems. The main measurement of the WCST is the ID/ED (intradimensional/extradimenional) shift, the difference in the number of trials required in changing strategy from using the same type of cues (in the intradimensional shift (IDS) discrimination) to the other (previously irrelevant) type of cue (termed an extra-dimensional shift, EDS). In control subjects, the ID/ED shift requires more trials to criterion than a shift between two consecutive IDS problems. If the number of trials to solve the EDS problem is not significantly greater than the previous IDS problem, then the data are interpreted as the lack of formation of the attentional set. Hence, the ID/ED shift is the metric to compare strategy shifting in animal models of human disease.

Neurotoxic lesions in monkeys using an automated visual discrimination analogue of the WCST revealed the prefrontal cortex as a critical neural substrate for the attentional set-shift (Dias, Robbins et al. 1996; Dias, Robbins et al. 1996). The impairments observed in the non-human primates were similar to abnormal responses reported in humans with prefrontal damage (Goldstein et al 2003, Chase et al 2007, Tsuchida et al 2010) The automated visual discrimination test has been adapted for the rat and mouse to test attention, along with discrimination and reversal learning (Bussey, Muir et al. 1997; Brigman, Bussey et al. 2005). Testing in the male B6 mouse did not yield the ID/ED shift, leading to the conclusion that mice were not capable of complex learning, such as

attentional set shifting (Brigman, Bussey et al. 2005). However, by including additional discrimination tests, the same group has shown a significant ID/ED shift in mice: the *Reelin* heterozygote on the B6C3Fe background (Brigman, Padukiewicz et al. 2006). *Reelin* haploinsufficiency did not impair set-shifting.

A more common version of the WCST for rodents is the two cup digging task (Colacicco, Welzl et al. 2002; Garner, Thogerson et al. 2006). Originally designed and validated for rats, lesions to the medial wall of either rat or mouse lead to impaired performance (Birrell and Brown 2000; Bissonette, Martins et al. 2008). The mouse version of the task employs multiple days of testing in order to avoid satiety of the reward and to accommodate the ethological differences between species. The initial adaptation demonstrated an ID/ED shift for males of the B6 strain, but not for 129/SvEv or first generation of the B6x129/SvEv cross (Colacicco, Welzl et al. 2002); again questioning whether mice are capable of forming the attentional set. Subsequent studies demonstrated that increasing the number of discriminations problems, either with additional new problems or with overtraining, led to the formation of the attentional set (Garner, Thogerson et al. 2006; Bissonette, Martins et al. 2008). Thus, with either the touchscreen or two cup choice task, mice are able to form an attentional set, as defined by the ID/ED shift, but mice require additional discriminations, as compared to the rat versions of the task.

Several adaptations of the two cup digging tasks with numerous odors, media textures and configurations have been reported to assess the role of pharmacological and genetic

manipulations. Acute administration of subchronic doses of PCP altered the ID/ED shift at the highest dose, but the control and low dose B6 males did not show a significant ID/ED shift (Laurent and Podhorna 2004). A possible explanation for lack of formation of the attentional set in the control mice is that only three discrimination problems were presented, and the high dose of PCP strengthened the association between dimension and reward on the compound discriminations, inadvertently forming the attentional set. A similar disparity in formation of the attentional set in control and treated mice was reported with acetylcholinesterase inhibitor diisopropylfluorophosphate (DFP); no ID/ED shift was observed, but there was a significant increase in trials to criterion for the DFP treated group (Levi, Kofman et al. 2008). In a task which included two intradimensional shift and three reversals, the attentional set formation was impaired by ketamine, but reversed with the addition of sertindole (Kos, Nikiforuk et al. 2010). However, the major summary of all of this literature reveals that mice are viable models for testing attentional set-shifting, though there are apparent species differences in terms of the numbers of trials, task exemplars and repeats of behaviors that are required for the formation of an attentional set.

Chapter 2

Dissociable roles of the murine Medial Prefrontal Cortex and Orbital Frontal Cortex.

2.1 Introduction

Cognitive inflexibility is a hallmark of many neuropsychiatric diseases, especially those that notably affect the prefrontal cortex. This cognitive rigidity is observed in developmental disorders ranging from autism to schizophrenia. While there are many cognitive tests for prefrontal cortical function, one of the best and most widely used is the Wisconsin Card Sorting Test (WCST) (Nyhus & Barcelo 2009) which allows the experimenter to test both reversal learning and attentional set-shifting, and provides a measure of an individual's ability to adapt a behavioral strategy to changing task contingencies.

Transgenic mouse models hold promise for elucidating the genetic basis of human neuropsychiatric disorders, including addiction, schizophrenia, autism spectrum disorders, and degenerative disorders. However, these diseases often involve changes in cognitive flexibility, dependent upon prefrontal cortical areas (Shad, Tamminga et al. 2006; Verdejo-Garcia, Bechara et al. 2006; Clarke, Walker et al. 2007; Thoma, Wiebel et al. 2007). Currently, there are no good mouse models for testing prefrontal function. Many behavioral tasks originally developed for the rat have been successfully modified

to assess similar function in the mouse (Crawley 2004). Here, we evaluated the rat reversal and set-shifting task of Birrell and Brown (Birrell and Brown 2000; Colacicco, Welzl et al. 2002; McAlonan and Brown 2003) for its suitability to test prefrontal cortical functioning in mice.

There is significant debate whether mice even exhibit key functions thought to be mediated by prefrontal areas in other species (Preuss 1995; Uylings, Groenewegen et al. 2003). For example, the ability to shift away from acquired affective and attentional sets is dependent upon prefrontal cortex (Dias, Robbins et al. 1996; Birrell and Brown 2000; McAlonan and Brown 2003; Clarke, Dalley et al. 2004; Clarke, Walker et al. 2005; Floresco, Magyar et al. 2006). While mice do seem to form affective sets, as evidenced by increased trials to acquire simple reversals (Colacicco, Welzl et al. 2002; Lidow, Koh et al. 2003; Brigman, Bussey et al. 2005; Glickstein, Desteno et al. 2005; Izquierdo, Wiedholz et al. 2006), initial attempts concluded that mice do not seem to form attentional sets (Colacicco, Welzl et al. 2002; Brigman, Bussey et al. 2005). The addition of repetitive training (overtraining) (Garner, Thogerson et al. 2006) suggests that mice may be able to form attentional sets, although differently than rats and primates.

Classically, lesion studies have been used to assign function to specific brain areas. Frontal lobe lesions in the rat led to behavioral deficits comparable to those observed in primates (Kolb 1984; Schoenbaum, Setlow et al. 2003; Uylings, Groenewegen et al. 2003). Lesions to the OFC regions (Schoenbaum, Setlow et al. 2003; Schoenbaum and Roesch 2005) impaired goal-directed behaviors and reversal learning, whether the

choices were presented as visual stimuli in the primate (O'Doherty, Critchley et al. 2003; Remijnse, Nielen et al. 2005) or rat (Chudasama and Robbins 2003) or as odor-mediated rewards in the rat (McAlonan and Brown 2003; Schoenbaum, Setlow et al. 2003). Similar conclusions have been drawn about the parallel mPFC areas. Lesions to the mPFC areas reduced formation of an attentional set, as measured by the WCST in primates (Nelson 1976; Hansgen, Podhaisky et al. 1981; Dias, Robbins et al. 1996; Pantelis, Barber et al. 1999; Goldstein, Obrzut et al. 2004) or by the perceptual attentional set-shifting task in rats (Birrell and Brown 2000). In summary, lesion studies have demonstrated correlations between structure and function of prefrontal cortical areas.

The mouse literature, however, lacks lesion studies to show that specific correlations between prefrontal regions and cognitive function. Using a modified reversal learning and set-shifting task that was developed for the rat (Birrell and Brown 2000; McAlonan and Brown 2003), this chapter shows that mice form both affective and attentional sets and that their ability to shift away from these sets depends critically on the OFC and mPFC regions. These results are identical to those reported in rats and similar to those reported in marmosets, providing a behavioral model in which to assess prefrontal function in mice.

2.2 Results

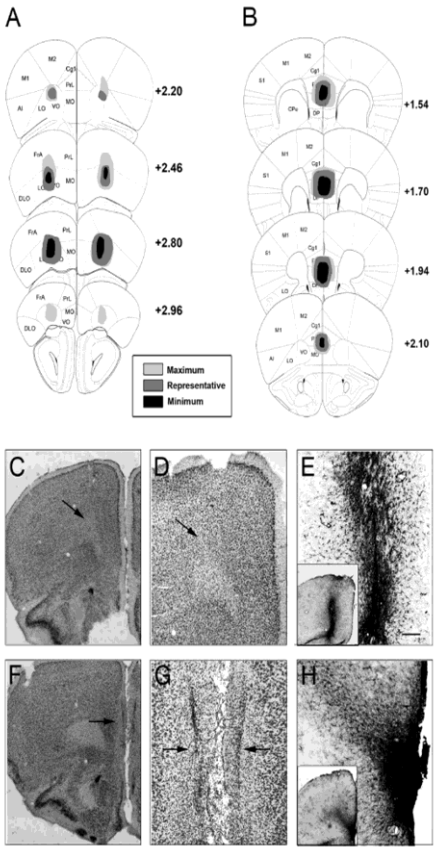
2.2.1 Area specific lesions impair reversal and set-shifting ability

Studies in rats and primates show that reversal learning and set-shifting reflect functions in specific prefrontal subdivisions. To assess parallel functioning in the murine

prefrontal cortex, the OFC or mPFC regions, as anatomically defined (Paxinos and Franklin 2001) were lesioned with NMDA. Sham-lesioned control mice received injections of saline vehicle. The neurotoxic lesions were characterized by anatomical methods. Of the group of 10 mice receiving NMDA lesions targeted to the OFC, 8 had damage within the OFC. For the mPFC-lesion group, 8 out of 10 mice had selective damage within the medial wall. Representations of the lesioned areas and the range of the extent of damage for these mice are shown in Fig. 2.1A and B. Cresyl violet histology demonstrated cell loss in both OFC (Fig. 2.1C, D) and mPFC (Fig. 2.1F, G) areas. Immunoreactivity for the gliotic scar marker, glial fibrillary acidic protein (GFAP) was observed in the lesioned OFC (Fig. 2.1E) and mPFC (Fig. 2.1H).

Figure 2.1 Lesion and sham histology.

Lesions demonstrate selective impairments in reversal learning and set-shifting ability. A, B Distribution of lesioned areas. Cresyl violet staining was used to determine lesioned regions. The maximum extent of lesions is denoted by the lightest gray shading, and the minimum extent of lesions in all mice is shown by black shading. Representative affected areas, present in at least 50% of the subjects, are shown by medium gray. Drawings were adapted from Paxinos and Watson atlas (Paxinos and Franklin 2001). C,D show cresyl violet staining demonstrating OFC damage in sham and lesioned animals, while F,G illustrates damage to the mPFC in sham and lesioned animals, respectively. E,F demonstrate immunoreactivity for gliotic scar using GFAP in lesioned OFC and mPFC areas, respectively.



2.2.2 Reversal Set-shifting task

Mice readily performed the discriminations on the digging task (see Tables 2.1 and 2.2, and also Appendix 1), as described in previous rat studies (Birrell and Brown 2000; McAlonan and Brown 2003), indicating mice respond to the rules set forth by these exemplars (Fig. 2.2). ANOVA indicated main effects of group ($F(2, 144) = 3.86$, $p = 0.0232$) and task ($F(7,144) = 12.92$, $p < 0.0001$). All groups learned the tasks, however, the mPFC-lesioned group required more trials to reach criterion on the Simple Discrimination (SD) (compare 13.5 ± 1.9 (mPFC) with 10.6 ± 1.0 (Sham) and 10.3 ± 1.2 (OFC), $p = 0.0017$, for both post-hoc comparisons). No differences among groups were found in the rest of the training discriminations, from Compound Discriminations (CD) in which one of two exemplars within the correct dimension is rewarded, regardless of whether it is with either of the other two exemplars in the non-rewarded dimension, and on subsequent CDs, called Intradimensional Shifts (IDS), so called because the rewarding pattern is the same as any CD, but the exemplar pairs are novel. (CD ïIDS IV, $p > 0.39$). The relevant dimension, either odor or medium, was counterbalanced within the experimental subjects, and the same results were obtained when dimension was considered as a variable ($F(1, 144)$, $p = 0.81$), suggesting equivalent valence for both odor and medium.

Table 2.1. Order of discrimination tasks.

The order of exemplars presented was the same for all mice, but the actual rewarded cues were randomized and counterbalanced within dimensions and between.

Task	Dimension		Exemplar combinations	
	*Relevant	Irrelevant	Correct	Incorrect
SD	Odor	Medium	O1, M1	O2, M1
CD	Odor	Medium	O1, M1, M2	O2, M1, M2
IDS I	Odor	Medium	O3, M3, M4	O4, M3, M4
IDS II	Odor	Medium	O5, M5, M6	O6, M5, M6
IDS III	Odor	Medium	O7, M7, M8	O8, M7, M8
IDS IV	Odor	Medium	O9, M9, M10	O10, M9, M10
IDS IVrev	Odor	Medium	O10, M9, M10	O9, M9, M10
EDS	Medium	Odor	M11, O11, O12	M12, O11, O12

Mice had more difficulty meeting criteria when the associations were reversed (Fig. 2.2, IDS IVrev). Sham mice demonstrated reversal learning, by the increased numbers of trials required for the IDS IVrev (15.6 ± 1.7) as compared to IDS IV (9.5 ± 0.8, $p < 0.0001$, Fig. 2.2). Mice were able to perform multiple reversal discriminations (Figure 2.4). Neurotoxic damage to the murine OFC impaired ability to reach criterion on reversal associations, (compare 22.4 ± 2.0 trials (OFC) for IDS IVrev, $p = 0.0017$). The effect was specific to the OFC area, as mice with mPFC-lesions performed similarly to sham animals (16.3 ± 2.3 trials, $p = 0.6959$), but were significantly different from the OFC-lesioned group ($p = 0.0057$).

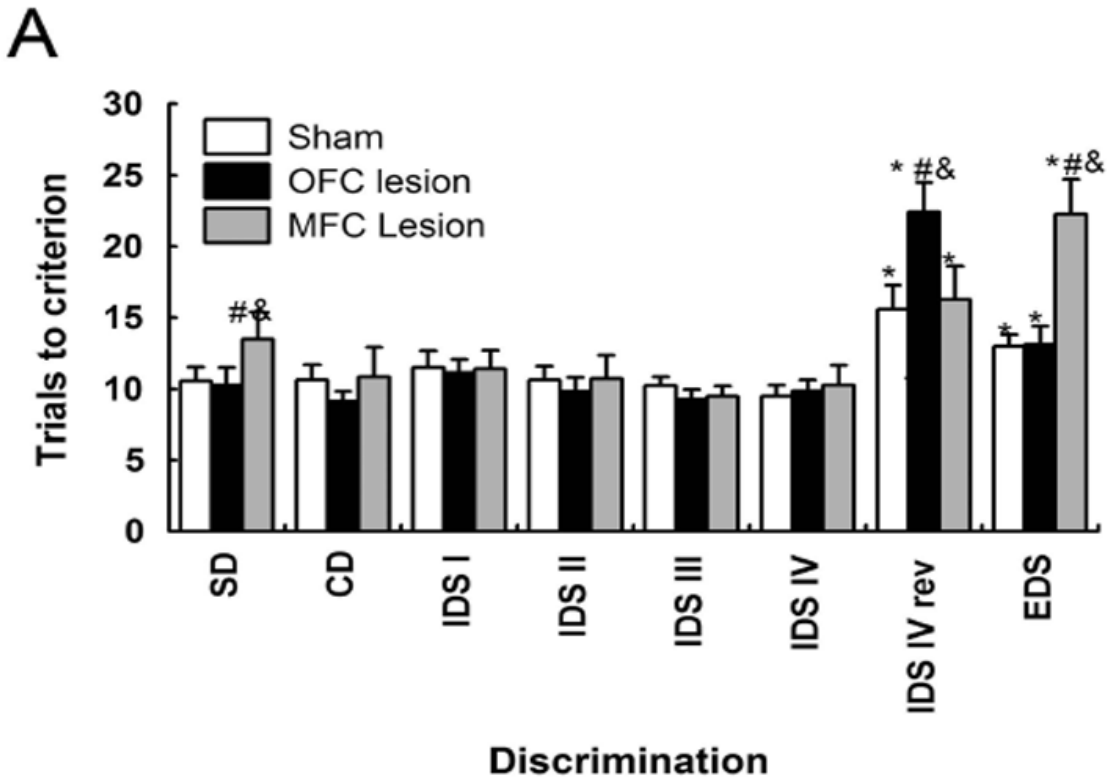
Table 2.2: Exemplar combinations.

The following are the exemplar combinations and order used for this and subsequent studies in this thesis. The order of presentation was the same for all animals, but the actual rewarded cue was counterbalanced and randomized between mice.

Pair	Exemplar	Dimension	
		Odor (O)	Medium (M)
1	1	Rosemary	Aspen bedding
	2	Cloves	Gravel
2	3	Cinnamon	Kaykob bedding
	4	Sage	Moss
3	5	Onion	Perlite
	6	Paprika	Bark
4	7	Garlic	Cat litter
	8	Coriander	Feathers
5	9	Thyme	Plastic pellets
	10	Black pepper	Cotton balls
6	11	Cumin	Shredded paper
	12	Cardamom	Packing peanut piece

Figure 2.2 OFC and mPFC lesions yield selective deficits.

A. shows trials to criteria from simple discrimination to the extra-dimensional shift. The number of trials to reach criterion was the same for training, whereas more trials were needed for the reversal learning (IDS IVrev) and for the ID-ED shift. The OFC-lesion group required more trials to complete the reversal task (IDS IVrev), and the mPFC-lesion required more trials for the ID-ED shift. The single asterisks (*) denote significant difference between IDS IV and either IDS IVrev, EDS. The pound sign (#) denote a difference between control sham group and either the OFC or mPFC-lesioned group for the specific discrimination, whereas the ampersand (&) signifies a difference between the mPFC and OFC lesioned groups for the specific discrimination. Significance is $p < 0.05$. Bars represent groups of n > 7 mice per group.



To test attentional set-shifting, the reward-predicting dimension was changed, from odor to medium (or vice versa) in the EDS discrimination. Sham mice demonstrated an increase in trials necessary to achieve criterion, comparing the IDS IV (9.5 ± 0.8; Fig 2.2) and the EDS (13.0 ± 0.8, $p < 0.002$). OFC-lesioned mice were similar to the sham group (13.1 ± 1.3 trials, $p = 0.65$) However, the formation of the attentional set was impaired in the mPFC-lesioned subjects, as the mPFC-lesioned mice needed 22.3 ± 2.4 trials to complete the EDS ($p < 0.0003$ compared to either sham or OFC).

We also compared the numbers of errors for each task (Fig. 2.3). While there were effects of task ($F(7,143) = 27.7$, $p < 0.0001$), *post-hoc* comparisons showed no differences between groups were observed in errors made during training phase (SD ï IDS IV). In agreement with the trials needed for criterion, the number of errors to complete the reversal task increased significantly from 0.75 ± 0.4 (IDS IV) to 5.13 ± 1.1 (IDS IVrev, $p < 0.0001$) in the sham mice. As expected, the number of errors made by the OFC-lesioned mice was greater than the sham and mPFC groups (OFC: 7.6 ± 1.1, sham: 5.1 ± 1.1, and MFC: 3.43 ± 0.78 errors). *Post-hoc* analysis indicated that the difference in errors between the sham and OFC groups was significant ($p = 0.0021$), but not between the sham and mPFC groups. In summary, only the OFC-lesioned group made more errors on the reversal task, indicating that mouse OFC area contributes to the formation of affective learning sets.

For the sham group, the number of errors (Fig. 2.3) also increased significantly for the EDS discrimination from 0.75 ± 0.4 (IDS IV) to (2.1 ± 0.4, $p < 0.02$), indicating set-

shifting. The numbers of errors between sham (2.13 ± 0.40) and OFC (1.86 ± 0.51) groups were the same for the EDS task (p = 0.4614). The errors made by the mPFC-lesioned mice were different from the sham and OFC lesioned groups on the set-shifting task (Fig. 1D, EDS, p = 0.0003). These data indicate that lesions to the mPFC selectively alter formation of the attentional set and impair shifting between dimensions.

Figure 2.3 Selective OFC/mPFC deficits are the result of increased errors.

A. The numbers of errors recorded were similar between all groups during the learning phase. The OFC-lesion group had significantly more errors than the sham group (#) or the mPFC group (&) for the IDS IVrev task, whereas the mPFC lesion group was similar to the sham group for the IDS IVrev. The mPFC-lesion group had significantly more errors on the EDS task, as compared to the sham group (#) or the OFC group (&). OFC lesions did not affect performance on the EDS task. Symbols signify statistical differences at $p < 0.05$.

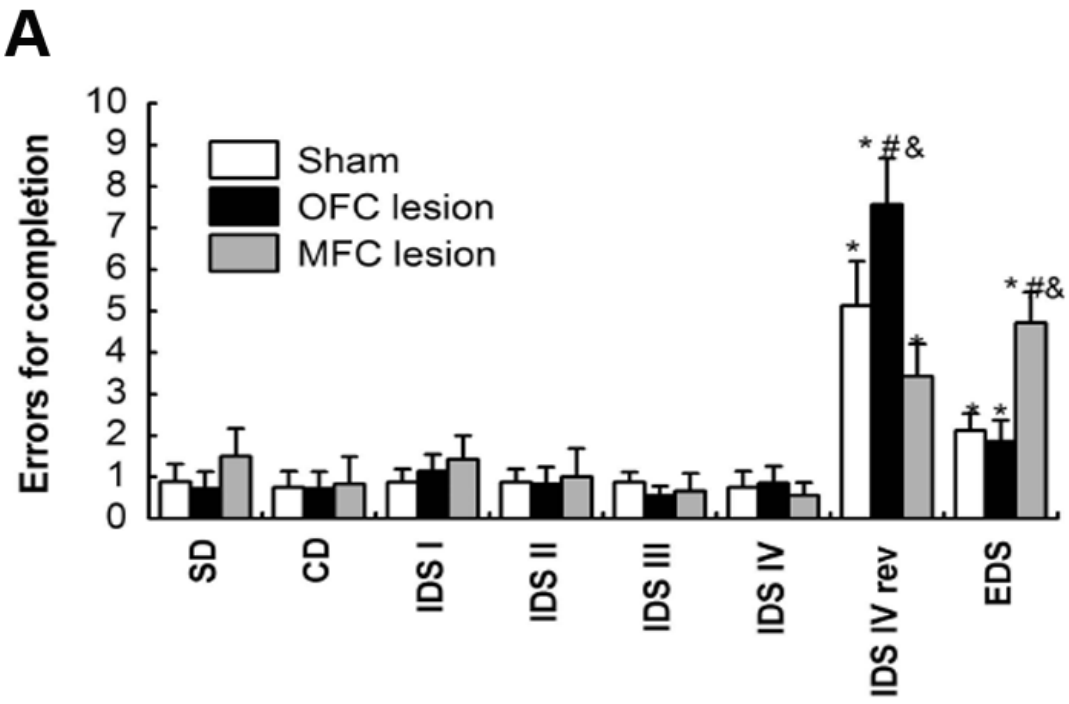
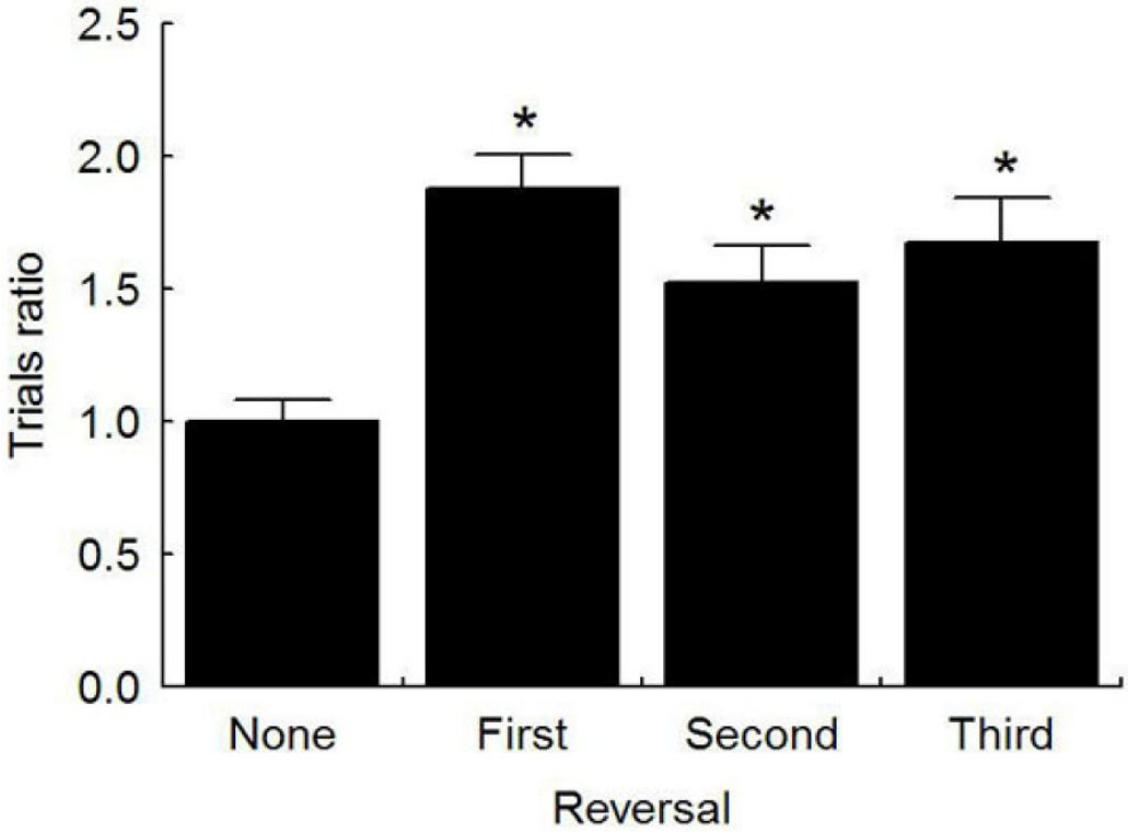


Figure 2.4 Reversal trials ratio verifies the difficulty of the reversal task.

Trials ratio portrays the fact that regardless of number or portion of the reversal, the reversal task is significantly more difficult than the previous discrimination.

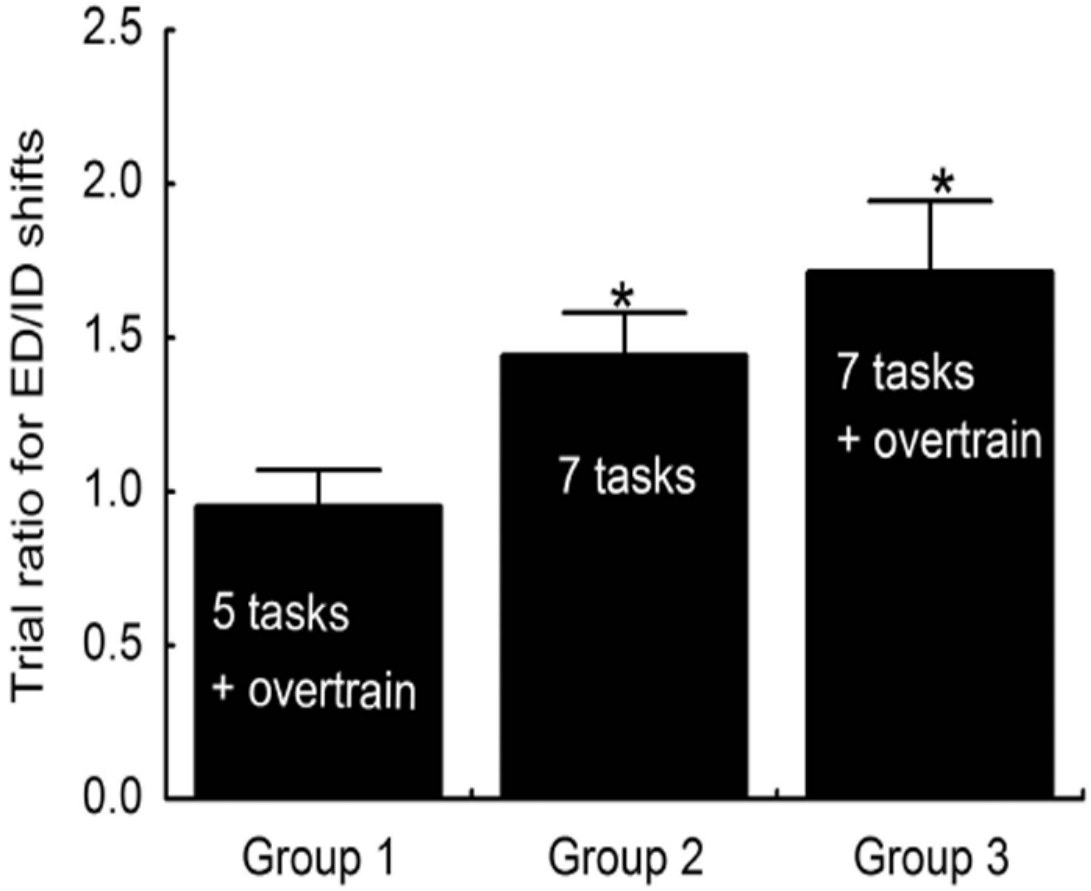


2.2.3 Set-shifting is dependent upon number of tasks and overtraining

In the lesion experiments (Fig. 2.2A), the mice were presented with seven discriminations (CD ï IDS IVrev) in succession, with the same relevant dimension prior to being challenged by the set-shifting rule. The paradigm demonstrated successful set-shifting ability. However, literature reports with fewer discriminations suggested that mice were unable to form attentional sets (Colacicco, Welzl et al. 2002), and that additional training may strengthen the formation of the attentional set (Garner, Thogerson et al. 2006). Initially, we performed the task as outlined in the literature (Birrell and Brown 2000; Colacicco, Welzl et al. 2002), and the mice demonstrated reversal learning (Figure 2.5), but not formation of the attentional set. Examination of the trial and latency data indicated that the mice did not learn in the same manner as rats, leading us to further examine multiple components of the task. We systematically examined the effects of task number, presence and location of reversals, and inclusion of additional training on evidence of set-formation and set-shifting in mice. To compare experimental groups, we calculated the ratio of trials needed for criterion for the EDS to those needed for the preceding IDS (Fig. 2.5). The main effect was observed with task number ($F(2,32) = 65.4$, $p < 0.0001$). The presence or location of reversal discriminations had no effect on set-shifting ability. The additional training increased the ratio of trials (Group 3, 1.72 ± 0.23, Fig. 2.5), but this increase was not significant (compared to Group 2, 1.44 ± 0.14, $p = 0.25$). Therefore, mouse set shifting ability is dependent upon the number of previously encountered discrimination problems within the same dimension. In summary, these experiments yielded outcomes that suggest that mice form affective and attentional sets to solve problems.

Figure 2.5 Determining sufficient number of tasks to form an attentional set.

We systematically examined the effects of problem number, presence and location of reversals, and inclusion of overtraining (additional problem sets) on set-shifting ability in mice. To compare experimental groups, we calculated the ratio of trials needed for criterion for the EDS to those needed for the preceding IDS. In Group 1, 5 separate tasks were insufficient to form as attentional set, as indicated by a ratio of ED/ID trials of 1. In Groups 2 and 3, 7 tasks were sufficient to form an attentional set. The main effect was observed with task number ($F(2,32) = 65.4$, $p < 0.0001$). The presence or location of reversal discriminations or additional problem sets had no effect on set-shifting ability. Asterisks denote significant different from Group 1 ($p < 0.04$).



2.3 Discussion

This study demonstrates that mice, like rats and primates, are capable of forming affective and attentional sets (Dias, Robbins et al. 1996). Sham-lesion control mice exhibited significant increases in the trials to acquire reversal learning and also shift attention between learning rules. These increases in trials show that the mice, like other species, attempt to learn rules to allow them to generalize from one problem to the next. When these rules are violated, by changes either in a previously acquired problem or attentional set, then mice require more trials to successfully complete the new problem, because they must overcome the influence of these normally helpful rules. Interestingly, in agreement with Garner *et al*. (Garner, Thogerson et al. 2006), mice appear to form these rules less efficiently than rats, and therefore additional presentations of similar problems are required to strengthen the formation of the attentional set. The murine response to rule changes is directly analogous to the difficulty experienced by rats and primate species when presented with reversal problems or set-shifts in similar tasks.

Furthermore, data presented in the current report show that, like rats and primates, the ability to overcome these rules depends in part on subdivisions within the frontal pole of the murine brain. Lesions to the OFC caused a selective deficit in reversal learning, indicating that the OFC-lesioned mice had more trouble than normal overcoming the affective rule. By contrast, lesions to the medial wall (mPFC) caused a selective deficit in set-shifting, indicating that the mPFC-lesioned mice had more trouble than normal switching the attentional rule. These results are identical to what has been reported in rats, where damage to the OFC and mPFC cause a double dissociation in impaired

reversal learning and set-shifting respectively (Birrell and Brown 2000; McAlonan and Brown 2003). Similarly in marmosets, it has been shown that OFC lesions impair reversal learning, while damage to lateral PFC disrupts set-shifting (Hansgen, Podhaisky et al. 1981; Dias, Robbins et al. 1996; Clarke, Walker et al. 2005). While rodent frontal areas do not share the anatomical complexities of the primate, these areas are defined based upon similarities in connectivity and function (Guldin, Pritzel et al. 1981; Uylings, Groenewegen et al. 2003). The behavioral deficits in affective and attentional sets exhibited by the mouse after circumscribed lesions to the frontal pole imply that the murine brain shares a subset of rule acquisition and problem solving abilities with the rat and primate brain. The availability of a mouse model enables rapid analysis of the cognitive consequences of genetic and developmental manipulations responsible for human neuropsychiatric disorders.

Our studies demonstrated that a single IDS discrimination is insufficient to form the attentional set in mice. However, training on multiple discriminations and exemplar sets with the same dimension yields consistent formation of the attentional set; additional IDS tests did not improve formation of the attentional set. The need for multiple discriminations is supported by response latency data that demonstrates a significant decrease only after four discriminations. This requirement may explain why our results differ from two prior reports, which have used such brief training procedures and reported an inability to generate attentional sets in mice (Colacicco, Welzl et al. 2002; Brigman, Bussey et al. 2005). Our results agree with Garner *et al.* (Garner, Thogerson et al. 2006), that additional presentation of the same dimension, strengthens the formation

of the attentional set. The cohort of mice used by Garner *et al.* included a mix of males and females of varying ages with the task performed over several months, while the data presented in this report are male mice. The difference in effect of overtraining may be due to inclusion of the female subjects, as females were observed to respond to the discrimination tasks significantly differently than their male counterparts (data not shown). Overall, mice perform the tasks similarly, but not identically, to their rat counterparts.

Here, the mice required the same range of numbers of trials on the discriminations to reach criterion as reported for rats (Birrell and Brown 2000; McAlonan and Brown 2003) and C57Bl/6 mice, from different sources (Colacicco, Welzl et al. 2002; Garner, Thogerson et al. 2006). The exemplars of texture and odor are easily discriminated by the rodents and require few trials over criterion to learn the initial training tasks, in contrast to two visual cues (Brigman, Bussey et al. 2005; Brigman, Padukiewicz et al. 2006; Izquierdo, Wiedholz et al. 2006) In many reported versions of this task, learning does not appear to be reflected by decreasing trials to criterion as more discriminations are presented (Birrell and Brown 2000: McAlonan, 2003 #462; Colacicco, Welzl et al. 2002; Tunbridge, Bannerman et al. 2004; Glickstein, Desteno et al. 2005; Black, Maclaren et al. 2006; Lapiz and Morilak 2006). However, a decrease in latency to choice was used as an indicator of improved performance (Colacicco, Welzl et al. 2002). Our data demonstrated a similar decrease. Thus, our mice performed the task similarly to rats and demonstrated learning by two measures: first, the decreased latency to choice on

multiple consecutive compound discriminations (CD ïIDS IV) and second, the increased trials for the reversal and set-shifting discriminations.

The mPFC-lesioned group demonstrated impaired learning on the first discrimination (SD), but similar performance on subsequent training discriminations. These results are in agreement with rat lesion studies using the continuous spatial-delayed alternation task (Schwabe, Enkel et al. 2004) and in instrumental conditioning (Ostlund and Balleine 2005). Data with mPFC-lesioned rats on the eight arm-radial maze (McDonald, Foong et al. 2007) and on this reversal set-shifting task after cocaine administration in rats (Black, Maclaren et al. 2006) show a similar trend, suggesting that impaired mPFC function can delay acquisition of a task. Thus, the mPFC-lesioned mice appeared to have delayed acquisition of the initial discrimination, but eventually learned the task, as the numbers of trials to criterion and response latencies were the same as sham controls in later discriminations.

The order of the presentation of the reversal task did not alter the ability to form the attentional set, also in agreement with overtraining concept that the EDS is dependent upon the repeated presentations of the relevant dimensions (Garner, Thogerson et al. 2006). Several details of our test are slightly different from previous reports, including different exemplar pairs due to availability or response. All of our materials were tested for equivalent valence, independent upon type of discrimination. Sand and dirt, which are very naturalistic media, were avoided because the mouse stopped digging in the medium to clean its whiskers. However, these changes do not appear to significantly

alter the ability of the mouse to perform the reversal learning or the set-shifting. The key factor is the number of presentations of the discriminations to strengthen the learning rules.

Proper functioning of the prefrontal cortical areas is dependent upon multiple neurotransmitter systems including catecholamines, serotonin, and GABA. Depletion of serotonin impairs OFC-mediated reversal learning in non-human primates (Clarke, Walker et al. 2005), whereas loss of dopamine in the OFC has no effect on reversal learning (Clarke, Walker et al. 2007). In the MFC, imbalances in catecholamines, mainly dopamine, impaired set-shifting in marmosets and rats (Crofts, Dalley et al. 2001; Tunbridge, Bannerman et al. 2004). In humans, loss of dopamine, along with GABA, has been implicated in decreased working memory (Lewis, Hashimoto et al. 2005; Hashimoto, Arion et al. 2008). Modulation of the balance of inhibitory to excitatory output appears to be critical in all species for proper prefrontal function (Wilson, O'Scalaidhe et al. 1994; Rao, Williams et al. 2000; Constantinidis, Williams et al. 2002; Schwabe, Enkel et al. 2004; Tunbridge, Bannerman et al. 2004; Kim and Ragozzino 2005; Black, Maclaren et al. 2006; Floresco, Magyar et al. 2006; Lapiz and Morilak 2006).

Chapter 3

Fast-spiking Parvalbumin interneurons mediate reversal learning

3.1 Introduction

Many psychiatric and neurological disorders present persistent neuroanatomical abnormalities in multiple brain regions that may reflect a common origin for a developmental disturbance (Benes et al., 1991; Lewis et al., 2005; Verte et al 2005, Campbell et al, 2008, Sebe & Baab et al 2010). In mammals, many of the local GABAergic inhibitory interneurons arise from a single subcortical source (Corbin 2001, Anderson 1997, Pleasure et al, 2000). Perturbations in the ontogeny of the GABAergic interneurons may be reflected in the adult by interneuron deficits in both frontal cerebral cortical and striatal regions (Powell et al 2003, Bissonette et al 2010) Disrupted GABAergic circuitry has been reported in patients with schizophrenia and frontal lobe epilepsy (Lewis et al., 2005; Piau et al, 2010), and may contribute to their associated impairments in behavioral flexibility (Woo et al 1997, Lewis 2000). The present study demonstrates that one type of behavioral flexibility, reversal learning, is dependent upon proper numbers of GABAergic interneurons. Mice with abnormal interneuron ontogeny have reduced numbers of parvalbumin-expressing GABAergic local interneurons in the orbitofrontal cortical and striatal regions and also have impaired reversal leaning (Bissonette 2010). Using a genetic approach, both the anatomical and functional deficiencies are restored with exogenous postnatal growth factor supplementation.

These results show that GABAergic local circuitry is critical for modulating behavioral flexibility. They also suggest that birth defects can be corrected by replenishing crucial growth factors.

In schizophrenia and frontal lobe epilepsy, the age of onset reflects a developmental origin, with disruptions in GABAergic neuron ontogeny as a possible cause (Porter, Brooks-Kayal et al. 2002; Tamminga, Hashimoto et al. 2004; Hashimoto, Arion et al. 2008). During embryogenesis, forebrain GABAergic neurons are generated subcortically and interneurons migrate to the cerebral cortex, hippocampus, amygdala and olfactory bulbs, while the medium spiny projection neurons and local interneurons remain in the striatum (Anderson, Marin et al. 2001; Letinic, Zoncu et al. 2002; Nery, Fishell et al. 2002; Haiat, Padilla et al. 2005 Marin, 2000 #12). Studies in transgenic mice report that perturbations in GABAergic ontogeny lead to anatomical deficits and abnormal behaviors, similar to those found in human psychiatric and neurological disorders (Stork, Ji et al. 2000; Powell, Campbell et al. 2003; Cobos, Calcagnotto et al. 2005).

Multiple molecules contribute to the ontogeny of the cerebral cortical GABAergic interneurons (reviewed in (Wonders and Anderson 2006). In particular, transgenic mice lacking the urokinase plasminogen activator receptor (*Plaur*) have selective loss of GABAergic interneurons in anterior cingulate and somatosensory cortical areas (Powell, Campbell et al. 2003). These defects are specific for the parvalbumin-expressing (PV^+) GABAergic interneuron subtype, whereas neurons expressing the somatostatin and calretinin markers are unaffected (Powell, Campbell et al. 2003; Eagleson, Bonnin et al. 2005). The cerebral cortical PV^+ interneuron populations are fast spiking cells that are

reported to be reduced in human epilepsy and schizophrenia (Ferrer, Oliver et al. 1994; Beasley and Reynolds 1997; Hashimoto, Arion et al. 2008). Therefore, the *Plaur* null mice display anatomical deficits observed in human disorders.

Previous reports have demonstrated that *Plaur* mice have grossly normal sensory and motor function, as well as exploratory behavior (Powell, Campbell et al. 2003). *Plaur* mice displayed increased anxiety, as measured by the light-dark avoidance and elevated plus maze tests. However, the localized interneuron defects in the *Plaur* mice suggest that behaviors relying on the frontal cortex may also be impaired. One task that evaluates prefrontal cortical function is reversal learning, which is a measure of behavioral flexibility or ability to adapt to the changing environment. In primates and rodents, reversal learning is dependent upon intact OFC and dorsal striatal regions (Dias, Robbins et al. 1996; McAlonan and Brown 2003; Brigman, Bussey et al. 2005; Bissonette, Martins et al. 2008). Local inhibitory circuitry for the OFC and striatum is provided by the GABAergic interneurons that arise embryonically, and interruptions in ontogeny are predicted to impair reversal learning. This study tests the hypothesis that the GABAergic interneuron deficits in the *Plaur* mice extend to the OFC and striatal regions and correlate with impaired performance on a reversal learning task.

In the absence of *Plaur*, reduced levels of hepatocyte growth factor/scatter factor (HGF/SF) and of its receptor Met appear to limit embryonic cell migration and survival (Powell, Mars et al. 2001; Bae, Bissonette et al. 2009). Thus, it is predicted that postnatal supplementation of HGF/SF may prevent the GABAergic interneuron loss and rescue the functional deficits observed in the adult. In this report our data demonstrate a role for

GABA in reversal learning, and a possible mechanism to correct deficits in neuropsychiatric disorders.

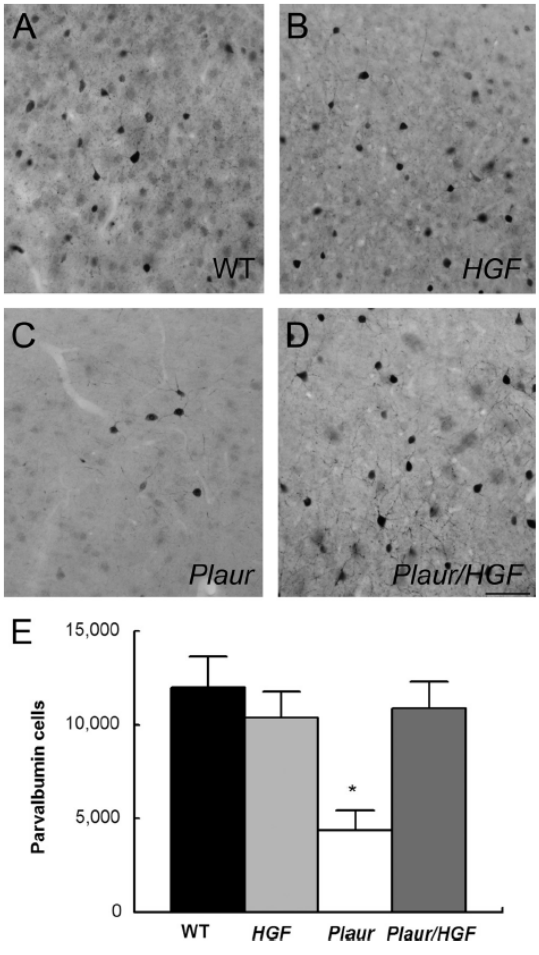
3.2 Results

3.2.1 HGF/SF levels alter forebrain interneuron numbers

Immunohistochemistry for PV shows a marked decrease in OFC neurons in the *Plaur* mouse (Fig. 3.1). Comparison of total PV^+ cells in the OFC demonstrates a main effect of genotype [$F(3,10) = 4.50$, $p = 0.03$]. The number of PV^+ cells in the *Plaur* mice is about 36% of WT cells (*Plaur*: 4,346 ± 1,090 cells, Fig. 1*C,E*, WT: 11,965 ± 1,675, Fig. 3.1*A,E* $p = 0.03$). The number of OFC PV^+ cells in the *HGF* mice (10,379 ± 1,568, Fig. 3.1*B,E*) is similar to that in WT mice ($p = 0.77$). Increased postnatal HGF/SF expression in the *Plaur* mice eliminates the deficit observed in *Plaur* (Fig. 3.1*D,E*), and the number of PV^+ cells in the *Plaur/HGF* mice (10,885 ± 1,413) is similar to that in WT mice ($p = 0.65$). The volumes of the OFC regions were similar in all genotypes [$F(3,10) = 0.72$, $p = 0.56$]. In all mice, the distributions of PV^+ cells are similar to that in WT mice. In summary, the deficit of PV^+ interneurons in the *Plaur* mice was corrected by the postnatal addition of HGF/SF, as shown in the *Plaur/HGF* mice.

Figure 3.1. HGF/SF levels affect the number of GABAergic neurons in OFC.

Immunohistochemistry of PV cells in OFC of adult WT A, *HGF* B, *Plaur* C, and *Plaur/HGF* mice D. Bar =200µm. E The numbers of PV^+ cells were stereologically counted in orbital frontal cortex. Each ba SEM. The PV^+ cells were severely decreased in the *Plaur* mice. Over expressing HGF/SF increases the number of interneuron as compared to that in WT mice. In the *HGF* mice, PV^+ cells are similar to those in WT littermates. An asterisk denotes statistical significance as compared to WT mice ($p < 0.05$). (From Bissonette 2010, results from this figure were performed by Mihyun Bae as part of her thesis).



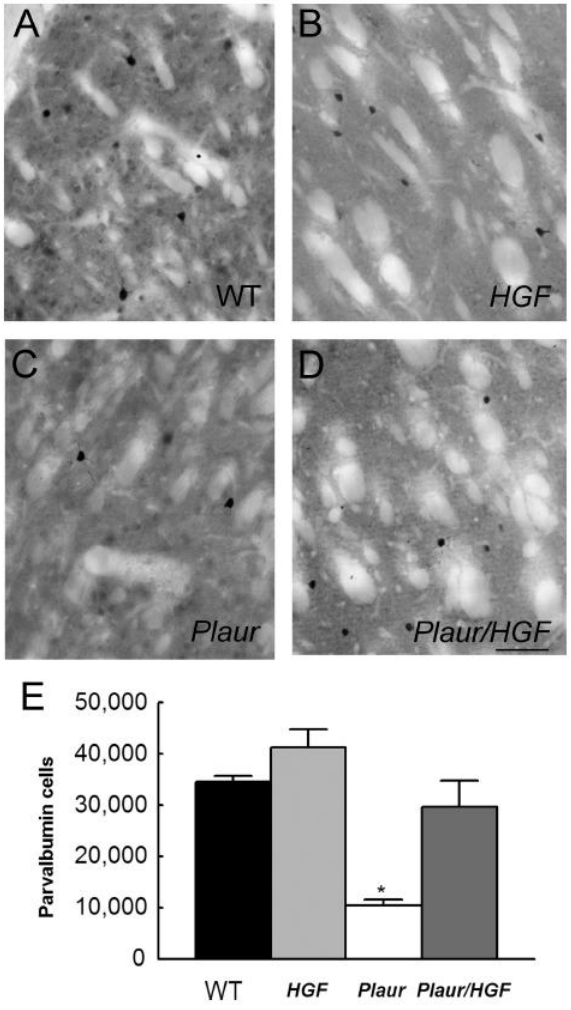
Forebrain GABAergic interneurons arise from the developing basal ganglia and the deficit in cerebral cortical interneurons in the *Plaur* mice suggested that other areas may have similar deficiencies. The anatomical studies were restricted to areas known to interact with the OFC to modulate behavior. Neurons in the OFC share reciprocal connections with the basal lateral amygdala (BLA). Previously the numbers of GABAergic (GAD67-expressing) neurons in the BLA were reported to be unchanged in the *Plaur* mouse as compared to those in WT (Eagleson, Bonnin et al. 2005). In agreement, the numbers of PV^+ cells were similar in WT ($924x10^3 \pm 200$) as compared to those in *Plaur* mice ($839\ x10^3 \pm 197$, Fig. S1). The addition of HGF/SF did not change the number of PV^+ cells in the *HGF* ($760\ x10^3 \pm 101$) and *Plaur/HGF* mice ($833\ x10^3 \pm 110$). No effect of genotype was found in the number of BLA interneurons [$F(3, 9) = 0.22$, $P = 0.87$]. There was no effect of genotype with regard to BLA volume [$F(3,9) = 0.18$, $p = 0.90$]. The numbers of PV^+ interneurons were not altered in the mutant mice.

Lastly, the interneuron profiles in the striatum were examined in the mouse cohort. Figure 3.2 shows a robust decrease in the number of PV^+ cells in the *Plaur* mouse (*A*), which is restored in the *Plaur/HGF* mouse (*D*). The WT and *HGF* mice displayed similar numbers of PV^+ cells. Stereological counts confirm these observations (Fig.3. 2*E*), with an effect of genotype [$F(3,9) = 22.10$, $p < 0.0001$]. The WT ($34,620 \pm 1,129$ cells), *HGF* ($41,354 \pm 3,475$), and *Plaur/HGF* mice ($29,651 \pm 5,204$) had similar numbers of cells, whereas the *Plaur* mice had a 70% decrease ($10,444 \pm 1,156$, $p < 0.001$). Estimates of striatal volumes were similar in all genotypes [$F(3,9) = 2.07$, $p =$

0.17]. The addition of HGF/SF in the *Plaur/HGF* mice corrected the PV^+ interneuron deficit in the striatum.

Figure 3.2. Striatal interneuron number is dependent upon *Plaur* and *HGF*.

Immunohistochemistry of PV^+ cells in the dorsal striatum of adult WT A, *HGF* B *Plaur* C, and *Plaur/HGF* D mice. Bar = 250 µm. E. Estimation of the PV^+ cells shows decreased numbers in the *Plaur* mice and a partial restoration of cell numbers in the *Plaur/HGF* mice. Each bar represents n Ó 3 mice asterisk denotes statistical significance as compared to WT mice ($p < 0.05$). (From Bissonette 2010, results from this figure were performed by Mihyun Bae as part of her thesis).

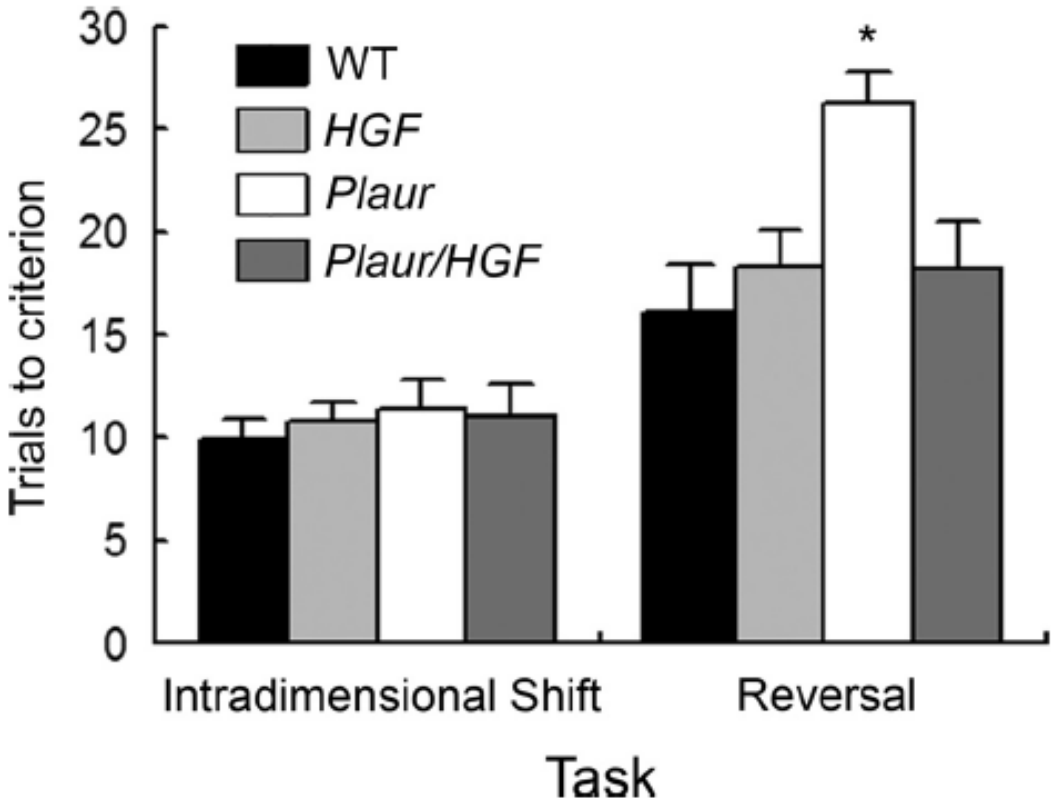


3.2.2 Reversal learning is correlated with GABAergic interneuron number

Previously, we have shown that reversal learning in mice, like in rats and primates, is mediated through the OFC region (Bissonette, Martins et al. 2008). We tested all four genotypes of mice using the same paradigm: a series of training discriminations with a final reversal problem (Fig 3.3). An ANOVA demonstrated an effect of task [$F(6, 323) = 21.56$, $p < 0.0001$] and interaction of task*genotype [$F(18,323) = 60.55$, $p < 0.004$]. There was no effect of genotype [$F(3,323) = 2.25$, $p = 0.082$], as all four groups performed similarly on the training tasks. For the test discrimination, the *Plaur* mice completed the task with the same number of trials (11.4 ± 1.4) as the WT mice (9.9 ± 1.0, $p > 0.99$, Fig. 3.3). For the reversal discriminations, the *Plaur* animals required significantly more trials (26.3 ± 1.5) to learn the reversal task than WT mice (16.2 ± 2.3, $p < 0.0001$). The *Plaur* mice with the GABAergic interneuron deficits demonstrated impaired reversal learning.

Figure 3.3. Impaired reversal learning in *Plaur* mice is recovered with addition of HGF/SF.

All mice performed similarly on the compound odor and texture discrimination task. The reversal task was more difficult for all groups, as shown by the increase in trials to criterion. The *Plaur* mice were impaired on the reversal task, as compared to WT mice, but the deficit was recovered in the *Plaur/HGF* group. The *HGF* mice performed similarly to the WT group. An asterisk denotes statistical significance as compared to WT mice ($p < 0.05$).



The *HGF* and *Plaur/HGF* mice were also tested on the reversal task. The presence of the *HGF* allele did not affect the ability of the mice to solve the problem, as *HGF* mice (10.9 ± 0.9 trials) and *Plaur/HGF* mice (11.0 ± 2.3 trials) performed similarly to WT mice ($p > 0.92$). On the reversed discriminations, the *HGF* (18.3 ± 1.8 trials) and *Plaur/HGF* mice (18.2 ± 2.4 trials) performed similarly to WT mice ($p > 0.45$). The *Plaur/HGF* mice performed the reversal discriminations differently than their *Plaur* littermates ($p < 0.001$). The addition of HGF/SF to the *Plaur* mouse reduced the number to trials needed to reach criterion and restored the problem solving ability to WT levels. The improved performance of the *Plaur/HGF* mouse on reversal testing corresponds to the amelioration of the GABAergic interneuron deficit in the OFC and striatum.

3.3.3 Discussion

The present study demonstrates that reversal learning is dependent upon proper numbers of GABAergic interneurons. Mice with a null mutation in *Plaur* have reduced numbers of PV^+ GABAergic local interneurons in the OFC and striatal regions, with normal numbers of interneurons in the BLA. The PV^+ interneuron populations in the OFC and striatum along with the reversal learning impairment are restored with postnatal HGF/SF supplementation in the *Plaur/HGF* mice. These results show that GABAergic local circuitry in the OFC and striatum are critical for modulating behavioral flexibility and that birth defects can be corrected by replenishing crucial growth factors.

Through lesion studies, specific forms of cognitive flexibility are attributed to distinct brain regions and circuits. Reversal learning in primates and rodents is dependent upon

intact OFC and striatum (Dias, Robbins et al. 1996; McAlonan and Brown 2003; Bissonette, Martins et al. 2008). Animals with neurotoxic lesions to the OFC acquire discriminations normally but require ~40% more trials to switch responding when reward contingencies are reversed. In comparison, the *Plaur* mice needed 62% more trials to complete the test. The OFC is reciprocally connected to the BLA, and lesions to BLA alone have no effect on reversal learning (Schoenbaum, Setlow et al. 2003; Izquierdo and Murray 2007). Thus, the BLA is downstream of the OFC with regards to reversal learning. In the case of interrupted local OFC circuitry, but normal GABAergic BLA interneurons of the *Plaur* mice, impaired reversal learning is expected and subsequently observed. The postnatal intervention of HGF/SF in the *Plaur/HGF* mouse allows the GABAergic interneurons to survive (Bae, Harmon et al. 2005; Bae, Bissonette et al. 2009) and thus the resulting OFC neural circuitry provides the correct information to the BLA.

Information from the OFC flows through the basal ganglia, and lesions in this area in the human, primate and rat impair reversal learning (Bellebaum, Koch et al. 2008; Clarke, Robbins et al. 2008; Tait and Brown 2008). Studies in monkeys report that lesions in the striatum yield similar reversal learning deficits as do lesions to the OFC, but that the response to feedback differs, suggesting a hierarchy in the processing of information (Clarke, Robbins et al. 2008). The dorsal striatum has a major role in signaling reward prediction (Seymour, Daw et al. 2007), whereas the OFC represents both positive and negative outcome expectancies in the task, driving changes in behavior as needed (Schoenbaum, Chiba et al. 1998; O'Doherty, Kringelbach et al. 2001). Damage to the

ventral striatum has often been reported to alter reversal learning in primates and rodents (Cools, Clark et al. 2002; Tait and Brown 2008). However, experimental ventral lesions have the potential to simultaneously compromise the dorsomedial striatum, which is the division involved in cognition. In fact, in patients, lesions in the dorsal striatum caused the greatest impairments (Seymour, Daw et al. 2007; Bellebaum, Koch et al. 2008). In addition, current reports in primates and rats support the role of the dorsomedial striatum in reversal learning (Ragozzino 2007; Clarke, Robbins et al. 2008).

The anatomical deficits presented in this study affect both dorsal striatum and OFC areas, because the OFC interneurons arise in the striatum during embryogenesis and the *Plaur* mutation alters both GABAergic interneuron populations. In the striatum, the PV^+ interneurons receive direct inputs from different cortical regions, including the OFC, and synapse on the medium spiny output neurons. Loss of PV^+ neurons in the striatum may lead to rerouting of the OFC afferents to directly synapse on the medium spiny neurons or to reduce the number of OFC inputs. Similar effects are observed in studied using direct insults to the developing brain though lesion studied, typically investigating the connections between the motor cortex and muscles, or muscles to motor cortex(Z'Graggen, Fouad et al. 2000). Also, the present methodologies cannot fully dissociate a severe down-regulation of GABA or PV in these interneurons, though work done by another student in the lab has demonstrated decreased presence of Peri-neural nets which surround PV^+ interneurons, through wisteria toxin staining, as well as a decreased HGF/SF expression level in adults. Tunnel staining for apoptotic tissue specifically looking to address this question, also performed by a former graduate student

(Bae, Unpublished data) was inconclusive. The present system cannot dissociate the individual contributions of the OFC and striatum with regards to the deficit in reversal learning. Nonetheless, it is li(Z'Graggen, Fouad et al. 2000)(Z'Graggen, Fouad et al. 2000)(Z'Graggen, Fouad et al. 2000)(Z'Graggen, Fouad et al. 2000)(Z'Graggen, Fouad et al. 2000)kely that human neurological disorders, such as obsessive compulsive disorder (OCD) and schizophrenia, have altered circuitry in multiple regions due to developmental perturbations. In the case of the *Plaur* mice, the anatomy in each region was restored after postnatal supplementation with HGF/SF indicating a possible candidate for therapy.

Pharmacological depletion of selective neurotransmitters has revealed specific candidates for regulating reversal learning in the OFC and striatum. Direct blockage of GABAergic transmission, using muscimol infusions in rats, demonstrated increased errors on an odor discrimination task that was similar to the present study (Kim and Ragozzino 2005). The 70% reduction of PV^+ interneurons in the *Plaur* mice supports the muscimol study and leads to similar behavioral dysfunction. Our results suggest that supplementation of HGF/SF maintained the interneuron population and allowed for normal synaptogenesis by restoring the OFC circuitry and function.

The OFC and striatal interneurons are specified in a division of the ganglionic eminence that is found medially, whereas the BLA interneurons are derived from a more caudal division (Nery, Fishell et al. 2002). The *Plaur* mutation and changes in HGF/SF levels specifically influence the neurons derived from the medial ganglionic eminence (Eagleson, Bonnin et al. 2005; Martins, Plachez et al. 2007; Bae, Bissonette et al. 2009)

(also Martins and Powell, unpublished data). Finally, the interneuron deficits in the *Plaur* mouse cerebral cortex were more severe in rostral forebrain regions and not present in occipital areas (Powell, Campbell et al. 2003; Eagleson, Bonnin et al. 2005). One possible explanation for this phenotype is the gradient of HGF/SF expression, high caudal to low rostral (Achim, Katyal et al. 1997), leading to sub threshold HGF/SF levels in frontal areas in the *Plaur* mice, but having little effect on the occipital pole. The anatomical deficit in the *Plaur* mice reflects the reduced HGF/SF expression and can be corrected by exogenous perinatal HGF/SF supplementation (Bae, Bissonette et al. 2009).

The anatomy of the *HGF* mouse appeared to be grossly normal, indicating that exogenous HGF/SF did not alter postnatal development. Based on the roles of HGF/SF in forebrain ontogeny (Achim, Katyal et al. 1997; Thewke and Seeds 1999), we suggest that it may be involved in cell survival and possibly maturation and synaptogenesis. In another study, HGF/SF levels were measured in the four groups of mice, and the supplementation in mice with the *HGF* allele was measured at birth (Bae, Bissonette et al. 2009). The *Plaur* mouse demonstrated a 40% decrease in HGF/SF levels in the cerebral cortex at birth and in the adult, whereas HGF/SF levels in the *Plaur/HGF* mouse were similar to those in the WT mouse at both ages. The anatomical counts are based on PV immunoreactivity, and PV levels may be regulated by HGF/SF. However, immunohistochemistry for GABA, glutamic acid decarboxylase (Gad67), and perineural nets revealed similar results ((Bae, Bissonette et al. 2009) and data not shown), indicating loss of the GABAergic cells. The striking anatomical and behavioral similarities in the

WT and *Plaur/HGF* mice indicate that the *Plaur* phenotype may be due, in part to a HGF/SF deficiency.

The interneuron deficit of the *Plaur* mouse is not evident in the *Plaur/HGF* mouse. Yet, multiple other cell types are likely affected by the loss of *Plaur*, particularly in regards to synaptic connectivity. In the cerebral cortex, the PV subpopulation of GABAergic interneurons seems to be uniquely affected, as the numbers of somatostatin and calretinin expressing cells is the same in all genotypes (Bae, Bissonette et al. 2009). In addition, multiple other factors may be involved (Berghuis, Dobszay et al. 2004; Galloway, Woo et al. 2008). The increased levels of HGF/SF in the *Plaur/HGF* mice may be sufficient to overcome many deficits and mask others.

Neurological disorders including schizophrenia and epilepsy have similarities in loss of GABAergic inhibition (Benes, McSparren et al. 1991; Beasley and Reynolds 1997; Avoli, Bernasconi et al. 1999; Baulac, Huberfeld et al. 2001; Lewis, Hashimoto et al. 2005). In our experiments, through manipulating development of GABAergic interneurons, we were able to show selective anatomical deficits that alter specific circuits with parallel behavioral dysfunction. The agreement of behavioral alterations with changes in GABAergic tone in specific forebrain structures indicates that shifting away from an optimal level of GABA yields cognitive impairments. Additional studies should be done to demonstrate if the recovery of the PV^+ interneuron deficit by HGF/SF is specific to the OFC and striatum or more general throughout the forebrain. Along the same lines, the behavioral analysis in the *Plaur/HGF* mice is a broad measurement of

function. Future studies with electrophysiological recordings in awake mice should provide more detailed information about the circuits in the *Plaur* and *HGF* mutants.

Chapter 4

Fast-spiking interneurons sculpt orbitofrontal cortical oscillations critical for reversal learning

4.1 Introduction

Local interneuron dysfunction is hypothesized to be a source of cognitive deficits associated with multiple human psychiatric disorders including autism, epilepsy and schizophrenia (Benes and Berretta 2001; Levitt, Eagleson et al. 2004; Magloczky and Freund 2005; Lewis and Moghaddam 2006; Aronica, Redeker et al. 2007; Lawrence, Kemper et al. 2010). GABAergic interneurons, especially fast-spiking PV-expressing (PV^+) interneurons, regulate the activity of small local cortical networks to coordinate the formation of emergent cortical ensembles (Preuss, Faber et al. 2009; Mizoguchi, Shoji et al. 2010; Mizoguchi, Shoji et al. 2011). Recent evidence in human subject and tissues indicate that network activity, in particular high frequency oscillations, are perturbed in many psychiatric illnesses with developmental origins (Rojas, Maharajh et al. 2008; Weiss, Preuss et al. 2008; Haenschel, Bittner et al. 2009; Gandal, Edgar et al. 2010; Minzenberg, Firl et al. 2010).

We have employed a transgenic mouse model, the null mutant of *Plaur*, the B6.129 ï $Plaur^{tm1/Mlg}/Plaur^{tm1/Mlg}$ mouse (Dewerchin, Nuffelen et al. 1996), which is missing urokinase plasminogen activator receptor (also known as uPAR, CD-87), to understand consequences of specific anatomical deficits in interneuron development on neural

network function and cognitive flexibility. *PLAUR* is associated with rolandic epilepsy, impaired language and cognition and autism spectrum disorder (Royer-Zemmour, Ponsole-Lenfant et al. 2008; Szabo, Brookings et al. 2008; Liu, Zhang et al. 2010; Roll, Vernes et al. 2010), and interacts with receptors linked to schizophrenia including *MET* (Bertram, Bernstein et al. 2007; D'Alessio and Blasi 2009; Burdick, DeRosse et al. 2010; Archinti, Britto et al. 2011). The *Plaur* mutant mouse has been shown to have sensory cortical and striatal deficits specific to the PV^+ interneurons leading to behavioral dysfunction (Bissonette, Bae et al. ; Powell, Campbell et al. 2003; Rosen, Farberg et al. 2008).

The OFC plays an important role in mediating reversal learning in primates and rats, and has recently begun to be studied in mice (Dias, Robbins et al. 1996; Preuss, Koller et al. 2001; Preuss, Fischer et al. 2006; Carlson, Rudgers et al. 2007; Bissonette, Martins et al. 2008). We tested our mice on a rodent version of the Wisconsin Card Sorting Task (WCST)(Colacicco, Welzl et al. 2002; Bissonette, Martins et al. 2008), modified to feature multiple reversals while simultaneously recording single unit (SU) and local field potential (LFP) from OFC. Like previous rat studies (Schoenbaum, Chiba et al. 1999; Schoenbaum, Setlow et al. 2003), we found selective activation of SUs in murine OFC for task elements was minimally affected in the *Plaur* mice. However, we observed a paucity of putative fast-spiking (FS) PV^+ cells, supporting the anatomical data that the PV^+ interneurons are missing.. The initial reversal learning task invoked dramatic increases in high frequency power in the beta and gamma ranges in control mice. Subsequent reversal tasks elicited changes in power similar to compound discriminations. The *Plaur* mice were unable to generate oscillations of significant strength in the first and

subsequent reversals. These data support the role of FS PV^+ interneurons regulating orbitofrontal mediated cognition, and suggest that adolescent loss of PV^+ interneurons hinders the generation of high frequency oscillations during decision-making. These results corroborate the role of interneurons in organizing prefrontal cortical neural circuitry, similar to studies in sensory and motor cortical areas (Preuss, Faber et al. 2009; Mizoguchi, Shoji et al. 2010), and show that loss of a gene associated with multiple developmental psychiatric and neurological disorders can produce shared dysfunctional cognitive consequences.

4.2 Results

4.2.1 Interneuron Ontogeny and Reversal Learning

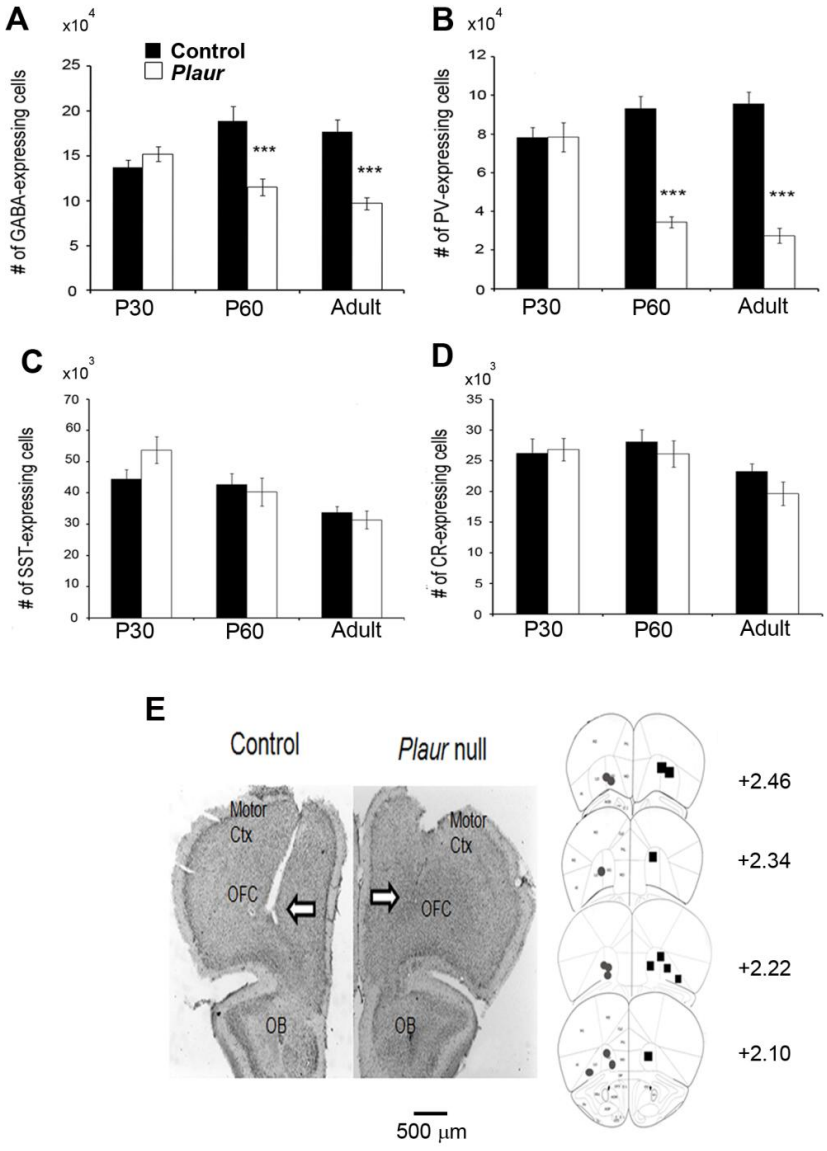
A specific PV^+ cell loss in the *Plaur* mouse occurs during adolescence, as shown using stereological estimation (Figures 4.1A and B). ANOVA for total GABAergic cells revealed an effect of genotype and age ($F(2,66) = 11.23$, $p = 0.0001$). Similar effects of age and genotype were found for PV^+ cells ($F(2,74) = 22.019$, $p = 0.0001$). Total cell counts of GABAergic and PV^+ cells in wildtype and *Plaur* null mice were not different on postnatal day 30 (P30) (Student-Newman-Keuls *posthoc*, $p = 0.36$, $p = 0.95$, respectively), while postnatal day 60 (P60) and adult (older than P90) brains show significant losses of GABAergic cells ($p = 0.001$) in the *Plaur* mice, mainly due to the loss of PV^+ cells, starting at P60 ($p = 0.001$). Other subpopulations of GABAergic interneurons were not affected, with ANOVA revealing no significant effects of genotype for the somatostatin-expressing (SST) population ($F(2,92) = 1.97$, $p = 0.15$) or calretinin (CR) expressing population ($F(2,87) = 1.503$, $p = 0.61$, Figures 4.1C and D).

We implanted drivable electrodes into the OFC (Figure 1E) and recorded SU activity and local field potentials LFPs from control mice as well as from *Plaur* null mice while they

performed serial compound discrimination and reversal tests using a previously described naturalistic foraging task (Bissonette, Martins et al. 2008).

Figure 4.1. Cortical PV interneuron ontogeny and electrode placement

A *Plaur* mice have significantly fewer GABAergic cells in early adulthood. B-D *Plaur* mice show a significant decrease in PV^+ cells in cortical areas, whereas the somatostatin (SST) and calretinin (CR) expressing GABAergic subtypes are not altered. E Electrode placements in mouse OFC of both control (filled circles) and *Plaur* null mice (filled squares), ranging from bregma level +2.10 to +2.46. *** $p < 0.001$; Scale bar = 500 µm.



To complete the task, mice must first learn to associate one cue with a food reward while ignoring a second unrewarded cue. After the animals successfully learned the association (8 consecutive correct digs), and completed a post-learning series of 15 trials, the identity of the rewarded cue was switched (or reversed) such that digging in the bowl associated with the previously unrewarded cue was rewarded. ANOVA revealed a main effect ($F(7,64) = 31.23$, $p = 0.001$) and *posthoc* Student-Newman-Keuls tests revealed a significant difference in trials to criterion on the first reversal (denoted as Reversal 1) for the *Plaur* null mice compared to control animals ($p = 0.001$). However, the impaired learning of the *Plaur* mice was not present on a second reversal discrimination (denoted as Reversal 2, $p = 0.81$, Figure 4.2A). In agreement with other studies of reversal learning (Clarke, Dalley et al. 2004);van der Plasse, 2008 #500;Bissonette, 2008 #3}, both *Plaur* and control mice continued to be challenged by the reversal tasks, and the mice demonstrated significant increases in number of trials to criterion on all subsequent trial reversals ($p = 0.001$ for control, $p = 0.01$ for *Plaur*). On Reversal 1, the increased number of trials to criterion in the *Plaur* mice reflected an increased number of errors, notably perseverative errors. Figure 4.2B demonstrates the difference between groups in total errors with a main effect of ($F(7,56) = 36.84$, $p = 0.001$). Student-Newman-Keuls *posthoc* tests revealed a significant difference between groups on Reversal 1 ($p = 0.001$) but not on Reversal 2 ($p = 0.074$). Figure 2C represents the number of errors that are preservative in nature. ANOVA revealed a significant main effect of genotype ($F(7,64) = 12.85$, $p = 0.001$) with *Plaur* mice having increased numbers of total and perseverative errors ($p = 0.001$) on the Reversal 1, but not on Reversal 2 ($p < 0.111$).

Figure 4.2. Behavioral alterations in mice with limited PV^+ neurons in OFC

A *Plaur* mice show significant impairment on the first reversal (Reversal 1), but not on subsequent reversal (Reversal 2) discriminations. B Compared to control group, *Plaur* mice have a significant increase in the number of errors on the first reversal. C The errors that the *Plaur* mice commit are perseverative in nature, and *Plaur* mice make significantly more perseverative errors than the control mice. *** $p < 0.001$.