

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 Parkinson's, Alzheimer's, and Huntington's diseases (Josiassen, Curry et al. 1983 ;

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 is 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 perseverative 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 (γ -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).

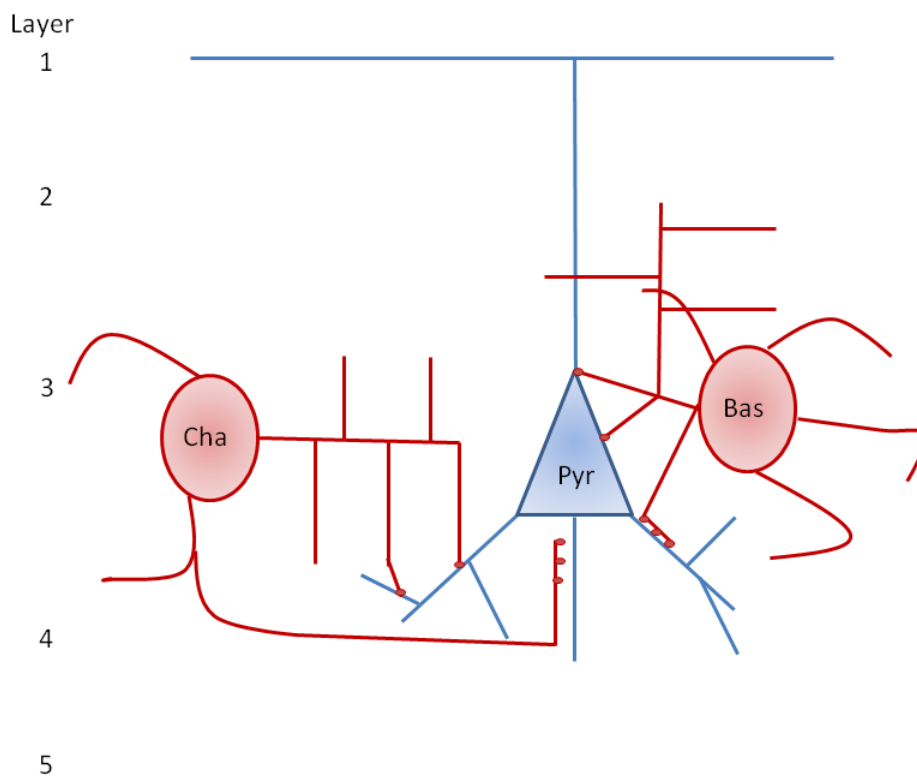
Additionally, long-term deficits in the GABAergic system in rodent and primate forebrain can lead to behavioral and cognitive symptoms commonly associated with mental retardation, mood disorders, schizophrenia, Tourette Syndrome , and ASD (Keverne, 1999; Benes and Berretta, 2001; Andres, 2002; Noebels, 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-hyperpolarization, 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-deltaGluR-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.

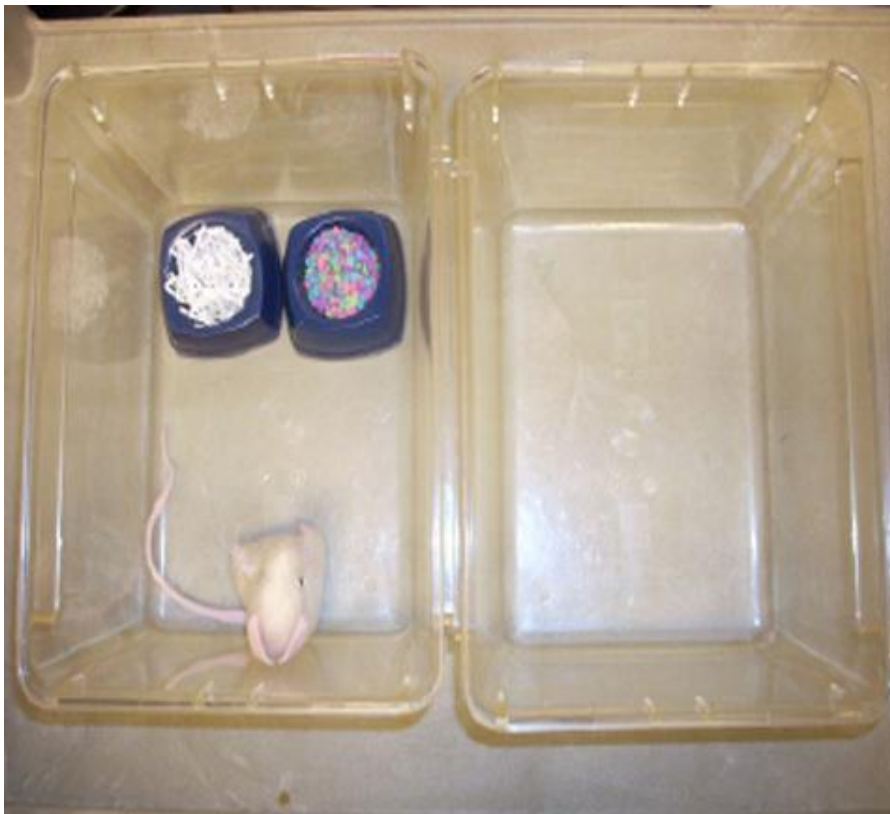
For reversal learning, the mouse must learn to discriminate between two cues. In the touchscreen task, the subject is trained to select between two images, and correct choices are rewarded (Bussey, Everitt et al. 1997; Brigman, Bussey et al. 2005). Once the mouse has reached criterion, usually 85% correct 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/extradimensional) 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; Remijne, 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

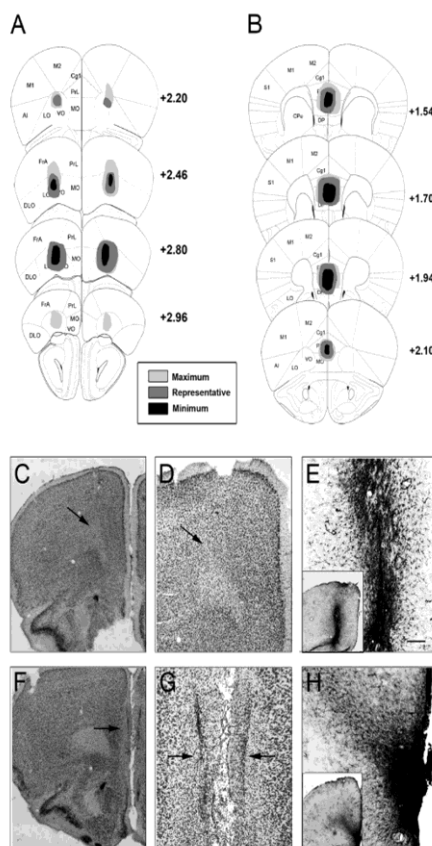
2.2.1 Area specific lesions impair reversal and set-shifting ability

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 – IDS 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 ($p = 0.0057$).

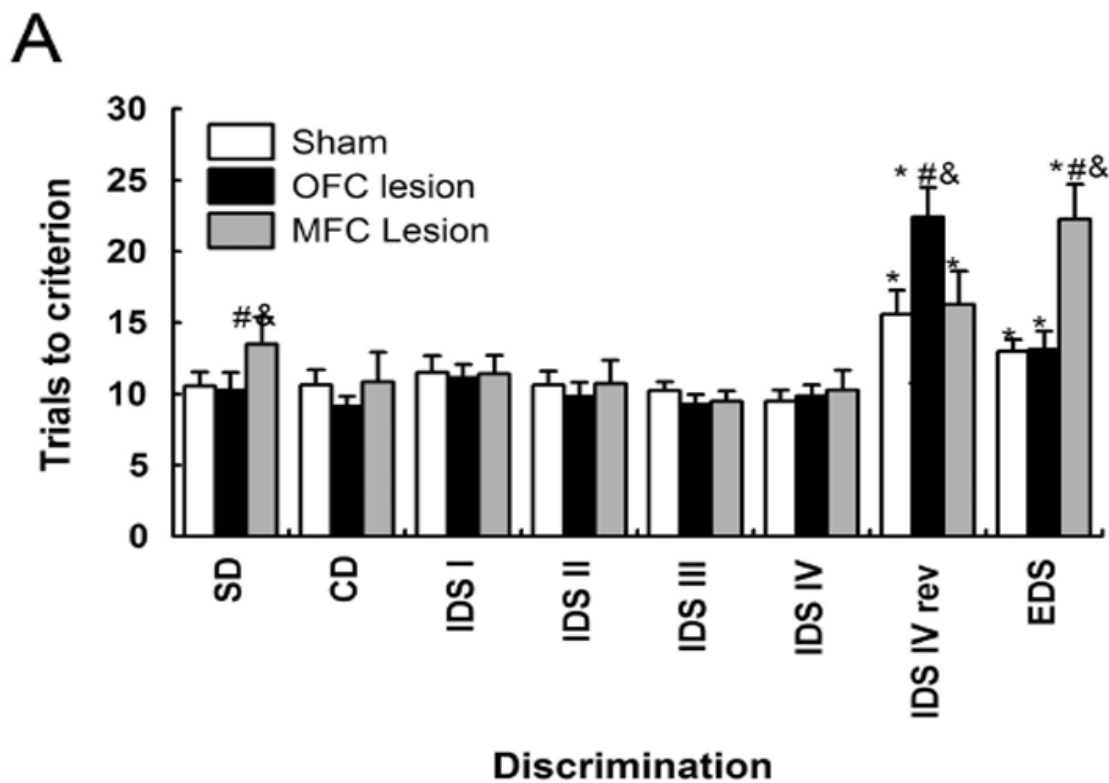
Table 2.2: Exemplar combinations.

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 pieces

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 – 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 IV_{rev}, $p < 0.0001$) 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 $p < 0.05$.

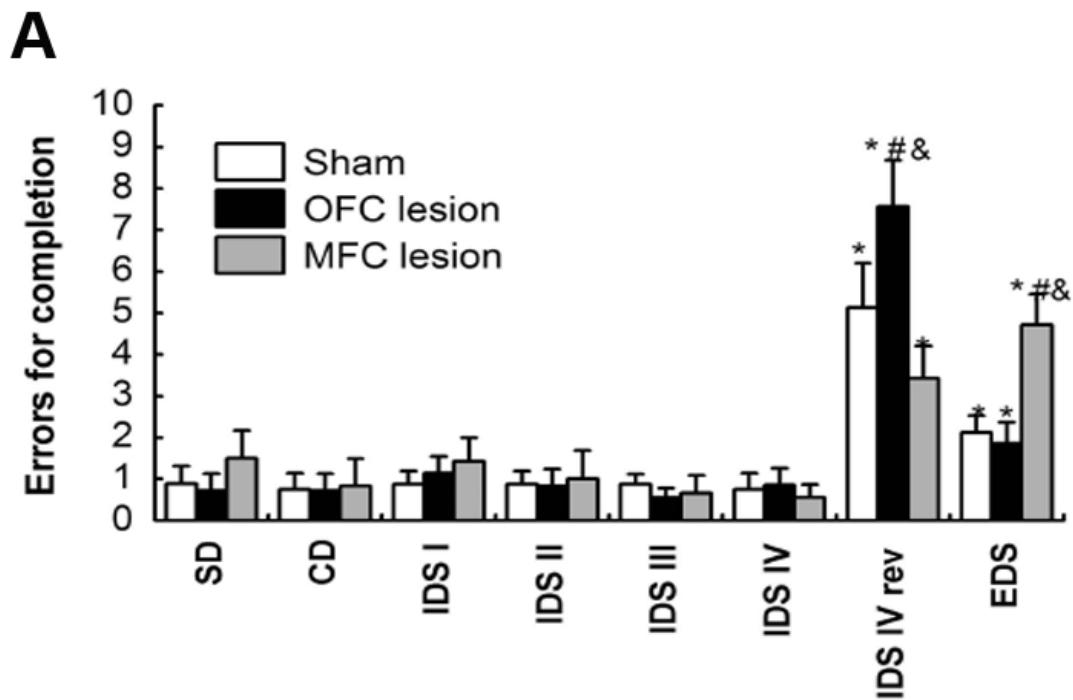
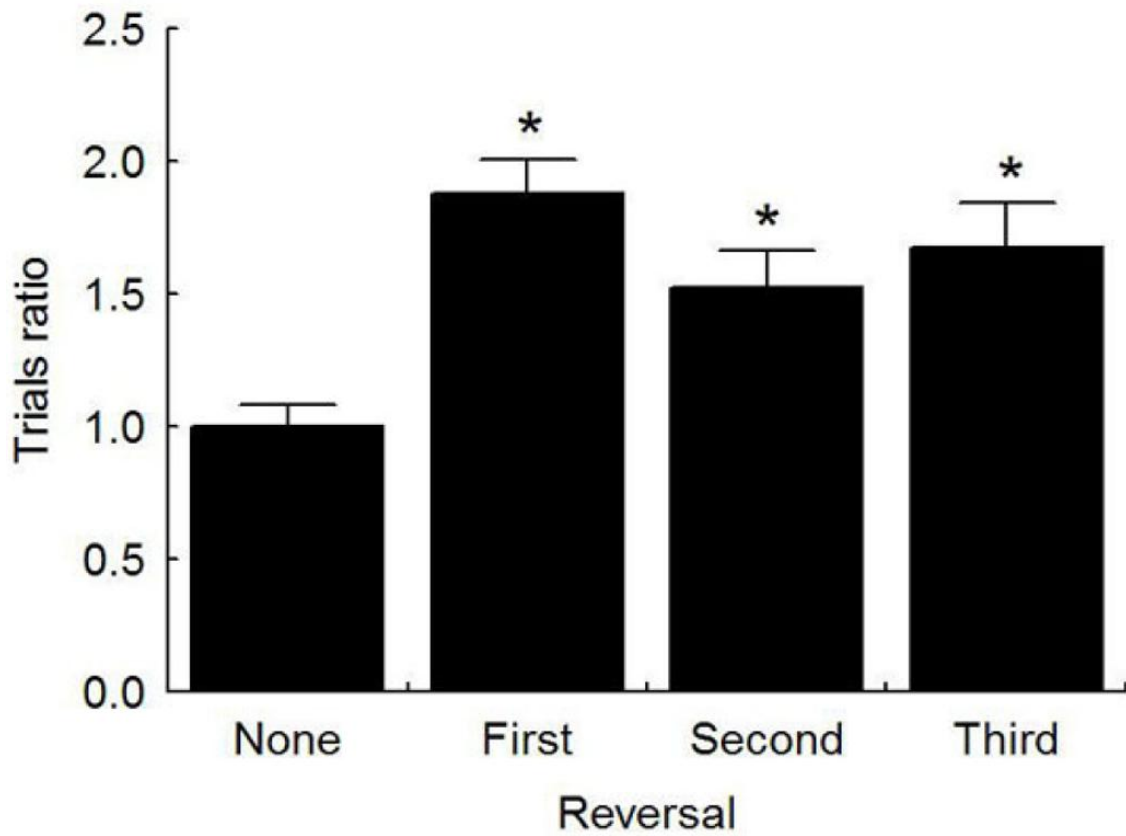


Figure 2.4 Reversal trials ratio verifies the difficulty of the reversal task.

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.

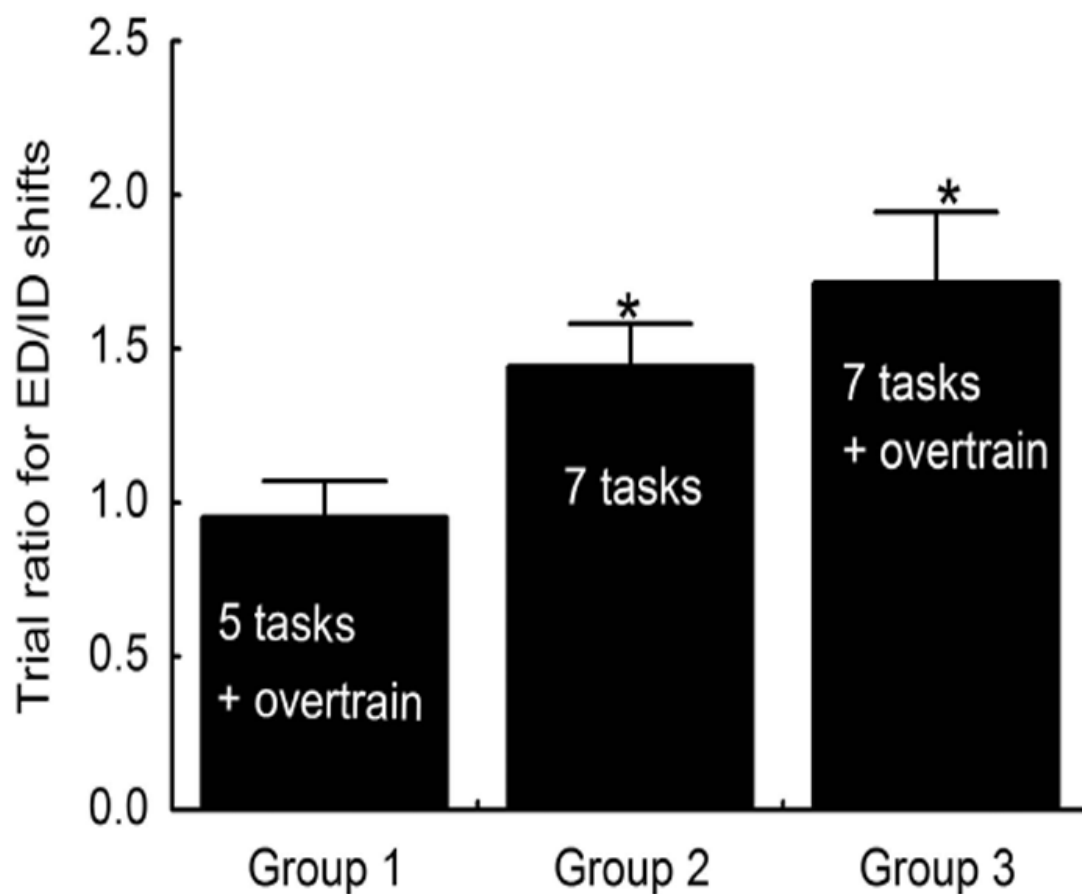


2.2.3 Set-shifting is dependent upon number of tasks and overtraining

In the lesion experiments (Fig. 2.2A), the mice were presented with seven discriminations (CD – 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, $1.44 \pm 0.14, p = 0.25$). 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 an 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 – IDS 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 & Baraban 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 learning (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.

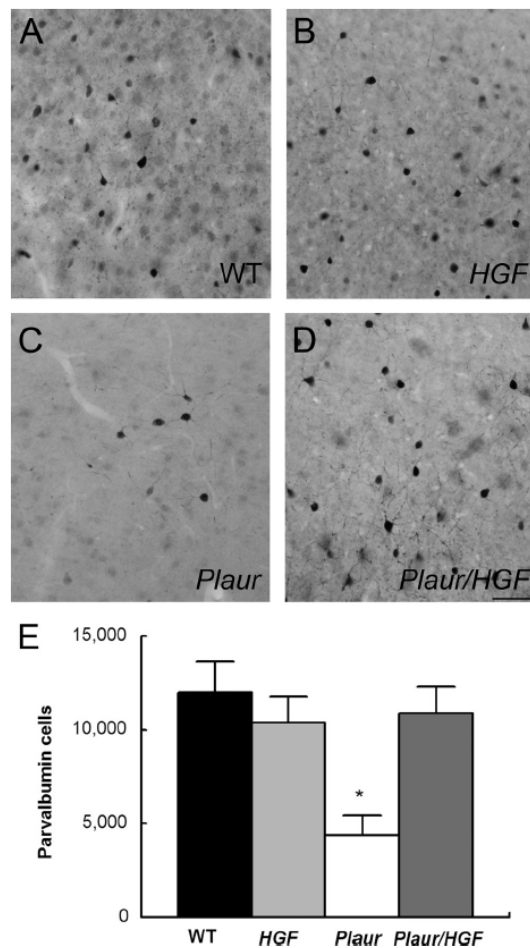
3.2 Results

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. 1C,E, WT: 11,965 ± 1,675, Fig. 3.1A,E $p = 0.03$). The number of OFC PV⁺ cells in the *HGF* mice (10,379 ± 1,568, Fig. 3.1B,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. 3.1D,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 =200 μ m. E The numbers of PV⁺ cells were stereologically counted in orbital frontal cortex. Each bar represents $n \geq 3$, with error bars denoting the 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).



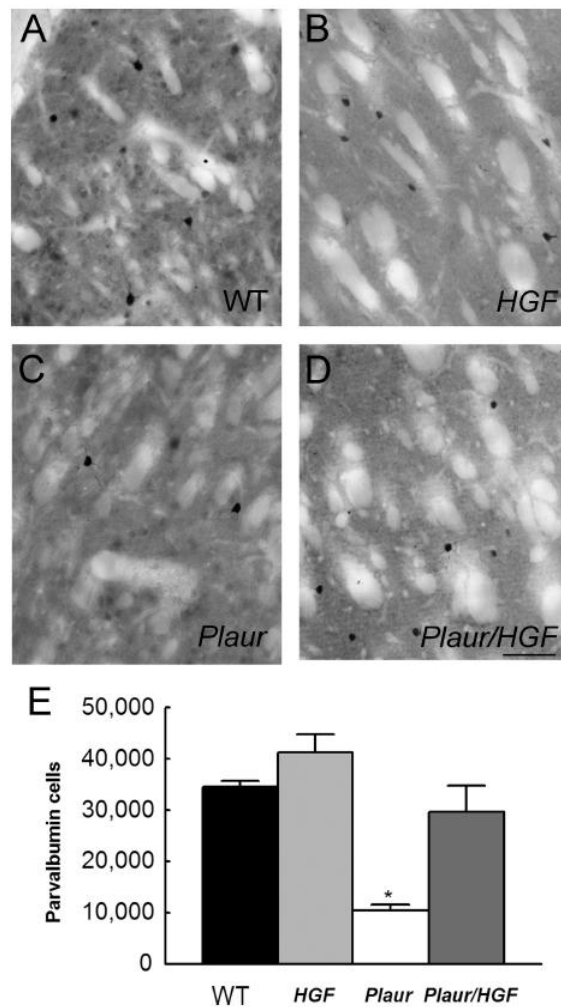
Forebrain GABAergic interneurons arise from the developing basal ganglia and the deficit in cerebral cortical interneurons in the *Plaur* mice suggested that other areas may have similar deficiencies. The anatomical studies were restricted to areas known to interact with the OFC to modulate behavior. 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 ($924 \times 10^3 \pm 200$) as compared to those in *Plaur* mice ($839 \times 10^3 \pm 197$, Fig. S1). The addition of HGF/SF did not change the number of PV⁺ cells in the *HGF* ($760 \times 10^3 \pm 101$) and *Plaur/HGF* mice ($833 \times 10^3 \pm 110$). 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. 3.2E), 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 μ m. 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 $n \geq 3$ mice, with error bars denoting the SEM. 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).

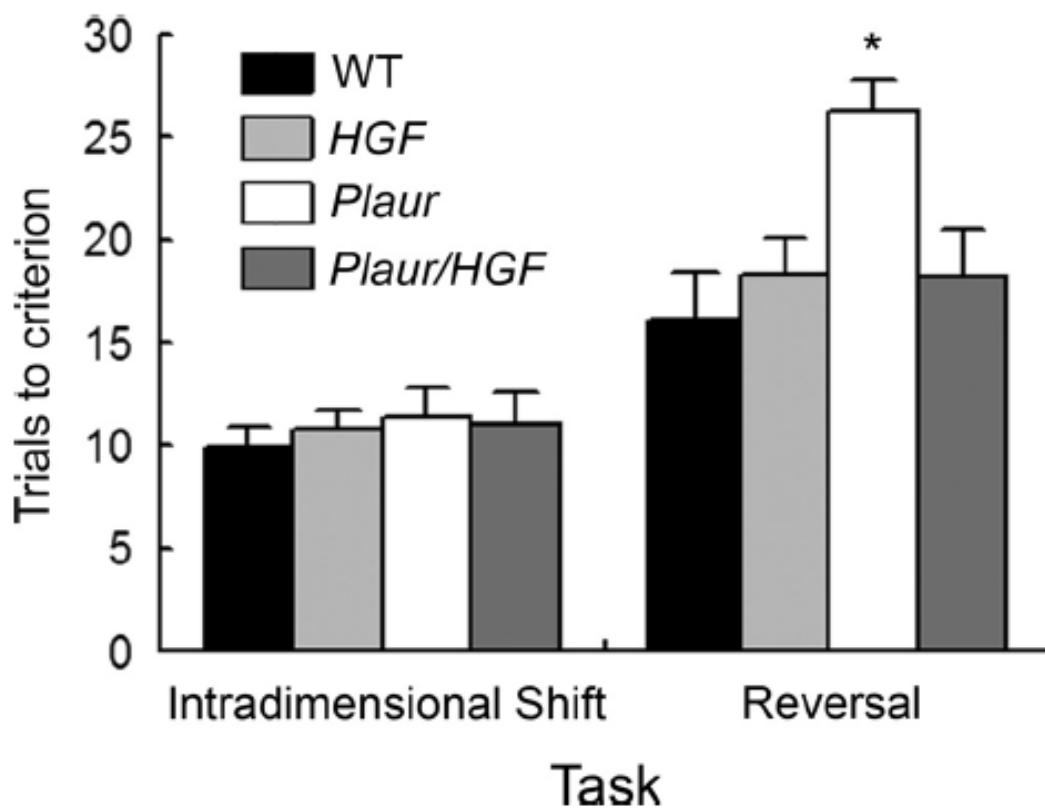


3.2.2 Reversal learning is correlated with GABAergic interneuron number

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 ($9.9 \pm 1.0, p > 0.99$, 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 ($16.2 \pm 2.3, p < 0.0001$). 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 studies using direct insults to the developing brain through lesion studies, 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 likely 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 – *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

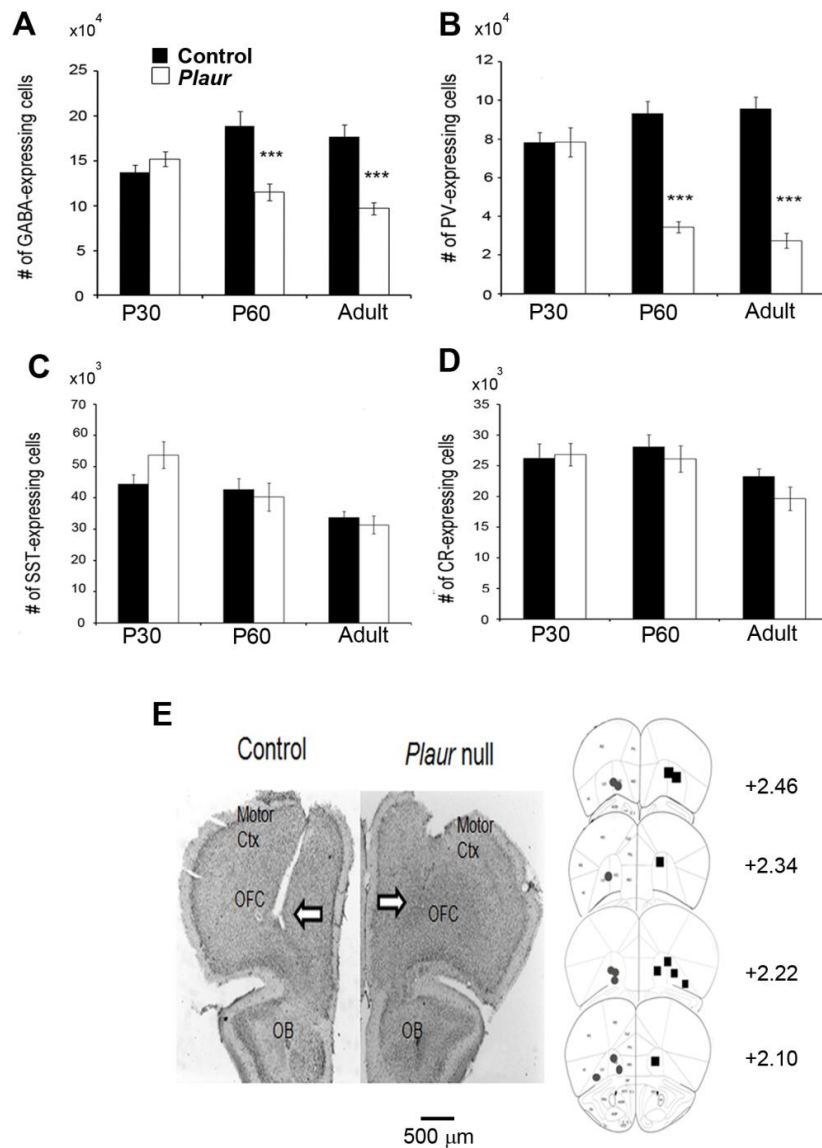
A specific PV⁺ cell loss in the *Plaur* mouse occurs during adolescence, as shown using stereological estimation (Figures 4.1A and B). ANOVA for total GABAergic cells revealed an effect of genotype and age ($F(2,66) = 11.23$, $p = 0.0001$). Similar effects of age and genotype were found for PV⁺ cells ($F(2,74) = 22.019$, $p = 0.0001$). 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

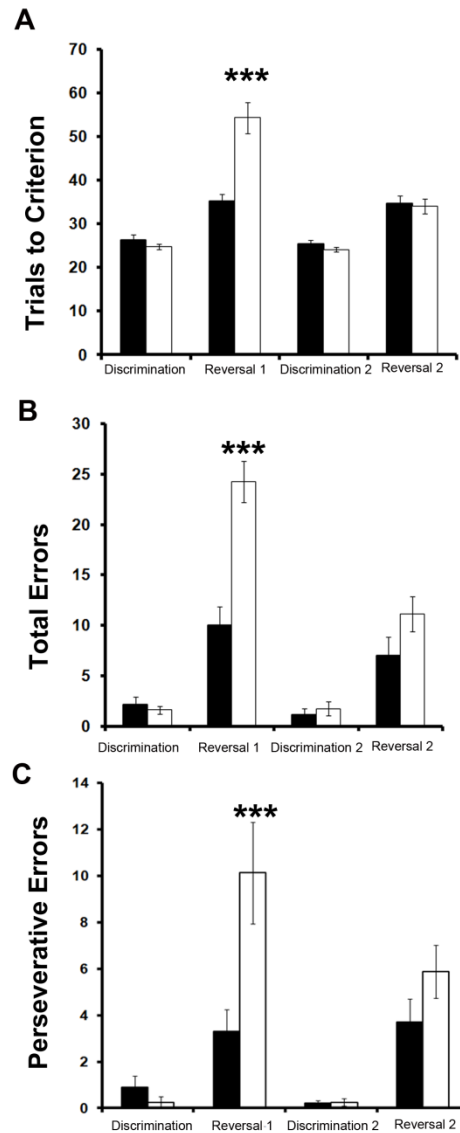
A *Plaur* mice have significantly fewer GABAergic cells in early adulthood. B-D *Plaur* mice show a significant decrease in PV⁺ cells in cortical areas, whereas the somatostatin (SST) and calretinin (CR) expressing GABAergic subtypes are not altered. E Electrode placements in mouse OFC of both control (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

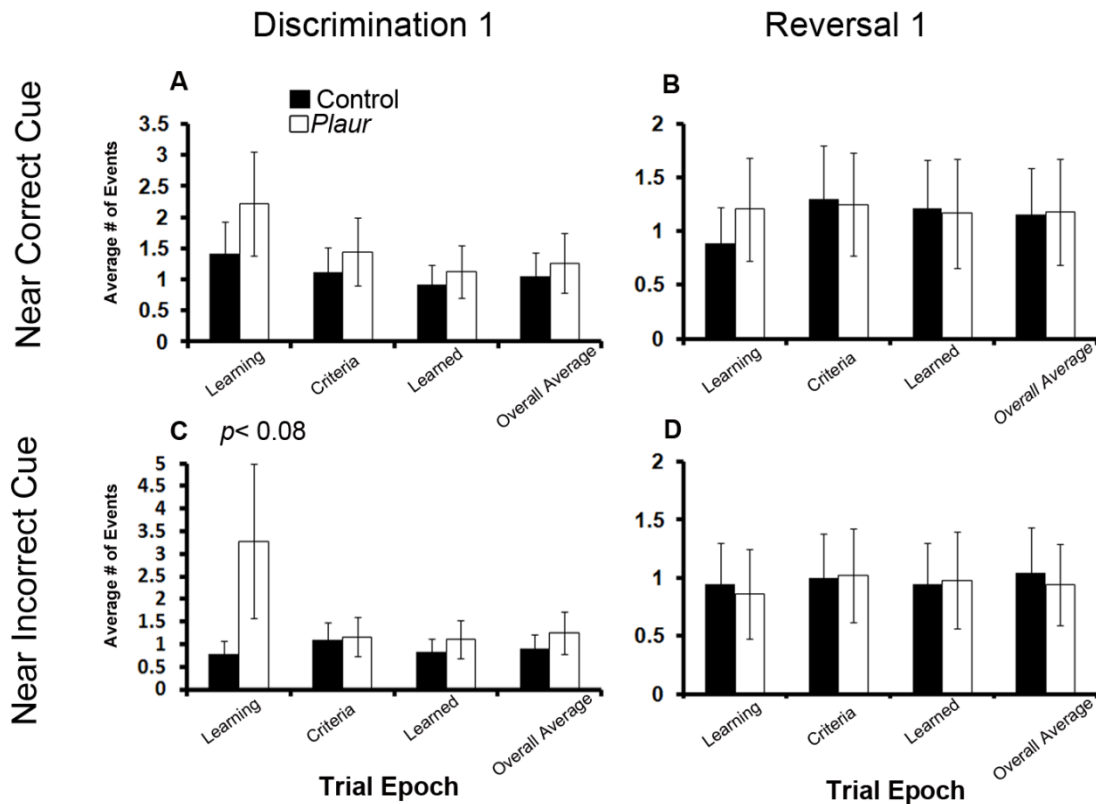
A *Plaur* mice show significant impairment on the first reversal (Reversal 1), but not on subsequent reversal (Reversal 2) discriminations. B Compared to control group, *Plaur* mice have a significant increase in the number of errors on the first reversal. C The errors that the *Plaur* mice commit are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



To ensure that both groups of mice were interacting similarly with the task cues, behavioral responses were compared for the overall task, separated into epochs representing initial training, consecutive correct trials to criterion, and additional 15 trials after criterion (overtraining). ANOVA revealed no main effects ($F(7,27) = 2.63$, $p = 0.08$) on approach or choice behavior during discrimination or reversal tasks ($F(3,24) = 0.338$, $p = 0.8$, Figures 4.3 A-D).

Figure 4.3. Analysis of interactions with each set of cues during the task

A-B Genotype had no effect on the number of times the mouse approached the correct cues during the initial trials prior to completing 8 correct choices (learning), during the 8 correct choices (middle 8), or after mastering the task (final 15). No differences were observed when all trials were grouped together (all). C-D The average number of times the control and *Plaur* animals were near an incorrect due was not significantly different for any testing epoch.



4.2.2 Neural Signals during Choice

The loss of the inhibitory tone predicted changes in the SU activity. Therefore, we collected and characterized SUs during the discrimination task (n = 206 in control mice, n = 229 for *Plaur* null mice) and the reversal task (n = 210 in control, n = 238 in *Plaur* null mice) as putative fast-spiking (FS), putative regular spiking (RS) or unclassified (UC) neurons based on spike rate and action potential duration (see Experimental Procedures). Average firing rate of RS cells was increased by more than 2-fold in *Plaur* null mice (6.91 spikes/s) compared to control animals (3.03 spikes/s) during the discrimination tasks (Mann-Whitney U, $p = 0.02$, Figures 4.4A and B), suggesting that loss of PV⁺ neurons leads to increased OFC excitation. RS unit mean firing rate nearly doubled for the control mice on the reversal tasks (n = 29, 5.34 spikes/s, $p = 0.99$ when compared to Discrimination task, Figure 3C), but remained the same in the *Plaur* null mice (n = 35, 5.9 spikes/s, Figure 3D). While the baseline firing rate of the *Plaur* mice was much higher than control during the Discrimination tasks, the *Plaur* mice did not modulate the firing rate when the task changed to a reversal.

As predicted from previous and anatomical data (Bissonette, Bae et al. ; Powell, Muhlfriedel et al. 2003), fewer FS cells were encountered in the *Plaur* null mice compared to the control in the awake recordings during the discrimination (n = 17 in *Plaur* null mice compared to n = 36 in control) or reversal tasks (n = 14 for *Plaur* mice, compared n = 32 for control, Table 4.1). K-means clustering supported the *a priori* classification of putative RS and FS cells and demonstrated an increased baseline firing rate of putative RS cells in the *Plaur* null mice, compared to control mice (k-means centroid of 1.5 spikes/s, 1100 μ s for control RS cells during discrimination and 2.43 spikes/s, 1100 μ s during reversal, compared to 3.23 spikes/s, 1000 μ s for RS cells in *Plaur* null mice on discrimination and 3.04 spikes/s, 1000 μ s on reversal). Thus, while the overall spike rate of RS cells appeared to have been initially elevated given the deficit

in overall GABAergic interneurons in the *Plaur* mice, the difference between groups in terms of firing rate was not significant upon reversal trials, as the *Plaur* mice did not accommodate to the change in the task.

Figure 4.4. Distribution of recorded units demonstrates FS PV⁺ interneuron deficit

A-B The distribution of single units during the first Discrimination demonstrates both fewer FS units (Red Diamonds) in *Plaur* mice and also elevated RS (Blue Diamonds) unit firing rate. Blue quad-diamond represents FS mean firing rate and AP duration and green quad diamond represents the mean RS firing rate and AP duration. C-D The distribution of single units during the first Reversal illustrates the continued lack of FS cells in *Plaur* mice, but also the elevated RS unit rate in controls. Blue quad-diamond represents FS mean firing rate and AP duration and green quad diamond represents the mean RS firing rate and AP duration.

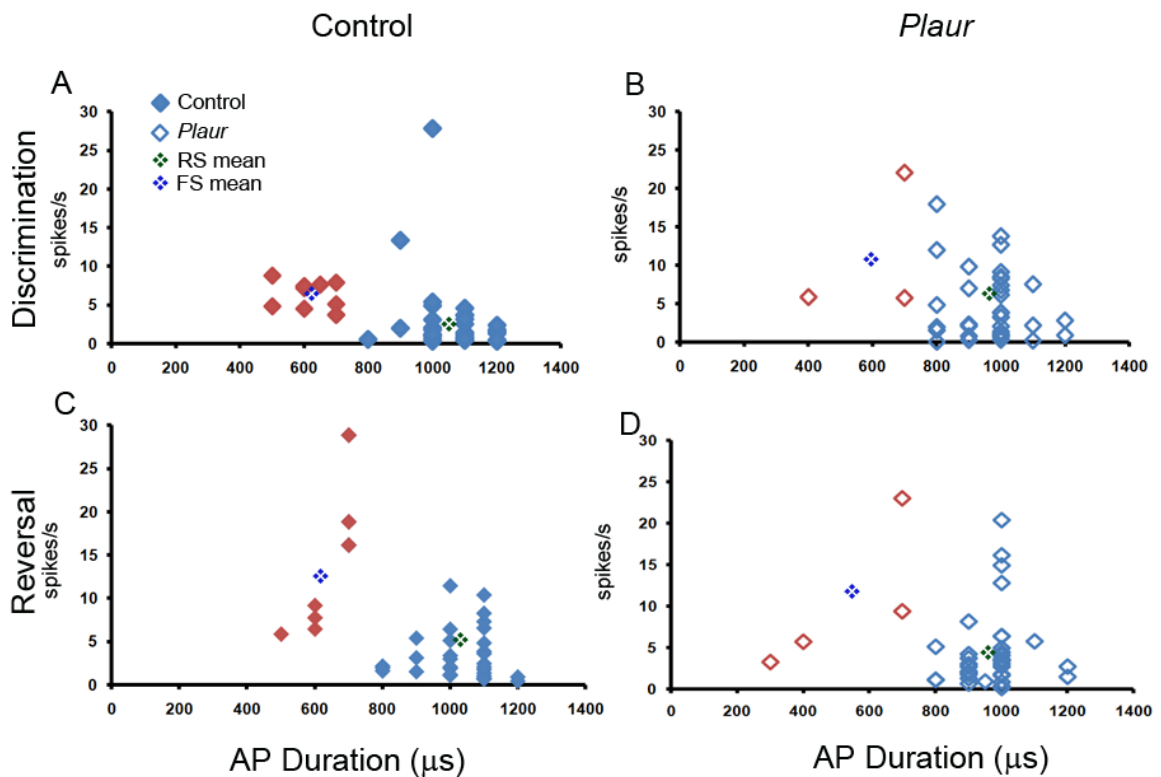


Table 4.1. Distribution of single units

Genotype	Discrimination			Reversal		
	RS	FS	UC	RS	FS	UC
Control	154	36	16	157	32	16
<i>Plaur</i>	201*	17*	9	214*	14*	10

Abbreviations: RS, Regular Spiking; FS, Fast Spiking; UC, Un-classified. Control (n = 206 for discrimination and n = 210 for reversal), *Plaur* (n= 229 for discrimination and n = 238 for reversal)

* Indicates significant χ^2 for alpha < 0.05

Using a different approach, we separated our single units into three groups: units that did not significantly alter their firing rate during task-related behavioral events (start/end trial, near correct/incorrect cues, correct/incorrect decisions and reward receipt/eating), units that significantly increased their firing rate (Figure 4.5), and units that significantly decreased their firing rate (Table 4.2). χ^2 analysis (significance set at $p = 0.05$) of the mice during the discrimination task revealed a decreased number of both activated and inactivated units in the *Plaur* mice, compared to control mice. Further comparisons on the reversal revealed an increased number of inactivated units in the *Plaur* mice at the time of reward. While overall SU behavior between the two groups was remarkably similar, the loss of the PV^+ neurons led to an elevated baseline firing rate in the *Plaur* mice along with altered responding to cues during learning and an increase in the number of cells that signal reward by being inactivated.

Figure 4.5. Raster plots and raw LFP traces during trials and behavioral time points show both similarities and disparities

A-B Raster plots show selective increased individual single unit activity for control and *Plaur* mice when near a correct cue (green triangle). C-D Raster plots demonstrate increased firing rate in Control and *Plaur* mouse OFC when a correct decision was made (red upside down triangle). Blue diamond symbols represent reward receipt. E Panel shows a raw LFP trace for a control and *Plaur* mouse during a trial. In the *Plaur* mouse, low frequency oscillations appear intact, but deficits in high frequency oscillations are easily observed.

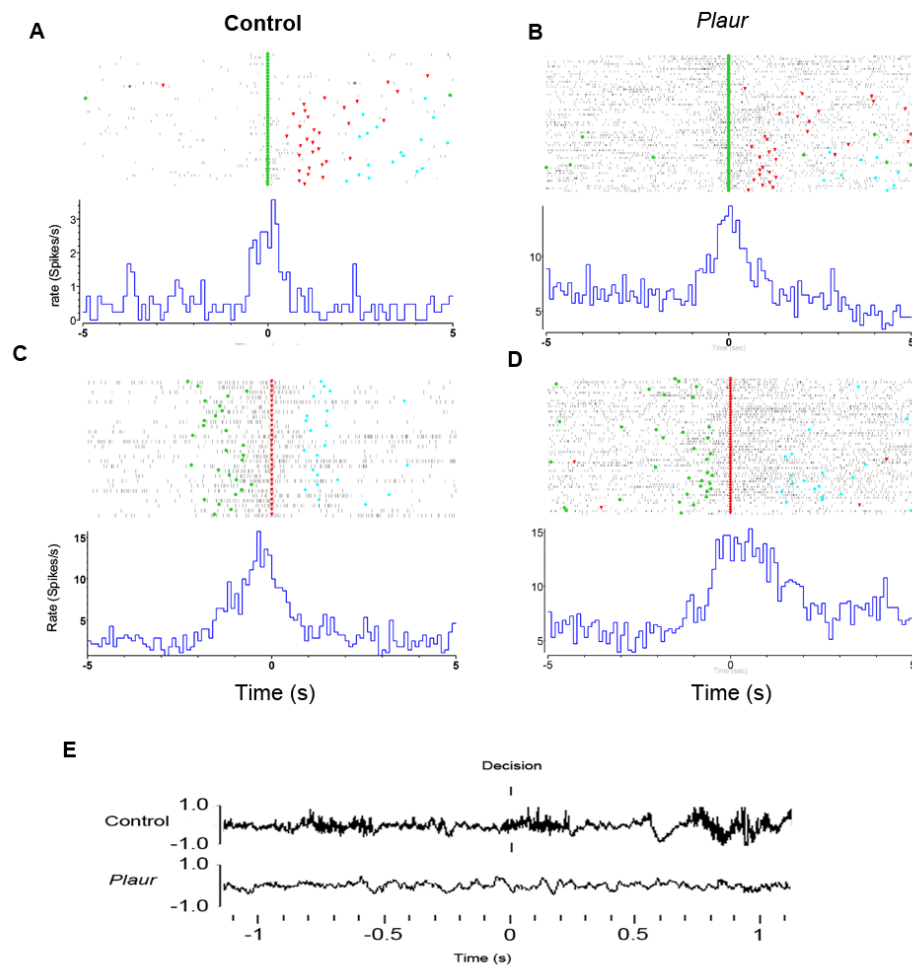


Table 4.2. Categorization of single unit significant firing patterns

Event	Discrimination				Reversal			
	Activated Units		Inactivated Units		Activated Units		Inactivated Units	
	Control (154)†	<i>Plaur</i> (201)	Control (154)	<i>Plaur</i> (201)	Control (157)	<i>Plaur</i> (214)	Control (157)	<i>Plaur</i> (214)
Near Cue	14.3%*	5.5%*	9.1%*	3.5%*	10.2%	6.5%	8.9%	5.1%
Decision	7.8%	9.0%	7.8%	10.9%	9.6%	6.1%	10.2%	13.6%
Reward	13.0%	12.4%	15.6%*	21.4%	6.2%	5.6%	3.8%*	20.5%*
Task	24.0%	21.4%	20.8%	15.9%	20.4%	22.0%	21.0%	17.3%

*significant at $p < 0.05$, ** $p < 0.01$, χ^2 analysis

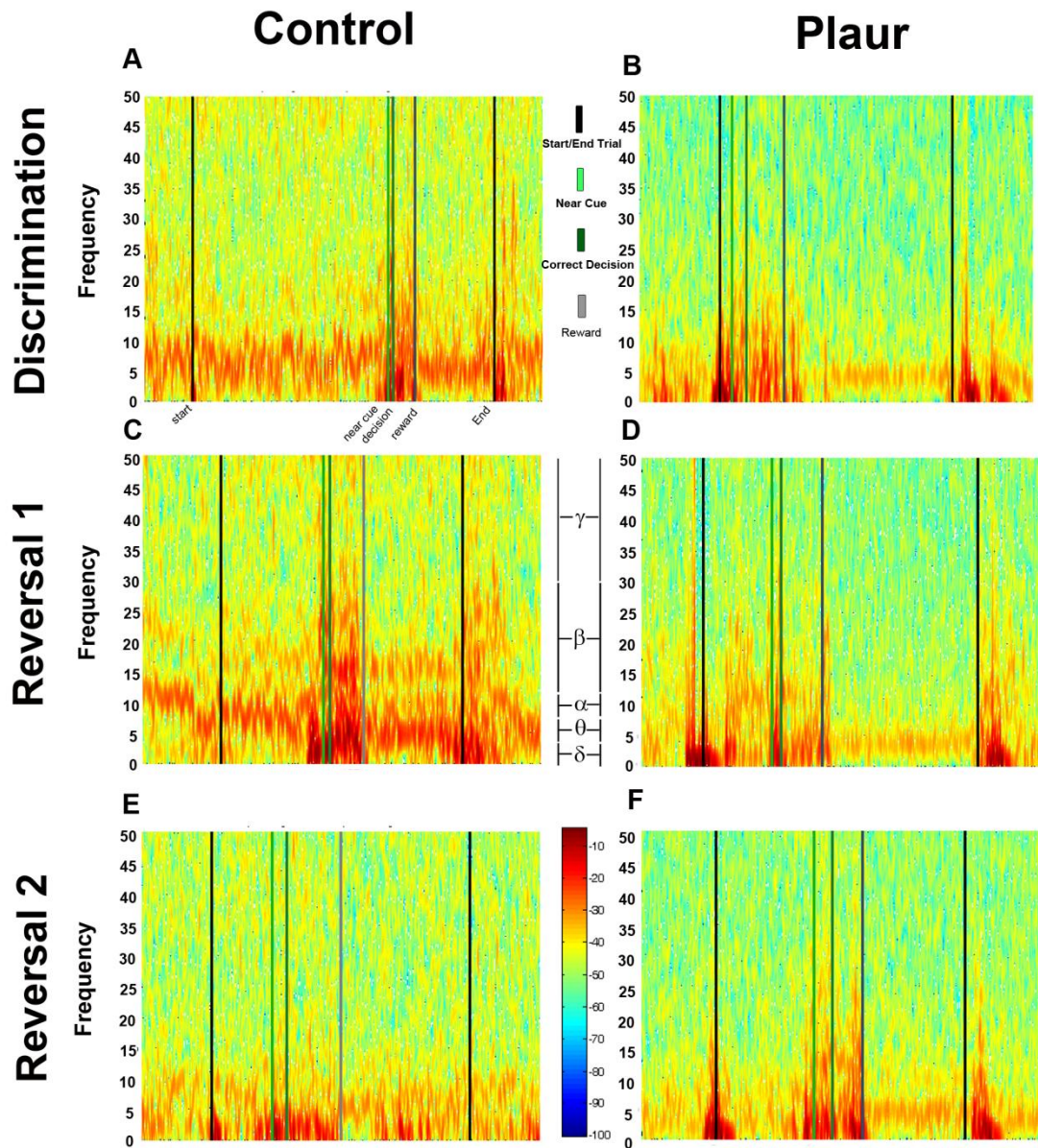
†Number of total units

4.2.3 Altered neural networks during reversal decisions

The network effects of a developmental alteration in GABAergic tone, notably loss of the PV⁺ interneurons, were investigated by comparing LFP recordings obtained during the task across the two groups. Initial trial-by-trial analysis showed easily discernable differences in patterns of wideband power spectral density (PSD) between the control mice and the *Plaur* null mutants (Figures 4.6A-F). Regions of increased power magnitude were visible in the control mice, especially when the mouse was near a cue or had made a correct decision (dark green lines in Figure 4.6). Compared to the control mice, *Plaur* null mice exhibited reduced alpha and beta frequencies during similar events, such as near a cue (Figures 4.6A and B). The PSD differences between the control and *Plaur* mice appear to be greatest during Reversal 1 (Figures 4.6C and D), where control mice demonstrated increased beta band power, particularly when near the correct cue and making a correct decision. The *Plaur* mice did not generate these beta frequencies. The PSD plots for reversal tasks after the initial Reversal 1 appear similar to the Discrimination for control and *Plaur* mice, as shown for Reversal 2 (Figures 4.6E and F). Only Reversal 1 evoked substantial high frequency oscillations in the control mice, with the *Plaur* mice appearing unable to generate significant increases in power in beta and gamma frequency bands for any of the tasks (Figures 4.6B, D, and F).

Figure 4.6. Parvalbumin interneuron deficient mice show task related differences in power spectra and frequency band deficits in OFC

A-F Comparison of task related changes in power across frequencies during Discrimination, Reversal 1 and Reversal 2.



Comparison of the summed power for each frequency band shows that *Plaur* null mice had a marked decrease in overall spectral power ($F(4,2109) = 161.58$, $p = 0.0001$), with significant decreases in the theta (*Plaur* null mouse at 60% control, $p = 0.002$) and alpha ranges (*Plaur* at 64% control, $p = 0.02$) during the discrimination task (Figure 4.7A). The power of the delta, beta and gamma frequency bands was not significantly different between control and mutant mice during the Discrimination ($p = 0.76$, $p = 0.39$, $p = 0.90$, respectively).

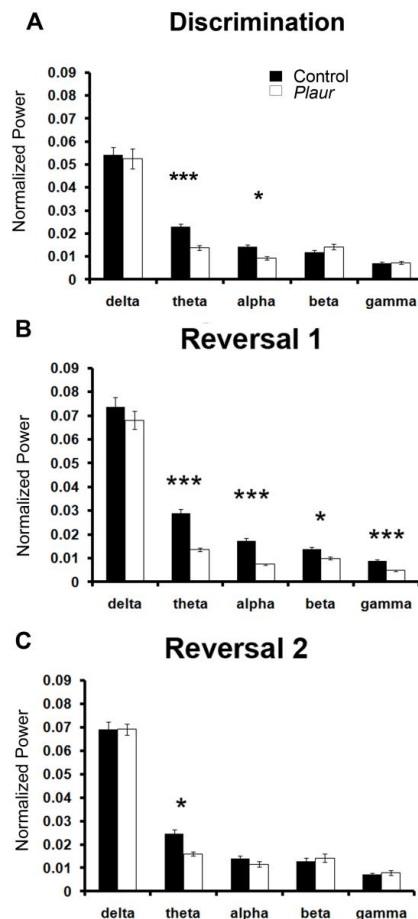
OFC has been reported to be a neural substrate for reversal learning, leading to the prediction that changes in PSD occur during the reversal task (Schoenbaum, Saddoris et al. 2007; Burke, Takahashi et al. 2009; Floresco, Zhang et al. 2009). We found control mice exhibited increases in every frequency band (ANOVA revealed main effect, $F(9,2915) = 93.19$, $p = 0.001$) during Reversal 1, with the greatest increases in alpha and beta bands ($p = 0.001$, Figure 4.7B). Comparisons between Discrimination and Reversal 2 indicate significant overall change (ANOVA main effect $F(9, 2745) = 111.67$, $p = 0.001$), likely due to differences in power in the delta band ($p = 0.001$), with other bands maintaining the same magnitude as during the Discrimination, (theta: $p = 0.53$, alpha: $p = 0.94$, beta: $p = 0.74$ and gamma: $p = 0.96$, Figure 5C).

By contrast, the *Plaur* null group did not increase overall spectra power during Discrimination, Reversal 1, or Reversal 2 tasks ($p = 0.25$). Although when the individual frequency bands were examined, power in the delta band tended to increase by ~29% during Reversal 1 (Figure 4.7B). Hence, comparison of the power during Reversal 1 in control and *Plaur* null mice demonstrated significant differences in individual overall power ($F(4,3189) = 412.99$, $p = 0.0001$, Figure 4B). The *Plaur* null mouse OFC generated significantly less power in theta (*Plaur* null theta at 47% of control, $p = 0.0001$), alpha (43% of control, $p = 0.0001$), beta (72%, $p = 0.016$), and gamma bands

(55%, $p = 0.0001$). However, when control and *Plaur* mice were compared on Reversal 2, the trends were more similar to the initial discrimination, though ANOVA revealed a significant main effect, ($F(9,2535) = 183.33$, $p = 0.001$) the only difference observed in individual power bands was in the theta band (*Plaur* was 64% of control, $p = 0.001$).

Figure 4.7. - Frequency band power is dependent on task and parvalbumin interneurons.

A Quantification of LFP during the compound discrimination. Mutant mice show decreased power in theta and alpha bands. B During Reversal 1 task the control mice demonstrate an increase in theta, alpha, beta and gamma average normalized frequency band power, as compared to the discrimination A. During the Reversal 1 task, mutant mice show lower power in theta, alpha, beta, and gamma bands, as compared to control mice. C During the second reversal task, when compared to control mice, the mutant littermates show lower average power in the theta range only. * $p < 0.05$, *** $p < 0.001$; All results are shown as mean \pm SEM



The data were normalized using z-scores to detect changes in variation within groups while engaging in the task. We chose a common data point, a correct decision, as the temporal center (3 s window) for comparing the average z-scores. These data are shown for the alpha, beta, and gamma frequency bands during the Discrimination, Reversal 1, and Reversal 2 tasks (Figure 4.8). The average variation around the correct decision was not different for the gamma band, but differences in alpha and beta bands were observed during Discrimination and Reversal 1. The data are represented as ratios of *Plaur*/control z-scores calculated at three separate time points: 0.25 s before a correct dig (decision), 0 s (at the correct decision), and 1 s after the decision when the mouse is consuming the reward. The gamma frequency band is relatively invariant (Figure 4.9). However, alpha and beta frequencies showed modulation on the Reversal 1 (Figures 4.9A and B) compared to the Discrimination or the Reversal 2 (Figures 4.9A and C).

Figure 4.8. Averaged z-score values vary with task

A-C Comparisons of difference of variances between control and *Plaur* animals during the correct dig (time = 0) in the discrimination task. D-F The first reversal leads to increased variability in the responses of the *Plaur* mice before a correct dig (time = 0) for α , β , and γ ranges, and decreases immediately afterward (time = 1 s). G-I During Reversal 2, the z-score values are similar between groups. Graphs are centered such that correct dig is the origin.

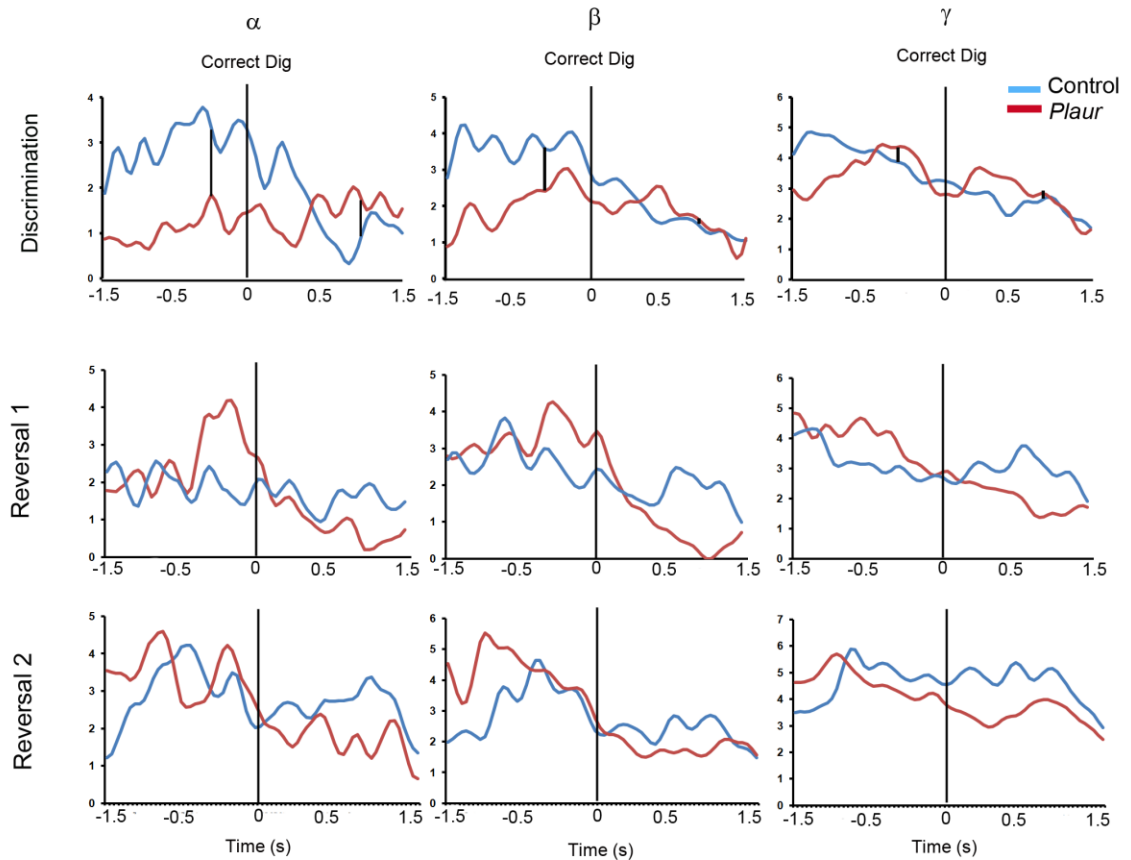
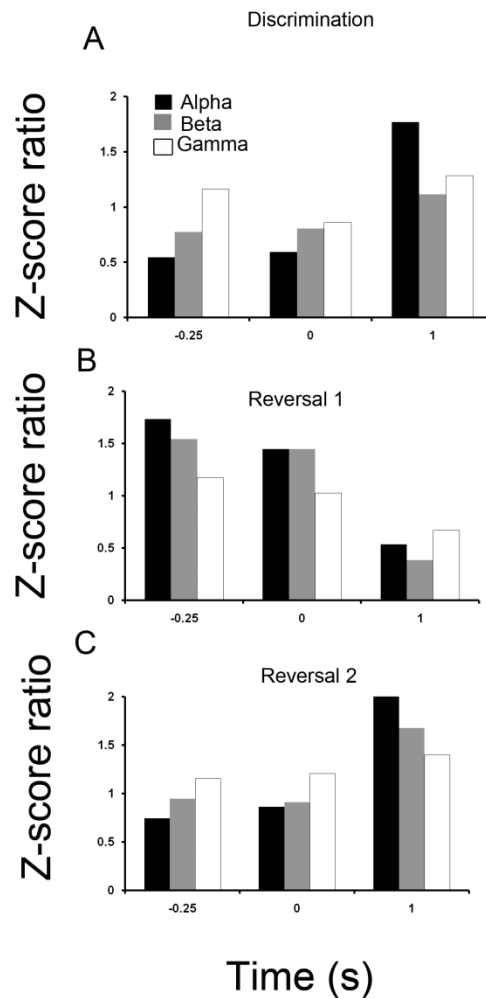


Figure 4.9. The ratio of z-scores *Plaur*/control mice at three time points demonstrate the selectivity of Reversal 1 compared to other trials

A z-score values from three time points illustrate a distribution around 1 of ratios from alpha, beta and gamma frequencies. B z-scores show an increase in variance in *Plaur* mice 0.25 s prior to the decision point, but not after the decision. Lowered ratios indicate an inability to 'keep up' with the cognitive demands of the reversal task in the *Plaur* mice compared to control mice. C Ratio values return to discrimination ratios on the Reversal 2, indicating the Reversal 1 requires more cognitive load than the Reversal 2.

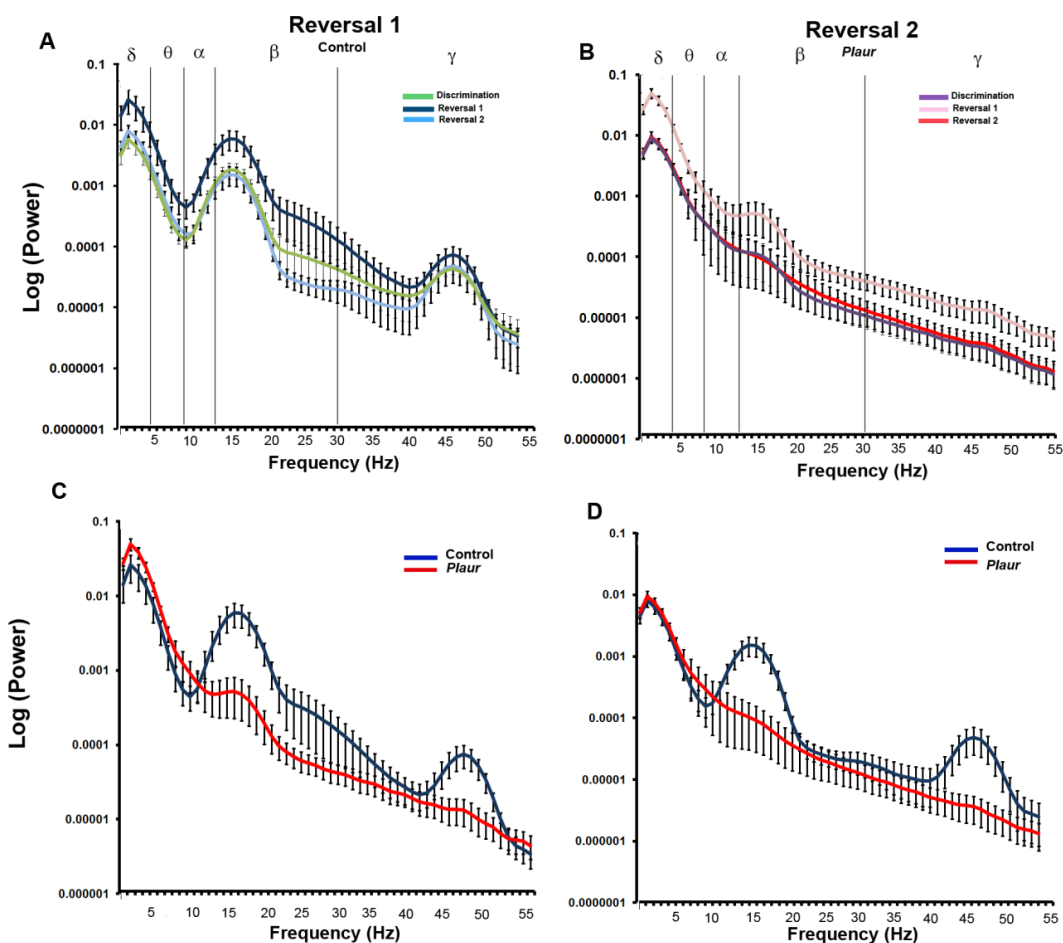


We compared the overall power spectral densities at the time of decision for each task and mouse genotype. PSD data from Discrimination, Reversal 1 and Reversal 2 are plotted on the same axis (Figure 4.10A). Reversal 1 requires a greater level of overall power compared to Discrimination (K-S test, $D = 0.5$, $p = 0.05$). The greatest increases in power in the control mice were seen in the alpha to gamma ranges, with one large peak beginning at around 12 Hz continuing to 22 Hz, followed by another peak from 42-53 Hz. Compared to Reversal 1, Reversal 2 has a 35% decrease in overall power (K-S test, $D = 0.4091$, $p = 0.0001$), with 32% less power in alpha, 17% less in beta and 42% less in gamma. In control mice, Reversal 2 and Discrimination are similar (K-S test, $D = 0.1786$, $p = 0.3$).

A similar trend for the *Plaur* mice demonstrates that Reversal 1 required increased power (Figure 4.10B). Comparing the control and *Plaur* mice during Reversal 1 shows differences between the two groups ($p = 0.05$, K-S test, $D = 0.2295$). With respect to the generation of high frequency oscillations, control animals show peaks in the beta and gamma ranges with a more broad ranging level of high beta power during this novel reversal (Figure 4.10C). *Plaur* animals do not show these robust peaks. During Reversal 2, the peaks in beta and gamma ranges are still evident in control mouse OFC, but not in the *Plaur* mice (K-S test, $D = 0.3036$, $p = 0.01$, Figure 4.10D). However the magnitude of the power in the beta and gamma bands during Reversal 2 is diminished, by almost an order of magnitude from Reversal 1 (K-S test, $D = 0.3214$, $p = 0.01$). In summary, control mice demonstrate the network load of a novel reversal upon OFC with the differences in high frequency power between Reversal 1 and Discrimination, and between Reversal 1 and Reversal 2. *Plaur* mice appear unable to generate higher frequency oscillations during discriminations, and also during reversal trials, demonstrating deficits in high frequency oscillatory power.

Figure 4.10. Power spectral densities highlight changes as OFC engages in the initial reversal task

A Average PSD from control and *Plaur* animals over first reversal demonstrates significantly less power in *Plaur* mouse OFC in high frequency ranges, starting in alpha and continuing to gamma. B Trial PSDs demonstrate a decreased difference between control and *Plaur* groups during the second reversal, as observed between higher frequencies. C Illustrates changes in power spectral with task for control mice. Control average spectral power in higher frequencies drops by an order of magnitude between Reversal 1 and Reversal 2. D The *Plaur* mice follow a similar trend as control mice, but the overall difference in power spectra is not as much less.



4.3 Discussion

Our findings integrate developmentally altered anatomy with behavioral and physiological data into a common mechanism to explain how fast-spiking PV⁺ GABAergic interneurons mediate decision making. A developmental deficit in number of cortical PV⁺ interneurons due to gene disruption alters ontogeny, culminating in abnormal cell loss during adolescence, leading to an adult mouse with increased baseline firing rate of putative RS cells and an inability to generate high frequency oscillations when required, such as during reversal learning.

Our data demonstrate that task-specific high frequency oscillations generated in the OFC are strongly correlated with tasks that are known to require intact OFC (Bissonette, Bae et al. ; Gross and Preuss 1954; Hansgen, Podhaisky et al. 1981; Schoenbaum, Setlow et al. 2003; Bissonette, Martins et al. 2008). Compared to learning a compound discrimination, the first reversal required nearly 10 fold more power. In control mice the power, across all frequency bands, was diminished in the second and subsequent reversal discriminations. These data suggest that murine OFC plays a critical role in helping an animal navigate a complicated change, when change is needed. Our data are in accordance with rat and primate literature (Hansgen, Podhaisky et al. 1981) Schoenbaum, 2000 #156; Schoenbaum, 2002 #456; Baxter, 2007 #459; Sul, 2010 #462}, which demonstrate using a variety of instrumental learning or even spatial learning tasks that the reversal of a previously learned cue association requires a functioning OFC. However, there appears to be a substantive difference between the first and subsequent reversals in a behavioral task. Monkeys that are repeatedly shown reversals acquire the subsequent reversals over a faster time course (Hansgen, Podhaisky et al. 1981; Boosen, Vetterkind

et al. 2005), as do rats (Boulougouris, Dalley et al. 2007). In our data, after the initial reversal, the *Plaur* mice perform no different than control mice, suggesting murine OFC's role lies in mediating initially cognitively difficult scenarios, but likely relying upon other neural structures for subsequent tests.

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, Gallagher et al. 2005; Talpur, Echard et al. 2005; Roesch, Calu et al. 2007). OFC activity correlates with cue related outcome-expectancies, and lesion studies in humans and non-human primates show perseverative reversal impairments (Fick, Barker et al. 1995; Schoenbaum, Setlow et al. 2003; Vetterkind, Boosen et al. 2005). OFC lesioned animals are able to acquire the initial discrimination, but when the task is changed, such that the subject must now choose the previously incorrect cue, as in a reversal task, the lesioned subjects require significantly more trials than control subjects to complete the task (Preuss, Gondal et al. 1995; Carlson, Rudgers et al. 2007). These behavioral data have been replicated here and in other reports in mice (Colacicco, Welzl et al. 2002; Bissonette, Martins et al. 2008).

It is interesting to note that, overall, the activity of single units between both control and *Plaur* mice were comparable. While there were a few distribution differences in terms of selectivity to firing on cues, with more cue-related cells observed in controls, and more reward sensitive cells observed in *Plaur* mice on the reversal, there were fewer differences observed than might have been expected. The individual ability of RS cells to generate action potentials is apparently not compromised in the *Plaur* mutant mouse,

though the firing rate and duration of action potentials were significantly different on the learning portion of the task. Similar numbers of RS units had activity that covaried with individual or multiple task events, indicating that the function of fast-spiking PV⁺ interneurons is not to sculpt individual neuronal responses to particular task-related events, but that these interneurons may be required for larger network cohesion (Preuss 1994; Preuss, Faber et al. 2009; Gomes Ade, Paula et al. 2010). These data are also the first to report an instance of elevated baseline firing in RS cells in the OFC while an animal learns a discrimination, though a similar effect was reported in the S1 of cortex (Mizoguchi, Shoji et al. 2010). Taken together, these data indicate the primary role of FS PV⁺ cells is that of a network facilitator, engaging large cortical networks through coordinating changes in oscillatory power rather than working on a cell by cell basis to sculpt cell specificity.

Awake recordings in the *PV-ΔGluR-A* mice, in which the AMPA receptor mediated innervations of PV⁺ cells is diminished, demonstrated that the hippocampal PV⁺ cells were integral in the coordination of pyramidal neurons to generate theta rhythms (Witt and Helmstaedter 2009). Gamma oscillations can also be suppressed or driven using optogenetic techniques *in vivo*, which impact local excitatory neurotransmission (Gomes Ade, Paula et al. 2010). While some studies have experimentally driven oscillations and others have modified PV cell receptivity to excitation, the common theme between our data and these studies is the need for PV⁺ interneurons to coordinate local and distant networks in order for learning to proceed efficiently. In terms of our experiment, mice

with a PV⁺ cell deficit were less efficient at modifying their behavior in the face of changing task contingencies.

The data from *Plaur* mice demonstrate a deficit in the generation of high frequency oscillations in the beta and gamma ranges, notably on the first reversal, though there are some inherent changes in LFP power observed both during the discrimination state and the subsequent reversal states, such as the altered theta rhythm. It is also important to note that *Plaur* mice still are able to complete the first reversal, albeit over a less efficient time course in terms of trials to criteria. Whether this ability to learn a reversal is due to the fact that *Plaur* mice still retain 20-30% of their fast spiking PV⁺ interneuron population or the activity of another neural structure such as the dorsal medial striatum is assuming responsibility in driving this behavioral change. Studies show the involvement of the striatum in reversal learning (Clarke, Robbins et al. 2008; Guo, Dey et al. 2009; Sanchez-Peinado Mdel, Gonzalez-Lopez et al. 2010), potentially by using dopamine to signal changes in outcome predictions related to cues (Jost 2008). Evidence to support the continued function of the few remaining PV⁺ interneurons may be found by observing the PSD for the first reversal in *Plaur* mice. In the beta frequency range, there was a minor increase in power in *Plaur* mice in the low beta (13-20Hz) which corresponded to the same frequency range that was altered in the control mice, although power in control mice was 10-fold stronger. Thus, the first reversal appears to require a functional OFC to efficiently navigate it, and this efficient application of behavioral flexibility is, at least in part, dependent upon FS PV⁺ interneurons.

The OFC has been implicated in animals to guide responding by mediating the meaning of cues from the environment as well as response inhibition (Preuss, Koller et al. 2001; Burke, Takahashi et al. 2009). Studies have shown alterations to OFC through inactivation or lesion cause preservative or impulse responding to cues (Clarke, Robbins et al. 2008; Nabholz, Glemin et al. 2008; Burke, Takahashi et al. 2009). However, the OFC does not act alone in cue association, but instead is an integral part of a complex network where basolateral amygdala and OFC interact initially to form associations (Saddoris, Gallagher et al. 2005; Satapathy, Krupadam et al. 2007; Nabholz, Glemin et al. 2008). The OFC functions to encode predicted outcomes of cues (Saddoris, Gallagher et al. 2005; Simoes and Simeao 2006; Stalnaker, Roesch et al. 2007) and communicates with the basal lateral amygdala dorsal striatum (Diniz-Filho and Torres 2006; Zhang, Hurek et al. 2007). In the *Plaur* mice, the dorsal striatum was found to have a selective deficit in PV⁺ interneurons (Bissonette, Bae et al.), while the amygdala was unaffected (Bissonette et al, in review). The striatum and amygdala are reported to have binary, all or nothing associations with cues, while OFC is active in a dynamic manner over the course of learning cues and associated outcomes (Leebens-Mack, Vision et al. 2006; Noar, Cole et al. 2006; Satapathy, Krupadam et al. 2007). The similarity of connections and function between human, primate and rodent neuroanatomical structures support the idea of OFC encouraging the learning of new behaviors and inhibiting old during tasks which require behavioral flexibility.

These findings may have important implications for our understanding of complex cognitive processes in both normal and disease conditions. For example, changes in the

GABAergic neurons in schizophrenia and frontal lobe epilepsy have been directly attributed to the PV⁺ population of interneurons (Castells, Rieta et al. 2005). Associations with *PLAUR* are reported in human populations with schizophrenia, autism and epilepsy (Andre 2004; Szabo, Brookings et al. 2008; Burdick, DeRosse et al. 2010). Many human neuropsychiatric disorders have developmental origins and have been linked to altered GABA (Skarja, Remic et al. 2004; Castells, Rieta et al. 2005; Szabo, Brookings et al. 2008). The specific anatomical and cognitive deficits seen in our mouse model strongly recapitulate those seen during human disease states, while control mice have functionally demonstrated murine OFC validity in terms of network activity during a classical OFC-mediated task (Charlin, Desaulniers et al. 2002; Murray, O'Doherty et al. 2007). Patients with schizophrenia demonstrate impaired performance and perseverative errors when tested on a probabilistic reward task, implying compromised OFC function (Preuss 2000; Chen, Lam et al. 2001; Drennan and Swartz 2002). Indeed, perseverative errors appear to be elicited as the cognitive effort required in a task increases. Patients learn new cue associations with relative ease, but when asked to reverse behavior in the face of a changing contingency, are unable to efficiently do so (Drennan and Swartz 2002).

The development of the GABA system may be subjected to multiple permutations of genetic and environmental perturbations. GABAergic interneurons leave the ganglionic eminence, the precursor for the adult striatum, and migrate to populate the forebrain, including the cerebral cortex, hippocampus and amygdala, or remain in the striatum (Marin, Yaron et al. 2001; Goto, Kaneko et al. 2005; Haiat, Padilla et al. 2005; Wagner,

Lacey et al. 2006). While the FS PV⁺ interneuron population is affected in the *Plaur* mice, this mutation only affects cortical and striatal FS PV⁺ cells, and not amygdala, hippocampal or caudal cortical areas (Bissonette, Bae et al. ; Powell, Campbell et al. 2003) (and Bissonette, et al. in review). In summary, it appears that OFC is necessary to efficiently modify existing behavioral responses and our data demonstrate that changing GABAergic tone in OFC will interfere with this function. Future studies will focus on how the inputs and the responses in the dorsal striatum are affected in the *Plaur* mice.

Recently, a postnatal loss of NR1 subunit was reported in all PV⁺ cells (Mizoguchi, Shoji et al. 2010). While this study did not investigate network dynamics of the cortex, they demonstrated that fully functional NMDA receptors on hippocampal PV⁺ interneurons are correlated while the mouse is engaged in a task. These data suggest that NMDA receptors are needed to enable PV⁺ cells to effectively synchronize nearby pyramidal neurons (Mizoguchi, Shoji et al. 2010). Without a critical number or ensemble of PV⁺ interneurons to facilitate coordination among neural networks, the rapid ability to learn in changing contingencies is diminished. For example, Sigurdsson et al, used a mouse model of a chromosomal microdeletion, the *Df(16)A^{+/-}* mouse, to demonstrate deficits in coherence between mouse PFC and hippocampus (Mizoguchi, Shoji et al. 2011). Data from our current study would suggest OFC is playing an important role in the first reversal and is perhaps doing so by using high frequency oscillations to enable coherence with extracortical regions. These data illuminate the role of fast-spiking PV⁺ interneurons in mediating cognitive processes and enable us to dissect the functional anatomy and molecular mechanisms governing the network behavior of the frontal cerebral cortex. As

our understanding of the development grows, so too will our understanding of developmental perturbations and how they alter the trajectory of different neuroanatomical substrates, leading to psychiatric disorders.

Chapter 5

Summary

5.1 Proposed 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 and that fast-spiking PV⁺ interneurons play an integral role in assisting OFC to guide animal behavior in the face of changing contingencies.*

5.2 Murine OFC is a neural substrate for reversal learning

While we know murine OFC is similar in terms of connectivity to rat and primate, the functional role of murine OFC has yet to be dissected. Rodent literature dissociates between OFC and the medial prefrontal cortex (mPFC) in terms of both function and connectivity, as regions of similar connectivity in humans are recognized as performing different cognitive functions. Lesions to OFC or mPFC yielded selective deficits on a rodent digging task that tests both reversal learning as well as attentional set-shifting. Sham mice and OFC lesioned animals were able to learn a global rule to guide their behavior while OFC lesioned animals exhibited a learning deficit on reversal trials. Sham mice and mPFC lesioned mice were able to efficiently modify responding during a reversal portion of a task but mPFC lesioned mice displayed an attentional set-shifting deficit. These data were validated both in trials to criterion as well as the number of errors committed on portions off the task. In fact, control mice tested on versions of this task which varied according to the number of discriminations prior to the ID/ED shift (Intradimensional/Extradimensional) demonstrated a critical number of tasks to be between 5 and 7 exemplar pairs. This behavioral test allowed me to demonstrate that mice are capable of forming attentional sets, while simultaneously affording me the opportunity to test reversal learning. Lesions to OFC and mPFC demonstrated selective

cognitive deficits, allowing me to use mice as an appropriate model for prefrontal-mediated cognition and to probe selective cerebral cortical areas for the affects of changes in GABAergic tone.

5.3 Fast-spiking PV⁺ interneurons in OFC are required for efficient reversal learning

If murine OFC functions similarly in terms of reversal learning in humans, this opens the door for mutant mice to test developmental alterations to neural substrates especially substrates linked to human disorders. To this end we used a mutant mouse with abnormal interneuron development that results in significant decreases of PV⁺ interneurons in OFC and striatum, as well as a mouse which constitutively over-produces a growth factor postnatally, recovering this interneuron loss (Chapter III). The loss of FS PV⁺ interneurons in the cortex and the striatum of *plaur*-null mice is manifested behaviorally in terms of a selective reversal deficit which did not impact learning of the intradimensional tasks. Mice which over-expressed HSF/SF performed no differently than WT animals and *Plaur/HGF* mice had normal numbers of FS PV⁺ interneurons in both OFC and striatum. These data support a role for FS PV⁺ interneurons in cortically-mediated cognition and the use of a transgenic mouse with genetic linkages to human disorders such as epilepsy, autism and schizophrenia, demonstrating similar cognitive deficits in a mouse model.

5.4 Fast-spiking PV⁺ interneurons coordinate high frequency oscillations during reversal learning in OFC

GABAergic interneurons are suspected to be responsible for coordinating both local networks and synchrony between multiple neuroanatomical areas during learning tasks. To understand the physiological implications behind an anatomical deficit in PV⁺ cells, as well as how murine OFC mediates reversal learning, we recorded both single units (SU) and local field potentials (LFPs) in OFC in both control and *Plaur* mice performing a task with multiple reversals. *Plaur* mice demonstrated a robust reversal deficit on the first reversal, as we have shown previously (Chapter III), but on reversal 2 and subsequent reversals, the deficit was abolished. *Plaur* mice had a physiological loss of FS PV⁺ interneurons in terms of the numbers of cells observed while recording and quantifying the single unit data, reinforcing the idea that PV⁺ cells are affected by the *Plaur* mutation and are, in fact, no longer present in the frontal cortex of adult mice. The anatomical deficit in the PV⁺ population also has a developmental onset, similar to the onset of many human psychiatric disorders in which GABA has been implicated. We investigated the correlation of regular spiking (RS) units with behaviorally relevant events and observed only mild differences between the two populations of cells from the two different types of mice. Overall the units observed were comparable in proportion between control and *Plaur* mice regarding which task-related elements they developed specificity for. There were a few instances where the two populations were different, such as a decreased number of cue selective units in *Plaur* during the discrimination and an increase number of reward-sensitive units in *Plaur* during the reversal. However, overall the two populations were remarkably similar, suggesting that coordinating activity that co-varies with behavior in RS cells is not one of the functions of FS PV⁺

interneurons. Control mice demonstrated a variable, wide-ranging change in spectral power during discrimination, reversal 1 and reversal 2. It is widely accepted that high frequency oscillations are a signature of learning, though a potential mechanism of assisting neural structures to synchronize and coherently communicate. Control mice demonstrated these learning hallmarks by robustly increasing the presence of high frequency oscillations in the alpha, beta and gamma ranges during reversal 1, as compared to those in the previous discrimination. The overall power was diminished in controls on a subsequent reversal, physiologically supporting the idea of OFC as an important neural structure to facilitate and instigate behavioral change. *Plaur* mice were unable from the start to generate high frequency oscillations of comparable magnitude to those of control groups, and were severally impoverished on the first reversal. The differences between these two groups were diminished on the second reversal, the time point where control and *Plaur* mice both showed comparable behavior.

5.5 Conclusions

It is possible to model particular elements of human cognition in the mouse. Mice are capable of performing behaviors of such complexity that they are comparable with rat, non-human primate and human. We have shown through neurotoxic lesions studies that separate regions of mouse frontal cortex are not only different connectivity-wise but also functionally and that they have readily dissociable cognitive functions. Further, perturbations in GABAergic cell ontogeny by manipulating growth factor sensitivity can produce a mouse with a 70% decrease in FS PV⁺ interneurons in the frontal cortical

regions and the striatum. This *Plaur* mouse demonstrates a reversal deficit on a digging task, suggesting a role for FS PV⁺ interneurons in mediating the reversal learning function of the OFC. These animals learned a task just as well as wild types, but had a reversal specific deficit. Breeding these mice with mice that over express the growth factor HGF/SF, we are able to recover the interneuron number deficit, and more importantly the cognitive reversal deficit as well. Our recording and behavior data suggest murine OFC is most functionally important on the first reversal, indicating the OFC's role in mediating novel behavioral situations. Our data from the *Plaur* mice demonstrate that a functional lack of FS PV⁺ interneurons leads to a less efficient ability of OFC to coordinate a behavioral change. The deficits in high frequency oscillations in *Plaur* mice corroborate previous research, indicating that PV⁺ cells are responsible for coordinating these oscillations, and that these high frequency oscillations play an integral part in driving the OFC's function. This thesis elucidates the role of mouse Orbitofrontal cortex and provides a functional role for fast spiking parvalbumin positive interneurons in organizing OFC mediated cognition. Improper OFC function has been demonstrated in numerous human psychiatric disorders, including epilepsy, autism and schizophrenia (Elliott et al., 1995; Verte et al., 2005, Lewis et al., 2005, Piau et al, 2010) and alterations in fast spiking PV cells have been linked to human psychiatric disorders as well (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). In this thesis we show that changes to the GABAergic tone of the OFC in a mouse model impairs cognition in a similar fashion to humans, providing a potential

model for studying the effects of altered GABAergic developmental trajectory on prefrontally mediated cognition.

Appendix 1

Materials and Methods:

A1.1 Animals

A1.1.1 Lesion Experiments

Adult male C57Bl/6J mice (>12 weeks old) were purchased from The Jackson Laboratory (Bar Harbor, ME). Experiments were conducted in accordance with Animal Care and Use Committee (IACUC) at University of Maryland approved protocols and the Policies on the Use of Animals and Humans in Neuroscience Research. Under sterile conditions, bilateral stereotaxic lesions were made in mPFC (AP: 1.9; ML: ± 0.3 ; V: 3.2 mm) or in OFC (AP: 2.6; ML: ± 1.2 ; V: 2.8 mm) of anesthetized (isoflurane) mice using established coordinates (Paxinos and Franklin 2001). At each lesion site, ~ 0.1 μ l of sterile N-methyl-D-aspartic acid (NMDA, 12.5 mg/ml in 0.9% saline, Sigma Chemical Co., St. Louis, MO) was injected using a pulled glass pipette and a picospritzer. For control sham lesions, saline vehicle alone was injected. Each group was tested on the reversal/set-shift task after a 2 wk recovery period.

A1.1.2 Transgenic Experiments

C57BL/6^J mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The B6.129-*Tg(Gfap-HGF)^{Ca}* (abbreviated as *HGF*) mice, in which the expression of human HGF is under the regulation of the glial fibrillary acidic protein (GFAP), and B6.129 – *Plaur^{tm1/Mlg}/Plaur^{tm1/Mlg}* lacking the gene that encodes the urokinase plasminogen activator receptor (uPAR) protein (abbreviated as *Plaur*) mice were

generous gifts from our collaborators (C. Achim, University of California San Diego) and P. Carmeliet (Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, KU Leuven, Belgium, respectively (Dewerchin, Nuffelen et al. 1996). All research procedures using mice were approved by the IACUC at University of Maryland and conformed to NIH guidelines. The *HGF* mice were genotyped via PCR using the primer sets: 5'-ggCCATgAATTTgACCTCTATgAA-3' and 5'-TTCAACTTCTgAACACTgAggAAT-3' (250 bp) for *Gfap-HGF* mice, and 5'-CCTCATCCTgggCCTggTTCTggTCT-3' and 5'- ggTTTTCCCCgCTgTggTCATCTgC-3' (200 bp) as a positive control. For genotyping *Plaur* mice, the primer sets were: 5'-gATgATAgAgAgCTggAggTggTgAC-3' and 5'-CACCgggTCTgggCCTgTTgCAgAggT-3' (145 bp) for *Plaur*, 5'-ATTgAAgAAgATggATTgCAC-3' and 5'-TTCgTCCAgATCATCCTgATCgAC-3' (500 bp) for neomycin resistant gene. Behavioral and anatomical analyses were performed on adult male littermates from at least 4 separate pedigrees. B6.129 male wildtype (WT) littermate mice were used as controls. All mice were bred from heterozygotes that had been backcrossed onto the C57Bl/6J (B6) background for >10 generations.

A1.1.3 Electrophysiology Experiments

B6.129-*Plaur*^{tm1/Mlg}/*Plaur*^{tm1/Mlg} mice lacking the gene that encodes the uPAR protein were genotyped as described above (Bae, Bissonette et al.). All mice were bred from heterozygotes that had been backcrossed onto the C57BL/6J (B6) background for >10 generations. Experiments were conducted in accordance with IACUC approved protocols and the *Policies on the Use of Animals and Humans in Neuroscience Research*. Behavioral and anatomical analyses were performed on adult male littermates from at

least 4 separate pedigrees. B6 or B6.129 male wild-type (WT) littermate mice were used as controls. Under sterile conditions, an electrode with a drivable microarray of 9, 25 μm diameter FeNiCr wires (A-M Systems, Sequim, WA) in 27 gauge thin wall cannula (Small Parts, Miami Lakes, FL) was implanted in OFC (AP: 2.6; ML: ± 1.2 ; V: 1.2 mm) using established stereotaxic coordinates (Paxinos and Franklin 2001). Prior to implantation, the wires were freshly cut and electroplated with platinum (H_2PtCl_6 , Sigma-Aldrich, Milwaukee, WI) to an impedance of approximately 300 $\text{k}\Omega$. After a recording session, the electrode bundle was advanced in 60 μm increments to access new neurons the following day.

A1.2 Behavior Tasks

A1.2.1 Reversal/Set-shift task

After recovery from surgery, mice were tested on a variant of the reversal/set-shifting task developed by Brown and colleagues for rats (Birrell and Brown 2000; McAlonan and Brown 2003) and adapted for mice (Colacicco, Welzl et al. 2002; Garner, Thogerson et al. 2006), also see Tables 1 and 2 in Chapter 2). Mice were food deprived to reduce body weight to 85% of free feeding weight and habituated with testing materials for 3 d prior to evaluation.

At the start of each trial, the mouse was placed in the testing arena to explore two bowls with combinations of odors and digging media until digging in one bowl to signify a choice. The bait was a piece of Honey Nut Cheerio cereal (~5 mg), and the cues, either olfactory (odor) or somatosensory and visual (texture of the digging medium which hides

the bait) were altered and counterbalanced (Table 2). All cues were presented in identical small animal food bowls (All Living Things Nibble Bowls, PetSmart) that were identical in color and size. Digging media was mixed with the odor (0.01% by volume) and Honey Nut Cheerio powder (0.1% by volume). All odors were ground dried spices (Penzeys, Milwaukee, WI; Hershey Chocolate Co, Hershey, PA; or McCormick Spice Co, Hunt Valley, MD) and unscented digging media was purchased from PetSmart (KayKob, bedding, wood chips, aquarium gravel, aquarium stone, kitty litter (2 types)) or local discount stores (cotton balls, feathers, moss, plastic pellets, shredded paper, perlite, bark, packing peanuts). The mice were housed in Softcard bedding. The digging media did not contain any components used in the animal bedding. On the first day of training, the mice were given 4 consecutive trials with the baited food bowl to ascertain they could reliably dig. All mice were able to dig for the reward. The testing was performed over a 4 d period.

Mice were tested through a series of discriminations where the exemplar pair was changed, but the dimension (odor or medium) of the correct choice remained the same. The dimension was relevant if its attributes predicted outcome. For example, if odor was the relevant dimension, then the mouse was required to choose the correct odor from each pair and ignore the attributes of the digging medium. In this example, the digging medium is considered the irrelevant dimension.

The discriminations were as follows:

- (1) A single series of simple discriminations (SD) in which the mouse was presented

- with two choices of the relevant dimension and one choice of the irrelevant dimension (i.e. two odors within the same medium);
- (2) A single series of compound discriminations (CD) in which the mouse was presented with the same choices of relevant dimension as in the SD and two choices of irrelevant dimensions (the exemplar used in the SD and a new exemplar);
 - (3) A series of four intradimensional shifts (IDS I – IV) in which the mouse was presented with compound discriminations using two novel exemplars from the relevant and irrelevant dimensions for each IDS. The relevant dimension of the correct choice (i.e. odor) was maintained throughout the discriminations.
 - (4) A reversal discrimination (IDS IVrev) in which the mouse was presented with the same set of exemplars as in IDS IV, but the stimulus-reward pairing was reversed within the relevant dimension.
 - (5) An extradimensional shift (EDS) in which the mouse was presented with a novel compound discrimination, except for the first time the correct choice was an exemplar that was previously from the irrelevant dimension. The previously relevant dimension has become irrelevant.

The baited bowl was randomly presented on either side of the testing cage, and the relevant exemplar was randomly presented with the irrelevant exemplars. The trial was stopped if the mouse did not dig within 3 min in the testing cage. Stopped trials were uncommon (< 3% of all trials), and they occurred most frequently during the SD. Aborted trials were not observed after completion of the CD and were not included in the

latency calculations. The order of discriminations and exemplars was the same for all mice (Tables 1 and 2), but the direction of the extradimensional shift (EDS, odor to medium or medium to odor) was counterbalanced within each experimental group. A criterion of eight consecutive correct trials was required to complete each task. The mice completed all discriminations within a 3-4 d period. Data are reported as the number of trials to criterion and the number of errors required for each discrimination.

A1.2.3 Behavioral methods in Electrophysiology studies.

Control (n = 10) and *Plaur* (n = 8) mice were tested on a modified naturalistic foraging reversal task as described in previous reports (Bissonette, Bae et al. ; Colacicco, Welzl et al. 2002; Bissonette, Martins et al. 2008). A reversal discrimination tasks was included after each compound discrimination. Two types of behavioral data were obtained: trials to criterion of 8 correct consecutive trials (learning) and on an additional 15 trials after criterion had been met, representing the learned behavior. Values are reported as the mean \pm standard error of the mean (SEM). For trials to criteria and errors, a two-way ANOVA was used to determine statistical significance between treatment groups and discriminations, with Student-Newman-Keuls *posthoc* testing. Behavioral analysis was performed with Statistica software package (Statsoft, Inc, Tulsa, OK). Statistical significance of $P < 0.05$ is denoted by asterisks, $P < 0.01$ is denoted by double asterisks, while $P < 0.001$ is denoted by triple asterisks.

A1.3 Histology and Cell Counting

The mice were transcardially perfused with buffered 4% paraformaldehyde and the brains were postfixed overnight at 4°C. Brains from electrophysiological studies were cut (50 µm) on a freezing microtome (Melville, NY) and stained with cresyl violet using routine laboratory protocols (Powell, Campbell et al. 2003; Martins, Plachez et al. 2007). Electrode placements were verified under light microscope and images were obtained with an upright light microscope (Leica DMRX microscope, Leica Microsystems GmbH, Wetzlar) and a digital camera (Retiga-2000R, Q Imaging Surrey BC Canada) using QCapture Pro 6.0 software and drawn onto plates adapted from the atlas of Paxinos and Franklin (Paxinos and Franklin 2001).

For immunohistochemistry, vibratome sections (50 µm; Leica) were cut and stained using routine laboratory protocols (Bae, Bissonette et al. 2009); Martins, 2007 #2596}. The sections were incubated with a mouse anti-PV antibody (1:1000, Sigma Chemical Co, St. Louis, MO) for 2 d at 4°C. For chromogenic staining, following incubation with primary antibodies, sections were incubated in the appropriate biotin-conjugated secondary antibodies (1:3000, Jackson Immunoresearch, West Grove, PA) for 2 h at room temperature and placed in Vectastain ABC solution (Vector Laboratories, Burlingame, CA) for 1 h. The antibody labeling was visualized with the 3'3'-diaminobenzidine substrate (DAB; Sigma, MO), enhanced with nickel sulfate. Images were obtained by Leica DMRX microscope (Leica Microsystems GmbH, Wetzlar) with Phase One image capture software version 3.04 (**Phase One A/S**, Frederiksberg, Denmark).

For each region of interest, the numbers of PV-immunoreactive cells were estimated at several anatomical levels by a blinded observer using the NeuroLucida system (MicroBrightField, Inc; Williston, VT). An estimate of cell number in each region was calculated using unbiased stereology (Inglis and Moghaddam 1999). The PV⁺ cells were counted in 2 sections in OFC (from Bregma level 2.86 mm extending caudally to 2.20 mm), and 5 sections along the striatum (Bregma level = 1.54 mm and extending caudally to -1.06 mm). Volume was estimated using the Cavalieri method. Data are provided as mean \pm standard error of the mean (SEM), with at least 4 mice for each genotype. The statistical significance among four different genotypes was examined using one-way ANOVA followed by Student-Newman-Keuls (SNK) *post hoc* analysis (Statistica version 8, StatSoft, Inc. Tulsa, OK).

A1.4 *In vivo* Electrophysiology Data acquisition and analysis

Experiments were performed in a behavioral chamber previously described (Schoenbaum and Roesch 2005). Action specific timestamps were recorded by an observer simultaneous to the task performance. We performed daily screening of active wires, and advanced the electrode assembly by $\sim 60\mu\text{m}$ per day at the end of the recording session. Neural activity was recorded using a Plexon Multichannel Acquisition Processor system, where signals from the electrode wires were amplified 20x by an op-amp headstage (Plexon Inc, HST/8o50-G20-GR). The signal was split, where the single units were amplified 50x and filtered at 150-9,000 Hz. Single unit data was then sent to the Multichannel Acquisition Processor box, where it was further filtered at 250-8,000Hz,

digitized at 40kHz and amplified 1-32x. Waveforms with a greater than 2.5:1 signal to noise ratio were recorded for Offline sorting. Continuous data was simultaneously recorded from 2-4 wires were also recorded. Continuous data was bandpass filtered from 0.77-300Hz, amplified 5,000x, sampled at 1kHz and recorded to a computer hard drive. Off-line sorter (Plexon, Dallas, TX) identified and sorted units, which were subsequently analyzed by Neuroexplorer software (Plexon). Data were exported and analyzed using statistical and graphing routines in MATLAB to examine firing activity to the experience of particular cues. Single unit average firing was calculated in a 3 second window around specified time events (1.5 seconds pre- and post-event) for both trial and ITI (inter trial interval) segments. A Mann-Whitney U test was performed on these populations, comparing all of the trial segments to all of the ITI segments for each file. Data from units that changed their firing exceeding a significance level of $p < 0.001$ were grouped by the corresponding behavioral timestamp and grouped by increased or decreased firing when significant. For unit quantification, units were separated into putative populations by action potential (AP) duration and spiking rate. Putative FS units were defined as having an AP duration of 700 μ s or less and spike rate greater than 3 spikes/s. Putative regular spiking (RS) units were defined as having an AP duration greater than 700 μ s. Unclassified units (UC) were units that fell outside these two inclusion criteria, generally having a spiking rate less than 3 spikes/s, and short AP duration (less than 700 μ s). Once grouped, the number of units observed which significantly changing their firing patterns were tested between control and *Plaur* mice by way of a χ^2 analysis to illuminate significant differences in distributions. Significance of $p < 0.05$ is denoted by an asterisk.

Continuous data in the form of LFPs were recorded simultaneously from the multiple array microwires. Spectrograms were constructed by using the Fast Fourier Transform (FFT), using notch filtered at 60Hz ($Q = 3$, 10th order Butterworth) to eliminate contributions from environmental electrical noise. To account for variations in gain across multiple animals and recording sessions, the overall means were normalized to 0.5 mV magnitude. For each mouse and task, the LFP was segmented into "trial period" and ITI. The Power Spectral Density (PSD) of all of the segments was calculated using the Multitaper method to obtain the average power. A spectrogram (power in log scale, Hamming window of length 500, window overlap of 455, processed using the Goertzel algorithm) was generated from the trial segments. Frequency bands were set prior to analysis as 1-4 Hz (delta), 4-8 Hz (theta), 8-14 Hz (alpha), 14-30 Hz (beta) and 30-50 Hz (gamma) as previously described (Buzsaki 2005; Gruber, Hussain et al. 2009). To compare differences in power across different bandwidths between the control and *Plaur* mice, for each trial, the power was summed within each frequency band, subsequently averaged to determine the mean and was compared statistically between control and *Plaur* groups for using a Student's *t*-test for each frequency band.

Power spectral density estimates were taken using Welch's method for power spectrum estimation on the continuous data. Data was segmented according to trial length, and values were averaged and plotted with standard error of the mean.

For z-score analysis, LFP data was processed as mentioned above except rather than normalized by voltage magnitude, a z-score was calculated. For the baseline, we used task-specific activity since the ITI was enormously different from trial activity in a non-

linear fashion. Each frequency bin (row) in the power spectrum was z-score normalized to the average spectral power during the baseline period (a 2 s window beginning 3 s before the cue). Normalized power spectra were averaged for control and *Plaur* animals. Z-score line graphs were generated by averaging the z-scores around behavioral time points and tested on Kolmogorov-Smirnoff (K-S) test for significance. Ratios of different time points around behaviors were taken between *Plaur*/control and plotted in histograms to better demonstrate the change in variation occurring around behaviorally relevant events. Power spectral density estimates were taken using Welch's method for Power Spectrum Estimation on the continuous data. Values were averaged and plotted with standard error of the mean (SEM).

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