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EXECUTIVE CONTROL IN THE ANTERIOR CINGULATE CORTEX

BY

LORI A NEWMAN

BS in Neuroscience, The College of William and Mary, 2004

MA in Psychology, University of New Hampshire, 2006

DISSERTATION

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

in

Psychology

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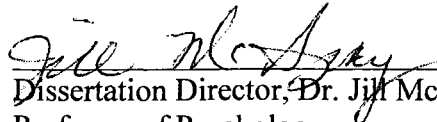
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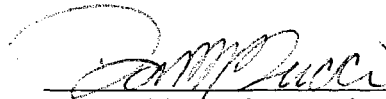
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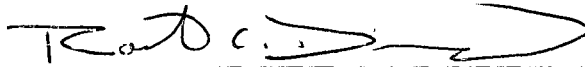
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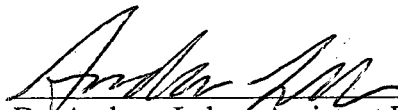
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DEDICATION

To my family, who always encouraged me to “think about it” and to Brian, who encouraged me to have faith in myself throughout this process.

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ABSTRACT

EXECUTIVE CONTROL IN THE ANTERIOR CINGULATE CORTEX

by

Lori A Newman

University of New Hampshire, September, 2009

Advisor: Dr. Jill McGaughy

Converging evidence supports the hypothesis that the prefrontal cortex is critical for executive control. One prefrontal subregion, the anterior cingulate cortex has previously been shown to be active in situations involving high conflict, presentation of salient, distracting stimuli, and error processing, i.e. situations that occur when learning new response contingencies, when previously learned response strategies fail, or when a shift in attention or responding is required. These situations all involve goal-oriented monitoring of performance in order to effectively adjust cognitive processes. Several neuropsychological disorders, for instance schizophrenia, attention deficit hyperactivity disorder, and obsessive compulsive disorder, are correlated with morphological changes in the anterior cingulate cortex. Individuals with these disorders show impairments on tasks that require goal-oriented monitoring. The current studies used multiple behavioral paradigms to assess the effects of anterior cingulate cortex excitotoxic lesions in rats on executive control. Animals with anterior cingulate cortex lesions showed greater decline in cognitive capacity

as tasks progressed, longer response latencies to conflicting stimuli, impaired reversal learning, impaired error processing, and impaired performance in the presence of previously relevant distractors. These results are consistent with the hypothesis that the anterior cingulate cortex is involved in executive control, specifically monitoring impairments in performance that signal the need to adjust cognitive control.

CHAPTER 1

INTRODUCTION

The ability to recognize problems and work to find new solutions can lead us to moments of inspiration and can help us reach our goals. However, as with individuals with obsessive/compulsive disorder, fixation on details that are unimportant or uncontrollable can create patterns that hinder progress (Aouizerate et al. 2004; Gu et al. 2008; Sachdev and Malhi 2005). The ability to discriminate vital information from what is inessential becomes a crucial process in learning to interact efficiently with the environment (Kennerley et al. 2006; Rudebeck et al. 2008; Walton et al. 2007). This process is a function of the prefrontal cortex (Brown and Bowman 2002; Carter et al. 2000; Dalley et al. 2004a). Known as executive control, the prefrontal cortex promotes efficient use of available information from memory as well as the current sensory input to respond accurately (Baddeley and Della Sala 1996; Dalley et al. 2004b; Goldman-Rakic 1996). While there are many important facets of executive control, the basic understanding that a change in behavior may be necessary is one critical part of the process. The anterior cingulate cortex (ACC) is an area of the prefrontal cortex that is active when cognitive flexibility is required and may

be responsible for this preparation to adjust attentional and behavioral processes (Botvinick et al. 2001; Brown and Braver 2005; Cole and Schneider 2007; Kerns et al. 2004; Ragozzino and Rozman 2007; Woodward et al. 2008).

Several situations can lead to activation of the ACC. Emitting an error or being in a situation where errors are likely has been correlated with activation of the ACC (Amiez et al. 2005; Brown and Braver 2005; Carter et al. 1998; Hester et al. 2005; Holroyd et al. 2004; Yeung et al. 2004). Activation of the ACC also occurs when subjects are presented with conflicting information (Botvinick et al. 1999; Botvinick et al. 2001; Chen et al. 2006; Kerns et al. 2004; Mitchell 2006; van Veen et al. 2001) or with the presentation of stimuli that indicate a shift in attention is necessary (Aarts et al. 2008; Luks et al. 2002). Finally the ACC has been implicated in decision making where evaluating the utility of stimuli and responses is required (Bush et al. 2002; Kennerley et al. 2006; Lee et al. 2007; Walton et al. 2003). The common link in all of these situations is a need to prepare for adjustments in cognitive control. While functional imaging studies help to identify potential situations in which the ACC is involved, they do not clearly identify whether the ACC is necessary for cognitive control. The current studies examine the effects of ibotenic acid lesions of the ACC in rats on multiple behavioral paradigms to determine the role of the ACC in executive control.

Neuroanatomical Organization

While there have been debates about the homology of the primate prefrontal cortex and the rodent prefrontal cortex (Preuss 1995), similar connective patterns and common functional properties have been demonstrated in the rat (Brown and Bowman 2002; Heidbreder and Groenewegen 2003). Two areas of the rat prefrontal cortex, the orbitofrontal cortex and medial prefrontal cortex have been associated with executive functions similar to those seen in primates (Birrell and Brown 2000; Brown and Bowman 2002; Dalley et al. 2004a; McAlonan and Brown 2003; Tait and Brown 2007). The medial prefrontal cortex can be further subdivided into the anterior cingulate, infralimbic, and prelimbic cortices based on functional differences (see Figure 1.1; Heidbreder and Groenewegen 2003). Connections of the ACC with other regions involved in attention, reinforcement assessment, emotional regulation, and memory support its hypothesized role in executive function and cognitive flexibility. For instance during decision making, activity in the ACC and orbitofrontal cortex are correlated with each other and high-risk decisions suggesting these regions have dense corticocortical connections related to functional connectivity (Cohen et al. 2005). Further discussions of functional connections of the ACC will be discussed below.

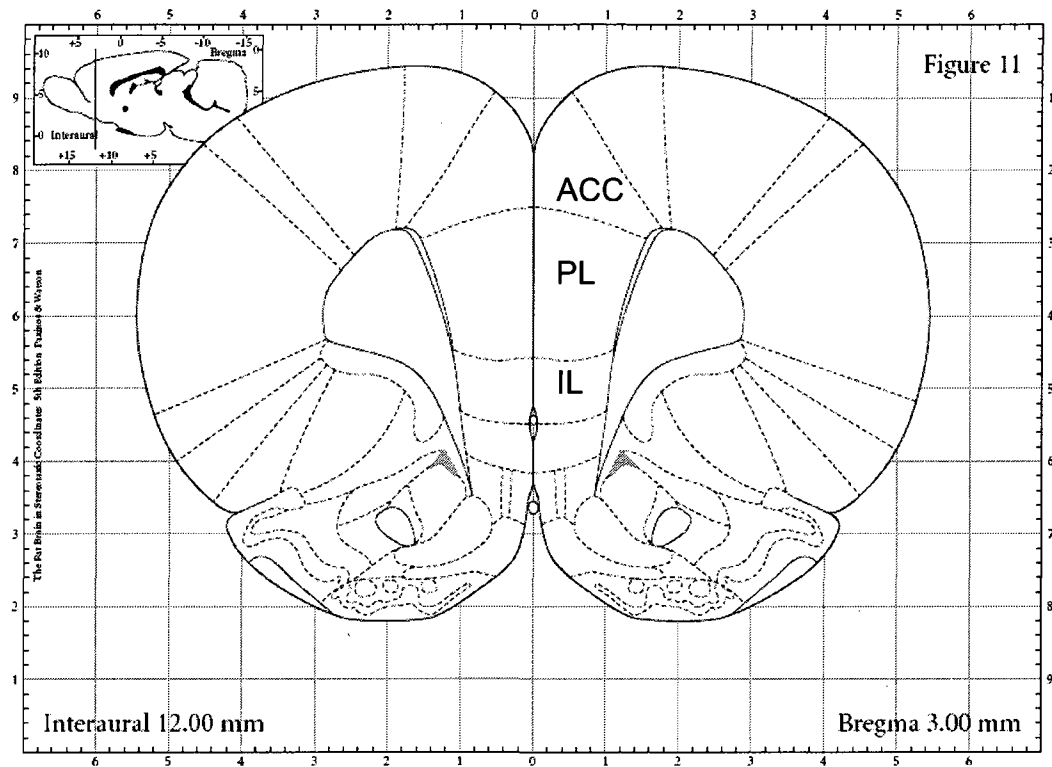


Figure 1.1 The anterior cingulate cortex in the rat brain. A coronal section is shown with the anterior cingulate cortex (ACC), prelimbic cortex (PL), and infralimbic cortex (IL) labeled in the right hemisphere (Paxinos and Watson 2005). Modified from: Paxinos G, Watson C (2005) *The rat brain in stereotaxic coordinates: the new coronal set*. Oxford: Elsevier Academic Press. 209 pp.

Clinical Relevance

ACC dysfunction has been demonstrated in several neuropsychological disorders. Patients with obsessive/compulsive disorder, schizophrenia or attention deficit hyperactivity disorder (ADHD) demonstrate morphological changes in the ACC (Benes et al. 1997; Makris et al. 2007; Manoach et al. 2007; Seidman et al. 2006; Stark et al. 2004; Yücel et al. 2002). These patients also

demonstrate deficits in attention and cognitive flexibility that can be linked to the dysfunction of the ACC.

Obsessive/Compulsive Disorder

Children with obsessive/compulsive disorder (OCD) show significant brain volume reductions in the ACC and adults with OCD show a trend towards lower ACC volumes (Atmaca et al. 2007; Carmona et al. 2007). Children with OCD show increased error related activity due to activation of the ACC (Santesso et al. 2006). Adult patients with OCD also show greater functional activation of the ACC during error processing and conflict situations (Aouizerate et al. 2004). This dysregulation may lead to the obsessive and compulsive symptoms in which they are unable to disengage from an often negative thought or action pattern (Sachdev and Malhi 2005). In other words this increased activation during errors may reflect an ineffective increased processing of errors, or an obsession with mistakes, and lead to the compulsive actions associated with this disorder.

Attention Deficit Hyperactivity Disorder

Adults with ADHD exhibit both structural and functional deficits in the ACC. MRI of the ACC reveals adults with ADHD have significantly less gray matter than controls in the ACC (Makris et al. 2007; Seidman et al. 2006). A

meta-analysis of functional neuroimaging shows individuals with ADHD consistently show hypoactivity in the ACC (Dickstein et al. 2006).

Children with ADHD show poor attentional control in paradigms where response conflict is present (van Meel et al. 2007). ADHD children emitted more errors than control subjects, and made shorter runs of correct responses (van Meel et al. 2007). These error rates significantly increased in children with ADHD when time pressure was increased (van Meel et al. 2007). This effect was amplified on conflict trials suggesting the ADHD children have greater difficulty when conflicting responses are present (van Meel et al. 2007). Individuals with ADHD are slower and less accurate than controls on the Stroop task and cued task switching paradigms (King et al. 2007; Shallice et al. 2002).

Schizophrenia

Patients with schizophrenia demonstrate decreased functional activation of the ACC as well as structural deficits in the ACC (Kerns et al. 2005b; Liddle et al. 2006; Neuhaus et al. 2007; Stark et al. 2004). In comparison to healthy controls, postmortem analysis of glial cell densities revealed glial cell densities in schizophrenic patients is lower in the ACC which could lead to dysregulation of the extracellular environment in the ACC in terms of neurotransmitter and ion concentrations (Stark et al. 2004). Moreover, levels of D2/D3 receptors in patients with schizophrenia in the cingulate gyrus is reduced compared to controls suggesting dysregulation of the dopaminergic system in the ACC

(Buchsbaum et al. 2006). Levels of D2/D3 receptors in the ACC correlates with performance on the Wisconsin Card Sorting Task which is a measure of executive function and suggests dysfunction of dopamine receptor binding in the ACC may contribute to executive function deficits in schizophrenic patients (Lumme et al. 2007).

Patients with schizophrenia have also demonstrated deficits on an intradimensional/extradimensional (ID/ED) set shifting task that tests executive function which, like the Wisconsin Card Sorting Task, requires the formation and shifting of an attentional set (Jazbec et al. 2007; Pantelis et al. 1999). In contrast to the Wisconsin Card sorting task, the ID/ED set shifting task uses a total changeover design that decreases the influence of prior reinforcement history and assesses performance in control tasks that allow a clear understanding of cognitive deficits (Dias et al. 1997; Owen et al. 1991). In addition to attentional set shifting deficits, patients with schizophrenia also show susceptibility to distracting information during performance of one of the control tasks tested during the attentional set shifting task (Jazbec et al. 2007; Pantelis et al. 1999). Additional studies have demonstrated deficits in orienting attention in patients with schizophrenia (Laurens et al. 2005; Liddle et al. 2006). Patients with schizophrenia are slower and less accurate at detecting task relevant novel stimuli in an auditory novelty oddball task as compared with controls (Laurens et al. 2005). Patients with schizophrenia also showed less activation of the ACC during target presentations (Laurens et al. 2005). In addition, patients with schizophrenia were more likely to be distracted by novel, salient stimuli that were

not relevant in the task (Laurens et al. 2005). This increased susceptibility to novel stimuli and decreased ability to detect relevant information is known as 'cognitive dysmetria' (Laurens et al. 2005). Similarly, schizophrenic patients also are slower to detect rarely presented familiar targets that was again correlated with decreased activation of the ACC as compared with controls (Liddle et al. 2006). These studies suggest the decreased activation of the ACC may indicate a decreased ability to attend to behaviorally relevant novel or rarely presented stimuli in schizophrenic patients in addition to creating an increased susceptibility to novel distracting stimuli (Laurens et al. 2005; Liddle et al. 2006).

Patients with schizophrenia often exhibit deficits in resolving conflicting response information that are partly due to dysfunction of the ACC. When performing the Stroop Task, schizophrenic patients show less activation of the ACC during high conflict portions of the task and when emitting errors (Kerns et al. 2005). While Kerns, et al. (2005) saw comparable error rates between the schizophrenic patient group and the comparison group, this similarity in performance may result from an increased time pressure because the experimenters encouraged individuals to respond faster if they were not making enough errors and required them to perform more trials if they did not have enough errors. Wang, et al. (2005) found significant increases in error rates and latencies for schizophrenic patients relative to controls on conflict monitoring tasks. When latencies on conflict trials, i.e. trials with simultaneous presentation of stimuli that elicit different responses, were compared to latencies on congruent trials, i.e. trials with simultaneous stimuli that elicit the same response, a greater

increase in latencies were seen in schizophrenic patients for conflict trials (Wang et al. 2005). In another study, while schizophrenic patients tended to show slower response times overall, the response latency difference when conflict trials were compared to congruent trials was significantly greater in controls than schizophrenic patients (Neuhaus et al. 2007). Neuhaus, et al. (2007) hypothesized the difference between these two studies lies in the nature of the schizophrenic symptoms with Wang, et al. (2005) having more patients with positive symptoms and Neuhaus, et al. (2007) having more patients with negative symptoms. The Neuhaus, et al. (2007) data suggests that while the ACC in the control participants may adjust cognitive processing to increase effortful processing only when conflict is high, the lack of ACC activity prevents this increased processing in schizophrenic patients resulting in an attenuated difference between conflict and congruent trials.

Conflict Monitoring

Functional imaging studies have shown activation of the ACC in the presence of conflicting stimuli or responses and this conflicting information leads to longer response latencies (Botvinick et al. 1999; Botvinick et al. 2001; Chen et al. 2006; Kerns et al. 2004; Mitchell 2006; van Veen et al. 2001). In particular, the activation of the ACC is seen when response conflict, or competition, is high (Nelson et al. 2003). For example, when competing stimuli are presented such as in the color-word Stroop task, where a participant has to respond to the ink

color of a word while ignoring what the word is (green printed in red ink = “red” verbal response; see Figure 1.2).

A Red Blue Green Brown Purple
B Green Purple Iron Blue Red
C Lot Goat Desk Fork Shirt

Figure 1.2. The Stroop color-word task requires participants to respond to color words presented in different ink colors by either identifying the ink color or identifying the word presented. **A.** Congruent trials where the ink color and written word match. **B.** Incongruent (conflicting) trials where the ink color and written word elicit competing responses. When the ink color and word are in conflict, participants take longer to respond and show activation of the ACC (Kerns et al. 2004). **C.** Neutral trials where the participant has to name the ink color and the written word is not in conflict or related to the ink color (Milham and Banich 2005).

In some conflict monitoring paradigms trials that have high response conflict, incongruent trials are intermixed with congruent trials. For instance Botvinick, et al.(1999) used a paradigm that requires the individual to respond by pushing a button on the left if an arrow appears in the center of the screen that points to the left and a button on the right if an arrow appears that points to the right (see Table 1.1). These are simple stimulus-response rules, however the task becomes slightly more difficult because the center arrow is flanked by arrows to the right and left of it on every trial. In some cases these arrows point in the same direction as the center arrow (congruent trials <<<<<) and in other trials the flanking arrows point in the opposite direction (incongruent trials <<>><<). On average, participants respond faster on congruent trials than

incongruent trials (Botvinick et al. 1999). However, as previously mentioned, ACC activity is seen when an incongruent i.e. conflict trial is presented. If the following trial is also a response conflict trial, the latency to respond on this subsequent trial decreases (Botvinick et al. 1999; Botvinick et al. 2001; Gratton et al. 1992; Kerns et al. 2004; Ridderinkhof et al. 2004). Stronger activation of the ACC was correlated with faster reaction times in the second conflict trial (Botvinick et al. 1999). It has been hypothesized that the activation of the ACC leads to increased top-down control and a narrowing of the attentional focus in order to disregard the irrelevant stimuli on the following trial (Botvinick et al. 1999). Other data suggests that the ACC is not necessary for the increase in top-down control, and the dorsolateral prefrontal cortex may be a more likely region to implement cognitive control in the presence of irrelevant information (Milham et al. 2003a; van Veen and Carter 2006). The difference in these viewpoints may be related to the stage of training during which activation is studied. Research has demonstrated the ACC is more likely to be active in the early learning stages of a task, suggesting the ACC may have a more preparatory role in top-down control, and once the task is learned it is not as active as the dorsolateral prefrontal cortex (Milham et al. 2003b).

Flanker Task		
Response	Congruent Trial	Incongruent Trial
Left Key Press	<<<<<	>><>>
Right Key Press	>>>>>	<<><<

Table 1.1. The flanker task requires the individual to respond by pushing the left key if an arrow appears in the center of the screen that points to the left and a button on the right if an arrow appears in the center of the screen that points to the right. The center arrow is flanked by arrows to the right and left of it on every trial. In some cases these arrows point in the same direction as the center arrow (congruent trials) and in other trials the flanking arrows point in the opposite direction (incongruent or conflict trials).

The Effects of Practice and Cognitive Demand on Conflict Monitoring

While the role of the ACC may decrease as practice of a task progresses, the presentation of conflicting information that is automatically processed may lead to greater activation of the ACC. The automatic processing of information is argued to be critical to creating interference effects in the Stroop task and flanker task (Cohen et al. 1990; Stolz and Besner 1999; Stroop 1935). In his original characterization of the task, J. Ridley Stroop (1935) found significant increases in the time it took to name the ink color when the color word differed from the ink color (see Figure 1.2B). This effect was not seen when participants were asked to read color words instead of naming the ink color. Stroop (1935) hypothesized participants were more likely to automatically read the word than they were to process the ink color when these two stimuli were in conflict. However, the interference of ink color could be induced with practice of ink color naming (Stroop 1935). Stroop (1935) had participants practice naming ink colors with

differing color words for eight days. He then tested them on naming color words on conflicting trials rather than naming the ink colors and found participants were significantly slower to name the word. This data suggests the amount of interference created by the conflicting stimuli may be influenced by how practiced the non-target information is (Stroop 1935).

While practice on the less automated dimension increases Stroop interference effects, evidence has also suggested the salience of the non-target dimension requires can also increase interference effects (Szmalec et al. 2008). Szmalec et al (2008) found if the irrelevant stimulus is degraded participants demonstrated a greater interference effect. Several other studies have shown individuals with dyslexia or poor reading skills tend to show significantly greater Stroop interference of a color word on naming a color suggesting as the cognitive processing demand of the non-target dimension increased the interference effect increased regardless of the response requirements (Cox et al. 1997; Everatt et al. 1997; Protopapas et al. 2007). These data suggest differences in task proficiency may lead to increased difficulties in conflict monitoring.

Several studies have been done to further characterize the role of attention in conflict monitoring. By only coloring a single letter in the color word, the Stroop interference effect is greatly reduced (Besner et al. 1997; Brown et al. 2002). In the single color letter paradigm, the ability to read the word is disrupted by focusing the attention on a letter rather than the whole word. Conversely, adding congruent trials where both stimuli elicit the same response increases Stroop interference on incongruent trials (Brown et al. 2002). This effect is

hypothesized to be due to an increased likelihood of voluntarily attending to and processing the non-target stimulus even though it has a probability of conflicting with the target stimulus (Brown et al. 2002). However, Brown et al. (2002) also found an interference effect of color words presented next to color shapes though the word was never congruent with the color shape suggesting that automatic processing of words still occurred.

Error Processing

Functional imaging has shown activation of the ACC occurs after errors are made (Carter et al. 1998; Holroyd et al. 2004; Yeung et al. 2004). Correct responses are significantly slower if they occur after an incorrect response (van Meel et al. 2007). This suggests the ACC may be involved in error processing and affect future behavior. Electroencephalogram (EEG) recordings have also identified event-related potentials (ERP) that have been localized as originating from the cingulate cortex (Neuhaus et al. 2007; Yeung et al. 2004). These ERPs are referred to as event related negativity (ERN) and are thought to promote inhibition of responding after errors (Yeung et al. 2004). One such ERP is the labeled N2 and is seen following no-go trials in a go/no-go task (Nieuwenhuis et al. 2003). In a go/no-go task participants make a response when a go stimulus is presented (circle). If a no-go stimulus is presented (square), the participant should inhibit responding. The N2 has been hypothesized to be an inhibitory signal which helps to prevent responding to the no-go stimulus (Nieuwenhuis et

al. 2003) and has also been seen in relation to conflict trials which also require the inhibition of certain responses (Yeung et al. 2004).

Other studies have shown lesions of the ACC can lead to errors in learning and implementing stimulus-response rules (Bussey et al. 1996; 1997; Cardinal et al. 2003). Animals with lesions of the ACC persist in using ineffective strategies longer than sham-lesioned animals, suggesting the ACC may be crucial for recognizing situations where the current attentional focus and behavioral response is not effective and a shift in cognitive processing is required (Dias and Aggleton 2000). In tests of reversal learning, rats whose ACC has been inactivated are more likely to respond to distracting, never reinforced stimuli. This increased responding to distracting stimuli suggests the animals with ACC inactivation have identified a change in reinforcement contingencies, however, they are less efficient at selecting the new reinforced stimulus (Ragozzino and Rozman 2007).

While there are several studies that suggest ACC's involvement in responding to errors, imaging studies further suggest that the actual errors are not needed to activate the ACC, just the awareness that increased errors are likely is enough to activate the ACC (Brown and Braver 2005). Brown and Braver (2005) used a modified stop-signal task and presented a cue to participants to indicate an increase in task difficulty, i.e. a longer delay between the go signal and the potential change signal. Presentation of the cue that signaled an increase in difficulty was sufficient to increase activity in the ACC (Brown and Braver 2005). In a go/no-go task, increasing frequency of no-go

stimuli (20% of trials or 80% of trials) has been shown to create N2 ERPs even in the presence of go stimuli (Nieuwenhuis et al. 2003). This suggests the ACC may create an overall dynamic change to increase cognitive control when the probability of a trial that requires no response increases (Nieuwenhuis et al. 2003). Electrophysiological recordings in the ACC of non-human primates have identified neurons that fire in response to errors in a saccade stop signal task, i.e. the failure to inhibit a saccade in the presence of a cue (Ito et al. 2003). Half of these error neurons were also active if the reward for a correct response was omitted (Ito et al. 2003). In other words, a lack of reinforcement causes neurons in the ACC to fire so that this increase in firing may potentially signal a change in reinforcement. This suggests the ACC is not just monitoring whether the actions were performed correctly, but monitoring whether the actions were reinforced. This again supports the hypothesis that the ACC is monitoring the effectiveness of behaviors and active in preparation for adjustments in cognitive control.

Inhibition of Choice Impulsivity

The ACC has been hypothesized to be involved in controlling impulsivity, but studies that have assessed this have produced contradictory results. The 5-choice serial reaction time task (5-CSRT) is a paradigm that has been used to assess attention and impulsivity in rodents (Carli et al. 1983; Dalley et al. 2004a; Muir et al. 1996; Robbins 2002). The 5-CSRT requires rats to detect a brief visual cue in one of 5 ports and nose-poke at that location to receive

reinforcement. In this task there is a fixed intertrial interval (ITI) and if an animal nose-pokes in a port during this ITI prior to the stimulus onset it is considered a premature response and suggests impulsivity (Barbelivien et al. 2001). Animals that emit high numbers of premature responses in the 5-CSRT show significantly less deoxyglucose uptake in the cingulate cortex when compared to well performing controls suggesting the cingulate cortex may help to prevent impulsivity (Barbelivien et al. 2001). However, lesions of the ACC rats did not produce significantly more premature responses in the 5-CSRT and instead produced greater accuracy impairments where animals with ACC-Lx were more likely to respond to ports adjacent to the target port instead of the correct port suggesting the ACC may be more involved in promoting accurate responses (Chudasama et al. 2003).

Individuals who receive scores on the Barratt Impulsiveness Scale indicative of greater impulsivity demonstrate higher activity levels of ACC during a go/no-go task (Brown et al. 2006). The authors suggest this may be a compensatory mechanism in impulsive individuals as they need greater cognitive control to inhibit responding (Brown et al. 2006). Further evidence for the role of the ACC in inhibiting impulsivity can be seen in individuals with diagnosed with impulsive aggression. Individuals diagnosed with impulsive aggression have reduced serotonin transporters in the ACC as compared with control subjects (Frankle et al. 2005). Low levels of serotonin have been correlated with increased aggression suggesting serotonin in the ACC that is crucial for controlling impulsive aggression (Frankle et al. 2005).

An inability to determine the value of an action can also lead to impulsive actions. Different factors can devalue reinforcement such as a delay to reinforcement, increased physical or mental effort to receive a reward (climbing a barrier, increasing the number of responses required; Walton et al. 2006). However if the reinforcement is large enough it can bias an organism to perform a more demanding task (Kennerley et al. 2006; Walton et al. 2006). Lesions of the ACC lead to an impaired ability to calculate the cost of a behavior and act in an economically sound fashion (Walton et al. 2003; Walton et al. 2009). Walton and colleagues have demonstrated ACC-Lx animals are less likely to perform tasks that require greater effort (more lever presses, or climbing a barrier) to receive higher reward (more food) than Sham-Lx rats when there is an option to get less food with less effort (Walton et al. 2003; Walton et al. 2009).

Memory

The ACC is active during complex tasks that require memory for and implementation of rules (Burgess et al. 2000). Burgess et al (2000) found individuals with damage to the left ACC remembered significantly fewer task rules than control participants after performing a multitasking test. The task used in the study was the Greenwich Test which requires participants to perform three open ended tasks in a way to score the greatest number of points in 10 minutes (Burgess et al. 2000). The rules for earning points are given to the participants before performing the task and participants are tested on these rules before

starting the task (Burgess et al. 2000). Individuals with ACC lesions do not perform significantly worse at the immediate recall of the rules before the start of the task but are impaired in a similar test of rule recall at the conclusion of testing (Burgess et al. 2000).

However the ACC does not seem to be involved in all aspects of memory. Some studies show the ACC is active during visuospatial working memory tasks (Garavan et al. 2000), while others fail to find effects of ACC inactivation on spatial working memory (Ragozzino et al. 1998; Ragozzino and Kesner 1998). Lesions of the ACC in rats impairs their ability to perform a series of conditional, visual discriminations but this accuracy impairment was not present when the animals only had one or two stimulus-response rules to remember (Bussey et al. 1997). The increased error rate seen by Bussey et al (1997) may result from a greater memory load produced by the larger number of items to be remembered. However, increasing the memory load has variable effects on the ACC, with some studies showing greater activation of the ACC as memory load increases (Gould et al. 2003), and other studies that showing a decrease in ACC activation as memory load increases (Habeck et al. 2005). The visuospatial working memory task used in the Garavan et al (2000a) study was designed to resist practice effects and individuals performing this task did not show significant improvements in accuracy even after 880 trials and the ACC remained active. Gould, et al. (2003) required participants to remember what objects were presented as well as what location they were presented in, which may account for the increases in ACC activity observed, while Habeck, et al. (2005) only

required the participants to remember what was presented which did not correlate with ACC activity. Therefore, similar to findings previously discussed, the ACC seems to be involved in memory for multiple associations or when mnemonic load is high and the role of the ACC lessens with practice.

Attentional Processes

The ACC has been implicated in determining what should be attended to (Dalley et al. 2004a). The connections between the ACC and dorsolateral prefrontal cortex and the ACC and the inferior parietal cortex may allow the ACC to recruit these areas following the detection of conflict to exert attentional control and reduce conflict (Banich et al. 2000b; Carter et al. 1998; Kerns et al. 2004; MacDonald et al. 2000). In the color-word Stroop task on trials where conflict is high (word meaning conflicts with ink color) greater activation is seen in the dACC and the dorsolateral prefrontal cortex (Milham and Banich 2005; van Veen and Carter 2005).

Tasks that require selective attention increase activity in the ACC (Smith and Jonides 1999). Activation of the ACC has been seen in preparation for shifts in attention (as indicated by a cue) suggesting a role for the ACC in guiding this process (Luks et al. 2002). Activation of the ACC is also seen in response to a valid cue in a spatial attention paradigm which allows the individual to orient attention to the proper location (Thiel et al. 2004). This preparatory activity suggests the ACC is not just involved in conflict and error processing but may be

involved in the preparation for spatially allocating attention. Activation of the cingulate cortex is seen when participants have to shift attention from previously relevant information (Nee et al. 2007), and studies have found animals with ACC inactivation have deficits in disregarding previously reinforced stimuli as well as distracting stimuli when reinforcement contingencies have shifted (Ragozzino and Rozman 2007). The ACC shows significantly greater activation when an individual performs a cross-modal divided attention task than when the task is unimodal (Vohn et al. 2007). This activation of the ACC indicates the cross-modal stimuli may uniquely recruit the ACC in monitoring performance (Vohn et al. 2007).

Decision Making

The ability of the ACC to integrate error, conflict, and reinforcement information has been hypothesized to lead to a critical role for the ACC in decision making (Kennerley et al. 2006; Lee et al. 2007). Kennerley, et al. (2006) found monkeys with anterior cingulate sulcus lesions had difficulty sustaining reinforcing behaviors in a paradigm that required the animal to continue a reinforced movement for 25 trials and then switch to a new movement. Even after making a correct response the anterior cingulate sulcus lesioned monkey was more likely than control animals to produce an error on the next trial (Kennerley et al. 2006). In a second experiment the probability of reward was manipulated for the two actions. With successive trials the monkey could

determine what the probability of reward was for each response and therefore which action was most likely to lead to a reward (Kennerley et al. 2006). Sham-lesioned animals took less trials to reach asymptotic performance than anterior cingulate sulcus lesioned animals (Kennerley et al. 2006). The authors hypothesize the anterior cingulate sulcus lesioned monkey is not able to utilize action-outcome history to choose the best course of action. Lee, et al. (2007) further hypothesize that the ACC, along with the orbitofrontal cortex, is specifically involved in assessing and updating the utility of specific actions and stimuli, while the lateral prefrontal cortex maintains information processing necessary for maintaining optimal performance in the current state.

This previous work provides evidence of a role for the ACC in monitoring behaviorally important changes in the environment and adjusting behavior to adapt to these changes. However, many of the functional imaging studies and studies with patients with neuropsychological disorders do not clearly delineate the role of the ACC in executive control. I hypothesize that the ACC is involved in monitoring performance and determining what information is relevant in the presence of potentially important stimuli (i.e. stimuli that has previously been associated with reinforcement). The following studies assess the effects of ibotenic acid lesions of the ACC in rats in performing tasks that require conflict monitoring, error processing, reversal learning, and sustained attention in the presence of salient distractors in order to determine the role of the ACC in executive control.

CHAPTER 2

EXPERIMENT 1: IS ACC NECESSARY TO PERFORM A CONFLICT MONITORING TASK?

In order to confirm the ACC is necessary for conflict monitoring, rats with excitotoxic lesions of the ACC were tested on a novel conflict monitoring paradigm. Multiple studies have implicated the ACC in conflict monitoring which requires adjustments in attentional focus and the inhibition of automatic responses (Botvinick et al. 2001; Kerns et al. 2004; Milham and Banich 2005; Nieuwenhuis et al. 2003). The current conflict monitoring paradigm allowed for the assessment of response accuracy and latency when two stimuli of different modalities are presented that require the same response (congruent trials) and when two stimuli of different modalities are presented that require different responses (incongruent trials). Within a session animals were reinforced for responding to stimuli of one modality referred to as the target modality and disregarding stimuli of the other modality in the case of incongruent trials. Previous conflict monitoring studies using human subjects have shown incongruent trials lead to longer response latencies and decreased response accuracy while individuals are faster and more accurate on congruent trials as compared to neutral trials, i.e. a single stimulus is presented (Lindsay and Jacoby 1994; Yeung et al. 2004). Functional imaging studies have also shown

increased activation of the ACC after incongruent trials (Botvinick et al. 1999; Botvinick et al. 2001; Chen et al. 2006; Kerns et al. 2004; Mitchell 2006; van Veen et al. 2001). Several studies have noted improved accuracy and faster reaction times on incongruent trials that were preceded by incongruent trials (II) as compared to incongruent trials that were preceded by congruent trials (CI) and slower reaction times on congruent trials that are preceded by incongruent trials (IC) as compared to congruent trials that are preceded by congruent trials (CC) (Botvinick et al. 2001; Gratton et al. 1992). This effect has been hypothesized to be due to the activation of the ACC on the first incongruent trial which speeds reaction times on successive incongruent trials and slows reaction times on successive congruent trials (Botvinick et al. 1999; Botvinick et al. 2001; Carter et al. 2000). A loss of these effects with lesions of the ACC would indicate the ACC is necessary for these changes in cognitive control.

The current experimental design is based on conflict monitoring paradigms (such as the Stroop task, see Figure 1.2, or a flanker task, see Table 1.1) used in humans (Botvinick et al. 2001; Bush et al. 2003; Nee et al. 2007b). Prior to these studies, only one other study has attempted to translate the conflict monitoring paradigms used in human studies to accommodate testing with rats (Haddon and Killcross 2006). Haddon & Killcross (2006) trained rats to respond to either auditory or visual signals which indicated which of two levers the rat should push to receive a reward (see Table 2.1). The signal was presented for 60 seconds; however, the rat only received a reward if he pressed the lever in the last 50 seconds of this minute presentation (Haddon and Killcross 2006).

Contextual cues (spotted or checkered operant box) indicated what type of reward the rat received (sucrose solution or food pellets), as well as whether the signals were auditory or visual (Haddon and Killcross 2006). After training on both the auditory and visual stimulus-response rules Haddon and Killcross (2006) presented visual and auditory signals at the same time. The simultaneous presentation was sometimes congruent eliciting the same response and sometimes incongruent eliciting competing responses (Haddon and Killcross 2006). Though this experiment was novel in translating response conflict tasks to rats, it has several limitations that hinder interpretation of the data. Animals were not reinforced for correct responses during the simultaneous presentation phase of the task (Haddon and Killcross 2006). This may lead to extinction of responding with repeated testing. It should also be noted there is a difference in the type of reinforcement given during training for the auditory and visual stimuli (i.e. sucrose or food pellets; Haddon and Killcross 2006). The effects of the different hedonic values for these reinforcements are never discussed by the authors but preferences for the different reinforcers may alter responding such that subjects focus solely on stimuli associated with the preferred reinforcer thus decreasing the effects of conflicting information. One of the major limitations in using this task in a translational manner to understand cognitive functions in humans is related to the dependent measures collected. Accuracy is calculated in a non-standard way by comparing the rate of responding to the correct lever versus the incorrect lever with a significantly higher rate of responding to the correct lever was considered evidence of accurate performance (Haddon and

Killcross 2006). Moreover though response latencies are a critical dependent measure in human studies (Botvinick et al. 1999; Kerns et al. 2004; van Veen et al. 2001), they were not assessed in this paradigm (Haddon and Killcross 2006). As a result, a novel conflict monitoring task was designed that assesses the effects of simultaneously presented incongruent or congruent stimuli on both response latency and accuracy.

Context	Biconditional Training	Extinction test sessions		
		Congruent	Incongruent	Single element
Checked	Tone: Left lever → sucrose	Tone/Steady light	Tone/Flashing light	Tone
	Click: Right lever → sucrose	Click/Flashing light	Click/Steady light	Click
Spotted	Steady light: Left lever → pellet	Tone/Steady light	Tone/Flashing light	Steady light
	Flashing light: Right lever → pellet	Click/Flashing light	Click/Steady light	Flashing light

Table 2.1. Response competition task used by Haddon and Killcross (2006).
Reproduced from: Haddon JE, Killcross S (2006) Prefrontal cortex lesions disrupt the contextual control of response conflict. *Journal of Neuroscience* 26:2933-2940.

Predicted Results

All animals should show longer response latencies on incongruent trials than congruent trials. Lesions of the ACC should augment this difference. All animals should show accuracy impairments on incongruent trials when they are compared to congruent trials. These impairments should be greater in ACC-Lx rats. Sham-Lx, but not ACC-Lx rats, should show higher levels of accuracy and

faster responding when tested on incongruent trials preceded by an incongruent trial.

Research Design and Methods

Apparatus and Materials

Operant chambers (Med Associates, St. Albans, VT) equipped with two retractable levers, a houselight (2.8 W), a 45 mg pellet dispenser, a 2900 Hz sonalert tone generator, and three panel lights (2.8 W) were used. The food dispenser, panel lights, tone generator, and retractable levers were all located on the same wall (see Figure 2.1). The houselight was located on the opposite wall. Records of signal presentation, lever operation, and food pellet (Dustless Precision Pellets, 45 mg; Bio-serv, Frenchtown, NJ) delivery were maintained using a personal computer with Windows XP (Microsoft, Seattle, WA) and the Med-PC IV software (Med Associates).

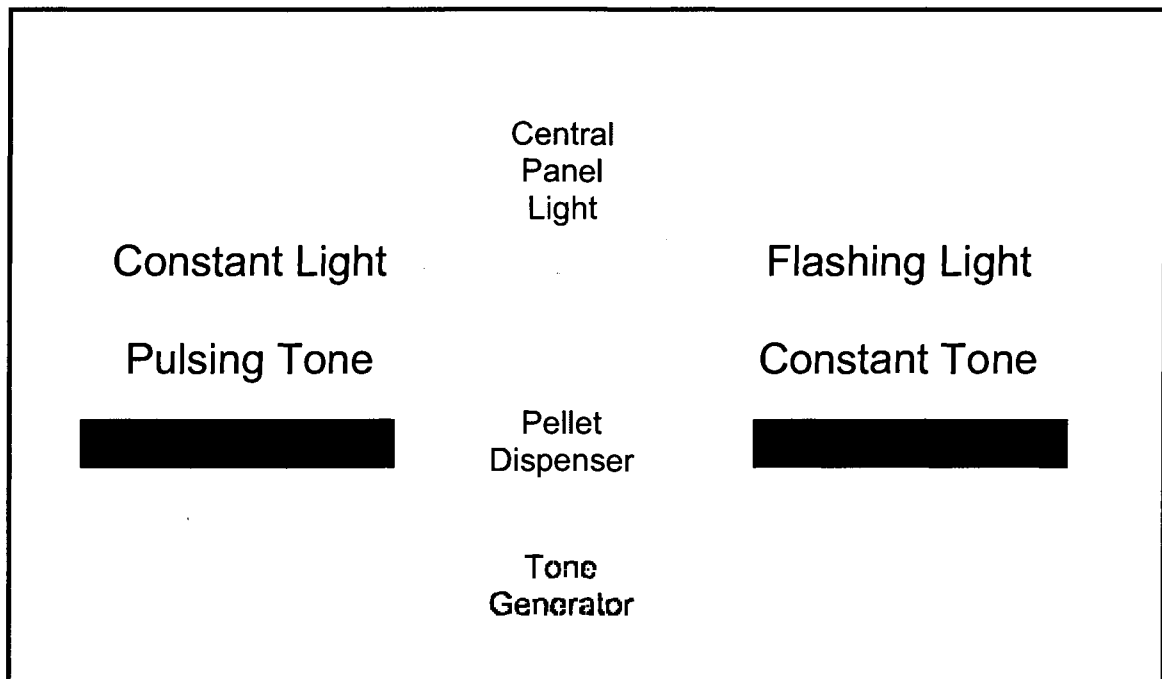


Figure 2.1. Stimulus response rules for both the conflict monitoring. The black bars represent the levers. Printed over the levers are the stimuli that are reinforced by pressing that lever. The flashing light and constant tone are presented at 20 Hz. Note the quality of the stimulus (constant vs. 20 Hz pulsing) is paired with the opposite lever across modality to prevent the animals from engaging in cross-modal matching where they learn to respond to the lever according to quality. Both the light and tone stimuli are located on the intelligence panel around the pellet dispenser.

Training

16 male Long Evans rats were trained in an operant chamber on two sets of conditional discriminations (see Figure 2.1). All animals were food restricted to ~80% of their free fed weight prior to training. In the first discrimination, the animals were reinforced for pressing the left lever after the presentation of a constantly illuminated light and the right lever when presented with a flashing light (20 Hz). Trials were presented in a pseudorandomized order and a fixed

intertrial interval (ITI) of 12 seconds was used. After achieving 65% accuracy on both stimulus-response rules, rats learned two stimulus-response rules with tones. When a pulsing tone (20Hz) was presented, the animal pressed the left lever to receive reinforcement and when a constant tone was presented, the animal pressed the right lever for a reinforcer. In this paradigm the same quality stimuli, e.g. constant light and constant tone, require opposite lever responses and prohibit the rats from generalizing response rules across modality. After achieving 65% accuracy in both the visual and auditory conditional discriminations, the animals were tested on the conflict monitoring task. The conflict monitoring task assesses the effects of presenting tones and lights simultaneously (see Table 2.2A). The rats first perform 20 unimodal trials. These trials will serve as a cue as to which modality the rat should attend, i.e. the target modality, and the conditional response rules that will be reinforced. The other modality will be referred to as the non-target modality. The subsequent 126 trials were a pseudo-randomized sequence of trials where simultaneous stimuli with incongruent response rules (e.g. **constant light** with constant tone → left lever press), congruent response rules (e.g. **flashing light** with constant tone → right lever press) or single modality trials of the target modality (neutral trials; e.g. **constant light** → left lever press) were presented. The pseudorandom presentation included 54 incongruent trials, 54 congruent trials, and 18 neutral trials with an inter-trial interval (ITI) of 12 seconds. On the day after a conflict monitoring session, animals were trained in unimodal sessions of the previously non-target modality. This allowed the animal to transition to focusing on this

modality as well as ensured that the animals still accurately recalled the stimulus-response rules of the previously non-reinforced modality. When the animal reached 65% accuracy on the unimodal session the following day the conflict monitoring task was administered again with the rats reinforced for responding to stimuli of previously non-target modality as it was now the target modality (incongruent trial: **constant tone**/constant light→ right lever press; congruent trial: **pulsing tone**/constant light→ left lever press; neutral trial **constant tone** → right lever press). Once an animal achieved > 75% accuracy on congruent trials in the light conflict monitoring task and the tone conflict monitoring task, they were considered eligible for surgery.

A

Conflict Monitoring Task				
Conflict Monitoring Block: 126 Pseudorandomized Trials				
Target Modality	Unimodal Trials (20 trials)	Congruent (54 Trials)	Incongruent (54 Trials)	Neutral (18 Trials)
Light	Constant light → Left lever Flashing light → Right lever	Constant light /pulsing tone → Left lever Flashing light /constant tone → Right lever	Constant light /constant tone → Left lever Flashing light /pulsing tone → Right lever	Constant light → Left lever Flashing light → Right lever
Tone	Constant tone → Right lever Pulsing tone → Left lever	Constant tone /flashing light → Right lever Pulsing tone /constant light → Left lever	Constant tone /constant light → Right lever Pulsing tone /flashing light → Left lever	Constant tone → Right lever Pulsing tone → Left lever

B

Conflict Monitoring Testing Procedure							
First Target Modality				Second Target Modality			
Target Unimodal Training	Conflict Monitoring Session	Non-target Unimodal Training	Conflict Monitoring Session	Target Unimodal Training	Conflict Monitoring Session	Non-target Unimodal Training	Conflict Monitoring Session
Light	Target Modality: Light	Tone	Target Modality: Light	Tone	Target Modality: Tone	Light	Target Modality: Tone
Tone	Target Modality: Tone	Light	Target Modality: Tone	Light	Target Modality: Light	Tone	Target Modality: Light

Table 2.2. Conflict monitoring task. **A.** A schematic of the conflict monitoring sessions. The conflict monitoring block presents 54 congruent, 54 incongruent, and 18 neutral trials in a pseudorandomized order. The conflict monitoring block was further divided into two blocks in order to assess performance across the session. **B.** The postsurgical conflict monitoring testing procedure. Half the animals received training with light as the target modality in the first two conflict monitoring sessions and half of the animals received training with tone as the target modality in the first two conflict monitoring sessions. After completion of these two conflict monitoring sessions, all animals went on to perform conflict monitoring sessions where the target modality was switched to that of the previously non-target modality.

Surgery

Animals were matched by accuracy and latency performance and one from each pair was randomly assigned to either receive the excitotoxin, ibotenic acid (0.06 M), or its vehicle, 0.1 M phosphate buffer, by infusion into the ACC. Rats were anesthetized using ketamine and xylazine (85 mg/kg ketamine and 8.5 mg/kg xylazine, i.m.). Infusions were made into the ACC via a 10 μ l, 26 gauge beveled tip microsyringe mounted on a stereotaxic frame. For each infusion the beveled tip was angled so the opening was pointing towards the midline. A small volume of the toxin or vehicle (0.2 μ l/site) was infused bilaterally at a rate of 125 nl per minute using an electronic infusion pump (Micro 4, Microsyringe Pump Controller, World Precision Instruments, Sarasota, FL). The needle was left in place for 4 minutes prior to and subsequent to infusion to minimize bleeding and undesired diffusion of the toxin. The following coordinates were used: AP: +2.7, ML: \pm 0.6, DV: -2.4; AP: +2.2, ML: \pm 0.6, DV: -2.2 with anteroposterior (AP) measurement relative to Bregma and dorsoventral (DV) measurement relative to the skull and the toothbar at -3.0 mm (modified from Chudasama et al, 2003). Animals received 4 days of ad libitum food and water before food restriction was reinstated. An additional 3 days of food restriction (~18 g/day) were established before postoperative training commenced.

Conflict Monitoring

After recovery the animals were retrained on the unimodal tone and light sessions. Once the animals achieved two days criterion performance (65%) on both unimodal tone and unimodal light sessions, the animals were tested on the conflict monitoring task (see Table 2.2B). Animals were counterbalanced so half received tone conflict monitoring first and half received light conflict monitoring first. Animals were tested in sequence of unimodal and conflict monitoring sessions to determine whether practice in the non-target modality prior to a conflict monitoring session would decrease accuracy on incongruent trials and increase the latency to respond on incongruent trials. Animals were trained to criterion on one modality, e.g. tone, in unimodal sessions. They were then presented with the conflict monitoring task of that target modality. After performing the conflict monitoring task they were placed in the unimodal sessions of the non-target modality, e.g. light. After 65% accuracy was achieved the animals were tested on the same conflict monitoring task presented previously i.e. tone conflict monitoring. Following this test session the animals were returned to training on the non-target modality, e.g. light, which now became the target modality and the pattern was repeated with conflict monitoring sessions of this modality (Table 2.2B).

Response latencies from the onset of the stimulus to the lever press were measured as well as accuracy to the target modality on congruent, incongruent and neutral trials. Because previous studies have suggested the role of the ACC

is reduced after practice (Besner et al. 1997; Milham et al. 2003b), the effects of practice within a modality were assessed by comparing latencies and accuracy of response incongruent and response congruent trials in the conflict monitoring sessions after target unimodal training sessions and conflict monitoring sessions after non-target unimodal training sessions. The effects of practice of the non-target stimulus-response rules were assessed by comparing latencies and accuracy of response incongruent and response congruent trials in the conflict monitoring sessions after practice of the non-target modality. If practice of the non-target modality affects the automaticity of rule processing, these unimodal practice sessions should reinforce the non-target stimulus-response rules to augment the impairments in accuracy and increase response latencies on incongruent trials. The impact of practice on the target modality within a session was analyzed by dividing the conflict monitoring block in half (63 trials in each block) and assessing performance on congruent and incongruent trials over time on task. Accuracy on incongruent trials should improve over time on task in Sham-Lx animals. Decreases in accuracy and increases in response latencies may suggest the cognitive demands of the task produce cognitive fatigue. Previous research has suggested activation of the ACC on incongruent trials can lead to enhanced performance on the following trial if that trial is also an incongruent trial (Botvinick et al. 2001; Carter et al. 1998; Gratton et al. 1992). Gratton et al. (1992) were the first to recognize the behavioral differences in trials based on the previously presented trials. They found if an incongruent trial precedes an incongruent trial (II) the performance on this second trial is faster

and more accurate than if a congruent trial precedes an incongruent trial (CI). Conversely, if an incongruent trial precedes a congruent trial (IC), the performance on the congruent trial is slower than when a congruent trial precedes a congruent trial (CC). Unlike the effects found on the performance of incongruent trials, successive presentation of congruent trials was not found to improve accuracy. Based on these findings the accuracy and latencies for congruent and incongruent trials were also assessed based on what type of trial preceded it to determine whether the ACC is critical for this facilitation of performance when the same trial type is presented successively.

Behavioral Assessment

Unimodal priming sessions. The sessions prior to conflict monitoring sessions were assessed to determine the ability of the animals to discriminate between the unimodal stimuli and perform conditional discrimination when the stimuli were presented without the simultaneous, non-target stimuli.

Distracting stimulus vs. conflicting response. In order to determine whether the ACC is critical for resolving conflicting responses or the presence of a distracting stimulus with no previous stimulus-response associations also requires the ACC, the effects of a non-reinforced visual stimulus was tested. In these sessions, the houselight was flashed at a rate of 5 Hz simultaneously with the presentation of the tone instead of the central panel light for which the animal had previously learned conditional response rules. This session followed a

similar design to the conflict monitoring sessions but the number of neutral trials was increased in order to compensate for the loss of congruent trials. The test session now consisted of 20 unimodal tone trials followed by a block of 126 distractor trials (tone and houselight, 63 trials) and neutral trials (tone only, 63 trials) presented in a pseudorandomized order. Because the flashing houselight stimulus is novel (presented in a different location and at a different rate) and has not been associated with a previous stimulus-response rule, it should serve as a distracting but not conflicting stimulus. Accuracy and response latencies were compared on trials where the flashing houselight is present and on trials from a session where the houselight remains constantly illuminated.

Open Field Test

Dysfunction in the ACC is hypothesized to underlie disorders that lead to emotional instability such as posttraumatic stress disorder and mood disorders (Bae et al. 2006; Fales et al. 2008; Hamner et al. 1999; Shin et al. 2001; Tang et al. 2007). In order to assess the reaction of animals to stressful situations all animals were assessed in an open field paradigm. This paradigm used a square wooden box (92 cm L X 92 cm W X 25 cm H). The box was painted black. Each animal was placed in the box for 10 minutes. Their behavior was recorded using a camcorder (Panasonic GS-19, Panasonic Corporation of North America, Secaucus, NJ) connected to a VCR recorder (Panasonic Monitor/VCR AG-520, Panasonic Corporation of North America, Secaucus, NJ). The video recordings

were then analyzed to determine the extent of thigmotaxis. Thigmotaxis was quantified by recording how much time the animal spent along the walls of the box (within 15 cm of the wall) which was further quantified by recording how much of this time was spent in the corners of the box (a 15 cm X 15 cm square). The total amount of time the animal spent freezing in the session was also assessed. To assess activity the box was divided into four quadrants and the number of quadrant crossings was recorded. Finally, the number of rears (lifting the front paws off the floor to explore the environment) for each animal were recorded (Brenes et al. 2008; Goddard et al. 2008; Sanberg and Ossenkopp 1977; Tayyabkhan et al. 2002).

Histology

After completion of testing, animals were given an overdose of Euthasol (6.5 mg/kg, i.p.). The rats were then transcardially perfused with 0.9% saline, followed by 4% paraformaldehyde in phosphate buffer. Brains were removed and placed in a 30% sucrose solution until they sunk and then sliced into 40 µm coronal sections. Sections were thionin stained for Nissl bodies after they were mounted with gelatin on gelatin-coated slides. Histological assessment of cell loss was made on an Olympus Optical BX51 microscope (Optical Analysis Corporation, Nashua, NH) using the 4X objective. Images of the sections were photographed using a SPOT Insight digital camera (Diagnostic Imaging, Sterling Heights, MI). Images were collected using Image Pro Plus version 6.0 software

(Media Cybernetics, Silver Springs, MD), and the area of damage was outlined using ImageJ version 1.41o (National Institutes of Health, Bethesda, MD, USA). The percentage of anterior cingulate cortex loss as compared to Sham-Lx animals was then calculated at bregma +3.7, +2.7, and +1.7 mm (Paxinos and Watson 2005). The amount of damage to the surrounding areas was also noted.

Statistical Analyses

All statistical analyses were performed with SPSS v. 17.0 (SPSS, Chicago, IL). All dependent measures excluding the open field testing dependent measures were analyzed using separate mixed-factor ANOVAs. The degrees of freedom in all analyses were corrected using the Huynh–Feldt correction in the case of a violation of sphericity and ϵ values not equal to 1 are reported below. Modified Bonferroni corrected alpha levels were used when necessary. All dependent measures of the open field test were analyzed using independent samples t-tests. The extent of the lesions was characterized with a repeated measures ANOVA with Hemisphere (2 levels) and Rostrocaudal position (3 levels) as within-subject factors.

Presurgical performance was compared to ensure there were no significant differences between Sham-Lx and ACC-Lx animals prior to surgery and to validate the task in comparison to human studies. Using a mixed factors ANOVA accuracy on congruent and incongruent trials were compared with Modality (2 levels, light vs. tone), Block (2 levels), and Trial (2 levels, congruent

vs. incongruent) as within subjects factors and Lesion (2 levels) as a between subjects factor. Two other separate ANOVAs were used to compare mean correct response latencies on congruent and incongruent responses and mean incorrect response latencies on congruent and incongruent responses following the same design. Unimodal sessions were assessed using separate mixed factors ANOVAs for accuracy, correct response latencies, and incorrect response latencies. Presurgical assessment included one within subject factor (Modality, 2 levels) and one between subjects factor (Lesion Assignment, 2 levels).

Evaluation of the previous trial type on accuracy of congruent trials was done using a mixed factors ANOVA with within subject factors of Modality (2 levels) and Trial (2 levels, CC vs. IC) and a between subjects factor of Lesion (2 levels).

A separate mixed factors ANOVA assessed the effects of the previous trial on accuracy of incongruent trials with within subject factors of Modality (2 levels) and Trial (2 levels, II vs. CI) and a between subjects factor of Lesion (2 levels).

Postsurgical performance was assessed using the same mixed factor ANOVAs described for presurgical performance with a few exceptions. Because we were interested in the effects of the practice in either the target or the non-target modality in the prior unimodal sessions on performance of the conflict monitoring task, the design of the task had all animals receive two conflict monitoring sessions in each modality when they first returned from surgery. Performance over these two sessions was analyzed by adding another within subjects factor of Session (2 levels) to the mixed factors ANOVAs. When analyzing the effects of the 5 Hz houselight distractor, no congruent trials were

present and this session was only tested with a visual distractor as no other auditory stimuli were present in the operant box to use as a distractor.

Therefore, the distractor trials were compared to incongruent trials from the baseline conflict monitoring session with tone as the target modality in a mixed factor ANOVA with Session (2 levels) as the within subject factor and Lesion (2 levels) as the between subjects factor.

In order to assess performance on unimodal trials, the unimodal session prior to a conflict monitoring session was analyzed. Due to the design of the testing procedure, the modality of the unimodal session varied while the target modality remained constant (e.g. unimodal sessions with target modality training were compared to unimodal sessions with non-target modality training. These unimodal sessions were analyzed with a mixed factor ANOVA that differed from previously described ANOVAs with Modality (2 levels, Light vs. Tone) and Repetition (2 levels) as a within subject factors and Lesion (2 levels) as a between subjects factor. Two other separate ANOVAs with an identical design were used to compare correct and incorrect response latencies.

Results

Histology

Animals that received ibotenic acid demonstrated significant excitotoxic damage in the ACC (Figure 2.2). There was a main effect of Rostrocaudal position ($F_{2,10} = 8.51$, $p=0.007$) due to greater excitotoxic damage (percent loss relative to Sham-Lx animals) in the ACC at +3.7 mm and +2.7 mm from bregma as compared to 1.7 mm from bregma (3.7 vs. 1.7: $t_6=4.45$, $p=0.007$, 2.7 vs. 1.7: $t_6=2.79$, $p=0.039$, percent loss at 3.7: $74.71\% \pm 11.22\%$, percent loss at 2.7: $60.21\% \pm 12.81\%$, percent loss at 1.7: $19.80\% \pm 11.22\%$). Damage was centered on the ACC and there were no significant hemispheric differences ($p>0.25$).

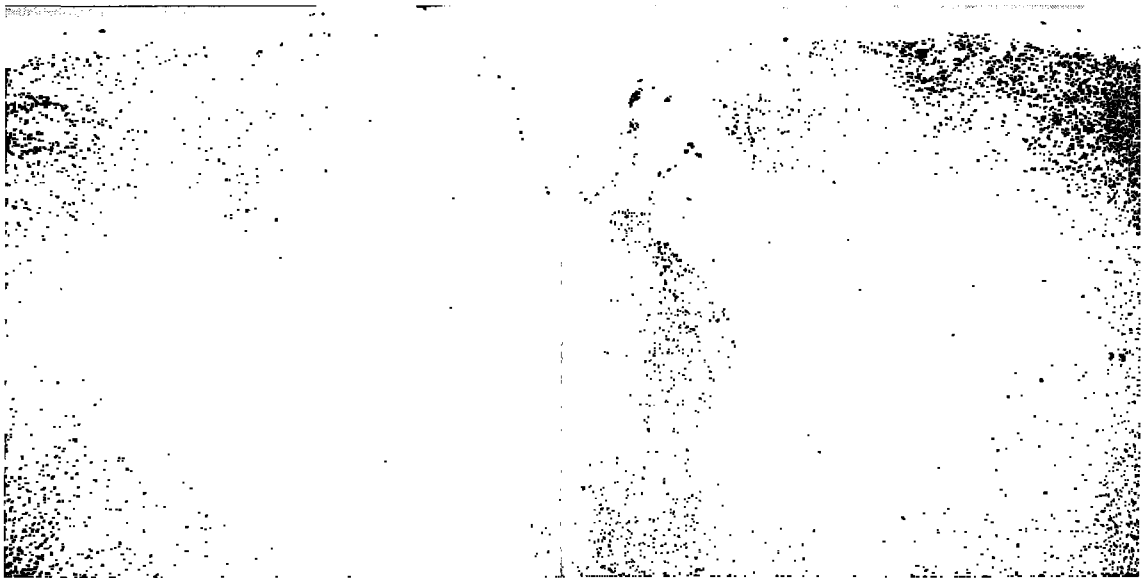
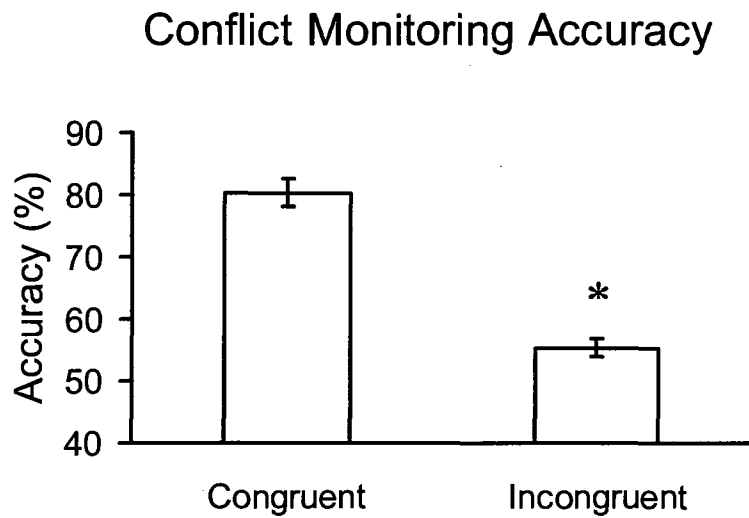


Figure 2.2. Excitotoxic damage of the ACC as elucidated by thionin staining.

Task Characterization

To allow comparisons of the current conflict monitoring task to those used with human subjects, the performance of all animals prior to surgery was examined. Rats were significantly more accurate on congruent trials relative to incongruent trials (Figure 2.3A; $F_{1,14} = 111.11$, $p < 0.001$; Congruent: $80.32\% \pm 2.29\%$, Incongruent: $55.36\% \pm 1.48\%$). Accuracy on incongruent trials was improved when two incongruent trials were presented consecutively (Figure 2.3B; $F_{1,14} = 8.49$, $p = 0.011$; II: $61.13\% \pm 3.02\%$, CI: $50.92\% \pm 1.77\%$). While response latencies did not significantly differ between incongruent and congruent correct responses ($p > 0.09$), all animals had significantly longer incorrect response latencies when responding to incongruent trials as compared to congruent trials (Figure 2.4; $F_{1,14} = 9.37$, $p = 0.008$; Congruent: $682.66 \text{ ms} \pm 87.92 \text{ ms}$, Incongruent: $856.20 \text{ ms} \pm 56.37 \text{ ms}$). There were no significant interactions or main effects between the assigned groups in terms of accuracy or response latency on the preceding unimodal sessions prior to surgery ($p > 0.2$).

A



B

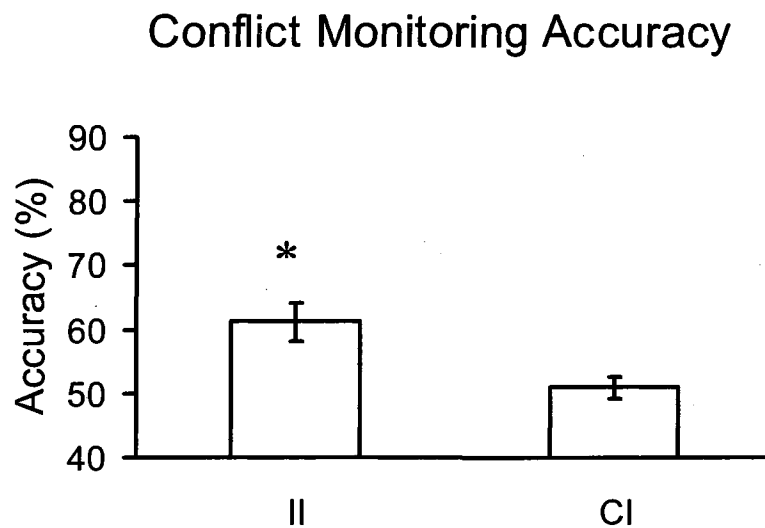


Figure 2.3. Accuracy on congruent and incongruent trials for all animals prior to surgery. **A.** All animals were significantly less accurate at incongruent trials than congruent trials. **B.** Accuracy was higher on incongruent trials preceded by an incongruent trial (II) relative to accuracy on incongruent trials preceded by congruent trials (CI).

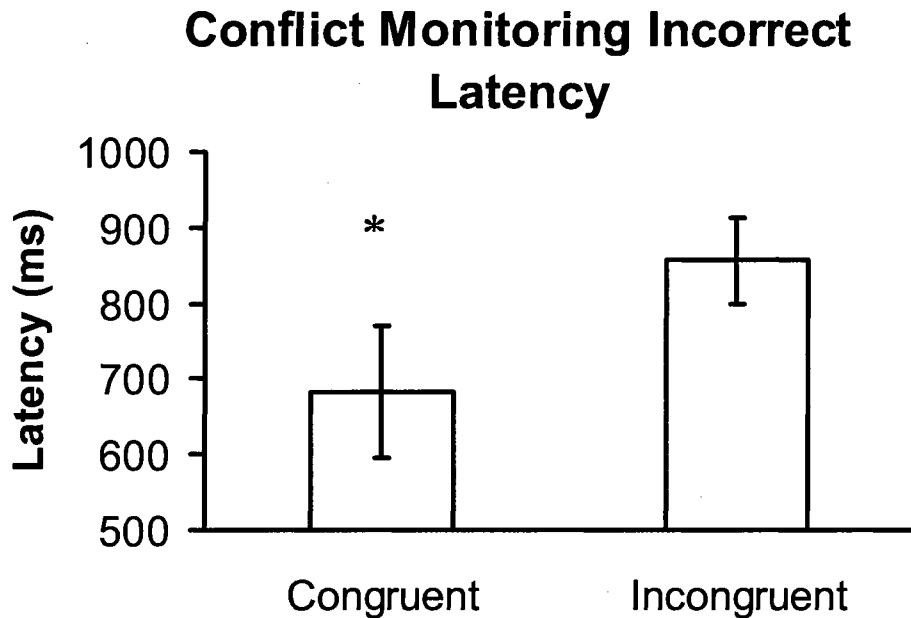


Figure 2.4. Latency to respond on incorrect incongruent trials is significantly greater than incorrect response latencies on congruent trials.

Postsurgical Performance

Performance on postsurgical unimodal priming sessions. There was a significant effect of Modality which revealed all animals were significantly less accurate at the unimodal light sessions than unimodal tone sessions ($F_{1,13} = 11.49$, $p=0.005$; Tone: $81.62\% \pm 1.37\%$, Light: $78.09\% \pm 0.89\%$). There were no other main effects or interactions of lesion on accuracy or latency analyses ($p>0.17$).

Postsurgical conflict monitoring. All animals showed a significant effect of trial type on accuracy ($F_{1,13} = 513.56$, $p<0.001$) with higher levels of

accuracy found on congruent trials than incongruent trials (congruent: $84.13\% \pm 1.51\%$; incongruent: $50.94\% \pm 1.08\%$). There was also a four way interaction (Modality X Session X Trial X Lesion: $F_{1,13} = 6.23$, $p = 0.027$). In order to understand the basis of this interaction the data was separated by modality. This was done because all animals were less accurate at light unimodal sessions than tone unimodal sessions, which may contribute to differential performance on conflict monitoring sessions targeting the two modalities. On conflict monitoring sessions with light as the target modality there was a significant interaction of Session X Trial X Lesion ($F_{1,13} = 11.29$, $p = 0.005$) that was not found in the analysis of tone conflict monitoring sessions ($p > 0.1$). This analysis revealed that a trend for ACC-Lx rats to be more sensitive to the effects of unimodal tone training prior to testing in a light conflict monitoring task. This training resulted in improved accuracy on congruent trials and impaired accuracy on incongruent trials relative to Sham-Lx rats during the test of conflict monitoring with light as the target modality (Figure 2.5; Congruent: $t_{13} = 2.61$, $p = 0.022$; Sham-Lx: $81.48\% \pm 1.11\%$, ACC-Lx: $87.53\% \pm 2.14\%$; Incongruent: $t_{13} = 1.98$, $p = 0.07$, Sham-Lx: $48.99\% \pm 2.84\%$, ACC-Lx: $41.53\% \pm 2.39\%$). There were no other significant main effects or interactions with lesion on accuracy performance of the congruent and incongruent trials ($p > 0.1$).

Conflict Monitoring Accuracy After Non-Target Modality Training

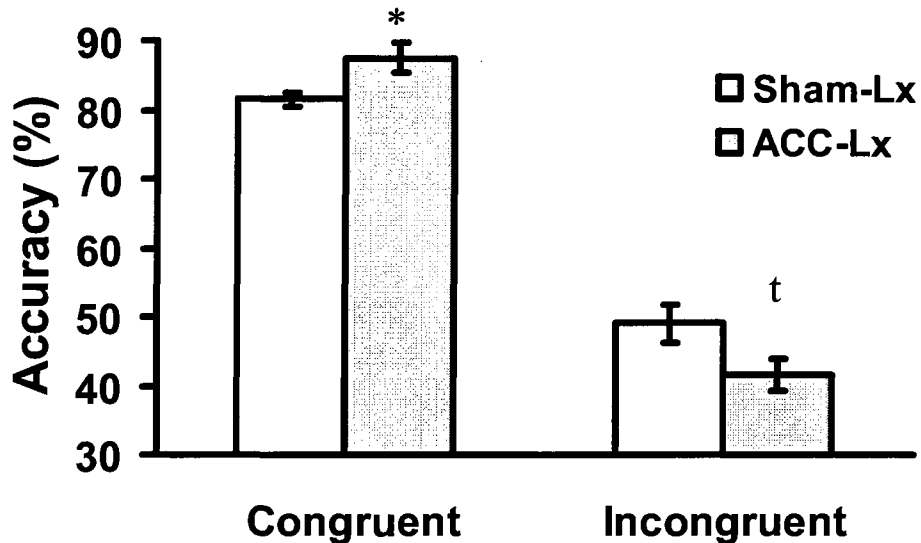


Figure 2.5. When light is the target modality within the conflict monitoring session, ACC-Lx animals show a trend to be more accurate on congruent trials than Sham-Lx rats after the previous day's training was on tone. This difference may indicate a greater influence of the tone stimulus in ACC-Lx animals while performing the conflict monitoring task.

There was a significant interaction of Trial X Lesion on correct latencies ($F_{1,13} = 6.58$, $p=0.030$) where ACC-Lx animals had significantly longer latencies on incongruent trials than congruent trials (Figure 2.6; ACC-Lx: $t_6 = 2.77$, $p=0.040$, Congruent: $950.71 \text{ ms} \pm 81.94 \text{ ms}$, Incongruent: $1125.57 \text{ ms} \pm 83.66 \text{ ms}$) and Sham-Lx animals did not (Sham-Lx: $t_7 = 0.25$, $p=0.81$, Congruent: $990.95 \text{ ms} \pm 73.63 \text{ ms}$, Incongruent: $983.83 \text{ ms} \pm 67.63 \text{ ms}$). In addition, correct response latencies increased significantly for ACC-Lx animals with increased time on task (Figure 2.7; Block X Lesion: $F_{1,13} = 23.35$, $p=0.001$; Sham-Lx: Block

1: 903.18 ms \pm 68.47 ms, Block 2: 1071.60 ms \pm 88.46 ms; ACC-Lx: Block 1: 886.67 ms \pm 69.90 ms, Block 2: 1089.60 ms \pm 92.56 ms). There were no other significant interactions or main effects of lesion ($p > 0.1$).

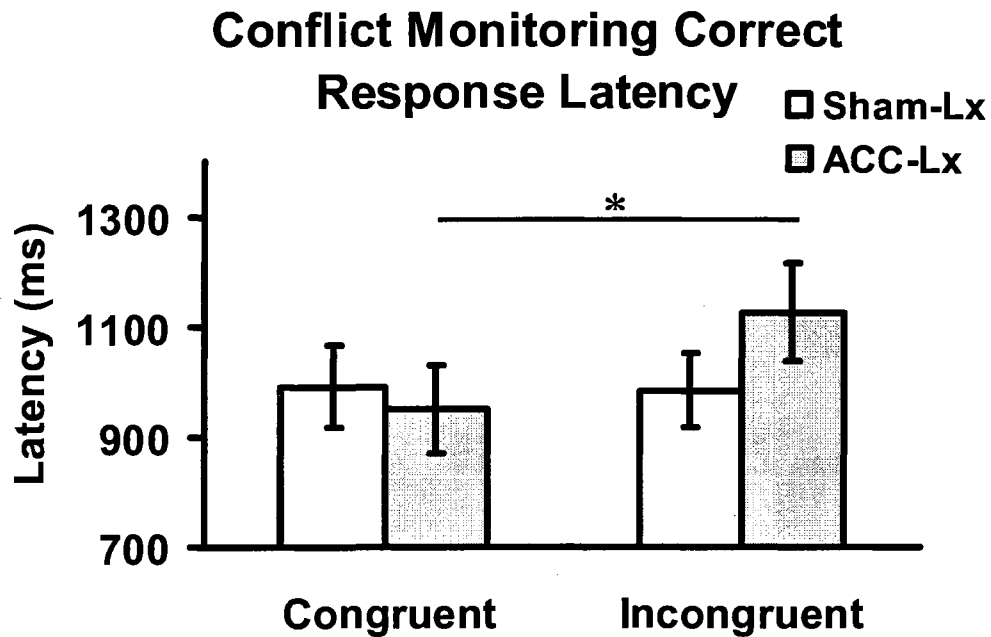


Figure 2.6. The difference between correct response latencies on congruent and incongruent trials is significantly different in ACC-Lx animals but Sham-Lx showed no significant difference.

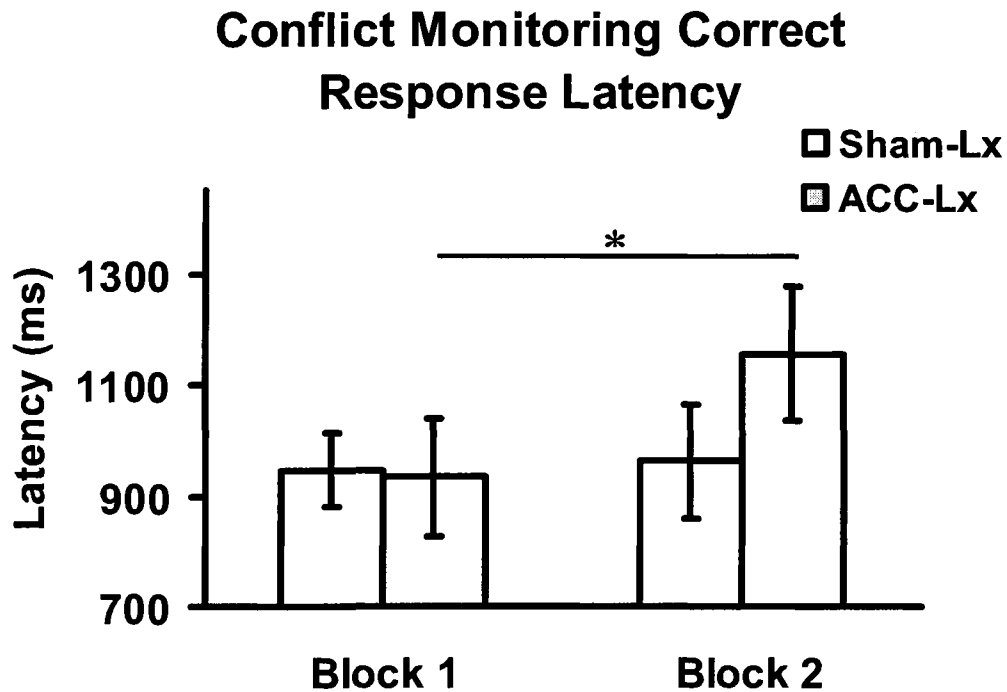


Figure 2.7. ACC-Lx animals show significantly slower correct response latencies as time on task increases. Sham-Lx animals do not show this difference.

On incorrect responses, latencies for both groups increased over time on task ($F_{1,13} = 16.66$, $p=0.001$) and there was an interaction between Block X Lesion ($F_{1,13} = 7.52$, $p=0.017$). Again, response latencies increased on the second block of trials for ACC-Lx animals but did not change over the course of the testing session in Sham-Lx rats (Figure 2.8; Sham-Lx: $t_7=1.35$, $p=0.22$, Block 1: $781.18 \text{ ms} \pm 67.98 \text{ ms}$, Block 2: $891.28 \text{ ms} \pm 94.91 \text{ ms}$; ACC-Lx: $t_6=3.53$, $p=0.010$, Block 1: $683.92 \text{ ms} \pm 86.63 \text{ ms}$, Block 2: $1168.97 \text{ ms} \pm 178.36 \text{ ms}$).

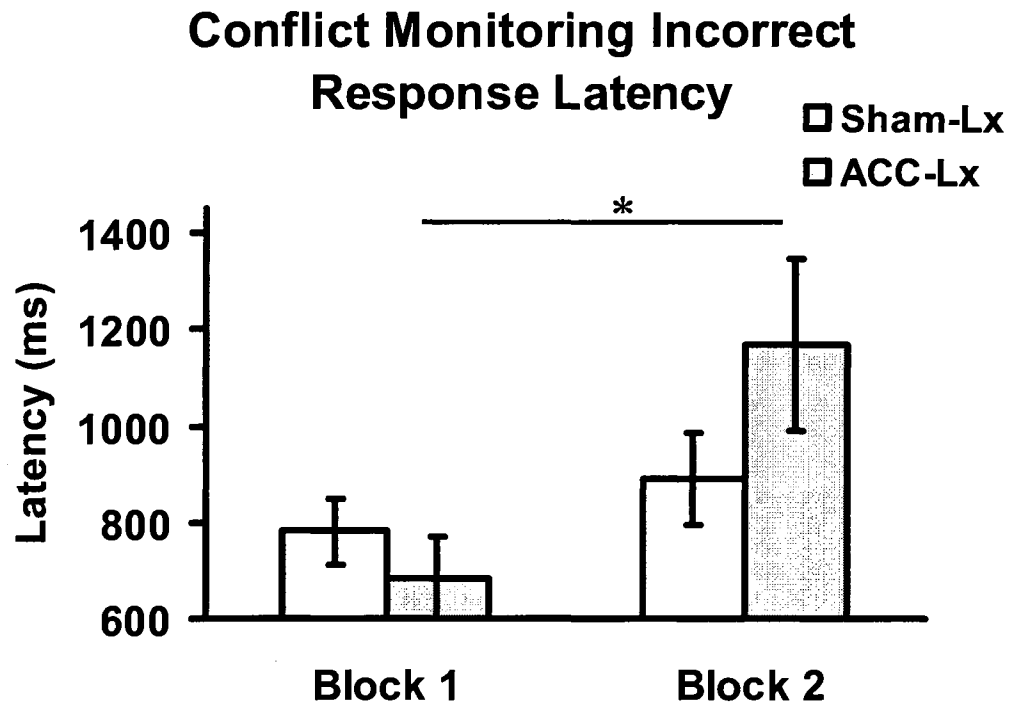


Figure 2.8. ACC-Lx animals show significantly slower incorrect response latencies as time on task increases. Sham-Lx animals do not show this difference.

Distractor. Both groups showed a significant improvement in accuracy when houselight distractor trials were compared to incongruent trials (Figure 2.9; $F_{1,13} = 48.60$, $p < 0.001$; Sham-Lx: distractor: $72.3\% \pm 3.7\%$, incongruent: $53.1\% \pm 3.0\%$; ACC-Lx: distractor: $78.2\% \pm 4.0\%$, incongruent: $53.5\% \pm 3.2\%$). Lesioning the ACC did not alter performance on this variant (all $p > 0.2$).

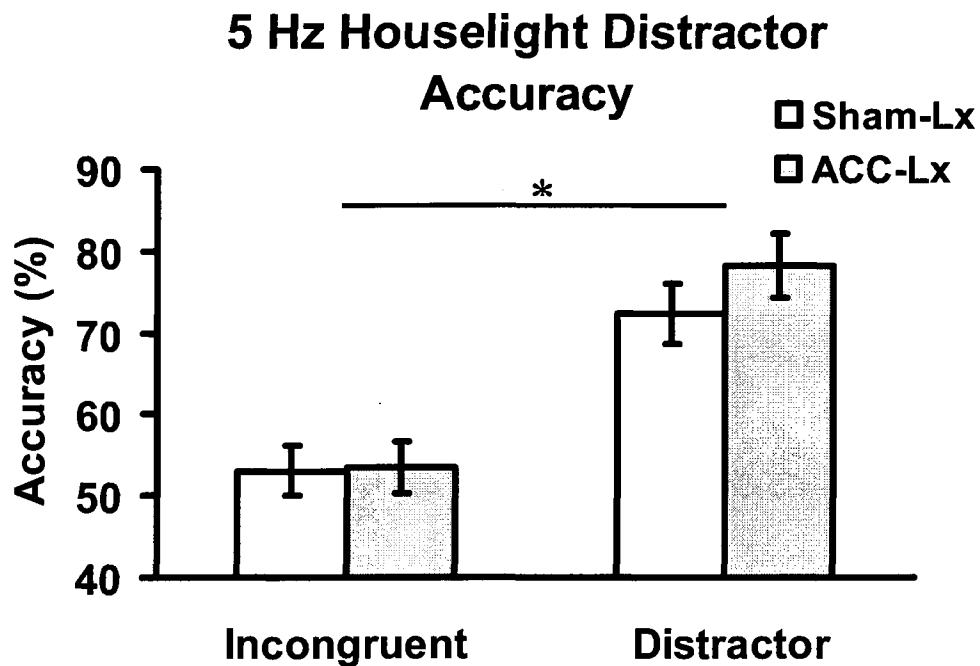


Figure 2.9. All animals were more accurate on sessions where a houselight distractor was presented with the target tone stimulus when compared to incongruent trials where the non-target light stimulus is presented with the target tone stimulus. This suggests the prior association of the panel light with a response rule is critical to disrupting performance on incongruent trials within the conflict monitoring task.

Open Field

Analysis of the open field recordings found no significant differences between ACC-Lx and Sham-Lx animals on any of the dependent measures ($p > 0.08$).

Discussion

The results of this experiment support this paradigm as a valid test of conflict monitoring in rats. The increased errors and increased response latencies on incongruent trials in comparison to congruent trials is similar to what has been seen in humans (Botvinick et al. 2001; Kerns et al. 2004; Yeung et al. 2004). Animals also showed accuracy facilitation on incongruent trials preceded by incongruent trials. This facilitation has previously been demonstrated in humans and has been hypothesized to be dependent on activity in the ACC (Botvinick et al. 2001; Gratton et al. 1992).

In addition the current conflict monitoring paradigm is sensitive to lesions of the ACC in rats. ACC-Lx animals showed greater latency impairments on correct responses to incongruent trials as compared to congruent trials than Sham-Lx animals similar to the effects of ACC dysfunction in humans (King et al. 2007; McNeely et al. 2003; Neuhaus et al. 2007). These results confirm previous research that suggests the ACC is involved in the processing of conflicting stimuli (Botvinick et al. 2001; Swick and Jovanovic 2002; van Veen et al. 2001). In addition, the response latency deficits seen in ACC-Lx animals were exacerbated by increased time on task. This effect may be due to a decreased cognitive capacity caused by the lesions, leading to greater cognitive fatigue with prolonged time on task. Many studies with clinical populations do not assess the performance of conflict monitoring tasks over time on task, however there is one

previous study that separated the task in half and found decreased ACC activity during mental fatigue (Lorist et al. 2005). This decreased ACC activity was correlated with poor cognitive control (Lorist et al. 2005).

There were no significant differences between groups on any dependent measures of the open field test. This suggests the ACC lesions are not increasing anxiety in the rat or affecting locomotor activity.

When ACC-Lx animals were trained on the non-target tone modality and then given a light conflict monitoring session there was a strong trend for a greater difference in accuracy between the congruent and incongruent trials. This could suggest a greater susceptibility of the ACC-Lx animals during the conflict monitoring task to be influenced by the previous day's training. The data suggest this improvement is a result of increased attention to the tone in the ACC-Lx rats that leads to improved performance on congruent trials and impaired performance on incongruent trials. This effect was only seen on the light conflict monitoring sessions. Analysis of the unimodal sessions revealed all animals were significantly less accurate on light trials. This suggests there is already a bias to process the tone stimuli more efficiently, which may contribute to this accuracy effect only on light conflict monitoring sessions. However as all rats were more accurate on tone sessions relative to light sessions, it is unclear why the effects of tone are greater in ACC-Lx rats as compared to the Sham-Lx rats.

In contrast to the conflict monitoring sessions, ACC-Lx animals performed on the distractor session as well as Sham-Lx animals. In the conflict monitoring

session the non-target modality has previously been associated with a reinforced response. In the distractor session, the 5 Hz houselight has never been associated with a reinforced response. The ability of ACC-Lx animals to perform the distractor session in contrast to the conflict sessions suggests the previous associations of the non-target modality contribute to the impairments seen in conflict monitoring. The results support the hypothesis the ACC is necessary for cognitive control in the presence of previously reinforced stimuli, but not in the presence of stimuli with no previous associations with reinforcement or behavioral response.

CHAPTER 3

EXPERIMENT 2: IS THE ACC NECESSARY FOR DISREGARDING DISTRACTORS, REVERSAL LEARNING AND ATTENTIONAL SET SHIFTING?

The ACC has previously been implicated in reversal learning (Ragozzino and Rozman 2007), attending in the presence of salient, novel information (Laurens et al. 2005), and the formation of an attentional set (Ng et al. 2007). Experiment 2 assessed the role of the ACC in attentional set shifting, reversal learning, and attending when distractors are presented using a task previously validated for use in rats (Birrell and Brown 2000). The attentional set shifting task is a task used in humans (Jazbec et al. 2007; Owen et al. 1991; Pantelis et al. 1999), primates (Dias et al. 1997) and rats (Birrell and Brown 2000; McGaughy et al. 2008; Newman et al. 2008a) to assess attentional set formation and shifting. The task employs a “total changeover design” which limits proactive interference from previously rewarded stimuli when shifts in attentional set are required (Birrell and Brown 2000). The attentional set shifting task allows for the assessment of attentional set formation, reversal learning, and susceptibility to distracting information at different stages of the task. Patients with schizophrenia have also demonstrated impairments in disregarding salient, distracting stimuli

which may be due to dysregulation of the ACC (Jazbec et al. 2007; Laurens et al. 2005; Pantelis et al. 1999). Animals with ACC inactivation have previously been shown to have difficulty disregarding distracting stimuli when performing reversal learning (Ragozzino and Rozman 2007) and potential impairments in the formation of an attentional set (Ng et al. 2007). Animals with lesions of the ACC persist in using ineffective strategies longer than sham-lesioned animals, suggesting the ACC may be crucial for recognizing situations where default strategies are not effective and a shift in behavior is required (Dias and Aggleton 2000). Lee, et al. (2007) hypothesized that the ACC, along with the orbitofrontal cortex, is specifically involved in assessing and updating the utility of specific actions and stimuli, while the lateral prefrontal cortex maintains information processing necessary for maintaining optimal performance in the current state. Based on these data, I hypothesize that animals with ACC-Lx will have difficulty performing reversals.

In the attentional set shifting task, subjects are given multiple discriminations that train them to focus attention on a particular dimension (Birrell and Brown 2000; Owen et al. 1991). If a subject has successfully focused their attention on this dimension, or formed an attentional set, when they are given an entirely new set of stimuli (intradimensional shift) performance should be facilitated by maintaining attention to this same dimension (Birrell and Brown 2000; Owen et al. 1991). If they are then given another new set of stimuli and reinforced for attending to a new dimension (extradimensional shift), they should take a significantly greater number of trials to shift attention to this new

dimension, i.e. to shift attentional set (Birrell and Brown 2000; Owen et al. 1991). In a study by Ng, et al. (2007) rats with excitotoxic lesions of the ACC took significantly more trials to reach criterion on the intradimensional shift than sham-lesioned animals. This suggests the ACC lesioned animals may not have formed an attentional set. Another explanation for this deficit could be the animals with excitotoxic lesions of the ACC have difficulty in discriminating rewarded actions from non-rewarded actions similar to the deficits seen in autoshaping, where animals have to learn to discriminate between stimuli that are rewarded and those that are not rewarded (Cardinal et al. 2003). However, if this was true ACC-Lx animals should be impaired at all stages of the task. This previous research suggests ACC-Lx animals in the current study should have difficulty forming an attentional set.

Predicted Results

The current experiment will assess the effects of ACC lesions in rats on the attentional set shifting paradigm which allows for the unique assessment of reversal learning, conditional discriminations in the presence of salient distractors, and the formation and shifting of an attentional set. Lesions of the ACC should impair performance on reversals, impair the formation of an attentional set, and impair performance in the presence of salient distractors. In addition, the repeated testing design used in this experiment will allow for the assessment of these abilities over multiple sessions.

Research Design and Methods

Apparatus and Materials

All training and testing was performed in a plastic testing box (91.44 x 45.72 x 25.40 cm; L x W x H, Sterlite Corporation, Townsend, MA). Rats were trained to dig in terra cotta pots with a height of 10 cm and an internal diameter of 10.2 cm. The outer surface of the pots were covered with textures and the inside of the pots were filled with digging media which were scented using diluted aromatherapy oils (essential oils diluted 1:100 in vegetable oil). Test stimuli were placed in either end of the testing box with access to these stimuli was prevented by a divider. The divider was then removed at the start of each trial to allow access to the test stimuli. Reinforcement for both training and testing was a 45 mg food pellet (Dustless Precision Pellets, Bio-serv, Frenchtown, NJ). For all stages of training and testing, the unbaited pot contained an equal, but crushed amount of reinforcer to prevent the rats from using the scent of cereal to identify the correct stimuli.

Training

16 male Long Evans rats were trained to dig in flower pots filled with pine shavings to retrieve a food reward (45 mg pellet). All animals were food

restricted to ~80% of their free fed body weight prior to training. After animals successfully retrieved 10 pellets in a row within a 90 second limited hold, they were considered eligible for surgery.

Surgery

Surgical procedures were identical to those described in experiment 1.

Postsurgical Training

After recovery from surgery the animal began testing in the attentional set shifting task. The animal was first given access to two exemplar pots, only one of which was baited. Three sets of exemplar pots were tested, one in each of the dimensions the rat would be required to attend to during the conditional discrimination tests. The pots were wrapped in fabric to give different textures (fuzzy vs. smooth fur), filled with different digging media (white vs. green paper), or scented with different scents (cherry vs. pine). A pair of pots was placed in the box behind a divider to prevent the rat's access. The experimenter removed the divider and started the timer. If the rat dug or displaced the digging medium with his nose or paw this was considered a response at which point the timer was stopped and the response latency and accuracy were recorded. For the first trial of every discrimination a pellet was placed on top of the digging medium to ensure the rat recognized one of the pots was reinforced. On subsequent trials

the pellet was buried. The first four trials of every discrimination were discovery trials. In these trials the animal was given 90 seconds to respond. If they responded incorrectly the latency was recorded, the trial was scored as a miss, and they were allowed to explore the correct pot. On trials subsequent to the discovery trials the limited hold was reduced to 60 seconds and the animal was no longer allowed to explore the correct pot if an incorrect choice was made. Once the rat achieved six correct responses in a row on each exemplar, he was ready to move on to testing in the ID/ED task. The exemplars were not used again.

Attentional set shifting. A schematic of the attentional set shifting task is provided in Figure 3.1. The first discrimination in the attentional set shifting task is a simple discrimination (SD) where the pots differ on one dimension (texture, digging medium, or odor). The animal was again given four discovery trials in which they were permitted to dig in both pots, and again only one pot was baited (i.e. patchouli scented pot). Again, once the animal achieved six consecutive correct trials the next discrimination was started.



CD Example

ID Example

ED Example

Task	Testing Pair 1	Testing Pair 2	Not Relevant Attribute
SD	Cinnamon vs. Patchouli	Cinnamon vs. Patchouli	Fake Fur
CD	Cinnamon /Light Shapes vs. Patchouli/Dark Shapes	Cinnamon / Dark Shapes vs. Patchouli/Light Shapes	Fake Fur
Rev 1	Patchouli /Light Shapes vs. Cinnamon/Dark Shapes	Patchouli / Dark Shapes vs. Cinnamon/Light Shapes	Fake Fur
ID 1	Lilac /Gold Buttons vs. Rose/Black Buttons	Lilac /Black Buttons vs. Rose/Gold Buttons	Ribbed side Corduroy
Rev 2	Rose /Gold Buttons vs. Lilac/Black Buttons	Rose /Black Buttons vs. Rose/Gold Buttons	Ribbed side Corduroy
ED 1	Metal Beads /Gardenia vs. Plastic beads/Jasmine	Metal Beads /Jasmine vs. Plastic Beads/Gardenia	Terrycloth
Rev 3	Plastic Beads /Gardenia vs. Metal beads/Jasmine	Plastic Beads /Jasmine vs. Metal Beads/Gardenia	Terrycloth
New Session Rep Rev 3	Plastic Beads /Rough terrycloth vs. Metal beads/smooth terrycloth	Plastic Beads /Smooth terrycloth vs. Metal Beads/Rough terrycloth	Gardenia
ID 2	Light pompoms /Sequin fabric vs. Dark pompoms/Smooth fabric	Light pompoms /Smooth fabric vs. Dark pompoms/Sequin fabric	Pomegranate
Rev 4	Dark pompoms /Sequin fabric vs. Light pompoms/Smooth fabric	Dark pompoms /Smooth fabric vs. Light pompoms/Sequin fabric	Pomegranate
ED 2	Velvet fabric /Light yarn vs. Smooth fabric/Dark yarn	Velvet fabric /Dark yarn vs. Smooth fabric/Light yarn	Linen
Rev 5	Smooth fabric /Light yarn vs. Velvet fabric/Dark yarn	Smooth fabric /Dark yarn vs. Velvet fabric/Light yarn	Linen

Figure 3.1. A schematic for the attentional set shifting task. Rewarded stimuli are in bold.

The next discrimination was a compound discrimination (CD) where the other two dimensions were introduced but the rat was still rewarded for attending to the previously reinforced dimension (patchouli). In order to minimize the

number of pairs of pots needed, the dimension that was irrelevant to ID and ED performance in the first session was held constant across both pots. In other words, rats trained to attend to odor in tests of the SD→REV2 that shifted responding to digging media in the ED→REV3 received two pairs of pots with identical texture cues. Any deficits seen at the CD would suggest deficits in ignoring new irrelevant stimuli which has previously been demonstrated in patients with frontal lobe damage and schizophrenic patients (Jazbec et al. 2007; Owen et al. 1991; Pantelis et al. 1999).

After six consecutive correct responses were made on the CD, the reinforcement contingencies within a modality were reversed (i.e. cinnamon scented pot is rewarded, not patchouli; REV 1). Deficits on reversal learning have been demonstrated after damage to orbitofrontal cortex (Dias et al. 1997; McAlonan and Brown 2003; Tait and Brown 2007). After six consecutive correct responses, a new set of stimuli (new odors, digging media, textures) is introduced. The animal was reinforced for attending to the same dimension as the previous discrimination (lilac scented pot). This discrimination is known as the intradimensional shift (IDS1). Learning at this stage should be facilitated by the animal maintaining attentional focus on the previously rewarded stimulus dimension. After the successful completion of six consecutive trials on IDS1, another reinforcement reversal was performed (REV 2; reinforced for digging in rose scented pot). The next discrimination is the extradimensional shift (EDS1) which used novel set of stimuli, and this time the animal was reinforced for attending to a previously unattended dimension, i.e. shifting attentional set

(digging medium; metallic beads). If the animal has formed an attentional set then they should take significantly more trials to achieve criterion performance on the EDS. Previous research has suggested excitotoxic lesions (Birrell and Brown 2000; Ng et al. 2007) and noradrenergic lesions (McGaughy et al. 2008; Newman et al. 2008a; Tait et al. 2007) of the prelimbic cortex impede the ability to shift attentional set. After successful completion of the EDS, the final reversal (REV 3; plastic beads) of the session was given.

In order to determine whether the deficits seen were consistent across time, the ID/ED shift and reversals were repeated. In the first discrimination of the second session all animals were given the same discrimination used in REV 3 of the previous session (digging medium; plastic beads). The animals were reinforced for responding to the same stimulus that was reinforced on the REV 3. However, because the animal will now have to shift attention at the ED to the dimension that was held constant across pots previously (texture) this previously constant dimension now varies between the pots and the other dimension (odor) is now held constant. If animals have successfully learned that this previously constant dimension e.g. texture is irrelevant, there will be no difference in performance on REV3 and its repetition. All animals were then given a novel set of pots and required to perform an intradimensional shift (IDS2). The alternate exemplar of the relevant dimension, e.g. the other digging medium, was then reinforced (Rev 4). The animals were again given a novel set of pots and required to perform an extradimensional shift, e.g. texture (EDS2). Finally the reinforcement was reversed within the relevant modality (REV 5). The order of

presentation of the dimensions throughout testing was counterbalanced across subjects and matched across Sham-Lx and ACC-Lx rats.

Serial Reversals

Previous research has suggested animals with ACC inactivation have difficulty with reversal learning only in the presence of distracting stimuli (Ragozzino and Rozman 2007). In order to determine whether ACC-Lx animals had difficulty on the reversals within the attentional set shifting task due to the presence of distracting stimuli, serial reversals were conducted after completion of the attentional set shifting task. These reversals were presented in each of the dimensions used in the attentional set shifting task without the presence of the other dimensions. New stimuli that had not previously been tested (terra cotta pots and Gladware cups plastic cups, diameter: 7 cm, height: 6 cm, Glad Products Company, Oakland, CA) were used in order to prevent interference effects. Testing was conducted in the same apparatus used in the attentional set shifting task. All animals were assessed on three discriminations for each dimension: the acquisition of the simple discrimination, the reversal of the reinforced pot, and the reversion of the reinforcement to the originally reinforced pot. These three discriminations were given in one session. All animals were given two more sessions to complete the serial reversals for the other two dimensions. All orders of dimensions were presented and counterbalanced

between groups. The testing procedure for each discrimination was identical to those used in the attentional set shifting task.

Open Field Test

In order to assess emotional reactivity all animals were tested in an open field using the same design described in experiment 1.

Statistical Analyses

All statistical analyses were performed with SPSS v. 17.0 (SPSS, Chicago, IL). Because performance at different stages of the task has been associated with functionally distinct areas of the frontal cortex, these separate stages were analyzed using separate mixed factors ANOVAs. The degrees of freedom in all analyses were corrected using the Huynh–Feldt correction in the case of a violation of sphericity. Epsilon (ϵ) values not equal to 1 are reported below. Alpha levels were modified using Bonferroni corrections when necessary. In order to ensure all animals were able to successfully discriminate in each dimension the number of trials needed to reach criterion performance in each exemplar was compared using a mixed factors ANOVA with a within subjects factor of Dimension (3 levels) and the between subjects factor Lesion (2 levels).

Attentional set shifting. In order to assess the initial acquisition of the attentional set a separate ANOVA with Test (2 levels, SD, CD) as a within

subjects factor and Lesion (2 levels) and Dimension (3 levels) as between subject factors. The CD is the first instance where the animal is required to focus attention in the presence of distracting, salient stimuli. Patients with schizophrenia have previously shown deficits at the CD which may be due to dysregulation of the ACC (Jazbec et al. 2007; Pantelis et al. 1999). This analysis will allow for the assessment of ACC in disregarding new, salient stimuli in the CD and dissociate impairments under these conditions from impairments due to an inability to discriminate two stimuli (SD). The number of trials needed to reach criterion performance in the ID was compared to the ED in a separate mixed factors ANOVA with within subjects factors of Session (2 levels) Test (ID vs. ED; 2 levels) and between subjects factor of Lesion (2 levels) to determine whether animals were able to repeatedly form an attentional set. An assessment of the effects of ACC lesions on the number of trials to reach criterion on the first reversal in a session (REV 1 and REV 4) as compared to the second reversal of the session (REV 2 and REV 5) was done using a separate mixed factors ANOVA with Session (2 levels), and Test (1st REV vs. 2nd REV; 2 levels) as within subject factors and Lesion (2 levels) as a between subjects factor. Finally REV 3 and its repetition on the following session were compared with a mixed factor ANOVA with Repetition (2 levels) as a within subject factor and Lesion (2 levels) as a between subjects factor in order to determine the effect of changing the irrelevant dimension that is held constant.

Impairments were found in the previously described analysis on reversal learning. In order to determine the nature of the impairments seen between the

groups on reversal learning, error analyses were conducted on reversal discriminations that showed significant differences between Sham-Lx and ACC-Lx animals on trials to criterion. Using methods similar to those described in previous studies, errors were categorized as either perseverative, suggesting continued attention to the previously reinforced stimulus, or regressive, suggesting a return in attention to the previously reinforced stimulus (Ragozzino et al. 2003; Ragozzino and Rozman 2007). All trials on a reversal discrimination were divided into blocks of four consecutive trials. Errors were categorized as perseverative if at least three out of the four trials in the block were errors. If less than three errors were seen in a block of four trials these errors were considered regressive. After a rat chose an incorrect pot less than three times in a row, all errors made after this point were considered regressive. The first error was also excluded from analysis as it is impossible for the animal to know reinforcement has shifted until after the first error. Independent samples t-tests were conducted to determine whether there were any differences between Sham-Lx and ACC-Lx animals on total errors, perseverative errors, or regressive errors.

Serial reversals. In order to assess the number of trials to criterion on acquisition and reinforcement reversal, a mixed factor ANOVA was used in which the within subjects factors were Dimension (3 levels) and Test (2 levels; acquisition vs. reversal) and the between subjects factor was Lesion (2 levels). Reversion back to the originally rewarded pot was assessed using a separate mixed factors ANOVA with Dimension (3 levels) and Test (2 levels; reversal and

reversion) as within subject factors and Lesion (2 levels) as a between subjects factor.

Results

Histology

Due to poor histological assessment one ACC-Lx animal was excluded from the study. All other animals that received ibotenic acid demonstrated significant excitotoxic damage in the ACC with lesions similar to those seen in experiment 1 (see Figure 2.4). There was a main effect of Rostrocaudal position ($F_{2,12} = 25.13$, $p=0.001$) due to greater excitotoxic damage in the ACC of ACC-Lx rats versus Sham-Lx baseline at +3.7mm and +2.7 mm from bregma than at +1.7 mm (3.7 vs 1.7: $t_6=4.81$, $p=0.003$; 2.7 vs 1.7: $t_6=5.64$, $p=0.001$; percent loss at 3.7: $91.87\% \pm 5.14\%$, percent loss at 2.7: $91.17\% \pm 5.53\%$, percent loss at 1.7: $57.05\% \pm 8.14\%$; corrected $\alpha = 0.017$). No other significant interactions or main effects were present (all $p>0.1$).

Attentional Set Shifting Task

ACC-Lx animals took significantly more trials to reach criterion on the compound discrimination than Sham-Lx rats (Figure 3.2; Test X Lesion: $F_{1,13}=6.57$, $p=0.031$; $t_{13}=2.89$, $p=0.022$; Sham-Lx: 17.25 ± 2.54 ACC-Lx: $39.43 \pm$

7.23) but were equally proficient on the SD ($t_{13}=1.26$, $p=0.23$; Sham-Lx: 9.13 ± 1.49 ; ACC-Lx: 7.00 ± 0.577). All animals took significantly more trials to reach criterion performance on the CD than the SD (Test: $F_{1,13}=15.63$, $p=0.003$).

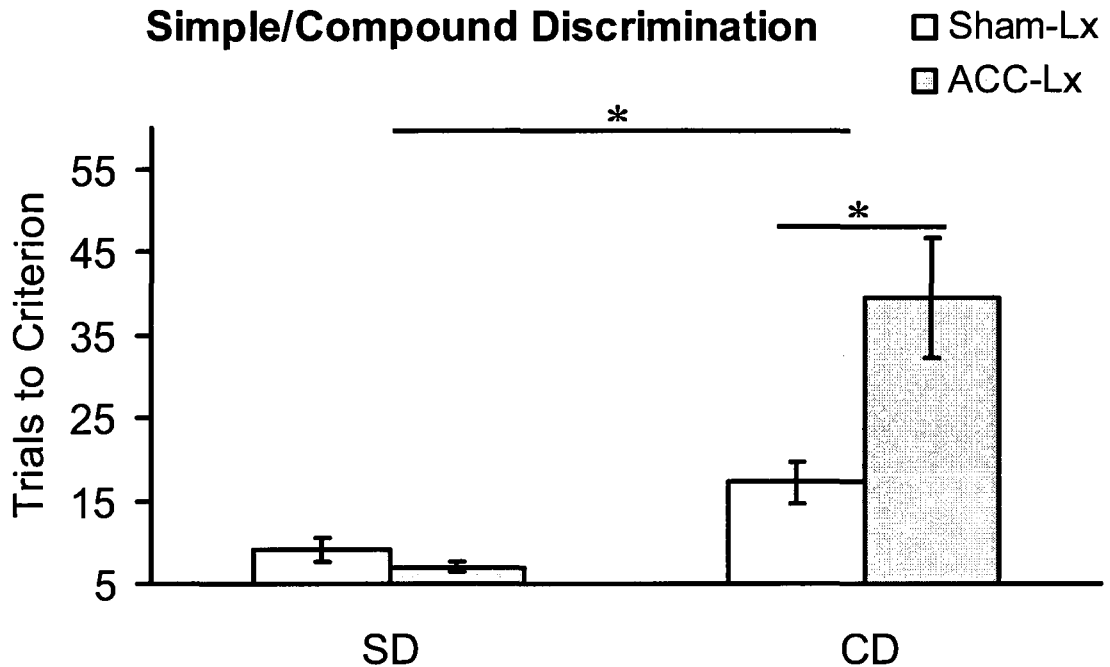


Figure 3.2. All animals took significantly more trials to reach criterion performance on compound discriminations (CD) than simple discriminations. ACC-Lx animals took significantly more trials to reach criterion on the CD than Sham-Lx animals.

Affective Shifting

Animals with lesions of the ACC also showed significant impairments on reversal learning (Lesion: $F_{1,13}=20.74$, $p=0.001$). This impairment was more severe on the first reversal versus the second reversal (Figure 3.3; Test X

Lesion: $F_{1,13}=27.49$, $p<0.001$). Further analysis revealed that the ACC-Lx animals took significantly more trials to reach criterion on the first reversals of each session (REV 1: $t_{13}=5.54$, $p<0.001$, Sham-Lx: 22.13 ± 2.80 , ACC-Lx: 47.43 ± 3.70 ; REV 4: $t_{13}=2.86$, $p=0.013$, Sham-Lx: 16.25 ± 1.62 , ACC-Lx: 24.86 ± 2.63) but were not significantly worse than Sham-Lx animals on the second reversal in a session ($p>0.07$). ACC-Lx rats showed improved performance on the first reversal of the second session, i.e. REV 4, relative to the first reversal of the first session, i.e. REV 1 (Session X Test X Lesion: $F_{1,13}=22.73$, $p<0.001$; $t_6=6.65$, $p=0.001$), but ACC-Lx rats still required more trials than Sham-Lx rats to reach criterion performance on this reversal. There was also a significant main effect of Session ($F_{1,13}=13.85$, $p=0.004$), a Session X Test interaction ($F_{1,13}=53.10$, $p<0.001$).

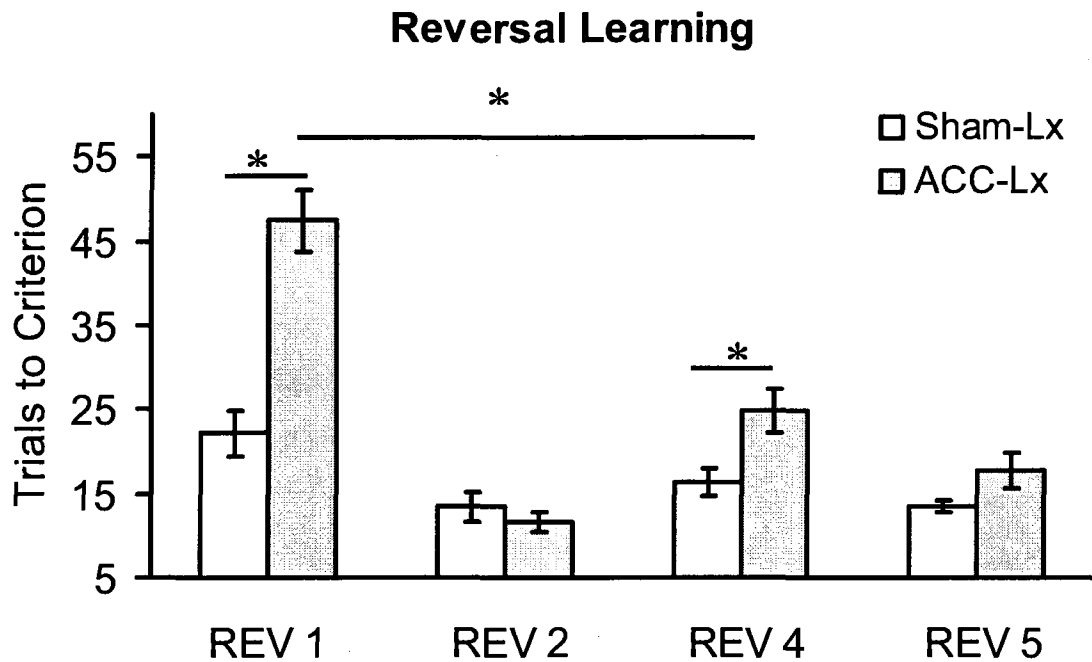
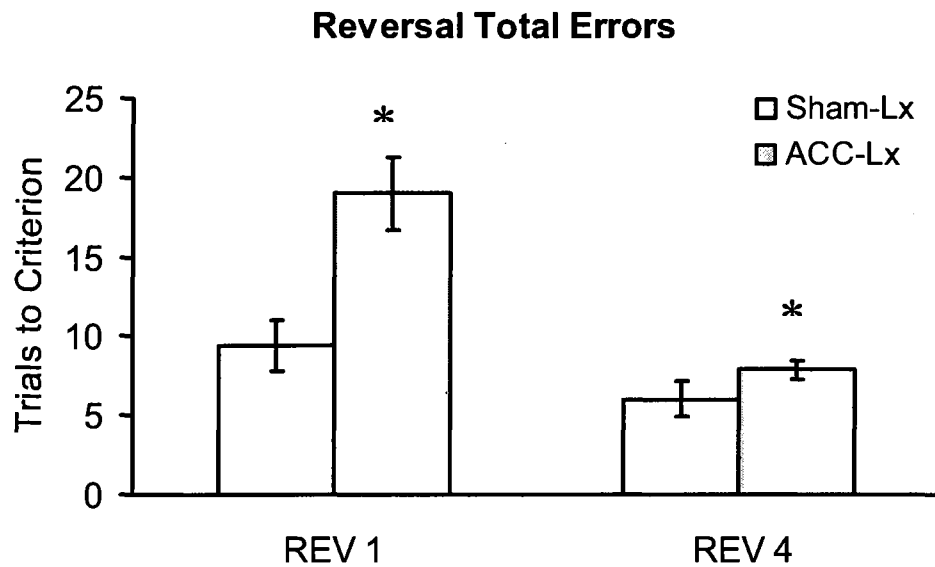


Figure 3.3. All animals took significantly more trials on the first reversals in a session (REV 1 & REV 4) than on the second reversals in a session (REV 2 & REV 5). This suggests all animals were able to learn how to perform reversals efficiently within a session. ACC-Lx animals took significantly more trials to reach criterion on the first reversals in each session (REV 1 & REV 4) than Sham-Lx animals. Even though ACC-Lx animals were impaired compared to Sham-Lx animals on REV 4, they did take significantly less trials to reach criterion on the first reversal of the second session (REV 4) as compared to the first reversal of the first session (REV 1).

Error analyses were only done on the first reversals of each session because these were the only reversals where ACC-Lx animals showed significant impairments as compared to Sham-Lx animals. Error analysis conducted on the first reversals of the session reveal ACC-Lx animals committed significantly more errors on REV 1 (Figure 3.4A; $t_{13}=3.51$, $p=0.004$; Sham-Lx:

9.38 \pm 1.59, ACC-Lx: 19.00 \pm 2.31) and REV 4 (t_{13} =2.86, p =0.013; Sham-Lx: 6.00 \pm 1.12, ACC-Lx: 7.86 \pm 0.595). The groups did not differ in the number of perseverative errors committed on each reversal (p >0.17) but ACC-Lx animals did show more regressive errors than Sham-Lx rats (Figure 3.4B. REV 1: t_{13} =5.02, p <0.001, Sham-Lx: 2.63 \pm 0.65, ACC-Lx: 9.86 \pm 1.35; REV 4: t_{13} =3.56, p =0.004, Sham-Lx: 2.00 \pm 0.54, ACC-Lx: 5.29 \pm 0.78).

A



B

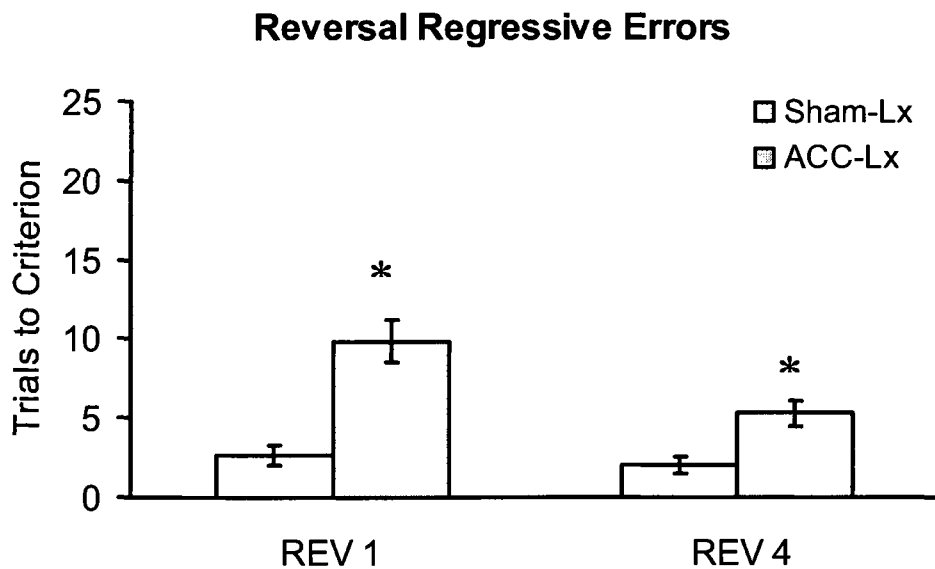


Figure 3.4. A. ACC-Lx animals committed significantly more overall errors on REV 1 and REV 4 than Sham-Lx animals. **B.** Further analyses revealed this increased in total errors was due to ACC-Lx animals committing significantly more regressive errors at REV 1 and REV 4 than Sham-Lx animals.

Analysis of the trials to criterion on the intradimensional/extradimensional shift showed all animals took significantly more trials on the extradimensional than the intradimensional shift (Figure 3.4; $F_{1,13}=42.28$, $p<0.001$). There were no other main effects or interactions with lesion ($p>0.1$) demonstrating both groups of animals were able to effectively form and shift an attentional set.

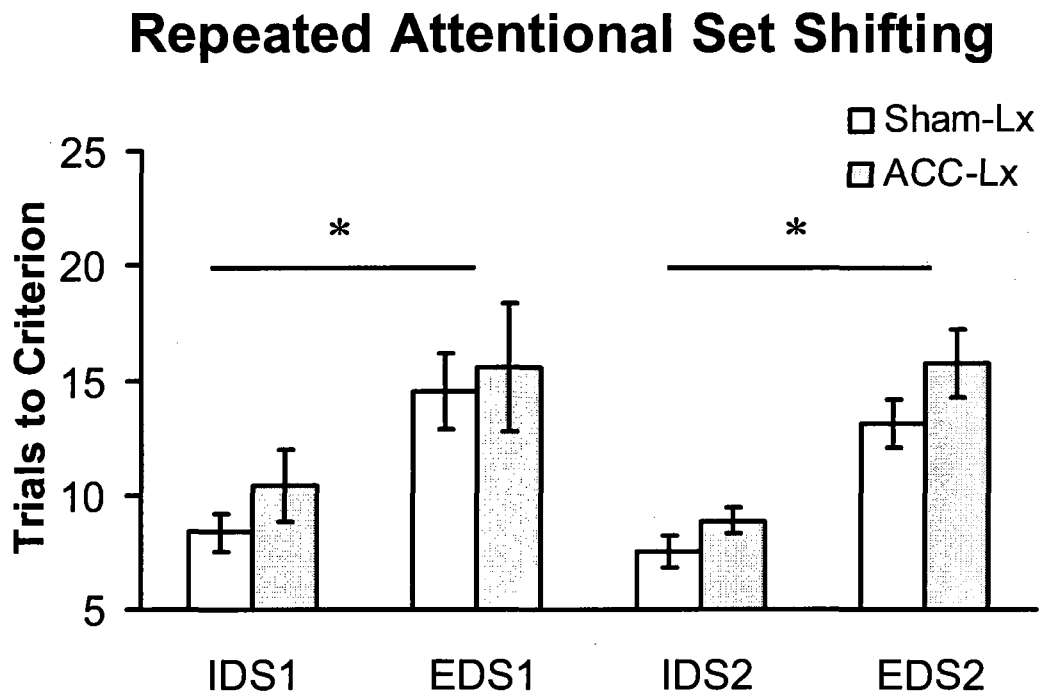


Figure 3.4. All animals more trials to reach criterion on extradimensional shifts than intradimensional shifts in both sessions.

Serial Reversals

Analyses revealed no significant interactions or main effects of lesion suggesting the performance of ACC-Lx animals did not differ from Sham-Lx animals on simple reversal learning. This finding is consistent with previous

research examining the affects of anterior cingulate lesions on reversal learning in two-choice discrimination tests (Bussey et al. 1997; Dias and Aggleton 2000).

Response Latencies

Response latencies were analyzed for all stages of the tasks (attentional set shifting task and serial reversals) however no significant differences were seen between the groups (all $p > 0.1$).

Open Field

No dependent measures revealed any differences between the groups on the open field task ($p > 0.5$).

Discussion

The results of this study suggest the ACC is not involved in the formation of or shifting an attentional set, but instead critical for disregarding irrelevant, novel stimuli that could potentially be associated with reinforcement and the effective processing of information in the presence of a shift in reinforcement. These impairments are unique compared to other studies involving excitotoxic lesions of the prelimbic cortex in rats which significantly increased trials to criterion on extradimensional shifts, i.e. impaired attentional set shifting (Birrell

and Brown 2000) or excitotoxic lesions of the orbitofrontal cortex which significantly increased trials to criterion on all reversals (McAlonan and Brown 2003; Tait and Brown 2007). Serotonergic (Clarke et al. 2007) and noradrenergic (unpublished data) lesions of the orbitofrontal cortex show similar impairments to those seen with ACC-Lx with greater trials to criterion on the first reversal. The interconnections of the ACC and the orbitofrontal cortex, and the consistent impairments of reversal learning in orbitofrontal lesioned animals suggest the orbitofrontal cortex is more likely to be responsible for the actual reversal learning required to perform the reversal discrimination. The ACC in contrast, may be involved in signaling that a change in reinforcement has occurred in order to engage the orbitofrontal cortex in preparing to perform the affective shift. The significantly greater number of regressive errors committed by ACC-Lx animals suggests they are not effectively engaging the processes necessary for an affective shift. It is possible that the loss of ACC leads to an inefficient processing of this error information in order for the animal to determine which stimuli is now reinforced. This impairment is unlike the deficits seen with serotonergic lesions of the orbitofrontal cortex where subjects had more perseverative errors (Clarke et al. 2007). After reaching criterion performance on the first reversal, ACC-Lx rats are able to perform as well as Sham-Lx rats on the second reversal. This suggests that if the animal has learned how to respond to a shift in reinforcement contingencies, the ACC is not as necessary to perform this action.

Deficits in reversal learning using a four choice odor discrimination paradigm have also been demonstrated in rats with ACC inactivation (Ragozzino and Rozman 2007). Ragozzino and Rozman (2007) found that animals with ACC inactivation were more likely to dig in odors that were never associated with reinforcement when reinforcement contingencies were reversed. In the current paradigm, though there are only two pots, these pots have multiple stimulus dimensions. It is possible that the deficits seen on the first reversal are due to the rat responding to these other distracting stimulus dimensions. The lack of a deficit on the serial reversals supports this hypothesis.

Both ACC-Lx and Sham-Lx animals show impaired performance on the first reversal of the second session suggesting the break in testing may reset the animals' ability to perform an affective shift. The reinstatement of impairments in ACC-Lx animals in comparison to Sham-Lx animals at the start of a new session on the first reversal suggests ACC may play a role in the retrieval of how to respond when reinforcement contingencies change. This fits with previous research on the role of ACC in memory for procedures and the reduction of ACC activity with practice (Burgess et al. 2000; Garavan et al. 2000). In addition, while the ACC-Lx animals require significantly more trials to reach criterion on REV 4 as compared to Sham-Lx animals, ACC-Lx rats show an improvement in performance on REV 4 relative to REV 1 suggesting this early learning influences later learning. These data suggest that ACC lesions do not impair the ability of the rat to maintain information over the course of a testing session, but do impair rats at the start of a new testing session though these deficits are not as great as

those found in rats' first ever encountered change in reinforcement contingencies.

The significant increase in trials to criterion on the CD corresponds with previous studies testing patients with schizophrenia on a human version of this attentional set shifting paradigm (Jazbec et al. 2007; Pantelis et al. 1999).

Patients with schizophrenia also demonstrated impairments on the CD suggesting that deficits in the ACC may be responsible for the impairments seen in schizophrenic patients in disregarding salient, novel stimuli. Novel stimuli are presented at several other points in the testing procedure (IDs and EDs) but again the ACC-Lx animals show no impairment at any of these stages. The CD may be unique because this is the first time irrelevant stimulus dimensions are introduced in conjunction with the target dimension. Previous research has suggested patients with schizophrenia are susceptible to novel, rarely presented stimuli (Laurens et al. 2005; Liddle et al. 2006). Animals with ACC-Lx may be more susceptible to the distracting dimensions on the CD because they have not previously had to disregard any irrelevant stimuli. Again, this suggests the ACC is involved in the preparation to engage specific attentional processes. In addition, these dimensions were previously associated with reinforcement in the exemplars. Therefore, adding these dimensions in the CD is not simply adding novel distractors but adding dimensions that have previous associations with reinforcement. While the results of the conflict monitoring task suggest ACC-Lx animals have difficulty with stimuli that are associated with reinforcement, the deficits at the CD indicate the probability of reinforcement also contributes to

impairments in ACC-Lx animals as the specific stimuli used in the CD were similar but not identical to those stimuli paired with reinforcement in exemplar training. For example, all animals were trained to discriminate between pine and cherry scents in the odor exemplar. When the odor dimension is added into the CD, the specific odors used in the first discrimination are not used (now patchouli and cinnamon) but the presentation of a dimension that was previously reinforced creates the distracting effect because the animal has learned this dimension might potentially be reinforced.

In Ng, et al. (2007) rats with excitotoxic lesions of the ACC took significantly more trials to reach criterion on the intradimensional shift than sham-lesioned animals. The current experiment did not confirm these results. Analyses found all animals showed a significant difference between the ID and the ED in trials to criterion confirming the formation of an attentional set. While Ng et al (2007) found ACC-Lx animals took significantly more trials to criterion on the ID as compared to Sham-Lx animals, the authors did not compare the trials to criterion on the ID to the trials to criterion on the ED to determine if animals formed an attentional set. Therefore, while the significant difference between ACC-Lx and Sham-Lx animals on the ID suggests the failure to form an attentional set, the critical analyses to determine if there is a difference between the ID and the ED was not conducted (Ng et al. 2007). In addition, Ng et al. (2007) did report increased trials to criterion in ACC-Lx animals on the first reversal of some but not all stimulus dimensions. The deficits seen on reversals in the present study could also be due to differences in task design. While all

three dimensions are present in the current study, Ng et al. (2007) only had two dimensions present which would limit the distraction on the first reversal.

The results of the current experiment suggest the ACC is involved in the preparation to disregard novel, distracting stimuli that are potentially reinforced and to perform an affective shift. The corticocortical connections between the ACC and the orbitofrontal cortex seem to be critical in engaging the processes necessary for reversal learning and loss of the ACC disrupts these connections, leading to impairments in performing an affective shift.

CHAPTER 4

EXPERIMENT 3: IS THE ACC NECESSARY FOR DISREGARDING NOVEL DISTRACTING STIMULI?

In conflict monitoring paradigms there are both irrelevant stimuli and incorrect responses that must be ignored. While there have been some studies suggesting the ACC is only involved when responses are in conflict (Milham et al. 2003a), others have demonstrated the potential for ACC in determining the relevance of stimuli and ignoring irrelevant stimuli (Kennerley et al. 2006; Lee et al. 2007; Walton et al. 2007). One of the symptoms of schizophrenia is a disturbance in processing incoming information or 'cognitive dysmetria' which can lead to increased distraction as well as inefficient attention to relevant stimuli. When performing an auditory oddball task, patients with schizophrenia have not only been shown to respond less to task-relevant auditory stimuli, but they also are more susceptible to distracting auditory stimuli (Laurens et al. 2005). In addition these patients also show reductions in activity in the ACC during these tasks (Laurens et al. 2005). It has been hypothesized that the ACC can lead to recruitment of other areas of the brain that may be useful in either implementing greater top-down control to increase attentional control (Sarter et al. 2006) or

promote shifting to new cognitive strategies (Aston-Jones and Cohen 2005; Wager et al. 2005). Experiment 3 was designed to assess the effects of newly introduced stimuli that are similar to target stimuli but have never been associated with a specific response or reinforcement. These distractors are presented within the same modality as the target or cross-modally. Previous research has shown greater activity in the ACC in tasks that require cross-modal divided attention than tasks that require unimodal divided attention (Vohn et al. 2007). This activity may be uniquely driven by the presentation of cross-modal information. If this hypothesis is true, ACC-Lx animals should be more susceptible to the cross-modal distractor than Sham-Lx rats. In contrast to the response conflict paradigm, the sustained attention task allows for the assessment of salient, distracting stimuli without response conflict.

Predicted Results

In the presence of salient distractor stimuli, ACC-Lx animals should show impaired performance in comparison to Sham-Lx animals. If they are particularly susceptible to cross-modal distractors they may show impaired performance in the presence of a distracting tone when Sham-Lx animals do not.

Research Design and Methods

Apparatus and Materials

All animals were trained and tested in the same operant chambers described in experiment 1.

Shaping

After completion of the attentional set shifting task and serial reversals, rats were trained on a previously validated test of sustained attention (McGaughy and Sarter 1995), which required animals to discriminate between signal (panel light illumination) and non-signal (the absence of panel light illumination) trials presented in a temporally unpredictable manner (12 ± 3 seconds). Training sessions consisted of a total of 162 trials. Subjects were placed in the operant chambers and given 1 min to acclimate to the environment. The animals were initially trained to discriminate between signal and nonsignal trials with the houselight on throughout the session. Signal and nonsignal trials were presented in a pseudorandomized sequence so that each block of 54 trials consisted of an equal number of signal and nonsignal events. Signal trials consisted of illuminating the central and left panel lights for 1 second. The lights were not illuminated for nonsignal trials. Two seconds after the presentation of the signal or the nonsignal event, both levers were extended into the box and remained for

4 seconds or until a lever press occurred. Animals were reinforced for responding to the presence of the light stimuli by depressing the left lever (hit) and the absence of the lights by pressing the right lever (correct rejection). Incorrect lever presses were defined as misses when they occurred on a signal trial and false alarms when they occurred on a nonsignal trial. If the animal failed to respond or responded incorrectly, the levers were retracted and the ITI was reinstated. After an incorrect response, the trial was repeated up to three times (correction trials). If the animal failed to respond correctly after three correction trials, a forced-choice trial was initiated. In forced-choice trials, the event (signal or nonsignal) was repeated but only the correct lever was extended and remained active for 90 seconds. On forced-choice signal trials the lights remained illuminated for 90 seconds. These trials facilitated discriminative conditioning and blocked the development of a side bias. After the animals responded correctly to >70% of both the signal and nonsignal events for at least 2 consecutive testing days, they entered a second shaping task.

In this task, only the central panel light was illuminated for 1 second. All other aspects of the task were the same as the previous shaping task. After the animals responded correctly to >75% of both the signal and nonsignal events for at least 2 consecutive testing days in this phase of shaping, they entered the final task.

Sustained Attention Task: Baseline Task

A schematic of the final version of the task is provided in Figure 4.1. In the final version of the task, the length of the signal duration was changed from 1 s to 25, 100, or 500 ms. Sessions consisted of 27 trials of each of the three signal lengths and 81 trials of the nonsignal trials, yielding a total of 162 trials per session. Because it was planned to analyze performance changes across three blocks of 54 trials each, the sequence of signal and nonsignal trials was pseudorandomized so that one block consisted of 27 signal and 27 nonsignal trials, with each signal length being presented nine times. In addition, both correction and forced choice trials were discontinued. Animals were trained to a criterion of >75% hits to 500 ms signals and >75% correct rejections to nonsignal trials for at least two consecutive sessions, at which point they were considered ready for manipulations. The presentation of all manipulations was counterbalanced across groups.

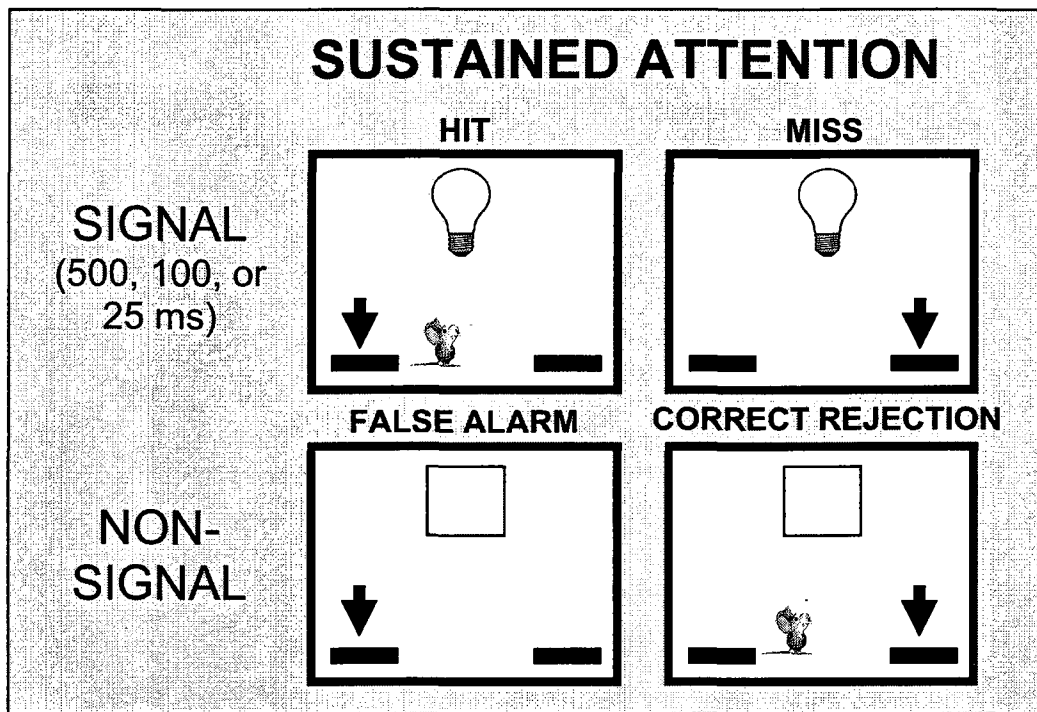


Figure 4.1. Schematic representation of the sustained attention task. Rats must discriminate brief, temporally unpredictable lights of varying durations (500, 100 and 25 msec) from non-signals. Rats respond by the extension of levers into the box. Rats are reinforced for responding on the right lever after a non-signal trial and the left after a signal trial. There are 162 total trials which are divided into 3 blocks of 54 trials

Houselight Distractor

Previous research has indicated as a distractor increases in similarity to the target stimulus it becomes increasingly difficult to disregard (Folk et al. 2008; Friedman-Hill et al. 2003; Newman et al. 2008b). In this task a a houselight distractor was added that flashed at a rate that corresponded with the target stimulus durations throughout the entire session (500, 100, 25 ms on/off pseudorandomly presented). Previous research in our lab has suggested

animals with ibotenic acid lesions of the posterior parietal cortex are more susceptible to the presence of this distractor than sham-lesioned animals (Newman et al. 2008b).

Tone Distractor

In order to assess the effect of a crossmodal distractor that is presented at equivalent rates to the target stimulus durations (500, 100, 25 ms on/off) was introduced. The addition of this variable tone increases task irrelevant information. Increased activity in the ACC has been seen in response to task irrelevant, salient stimuli (Laurens et al. 2005; Mohanty et al. 2007; Szucs and Soltesz 2008) and to crossmodal stimuli (Vohn et al. 2007). However, these findings are not consistent (Banich et al. 2000a). The present experiment will clearly identify whether ACC is important for ignoring task irrelevant information and if cross-modal presentation of stimuli represents a unique cognitive demand that requires an intact ACC.

Statistical Analyses

From each test session, the number of hits, misses, correct rejections, false alarms, and errors of omissions were recorded. Response latencies from the extension of the levers to when a lever was pressed were also recorded. The relative number of hits ($\text{hits}/(\text{hits} + \text{misses})$) was calculated for each signal length.

Furthermore, the relative number of correct rejections (correct rejections/(correct rejections + false alarms)) was computed. All statistical analyses were done on SPSS 17.0 for Windows.

Baseline sessions. All dependent measures were analyzed using separate mixed-factor ANOVAs. The degrees of freedom in all analyses were corrected using the Huynh–Feldt correction in the case of a violation of sphericity. Epsilon (ϵ) values not equal to 1 are reported below. The mean performance on the baseline days before each attentional variation day were compared with each other to determine whether there was any difference in the performance of ACC-Lx and Sham-Lx rats in the standard task over time. For the analysis of the effects of time on task (vigilance decrement), test sessions were divided in three blocks of 54 trials (see above). The effects of signal length and block over the days of baseline on hit accuracy were analyzed using a mixed factors ANOVA with one between subjects factor (Lesion, 2 levels) and three within subject factors (Day, 2 levels; Block, 3 levels; Signal, 3 levels). Mean latencies on hits were also analyzed using a separate mixed factors ANOVA with one between subjects factor (Lesion, 2 levels) and two within subjects factors (Day, 2 levels, Block, 3 levels, Signal, 3 levels). The effects of block on correct rejection accuracy were analyzed using a mixed-factors ANOVA with one between subjects factor (Lesion, 2 levels) and two within-subject factors (Day, 2 levels; Block, 3 levels). A separate mixed factor ANOVA was used to analyze correct rejection latency using the same designs described for the correct rejection accuracy analysis.

Distractor sessions. Both distractor sessions were compared to the baseline performance of the session immediately prior to the test day so that each ANOVA has a Session within subjects factor (2 levels), allowing a comparison of the mean baseline performance to the test session. The effects of varied attentional demand were assessed in independent, mixed-factors ANOVAs with one between-subjects factor (Lesion, 2 levels) and three within subject factors (Session, 2 levels; Block, 3 levels; Signal, 3 levels). The effects on correct rejection accuracy were analyzed using a mixed factors ANOVA with one between subjects factor (Lesion, 2 levels) and two within subject factors (Session, 2 levels; Block, 3 levels).

Results

Histology

See experiment 2 for a detailed description of the histological results.

Baseline Accuracy

ACC-Lx animals showed a significant vigilance decrement as evidenced by a decrease in hits over blocks of trials on the baseline task as compared to

Sham-Lx animals (Block X Lesion: $F_{2,26}=7.44$, $p=0.003$, $\epsilon=0.966$). Further analyses revealed ACC-Lx rats were significantly less accurate at signal detection in the second and third blocks of trials as compared to the first block of trials while Sham-Lx animals did not show this decrement (Figure 4.2; ACC-Lx: Block 1 vs. Block 2: $t_6=3.27$, $p=0.014$ and Block 1 vs. Block 3: $t_6=2.82$, $p=0.026$; Sham-Lx: Block 1 vs. Block 2: $t_7=0.22$, $p=0.83$ and Block 1 vs. Block 3: $t_7=0.29$, $p=0.78$, corrected $\alpha=0.017$). All animals showed signal-length dependent performance with accuracy being greatest at the 500 ms signal length and worst at the 25 ms signal length ($F_{2,26}=168.76$, $p<0.001$, $\epsilon=0.931$). This effect remains consistent throughout all variants of attentional demand. There were no other main effects or interactions with lesion in the analyses of hits ($p>0.1$). Lesions of the ACC did not impair accuracy on non-signal trials nor did the effects of lesion vary with increased time on task ($p>0.09$).

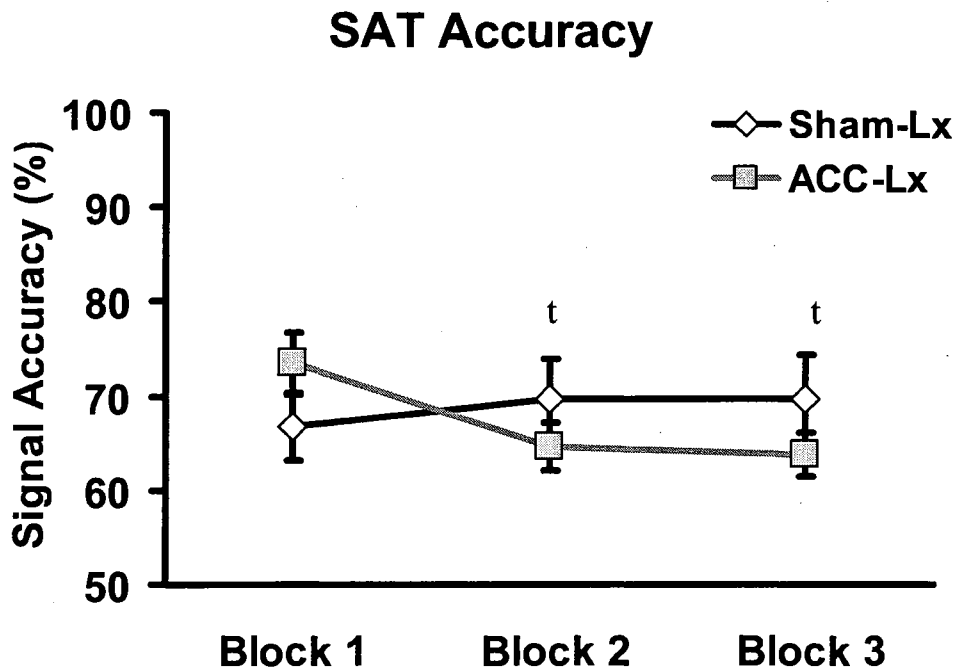
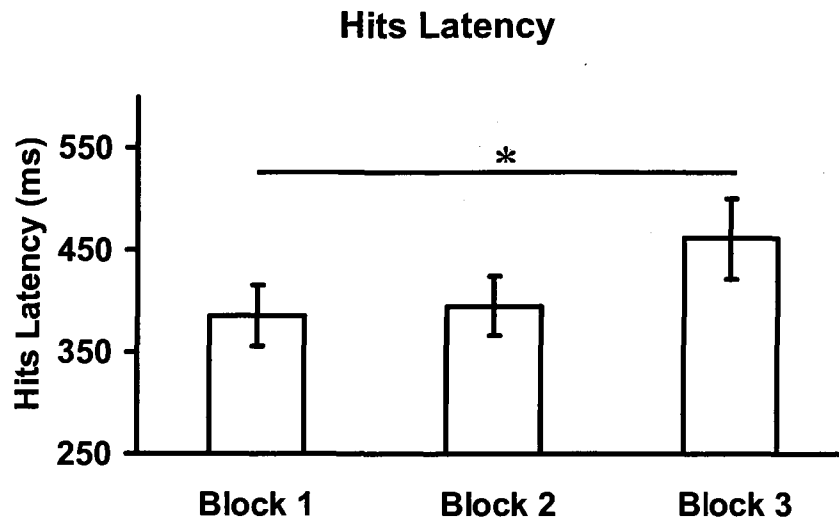


Figure 4.2. ACC-Lx animals were significantly impaired on the second and third blocks of trials when detecting signals as compared to Sham-Lx animals. This suggests a vigilance decrement due to ACC lesions.

Baseline Response Latencies

There was an effect of Block on hits and correct rejection latencies where all animals showed an increase in average latency in the third block of trials relative to response latencies in the first block of trials (Figure 4.3A, Hits: $F_{2,26} = 4.88$, $p = 0.015$; Figure 4.3B: Correct Rejection: $F_{2,26} = 18.25$, $p < 0.001$). ACC-Lx animals were also significantly slower to respond on correct rejections than Sham-Lx animals ($F_{1,13} = 5.20$, $p = 0.039$). There were no other main effects or interactions with lesion ($p > 0.2$).

A



B

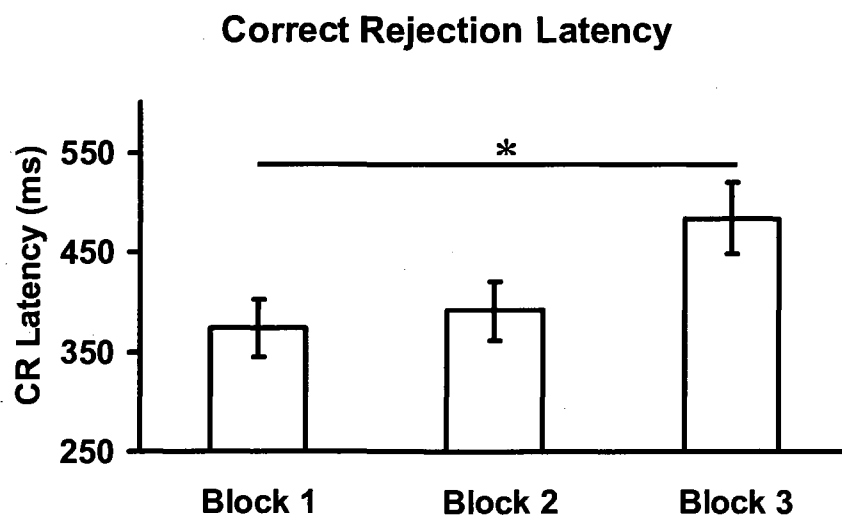
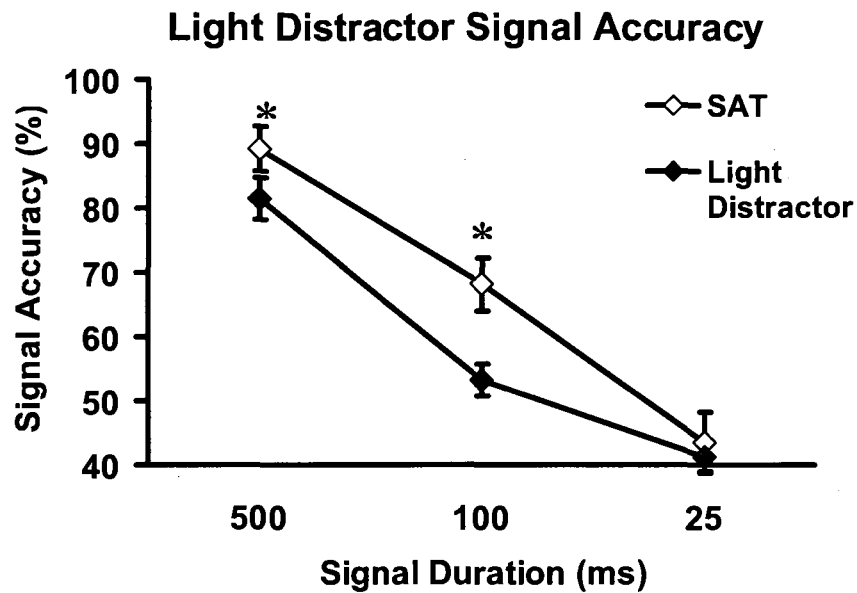


Figure 4.3. **A.** All animals had slower hit response latencies over time on task where the latencies in block 3 were significantly longer than those in block 1 ($t_{14}=2.70$, $p=0.016$). **B.** All animals had slower correct rejection response latencies over time on task where the latencies in block 3 were significantly longer than those in block 1 ($t_{15}=4.85$, $p<0.001$) and block 2 ($t_{15}=4.60$, $p<0.001$).

Houselight Distractor Accuracy

ACC-Lx rats were not more susceptible than Sham-Lx rats to the presence of a flashing houselight ($p>0.08$). All rats were impaired at signal detection in the presence of this distractor ($F_{1,13} = 13.59$, $p=0.002$). Detection of the 500 and 100 ms stimuli but not the 25 ms stimulus were impaired by the presence of the visual distractor (Figure 4.4A; Distractor X Signal Duration: $F_{2,26} = 6.32$, $p=0.006$; 500 ms: $t_{15}=3.50$, $p=0.003$; Baseline: $89.23\% \pm 1.12\%$, Distractor: $81.42\% \pm 2.19\%$; 100 ms: $t_{15}=3.75$, $p=0.002$; Baseline: $68.14\% \pm 2.52\%$, Distractor: $53.20\% \pm 5.26\%$; 25 ms: $t_{15}=0.88$, $p=0.39$, Baseline: $43.53\% \pm 3.35\%$, Distractor: $41.31\% \pm 3.16\%$, corrected $\alpha=0.017$). The houselight distractor led to an increase false alarm responding thereby decreasing non-signal accuracy particularly in the first block of trials (Figure 4.4B; Distractor: $F_{1,13} = 11.33$, $p=0.005$; Distractor X Block: $F_{2,26} = 5.87$, $p=0.011$; Block 1: $t_{15}=3.37$, $p=0.004$; Baseline: $85.95\% \pm 1.28\%$, Distractor: $71.33\% \pm 3.77\%$; Block 2: $t_{15}=0.495$, $p=0.628$; Baseline: $88.40\% \pm 0.89\%$, Distractor: $87.22\% \pm 2.34\%$; Block 3: $t_{15}=2.59$, $p=0.021$, Baseline: $85.72\% \pm 1.09\%$, Distractor: $80.09\% \pm 2.48\%$, corrected $\alpha=0.017$). The effects of the distractor on non-signal accuracy did not differ between the ACC-Lx and Sham-Lx rat nor did this effect change over the course of the testing session ($p>0.3$).

A



B

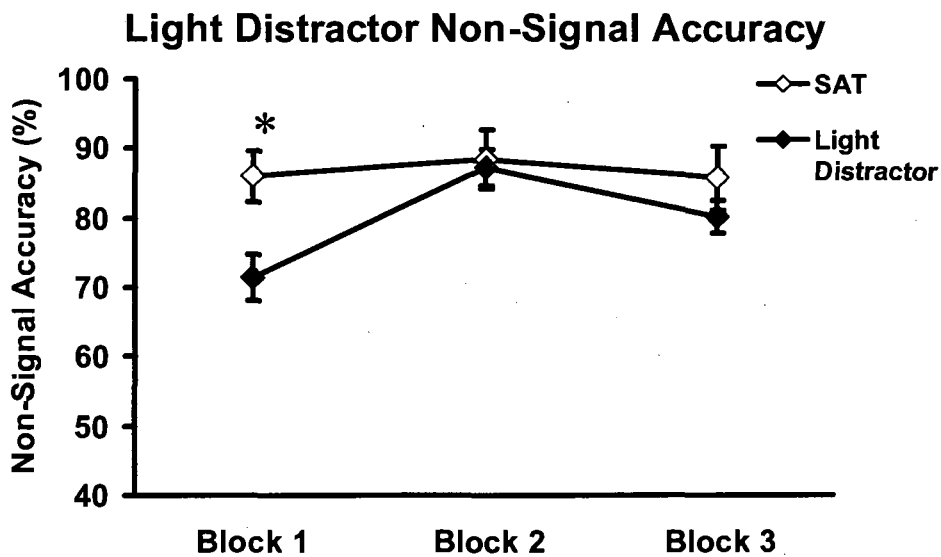


Figure 4.4. **A.** All animals were significantly impaired on the 500 and 100 ms signal durations in the presence of a houselight distractor. **B.** All animals were significantly impaired on non-signal trials in the first block of trials.

Houselight Distractor Response Latencies

ACC-Lx animals remained significantly slower to respond to non-signals than Sham-Lx animals in the presence of the houselight distractor ($F_{1,13} = 6.70$, $p=0.021$). There were no other significant main effects or interactions with lesion ($p>0.2$).

Tone Distractor Accuracy

Sham-Lx rats, but not ACC-Lx rats, showed an improved ability to detect signals in the presence of the tone distractor (Figure 4.5; Session X Lesion signal accuracy: $F_{2,26} = 4.36$, $p=0.055$; Sham-Lx: $t_7 = 2.68$, $p=0.032$; Baseline: $63.94\% \pm 3.83\%$, Distractor: $72.99\% \pm 5.22\%$; ACC-Lx: $t_6 = 0.42$, $p=0.69$; Baseline: $67.66\% \pm 2.93\%$, Distractor: $66.05\% \pm 4.14\%$). There were no other interactions or main effects of lesion on hits or correct rejection accuracy ($p>0.1$).

Tone Distractor Hits Accuracy

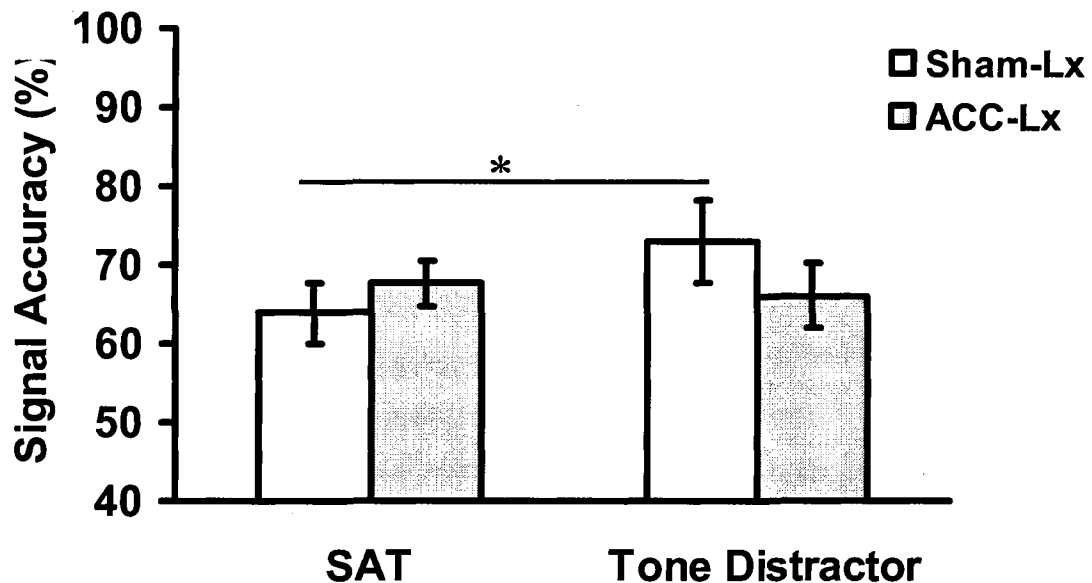


Figure 4.5. Sham-Lx animals significantly improved signal detection in the presence of the tone distractor while ACC-Lx animals did not.

Tone Distractor Response Latencies

All animals to have faster hit latencies in the presence of the tone distractor as compared to baseline performance (Figure 4.6; $F_{2,26} = 6.45$, $p=0.024$). Similar to their baseline performance, ACC-Lx animals continued to be slower to respond in the presence of a tone distractor than Sham-Lx animals on correct rejections (Lesion: $F_{1,13} = 6.56$, $p=0.023$). All other main effects and interactions with lesions were not significant ($p>0.1$).

Tone Distractor Hits Latency

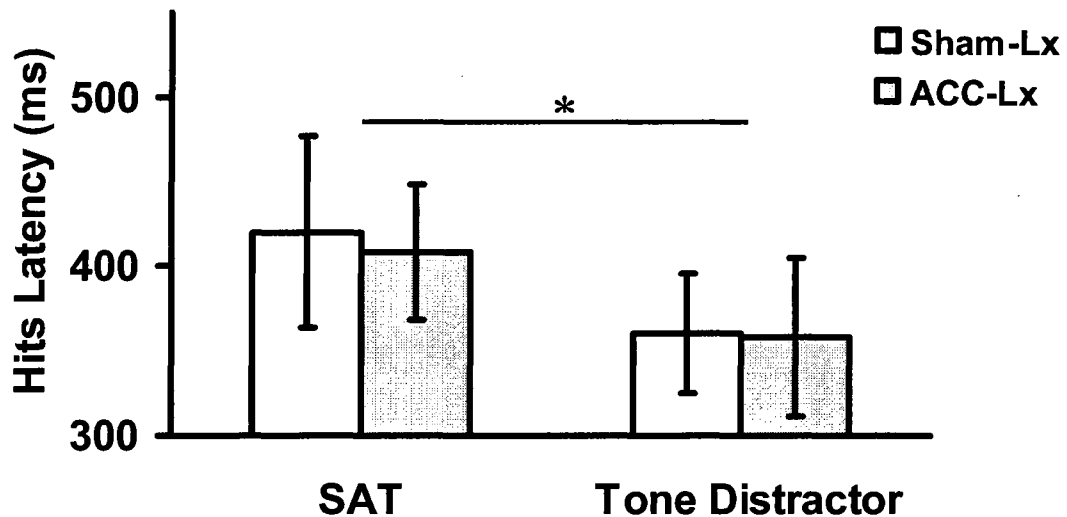


Figure 4.6. All animals were significantly faster to make hits in the presence of the tone distractor.

Discussion

Evidence from the current study that suggests ACC-Lx animals are more susceptible to cognitive fatigue than Sham-Lx rats on an attentionally demanding task. These results are also in line with the findings from experiment 1. ACC-Lx animals show impaired signal detection in the second and third blocks of testing on the SAT which was not seen in Sham-Lx animals suggesting the ACC is involved in maintaining vigilance.

In the presence of a cross-modal distractor, Sham-Lx animals significantly improved accuracy on signal trials as compared to baseline performance. This may be due to the placement of the tone generator. As the tone generator is

located on the intelligence panel below the central panel light which is where the signal will appear, the presentation of the tone may help to orient the animal to the central panel light, thereby improving their ability to respond correctly. This facilitation may be limited to signal performance because many of the animals obtain a ceiling effect on non-signal trials whereas on signal trials there is more room for improvement due to the effects of the dynamic stimulus range. A lack of an impairment in non-signal detection suggests there is not an overall response shift to prefer the non-signal lever. Unlike Sham-Lx animals, ACC-Lx animals do not show a significant change in accuracy in the presence of a tone distractor either on hits or correct rejections. This suggests ACC-Lx animals are not impaired by the presence of a cross-modal distractor but do not benefit from its presentation either.

In contrast, the visual distractor did not dissociate accuracy performance of ACC-Lx and Sham-lx rats as both groups show deficits in signal detection and correct rejections in the presence of the flashing houselight. These results suggest ACC lesions do not impair sustained attention in the presence of irrelevant distractors. In addition, the results do not support a specific role for the ACC in disregarding cross-modal distractors. ACC-Lx may lead to an inability to integrate potentially relevant information that is not necessary for the completion of a task. These results support the findings in experiment 1 suggesting distractors that are not associated with reinforcement do not impair or facilitate performance of ACC-Lx animals.

CHAPTER 5

GENERAL DISCUSSION

The current studies support the role of the anterior cingulate cortex in executive control. In particular, the ACC is involved in adjusting cognitive control in order to prepare for increases in attentional demand such as the presentation of conflicting stimuli or distracting stimuli that are potentially relevant either because the particular stimulus has been associated with reinforcement or in the case of the attentional set shifting task, a stimulus within that dimension, has been associated with reinforcement. When distracting stimuli are presented that have no previous reinforcement or response associations, such as in the distracting light within the conflict monitoring session in the first experiment and the light and tone distractors in the third experiment, ACC-Lx animals show no impairments in comparison to Sham-Lx animals and both groups are better able to disregard these stimuli as compared to the conflicting stimuli. In the conflict monitoring paradigm the non-target modality has been strongly associated with a particular response and in the compound discrimination in Experiment 2 the newly added dimensions have previously had reinforcement associated with them in the exemplar training. Thus in both of these cases the animals have

learned that these stimuli may be relevant to reinforcement and in both experiments ACC-Lx animals showed deficits.

Further evidence of the ACC's involvement in disregarding previously relevant stimuli can be found in the deficits on reversals in Experiment 2. Because there is no difference in perseverative responding between the ACC-Lx and the Sham-Lx group, this suggests the ACC-Lx animals recognize the previously reinforced pot is no longer reinforced just as quickly as Sham-Lx animals. However, the increase in regressive errors suggest the ACC-Lx animals have difficulty discriminating which response they should make once they have begun to adjust their response pattern. A lack of impairment on serial reversals suggests these previously reinforced dimensions may be distracting the ACC-Lx animals in the process of learning the affective shift.

Loss of the ACC leads to an increase in cognitive fatigue and increased vigilance decrements. This suggests the ACC may play a role in maintaining cognitive capacity, potentially by promoting the efficient use of cognitive resources. The variations in ACC activity in response to changes in cognitive demand such as those seen in the conflict monitoring paradigm (Botvinick et al. 2001; Carter et al. 2000; van Veen et al. 2001) may promote variations in cognitive processes to most efficiently process the current information and respond accordingly. The loss of this function creates inefficient processing of stimuli leading to longer latencies on incongruent trials as well as increased cognitive fatigue.

The results of these studies also validate the novel conflict monitoring paradigm used in experiment 1 on multiple dependent measures. In addition, lesions to the ACC in rats have similar effects to those seen in clinical populations with dysregulation of the ACC (Bedard et al. 2002; King et al. 2007; Wang et al. 2005). This paradigm can help to further elucidate what systems are involved in conflict monitoring. A better understanding of the underlying neural networks will help to provide a better understanding of neuropsychological disorders that lead to impairments in conflict monitoring. This can also help in testing out new treatments for these neuropsychological disorders. Furthermore, because this paradigm is available for use in the rat model, we can modify the task to increase or decrease cognitive demands (for example, the houselight distractor session) in order to better understand what aspects of cognition are necessary to perform conflict monitoring.

The current data suggests the ACC is uniquely involved in disregarding information that potentially has relevance due to previous associations with reinforcement. The results of the current studies suggest the role of the ACC is dissociable from the prelimbic cortex which is involved in disregarding salient, irrelevant stimuli (Newman and McGaughy 2008). The role of the ACC also differs from the orbitofrontal cortex which promotes shifting away from a previously reinforced stimulus when it is no longer reinforced (Clarke et al 2007, McAlonan and Brown 2003) while the ACC is involved in promoting responding to a newly reinforced dimension in the presence of multiple stimuli that were

previously reinforced. In addition, the ACC promotes the efficient cognitive control in order to prevent cognitive fatigue.

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APPENDIX

University of New Hampshire

Research Conduct and Compliance Services, Office of Sponsored Research
Service Building, 51 College Road, Durham, NH 03824-3585
Fax: 603-862-3564

25-Jun-2008

McGaughy, Jill A
Psychology, Conant Hall
10 Library Way
Durham, NH 03824

IACUC #: 080403

Project: The role of anterior cingulate acetylcholine in attention

Category: D

Approval Date: 25-Apr-2008

The Institutional Animal Care and Use Committee (IACUC) reviewed and approved the protocol submitted for this study under Category D on Page 5 of the Application for Review of Vertebrate Animal Use in Research or Instruction - *the research involves chronic maintenance of animals with a disease/functional deficit and/or procedures potentially inducing moderate pain, discomfort or distress which will be treated with appropriate anesthetics/analgesics.*

Approval is granted for a period of three years from the approval date above. Continued approval throughout the three year period is contingent upon completion of annual reports on the use of animals. At the end of the three year approval period you may submit a new application and request for extension to continue this project. Requests for extension must be filed prior to the expiration of the original approval.

Please Note:

1. All cage, pen, or other animal identification records must include your IACUC # listed above.
2. Use of animals in research and instruction is approved contingent upon participation in the UNH Occupational Health Program for persons handling animals. Participation is mandatory for all principal investigators and their affiliated personnel, employees of the University and students alike. A Medical History Questionnaire accompanies this approval; please copy and distribute to all listed project staff who have not completed this form already. Completed questionnaires should be sent to Dr. Gladi Porsche, UNH Health Services.

If you have any questions, please contact either Dean Elder at 862-4629 or Julie Simpson at 862-2003.

For the IACUC,



Jessica A. Bolker, Ph.D.
Chair

cc: File