THE ROLE OF THE ANTERIOR CINGULATE CORTEX IN EXTINCTION LEARNING OF AVOIDANCE BEHAVIOR AND IN THE RETRIEVAL OF ITS EXTINCTION MEMORY

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To my loving and patient son,
Trevor Curtis Pasley

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Magdeburg, den 24 Juni 2011

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Titel: Die Rolle des anterioren cingulären Cortex (ACC) bei der Extinktion des Vermeidungslernens und beim Abruf des Extinktionsgedächtnisses

Zusammenfassung

Extinktionslernen führt zu einer Änderung des Verhaltens, d.h. zur Unterdrückung einer erlernten Reaktion, wenn ein vorher bedeutendes Signal nicht mehr relevant ist bzw. eine andere Bedeutung erlangt. Verschiedene Hirnregionen sind bei der Extinktion von Verhalten beteiligt, insbesondere der anteriore cinguläre Cortex (ACC). Während die Rolle des ACC bei der Extinktion von appetitiver Konditionierung anhand von Inaktivierungsexperimenten nachgewiesen wurde, ist noch wenig über seine Rolle bei der Extinktion von aversiver Konditionierung bekannt. In der vorliegenden Arbeit wird die Rolle des ACC bei der Extinktion des Vermeidungsverhaltens untersucht. Im ersten Experiment wurden Rennmäuse trainiert, einen Fußschock in einer Shuttle-Box durch Sprung über eine Hürde zu vermeiden, wenn ein Ton als bedingter Reiz (CS) gegeben wurde. Nach acht Konditionierungssitzungen und weiteren drei Tagen der Ruhe wurde eine ACC-Läsion bzw. ACC-Scheinläsion durchgeführt. Nach einer Erholungspause von einer Woche erfolgte eine weitere Konditionierungssitzung, um die Gedächtnisfunktion nach der Läsion zu überprüfen. Danach erfolgten acht Extinktionssitzungen, in denen nur der Ton-CS, nicht aber der Fußschock gegeben wurde. Sieben Tage nach der letzten Extinktionssitzung wurde die spontane Erholung (Recovery) der früher gelernten bedingten Reaktion getestet. Eine Woche später wurde den Versuchstieren nur der Fußschock allein gegeben, um am folgenden Tag das Wiederauftreten (Reinstatement) der Reaktion zu prüfen. Es zeigte sich, dass die ACC-Läsion, die nicht zu perseverativem Verhalten führte, eine Abnahme der Anzahl

der erlernten Reaktionen während der Extinktionsphase und in der nachfolgenden Prüfung der Gedächtnisabrufung bewirkte.

Im zweiten Experiment wurden Rennmäuse trainiert, in der Shuttle-Box zwei Töne mit unterschiedlicher Frequenz zu unterscheiden, d.h. sie mussten, um den Fußschock zu vermeiden, bei einem Ton über die Hürde springen, bei dem anderen Ton aber nicht springen (Go/No-Go Diskriminierung). Die Hälfte der Tiere wurde darauf trainiert, bei dem hohen Ton zu springen und bei dem tiefen Ton sitzen zu bleiben, für die andere Hälfte der Tiere erfolgte die umgekehrte Zuordnung der Verhaltensbedeutungen zu den Tönen. Ähnlich wie in Experiment 1 wurden zunächst acht Konditionierungssitzungen durchgeführt. Nach drei Tagen Ruhe erfolgte die ACC-Läsion bzw. –Scheinläsion und, ebenso wie in Experiment 1, erfolgte nach einer Erholungspause von einer Woche eine weitere Konditionierungssitzung, um die Gedächtnisfunktion nach der Läsion zu überprüfen. Danach erfolgten acht Extinktionssitzungen, in denen nur die Tonreize, nicht aber der Fußschock gegeben wurde. Sieben Tage nach der letzten Extinktionssitzung wurde die spontane Erholung (Recovery) der früher gelernten bedingten Reaktion geprüft und am nächsten Tag wurde die Erneuerung (Renewal) der Reaktion getestet. Im Renewal-Test wurde die Shuttle-Box mit Papier bedeckt und um 90 Grad gedreht um einen anderen, d.h. von der Extinktionsphase verschiedenen, Kontext zu schaffen. Am folgenden Tag wurde der Fußschock allein im Extinktionskontext gegeben und einen Tag später wurde das Wiederauftreten der Reaktion (Reinstatement) geprüft. Die Ergebnisse zeigen, dass die ACC-Läsion nicht zu perseverativem Verhalten während der Extinktion des diskriminativen Vermeidungslernens führte, dass aber die Extinktionsleistung und die Abrufbarkeit im Renewal- und im Reinstatement-Test in Abhängigkeit von der Tonhöhe des Go-Reizes beeinflusst wurde: Tiere mit ACC-

Läsion, die trainiert wurden, auf den tiefen Ton zu springen (und auf den hohen Ton nicht zu springen), zeigten eine höhere Diskriminationsrate als die Tiere mit umgekehrter Zuordnung der Töne bzw. als die Tiere mit Scheinläsion. Zusammengefasst sprechen die Ergebnisse für die Rolle des ACC bei der differenziellen Modulation der motivationalen Bedeutung des bedingten Reizes. Der konsistente Läsionseffekt im Test auf die spontane Recovery sowohl bei der einfachen als auch bei der diskriminativen Vermeidungsreaktion spricht für eine wesentliche Rolle des ACC bei der zeitlichen Verarbeitung. In zukünftigen Studien könnte geklärt werden, worin genau diese Rolle bei der zeitlichen Verarbeitung besteht: ist es die Verarbeitung des Intervalls zwischen den Trainingsereignissen, die Perzeption des Ablaufs der Zeit, oder aber die unterschiedliche Ausprägung der initialen und der späteren Bedeutung das bedingten Reizes wenn der zeitliche Kontext sich ändert. Weitere Studien sollten sich mit der Rolle des ACC bei autonomen Reaktionen während des Lernens (z.B. Aufzeichnung der Herzfrequenz) und mit den unterschiedlichen Aspekten der Bedeutung des bedingten Reizes in verschiedenen Lernsituationen beschäftigen.

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Abbreviations

ACC	anterior cingulate cortex
ANOVA	analysis of variance
AP	anteroposterior
Cg1	cingulate area 1
Cg2	cingulate area 2
CJM	conditioned jaw movement
CR	conditioned response
CS	conditioned stimulus
CVD	conditional visual discrimination
dB	decibel
DV	dorsoventral
e.g.	exempli gratia
et al.	et alia
ECG	electrocardiograph
fMRI	functional magnetic resonance imaging
g	gram
GABA	gamma aminobutyric acid
GSR	galvanic skin response
HR	heart rate
HRV	heart rate variability
i.e.	id est
IL	infralimbic cortex
kHz	kilohertz
M2	motor area 2

mg	milligram
ml	milliliter
mm	millimeter
ML	mediolateral
mPFC	medial prefrontal cortex
NMDA	N-Methyl-D-aspartate
OFC	orbitofrontal cortex
PCC	posterior cingulate cortex
PIT	Pavlovian to Instrumental Transfer
Rei	reinstatement
Ren	renewal
SPSS	Statistical Program for the Social Sciences
S-R	stimulus-response
SR	spontaneous recovery
TIA	training-induced activity
µA	microampere
µl	microliter
UR	unconditioned response
US	unconditioned stimulus

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Preface

Behavioral extinction is an important learning process that allows an organism to adapt its behavior according to the relevance of present cues that would call for inhibition of prepotent responses. Insights into the different mechanisms of this behavioral process are of great clinical relevance given the prevalence of behavioral perseveration apparent in pathological fear and anxiety as well as drug abuse. Present work explores the neural mechanism involved in the extinction of avoidance response to an auditory cue that had been associated with footshock. Specifically, the role of the anterior cingulate cortex (ACC) in extinction learning of avoidance behaviour in a shuttlebox is investigated using an ibotenic acid lesioning technique in a gerbil model. Present written report of the work is comprised of four chapters that include a general introduction, the summaries of the two experiments and finally the general discussion of present findings and what they collectively suggest the role of the ACC may be.

The first chapter of the current written work describes the behavioral definition of extinction along with the neural substrates that have been so far identified to be involved. While the generally used behavioral paradigm in studying extinction has been fear conditioning, avoidance behaviour is instead used here for reasons that are discussed in this chapter. Anatomical and functional definitions of the ACC are accounted as well.

The second and third chapters start with a brief introduction followed by a summary of the methods, results and conclusions of the extinction of an active avoidance response and the extinction of discriminative avoidance behaviour, respectively. The

final chapter presents a general discussion of current findings and how they relate to results of other animal as well as human studies of the functional significance of the ACC.

Chapter 1
GENERAL INTRODUCTION

1.1 Extinction

Much of optimal behavior calls for continuous monitoring and updating information considering the dynamics of our environment where the relevance of cues could change in any given time and place, even moment to moment. In retroactive inhibitory learning such as extinction for instance, a cue that once predicts danger thus calling for avoidance behavior, subsequently signals safety hence leading to response inhibition. Response inhibition has been pointed out to be a key determinant of successful cognitive and motor control (Chambers et al., 2009). Prepotent responses acquired from previously learned associations may lead to maladaptive behavior when there is a failure to suppress actions no longer appropriate or relevant. While cues act as excellent signposts to the next course of action to take, they may consequently gain incentive salience and drive behavior as if they in themselves have biological significance. Inhibitory processes serve to maintain behavioral flexibility so that a dysfunction could translate to behavioral rigidity as those seen in anxiety disorders and even in substance use disorder. As a model of inhibitory learning, behavioral extinction bears clinical relevance for the intervention of psychiatric disorders.

1.1.1. Definition of Extinction

Extinction is a behavioral phenomenon in which the weakening of the expression of a conditioned response (CR) to a conditioned stimulus (CS) becomes apparent in the absence of the presentation of the unconditioned stimulus (US). In a typical Pavlovian fear conditioning, an organism is exposed to an initially neutral stimulus

(becoming the conditioned stimulus; CS), e.g. a tone, that is followed by an aversive stimulus (the unconditioned stimulus; US) which is usually a footshock. Subsequently, the pairing of the CS with the US leads to a CS-US association so that presentation of the CS elicits fear responses such as freezing or fear-potentiated startle. Typically, even after as few as one conditioning session, the CS can elicit a fear state of autonomic and behavioral responses that include changes in heart rate as well as skin conductance, and freezing responses. However, repeated presentation of the cue without the previously paired aversive stimulus will lead to extinction of fear or a reduction in fear responses. The decline in the behavioral response reflects an inhibition of the initially learned association between the CS and US by the new mental representation of a subsequent meaning of the CS, i.e., the CS- no US association. Described as an example of retroactive inhibition phenomenon in which new learning inhibits old (Bouton, 2004), extinction has once been viewed as the erasure or the forgetting of the original CS-US association (McClelland and Rumelhart, 1985; McCloskey and Cohen, 1989; Rescorla and Wagner, 1972). However, the return of the CRs with the passage of time in the classical dog experiment of Pavlov (1927) together with subsequent studies that manipulated the extinction context[1] (Bouton, 1993; Rescorla and Heth, 1975) indicate that extinction is instead a new form of learning that is inhibitory in nature, allowing the original conditioned memory to remain intact.

1.1.2. Extinction as New Learning

Similar to most types of learning, extinction occurs in three phases: acquisition, consolidation and retrieval. During acquisition, the CRs decline within an extinction

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[1] context – the surroundings and circumstances in which an event takes place (Dudai, 2004)

training session as the CS that used to be followed by the US is instead presented without the US. The CS-no US association starts to develop, which is then consolidated into long-term memory. Subsequent presentation of the CS in the absence of the US will trigger retrieval of extinction memory that becomes apparent in the low rate of CRs displayed. Further as a form of learning, extinction also shares similar molecular mechanisms as other types of learning such as the NMDA receptor that is involved in the initiation of synaptic strengthening (Baker and Azorlosa, 1996; Falls et al., 1992; Walker et al., 2002). The adrenergic system also seems to be involved in extinction learning by playing a positive modulatory role (Cain et al., 2004) just as it facilitates other forms of learning. On the flip side of the coin, there are also differences between extinction and other forms of learning. For instance, the early phase of fear extinction learning seems to depend on L-type voltage-gated calcium channel activity (Cain et al., 2002; Suzuki et al., 2004) and on GABA(A) receptors (Harris and Westbrook, 1998) which are not necessary in the acquisition of excitatory learning.

While it has been generally accepted that extinction is new inhibitory learning established in parallel with the original associative learning, some findings seem to imply that under certain circumstances, extinction may be deemed as erasure of the original learning or unlearning. It has been suggested that there may be differing mechanisms of extinction which may be determined by when extinction training is initiated (Barad, 2006). While L-type voltage-gated calcium channel antagonists have been found to block extinction when extinction started an hour or later after fear conditioning, the antagonists had no effect when extinction started shortly after conditioning (Cain et al., 2005). Myers and colleagues (2006) demonstrated that when extinction training commenced ten minutes after fear conditioning, there was no

evident return of the extinguished fear when animals were later tested hence implying deletion of the conditioning memory. However, conflicting human and rat studies have shown the return of fear responses of the subjects during tests for spontaneous recovery, renewal and reinstatement (later defined in subsection 1.1. 4.) despite immediate extinction training done after fear conditioning (Alvarez et al., 2007; LaBar and Phelps, 2005; Milad et al., 2005a; Schiller et al., 2008). Using aversive and appetitive conditioning paradigms, the effects of immediate versus delayed extinction training were further investigated by Woods and Bouton (2008) whose results parallel those of older studies demonstrating that immediate extinction training instead produced poorer retention of extinction memory (Maren and Chang, 2006; Rescorla, 2004b). In other words, while immediate extinction improves expression of the CS-no US memory, it does not necessarily improve its expression outside the extinction context. Better extinction learning does not translate into attenuated return of extinguished CRs. Considering that retrieval renders a memory labile until its reconsolidation, it is possible that extinction training done right after a conditioning session attenuates the expression of the primary associative memory by amending the existing memory representation with a more recent meaning of the CS. The updating then enables better expression of the secondary associative memory. Thus, the original associative memory is not necessarily erased but just transiently muted while in its labile state as evidenced by its subsequent expression in circumstances different from the extinction context.

1.1.3. Reconsolidation versus extinction

A fine line serves as a boundary between reconsolidation and extinction. Extinction of learned behavior requires retrieval of the conditioned memory. During retrieval, two competing processes: reconsolidation or extinction (Eisenberg et al., 2003; Lee et al.,

2006; Nader et al., 2000), may occur depending on the length of the memory reactivation triggered by exposure to reminder cues. Short retrieval sessions lead to reconsolidation of the original conditioned memory while extinction occurs when retrieval sessions are long (Myers and Davis, 2002; Pedreira and Maldonado, 2003; Suzuki et al., 2004). For example, after conditioning an animal is briefly exposed to a CS within a 2-minute session that then leads to reconsolidation compared to 10 CS presentations within a 20-minute session that results in extinction (Lee et al., 2006). Until reconsolidated, a retrieved memory enters and stays in a labile state that makes it susceptible to either enhancement or disruption (Nader et al., 2000; Tronson et al, 2006) as in the case when new information is introduced during this state. This connotes an adaptive significance for reconsolidation as it allows updating of memory representation (Alberini, 2005; Hupbach et al., 2007; Nader et al., 2000). Monfils and colleagues (2009) found that when extinction training was done within the lability window of 6 hours from the time retrieval occurred, freezing responses upon testing were significantly less than that of the control group as well as the groups given extinction training past the lability window. The authors explained that extinction done during the lability window updated the meaning of the CS with the new valence thereby weakening the initial valence associated with the conditioning session.

1.1.4. Context-dependence of Extinction

Extinction memories are widely accepted to be context-dependent (Bouton and Ricker, 1994; Bouton, 2002). The CS gains two meanings and the context therefore becomes the occasion-setter that determines which memory will be prevalently expressed. Noteworthy is the finding that after extinction training, retrieval of conditioning memories become context-dependent as well (Effting & Kindt, 2007; Harris et al., 2000). When a retention test for spontaneous recovery is done in the

extinction context that is different from the conditioning context, the CS-no US memory is activated (Herry and Garcia, 2002). Most studies have shown however that expression of the CS-no US association is more context-dependent than the original CS meaning. Context-specificity of extinction is not necessarily because of its nature of being inhibitory but has been suggested as perhaps due to being a secondary associative representation of the CS meaning (Bouton, 2004). Case in point, rats trained in feature-negative discrimination paradigm[1] showed transfer of inhibitory learning to a new context (Bouton and Nelson, 1994; Nelson and Bouton, 1997). In counterconditioning[2], Nelson (2002) found that regardless whether the conditioning was excitatory or inhibitory, the association easily transferred to different contexts. When the CS has been previously trained as an excitor or inhibitor, expression of the second opposing associative meaning becomes context-specific similar to that seen in extinction.

The same context-dependence evident in extinction has been observed in other retroactive interference paradigms such as discrimination reversal learning and latent inhibition (Bouton and Peck, 1992; Bouton and Swartzentruber, 1989; Kraemer et al., 1991; Peck and Bouton, 1990; Spear et al., 1980; Talk et al, 2005; Thomas et al., 1984). These examples of the interference phenomena illustrate that when a CS becomes ambiguous by gaining more than one meaning, the particulars of the

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[1] feature-negative discrimination paradigm - a conditioning procedure in which a conditional stimulus is presented with the unconditional stimulus on some trials and without the unconditional stimulus on other trials; a second conditional stimulus is added to signal when the unconditional stimulus will *not* occur (Bouton, 2007).

[2] counterconditioning - a conditioning procedure that reverses the organism's response to a stimulus; for example, by pairing the stimulus with a positive event, an organism may be conditioned to respond positively to a stimulus that would otherwise conditionally or unconditionally elicit fear (Bouton, 2007).

context determine which associative memory becomes expressed. When an animal learns the first association, it makes an inference that the initial association is the rule and that the second association is considered an exception to that rule (Bouton, 2004) taking into account under what circumstances the exception occurs. Retrieval of the secondary meaning of the CS therefore depends on the specifics of the context. Expression of the extinction memory is highly context-sensitive so that the return of extinguished responses would even occur when there is a change in the usual temporal interval between sessions.

Weak expression of extinction memory is reflected in the return of the extinguished CR evident when an animal is exposed to the CS under conditions dissimilar from the extinction training context. In fear conditioning studies, the three most cited phenomena that demonstrate the return of the extinguished CRs in a context that does not precisely match the extinction context are: *Spontaneous recovery*, *renewal* and *reinstatement*. Spontaneous recovery refers to a change in temporal context. Extinguished CRs reappear after a considerable passage of time between the last extinction session and the test session. The degree of the recovery of the CRs is determined by the length of the interval so that higher return becomes evident the longer the lapse is between sessions (Robbins, 1990). Renewal refers to a change in spatial context. When an animal is presented with the CS alone in a spatial context different from where extinction training took place, the extinguished CRs reappear (Bouton and King, 1983; Bouton and Brooks, 1993). Reinstatement of the extinguished CRs occurs when an animal is given an extinction test session after being exposed to the US alone. The context where the US was presented becomes associated or conditioned with the US so that when the CS is presented in the relevant (same) context, reinstatement of the extinguished CRs is generated.

Otherwise, no reinstatement can be observed if the CS is tested in a different context or if an animal is given an extinction exposure to the relevant context after the reinstating US presentations have been given (Baker et al., 1991; Bouton and Bolles, 1979).

1.1.5. Basic Extinction Circuit

Fear conditioning has been the commonly used behavioral paradigm for studying the neural mechanisms of emotional learning and behavioral extinction (e.g., LaBar and Phelps, 2005; La Bar et al., 1998; Norrholm et al., 2006; Schiller et al., 2008) since functional and neural mechanisms of conditioned fear are similar to that of anxiety disorders (Rosen and Schulkin, 1998). Based on a number of studies, Quirk and

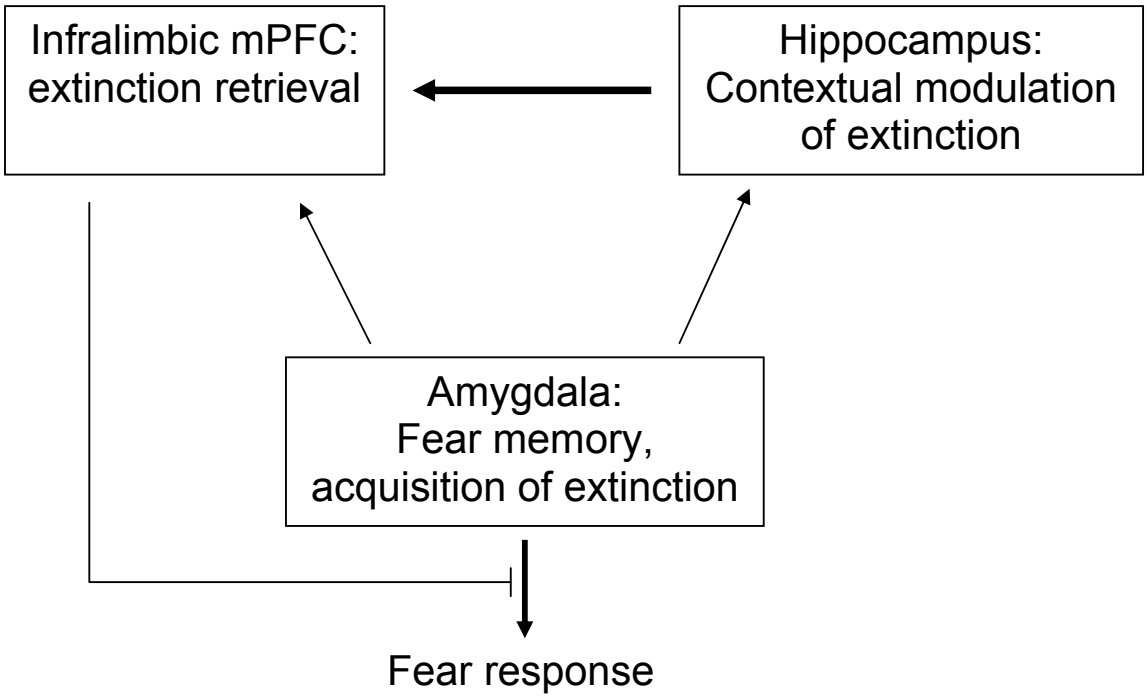

Figure 1. *Basic fear extinction circuit (and figure caption) based on a schematic diagram by Quirk and Mueller (2008).* Conditioned fear and extinction memories are stored in the amygdala. CS presented within the extinction context is integrated with contextual information from the hippocampus, leading to IL inhibition of amygdala output that reduces

expression of fear response. Otherwise, amygdala output is uninhibited when the CS is presented outside of extinction context.

Mueller (2008) proposed a basic fear extinction circuit that includes the amygdala, hippocampus and the infralimbic cortex (IL; Fig.1). In the basolateral amygdala, administration of NMDA receptor antagonists and kinase inhibitors blocked extinction of fear memory (Falls et al., 1992; Lin et al., 2003; Lu et al., 2001) while its consolidation was modulated by the noradrenergic system (Berlau and McGaugh, 2006) suggesting involvement of the amygdala in the acquisition and consolidation of extinction memory. Muscimol inactivation of the dorsal hippocampus before extinction training led to poor retrieval of extinction memory the following day (Corcoran et al., 2005) and the MAPk cascade (Fischer et al., 2007) as well as actin rearrangement (Fischer et al., 2004) in the hippocampus were found to be essential in the extinction of contextual fear. Hippocampal inactivation before a renewal test disrupted extinction retrieval (Corcoran and Maren, 2001 and 2004; Hobin et al., 2006) although some studies found no renewal effect (Frohardt et al., 2000; Wilson et al., 1995) suggesting that the hippocampus is essential for only some types of contextual processing of extinction memory. Projections from IL to amygdala (McDonald et al., 1996; Chiba et al., 2001; Ghashghaei and Barbas, 2002) have been suggested to mediate its inhibition of amygdala output that determines expression of fear responses. Stimulation of the IL just before CS presentation reduced freezing responses to CS as if simulating extinction learning (Milad and Quirk, 2002). Retrieval of extinction memory is decided when the IL cortex integrates CS information with the contextual information from the hippocampus within the extinction context, leading to inhibition of amygdala output thus, the behavioral suppression (Quirk and Mueller, 2008).

Other neural substrates found to be involved in extinction include but not limited to: the ventrolateral periaqueductal gray, a site of expression of fear responses (De Oca et al., 1998; Le Doux et al., 1988) that has been implicated in the acquisition of extinction (McNally et al., 2004; 2005); the orbitofrontal cortex whose volume is correlated with the retention of extinction memory (Milad et al., 2005b; Rauch et al., 2005) and whose inactivation leads to behavioral perseveration (Butter et al., 1963) and uncoupling of conditioned behavioral and autonomic responses (Reekie et al., 2008); and the prelimbic (PL) cortex whose neuronal activity correlates with extinction failure (Burgos-Robles et al., 2009). To further elucidate the neural circuitry of extinction, the present work investigates the role of the anterior cingulate cortex (ACC) in the extinction of avoidance behavior and its expression outside the extinction context. Beyond mere fear conditioning, this paradigm allows us to probe into the subsequent development of instrumental avoidance behavior that is driven by the fear response.

1.2. Avoidance Learning

Avoidance conditioning is a signaled form of escape conditioning where an animal performs a behavior that terminates an unpleasant ongoing event. The early part of avoidance learning is similar to fear conditioning such that a response is elicited upon presentation of a once neutral cue (subsequently becoming a conditioned stimulus or CS) that has been associated with an unconditioned stimulus (e.g., shock), thus reflecting conditioning. Just like the US, the CS (e.g., tone) through classical conditioning elicits a conditioned emotional response, fear, thereby becoming aversive in itself. The aversion towards the CS is then what drives the animal to make the instrumental avoidance response in order to escape the aversive internal state produced by the CS. In this regard, avoidance learning has been described as

a two-process learning (Mowrer, 1947). The theory explains the underlying learning phenomenon as an escape from conditioned fear which is what reinforces the behavior rather than the shock presentation that is absent in successful avoidance trials. D'Amato's theory (1967) takes into account the notion that both pain and relief motivate avoidance. The CS elicits an anticipatory pain response that motivates an escape behavior which leads to the conditioning of an anticipatory relief response. In either sense, the study of avoidance learning and its extinction could provide theoretical implications as well to understanding drug-taking behavior (an approach-related behavior that is counter to the action tendency of avoidance behavior) and its relapse after abstinence since drug use could be deemed as a form of avoidance response to a state of unpleasant emotions.

Being a step or so beyond fear conditioning, more associative memory representations are formed during avoidance learning. In such a conditioning preparation, an animal forms a Pavlovian association of the CS with the US (stimulus-outcome association) that earns a CS the ability to elicit fear (stimulus - Pavlovian response) which is relieved as execution of the avoidance response turns the CS off (instrumental response -outcome association). Theoretically, a parallel stimulus – response association develops (White and McDonald, 2002) which becomes prevalently expressed over a number of sessions so that when the CS is presented, an automatic instrumental response is displayed. A cognitive, goal-directed (action-outcome) behavior may be overruled by a stimulus-response strategy or habit learning with increased training (Chang and Gold, 2003; Hicks, 1964; Noblejas, 2005; Packard and McGaugh, 1996; Ritchie et al., 1950). Thus, the general CS-US associative memory of the avoidance behavior may be multiply represented albeit in segments by the basic associations formed that interconnect to

lead to the behavioral outcome. Hypothetically, this may mean that disruption or alteration of one (or some) of its basic associative memory representation may be survived by the remaining others. Extinction of avoidance behavior might therefore command that closer attention be paid to what may be extinguished in a given extinction training preparation.

1.3. Anterior Cingulate Cortex

1.3.1. Anatomical Definition

As part of the prefrontal cortex by definition of its connection with the mediodorsal thalamus, the anterior cingulate cortex (ACC) has been extensively studied for its role in learning and memory and classically associated with emotion. The ACC is the frontal part of the cingulate cortex located below the cingulate sulcus and above the corpus callosum. It is a heterogeneous structure that has been anatomically subdivided into the dorsal part that has connections with lateral prefrontal cortex, parietal cortex and premotor and supplementary motor areas; and the ventral part that is interconnected with the amygdala, periaqueductal grey, nucleus accumbens, hypothalamus, anterior insula, hippocampus and orbitofrontal cortex (Carmichael and Price, 1994; Devinsky et al., 1995; Divac and Diemer, 1980; Sripanidkulchai, Sripanidkulchai & Wyss, 1984; Vogt and Miller, 1983). Corresponding connections of each of the anatomical subdivisions reflect functional differentiation between the two subdivisions. Cognitive functions of the dorsal aspect of the ACC include modulation of attention or executive functions, monitoring competition, novelty and error detection, motivation and working memory (Botvinick et al., 2001; Bush et al., 1999; Bush et al., 2000; Carter et al., 1999; Devinsky et al., 1995; Fan et al., 2003; Gehring & Fencsik, 2001; Mohanty et al., 2007; Polli et al., 2008; Posner and DiGirolamo,

1998; Swick & Turken, 2002; Vogt et al., 1992). Affective processing of the ventral aspect involves assessing salience of emotional and motivational information as well as regulation of emotional responses (Devinsky et al., 1995; Drevets and Raichle, 1998; Vogt et al., 1992; Whalen et al., 1998). Imaging studies have shown that the ventral part of the ACC is activated in situations where healthy subjects are asked to imagine emotionally laden situations (Dougherty et al., 1999; Pardo et al., 1993). Damage to ACC has been found to disrupt generation of autonomic arousal responses (Critchley et al., 2003; Zahn et al., 1999) while its electrical stimulation in animals as well as humans could elicit autonomic responses (Burns and Wyss, 1985; Chefer et al., 1997; Kaada et al., 1949; Pool and Ransohoff, 1949; Ward, 1948). Critchley (2004) suggested rather cautiously that activity found in certain regions of the ACC may be a result of where the input is coming from. For example, input from somatosensory and motor cortices to the caudal region of the ACC may reflect the arousal found there during pain or physical effort. Counter to this notion (hence his caution), a region such as the subgenual ACC which is more strongly anatomically connected to autonomic control centers than the dorsal ACC (Barbas et al., 2003; Kaada, 1951), has been found to be more active during baseline resting state or vegetative states such as sleep while the dorsal ACC has been found to be more active during effortful tasks. An inference that had been drawn from this is that the subgenual ACC underpins parasympathetic versus sympathetic autonomic drive. To sum it up, gaining insight into the functional role of the ACC would require understanding its anatomical connections and their respective functional implications.

1.3.2. Putative Roles

Considering the myriad of functional implications attributed to the ACC (see review by Bush et al., 2000), several hypotheses have been put forth to define the role of the

ACC from it being part of a cingulo-frontal network of working memory (Smith et al., 1998); to being a part of a circuit used when effortful control is necessary in switching response pathways (Raichle et al., 1994); to error detection (Carter et al., 1998) that is separate from the competition monitoring hypothesis (Botvinick et al., 1999); to executive attention theory (Norman and Shallice, 1986). However, none of these hypotheses have explained an encompassing role of the ACC that integrates both its involvement in cognitive and emotional processing. Ward (1948) had described the ACC as an autonomic effector region. Following ablation of the anterior cingulate area done in monkeys, he had observed behavioral changes that included tameness and loss of the usual fear towards humans. Luu and Posner (2003) suggested that cognitive processes such as conflict and error monitoring which involve the ACC produce autonomic reactions that signal the need for behavioral modification. They further indicated that this is compatible with previous finding associating theta activity (an index of cognitive control), putatively generated by the ACC, with autonomic functions during sustained attention (Kubota et al., 2001). Moreover, other studies have shown that the ACC receives nociceptive information and plays a role in the coordination of autonomic responses (Fisk & Wyss, 1997; Hsu & Shyu, 1997; Neafsey et al., 1993).

It has been conjectured that the autonomic responses that develop during classical conditioning tasks represent an early aspect of learning related to the attachment of emotional significance of the CS-US contingency (Buchanan and Powell, 1993; Gantt, 1960, Konorski, 1967). Lesions of the ACC have been reported to attenuate conditioned heart rate decelerations involved in Pavlovian conditioning (Buchanan and Powell, 1982a, 1982b). Gabriel and colleagues (1991) found that lesions of the ACC in rabbits mildly retarded acquisition of learning. In a subsequent study (Gabriel,

1993), an absence of early- but not late-developing training induced neuronal activity (TIA) in the posterior cingulate cortex (PCC) was found which suggested that the ACC is a source of early-developing plasticity in the PCC. Gabriel (1993) described two phases of learning in a discriminative avoidance paradigm: During the early part of training, the ACC together with the mediodorsal thalamus, was found to encode training induced neuronal activity[1] (TIA) while the PCC together with the anteroventral thalamus, encoded TIA during the later stages of training. TIA in the ACC has been described as easily gained and modifiable in response to the new CS-US association while TIA in the PCC develops more slowly and not as flexible when obtained. Hence the implications of the ACC and PCC as part of the recency or primacy system respectively. Their differential involvement during the early and later part of learning is not limited to discriminative avoidance learning, but may also be observed behaviorally in visual attention task (Bussey et al., 1996) and in spatial task (Meunier, Jaffard & Destrade, 1991).

Implicated in reinforcement-guided decision making (Rushworth and Behrens, 2008), the ACC has been found to be involved in executive functions necessary for behavioral extinction such as inhibition of prepotent responses[2] and behavioral flexibility (Bussey et al., 1996; Ng et al., 2007) as well as affective aspects of behavior (Bush et al., 2000). Anatomical studies in rodents and primates have revealed ACC connections with the amygdala (Divac and Diemer, 1980; Ghashghaei

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[1] training induced neuronal activity – the occurrence of changes in the tone-elicited discharges that develops during learning or conditioning (Gabriel, 1993)

[2] prepotent response – a predominant behavioral reaction acquired through its association with reinforcement

et al., 2007; Sripanidkulchai et al., 1984), a substrate involved in emotional conditioning as well as its extinction (Akirav et al., 2006; Davis, 1992; Falls et al., 1992; Fanselow and LeDoux, 1999; Kim et al., 2007; Kim et al., 2008; LeDoux, 1993, LeDoux et al., 1988; Maren, 1999; McGaugh et al., 1993). Disruptions of the ACC have been found to retard acquisition of avoidance learning (Gabriel et al., 1991; Kimble and Gostnell, 1968; Peretz, 1960) and impair extinction of non-aversive tasks such as conditional visual discrimination (Bussey et al., 1996) and conditioned jaw movement (Griffin and Berry, 2004).

1.3.3. Previous Extinction Studies

Animal and human studies have reported a role of the ACC in extinction. In a conditional visual discrimination task, excitotoxic lesions of the ACC of rats impaired extinction of lever press responses (Bussey et al., 1996). In a differential context conditioning procedure, fMRI showed enhanced activation in the human ACC during extinction to the context that served as CS+ compared to the one that did not, the CS- (Barrett and Armony, 2009; Lang et al., 2009). Griffin and Berry (2004) found that ACC inactivation led to a persistence of the conditioning-related hippocampal unit activity that would have otherwise be inhibited during extinction of conditioned jaw movement. Despite that there are no known direct anatomical connections between the ACC and the hippocampus, the ACC apparently have an inhibitory influence on the activity of the hippocampus, a substrate implicated in contextual processing as well as consolidation of extinction (Corcoran and Maren, 2004; Fisher et al., 2007; Hall et al., 2001; Heldt et al., 2007; Lang et al., 2009; Malin and

McGaugh, 2006). This in turn can affect expression of extinction memory especially when its expression is called for outside the context such as in tests for spontaneous recovery, renewal and reinstatement. We therefore seek to explore the role of the ACC in extinction learning and its expression outside of the extinction training context. In the present work, lesions of the ACC were made after conditioning and before extinction since previous avoidance learning studies have shown that pretraining lesions of the ACC produced retardation in avoidance learning (Gabriel et al., 1991; Kimble and Gostnell, 1968; Peretz, 1960). Peretz (1960) found no difference between the sham and the cingulectomized groups in the extinction of avoidance learning. This would most likely mean that no extinction learning deficits would be apparent that is in line with what Peretz (1960) had reported since the behavior to be extinguished is not robust to begin with. Non-aversive extinction studies have shown that ACC inactivation produced perseverative behavior which makes it reasonable to ask whether the same can be said in aversive studies given the ACC implication in the acquisition of associations.

Chapter 2
Effects of Anterior Cingulate Cortical Lesions on Extinction, Spontaneous Recovery and Reinstatement of an Active Avoidance Response

2.1. Introduction

Avoidance behavior is a defense mechanism that an organism displays in order to escape or prevent unpleasant situations or feelings such as fear (Avoidance Behavior, the free dictionary.com, 2011). At best, it is adaptive such as using an umbrella to avoid getting wet or putting on sunglasses to keep the sunshine directly away from the eyes especially when driving and so on. However, such a behavioral strategy can become aberrant as sometimes brought about by traumatic events that consequently instill fear. Fear serves a biological purpose as it motivates one to observe safety practices for survival. But just as with anything else in excess, excessive feelings of fear or anxiety can become detrimental to normal daily functioning; or at worst, to one's survival which the motivational component of fear has evolutionarily set to promote. An attack in an alley by a masked person wielding a knife should not keep the patient from allowing a masked person wielding a knife in an operating room to treat him. In this case, a healthy cognitive system would process the difference in context and subsequently, the final outcome (safety in the form of medical treatment) that would then result in modification and updating of the existing associative memory.

Various manipulations of the ACC suggest that it plays a role in the acquisition of learning whether in excitatory or inhibitory learning that includes extinction and

passive avoidance (Bussey et al., 1996; Gabriel et al., 1991; Griffin and Berry, 2004; Riekkinen et al, 1995). Extinction studies of approach-related behavior[1] have shown that lesions of the ACC lead to impairment in extinction learning (Bussey et al., 1996; Griffin and Berry, 2004). Little is known about its role in the extinction of avoidant behavior which turns out to be pathological in patients suffering from anxiety disorders such as post-traumatic stress disorder (PTSD). Persistence of traumatic memories in such patients renders them sensitive (reactive) to trauma reminder stimuli despite that these are no longer threat-related, which is indicative of a failure of an executive function that putatively is mediated by the anterior cingulate cortex (see Hamner et al., 1999).

Present study explores the role of the ACC in the extinction of an active avoidance behavior given its implications in action-outcome associations (Kennerley et al., 2006; Matsumoto and Tanaka, 2004; Oliveira et al., 2007; Rushworth et al., 2004). Rushworth et al. (2004) had suggested that not only does the ACC encode the action that leads to a specific outcome and the likelihood that it will lead to an error, but also the cost-benefit of an action in relation to the value of its intended outcome. For instance, rats with ACC lesions would choose an easily accessible goal arm with fewer food pellets in a T-maze rather than the alternative arm that contained more pellets but required them to climb over a barrier to obtain the reward (Walton et al, 2003). In a monkey study, lesion-induced performance impairment in sustaining rewarded responses in a reward-guided choice task led to the suggestion of a role of

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[1] approach-related behavior – basic response associated with appetitive motivations that elicit an approach behavior compared to avoidance-related behavior associated with aversive motivations that elicit avoidance (Marsh et al, 2005)

the ACC in integration of reinforcement history to guide choice behavior (Kennerley et al., 2006). This suggests that the ACC is involved in processing the likelihood of choosing a correct response that involves calculating the value of a response based on how often it was previously reinforced. Thus, the present study sought to investigate the effects of lesions of the ACC on extinction learning where a previously learned action-outcome is no longer reinforced. Lesions of the ACC would be expected to lead to behavioral perseveration during extinction of an avoidance behavior given that without the ACC, learning of the new meaning of the CS would be impaired. However present results show that ACC lesioned animals displayed better expression of extinction learning and its retrieval during retention test. This suggests a regulatory role of the ACC in the rate of expression of extinction memory that may depend on the motivational salience attached to the CS.

2.2. Materials and Methods

2.2.1. Subjects. Subjects were 21 male Mongolian gerbils (*Meriones unguiculatus*) from Tumblebrook Farms, Westbrook, MA USA (65 - 85g, 3-6 months old), fed ad libitum and individually housed in a temperature-controlled environment on a 12-hour light-dark cycle with the lights on from 7 a.m. to 7 p.m. Of the 21, 13 were given lesions of the ACC while the rest were given sham surgery after conditioning.

2.2.2. Surgical procedures. Prior to surgery, the gerbils were anesthetized with a cocktail of Ketamine (500mg/10ml, Ratiopharm GmbH), Rompun (2%, Bayer Vital, GmbH) and isotonic NaCl (0.9%, Braun) with a ratio of 10: 9: 1 (dose of 0.30 ml/70g body weight) administered intraperitoneally. Treatment of the animals and surgical procedures were in accordance with the rules of the Ethics Committee of the state of Sachsen-Anhalt, Germany. Injections of ibotenic acid (Sigma, 0.3 μl per injection,

5mg/ml in PBS) were administered into both the right and left hemispheres of the ACC through a 28g, 5µl - Hamilton syringe. After the injection, the needle was left imbedded in each site for at least five minutes to allow for diffusion of the solution. The ACC lesion group had a total of 12 injection sites per gerbil with surgical coordinates derived from Paxinos and Watson (1998) since the existing gerbil atlas does not provide adequate coordinates for a skull oriented on a leveled plane.

The brain coordinates for the ACC lesions were from bregma, anteroposterior (AP) = +0.9 mm, mediolateral (ML) = ±0.4 mm and dorsoventral (DV) = -1.0/-1.7 mm; AP = +0.1 mm, ML = ±0.4 mm and DV = -0.9/-1.3 mm; AP = -0.7 mm, ML = ±0.4 mm and DV = -0.7/-1.0 mm. The sham group did not receive any ibotenic injection but holes were drilled on the skull of the sham group where the coordinates for the anterior cingulate cortex lesions would be located.

2.2.3. Apparatus. Gerbils were trained in a two-compartment shuttlebox (38×19×22.5 cm, HASOmed GmBH) that had a hurdle (4 centimeters high) in the middle which the animal had to go over to avoid the electric shock delivered through the grid floor during the conditioning sessions.

2.2.4. Behavioral Procedures. Gerbils were trained between 1:00 – 7:00 pm. Figure 2a-b illustrate the schematic summary and the complete timeline of the training procedure. There were eight conditioning sessions that included sixty trials each. A session lasted for 25 minutes and was done one per day. A series of pure beeping tones (2 kHz, 65 dB, 200 ms per beep with an interval of 300 ms in between beeps) served as a conditioned stimulus (CS) that signaled the occurrence of the delivery of footshock (600 µA), the unconditioned stimulus (US), if a gerbil did not go over the

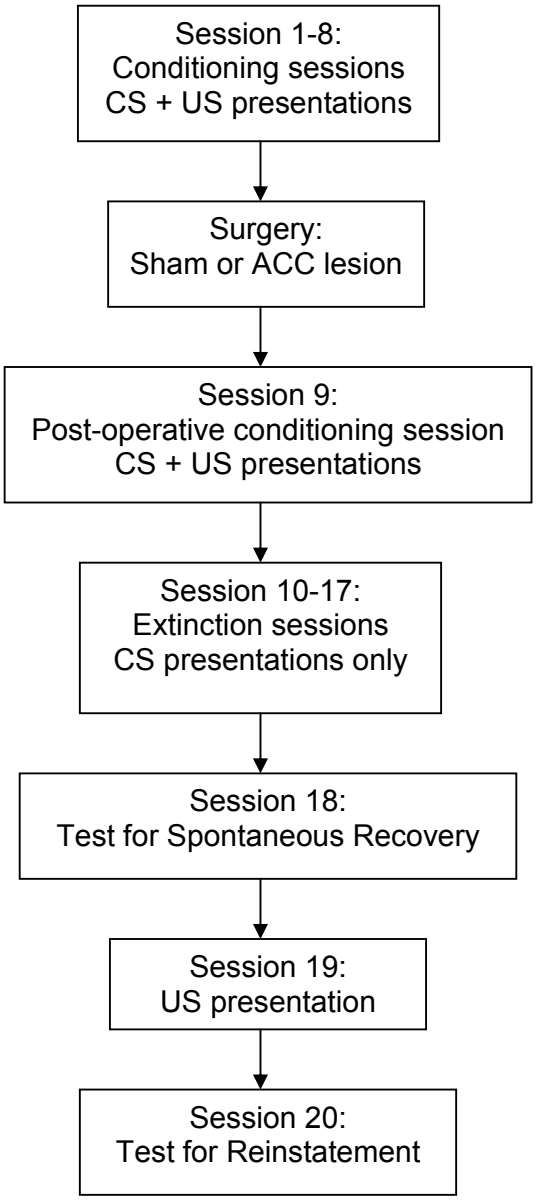

Figure 2a. *Schematic diagram depicting the different phases of the behavioral training.* Gerbils were trained for eight days to respond, by going over a hurdle, to a tone that predicts the delivery of a footshock. Three days after the last conditioning session, either sham or lesion surgeries were done on the animals. After a recovery period of about seven to ten days, animals were given a postoperative conditioning session to make sure the memory representation of the CS-US association remained intact. The following day, extinction training commenced and went on for a total of eight days where the gerbils were presented with just the tone in the same shuttlebox used during the conditioning sessions. Seven days after the last extinction session, the animals were given another extinction session to test for spontaneous recovery of the extinguished CR. The animals were then given presentations of shock alone followed by a test for reinstatement the following day.

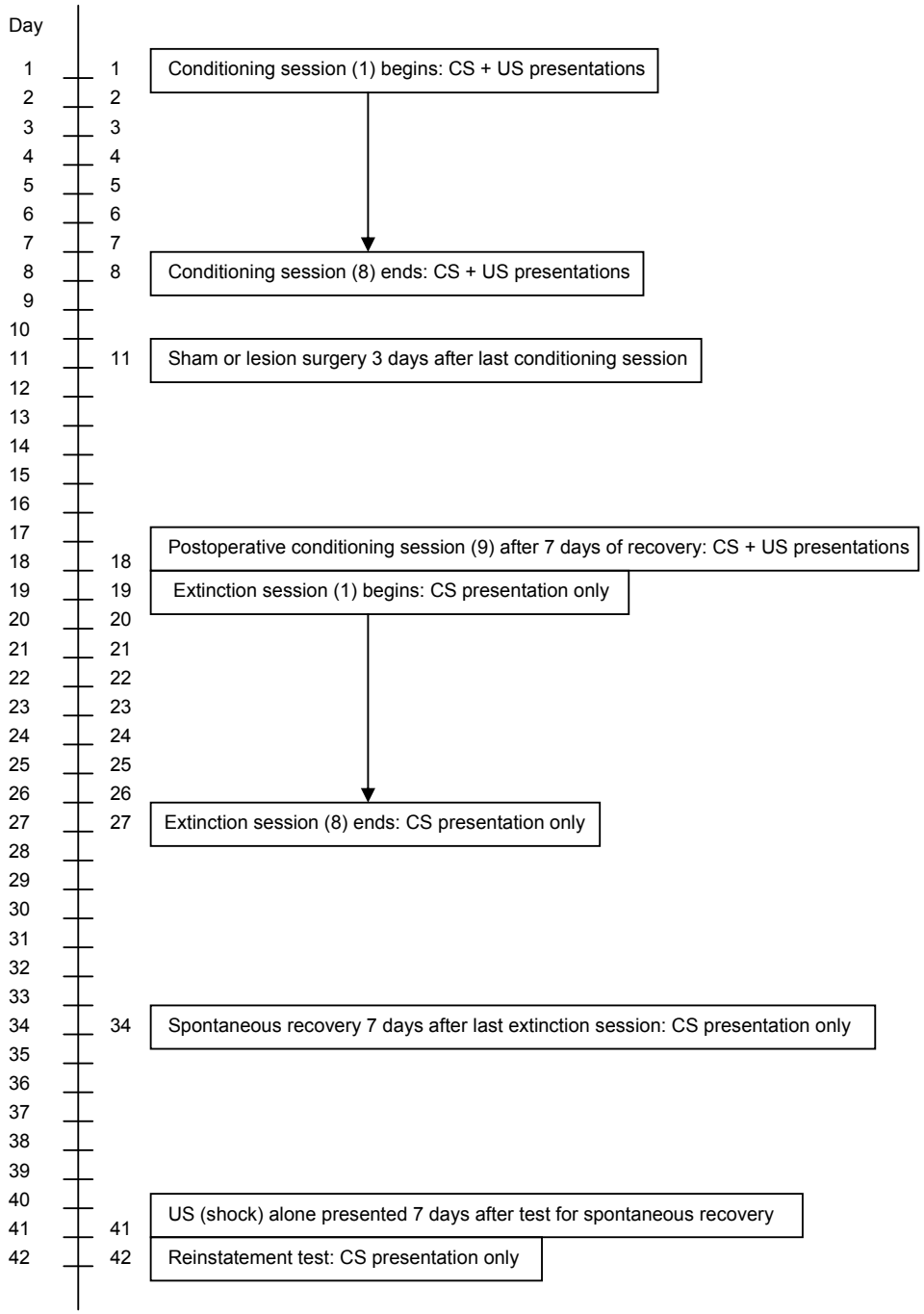

Figure 2b. *Schematic diagram depicting the complete timeline of the training.* The timetable illustrates the duration of the different phases and the gaps (number of days) in between which altogether takes forty two days from start to finish.

hurdle (the conditioned response; CR) during the six-second CS presentation. In such case, a 4 s footshock (600 uA) delivery would occur upon CS offset. If the

gerbil responded before CS offset, CS presentation was terminated. Intertrial interval was 16 to 20 seconds. Three days after the last conditioning session, a gerbil was given either a lesion or sham surgery in the ACC. Recovery period was seven to ten days after which an animal was first given a postoperative conditioning session to ensure that the memory of the CS-US association remained intact. Extinction training commenced the following day where an animal was given a session of 60 trials of the CS presentation without the US. A gerbil went through extinction training one session a day for eight days. Seven days after the last extinction session, animals were tested for spontaneous recovery where gerbils were given 60 presentations of CS alone. After a week, animals were then exposed to a presentation of US (shock) alone for one session (60 trials). The following day, the animals were tested for reinstatement of the extinguished CRs in a session of 60 presentations of CS alone.

2.2.5. Histology. After the behavioral training, the gerbils were decapitated and the brains were taken out and frozen in liquid nitrogen (Linde, Germany) for 10 minutes. All brains were stored in a freezer at -80° Celsius. The brains were sliced into coronal sections of 40 μm thick which were stained with thionin, a Nissl stain for cell bodies to determine the extent of the ibotenic acid lesions. To quantify the size of lesion damage, a grid transparency was used and the number of grid squares covering the damaged portion of the ACC was divided by the number of squares of the intact targeted lesion area multiplied by 100. The targeted ACC areas were cingulate cortex area 1 and 2.

2.2.6. Data Analysis. Analyses were done using the statistical program Statistical Program for the Social Sciences (SPSS), USA. The rate of CRs (related to the total number of trials), the number of CRs and response latencies during conditioning,

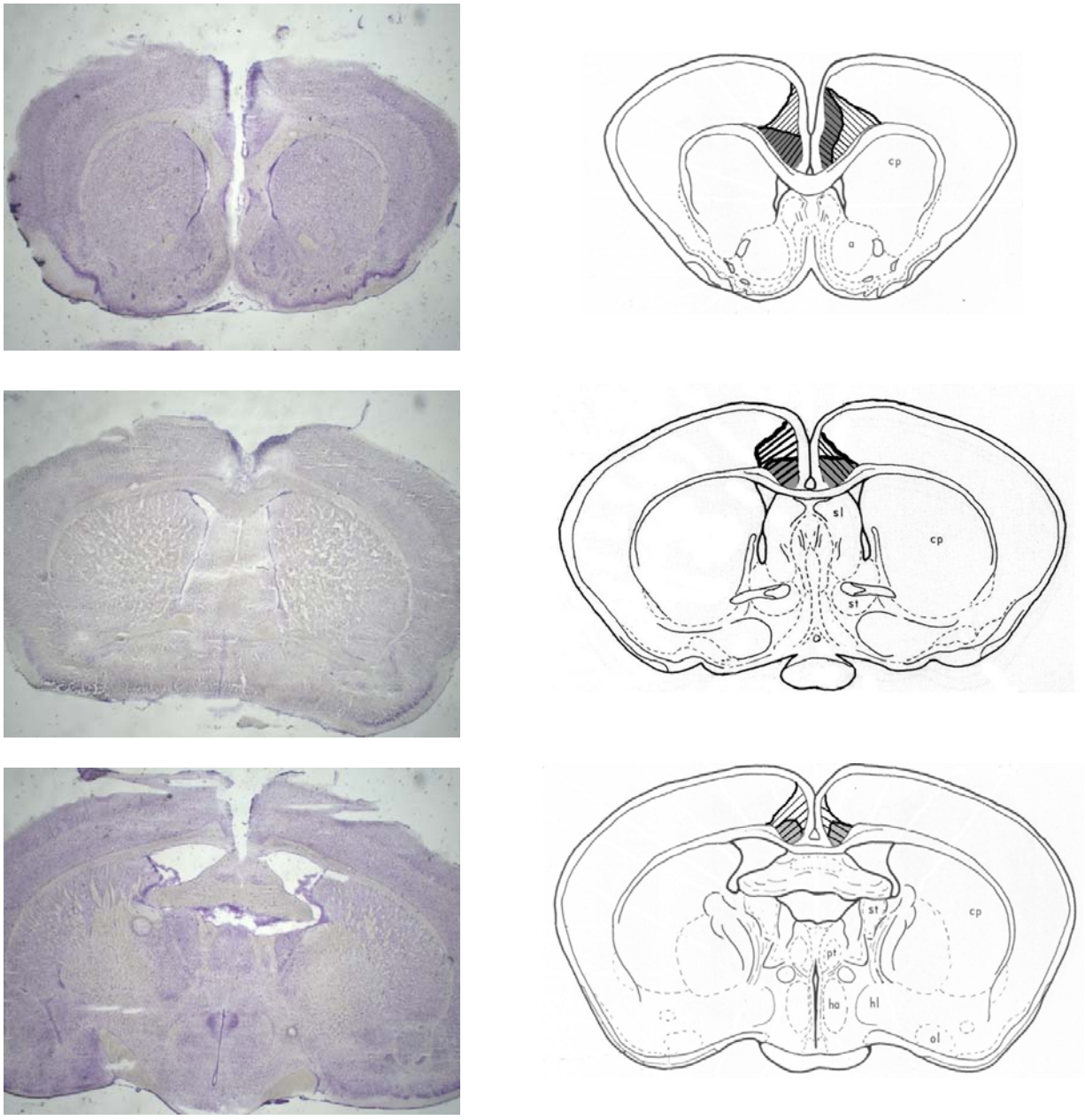

Figure 3. *Coronal sections of the lesion area.* Representative photographs of ACC lesions (left panel) with the corresponding schematic diagrams on the right, depicting ACC lesion placements at from top to bottom: AP +1.1 mm, +0.1 mm and -0.4 mm from bregma. Gray-shaded areas represent the extent of damage in the gerbil brain with the smallest lesion damage while areas with hatched bars plus the gray-shaded areas represent the gerbil brain with the largest lesion damage.

extinction training as well as the test for SR were analyzed by using general linear model repeated measures ANOVA. The reinstatement test was analyzed using unpaired t-test.

2.3. Results

2.3.1. Histological Analysis.

Targeted lesion area of the ACC (Fig. 3) was from AP +1.1 mm to –0.4 mm from bregma according to the gerbil atlas (Loskota et al., 1973). Bilateral lesions in the ACC were mainly on the Cg1 and Cg2 area with minimal damage to M2 in some gerbil brains. One gerbil brain had damage extending slightly to the PL cortex. The extent of damage measured in the gerbil with the smallest ACC lesion was 69% while the largest ACC lesion was 92%.

2.3.2. Behavioral Analyses

2.3.2.1 Conditioning. Repeated measures ANOVA showed a main effect of session (F (7, 133) = 41.925, P = .000) in the rate of CRs displayed which was indicative of the learning acquisition (fig. 4A). There was no main effect of group (F (1, 19) = .840, P = .371) or session by group interaction effect (F (7, 133) = .257, P = .969). There was also a main effect of session in the response latencies displayed by both groups (F(7, 133) = 6.791, P = .000; fig. 4B). No main effect of group (F(1, 19) = 1.819, P = .193) or session by group interaction effect (F(7, 133) = .440, P = .875) was apparent.

2.3.2.2. Postoperative conditioning. There was a main effect of session (F (1, 19) = 4.768, P = 0.042) but no significant session by group interaction effect (F (1, 19) = 3.055, P = 0.097) or main group effect (F (1, 19) = 0.002, P = 0.965) was evident

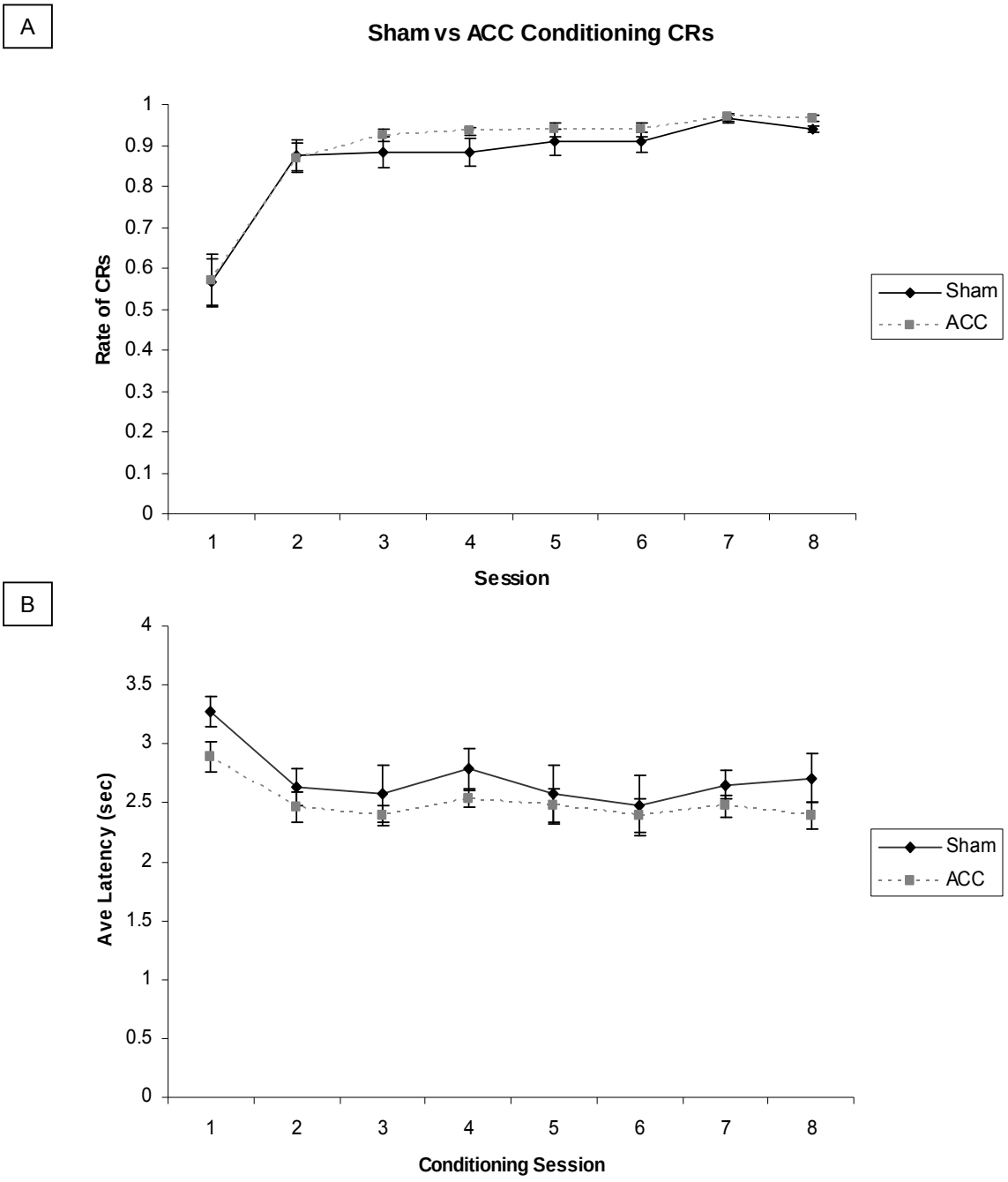

Figure 4. *Performance summary of Sham versus ACC groups during conditioning*. Line graphs showing the (A) rate of conditioned responses and (B) response latencies displayed by the sham (n=8) and ACC (n=13) groups (mean ± SEM). Black diamonds with solid lines illustrate the performance displayed by the sham group in each session while gray squares with broken lines illustrate that displayed by the ACC group. Analyses of the performance and response latencies showed a significant main effect of session indicating learning acquisition.

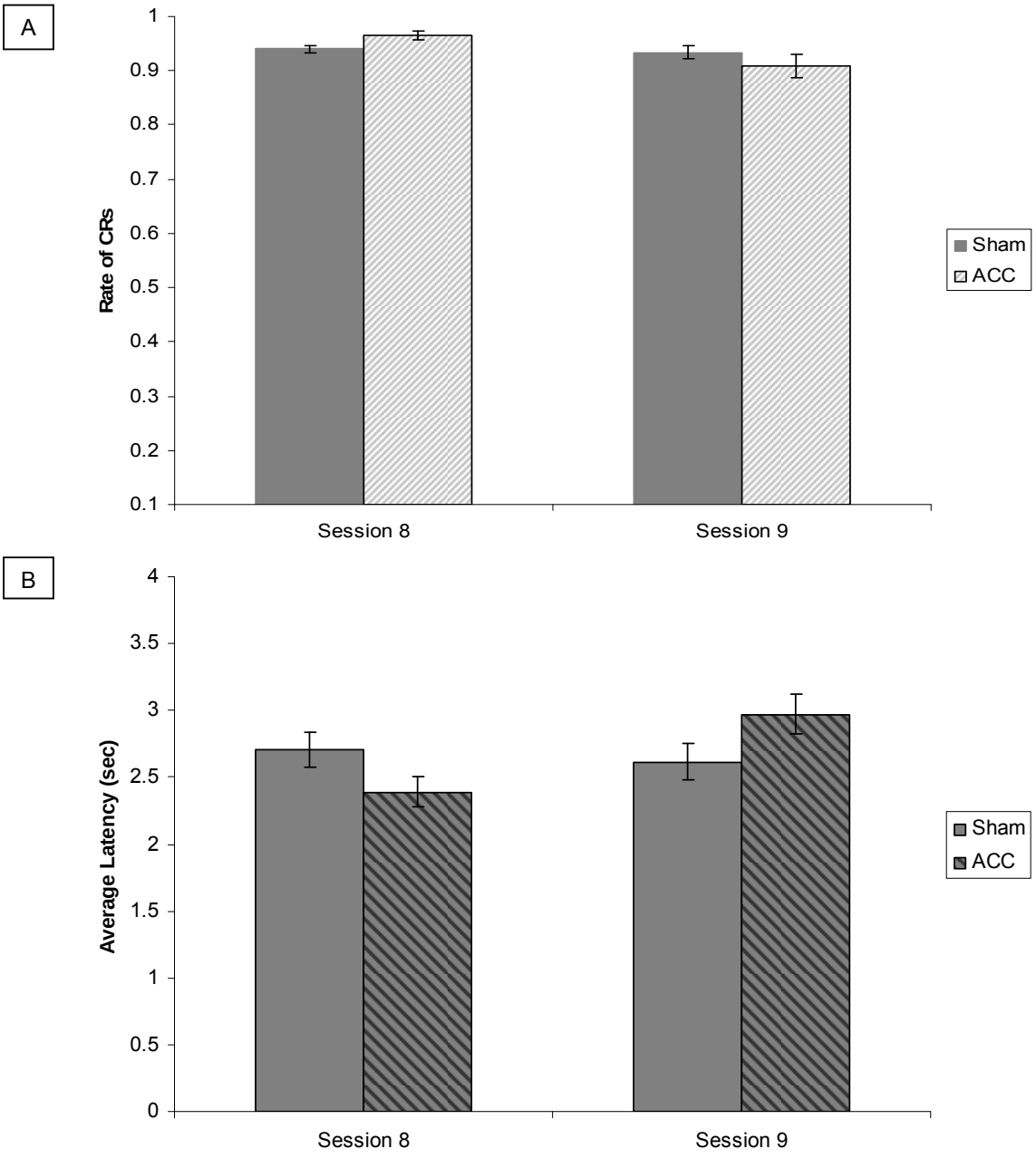

Figure 5. *Conditioning sessions before and after surgery*. Bar graphs showing the average performance displayed by the sham and ACC groups (mean ± SEM) on the last conditioning session before- and the conditioning session after surgery. Solid gray bars illustrate the performance of the sham while diagonally striped gray bars illustrate the performance of the ACC group. (A) There was no significant difference between the sham and ACC groups in the CRs displayed on pre-operative session 8 and post-operative session 9. (B) However, analysis of the response latencies showed a significant group by session interaction effect

indicating that the lesioned animals responded slower than their regular pre-lesion response time to the CS even prior to extinction training.

when CRs displayed by the two groups during the last conditioning session and the postoperative conditioning session were compared (Fig. 5A). Interestingly, there was a significant session by group interaction effect when the response latencies of the two groups were compared ($F(1, 19) = 7.555$, $P = .013$; Fig. 5B). This was from the lesioned group responding slower during the post-operative conditioning session compared to their performance on the last conditioning session. No main effect of session ($F(1, 19) = 3.949$, $P = .062$) or group ($F(1, 19) = .015$, $P = .905$) was found. This indicated that the disruptive effect of the lesion was relatively minor so that it did not render the lesioned group to respond slower than the sham group.

2.3.2.3. Extinction learning. There was no session by group interaction effect (F (7, 133) = 1.925, $P = 0.070$) but main effects of session (F (7, 133) = 49.465, $P = 0.000$) and group (F (1, 19) = 5.190, $P = 0.034$) were evident. Gerbils with lesions of the ACC displayed less CRs compared to the sham group although it did not necessarily facilitate a faster extinction learning rate (Fig. 6A).

Analysis of response latencies showed no main effect of session (F (7, 133) = 1.219, $P = .297$) or session by group interaction effect (F (1, 133) = 1.038, $P = .408$). There also was no significant difference between the groups in their response latencies (F (1, 19) = 3.981, $P = .061$). However, considering that the difference approached significance, it reflects a tendency of the lesioned group to respond slower than the sham group (Fig. 6B). This tendency of the lesioned animals to respond slower leads to the question of whether it is possible that the lesioned animals jumped more in response to the CS, but may not have been apparent due to the delay in response by a few milliseconds after CS offset. To determine this, the jumps an animal displayed

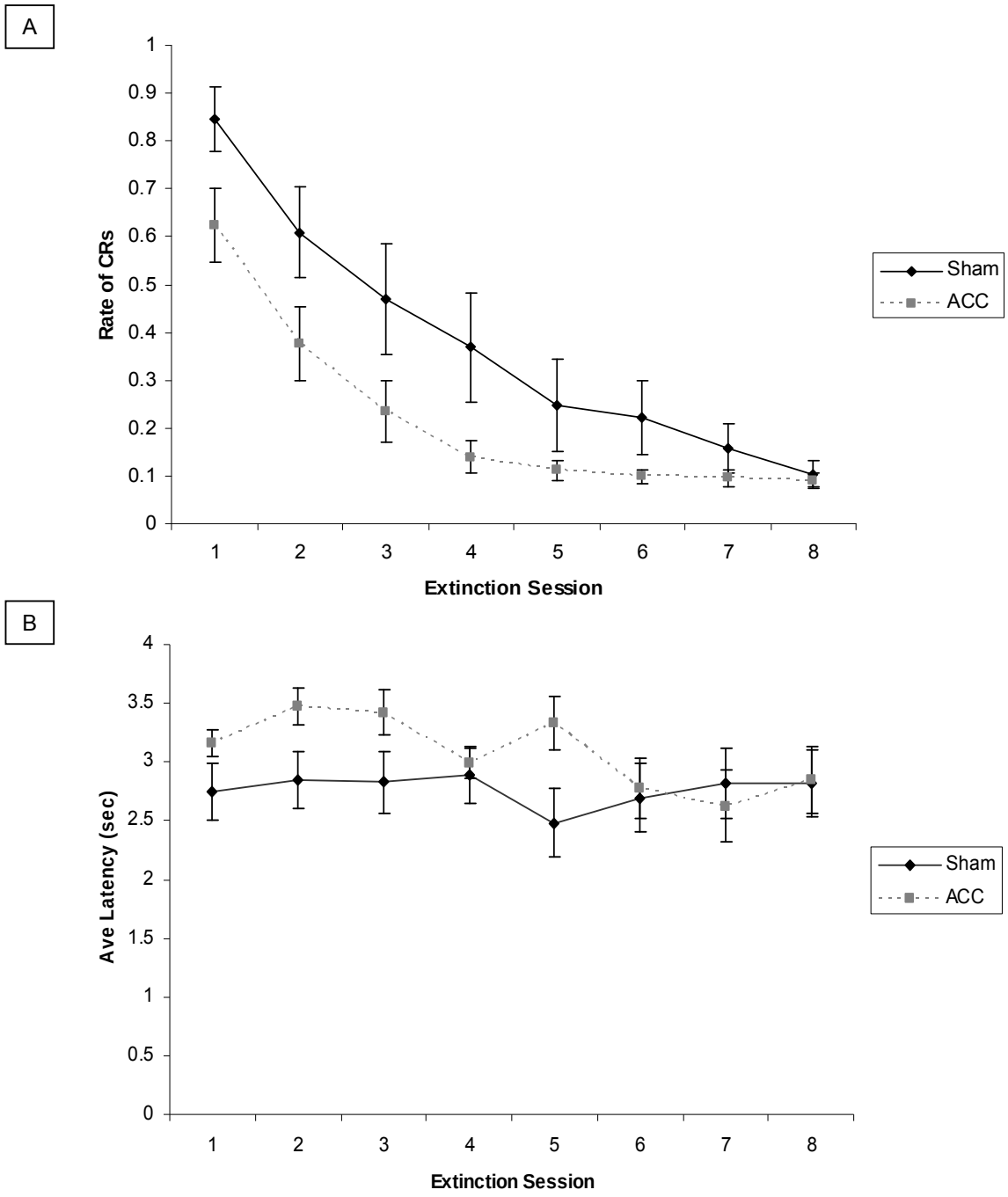

Figure 6. *Performance summary of Sham versus ACC Groups during extinction training.* Line graphs with black diamonds and solid connecting lines depict the performance of the sham group while line graphs with gray squares and broken lines depict the performance of the lesioned group. (A) Lesioned animals displayed better performance (rate of CRs) than the sham group as indicated by a significant main group effect in the CRs but did not necessarily learned faster as indicated by a lack of significant interaction effect between the

two groups. (B) The difference in their response latencies approached significance (p = 0.061) indicating that the lesioned animals had a tendency to respond slower than the sham.

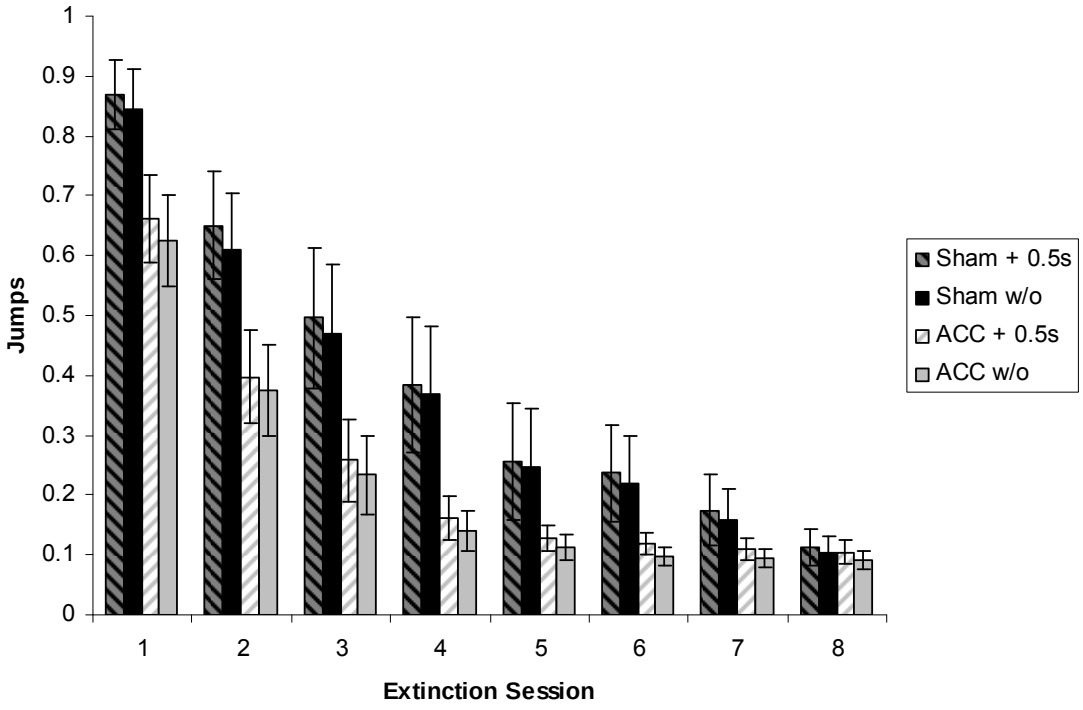

Figure 7. *Jump summary of the Sham and ACC groups upon CS presentation and within CS onset up to 0.5s after CS offset.* Bars with solid black or gray color represent the CRs displayed by the sham and lesioned group, respectively. Bars with black or gray diagonal stripes represent the jumps displayed by the sham and lesioned groups respectively within CS onset up to 0.5s after CS offset. Performance displayed by the lesioned group remained significantly weaker than the sham group even when comparing the recorded jump response 0.5s after CS offset.

upon CS presentation plus 500 milliseconds after tone offset were compared between groups. There was no significant difference between the CRs and the jumps displayed by the lesion group recorded up to 500 milliseconds after tone offset (F(1, 24 = 0.188, P = 0.668). There was a significant main effect of session (F(7, 133) = 51.954, P = 0.00) and group (F(1,19 = 4.989, P = 0.038) just like the group

comparison of the CRs indicating that the jump displayed by the lesioned group remained significantly less than the sham group (Fig. 7).

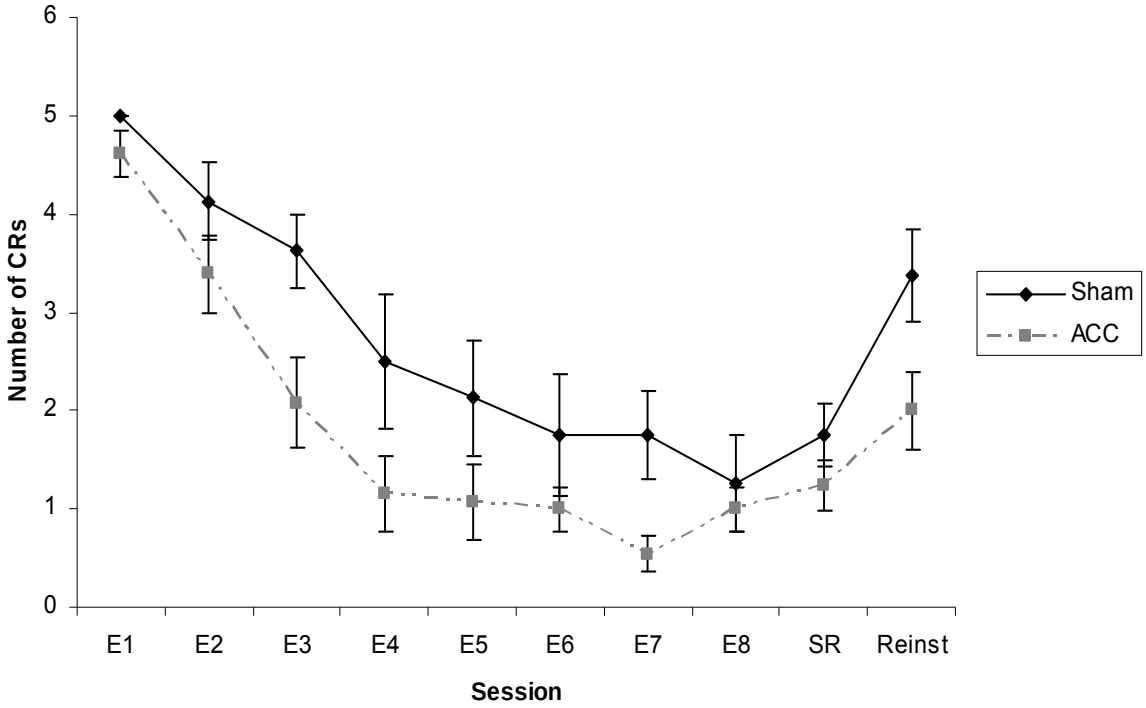

Figure 8. *Conditioned responses displayed during the first five trials of all the extinction training sessions as well as during the retention test for spontaneous recovery and reinstatement*. Line graph with black diamonds and solid connecting lines represents the performance (number of CRs) of the sham group while line graph with gray squares and broken lines represents the performance of the lesioned group. A main effect of session during extinction training indicated that improvement in performance between sessions occurred even at the start of the following session. There was a significant difference between the groups during Rei seemingly indicating greater contextual conditioning in the sham group compared to the lesioned group.

Analysis of the first five trials of all the sessions allows us insight into whether the animals are able to maintain the benefit of the previous training session despite the passage of time that occurs between sessions. A main effect of session (F (7, 133) = 34.625, P=.000) as well as group (F (1, 19) = 6.120, P=.023) was evident indicating

that both groups responded less and less as the training progressed; and that the lesioned group responded less than the sham group during the first five trials (Fig. 8). Both groups are able to benefit from the previous training as reflected in a decrease in number of jumps during the first five trials of the following extinction session.

Performance evaluation during the 60 trials of the first extinction session allows a comparative analysis of the behavioral acquisition between the two groups. This can reveal whether the responses on the first day of extinction learning taper off or otherwise. The 60 trials of the first extinction session were grouped into four blocks of 15 trials to allow for a within session analysis (Fig. 9). Repeated measures ANOVA showed a main effect of block ($F(3,57) = 16.400$, $P = .000$) and a significant block by group interaction effect ($F(3, 57) = 15.057$, $P = .030$). These indicate a decrease in the response of both groups to the CS presentation, but with the lesioned group learning faster than the sham group.

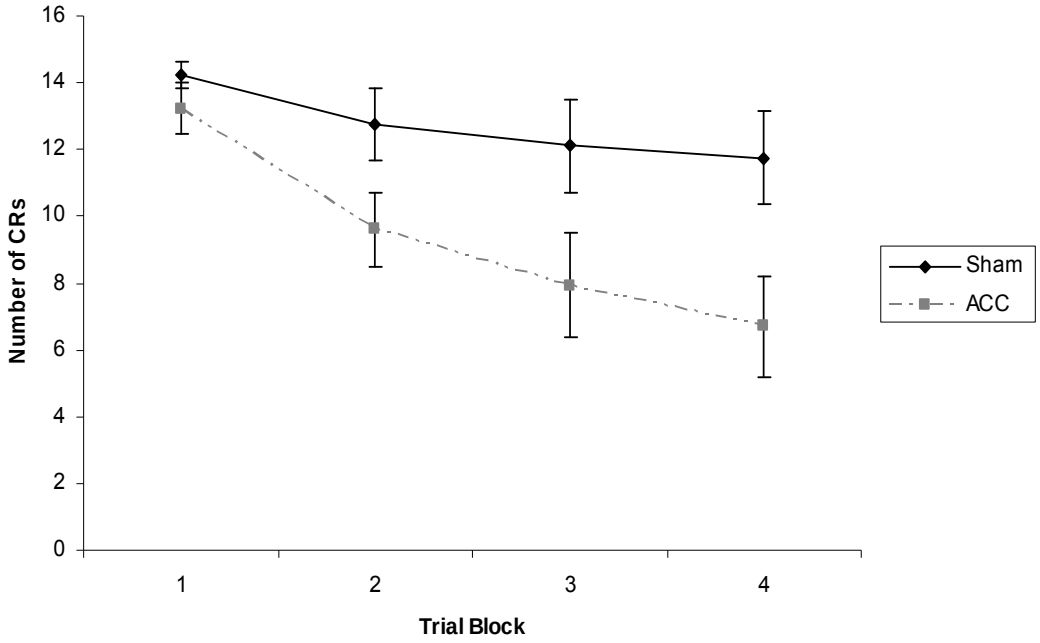

Figure 9. *Conditioned responses in blocks of fifteen trials displayed during the first extinction training session.* Line graph with black diamonds and solid connecting lines represents the performance of the sham group while line graph with gray squares and broken lines

represents the performance of the lesioned group. Lesioned animals learned faster than the sham group during the first extinction session as indicated by a significant block by group interaction effect.

2.3.2.4. Spontaneous recovery. There was a significant session by group interaction effect (F (1, 19) = 6.253, P = 0.022) when retrieval of extinction memory of the gerbils was tested seven days after the last extinction session (Fig. 10). Tests of within-subjects effects showed a main effect of session (F (1, 19) = 5.529, P = .030) while tests of between-subjects effects showed a main effect of group (F (1, 19) = 5.184, P = .035). While the sham group showed SR when the temporal context changed, the lesioned group showed a lack of SR of the extinguished CRs despite the passage of time. There was no significant difference between groups in their response latencies (F(1, 19) = .084, P = .775)

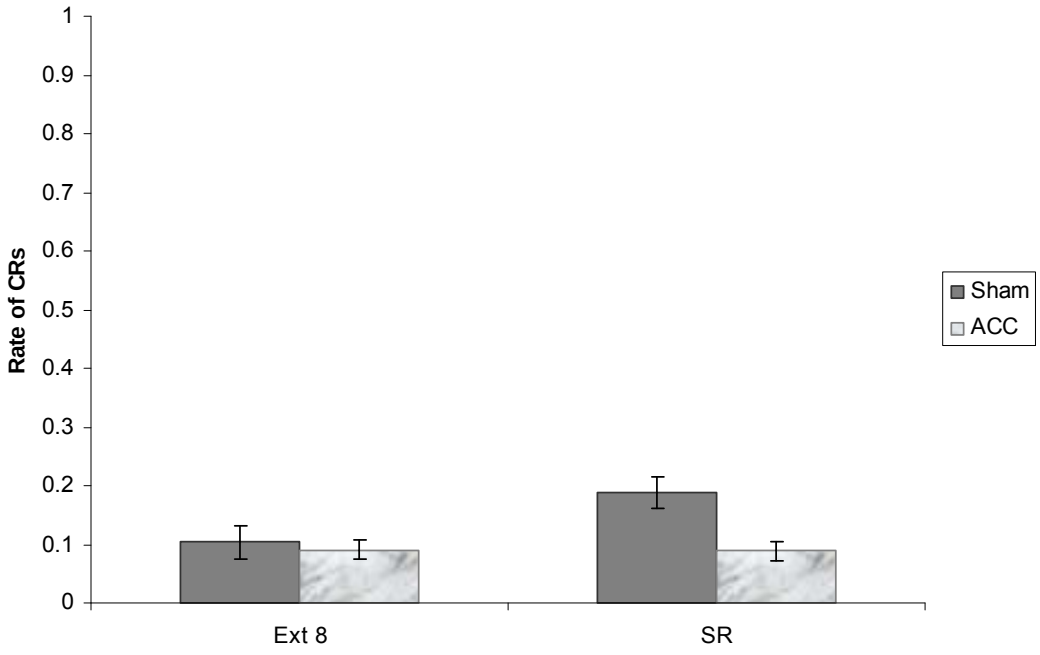

Figure 10. *Conditioned responses during SR*. Solid gray bars represent the performance of the sham group while marbled gray bars represent the performance of the lesioned group. Lesioned animals displayed significantly less return of extinguished CRs compared to the sham animals.

2.3.2.5. Reinstatement. Conditioned responding displayed by the ACC group was significantly less than that displayed by the Sham group (*t* (19*)* = 2.444, *p* = 0.024) when tested after exposure to shock (US) alone in the shuttlebox (Fig. 11). There was no significant difference in the latency to respond (*t* (19) = -.186, *p* = 0.855).

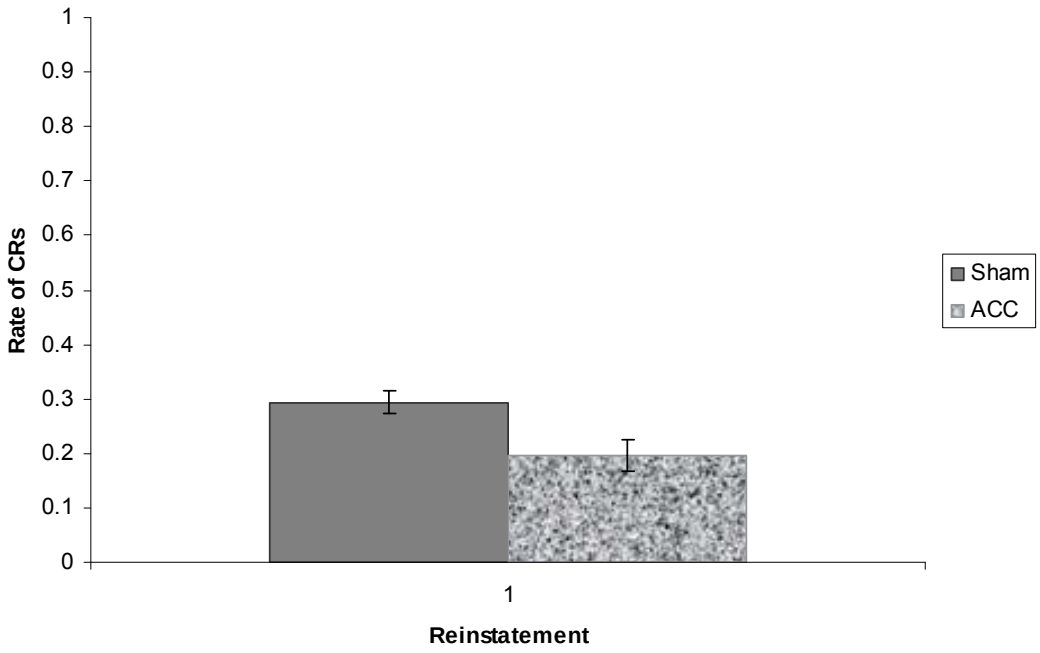

Figure 11. *Conditioned responses during Reinstatement*. Solid gray bar represent the score of the sham group while densely spotted gray bar represent the score of the lesioned group. Lesioned animals displayed significantly less return of extinguished CRs compared to the sham animals.

The ACC lesioned group displayed significantly less CRs than the sham group (*t* (19) = 2.227, *p* = .038) during the first five trials of this session (Fig. 12) suggesting that the contextual conditioning of the lesioned group with the shock alone exposure in the shuttlebox was weak. Paired t-test analysis of the CRs displayed by the ACC group during the test of SR and reinstatement showed a significant difference (*t* (13) = -4.882, *p*= 0.020) indicating that the lesioned animals still conditioned.

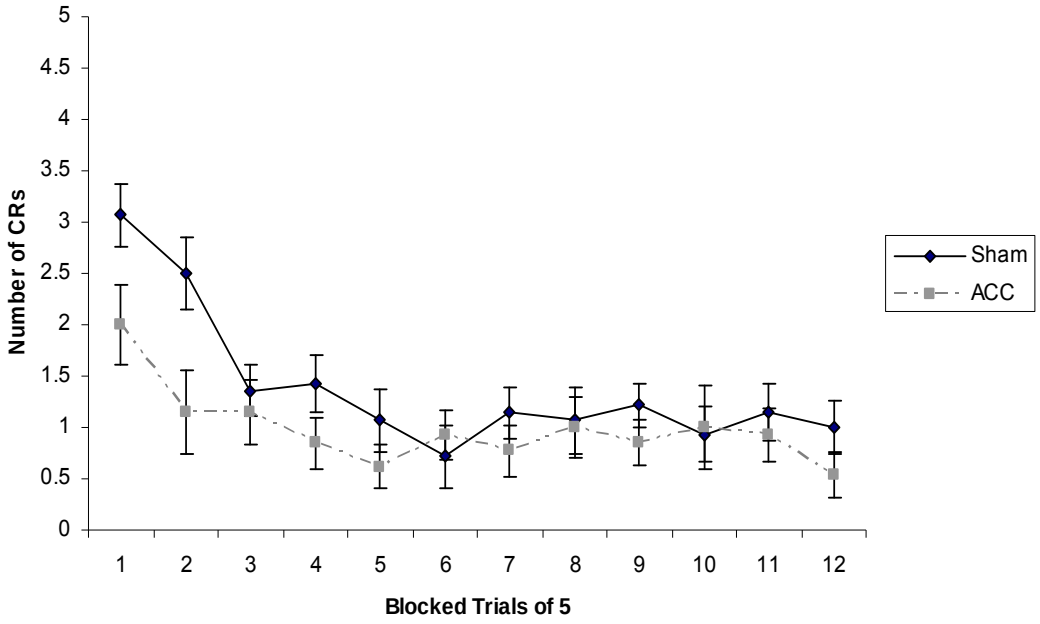

Figure 12. *Conditioned responses in blocks of 5 trials during the reinstatement session*. Line graph with black diamonds and solid connecting lines represent the performance of the sham group while gray squares with broken connecting lines represent the performance of the lesioned group. Significant main effect of group indicated that the lesioned animals displayed less return of extinguished CRs than the sham animals.

2.4. Conclusion

Present findings show that lesions of the ACC lead to better expression of the extinction memory of an active avoidance behavior and its subsequent retrieval in tests of SR and reinstatement. Earlier lesion and imaging studies have shown involvement of the ACC in behavioral flexibility when there is a change in the relevance of existing information such as during extinction learning (Barrett and Armony, 2009; Bussey et al, 1996; Griffin and Berry, 2004; Yaguez et al., 2005), switching strategies (Ragozzino et al, 1999) as well as reversal learning (Ragozzino and Rozman, 2007). Based on previous electrophysiological studies, Gabriel (1993) has proposed that the ACC is part of the recency network that updates the meaning of current CS-US relationships. Thus, ablation of the ACC should lead to a deficit in

the acquisition of extinction learning. Results of the present study were unexpected since these indicate that under certain conditions, ACC inactivation could lead to better performance instead of a deficit during learning. Similarly, this was evident as well when ACC-lesioned rats learned faster in appetitive conditioning tasks (Bussey et al., 1996; Peretz, 1960). Given that the ACC has been implicated in assigning emotional valence and motivational assessment (Devinsky, 1995), disruption of the ACC may have attenuated the negative motivational salience of the CS so that in the absence of the US, the lesioned group displayed less CRs compared to the sham group. That the lesioned group also showed less CRs during the postoperative conditioning session compared to how the group performed prior to surgery may also reflect this attenuation which is further supported by an increase in response latency during this session and a tendency to respond slower during extinction training. The attenuated salience may be related to a reduced arousal level which is consistent with Critchley's (2004) suggestion of a role of the ACC in generating arousal. Despite the attenuated display of CRs, the apparent progressive decline in the expression of CRs during extinction training suggests that the lesioned animals were still capable of learning. This was evident as well when the lesioned animals showed contextual conditioning, i.e., the lesioned animals jumped significantly more during the reinstatement test than they did when tested for SR. Thus, while better expression of extinction learning during training and retention tests may be mediated by an attenuated level of arousal, it would not be due to a general anxiolytic effect since the lesioned animals still displayed a capacity to learn.

Consistent with earlier implication of ACC involvement during the early phase of learning, the lesioned animals displayed faster learning rate only during the first extinction session compared to the sham group. Better performance displayed by the

lesioned group compared to the sham even though there was no difference in their overall learning rate may have come from this initial faster learning rate revealed by the trial by trial analysis. This is in line with previous electrophysiological implications of the ACC in the acquisition of the CS-US relationship (Gabriel, 1993). Gabriel had described how neurons in the ACC would display rapid development of discriminative training-induced activity (TIA; greater firing to CS+ than to CS-) that is evident in the first conditioning session and a rapid decline of excitatory TIA (greater firing of CS during conditioning compared to its pretraining activity) early in training. The initial facilitation of learning during the first extinction session may reflect an initial influence of ACC processing (that otherwise would be there) on autonomic modulation that corresponds with the acquisition of the new meaning of the CS.

Lesioned animals did not show recovery in the expression of extinguished CRs when given an extinction test session seven days after the last one suggesting a lack of perception of the passage of time. Spontaneous recovery is defined as restoration, albeit partial, of the extinguished CRs evident in a delayed subsequent testing (Rescorla, 2004). Significance of the phenomenon of SR may bear survival value when an organism is back in the same precarious spatial context after a given period of absence during which conditions may have changed and the presentation of the once predictive cue then signals some inconspicuous threat that may be lurking. On the surface, the return of extinguished CRs with the passage of time suggests the decay of extinction memory, reflected by its instability with time. However, Quirk (2002) found that rats re-extinguish significantly faster than they extinguished on the first day of extinction training thereby, implying savings of extinction memory. The increase in CRs when an animal is tested for SR may instead be explained as a failure to retrieve extinction memories outside its temporal context (Bouton, 1993;

2004). In the present study, the ACC-lesioned animals displayed a lack of spontaneous recovery indicating that the ACC is involved in processing temporal context which is in line with its previous implications in the temporal organization of behavior (Meunier et al., 1991; Sutherland et al., 1988) What is unclear however is if the effect reflects a role of the ACC in encoding the interval between training sessions so that a longer interval is recognized as a change in temporal context, or if the ACC is involved in the ability of an animal to sense the amount of time that has passed.

Current finding further provides additional support that extinction memory does not dissipate over time and that an otherwise intact ACC mediates the expression of extinguished CRs with the passage of time. While one may argue that the lack of responding may instead represent the decay of the CS-US memory representation induced by lesions of the ACC, return of the extinguished CRs in the reinstatement test although weak proves otherwise. Existing parallel memory representations of CS-US and CS-no US associations may thus be inferred from the finding. Notably, the lack of SR may go beyond a mere inability to sense the passage of time. It may represent another facet of the ACC such as a possible gating role on which associative memory, whether the primary or the recent association of CS, is selectively expressed when the context has changed.

In a reinstatement test, US reexposure with the relevant spatial context bestows upon it an excitatory property that then produces reinstatement (Bouton, 2004) as the conditioned strength of the context summates with that of the CS (Bouton and Bolles, 1979). Reinstatement was evident in the sham group in the present study as expected, but was attenuated in the ACC group which could be explained in terms of

ACC involvement in affective processing (Bush et al., 2000; Devinsky et al., 1995; Vogt et al., 1992). Although the lesioned group displayed significantly less CRs than the sham, it was evident nonetheless that the group still experienced contextual conditioning as revealed by their display of more CRs during the first five trials of the Rei test compared to the first five trials of the SR test. Malin and McGaugh (2006) found that infusion of a muscarinic agonist into the ACC after footshock training enhanced retention latencies in rats in an inhibitory avoidance task. A damaged ACC would presumably then compromise information processing of the US (footshock) and the context (the occasion-setter). Without an intact ACC that assigns emotional valence to stimuli and assesses motivational information (Devinsky et al., 1995), the reinstating footshocks would fail to attach motivational significance to the context, rendering a lesioned animal less likely to respond to a CS once predictive of danger. This implies that the ACC, with its modulatory influence on the autonomic nervous system (Matthews et al., 2004), may play a role in strengthening the association between the footshock and context representation.

Chapter 3

Effects of Anterior Cingulate Cortical Lesions on Extinction, Spontaneous Recovery, Renewal and Reinstatement of Discriminative Avoidance Behavior

3.1. Introduction

Tasks are better performed when attention is allocated to relevant cues which at times can be characteristically similar but may behaviorally require competing responses such as a green traffic light that signals a go response versus a red traffic light that signals a no-go response. This is attained through discrimination learning that trains an organism to differentially respond to cues by their association with reinforcement such as in a Go/No Go training paradigm. Such type of learning paradoxically engages opposing mechanisms: differentiation and unitization. Differentiation involves separating similar stimuli into different categories or isolating perceptual components psychologically fused together; while unitization involves creating perceptual units by grouping stimuli or combining object components that co-occur (Goldstone, 2004). Transfer of learning may thus be gained so that what is learned in one context may be transferred to another; from simple tasks such as applying the basic concept of using the dictionary when using the phonebook, to more complex tasks such as when special operations forces are trained to distinguish between innocent civilians versus the hostiles and their undercover cohorts.

The ability to distinguish and selectively respond to cues would suggest the need for error detection and conflict monitoring in information processing which the ACC has both been implicated in (Botvinick et al., 2001, Braver et al, 2001; Carter et al., 1998;

Wang et al., 2005). In an attentional set-shifting task, lesions of the ACC in rats have produced deficits in intradimensional shifting, i.e., shifting attention between stimuli of the same perceptual dimension that is associated with reward (Ng et al., 2007). Electrophysiological studies have demonstrated differential training-related activity in the ACC of rabbits in response to presentation of CS+ or CS- in a running wheel avoidance task (Foster et al., 1980; Gabriel, 1993); and that lesions of the ACC disrupted extinction-related inhibition of neural activity in the hippocampus underlying conditioned jaw movement (Griffin and Berry, 2004). Moreover, it has been shown that inactivation of the ACC impaired reversal learning in an odor discrimination task and that irrelevant stimuli were more likely to interfere in the performance of an organism (Ragozzino and Rozman, 2007). These indicate that the ACC is engaged when distinguishing the meaning or significance of multiple stimuli and prepotent responses need to be overruled. Thus in the present experiment, the role of the ACC is investigated in the extinction of competing conditioned avoidance behavior.

Unlike most Go/No go paradigm, the No go trials in the current paradigm are reinforced such that an animal is required to make a passive response such that an animal will receive a footshock if it would display a Go response in such a trial (false alarm in signal detection theory). The CS presented in No go trials does not serve as a safety signal, i.e., an absence of an aversive reinforcement regardless if an animal goes over the hurdle or stays put. Thus to solve the discriminative task, an animal must learn to discriminate between two CSs of different tone frequency and form associations as to which avoidance behavior, excitatory or inhibitory, is required to avoid the shock outcome. Animals in the present discriminative avoidance paradigm therefore concurrently learned active and passive avoidance behaviors that elicit competing responses, the monitoring of which has been suggested to involve the

ACC (Posner and Digirolamo, 1998). Although lesions of the ACC in the earlier study (Chapter 2) did not lead to behavioral deficits, we have hypothesized that the same lesions would disrupt extinction of a more challenging avoidance task that required an animal to discriminate between cues in order to determine which of the competing response to make to avoid shock.

3.2. Materials and Methods:

3.2.1. Subjects. Subjects were 22 male gerbils from Tumblebrook Farms, West Brookfield, MA (73 - 91g, 3-6 months old) fed ad libitum and individually housed in a temperature-controlled environment on a 12-hour light-dark cycle with the lights on from 7 a.m. to 7 p.m. The animals were divided into a sham group of 10, and an ACC lesion group of 12 gerbils.

3.2.2. Surgical procedure. This is similar to Chapter 2, so please refer to subsection 2.2.2.

3.2.3. Apparatus. This is similar to Chapter 2, so please refer to subsection 2.2.3.

3.2.4. Behavioral Procedure. Gerbils were trained between 1:00 – 7:00 pm. Figure 13a-b illustrate the schematic summary and the complete timeline of the training procedure. There were eight conditioning sessions that included sixty trials each. The sixty trials consisted of thirty Go trials and thirty No Go trials which are presented according to a randomized schedule of Gellerman (1933). A session lasted for 25 minutes and was done one per day. A gerbil was trained to discriminate between two tones. A high tone served as a conditioned stimulus (CS) that signaled the occurrence of the delivery of electric shock, the unconditioned stimulus (US), if a

gerbil did not go over the hurdle within a certain time window (Go trial). The CS was a series of beeping pure tones (4 kHz, 65 dB, 200 ms per beep with a 300 ms interval in between beeps) that would last for six seconds upon which a 4 s footshock (600 μA) would be given in the event of a lack of the required response from the animal. Termination of the CS occurred if a gerbil went over the hurdle. A second tone (1 kHz, 65 dB, 200 ms per beep with a 300 ms interval in between beeps) that served as another CS required a gerbil to withhold the response of going over the hurdle (No Go trial). Otherwise if a gerbil jumps during this trial (false alarm), shock would be presented for 1.5 ms. Intertrial interval was 16 to 20 seconds. The tone assignment of the two CSs was counterbalanced with the respective required responses so that in approximately half of either the sham or lesion group, gerbils were trained to go over the hurdle in response to a low tone and to stay put when a high tone was presented. Three days after the last conditioning session, a gerbil was given either a sham or lesion surgery in the ACC. Recovery period was seven to ten days after which an animal was first given a postoperative conditioning session to ensure that the memory of the CS-US association remained intact. Extinction training commenced the following day where a gerbil was given a session of 60 trials of the CS presentation without the US. A gerbil went through extinction training one session a day for eight days. Seven days after the last extinction session, gerbils were tested for spontaneous recovery in one session of 60 presentations of CS alone. Gerbils were tested for renewal the following day, again in a session of 60 CS presentations. During the renewal session, the shuttlebox where a gerbil was tested, was turned 90° from its usual location, and the sides of the box were covered with white sheets of paper that had blue pasted geometric figures. Animals were then exposed to a presentation of US (shock) alone for one session (60 trials) the day after. The following day, the animals were tested for reinstatement of the extinguished CRs.

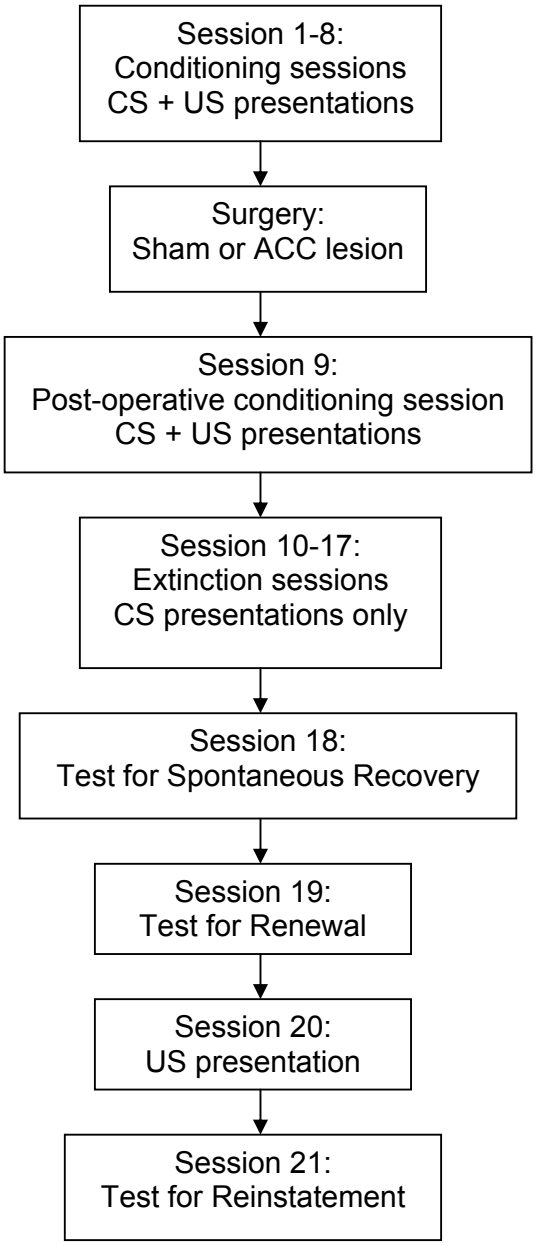

Figure 13a. *Schematic diagram depicting the different phases of the behavioral training.* Gerbils were trained for eight days to respond, by going over a hurdle, to a tone that predicts the delivery of a footshock. Three days after the last conditioning session, either sham or lesion surgeries were done on the animals. After a recovery period of about seven to ten days, animals were given a postoperative conditioning session to make sure the memory representation of the CS-US association remained intact. The following day, extinction training commenced and went on for a total of eight days where the gerbils were presented with just the tone in the same context used during the conditioning sessions. Seven days after the last extinction session, the animals were given another extinction session to test for spontaneous recovery of the extinguished CR. Renewal test was done the next day. The

animals were then given presentations of shock alone followed by a test for reinstatement the following day.

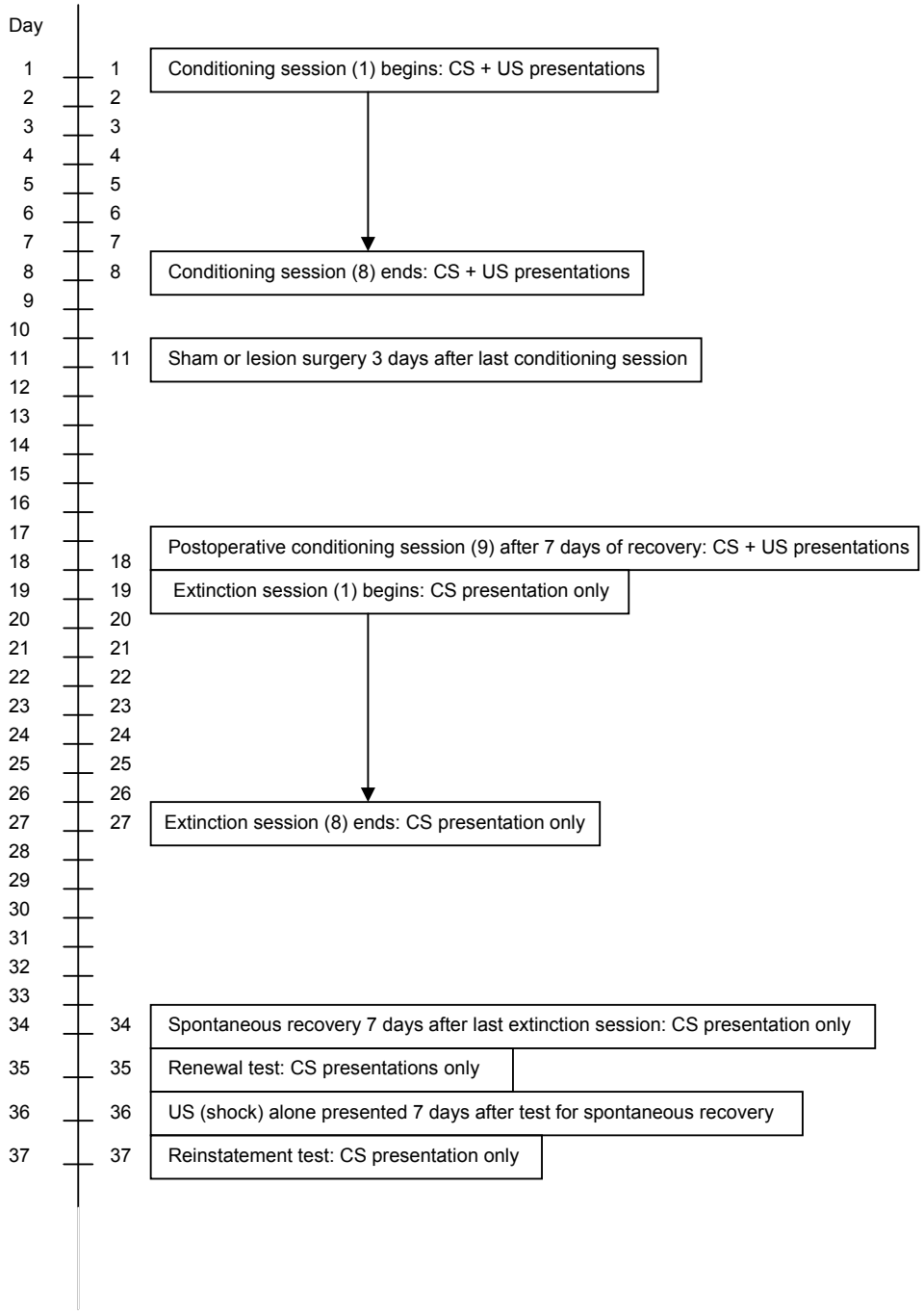

Figure 13b. *Schematic diagram depicting the complete timeline of the training.* The timetable illustrates the duration of the different phases and the gaps (number of days) in between which altogether takes thirty seven days from start to finish.

3.2.5. Histology. After the behavioral training, the gerbils were decapitated and the brains were taken out and frozen in liquid nitrogen (Linde, Germany) for 10 minutes. All brains were stored in a freezer at -80° Celsius. The brains were sliced into coronal sections of 40 μm thick which were stained with thionin, a Nissl stain for cell bodies to determine the extent of the ibotenic acid lesions. To quantify the size of lesion damage, a grid transparency was used and the number of grid squares covering the damaged portion of the ACC was divided by the number of squares of the intact targeted lesion area multiplied by 100. The targeted ACC areas were cingulate cortex area 1 and 2.

3.2.6. Data Analysis. Analyses were done using the statistical program Statistical Package for the Social Sciences (SPSS) version 16.0, USA. The rate of conditioned discriminative responses during conditioning or extinction training sessions was analyzed by using general linear model repeated measures ANOVA. SR, Ren and Rei tests were analyzed using univariate analysis. *d* value is calculated by subtracting the number of incorrect responses during No Go trials from the correct responses during the Go trials and then multiplied by a 100.

3.3. Results

3.3.1. Histological Analysis Targeted lesion area of the ACC was from AP +1.1 mm to –0.4 mm from bregma according to the gerbil atlas (Loskota et al., 1973). Bilateral lesions in the ACC were mainly on the Cg1 and Cg2 area with minimal damage to M2 in some gerbil brains. There were five animals that had lesions extending more anterior than the intended area but still within Cg1. Two of those animals had been trained with high tone as CSgo while the other three with low tone as CSgo. The

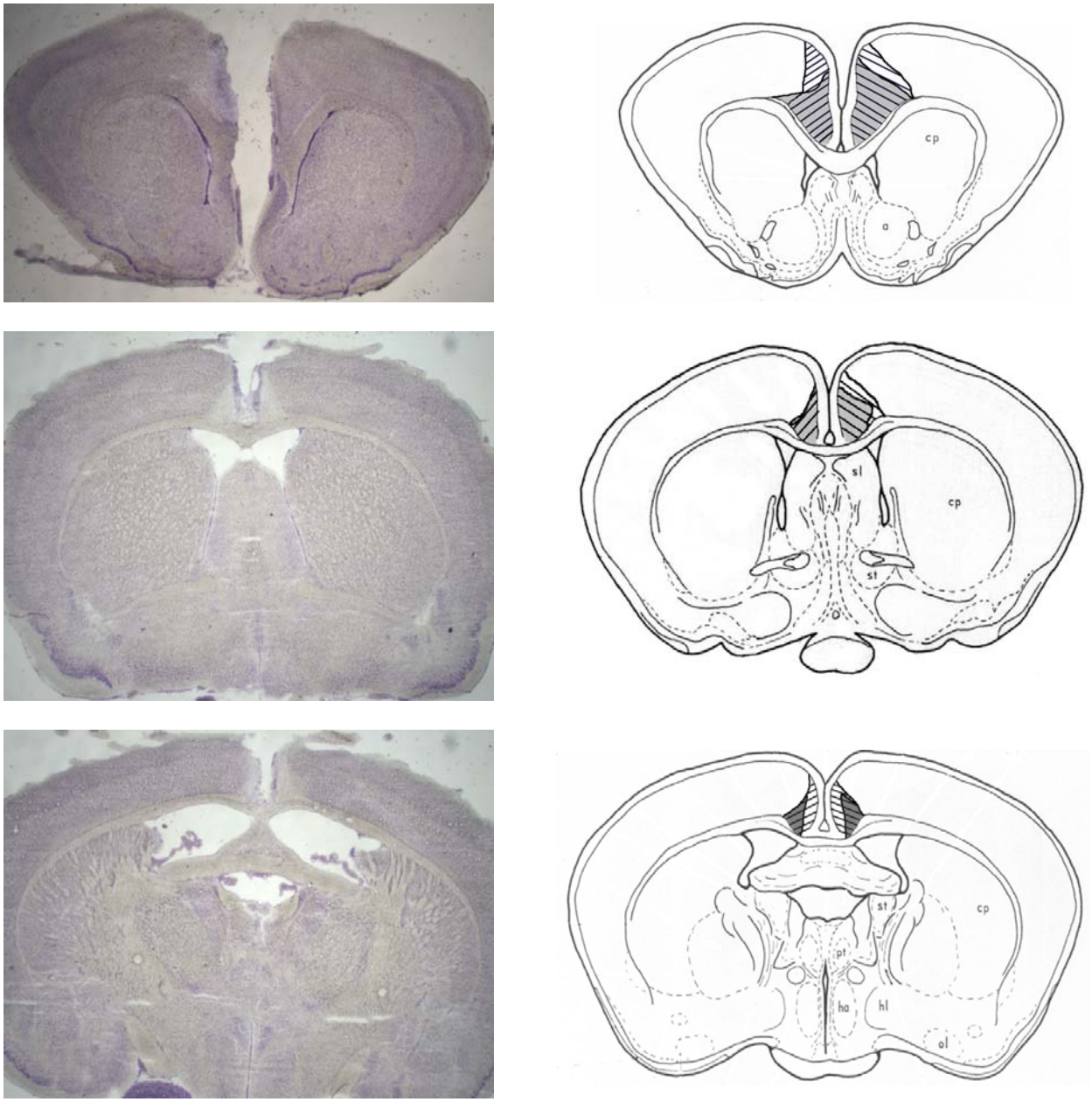

Figure 14. *Coronal sections of the lesion area.* Representative photographs of ACC lesions (left panel) with the corresponding schematic diagrams on the right, depicting ACC lesion placements at, from top to bottom: AP +1.1 mm, +0.1 mm and -0.4 mm from bregma. Gray-shaded areas represent the extent of damage in the gerbil brain with the smallest lesion damage while areas with hatched bars plus the gray-shaded areas represent the gerbil brain with the largest lesion damage.

extent of damage measured in the gerbil brain with the smallest ACC lesion was 66% while the largest ACC lesion was 93% (Fig. 14).

3.3.2. Behavioral Analyses. Analyses of the jumps during the Go trials and the No Go trials showed no significant tone or group effects in the ANOVA but a session effect during the conditioning Go trials (F (7, 12) = 74.966, P = .000) and extinction training Go trials (F (7, 12) = 7.885, P = .001) as expected. The following results are the analyses of the discriminative responses (d value) displayed by the groups.

3.3.2.1. Conditioning. As expected, the only significant difference found was a main effect of session (F (7, 12) = 54.052, P = .000; Fig.15) indicating the acquisition of learning. There was no significant interaction effect of session by group (F (7, 12) = .362, P = .908) session by tone (F (7, 12) = 1.036, P = .456) or session by group by tone (F (7, 12) = .918, P = .525). There was no main effect of group (F (1, 18) = .042, P = .841) or tone (F (1, 18) = .489, P = .493) and no significant group by tone interaction effect (F (1, 18) = .069, P = .796).

3.3.2.2. Postoperative Conditioning. There was a main effect of session (F (1, 18) = 9.971, P = .005) but no interaction effect of session by group (F (1, 18) = .007, P = .935), session by tone (F (1, 18) = 0, P = .985) or session by group by tone (F (1, 18) = 1.161, P = .295) when performance on the last conditioning session and the postoperative session between the ACC and Sham groups were compared (Fig. 15). This may reflect a transient side effect of the surgery on the performance of the animals. There was no main effect of group (F (1, 18) = .210, P = .652) or tone (F (1, 18) = .007, P = .936) or group by tone interaction effect (F (1, 18) = .397, P = .537).

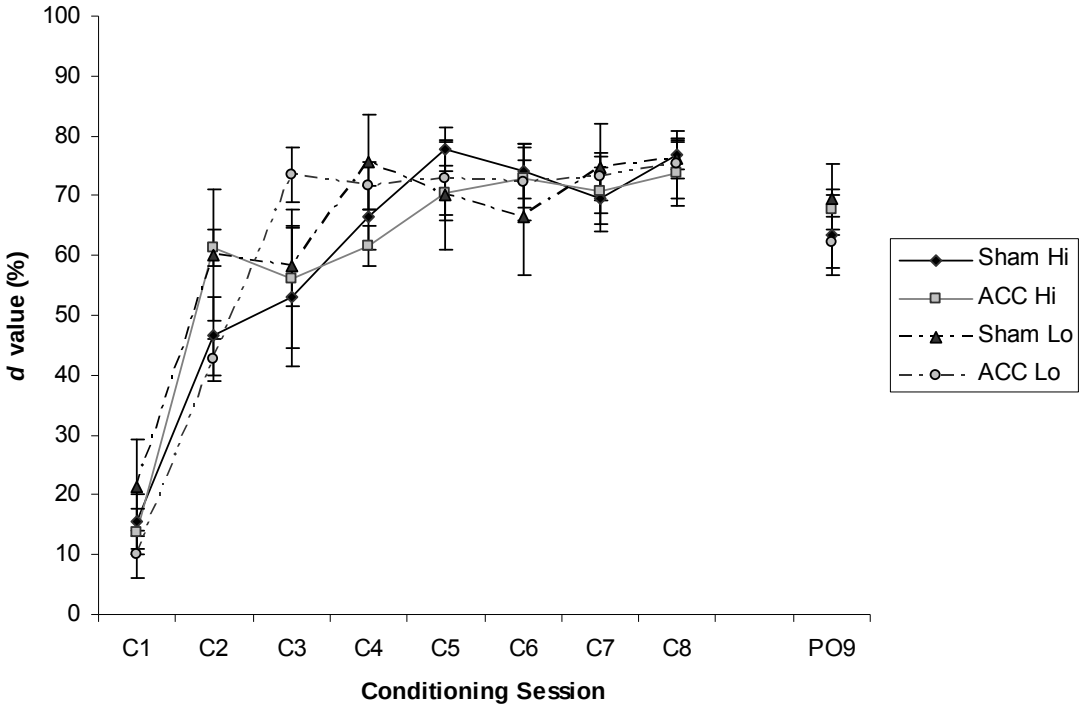

Figure 15. *Group performance summary according to tone frequency used as CSgo and CSno- go during conditioning.* Data points with black geometric shapes and lines represent the scores of the Sham subgroups (n=5 for each subgroup) while points with gray geometric shapes and lines represent the ACC subgroups (n=6 for each subgroup). Data points with solid lines represent scores of gerbils trained with high tone as CSgo and low tone as CSno-go and vice versa for points with broken lines. Only a main effect of session was found during conditioning indicating the acquisition of learning. There was also a main effect of session when performance during the last conditioning session (C8) and the postoperative session (PO9) were compared seemingly indicating a transient effect of the surgery on the performance of the animals.

3.3.2.3. Extinction Learning. Analysis of extinction learning using repeated measures ANOVA showed a significant main session effect (F (7, 12) = 8.295, P = .001) and a session by group by tone interaction effect (F (7, 12) = 3.613, P = .025). There was no session by group (F (7, 12) = .717, P = .660) or session by tone effect (F (7, 12) = 2.009, P = .138) or group by tone effect (F (1, 18) = 1.882, P = .187). There was no main effect of group (F (1, 18) = .010, P = .920) or tone (F (1, 18) = .004, P = .948)

indicating that by themselves, the lesions or the type of tone frequency used as CSgo or CSno-go did not have an effect on extinction learning. However, the effect of the lesion depends on the tone frequency used. As evident from the graph (Fig. 16), lesioned animals trained with high tone as CSgo displayed less discriminative responses on average during extinction training than their Sham counterpart did. Lesioned animals trained with low tone as CSgo displayed more discriminative responses on average compared to their Sham counterpart.

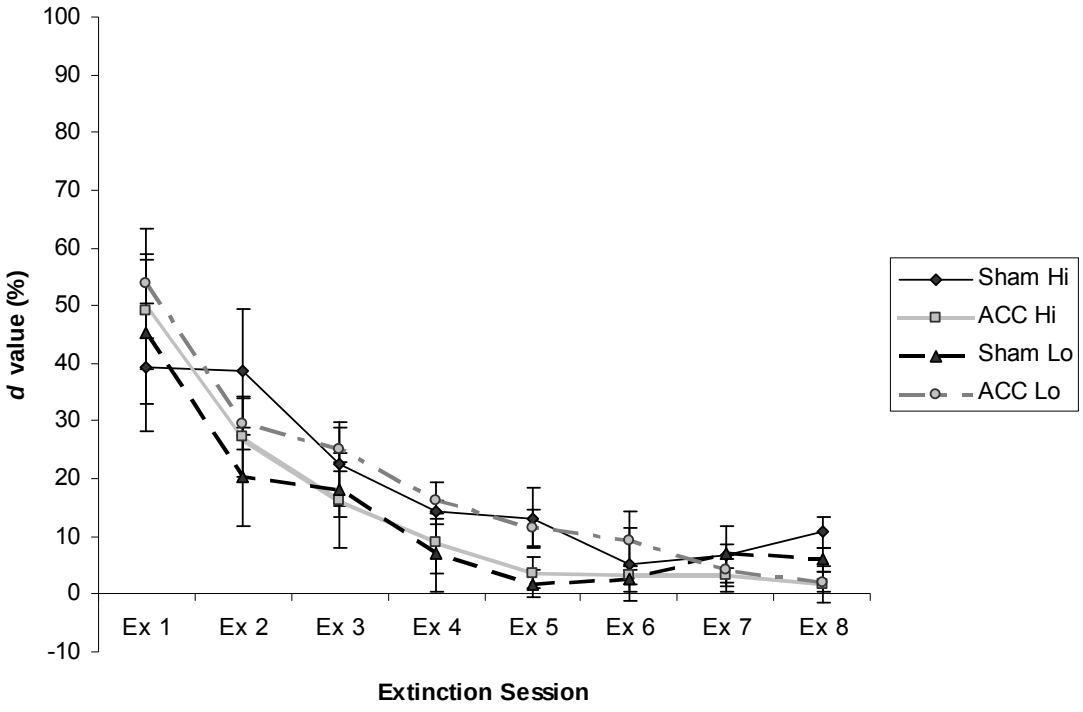

Figure 16. *Group performance summary according to tone frequency used as CSgo and CSno-go during extinction training*. Data points with black geometric shapes and lines represent the scores of the Sham subgroups while points with gray geometric shapes and lines represent the ACC subgroups. Data points with solid lines represent scores of gerbils trained with high tone as CSgo and low tone as CSno-go and vice versa for points with broken lines. Significant session by group by tone interaction effect indicated that performance of the groups was affected by the tone assignment each had.

3.3.2.4. Spontaneous Recovery. Univariate analysis showed no significant group by tone interaction effect (F (1, 18) = .367, P= .552) but a significant main effect of group (F (1, 18) = 5.346, P = .033) and of tone (F (1, 18) = 4.777, P= .042) when gerbils were tested seven days after the last extinction session. The ACC group displayed less extinguished discriminative CRs compared to the sham group regardless if they were either conditioned to jump when the high or low tone is presented (Fig. 17). This effect is consistent with the ACC lesion effect on the spontaneous recovery of an extinguished active avoidance response in a detection task. Interestingly, ACC animals conditioned to jump when a low tone is presented displayed more extinguished discriminative response compared to their high tone counterpart.

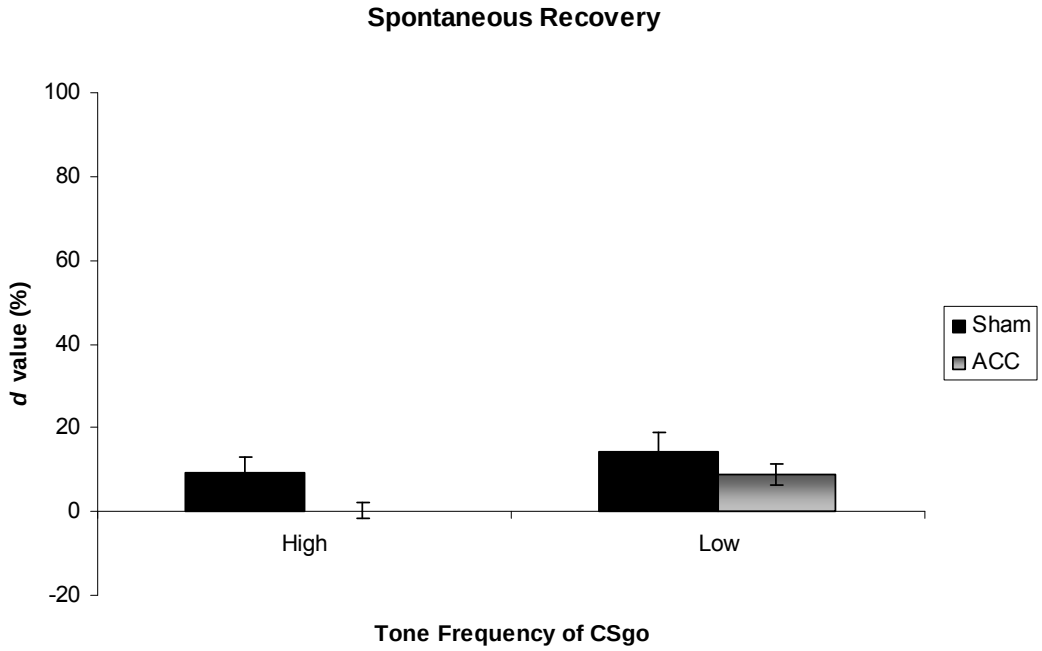

Figure 17. *Discriminative CRs of ACC versus Sham groups during the test for spontaneous recovery*. Solid black bars represent the scores of the Sham subgroups while the two-toned black-gray bar represents the score of an ACC subgroup. The average score of the ACC high tone subgroup is less than one thus, its representative bar is not evident. Lesioned animals displayed significantly less return of extinguished discriminative CRs compared to their sham counterpart.

3.3.2.5 Renewal. Univariate analysis showed a significant group by tone interaction effect (F (1, 18) = 7.782, P = .012) but no main effect of either group (F (1, 18) = .120, P = .733) or tone (F (1, 18) = 3.859, P = .065) when the gerbils were tested in a different spatial context. ACC -lesioned animals trained to jump when a high tone is presented showed less return of discriminative responses on average compared to their sham counterpart while lesioned animals that were trained to jump when a low tone is presented showed more discriminative responses compared to their sham counterpart (Fig. 18).

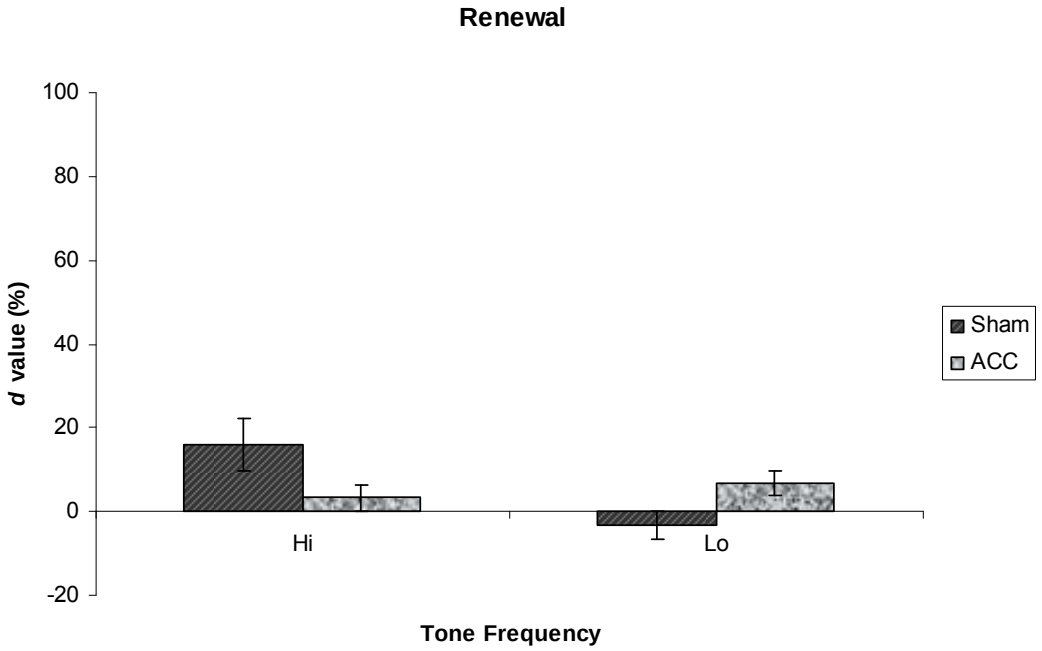

Figure 18. *Discriminative CRs of ACC versus Sham groups during the test for renewal*. Bars with black and gray diagonal stripes represent the Sham subgroups while bars with grayish black spots represent the ACC subgroups. There was no main effect of group nor tone but a group by tone interaction effect. On average, the ACC subgroup trained to jump when a high tone is presented responded less than its sham counterpart while the ACC subgroup trained to jump when a low tone is presented responded more than its sham counterpart when the spatial context is changed.

3.3.2.6. Reinstatement. Univariate analysis showed a significant group by tone interaction effect (F (1, 18) = 9.411, P = .007) but no main effect of group (F (1, 18) = .002, P = .962) or tone (F (1, 18) = 3.940, P = .063) when gerbils were tested after being exposed to shock alone the day before. On average, ACC-lesioned animals that were trained to jump when the high tone is presented responded more than their sham counterpart while lesioned animals trained to jump when the low tone is presented displayed more extinguished discriminative behavior than their sham counterpart (Fig. 19).

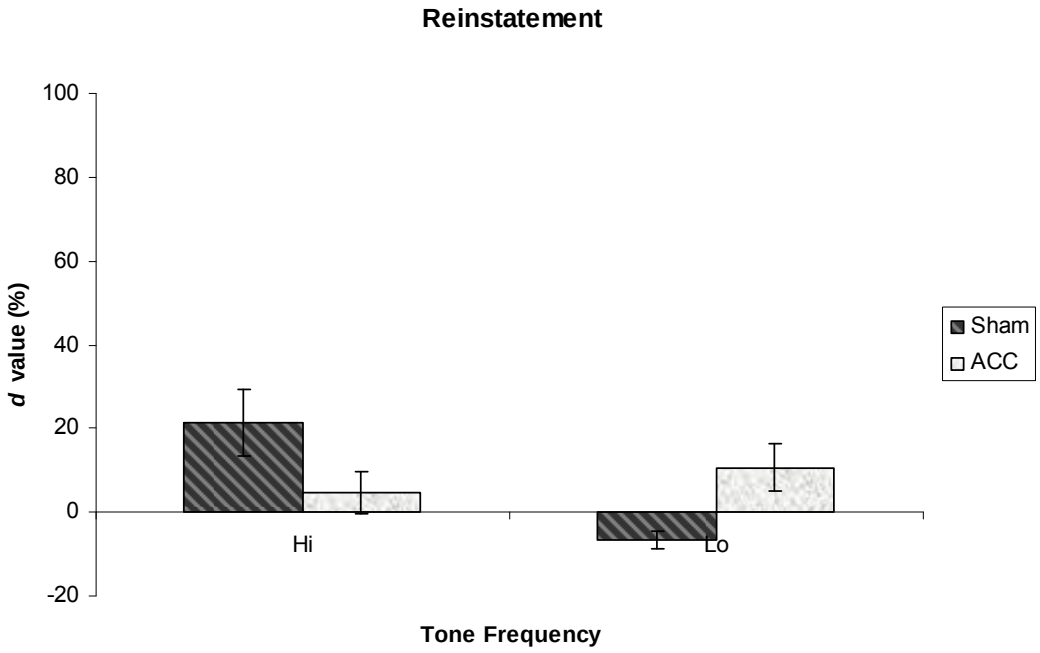

Figure 19*. Discriminative CRs of ACC versus Sham groups during the reinstatement test.* Bars with black and gray diagonal stripes represent the score of the Sham subgroups while bars with light gray spots represent the scores of the ACC subgroups. There was a group by tone interaction effect. On average, the ACC subgroup trained to jump when a high tone is presented responded less than its Sham counterpart while the ACC subgroup trained to jump when a low tone is presented responded more than its Sham counterpart.

3.4. Conclusion

Apparently, lesions of the ACC have a modulatory effect on the discriminative responses displayed by the animals depending on the type of frequency tone used as CSgo and CSnogo during concurrent training of active and passive avoidance. This was evident during extinction training and subsequent retrieval tests. To our knowledge, this is the first time that lesions of the ACC displayed such an effect on the expression of extinguished discriminative behavior based on the tone frequency type used as a signal for an aversive event. Relevant literature that could help explain such results are lacking and thus, the simplest explanation could be that it implies a role of the ACC in modulating the acquired motivational property of a sensory cue to reduce irrelevant responding. It has been noted that unlike high frequency sounds that attenuate with distance, low frequency sounds travel far (Morgan and Hanson-Abbott; 2008) so it is possible that motivational salience of a low frequency tone may need to be modulated so that an organism is less likely to react to it inappropriately. Considering that its source may come from a distance, the probability of an immediate threat would most likely be low unlike when a high tone presentation is perceived. After an extended training such as in avoidance learning, an animal would theoretically display an S-R strategy to solve a task (Packard and McGaugh, 1996). This would mean that once an animal has been well-trained to respond to a CS, its behavior would tend to be more rigid and insensitive to changes in the reinforcer status. So that when the CS is presented, an animal would be more likely to be governed by its habit of responding and thus be more prone to irrelevant responding if left unmodulated by a parallel but competing cognitive system. It has been previously suggested in a CVD study that in the absence of the ACC, an S-R strategy could preside over a more purposeful stimulus-reinforcement strategy (Bussey et al., 1996) which the ACC has been implicated (Bussey et al., 1996;

Bussey et al., 1997, Schweimer and Hauber, 2005) in guiding behavior. In the present experiment, the action tendency of a lesioned animal to 'flee' upon a low tone presentation was specifically training-related since an animal trained with a low tone to stay did not display the same propensity for a flight response. This implication of the ACC modulating the acquired salience with regards to a physical property of a sensory cue is a degree or so away from implications of previous electrophysiological findings that showed increased training-induced activity (TIA) in the ACC when the duration of a CS+ used in a previous training was shortened from 500 ms to 200 ms compared to the TIA in response to a 5000 ms CS (Gabriel, 1993). The author has explained the increase in TIA as a compensatory mechanism for a possible loss of salience due to its diminished duration. Current and latter findings when taken together imply an involvement of the ACC in processing the salience of a CS with consideration to the physical properties of the CS and how these properties can interact with the expression of the associated behavior.

Another explanation could be that association of high frequency sounds with a flight response could be more biologically relevant to the survival of gerbils, thus inherent neural wiring are organized so that responsivity to low frequency sounds are selectively suppressed presumably by a system that involves the ACC. Evolutionary adaptation endows organisms with inherent behavioral predispositions that are compatible with their natural habitat, reflective of how they live. For example, comparative studies have found that marmosets are more likely to discount rewards spatially compared to tamarins who are more likely to discount rewards temporally (Stevens et al., 2005a, 2005b). This was explained in terms of the appropriate behavioral strategies adapted by the species that support the foraging opportunities afforded by their environment. Marmosets subsist on a spatially localized resource

that is replenished at regular intervals while tamarins feed on insects that are spatially distributed. Thus similarly, it is then possible that the present results may reflect biological adaptations as far as the efficiency in how gerbils respond to sounds that signal danger. In their natural habitat, high frequency sounds may have come to serve as cues for a flight response, thus a behavioral predisposition to do so may have developed accordingly. The interaction effect found during the renewal and reinstatement tests seem to allude to this. While the ACC trained with low tone as CSgo displayed more discriminative CRs than its sham counterpart as well as its high-tone counterpart, it is remarkable how the sham counterpart displayed negative discriminative scores during these tests. This means that the sham group trained with low tone as CSgo jumped more in response to the high- than to the low tone. When the context has become ambiguous such that some unfamiliar elements have been introduced to the situation, the performance of the sham-low group may reflect a competition between a propensity to 'flee' when a high tone is presented and its conditioned behavior to 'flee' when a low tone is presented. The behavioral predisposition wins since the conditioned behavior to 'flee' when a low tone is presented has not been well-trained yet in a renewal or reinstatement context. This effect seems to occur when there is an obvious change in context and insensitive to the passage of time with time being an abstract concept which may be why the test for SR did not yield the same outcome.

Consistent with the detection experiment, lesioned animals in the present study showed significantly less return of extinguished (discriminative) CRs compared to the Sham despite the passage of time when tested for SR. This lends further support to a role of the ACC in the expression of extinguished CRs when temporal context has changed. It is rather curious to note that whereas the lesioned animals displayed

significantly less discriminative CRs during SR, this effect was not evident during Ren or Rei, thereby reflecting a diverging mechanism between SR and Ren as well as Rei. Despite that all three phenomena are variants of contextual change, the effect of a change in temporal context in this case, is different from that produced by a change in spatial context or by Rei. The weak return of extinguished CRs displayed here by the lesioned animals that was not only found in the first experiment but in another ACC study that is yet to be completed may be representative of a vital involvement of the ACC in the expression of extinguished CRs when temporal context has changed. By comparison, the lesion effect found during Ren or Rei might instead reflect an incidental ACC function.

Chapter 4
General Discussion

4.1. The role of the ACC in extinction learning

4.1.1. Implications of present findings: Involvement of the ACC in the extinction of avoidance behavior

Current findings suggest a role of the ACC in regulating the expression of extinction memory and its retrieval by modulating the motivational salience of the conditional stimuli. Though the two experiments shared a common lesion technique and behavioral paradigm, the similarities and differences of the results may illustrate the nuances of ACC functioning in the expression of extinction memory of avoidance behavior. Both experiments showed a lack of perseverative behavior that otherwise were apparent in other extinction studies and both have consistently demonstrated ACC involvement in processing the passage of time as a contextual cue. However, though neither showed lesion effects of perseverative behavior, the lesion produced differential effects during extinction learning and the subsequent retrieval of extinction memory. In the extinction of a simple active avoidance behavior, the results showed that without the ACC, expression of behavior deviates from the norm in the negative direction, i.e., the CRs were less than that displayed by the intact animals. Performance of the lesioned animals appeared to not have been weighted by expectancies derived from the previous experience of the animals with the initial CS-US association. Lesioned animals seemed to display a lack of regard for the risk or probability of being shocked. On the other hand, extinction performance of the lesioned animals during extinction of discriminative avoidance responses was comparable to that of the sham animals. It thus cannot be said that ACC lesions

generally produce diminished drive or emotionality that becomes evident during extinction of avoidance behavior. In the extinction of discriminative avoidance behavior using tone frequencies as auditory cues, disruption of the ACC made apparent the differential influence of tone frequency assignment on extinction learning and its memory retrieval during renewal and reinstatement tests. The results of both experiments when taken together indicate a role of the ACC in processing the motivational salience of a CS during extinction of avoidance behavior which carries over to retrieval of its memory when the context has changed. Disruption of the ACC did not lead to any cognitive deficits as at least evidenced by the capacity of the lesioned group to display normal learning during extinction training; or even in contextual conditioning which was tested during reinstatement. These results illustrate situations where the ACC is not critical in stimulus-reinforcement association and updating of CS-US contingency, both of which it has been previously implicated in. What remains to be clarified is how the ACC regulates CS salience.

4.1.2. Implications of earlier findings: Modulatory role of the ACC in emotional processing and motivated behavior

Behavioral changes in terms of emotionality have been observed in animals given lesions in the ACC such as tameness, loss of fear as well as aggression and even dysregulation of autonomic functions (Glees et al., 1950; Smith, 1945; Ward, 1948). However, it should be noted that Pribram and Fulton (1954) reported contradicting results when cingulectomy was done in their monkey study. Later study of patients treated with cingulotomy for intractable pain reported a role of the ACC in modulating emotional experience (Cohen et al., 2001). Disruption of ACC functioning decreased the subjective experience of chronic pain but leaving unimpaired the objective perception of stimulus location and intensity of the pain (Foltz and White, 1962; Hurt

and Ballantine, 1974). This is further supported by a rodent pain assay. Johansen and colleagues (2001) have found that although ACC-lesioned rats still displayed formalin-induced nociceptive behavior such as paw lifting, licking and flinching, they however displayed weak formalin-induced conditioned place avoidance. An imaging study by Yaguez and colleagues (2005) has shown activation of the mid-ACC during a learning phase where a visual cue was paired with an aversive event, a painful esophageal distention. When situations are associated with expectations of decreased pain, Koyama and colleagues (2005) found a reduction in the subjective experience of pain and ACC activation. These illustrate a role of the ACC in regulating basic arousal and the affective processing of sensory stimuli. While disruption of the ACC retarded acquisition of avoidance learning (Gabriel, 1991; Kimble and Gostnell, 1968; McCleary, 1961; Peretz, 1960; Thomas and Slotnick, 1962), it however facilitated appetitive conditioning (Bussey et al., 1996; Peretz, 1960). Interestingly, food deprivation ameliorated the deleterious effect of the lesion on avoidance learning so that the avoidance performance of the lesioned group was comparable to the control group (Thomas and Slotnick, 1963). The authors explained that heightened activity of the animals due to hunger countered their tendency to freeze that then led to a normal acquisition of avoidance learning. Increased anticipatory responding was observed by Bussey and colleagues (1996) in their food-restricted lesioned rats which could suggest hyperactivity. It should be noted that lesions of the ACC though do not necessarily produce hyperactivity or enhance its effects on behavior. Lack of perseverative behavior during extinction in the present study contradicts a notion of a general hyperactivity produced by the lesions. Instead, contradictory results reported by various studies of ACC lesions seem to be in line with the response-modulating hypothesis put forth by McCleary (1961, 1966) that refers to the role of the ACC in response initiation and facilitation.

The differential effects produced by lesions of the ACC on avoidance and appetitive learning may provide a clue behind the lack of perseveration found during extinction learning in the present study that is contrary to lesion effects on the extinction of approach-related behavior. The valence of the reinforcement involved in the conditioning may determine the autonomic influence the ACC may exert. Buchanan and Powell (1993) had listed the various classes of autonomic responses (gastrointestinal motility, papillary dilatation and constriction, thermoregulatory and skin conductance response) evoked by stimulation of the ACC and medial prefrontal cortex (mPFC) as shown by earlier studies (Bailey and Sweet, 1940; Darrow, 1937; Delgado and Livingston, 1948; Kaada, 1951; Hurley-Guis and Neafsey, 1986; Smith, 1945; Ward, 1948; Wilcott, 1968). Critchley (2009) has suggested a close relationship between dorsal ACC activity and enhancement of autonomic arousal. Based on imaging and electrocardiograph (ECG) studies that linked autonomic arousal to mental tasks (Critchley et al., 2003; 2005) he suggested that the ACC mediates changes in sympathetic arousal (including cardiovascular and electrodermal responses) coupled to cognitive processing. It is then plausible that when aversive or appetitive reinforcement is involved, ACC would enhance sympathetic activity to promote a 'fight or flight' response or parasympathetic activity to promote a 'rest and digest' state of learning. Hence when the ACC is ablated, an animal would experience reduced arousal during avoidance conditioning leading to retardation of learning and better extinction performance; and increased arousal leading to facilitation of learning an appetitive task and perseveration during extinction of the behavior. Arguably, these are very simplistic explanations of the contradicting effects produced by the lesions on aversive and appetitive conditioning together with their behavioral extinction. Reduced or increased arousal does not

necessarily lead to impairment or facilitation of learning. For instance, ACC-lesioned rats displayed normal conditioned freezing behavior when fear conditioned (Cardinal et al., 2003). Discrimination studies that require animals to discriminate which CS is associated with reward (Cardinal et al., 2002; Parkinson et al., 2000; Bussey et al., 1997) have shown that ACC-lesioned animals displayed learning deficits by responding to the irrelevant stimuli (CS-) as much as the relevant stimuli (CS+). Cardinal and colleagues (2003) had emphasized that this was not particularly due to a decrease in responding to CS+. This is a rather significant detail since it clarifies that the learning deficit produced by the lesion is not necessarily the inability of the animals to learn the relevance of a (reinforced) CS but to discriminate which feature of presented cues is predictive of reinforcement. This leads to a question of what the basis is of the failure to discriminate when the ACC is disrupted considering its implications in cognitive and emotional processing. Does the lesion produce a failure to attend to the different features of a CS which facilitates discrimination? Or is it possible that the lesion induces an exaggeration of the motivational salience of the reinforced CS so that other cues that share some of its features also become imbued with a reinforcing quality? A similar phenomenon has been found apparent in drug use when Henry, a heroin addict who has been going through treatment, would crave to get high at the sight of any white powder even as innocuous as confectioner's sugar (Friedman and Rusche, 1999).

Counter to lesion effects on discrimination learning revealed in appetitive conditioning, the lesion-induced learning deficit found in avoidance learning was a matter of diminished avoidance responding and not a failure to refrain from responding to an irrelevant stimulus (Gabriel, 1993). While this could be easily interpreted as a failure to learn CS-US contingency, this could alternatively be due to

an induced state of ‘non-action’ that could be related to behavioral initiation which the ACC has been implicated in (Devinsky et al., 1995). This lesion effect is compatible with the freezing responses associated with fear-conditioning which could be the reason why there is no apparent learning deficit displayed by the ACC-lesioned rats when fear-conditioned (Cardinal et al., 2003). This additional piece of descriptive information regarding the ACC lesion effect on aversive conditioning further alludes to plausible antagonistic functional influence of the ACC on appetitive versus aversive conditioning.

An enigma, which would provide the tone in planning future studies, is how ACC functions to regulate CS-driven motivated behavior. Expression of extinguished discriminative CRs in the present study even suggests that salience depending on the physical attributes of a cue and the behavioral significance it has earned may be modulated by the ACC. This was shown as well in an earlier study (Gabriel, 1993) that suggested ACC engagement of compensatory mechanism to promote salience of a behaviorally relevant cue that otherwise would not be so prominent or conspicuous due to its short duration. The implied role of the ACC in the modulation of motivational salience and regulation of motivated behavior in the present study is in line with similar implications in earlier studies.

4.1.3. Motivation and emotions – impetuses to behavior

Through association, neutral cues gain relevance that enables them to direct motivated behavior. Motivation is a driving force that directs goal-oriented behavior. Clark Hull explained motivation in terms of drive reduction or homeostasis that refers to self-regulation of biological variables within a set-point or normal range as explained by Cannon (1939). Sensations such as hunger, thirst and pain motivate

behavior that in turn reduces the drive, thereby placing an organism in an optimal state. Besides sharing a common Latin root word ‘movere’ which means to move, motivation and emotion are closely linked in that emotions have been defined as precursors of motivational phenomena (Parkinson & Colman, 1995) which is exemplified in how reduction of fear, an emotion, drives avoidance behavior. Thus, an emotion can motivate you to perform behaviors based on the valence of the consequences such as an avoidance behavior towards something repulsive or an approach behavior towards something rewarding. A neutral cue that is paired with a consequence (reinforcement) not only serves to predict an event but subsequently earns an emotional quality that is associated with the event. The motivational salience of a cue becomes embedded into the cue so that an animal would even approach the cue predictive of reinforcement despite that its behavior does not influence the occurrence of the reinforcement as seen in autoshaping tasks (Bussey et al., 1997). In pathological cases, exposure to cues associated with the pleasurable experience of drug use could produce intense craving that has been found to activate the thalamo-orbital circuit and the ACC (Volkow et al., 1999).

Emotion has more or less remained an abstract term as it implies a subjective experience that sometimes is not directly or immediately observable. Difficulties exist in distinguishing a raw experience of emotion versus an emotional experience that is colored by the context and its relative meaning to a subject. Different theories of emotions abound but the three more known ones include those of James-Lange, Cannon-Bard, and the unhyphenated Schachter and Singer. William James (1884) and Carl Lange separately arrived at a similar position, now known as the James-Lange theory, regarding how emotions arise. Their view proposes that as the physiological responses to an event arise, one physically reacts and then feels the

emotion so that one is afraid because he is running away from the bear contrary to the common sense view that one runs because s/he is afraid. Their view was countered by Walter Cannon (1927) and Philip Bard saying that the physical event evokes physiological responses and the emotion at the same time. Cannon (1929) explained that visceral reactions that constitute emotions are non-specific so that based on physiological responses, one cannot distinguish between fear and anger since both have identical visceral responses. Schachter and Singer (1962) proposed that with the physiological responses to the event, the feeling is labeled based on what is happening at the time. This was derived from their epinephrine study where the arousal induced by the drug led to an emotional experience (and self-interpretation) that was congruent with the situation they were currently in: subjects that were in a room with an angry actor interpreted their arousal as anger while those who were in a room with a euphoric actor interpreted their arousal as euphoria. A further support of this theory, Dutton and Aron (1974) had shown that men who were interviewed by an attractive woman while they were swaying on a rope bridge mistook the arousal they felt at the time as attraction towards the woman so that 60% of them called her back versus the 30% of the men who were interviewed on solid ground. A fourth theory, the Lazarus theory (1991) builds on the Schachter and Singer theory by stating that an emotion follows after the cognitive appraisal of an event.

Despite all the different theories on what emotions are, it is at least generally agreed that emotions are made up of physiological responses, physical (behavioral) response and the subjective feeling. Biologically, emotion is defined as the complex psychophysiological experience of an individual's state of mind as interacting with biochemical (internal) and environmental (external) influences (Emotion, 2011).

Papez (1937) proposed an underlying neural circuit involved in the integration of emotion and cognition that included the hypothalamus, cingulate gyrus, cingulate bundle, hippocampus, fornix, mamillary bodies, mamillothalamic tract, and anterior thalamic nuclei. MacLean later expanded this circuit to include the limbic lobe (1949) and finally labeling them collectively as the limbic system (1952) which is associated with learning and memory. Being part of the limbic system, the ACC has been considerably implicated in emotional processing. Cingulectomy done in primates led to diminished expression of negative emotions such as loss of aggression and fear (Glees, 1950; Smith, 1945; Ward, 1948). Additionally, the ACC has been implicated in pain processing by electrophysiology (Rios et al., 1999; Sikes and Vogt, 1992; Tarkka and Treede, 1993), imaging (Casey et al., 2001; Coghill et al., 1999; Craig et al., 1996; Davis et al., 1997; Derbyshire et al, 1998; Ploner et al., 2002; Rainville et al. 1997; Tolle et al., Vogt et al., 1996) and inactivation studies done in rats (Johansen et al., 2001; Vaccarino and Melzack, 1989) as well as ablation done on human patients for pain relief (Ballantine et al., 1967; Corkin, 1980; Hurt and Ballantine 1974). Suffice it to say that implication of the ACC in the subjective experience of pain and affective responses to noxious stimuli therefore extends to avoidance learning since the former produces the latter. A cue that signals a painful event would elicit affective responses that are modulated by the ACC. In the extinction of what has been conditioned during avoidance learning, engagement of the ACC would include the cognitive and the emotional updating of the available information considering its previous implications in both processes. But this dual processing attributed to the ACC would suggest an interplay between the two that in turn provides a challenge in characterizing its role more specifically.

4.2. Extinction of Avoidance Behavior – What is extinguished?

The formation of an association during conditioning and extinction training has been basically defined as CS-US memory representation and inhibitory CS-US (CS-no US) memory representation, respectively. Simply put, presenting the CS in temporal proximity with the US endows the CS a predictive property that conditions an animal to make the appropriate response to avoid the unpleasant event. In a subsequent training, presenting the CS without the US leads to extinction that is evident in the decline of the display of CRs; thereby indicating a modification of the CS-US memory representation. Perhaps because of its utility in explaining avoidance behavior in terms of the influence of negative affect, Mowrer's two-factor theory remains to be influential despite criticisms raised against it. The theory takes into account the interplay of two types of conditioning, Pavlovian and instrumental, that occur during avoidance learning which helps explain the persistence of behavioral response despite the absence of concrete reinforcement in successful trials. Through its association with an aversive event, the CS gains a motivational property as it induces fear. Thus the resulting avoidance behavior has been explained as being reinforced by a reduction of fear. However, since successful avoidance trials keep an animal from experiencing fear, it becomes questionable to what extent this holds true. Further, the behavior may be more out of habit than fear since an animal would develop a more automatic S-R behavior with continued practice. This is not far-fetched since it is compatible with the theory of parallel memory systems (White and McDonald, 2002) and findings that illustrated a shift from a goal-oriented cognitive behavioral strategy during the early phase of training towards an automatic S-R strategy as an animal is given extended training (Chang and Gold, 2003; Hicks, 1964; Noblejas, 2005; Packard and McGaugh, 1996; Ritchie et al., 1950) even in nonmotor learning such as a verbal response selection task (Raichle et al., 1994). It

has been pointed out that an S-R strategy bears adaptive significance in terms of saving cognitive resources in procedural tasks which do not require much mental effort. Moreover, it serves functional efficacy when a delay in response or reaction time could prove fatal had one resorted to a slow(er), calculating cognitive strategy. For example, as one sits in the middle of an intersection waiting for his turn to turn left, a fast(er) S-R strategy will help a motorist take advantage of the soonest opening he could get to make his turn. In this case, even a few milliseconds of delay can spell disaster (accident) at the very worst or angry honking by fellow motorists at the very least.

Considering the two types of conditioning involved in avoidance behavior, it is rather a reasonable question to ask whether both types of CRs, an emotional Pavlovian response (fear) and a mechanistic instrumental response (avoidance behavior) to the CS get extinguished in any given extinction paradigm. Dissonance between cognition and the related autonomic conditioning have been illustrated in a study where the galvanic skin response (GSR) of subjects previously conditioned to a tone-shock contingency, actually increased during extinction when the CS and the US were unpaired (see Gray, 1975). Despite that the subjects were cognizant of the unpairing, the related autonomic response seem to belie that. In another tone-shock conditioning study, a similar result was found where there was a lack of discriminative GSR displayed by the subjects in response to two different control stimuli, a stimulus that was randomly paired with the US and another that was not paired at all (Furedy et al., 1977). There was a difference in the performance of the subjects (as measured by the subject contingency index that indicates their expectation of the occurrence of the US) in their response to the two control stimuli yet their corresponding GSRs did not differ. These studies illustrate dissociation between autonomic and cognitive

processes that justifies examination whether the memory representation of both types of CRs in avoidance learning do get modified during any given extinction training paradigm.

Standard extinction training involves the presentation of the CS without the subsequent US presentation versus the random pairing (unpairing) of the CS and the US. The omission of the US has been considered non-associative and deemed as an inadequate extinction procedure when addressing the extinction of Pavlovian CRs since the absence of the US does not allow the loss of the motivational property of the US (Rescorla, 1967). In a Pavlovian conditioning preparation, the strength of the extinction memory acquired by the omission of the US has been challenged by the more durable representation of the extinction memory acquired by the subsequent unpairing of the CS-US. Frey and Butler (1977) have shown in eyeblink conditioning experiments that while the former paradigm results in a faster rate of response loss compared to the latter during extinction training, it also brought about greater responding during reacquisition compared to the latter design. The result was robust even when the CS-US interval was increased from 400 ms during the conditioning phase to 1000 ms during the reacquisition phase after extinction training. The study demonstrated that explicit unpairing of the CS and US was more effective in weakening the motivational salience of the CS even when the original temporal interval was manipulated. However, while it may seem like a more sophisticated approach to weakening the initial CS-US memory representation, its applicability to clinical applications is for obvious ethical reasons null. Besides, it may not yield comparable results when the degradation of the initial CS-US memory representation is tested outside of the extinction training context that in a natural setting does not include the US. Perhaps then, this would serve as a true test of the superiority of the

unpairing paradigm if the same weaker display of CRs would be evident as well. In any case, the comparative study gives one pause for thought. The phenomenon may depict a distinction between true updating of the CS-US association that occurs during the subsequent unpairing of the CS-US versus new learning that occurs in the presentation of the CS alone. This may spell a difference in how each is neurally represented. In the unpairing extinction preparation, the original memory trace of the CS-US association may have been modified, hence effectively undermining the initial CS-US relationship resulting in weaker retrieval. In the CS-alone extinction preparation, a parallel CS-no US memory trace is formed that may explain the faster decline of CRs during extinction training compared to the unpaired design.

4.3. Contextual modulation of extinction memory retrieval – but what about the CS?

The power of cueing is illustrated in so many situations, sometimes even subtle in its assertion yet effecting an undeniable impact. For example, food intake of college students seated at unbussed tables (wing bones left on the table) were less than those seated at bussed tables in a sports bar that served chicken wing buffet (Wansink and Payne, 2007). Apparently, a representation of food consumption is enough to modulate the drive to eat which may reflect the activation of a personal memory representation of a similar experience and what it stands for. Despite being immersed in an ambience of dining, discrete cues that represent completion of the intended behavior apparently dampened the drive. A key factor that drives behavior is the associative valence a cue earns that is modulated by context. In extinction, much focus has been given to the contextual control of the expression of the extinction memory. The generally accepted notion of the context-dependence of extinction memories as the limiting factor in its retrieval has been supported by its

weak expression evident when extinguished responses return in tests such as spontaneous recovery, renewal and reinstatement. Despite that a conditioning memory after extinction training also becomes context-dependent, not much attention has been directed towards the significance of this finding (Harris and Westbrook, 1998; Effting and Kindt, 2007). For example, when conditioning is done in context A, extinction in context B, and retrieval test in context B, the CS-no US is activated (Herry and Garcia, 2002) despite that there is a change in temporal context that could impede its retrieval. This leaves room for questioning whether it means that information about the spatial context carries more weight than the temporal context in governing behavior; or if it is enough that only one cue that is familiar with the extinction context be present during a retention test for the extinction memory to be expressed. In another situation of contextual change, comparative analysis of a difference in a renewal design has revealed a weaker return of the extinguished CRs in an ABC design compared to an ABA design. As a secondary associative memory, if retrieval of extinction memory is indeed inferior to that of conditioning memory and is context-dependent, then there should not be a difference in the magnitude of the return of extinguished CRs during a renewal test in a specially unfamiliar context that an ABC design is. In still another example, the effect of reinstatement may only be apparent if the test is done in the same context as where the US is presented (Baker et al., 1991; Bouton and Bolles, 1979). This means that mere exposure to the US alone does not retrieve the associative memory linked to the US (the conditioning memory) despite that the alternative associative memory (the extinction memory) has no direct link to the US. These findings indicate that the conditioning memory is just as context-dependent as the extinction memory; and that the well documented context-dependence of the extinction memory may perhaps be partly due to the lack

of sensitivity of the task design to detect the context-dependence of a conditioning memory.

Performance of an animal during retrieval tests of extinction memory reflects the influence of its prior experience with the CS, both the excitatory and inhibitory associations. Recognizing the context as an occasion-setter and investigating the effects of contextual change on the expression of extinction memory have provided us insights into how to manipulate aspects of the extinction context to improve retrieval of extinction memory. For instance, widely spacing extinction trials during extinction treatment of conditioned fear has been found to weaken the effect of SR and Ren (Urcelay et al., 2009). An overlooked issue however, is how the CS and its different properties may influence performance. Emphasis on contextual modulation of behavioral expression has inadvertently taken away much needed attention to understanding how a conditioned cue could drive behavior. In autoshaping for instance, repeated pairing of a lever CS with a food US would lead an animal to act to a lever like it would to food: grasping the lever with its paws, licking and chewing it as if it were food (Tomie et al., 1989); and this is despite that US presentation is independent of its behavior towards the CS. Moreover, CS presentation has been found to elicit feeding in sated rats within five seconds in a test that allowed ad lib access to food (Weingarten, 1983), thereby countering the drive reduction theory of behavior. A cue becomes endowed with motivational salience that is apart from its predictive attribute. By association, a CS gains a signaling property as it becomes predictive of an event and an affective property that is determined by the valence of the reinforcement it has been associated with. Dissociating these properties and how these could influence performance in a new situation could provide better understanding on how to manipulate aspects of the CS or counter its effects for

better retrieval of extinction memory when the context has changed. Perchance we could identify the wing bones that could dampen the expression of conditioned memories no longer adaptive.

4.4. Study Proposal

4.4.1. When the avoidance conditioning model is used, do both the Pavlovian and instrumental conditioned responses get extinguished in any given extinction paradigm?

The decline in the display of CRs during extinction training is indicative of extinction of the instrumental behavior but inferences can only be made that the same holds true of the physiological Pavlovian responses which are involuntary and internally generated. In the first experiment, the ACC-lesioned group consistently displayed less CRs compared to the sham group during extinction training and even during retrieval tests, thus suggesting attenuation of the negative motivational salience of the CS evident in different contexts. However in the second experiment, ACC-lesioned gerbils displayed a weak return of the extinguished CRs during the test of SR in contrast to their performance during extinction learning and other retrieval tests. The differential influence of the extinguished CS on the behavior of the animals suggests that the extinguished CS may bear different degrees of emotional salience. To address this query, a future study will be conducted that measures autonomic responses during avoidance learning, extinction training and subsequent memory retrieval tests. Specifically, an autonomic index of interest is heart rate (HR) which can be recorded by an ECG. While it has been shown that HR CRs are reduced when the CS is presented alone in fear conditioning preparation (Burhans et al., 2010), it would be of interest how the HR CRs may be characterized not only during extinction but during avoidance training as well. While fear reduction is posited to

drive the avoidance behavior at least perhaps initially, an animal may subsequently develop cognizance of its control over the experience of shock exposure through its avoidance response. This would degrade the role of fear (reduction) as a primary motivator. Moreover with extended training, an S-R memory (habit-driven) system theoretically may prevail in governing behavior thus there may be a difference in the quality of HR CRs during the early and latter part of training. This may be relevant in examining the decline of HR CRs during extinction and its return during subsequent extinction memory retention tests.

Another set of information that ECG recordings could afford us is the analysis of heart rate variability (HRV) during training. The different components of HRV such as the high frequency and the very low frequency have been found to be markers of parasympathetic and sympathetic activity, respectively. With the aid of electrical stimulation, this will allow us to elucidate the kind of autonomic influence the ACC may have on appetitive and aversive conditioning that may be differential as suggested by the lesion effects revealed in earlier studies.

4.4.2. How do the different properties of a CS influence performance when placed in an unfamiliar context where the previously experienced CS is presented?

The enigma that the return of extinguished behavior presents whenever an animal experiences the CS in a context different from the extinction context may be related to a form of transfer of learning. Previous findings have shown that disruption of the ACC would lead to deficits in discrimination where lesioned animals would respond to irrelevant stimuli which share some characteristics with the relevant stimuli. Moreover, it has been shown that human participants are quick to respond to a target that has a color recently associated with reward (Hickey et al., 2010). These findings

suggest that perceptual features or properties of the CS may become imbued with motivational salience that enables the CS to elicit a similar reinforcement-related response when the CS is presented in a novel or unfamiliar context. Characterizing the different properties of a CS merits investigative consideration since it gains not only a signaling property related to its perceptual features but also an affective property which is related to its motivational significance. A variant of a Pavlovian to instrumental transfer (PIT) paradigm could prove to be helpful in assessing which of the CS properties would guide behavior of an animal in a new learning situation. The two stages of learning in this paradigm will allow an opportunity to investigate which property of the previously experienced CS during the first stage will an animal use when faced in a new learning situation in the second stage. For example, a high tone will be used as CS+ which upon presentation will lead to an automatic dispensing of food pellets while presentation of CS- will be of no consequence. Once an animal reliably shows discriminative approach it will be exposed to another outcome (this time unsignaled), i.e. shock, in a different context. After which the animal will be given signaled trials with the CSs that it had previously experienced in the first stage of training. A group of animals will be trained to jump to the CS that used to be predictive of food but now will be a signal for a different event, i.e. shock, while the CS- is of no consequence. Another group of animals will be trained to jump to the previous CS- that used to be of no consequence while the previous CS+ will now be of no consequence. This paradigm will allow us to examine if an animal will use the predictive or the affective property of the CS to guide its behavior in a new learning situation that uses the same CSs it had previous experience with. If the first group learns faster, then it indicates that an animal when presented with previously conditioned stimuli in a new context, will use the predictive property of the CS; i.e., the memory representation of the CS+ as predictive of an event no matter the

outcome. If the second group learns faster, then it indicates that an animal will use the affective property of the CS; the positive valence attached to the former CS+ which is now CS- serves as a signal for a positive event which in this training stage means safety while the negative valence (the absence of food) attached to the former CS- which is now CS+ serves as a signal for a negative event which in this training stage is a shock presentation.

It is possible that there may be no difference between the two groups. But after a subsequent extinction training, we can examine if the same CS property that drove behavior during learning transfer would also do the same during retrieval of extinction memory. Would the animals that used the signalling property of the CS to guide its behavior in a new learning context show greater return of extinguished CRs during Ren? Would the animals that used the affective property of the CS to guide its behavior in a new learning context show greater return of extinguished CRs during Rei?

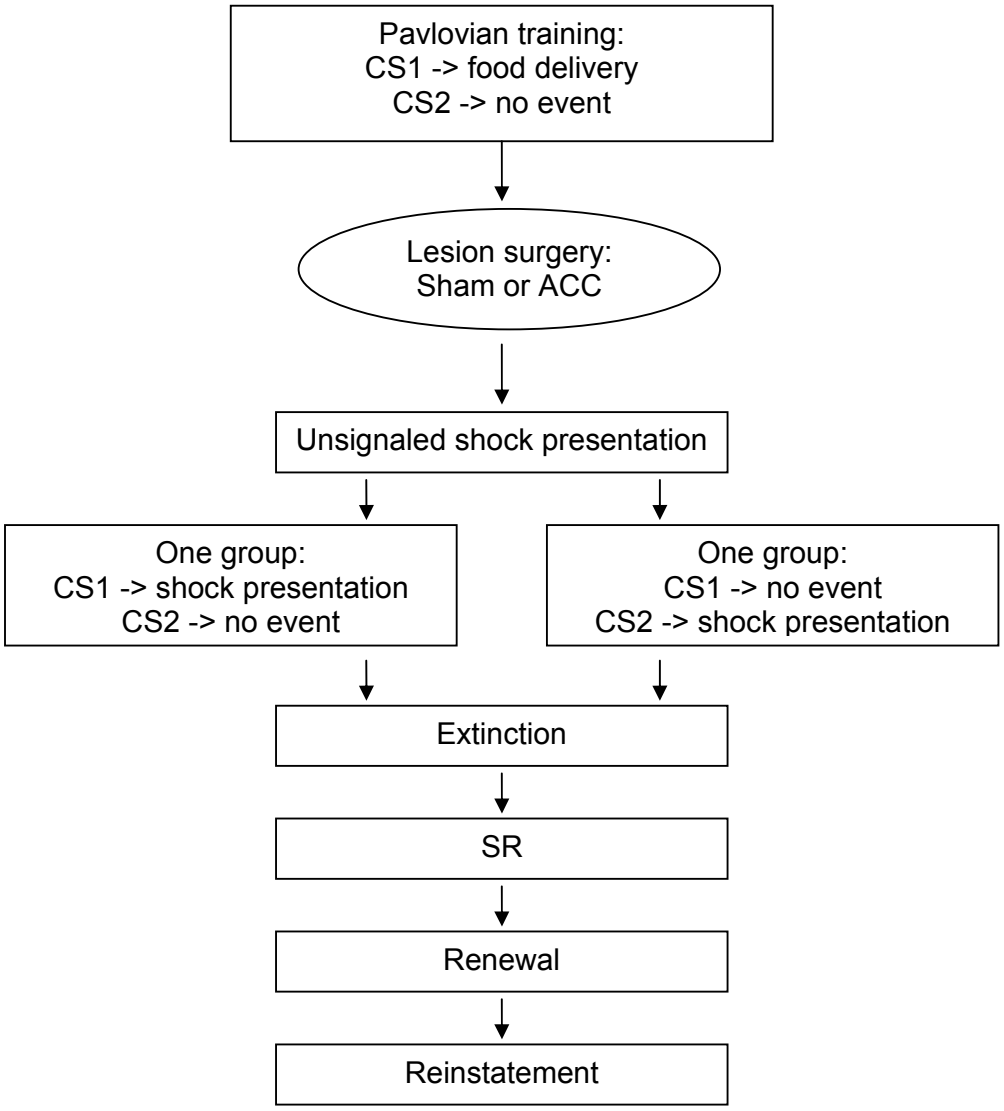

Figure 20. *Schematic diagram of a proposed Pavlovian to Instrumental Transfer (PIT) paradigm.* A PIT variant would be used to examine which CS property influences behavioral response of an animal in a new learning situation (please refer to 4.4.2 for further explanation).

4.5. Summary

Extinction learning allows modification of behavior, i.e. response suppression, when a previously relevant cue later gains a second meaning of being no longer relevant.

Various brain structures have been identified to be involved in behavioral extinction including the anterior cingulate cortex (ACC). However, while the role of the ACC in the extinction of appetitive conditioning has been explored through its inactivation, little is known about its role in the extinction of aversive conditioning. The present study explores the role of the ACC in the extinction of avoidance behavior. In the first experiment, gerbils were first conditioned to avoid footshock in a shuttlebox by jumping over the hurdle when a CS is presented. After eight conditioning sessions, gerbils were given three days of rest before either sham or ACC lesion surgery was done. After a week of recovery period, gerbils were first given a conditioning session to ensure the conditioning memory remained intact before extinction training was commenced. During the extinction training, the CS is presented again but this time without the subsequent shock presentation. Gerbils were trained for eight extinction sessions. Spontaneous recovery was tested seven days after the last extinction session and the following week, gerbils were exposed to shock alone before being tested for reinstatement the next day. Lesions of the ACC did not lead to perseverative behavior but instead to less hurdle jumping during extinction of an active avoidance task as well as during its subsequent memory retrieval tests.

In the second experiment, gerbils were trained in a shuttlebox to discriminate between two pure tones (of high or low tone frequency) that signaled them to either jump over the hurdle or stay depending on the tone assignment, to avoid footshock (Go/No Go discrimination). The tone assignment was counterbalanced so that half of the gerbils were trained to jump when a high tone was presented and to stay when a low tone was presented while the other half were trained to jump when a low tone was presented and to stay when a high tone was presented. Gerbils were given eight conditioning sessions and three days of rest afterwards before either sham or ACC

lesion surgery was done. After a week of recovery, gerbils were first given a conditioning session before extinction commenced the next day. Extinction training went on for eight sessions where the two tones were presented without the subsequent shock presentation. Seven days after the last extinction session, gerbils were tested for spontaneous recovery then renewal the following day. During the renewal test, the shuttlebox was covered with paper and turned 90º to create a context different from the extinction training context. The following day, gerbils were presented with footshock in the same context as that during extinction training to test for reinstatement the next day. Results indicate that effects of ACC lesions on the extinction of discriminative avoidance responses did not include perseverative behavior but however differentially modulated extinction performance and its memory retrieval during the renewal and reinstatement tests depending on the tone assignment. Animals trained to flee when low tone is presented and to stay when high tone is presented tend to display more discriminative CRs on average than their high CSgo - low CSnogo counterpart or sham counterpart. The results taken together imply a role of the ACC in differentially modulating the motivational salience of CSs. The consistent lesion effect during the test of SR of both simple avoidance and discriminative avoidance behavior indicates a significant role of the ACC in temporal processing. However, it remains to be examined what its role is in temporal processing: whether it is in encoding the interval between training events, the perception of the passage of time or a gating role in the expression of the initial or secondary meaning of a CS when temporal context has changed. Future studies will delve more into the role of the ACC in the autonomic aspect of learning by recording heart rate and in learning transfer as the CS gains more than one meaning, paying closer attention to delineating the different properties of a CS that would guide behavior in a different learning situation.

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