

**ROLE OF RAT ANTERIOR CINGULATE CORTEX IN EFFORT- AND  
COURAGE-BASED DECISION MAKING**

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BACHELOR OF ARTS (HONS.), UNIVERSITY OF BRITISH COLUMBIA, 2010**

A Thesis  
Submitted to the School of Graduate Studies  
of the University of Lethbridge  
in Partial Fulfillment of the  
Requirements for the Degree

**MASTER OF SCIENCE**

Neuroscience  
University of Lethbridge  
LETHBRIDGE, ALBERTA, CANADA

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## Abstract

When given a choice between getting a high reward that requires climbing a high ramp or pressing a lever multiple times, versus freely obtaining a low reward, healthy rats prefer the former, while rats with lesions to the anterior cingulate cortex (ACC) prefer the latter. We developed two novel effort tasks to examine if ACC mediates other types of physical effort (weight-lifting) as well as emotional effort (courage). We replicated previous findings on a modified version of the ramp-climbing task, showing that ACC lesions impair these decisions. Lesions of ACC did not impair weight-lifting effort, even when higher levels of effort were used and training on the task was eliminated. Initially, lesions of ACC did not impair courage effort. When the task effort was subsequently increased, rats with ACC lesions showed a failure to adapt to novelty throughout testing. This research indicated that not all effort is mediated by ACC.

## Acknowledgements

Special thanks goes to the following people who consistently contributed to the implementation of this project and/or my academic development over the past two years: Dr. Jonathan Brown for assisting with statistics and unconditional support, Nathan House for programming all in-house maze software programs; Jenni Karl for data brainstorming; Valerie Lapointe for assisting in histological procedures; Amanda Mauthe-Kaddoura for alleviating bureaucratic burdens and for moral support; Geoffrey Minors for assisting with design of mazes and electronics; Heather Pirot for assisting with surgery, histology and running experiments; David Sessford for adjusting and writing analysis files and maze software; Dr. Hendrik Steenland for providing guidance with surgical procedures; Jennifer VanOyen for writing analysis programs, maze software, and running experiments; Melinda Wang for teaching me stereological and histological methods; Dr. Ian Whishaw for providing mentorship; Dr. Aaron Wilber for providing guidance with temporary inactivation and histological procedures; and Hiroe Yamazaki for assisting with surgery and stereology.

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## List of Abbreviations

ACC = anterior cingulate cortex

BLA = basolateral amygdala

Cg1 = field 1 of anterior cingulate cortex

Cg2 = field 2 of anterior cingulate cortex

DA = dopamine

dIPFC = dorsolateral prefrontal cortex

HRA = high-reward arm

HRL = high-reward lever

IL = infralimbic cortex

LRA = low-reward arm

LRL = low-reward lever

M2 = secondary motor cortex

mPFC = medial prefrontal cortex

NAcc = nucleus accumbens

OFC = orbitofrontal cortex

PFC = prefrontal cortex

PL = prelimbic cortex

SEM = standard error of the mean

SME = simple main effect

# **Role of Rat Anterior Cingulate Cortex in Effort- And Courage-Based Decision Making**

## **Chapter 1: Introduction**

In a world that continually presents us with innumerable choices, we are faced with having to make decisions on a daily basis. Consequently, without the capability of deciding which brand of cereal to select from the shelf or which shirt to wear in the morning, we would never get out of the store or be on time for work. Because of their significance in everyday life, decision-making processes have been extensively studied over the past three decades. It is now known that decision making, like other executive functions (Uylings, Groenewegen, & Kolb, 2003), recruits prefrontal cortex (PFC) in humans (Walton, Kennerley, Bannerman, Phillips, & Rushworth, 2006), non-human primates (Bechara, Damasio, Damasio, & Anderson, 1994) and rodents (Walton, Bannerman, & Rushworth, 2002) . Many early conjectures about PFC function came from work on patients with frontal lobe damage. The widely cited case of Phineas Gage (Harlow, 1848) marks a milestone in the literature by deducing the first conclusions about frontal lobe injury and its implications. Harlow (1848) recounted his medical assessment of Mr. Gage who, working on a railroad track, was in a blasting accident that caused an iron rod to pass through his frontal lobe. In detail, the 3-foot-7-inch long tamping iron entered at the left cheek, passed through the lower orbit of the eye into the skull and brain, and exited at the intersection of coronal and sagittal sutures close to the midline. Despite a physical recovery, Gage's coworkers described that he was no longer himself, had a changed personality, was obnoxious, unable to uphold his commitments, and drawn to making poor decisions (Burns & Bechara, 2007; Damasio, Grabowski, Frank,

Galaburda, & Damasio, 1994; Harlow, 1868). Overall it seemed Gage was no longer able to plan or execute rational behaviours (Harlow, 1868). Damasio et al. (1994) described it as an inability to make rational decisions and to process emotion. In an effort to characterize the locus of brain damage more precisely, Damasio and colleagues (1994) reconstructed the injury and concluded that Gage had received extensive damage to the anterior portion of the orbital frontal cortex, polar and anterior medial frontal cortices, and most anterior portion of the anterior cingulate gyrus.

The behavioural deficits Gage exhibited were largely confined to executive functions, while other behaviours remained relatively intact. This account of damage to PFC and the specific resulting deficits spiked an interest in investigating PFC function. Later work has found that these prefrontal regions play a key role in decision making (Fellows, 2007; Kennerley & Walton, 2011) and impulsivity (Chudasama et al., 2003; Dalley, Cardinal, & Robbins, 2004). Prefrontal functions have since been heavily investigated. While this brief anecdote of Phineas Gage highlights the overall importance of PFC, the main focus of this thesis will be on the role of PFC in decision making.

This thesis will explore the role of rat anterior cingulate cortex (ACC), a subregion of PFC, in cost-benefit decision making. To understand the role of PFC in general, and ACC in particular, in decision making, a discussion of the anatomy and connectivity of PFC is necessary. Thus, Chapter 1 serves the purpose of elucidating PFC's interconnectedness with other areas of the brain important to decision making, such as reward centres (e.g., the striatum), emotional centres (e.g., the amygdala), and structures that integrate and relay other inputs to PFC (e.g., the thalamus). In Chapter 2, the part of PFC that is hypothesized to mediate certain decisions, the ACC, will be

discussed with focus on its functions in pertinent decision-making paradigms, including the type of cost-benefit decisions studied in the present thesis, effort-based decisions. Existing literature on effort-based decision making will be discussed and questions from these studies will be raised. In Chapter 3, a detailed rationale for the present experiments will be provided. Further, Chapters 3, 4, and 5 will present the three experiments conducted for this thesis and discuss their findings. Lastly, Chapter 6 will provide a conclusion of what the present experiments mean in the broader context of the decision-making literature.

### **1.1 Anatomy of Prefrontal Cortex (PFC) in the Rat**

Before discussing PFC function, some anatomical connections will be discussed. This is necessary, as it highlights the rich interconnections PFC has with cortical, subcortical, and limbic systems. It is because of this interconnectedness that PFC plays such a central role in a vast array of behaviours. The scope of anatomical studies on PFC is very broad. Because PFC is not a homologous entity across species, this section will focus on the anatomy in the rat. Parallels to human and non-human primates will be drawn where comparison of these species might aid the reader.

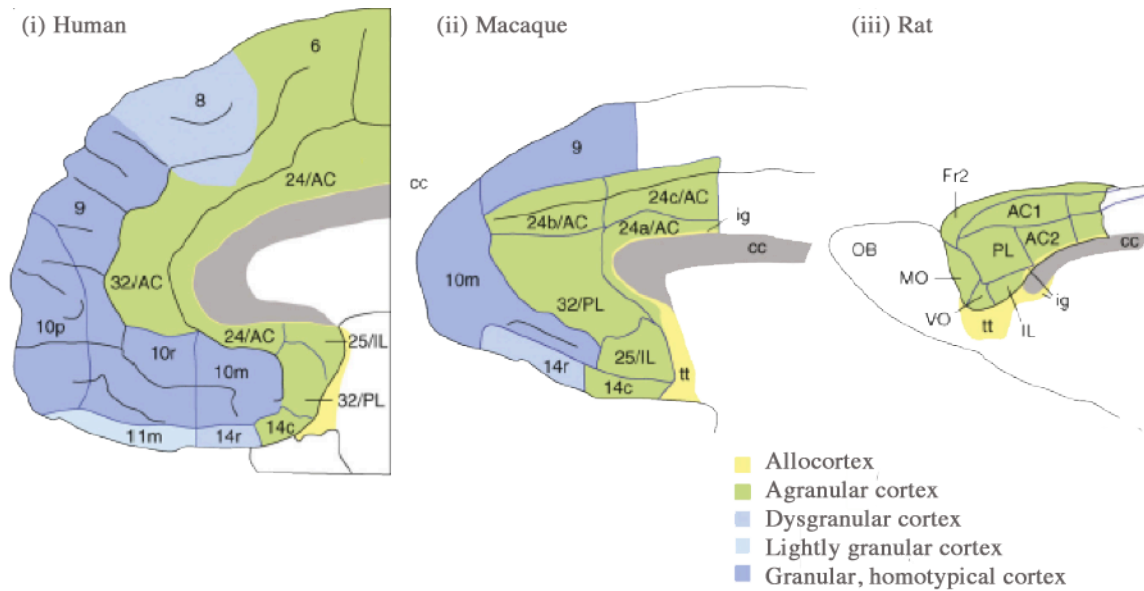
Uylings and colleagues (2003) asked the question of whether rats have a PFC, because the anatomical criteria used to define PFC show large variations across species, and there has been considerable dispute over the question of which subregions should be collectively classified as PFC in different species (Dalley et al., 2004; Kolb, 1984; Uylings et al., 2003; Uylings & van Eden, 1990). It has been suggested that rat PFC can be distinguished in terms of cytoarchitecture and connectivity (Heidbreder & Groenewegen, 2003). Although terminology is slightly different from the rat atlas of

Paxinos and Watson (2009), Dalley et al.'s (2004) overview of structural organization is appropriate for this thesis. Abbreviations mentioned here correspond to Paxinos and Watson (2009) unless otherwise mentioned. In the rat, three main subregions have been identified: (a) medial, (b) ventral, and (c) lateral (Dalley et al., 2004). The medial surface (mPFC) along the midline of the brain can be subdivided into a dorsal and ventral portion (Heidbreder & Groenewegen, 2003). The dorsomedial part of mPFC encompasses the anterior cingulate cortex (ACC) fields 1 and 2 (Cg1/Cg2) and secondary motor cortex (M2). The ventromedial part of mPFC includes medial orbital cortex (MO), prelimbic cortex (PL), and infralimbic cortex (IL). The ventral part of rat PFC includes the ventral orbital cortex (VO) and the ventrolateral orbital cortex (VLO). Finally, the dorsal and ventral agranular insular cortices (AID/AIV) and the lateral orbital cortex (LO) make up the lateral portion. Sometimes, the threefold division is based on medial parts as outlined above, orbital parts, including the orbital cortices (OFC), and lateral parts, including the insular cortices (Öngür & Price, 2000). Regardless of this difference in terminology, there is consensus in what constitutes mPFC, which is of particular interest to this thesis.

### **1.1.1 Medial PFC and anterior cingulate.**

In humans, ACC is situated adjacent to the corpus callosum, extending dorsally, ventrally, and rostrally (see Figure 1.1 for a comparative depiction of mPFC in humans, primates, and rats). In the rat, its location is largely dorsal and rostral to the corpus callosum. The Cg1 field of ACC extends rostrally to +4.20 mm anterior to bregma for about 2 mm before the fusion of corpus callosum and the appearance of the Cg2 field at +2.28 mm anterior to bregma. Both fields extend caudally to -1.56 mm posterior to bregma (Paxinos & Watson, 2009). Medial PFC can be divided into four main

subregions, including the most dorsolateral M2, dorsomedial Cg1, Cg2, and dorsal PL, and the ventromedial ventral PL and IL (Dalley et al., 2004; Heidbreder & Groenewegen, 2003; Kolb, 1984; Öngür & Price, 2000; Uylings et al., 2003; Uylings & van Eden, 1990).



*Figure 1.1.* From Wise (2008, Figure 2a). View of medial prefrontal cortex in (i) human, (ii) macaque monkey, and (iii) rat. AC = anterior cingulate, cc = corpus callosum, IL = infralimbic cortex, MO = medial orbital, PL = prelimbic cortex, VO = ventral orbital, r = rostral, c = caudal, m = medial, p = posterior, i = inferior, numbers indicate Brodmann areas.

### 1.1.2 Afferents and efferents.

Because the interconnections of mPFC are numerous and can be considered one of the chief reasons why mPFC is so functionally diverse, this section will focus on some important inputs (afferents) to and outputs (efferents) from mPFC. While the focus will be on mPFC, connections to PFC in general will be mentioned where it furthers the reader’s understanding. Further, this section will focus on the rat brain unless otherwise specified. Please refer to Figure 1.2 for a non-exhaustive schematic of connections mentioned here.

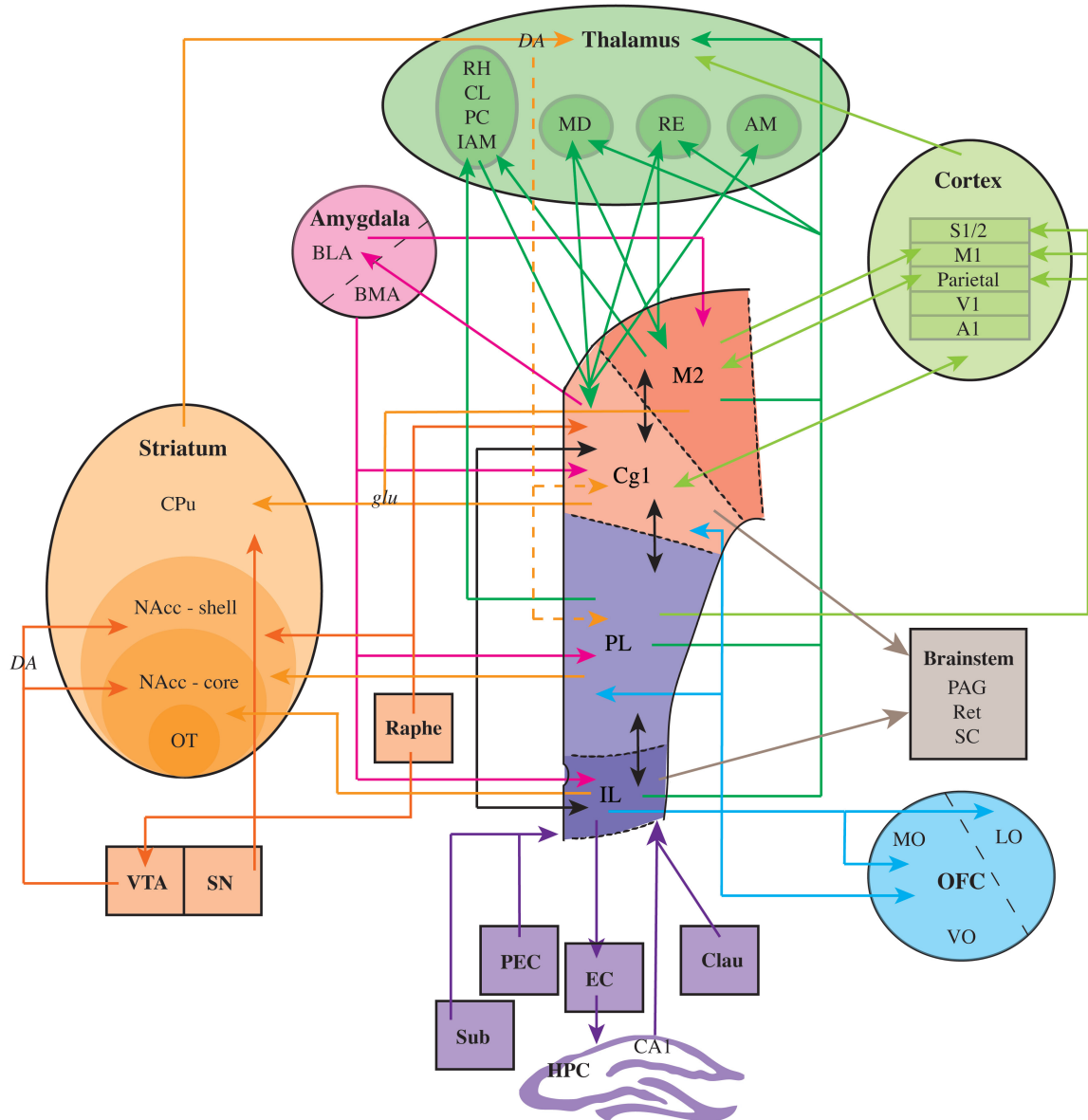
### ***1.1.2.1 Thalamic connections.***

#### *1.1.2.1.1 Afferents.*

Initially, the PFC was delineated as such based on its heavy projections from the mediodorsal nucleus (MD) of the thalamus, and was thus termed the MD projection cortex (Rose & Woolsey, 1948). That the entire structure was named after one source of afferents indicates how prominent these projections to PFC are. It was later determined that this criterion was not sufficient for classification of PFC (Kolb, 1984; Uylings et al., 2003; Uylings & van Eden, 1990), but that the pronounced projections from the thalamic nuclei are an important part of a thalamic-cortical loop that includes ACC. Apart from MD, several other thalamic nuclei project to different subregions of PFC (Uylings & van Eden, 1990). More often than not, these connections are reciprocal, going in both thalamocortical and corticothalamic directions. Thalamic nuclei projecting to ACC include the lateral MD, intermediodorsal nucleus (IMD), nucleus reuniens (RE) of the midline, central medial (CM) and central lateral (CL) intralaminar nuclei, paracentral nucleus (PC), rhomboid nucleus (RH), parafascicular intralaminar nucleus (PF), lateral posterior nucleus (LP), ventral medial nucleus (VM), and to a lesser extent paratenial nucleus (PT) of the midline, anteriomedial nucleus (AM), and interanteriomedial nucleus (IAM; Uylings & van Eden, 1990). It was later confirmed that particularly heavy projections stem from AM, CL, RE, RH, IAM, MD, and PC (Hoover & Vertes, 2007).

#### *1.1.2.1.2 Efferents.*

Many of the above-described thalamic afferents are reciprocal connections (Uylings & van Eden, 1990; Vertes, 2002). The strongest projections from ACC are to AM, lateral MD, and RE. Heavy projections from PL include IAM, medial and central MD, PT



*Figure 1.2.* Afferent and efferent projections in medial prefrontal cortex. Based on George & Koob (2010) ; Gruber & McDonald (2012); Hoover & Vertes (2007); Parker, Brock, Walton, & Brennan (2013); Vertes (2006). For abbreviations please see text, except: HPC = hippocampus, Sub = subiculum, PEC = perirhinal cortex, EC = entorhinal cortex, Clau = claustrum, Ret = reticular formation, A1 = primary auditory cortex, V1 = primary visual cortex, S1/2 primary/secondary somatosensory cortex, DA = dopamine, glu = glutamate.

paraventricular nucleus (PV), and RE, and projections from IL consist of medial MD, PT, PV, and RE. Finally, strongest projections from M2 encompass CL, RE, ventral anterior-

lateral nucleus (VA/VL), and VM. It is important to note that this is not an exhaustive review and does not include other moderate and light projections; cf. Vertes (2002).

#### *1.1.2.1.3 Significance.*

The heavy thalamic connections with ACC are often cited as a primary route through which mPFC receives limbic information (Vertes, 2006). Thalamic projections to mPFC further facilitate other inputs to mPFC, such as those related to sleep, autonomic functions, and sensory modalities (Hoover & Vertes, 2007). Medial PFC plays a role in many behaviours related to autonomic and visceral functions as well as in homeostatic behaviours.

#### *1.1.2.2 Cortical connections.*

##### *1.1.2.2.1 Afferents.*

Anterior cingulate in particular receives light input from IL directly, with most input indirectly reaching ACC through projections to PL, as PL and IL as well as different parts of PL are heavily interconnected (Heidbreder & Groenewegen, 2003; Jones, Groenewegen, & Witter, 2005). The dorsal/ventral anatomical division of mPFC corresponds to a division of afferent input from sensorimotor (dorsal; ACC, M2, dorsal PL) to limbic (ventral; IL, ventral PL; Hoover & Vertes, 2007; Vertes, 2004; 2006). The dorsal mPFC is said to receive input from all sensory modalities and their corresponding thalamic nuclei (Hoover & Vertes, 2007).

In primates, the frontal eye field area (FEF) lies adjacent to M1 and the dorsolateral PFC. This region is considered analogous to FEF in primates and plays a role in controlling eye movements. In the rat, M2, sits adjacent to M1 and ACC (Paxinos & Watson, 2009), and can be considered homologous to primate FEF, as it receives

projections from the mediodorsal nucleus of the thalamus and projects to the superior colliculus in both species and also has strong reciprocal connections with prefrontal cortex, specifically ACC, and parietal cortex (Erlich, Bialek, & Brody, 2011; Hoover & Vertes, 2007; Leonard, 1969). When stimulated, FEF/M2 elicits eye movements, and its role is thought to be one of guiding orienting movements that extend simply beyond eye movements (Guandalini, 1998).

Further, in primates, linkage between the mPFC and M1 is mediated via the pre-supplementary motor area (SMA) and the rostral cingulate motor area (CMAr; Takada et al., 2004 ). These areas are located on the medial wall and connect heavily and reciprocally with the dorsolateral prefrontal cortex. In rats, these areas do not exist analogously. However, it is thought that the dorsal mPFC mediates functions similar to those of SMA and CMar in rats, and is sometimes called the premotor area (Hicks & Huerta, 1991; Preuss, 1995).

#### *1.1.2.2.2 Efferents.*

The ACC projects most heavily to the other parts of the medial PFC (i.e., PL, IL, MO; Heidbreder & Groenewegen, 2003). Prelimbic cortex is often segregated into a dorsal and ventral part due to the afferent and efferent connections of these subregions. Dorsal PL projects to sensorimotor and parietal cortex, while ventral PL mainly projects to pyriform cortex (Heidbreder & Groenewegen, 2003). Infralimbic cortex sends projections to the adjacent MO and LO, PL, and ACC (Vertes, 2006).

#### *1.1.2.2.3 Significance.*

These cortical connections are of importance as they show how interconnected dorsal mPFC is with sensorimotor regions. Thus, ACC is situated in an optimal location

to integrate different sensorimotor information to guide behaviour. In particular, motor connections to ACC have been cited as playing a role in biasing motor outputs towards appropriate actions (Kurniawan, Guitart-Masip, & Dolan, 2011; Shackman et al., 2011)), and ACC has indeed been ascribed a role in action selection as will be discussed later (Alexander & Brown, 2011; Bailey & Mair, 2007; Camille, Tsuchida, & Fellows, 2011; Cowen, Davis, & Nitz, 2012; Euston, Gruber, & McNaughton, 2012; Hadland, Rushworth, Gaffan, & Passingham, 2003; Hayden, Heilbronner, Pearson, & Platt, 2011; Rushworth & Behrens, 2008; Rushworth, Walton, Kennerley, & Bannerman, 2004; Walton, Rudebeck, Bannerman, & Rushworth, 2007).

### ***1.1.2.3 Basal ganglia connections.***

#### *1.1.2.3.1 Afferents.*

The collection of structures and nuclei including caudate-putamen (CPu), nucleus accumbens (NAcc), and olfactory tubule (OT) are referred to as striatum (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004). Two structures that are part of the basal ganglia with heavy dopaminergic input into the striatum, the substantia nigra (SN) and the ventral tegmental area (VTA), will also be discussed. Medial PFC receives strong dopaminergic input from VTA directly and indirectly through NAcc and thalamic nuclei (Hoover & Vertes, 2007; Kolb, 1984; Voorn et al., 2004). More specifically, these projections go to both ACC and PL (Bardgett, Depenbrock, Downs, Points, & Green, 2009; Hoover & Vertes, 2007; Kolb, 1984). Voorn and colleagues (2004) discovered that the dorsal-ventral axis of the striatum corresponds to projection areas on the rostral-caudal axis in the cortex, including mPFC through the thalamus. According to this division, the most dorsal parts of the striatum project via thalamus to somatosensory

cortex (CPu) and anterior cingulate cortex (CPu and NAcc core), whereas the most ventral parts of the striatum connect to PL (NAcc core, shell) and IL (NAcc shell, OT).

#### *1.1.2.3.2 Efferents.*

Similar to the dorsal-ventral divide for afferents, there is a parallel division for efferents from mPFC to the striatum in that more dorsal parts of mPFC (ACC, M2) project to the dorsomedial striatum (CPu), respectively, and more ventral parts of mPFC (Pl, IL) project to the NAcc core and shell, respectively (Gruber & McDonald, 2012; Haber & Knutson, 2010; McGeorge & Faull, 1989; Voorn et al., 2004). The projections from mPFC to NAcc and CPu and from ventral mPFC to SN are mostly glutamatergic (Carter, 1982; Vertes, 2006; Walaas, 1981). Further, in primates, ACC has reciprocal connections to both NAcc and CPu, again along the dorso-ventral division mentioned above (Kurniawan et al., 2011).

#### *1.1.2.3.3 Significance.*

Medial PFC connections with the basal ganglia are crucial due to the dopaminergic innervation of this collection of nuclei. The basal ganglia dopamine (DA) system processes information about reward, motivation, as well as habit and goal-directed behaviour. The DA system innervates an important circuit which involves the striatum, basolateral amygdala, and ACC to guide reward-related behaviours, also referred to as the brain's *reward system* (Bailey & Mair, 2007; Cousins, Wei, & Salamone, 1994; Day, Jones, Wightman, & Carelli, 2010; Ghods-Sharifi & Floresco, 2010; Hauber & Sommer, 2009; Mai, Sommer, & Hauber, 2012; McKee, Kelley, Moser, & Andrzejewski, 2010; Salamone, 2011; Salamone, Correa, Farrar, & Mingote, 2007; Salamone, Correa, Mingote, Weber, & Farrar, 2006; Salamone, Cousins, & Bucher,

1994). Dopaminergic innervation of ACC and its relation to effort-based decision making will be discussed in section 2.2.

#### ***1.1.2.4 Other subcortical connections.***

##### *1.1.2.4.1 Afferents.*

As mentioned above, the ventral mPFC receives inputs from limbic areas such as medial basal forebrain, bed nucleus of stria terminalis (BST), and the basal amygdala (Hoover & Vertes, 2007; Vertes, 2006). Both basolateral (BLA) and basomedial (BMA) amygdala project to ACC, and more caudal BLA regions project most heavily to ACC (Floresco & Ghods-Sharifi, 2007). The deep layers of PFC receive afferents from hippocampus, perirhinal and entorhinal cortex, claustrum, and monoaminergic brainstem nuclei (Carr & Sesack, 1996; Hoover & Vertes, 2007). The hippocampal areas of ventral CA1 and subiculum are also said to project to PL/IL (Vertes, 2006; Vertes, Hoover, & Sherman, 2002). These memory-related structures may play a role in mediating reward- and action-related memory (Euston et al., 2012). Anterior cingulate cortex also receives heavy brainstem projections from the dorsal Raphé nucleus, locus coeruleus, nucleus incertus, and laterodorsal tegmental nucleus. Lighter projections from median Raphé nucleus and parabrachial nucleus also reach ACC, indicating a dorsal-ventral gradient along these projections, where more dorsal regions project more heavily (Heidbreder & Groenewegen, 2003; Hoover & Vertes, 2007). These structures are involved in more primal processes such as sleep/wakefulness regulation and homeostatic processes. Finally, mPFC receives projections from the retrosplenial cortex (Shibata, Kondo, & Naito, 2004).

#### *1.1.2.4.2 Efferents.*

There is a ventral-dorsal gradient in the mPFC in terms of projections to brainstem nuclei. Infralimbic cortex projects most heavily to brainstem nuclei, PL to a lesser extent, and ACC to the least extent. The more dorsal parts of the mPFC project to more dorsal parts of the brainstem, including to periaqueductal gray (PAG), reticular formation, and superior colliculus (Heidbreder & Groenewegen, 2003). Descending projections from ACC moreover reach the rostral part of the BLA (Floresco & Ghods-Sharifi, 2007). Anterior cingulate cortex also projects to the retrosplenial cortex (Shibata & Naito, 2008). Brainstem projections from IL include PAG, parabrachial nucleus and nucleus of the solitary tract (NTS; Vertes, 2006). It is hence not surprising that IL is involved in autonomic or visceral functions, as many of these latter projection sites directly control autonomic activity (Vertes, 2006). Infralimbic cortex also projects to several nuclei in the amygdala, taenia tecta of the olfactory cortex, anterior olfactory nucleus, anterior pyriform cortex, substantia innominata, BSR, lateral septum, midline thalamus, and nuclei of the hypothalamus (Endepols et al., 2010; Vertes, 2006). Stimulation of PL and M2 has also been found to elicit spikes in the cerebellum through the inferior olive (Watson, Jones, & Apps, 2009). These connections may be important in movement, balance, posture, and movement memory. Heidbreder & Groenewegen (2003) further cite the efferent projections of IL/PL to pons and medulla, and ACC projections to the autonomic intermediolateral cell column of the spinal cord. Thus, mPFC as well as insular cortices seem to be involved in autonomic, visceral functions, while OFC mediates stimulus discriminations its reward aspects (Gabbott, Warner, Jays, Salway, & Busby, 2005).

#### *1.1.2.4.3 Significance.*

While many of these connections are less pertinent for the focus of this thesis, there are two that deserve attention due to their functional roles. The first is the amygdala, which is involved in emotional processes including fear. Together with ACC and basal ganglia structures, it forms a loop to mediate emotional aspects of decision making, motivation, and reward- and effort-guided behaviours (Beckmann, Johansen-Berg, & Rushworth, 2009; Floresco & Ghods-Sharifi, 2007; Hauber & Sommer, 2009). Further, the Raphé nucleus plays an important part in pain processing. Anterior cingulate cortex receives projections from this structure, and has been linked in pain processing (Devinsky, Morrell, & Vogt, 1995; Hoover & Vertes, 2007).

#### **1.1.3 Homology in primates.**

In the following, some species-related structural differences in PFC will be briefly discussed. Rats have a much smaller PFC compared to the rest of their brains than do non-human primates and humans (Fuster, 2008). Further, the rat brain is virtually void of the characteristic gyri and sulci that make up the neocortex. Because of these differences, much debate has revolved around which structures can be considered homologous, or only analogous, in rats, non-human primates, and humans (Uylings et al., 2003).

Based on anatomy, connectivity, neurochemistry, and functionality, primate mPFC is largely considered homologous to rat mPFC (Öngür & Price, 2000; Seamans, Lapish, & Durstewitz, 2008; Uylings et al., 2003). According to Wise (2008), rats, monkeys, and humans share the agranular part of PFC (see Figure 1.2 for homologous structures). For instance, both monkey and rat mPFC lack a layer IV and receive dopaminergic projections from VTA; and thalamocortical afferents are also largely

parallel in both species (Seamans et al., 2008; Uylings et al., 2003). Further, mPFC projects to the same regions in the striatum in both rat and macaque (Wise, 2008). Both primate and rat mPFC also share connections with amygdala, peri- and entorhinal cortices, motor areas such as premotor cortex, and somatosensory cortices (Öngür & Price, 2000; Uylings et al., 2003).

The roles of the specific subfields of mPFC can be considered separately to further elucidate homology across species. It has been argued that rat PL is evolutionarily more closely related to dorsolateral PFC (dlPFC) in primates, yet it anatomically better resembles primate mPFC (Pandya & Yeterian, 1990; Seamans et al., 2008). In line with that, Vertes (2004) has described rat PL as functionally homologous to dlPFC, and IL to orbital medial cortex in primates. The Cg1 field of ACC and M2 have collectively been considered homologous to the frontal eye field (FEF) in macaques based on oculomotor projections and elicitation of eye movements after stimulation (Guandalini, 1998; Uylings et al., 2003; Vertes, 2004).

Whereas some authors stress that there is no structural dlPFC homolog in rats (Wise, 2008), others conclude that rat PFC has both functional and anatomical features that correspond to dlPFC in primates, mostly in ACC, M2, and dorsal PL (Uylings et al., 2003). Uylings and colleagues (2003) summarized the effects of lesions to PFC to bridge the functional parallels between primate and rat PFC. Impairments that commonly occur in both primates and rats following lesions include (a) working memory deficits, as well as impairments in behavioural planning and monitoring following lesions to dlPFC in primates and mPFC in rats; (b) changes in social and emotional behaviour, hyperactivity, impulsivity, and impairments in processing information related to gustation and olfaction

following OFC lesions in both species; and (c) reduced pain response, lethargy, and impaired motor initiation following ACC lesions (see also Kolb, 1984; Vertes, 2004).

The debate remains whether equivalent functions of dlPFC of primates can be found in the rat, but the above section can provide some insight into the functional correspondence between primate dlPFC and rat dorsal mPFC. For the purpose of this thesis, rather than focussing on homologs in anatomy, functions of mPFC, and ACC in particular, will be explored as they have become evident by individual studies. While the focus shall remain on the rat, functions in other species will be cited when available.

## **1.2 Overview of Prefrontal Cortex Functions**

After the preceding review of anatomy and homology, a very brief overview of PFC function will be given here. The purpose of this section is to briefly highlight the many behaviours PFC is involved in. Chapter 2 will subsequently explore the functional roles of ACC in particular.

Medial PFC has been implicated in a plethora of functions, including, but not limited to, action-outcome valuation (Bailey & Mair, 2007; Hadland et al., 2003), aggression (Rudebeck et al., 2007), anxiety (Frankland, Bontempi, Talton, Kaczmarek, & Silva, 2004; Morgan & LeDoux, 1995; Rudebeck et al., 2007; Steenland, Li, & Zhuo, 2012), attention (Chudasama et al., 2003; Rushworth, Kolling, Sallet, & Mars, 2012), behavioural flexibility and task switching (Rushworth, Hadland, Gaffan, & Passingham, 2003; Rushworth, Hadland, Paus, & Sipila, 2002), conflict and error monitoring (Beckmann et al., 2009; Botvinick, 2007), courage (Nili, Goldberg, Weizman, & Dudai, 2010), decision making and planning (Walton et al., 2002), delay monitoring (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; Denk et al., 2005; Ghods-Sharifi &

Floresco, 2010), pain (Wang, Luo, Chang, Woodward, & Han, 2003), goal-directed behaviour (Boorman, Rushworth, & Behrens, 2013), impulsivity (Ghods-Sharifi & Floresco, 2010; Kolb, 1984), memory (Euston et al., 2012), motivation (Kurniawan et al., 2011; Lydall et al., 2010; Mai et al., 2012), motor function (Cai & Padoa-Schioppa, 2012; Han et al., 2003; Turken & Swick, 1999), novelty and object recognition (Weible, Rowland, Pang, & Kentros, 2009), reward and value processing (Rushworth & Behrens, 2008), social valuation (Hillman & Bilkey, 2012; Rudebeck et al., 2007), stimulus discrimination (Cardinal et al., 2003; Schweimer & Hauber, 2005), and working memory (Beckmann et al., 2009). The fact that mPFC plays a role in such a vast number of behaviours corroborates its role of integrating and synthesizing various inputs to govern certain outputs such as those related to (a) monitoring actions; (b) ascribing value to stimuli, actions, and social situations; and (c) adjusting behaviour according to rules. This role of integrating sensory information about the world with internal sets and expectations will become more evident in the following chapter when ACC is discussed specifically.

## **Chapter 2: Anterior Cingulate Cortex and Decision Making**

The focus of the present chapter will be on ACC and its role in decision making, after some of its other functions are discussed briefly. Existing cost-benefit decision paradigms and relevant literature will be discussed. This chapter ends with a summary of the existing literature, and Chapter 3 will present the reader with the rationale of undertaking the present experiments.

### **2.1 Functional Diversity in ACC**

Medial PFC is sometimes called the brains *executive* or the *anterior executive region* (Devinsky et al., 1995). Vogt and colleagues explain that this terminology stemmed from ACC's involvement in many motor functions (Devinsky et al., 1995). However, knowing what is known at present about mPFC's role in general, and ACC's role in cognitive processes in particular, this designation may be more apt than ever. Medial PFC mediates a vast array of cognitive functions that regulate behaviour. Termed executive functions, these include goal-directed behaviour, behavioural flexibility, attention, and decision making. Anterior cingulate cortex is involved in more functions beyond simply executive functions. These functions can be broadly divided into three domains: (a) affective, (b) motor, and (c) cognitive. A brief overview of all three areas will be given, but the focus will be on ACC's role in decision making in the second part of the chapter.

#### **2.1.1 Emotional processing.**

Fear is one major emotional function in which ACC has been implicated using a variety of methods (Frankland et al., 2004; Han et al., 2003; Morgan & LeDoux, 1995; Steenland et al., 2012). In the rat, lesions of ACC have been found to increase fear response in a conditioned fear paradigm (Morgan & LeDoux, 1995). Moreover, activity

in subgenual ACC was elicited when courage was displayed in a functional magnetic resonance imaging (fMRI) study in humans (Nili et al., 2010). For the purpose of this thesis, as indicated by this study, courage is operationalized as the action of overcoming fear. In this study, participants could select whether to increase or decrease distance to a stimulus that was particularly fearful to them (i.e., a live snake). Activity in subgenual ACC was positively correlated with reported subjective fear only when courage was displayed (i.e., fear was overcome). In contrast, when participants succumbed to their fear, this activity was absent. This was the first neural correlate of courage in the human brain. Notably, subgenual ACC in humans has its functional correlate in IL in rats (Slattery, Neumann, & Cryan, 2011; Wise, 2008).

In addition to fear processing, ACC also seems to play a role in pain (Wang et al., 2003). For instance, pain sensitivity in monkeys is decreased after cingulate lesions (Devinsky et al., 1995). In fact, the midline thalamic nuclei that project to ACC also contain nociceptive neurons, at least in the monkey. When these thalamic nuclei are lesioned, nociception in ACC is blocked (Devinsky et al., 1995). Devinsky and colleagues (1995) proposed that ACC might specify the affective component of a painful stimulus and select a motor response to deal with such a stimulus, like the *fight-or-flight* response. Support for this came from a recent study of ACC recordings in mice (Steenland et al., 2012). The authors discovered that the *un-freezing* motor signal following freezing behaviour correlated with ACC activity. In that way, ACC might be crucial for integrating aversive stimuli with avoidance motor behaviour, or flight behaviour after initial freezing (Devinsky et al., 1995; Steenland et al., 2012).

Lastly, ACC has been implicated in emotional valence and social valuation of

information. In the macaque, pregenual ACC has been shown to mediate negative emotional valence as well as anxiety (Amemori & Graybiel, 2012). Anterior cingulate cortex plays a role in social valuation in primates (Rudebeck et al., 2007) and in rats (Hillman & Bilkey, 2012).

Based on the above studies, it seems to be the case that ACC is involved in modulating behaviour to aversive stimuli. These stimuli can either be fearful or painful. The role of ACC might be in determining which action to choose when faced with an aversive stimulus, such as the fight-or-flight response (Steenland et al., 2012). This is in line with findings that show involvement of ACC in aspects of the fight-or-flight response, such as courage (Nili et al., 2010).

### **2.1.2 Motor processes.**

Moreover, ACC has a distinct role in motor processing. Turken and Swick (1999) showed that a patient with a lesion to caudal ACC was unable to respond with motor commands, while decision making and performance on the same task remained stable when using vocal instead of motor responses. In monkeys, ACC has also been implicated in goal-based motor selection (Matsumoto, Suzuki, & Tanaka, 2003) and movement initiation (Cai & Padoa-Schioppa, 2012). In addition, there is the view that dorsal mPFC has functions like those of premotor areas in primates as elaborated when discussing cortical afferents in section 1.1.2.2.1 (Hicks & Huerta, 1991; Preuss, 1995). Motor processes play an important role for this thesis. Lastly, ACC is implicated in internally generated as opposed to externally cued actions (Passingham, Bengtsson, & Lau, 2010). Because two physical effort tasks are investigated, potential motor functions of ACC must be dissociated from effort and the potential role of ACC motor processes must be

kept in mind.

To explore ACC's role in motor tasks in more detail, selected studies will be reviewed. As previously mentioned, Matsumoto et al. (2003) found that ACC was implicated in motor selection in primates. The authors recorded from monkey mPFC and dlPFC on a Go/NoGo task where monkeys had to select motor responses depending on reward expectation. One of two visual cues was displayed and the monkey either performed a Go or a NoGo response. Only correct responses were rewarded. Combinations of motor response, visual cue, and reward were changed across blocks. When motor requirement or reward conditions were reversed, adjustment to the new rule was required. The authors found that monkeys chose a motor response based on anticipated reward. This finding was evident in both behavioural and neural data. Both mPFC and dlPFC neurons fired in anticipation of a reward immediately after the onset of the visual cue. In addition, mPFC also fired when specific combinations of motor response and reward condition were given. The authors concluded that mPFC holds a memory of an outcome associated with an action and initiates a goal-directed action plan. Action selection will be discussed in more detail in section 2.2.1.

When inactivating monkey pre-SMA, which is supposed to be a functional homolog of rat M2, monkeys made more errors in selecting the correct movement, and took more time to engage in a memory-guided sequence task (Shima & Tanji, 1998). They also made more errors on a novel action sequence that was learned prior to pre-SMA inactivation. This finding indicated that movement initiation leading to an action sequence might be mediated by ACC. It seems to be the case that ACC is involved in planning movement sequences or retrieving a correct movement sequence from memory,

as well as in the planning, initiation, and possibly execution of motor commands (Bailey & Mair, 2007). In summary, one of ACC's roles is that of planning movement sequences and selecting motor actions. Precisely, ACC may be responsible for integrating reward information with an appropriate response or strategy to lead to that reward (Matsumoto et al., 2003).

Interestingly, direct motor functions in ACC have been investigated exclusively in the monkey, with only distantly related studies in rodents (cf. Steenland et al., 2012). From the study by Steenland and colleagues (2012), one can conclude that mouse ACC may determine a motor plan to deal with aversive stimuli, such as the fight-or-flight response. Future research should be aimed at clarifying the role of ACC in motor processes in the rat.

### **2.1.3 Cognitive functions.**

Finally, and most pertinent to this thesis, the ACC has been shown to mediate several cognitive functions. One of these functions involves the learning, initiation, execution, and maintenance of action sequences, which ties the cognitive control processes to motor process discussed above. On a sequence-learning task, Bailey and Mair (2007) trained rats to nose-poke into illuminated ports, and varied task complexity and cue predictability. Rats received lesions to M1, M2, or dorsal mPFC (ACC/PL). Results showed that, for all types of lesions, reaction time for action initiation was increased, which led the authors to conclude that response organization depended on M1, M2, as well as dorsal mPFC. This finding implied that action-sequence learning seems to be in part mediated by dorsal mPFC, including ACC.

Anterior cingulate also plays a role in stimulus discrimination, but only in

complex scenarios when multiple stimuli needed to be discriminated (Cardinal et al., 2003; Schweimer & Hauber, 2005). In a simple, binary, two-stimulus discrimination, ACC was not needed (Schweimer & Hauber, 2005). This indicated, again, that ACC seems to mediate response selection even when selecting among stimuli rather than actions.

Further, ACC seems to be active when there is a response conflict, or when errors have been committed, as shown in electrophysiological recordings in rats and fMRI in humans (Botvinick, 2007; Botvinick, Cohen, & Carter, 2004; Swick & Turken, 2002; Totah, Kim, Homayoun, & Moghaddam, 2009 ).

One of the main cognitive functions relevant to this thesis is decision making. Therefore, the rest of this section will focus on different kinds of decision making in rats and primates. Finally, cost-benefit decision making will be discussed in detail.

## **2.2 ACC and Decision Making**

Research on ACC and decision making has exploded over the past two decades, and the focus has recently shifted to identifying the neural correlates responsible for this function. It seems to be the case that ACC is a vital part of a larger network responsible for reward-related decision making, which likely includes BLA and NAcc (Hauber & Sommer, 2009). In particular, all three regions are involved in the acquisition of tasks that involve stimulus-reward associations (Cardinal et al., 2002). Because all tasks discussed in the present experiments are first and foremost based on decisions about reward, this network seems particularly important, and its role will be discussed throughout this thesis. In the following, the role of ACC in action selection, reinforcement guided learning, and cost-benefit decision making will be elaborated.

### **2.2.1 Action selection.**

Anterior cingulate is said to have a central role in action selection, which constitutes one important form of decision making (Rushworth, Behrens, Rudebeck, & Walton, 2007). Often, a value is ascribed to an action, meaning that one action leads to better consequences than another. Camille et al. (2011) investigated the contributions of action-value and stimulus-value learning in humans with damage to either OFC or dorsal ACC. The authors found that these lesions have dissociable effects in that OFC is involved in value-based choice between stimuli, or stimulus selection, and dorsal ACC in value-based choice between actions, or action selection, but not vice versa.

Walton, Devlin, and Rushworth (2004) examined how decisions about actions affect outcome monitoring using fMRI in humans. Participants engaged in an action phase followed by an outcome-monitoring phase. Results showed that the brain region involved in performance monitoring correlated with the type of decision made. When participants freely decided which action to choose, dorsal ACC was activated. When participants were told which action to choose, OFC was activated. The same was found during outcome monitoring; if an internally guided action had been chosen, dorsal ACC was more active during this second phase, whereas, if an externally guided action had been chosen, OFC was more active. The authors concluded that ACC has a role in action selection, since this dorsal ACC region, including the rostral portion of the cingulate motor area, projects heavily to primary motor cortex and spinal cord (Heidbreder & Groenewegen, 2003).

Further support for ACC's role in action selection comes from Hadland et al. (2003) , who showed that monkeys with bilateral ACC lesions were impaired on selecting

actions when the action selection required taking into account the reward associated with the outcome. Action selection was also evident when a sequence of actions was involved (Procyk, Tanaka, & Joseph, 2000). Recording from monkeys, Procyk et al. (2000) found that dorsal ACC was involved in encoding motor (and sensory) events in a sequential way according to their temporal order relative to the reward at the end of the sequence. Croxson, Walton, O'Reilly, Behrens, and Rushworth (2009) postulated that ACC neurons encode the *integrated* value of an action associated with expectation of outcomes, whether they are beneficial (i.e., reward) or costly (i.e., effort or delay).

A contradictory finding to the action-value activity in monkey ACC comes from a study in rats. Cowen et al. (2012) demonstrated that based on electrophysiological recordings of rat ACC, action-value processing is not one of the tasks ACC is involved in, at least in the rat. During response selection, ACC neurons showed very little activity related to anticipated response values, but instead mainly exhibited sensorimotor activity, as has also been described in monkeys (Kennerley & Wallis, 2009). Only few cells encoded outcome value (< 4%).

In a stimulus-selection task, Amiez, Joseph, and Procyk (2006) recorded from ACC in two rhesus monkeys while they decided which of two novel stimuli was associated with the largest reward. This largest-reward stimulus was termed the *optimal stimulus*. Reward probabilities varied across trials, where the optimal stimulus was rewarded with a large juice reward 70% of the time, and with a small juice reward 30% of the time. For the non-optimal stimulus, these probabilities were reversed. Behaviourally, monkeys chose an optimal strategy by identifying the optimal reward in successive trials. Single cell activity was found to encode the stimulus onset and

movement execution correlated with the reward value. This study provided additional support for ACC's role in movement initiation and action selection based on reward expectancy.

It may be the case that in primates, ACC mediates action value as well as action selection (Kennerley, Walton, Behrens, Buckley, & Rushworth, 2006), whereas in rats this is not the case. However, it is also known that ACC function is highly context dependent, where it is often active in controlled laboratory settings, but not in dynamic settings (Walton & Mars, 2007), and lesions can produce different effects in different paradigms (Schweimer & Hauber, 2005). That is, small parameter changes within a task or changes across different paradigms introduce the question of how ACC might be involved anew. The fact that one task has found effects of ACC lesions or in ACC activity does not guarantee that these findings will be replicable in different paradigms or contexts. The higher the degree of dynamic in the environment or task, the less predictable is the effect of ACC involvement (Walton & Mars, 2007). This will become especially evident when cost-benefit decision making is discussed in section 2.2.3. In summary, ACC seems to encode multiple decision variables in general (Kennerley & Walton, 2011), and a common theme is that ACC is mostly concerned with actions, not stimuli, in relation to reward.

### **2.2.2 Reinforcement learning.**

Reinforcement learning (RL) describes a class of models of learning with the goal of reward maximization based on past experiences with positive and negative outcomes (Sutton & Barto, 1998). In what is now called *unsupervised learning*, it is through trial and error that an agent discovers which actions lead to the optimal outcome (i.e., highest

reward). Reinforcement learning models account for the following parameters of interest: (a) the history of past rewards, (b) the expected reward on the current trial, and (c) the discrepancy between the expected and actual reward. This expected reward is usually termed reward prediction, and may be encoded by ACC (Rushworth & Behrens, 2008; Schultz, 2000; Sutton & Barto, 1998). In the following, the role of ACC in terms of RL will be discussed as it pertains to this thesis. First, ACC's role in adapting to changing reward values will be highlighted. Second, reward prediction error mediation in ACC will be discussed. Lastly, a synthesis of these roles with respect to behaviour will be described.

Electrophysiological recordings have discovered that rat ACC may encode reward-related information dynamically during uncertainty, which fits well with RL (Hillman & Bilkey, 2010). In ACC, neurons encode reward prediction error similarly to what is classically seen in DA neurons in primate basal ganglia (Rushworth & Behrens, 2008; Schultz, Apicella, & Ljungberg, 1993). Recordings from macaque SN and CPU indicated that these neurons respond to unexpected delivery of reward and to cues that precede rewards, but they response is absent when the expected reward does not occur (Apicella, Legallet, & Trouche, 1997; Schultz et al., 1993; Schultz, Dayan, & Montague, 1997). However, in ACC, *different* neural populations encode positive and negative prediction errors, whereas in the DA system, the same population encodes both positive and negative prediction errors. In DA neurons, the direction becomes evident via a change in firing, where positive prediction errors are shown by an increase in firing, and negative prediction errors by the absence of firing (Rushworth & Behrens, 2008).

Anterior cingulate cortex seems to be active when there is some uncertainty or

dynamic in the environment (Kennerley et al., 2006; Rushworth & Behrens, 2008; Seo & Lee, 2009; Walton & Mars, 2007). This fits well with RL models and may indicate that ACC mediates allows for optimal updating of reward value in dynamic environments by biasing actions towards the history of the most recent trial (Rushworth & Behrens, 2008).

As briefly mentioned above, ACC mediates reward prediction error. According to RL, a reward prediction error occurs when the actual reward is different from the expected reward (Schultz et al., 1997). Even though it was initially thought that ACC only codes when an expectation was violated in a negative sense (i.e., outcome does not occur or is erroneous; Carter et al., 1998), it has since been shown that both positive (reward) and negative (error) outcomes of expectations are encoded in ACC (Hayden et al., 2011; Kennerley, Behrens, & Wallis, 2011; Matsumoto et al., 2003; Sallet et al., 2007). Even the degree of reward expectancy seems to be encoded by ACC in monkeys (Shidara & Richmond, 2002). A negative reward prediction error happens when reward was expected, but did not actually occur. This situation resembles the conflict monitoring function also ascribed to ACC, according to which action outcomes that yield unexpected or surprising consequences are monitored by ACC (Botvinick, Cohen, & Carter, 2004; Hayden et al., 2011) . It is now known that ACC also encodes positive reward prediction error, which occurs when a reward was not expected, but occurs.

Matsumoto, Matsumoto, Abe, and Tanaka (2007) trained monkeys to learn a visual discrimination task and recorded from mPFC and dlPFC. They found that PFC cells responded to both positive and negative feedback and the firing rate was correlated with the amount of differences between the anticipated action value and the feedback value. The authors concluded that PFC cells represented both the valence and magnitude

of error in the action value prediction (prediction error), which may be useful for adapting future responses. Again, this supports the role of ACC in outcome monitoring and action selection.

Iwata, Shima, Tanji, and Mushiake (2013) examined whether the monkey cingulate motor area (CMA) contributes to action selection in exploitative behaviour. Using a modified RL model which incorporated both context and outcome value, the authors suggested that the monkeys' behaviour could be explained with the model in that action selection occurred based on behavioural context as well as action outcome. The rostral portion of CMA was found to be involved in both the context and outcome action value encoding. Note that action outcome anticipation and action selection based on this outcome is again highlighted here as a function of ACC.

A reconciliation of the above findings including surprise, conflict, and reward is consistent with the fact that ACC seems to encode the expectation of reward and the outcome of that expectation, regardless of outcome valence (Botvinick, 2007; Kerns, Cohen, MacDonald, Cho, Stenger, & Carter, 2004; Procyk et al., 2000; Rushworth et al., 2004; 2009; Walton & Mars, 2007). To reiterate, RL models are concerned with (a) the history of past rewards, (b) the expected reward on the current trial, and (c) the discrepancy between the expected and actual reward. It seems to be the case that ACC encodes all of the above. However, as mentioned above, it could be the case that ACC primarily handles action-outcome monitoring, of which these functions are subcategories. This perspective is further consistent with the idea that ACC is part of a larger *reward system*, which also includes OFC, ventral striatum, and the midbrain DA system, all of which seem to be mediated by DA (Hauber & Sommer, 2009; Schultz, 2000). This

monitoring function may also take into account the task difficulty, or cost, as will be discussed further in the subsequent section (Botvinick et al., 2004).

### **2.2.3 Cost-benefit decisions.**

The effort-based decisions investigated in this thesis present one class of cost-benefit decisions. In a typical cost-benefit decision, the benefit is usually an incentive, such as a large food reward in non-human animals or a large gain of fictional money in humans, and the decision cost is associated with some form of disadvantage, such as a delay or physical effort in non-human animals, or loss of money in humans. When the benefits are equal, both non-human animals and humans tend to choose the less costly option (Kool, McGuire, Rosen, & Botvinick, 2010). That animals prefer to take the *easy way out* is a longstanding idea and was first formulated by Clark L. Hull (1943) in his *law of least work*:

If two or more behavioural sequences, each involving a different amount of energy consumption or work, have been equally well reinforced an equal number of times, the organism will gradually learn to choose the less laborious behaviour sequence leading to the attainment of the reinforcing state of affairs (p. 294).

However, in the real world, benefits associated with different decision outcomes are often not equal. Instead, different actions are associated with both, different costs and benefits. In fact, humans (Aronson & Mills, 1959) and rats (Lydall, Gilmour, & Dwyer, 2010) placed more value on outcomes when they had to invest more effort into obtaining them. Over the past three decades, cost-benefit decisions and their underlying neural mechanisms have received considerable attention in the literature. While both delay- and

effort-based decision making have been examined, the focus here will be on effort as a decision cost. In the following sections, accounts of effort-based cost-benefit decision making in the literature to date will be discussed.

### ***2.2.3.1 Humans.***

A behavioural manifestation of Hull's law of least work (1943) will be given here, followed by an exploration of neural correlates of cost-benefit decision making in humans. Kool et al. (2010) tested cognitive effort in human participants using a task-switching paradigm on a newly developed demand selection task (DST), which was initially modelled after reward-based tasks such as those used by Bechara et al. (1994). In the first of six experiments, subjects chose between two decks of cards, one of which was a high-demand deck, and the other a low-demand deck. The effort consisted of a higher cognitive demand imposed by many task switches from trial to trial versus a low-demand, low-switch alternative. Specifically, participants had to judge numbers based on their colours. If the colour was blue, numbers had to be judged by whether they were greater than 5. If the colour was purple, numbers had to be judged by whether they were odd or even. In the high-demand condition, the task was switched from one trial to the next 90% of the time. In the low-demand condition, this was only the case 10% of the time. Participants preferred the low-demand option, choosing the less demanding deck most of the time, and displaying longer reaction times and more errors on the high-demand deck. Interestingly, the authors also showed that avoidance of mental effort varied proportionally with task-relevant ability, meaning that those participants who were less resourceful were generally more biased towards the low-demand option. Results from all six experiments conducted in this study indicated that humans did in fact choose

the least effort (Hull, 1943) when presented with a cognitively high-demand versus (vs.) a low-demand option.

Decision-making studies in humans have revealed neural activity in NAcc and ACC, which corresponds to decisions about effort and reward. Botvinick (2007) reconciled his conflict monitoring theory of ACC with the statement that conflict can be seen as a cost in decision making. His lab earlier demonstrated in an fMRI study that this conflict correlates with activity in ACC (Botvinick & Huffstetler, 2007). Participants received monetary rewards on a cognitive demand task involving choices between a high-demand and a low-demand condition. The authors found that the level of demand involved in earning the reward modulated NAcc activity. Specifically, NAcc activity was stronger when rewards were easily earned compared to those that required high cognitive demand. The strength of this NAcc activation was preceded and predicted by ACC activity during task performance before the reward was received. Increased activation of ACC during earning of the reward predicted decreased activation of NAcc to the reward itself. This finding was later confirmed (Botvinick, Huffstetler, & McGuire, 2009). While effort was expended, ACC activity predicted reduced reward-response activity in NAcc. In addition to ACC, dlPFC also showed higher activation during cognitive demand, but dlPFC activity did not correlate with NAcc activity. Despite this evidence that conflict can serve as a cost in decision making, it is unclear whether physical effort and cognitive demand are indeed part of the same phenomenon.

In an fMRI study, Croxson and colleagues (2009) found that dorsal ACC together with ventral striatum indicated the level of reward as well as the amount of effort to be exerted in obtaining the reward. Here, ACC activity was dynamic; activation changed

according to the net value of the planned course of action. When the reward expectation increased, ACC activity increased. When the anticipated cognitive effort increased, ACC activity decreased. The authors concluded that ACC activity reflected the integrated value of a course of action. That is, ACC activity mediated not only the level of reward for an action outcome, but also the effort costs inherent in the action. Interestingly, Botvinick et al. (2009) found that ACC activity *increased* during task performance in high demand blocks compared to low demand blocks. Here, the activity was due to effort alone, and not due to reward. Nucleus accumbens, however, encoded both effort and reward. It could be the case that the discrepancy in ACC activity in these two studies was due to when the activity was measured. Botvinick et al. (2009) measured activity when the reward cue was presented, whereas Croxson et al. (2009) measured activity in the period post-cue in anticipation of effort and reward.

In a case study of a patient with a lesion to her left hemisphere ACC and part of OFC, Naccache and colleagues (2005) found that the patient had no subjective feeling of cognitive effort, including no physiological response that is evident in healthy subjects when faced with mental effort. This indicated that ACC is important to signal cognitive effort to other systems, such as subcortical structures that initiate the appropriate bodily response. Without perceiving an effortful task as effortful, dangers such as exhaustion from an activity may prevail, especially when no exhaustion signals can be translated into body responses (e.g., sweating, increased heart rate, increased respiration). This result thus may have constituted a failure of sending ACC effort signals to the brainstem and autonomic nervous system. The above studies indicated that human ACC has a role in decision making that includes effortful choices, such as those that require increased

cognitive demand, and may play a crucial role in relaying effort information to other systems, such as the NAcc.

### ***2.2.3.2 Non-human primates.***

Given the relatively large literature base on cost-benefit decision making in humans and rodents, it is surprising that this literature in non-human primates is rather sparse. Two recent studies investigating cost-benefit valuation in monkeys will be discussed here. Amemori and Graybiel (2012) tested macaques on an approach-avoidance task while recording from pregenual ACC, which is said to be involved in cost-benefit valuation. On this task, monkeys had to make decisions to approach or avoid cost-benefit situations involving combinations of positive (i.e., food reward) and negative stimuli (i.e., air puff). Monkeys adapted their responses based on the cost-benefit combinations. When stimulating ventral pregenual ACC during the approach-avoidance task, monkeys were biased towards avoiding the anticipated outcome. The authors concluded that hyper-activation of this region can lead to pessimistic evaluation of outcomes. This finding fits with the implication of pregenual ACC in depression and obsessive-compulsive disorder in humans. Amemori and Graybiel (2012) further suggested that this biased evaluation might represent a subjective over-estimation of costs compared to benefits. This was supported by the finding that the monkeys, when injected with an anxiolytic agent, showed a behavioural reversal of the stimulation-induced avoidance behaviour. It should be noted that pregenual ACC in human correlates to PL in rats (Wise, 2008). However, the fact that anxiolytic drugs reversed avoidance behaviour finds support in the rodent literature; Steenland et al. (2012) showed that ACC in mice responded to aversive stimuli and mediated fight-or-flight behaviour.

A recent study by Pasquereau and Turner (2013) investigated the activity of DA neurons in primate SN during a cost-benefit task. This task was a virtual reaching task in which the monkey had to align a cursor moved by his hand position with a target on a computer screen. Rewards varied between 1, 2, and 3 drops of juice, which was indicated to the monkey via a colour cue. Effort was varied by changing the amount of force (0, 1.8, or 3.2 N) required to move the cursor, which was indicated to the monkey via a shape cue. Thus, there were nine unique colour-shape combinations that yielded nine reward-effort outcomes. The authors found a subset of phasic DA neurons in SN that predicted the net utility of each action by discounting the reward signal by the physical effort invested. This finding indicated that DA neurons devalued rewards associated with physical effort.

The above studies indicate that non-human primates make cost-benefit decisions similar to humans and, as discussed below, rodents. When faced with varying levels of effort and reward, monkeys can adapt their behavioural responses accordingly. At the single cell level, both pregenual ACC and basal ganglia DA seem to be involved in these decisions. In the following section, cost-benefit decision making in rats will be discussed, as it most prominently pertains to this thesis.

### ***2.2.3.3 Rodents.***

This section will provide a chronological account of reports of cost-benefit decision making in rats. Behavioural experiments, pharmacological manipulations, lesion studies, and electrophysiological recordings will be discussed. While the focus remains on ACC, the chronological account will address some other findings as they have become available, including midbrain DA, and task-specific comparisons to other

prefrontal regions. This account serves the purpose of guiding the reader through the history of effort-based decision making towards what is presently known about this type of decision making in rats, will conclude with questions that remain open, and transition into the first section of the next chapter. This section will provide a rationale for the experiments conducted in this thesis. To understand this motivation, a detailed account of existing literature is pivotal.

An effort-based decision is a type of cost-benefit decision in which the cost is typically a physical exertion of effort. An initial account of effort-based decision making in rats came from a seminal study by Salamone and colleagues (1994). A T-maze was developed for a decision making task where the start arm, or stem of the T, led up to a decision region at the joint of the arms and the stem. Rats were placed in the start arm on each trial. On this task, rats chose between a low-reward arm (LRA), where they received two food pellets, and a high-reward arm (HRA), where they received four food pellets. Rats were trained on this maze in a no-effort and an effort condition. In the no-effort condition, rats simply chose between LRA and HRA. In the effort condition, a 44-cm-high vertical wire-mesh barrier was placed in the HRA. Rats were then divided into a control group, who received a vehicle injection, and a treatment group who received either (a) haloperidol to block DA receptors in NAcc or (b) 6-hydroxydopamine (6-OHDA) to deplete DA in NAcc. After haloperidol injections, rats in the treatment group now chose the HRA markedly less (69%) in the effort condition compared to the no-effort condition (94%), whereas HRA choices of control rats remained high even when obtaining the high reward meant having to climb the ramp (96% of HRA entries) compared to the no-effort condition (98%). When rats in the treatment group were then

injected with 6-OHDA, the bias away from the HRA was even more pronounced, as these rats only entered the HRA 15% of the time in the effort condition compared to 95% of the time in the no-effort condition when no barrier was present. Control rats remained at 90% HRA choices in the effort condition. Even three weeks following the DA depletion, rats in the treatment group only entered the HRA barely above 25% of the time when the barrier was present. Further, in both the no-effort and effort conditions, response latency increased after treatment with both haloperidol and 6-OHDA. The authors concluded that manipulating DA in NAcc did not impact motivation to obtain a food reward, because rats in both groups remained oriented towards food acquisition and consumption. However, DA depletions might result in a motor slowing or decreased motivation, which in turn diminish the effort that rats are willing and able to expend in order to obtain the high reward (Salamone et al., 1994). Nucleus accumbens core lesions have further been shown to reduce the preference for a large, delayed reward (Cardinal et al., 2001; Ghods-Sharifi & Floresco, 2010).

A second study by the same group (Cousins, Atherton, Turner, & Salamone, 1996) discovered that when rats had the choice between a HRA with a barrier and a LRA that actually contained no reward, NAcc DA depletions had little effect on HRA entries. Only if rats were presented with an actual choice between two reward magnitudes, and the presence of the ramp in the HRA, was the effect evident. Further, Cousins et al. (1994) also conducted an operant task in which rats were trained to press a lever for a preferred food reward on a fixed ratio (FR5) schedule, or to freely obtain standard lab chow. Different DA antagonists and agonists were then administered to rats. Results showed that the DA antagonist haloperidol, the D<sub>1</sub> receptor antagonist SCH-23390, a

non-selective DA antagonist (cis-flupenthixol), and the D<sub>2</sub> antagonist sulpiride decreased lever pressing. In almost all cases when a reduction in lever pressing was observed, chow consumption substantially increased. This study provides insight that DA is involved in effortful instrumental behaviours such as lever pressing on an FR schedule. Both studies mentioned here further support the notion that DA loss in NAcc may mediate effort-based decision making not by impairing task motivation or reward representation, as food consumption in all rats remained stable or increased. Rather, this impairment may be due to effort-reward weighing or decreased response vigor.

Because NAcc receives projections from the ACC, PL, and IL regions of mPFC, Walton and colleagues (2002) set out to test the role of mPFC in effort-based decision making. In a pre-training phase, rats were allowed to freely choose between the HRA (4 pellets) and LRA (2 pellets). After all rats chose the HRA at least 90% of the time, barrier training began. A wire-mesh barrier, which was increased by 5 cm every 3 days, was placed into the HRA, until rats learned to climb a final height of 30 cm and choose the HRA at least 80% of the time. Rats were then divided into a sham and a lesion group. Excitotoxic lesions to mPFC, including ACC, PL, and IL, were made using quinolinic acid. During each of three testing days, rats received two forced trials to sample each of the reward arms before the barrier was placed into the HRA, and then given ten test trials per day. Results showed that while sham animals remained at about 80% of HRA entries, mPFC lesion rats dropped to 5%. To rule out motor impairments, in a different testing block, an identical 30-cm-barrier was placed in the LRA so that effort was equated on both decision arms. Here, all animals improved from the initial three testing days; sham rats' performance went from 80% to 100% HRA entries, and lesion rats'

performance from 5% to 60% on day 1, and over 80% on days 2 and 3 of equated effort testing. This finding indicated that the difference in testing scores between groups in the first testing block was not caused by a motor impairment. When the LRA barrier was then taken out, lesion rats' performance declined from 50% on day 1, to under 25% on day 3.

To investigate how sensitive lesion rats were to barrier height and reward magnitude, two additional experiments were conducted. In a third testing block, the barrier height in the HRA was reduced to 20 cm. This was repeated to rule out interference from the original condition, so rats received the 30 cm in HRA for three days, then a 20 cm barrier for three days, and then the 30 cm barrier again for three days, followed by a final three days of the 20 cm barrier. Here, sham rats' performance remained at about 90%, whereas lesion rats' performance initially was 35% in the first 20-cm-block on day 1, and then remained stable at about 60% for the three days of the second 20-cm-block. In a final experiment, the reward ratio of HRA:LRA was varied from the initial 4:2 ratio to 5:1. During a 4:2 ratio baseline, sham animals performed at 85-90% HRA entries, while lesion animals performed at about 40%. When the ratio was changed to 5:1, sham animals performed over 95%, and lesion animals' performance also increased from 60% on day 1 to 85% by day 3. These results indicated that animals were sensitive to both barrier height and reward magnitude and initial findings could not be explained by an insensitivity to testing parameters. That is, the present results were due to the lesion rats' impairment of weighing effort and reward. It was previously established that if the LRA does not contain reward, all animals typically choose the HRA (Cousins et al., 1996).

Walton et al. (2002) were the first to show a direct involvement of mPFC in effort-based decision making. It must be emphasized that this effect was not due to a motor impairment, as lesion rats were physically able to climb the barrier at all stages of the experiment, but instead they *chose* not to do so. In a following study, Walton, Bannerman, Alterescu, and Rushworth (2003) sought to further clarify the respective contributions of different mPFC subregions to effort-based decision making. Specifically, they investigated ACC and PL/IL separately on the same T-maze effort task. After receiving lesions to ACC, rats dropped in performance as measured by HRA choices from 80% at baseline, to between 30% and 10% over three testing days. Oddly, however, both sham and PL/IL-lesioned rats dropped from the initial 80% baseline to about 50% of HRA choices. When the effort was equated by placing a second barrier into the LRA, all animals increased performance to over 80% by the third day of testing in this block. These findings showed that within mPFC, ACC seems to be the crucial region that allows for effort-related decisions, when the effort is a physical cost. Interestingly, although all regions tested project to NAcc and also to VTA, it is ACC (i.e., not other mPFC regions), and its midbrain connections, that play a critical role in this type of decision making (Walton et al., 2003).

Walton et al. (2003) were the first to show that ACC may not mediate all types of decision costs or effort. Whereas ACC seems to mediate physical effort, PL/IL seem to mediate delay effort (Cardinal et al., 2001; Delatour & Gisquet-Verrier, 1999). Delay presents a different type of decision cost, as the rat now has to wait longer for the reward to be delivered, rather than immediately obtaining a smaller reward or a less preferred food.

After the possibly contributory roles of NAcc DA and ACC in effort decisions were independently established, two research groups concurrently raised the question of whether the same effects of either DA depletion in NAcc or lesions of ACC could be obtained with DA depletions in ACC on the T-maze ramp-climbing effort task (Schweimer, Saft, & Hauber, 2005; Walton, Crosson, Rushworth, & Bannerman, 2005). Using 6-OHDA injections for DA depletion in ACC, Walton et al. (2005) showed that DA-depleted rats did not show a difference from vehicle-treated or unoperated control animals on the ramp-climbing effort task. This was the case even when reward magnitude ratio between HRA and LRA and barrier height on the HRA were increased. The authors concluded that ACC DA is not necessary for effort-guided decisions, as animals without ACC DA adjusted their effort-reward decision making in the same way as controls. After a systemic administration of haloperidol, a D<sub>2</sub> DA receptor antagonist, there was a shift from HRA to LRA choices in treated animals. This indicated that a systemic effect of DA reduction had the same effect as DA depletions in NAcc, but a focal loss of DA in ACC did not produce this effect.

In a nearly identical experiment, Schweimer et al. (2005) tested the same type of DA depletions in ACC using 6-OHDA on the T-maze ramp-climbing effort task. They found that there was a trend for DA-depleted rats to perform worse than controls, and this trend reached significance in a second testing block. However, the authors found a high variability in performance across individual rats and concluded that this high variability might have resulted in nonsignificance in the initial mean difference. It should be noted that Walton et al. (2005) did not give rats an additional block with a single barrier in the HRA following an equated effort block, whereas Schweimer et al. (2005) did. It was in

this second block that a group difference was found. Schweimer and colleagues (2005) also used a higher dose of 6-OHDA. However, Walton et al. (2005) concluded that drug dose of 6-OHDA did not affect results, as nearly no DA terminals remained in ACC even after a low dose as evidenced by immunohistochemical analysis. Schweimer and colleagues (2005) further conjectured that there might be a rat strain difference, as Lister hooded rats required a higher dose of haloperidol for the systemic D<sub>2</sub> receptor blockade to elicit LRA choice (Walton et al., 2005), whereas in Sprague Dawley rats a lower dose produced the effect (Salamone et al., 1994; Schweimer et al., 2005). It is unclear where the differences in these two studies stemmed from. They could indeed be due to strain differences, or to slight experimental variation. However, since the effect found by Schweimer et al. (2005) was small, results might simply indicate that ACC DA plays only a minor role in effort-based decisions.

In the same study, Schweimer et al. (2005) also tested a different effort paradigm in addition to the T-maze ramp-climbing task. A progressive ratio (PR) lever pressing task was used in which rats could choose to press a lever to obtain a preferred food (i.e., sucrose pellets), or to freely receive a less preferred food (i.e., lab chow). On the ramp-climbing task, rats performed as previously demonstrated (Walton et al., 2002; 2003), and ACC-lesioned rats discontinued to show a preference for the HRA, whereas sham animals continued to select the HRA. However, effort-based decision making was not impaired in the PR lever pressing task. Rats with ACC lesions still preferred the sucrose pellets even when effort expenditure was required, to freely available lab chow. When rats were tested on a breaking point paradigm using the PR lever-pressing task while eliminating any decisions, ACC-lesioned animals showed a similar breaking point to

sham animals. Both ACC-lesioned and control animals stopped pressing the lever when the effort became too high. This provided further confirmation that rats were sensitive to effort. Even when the decision to freely obtain lab chow instead of pressing the lever for sucrose pellets was introduced, both groups displayed the same reduction in breaking points, and the same amount of food consumption. Again, this study raised the possibility that ACC may not be involved in all types of effort.

Due to these contradictory results and following existing evidence for involvement of D<sub>1</sub> receptors in other mPFC mediated tasks, Schweimer and Hauber (2006) consecutively explored a more specific role of DA in ACC in effort-based decision making. Using microinfusions of the D<sub>1</sub> receptor antagonist SHC233390 or the D<sub>2</sub> receptor antagonist eticlopride into ACC, they found that only D<sub>1</sub> receptor blockade was involved in effort-based decision making on the ramp-climbing task. Rats receiving SHC23390 to ACC showed a marked decrease in HRA choices.

On a related note, Bardgett et al. (2009) systemically (subcutaneously) injected rats with different DA agonists and antagonists to further elucidate the role of D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> DA receptors in effort-based decision making on the ramp-climbing task. This experiment again followed after DA depletion of ACC had elicited conflicting results in the literature (Schweimer et al., 2005; Walton et al., 2005). It must be noted that the standard ramp-climbing paradigm was altered by using an effort-discounting protocol prior to testing. Here, rats were exposed to a discounting procedure for three days before the testing days during which DA agonists/antagonist injections were administered. On the first trial during discounting, rats were allowed to eat all the pellets on the maze (i.e., sample each arm). Thereafter, rats were only allowed to eat from the initially chosen arm

and could not sample the other arm. If the rat chose the LRA on any trial, testing was terminated. If the rat chose the HRA, one food pellet was taken away from it on the next trial. This was repeated and the number of trials it took to choose the LRA was noted. The following drugs were administered prior to testing: SCH23390 (D<sub>1</sub> antagonist), haloperidol (D<sub>2</sub> antagonist), 7-OHDPAT (D<sub>3</sub> agonist), and U99194 (D<sub>3</sub> antagonist), and combinations of any of the above with D-amphetamine (DA agonist). The authors showed that systemic blockade of D<sub>1</sub> and D<sub>2</sub> receptors resulted in a decrease in HRA entries, but D<sub>3</sub> receptor blockade had no effect. When D-amphetamine was administered in conjunction with DA antagonists, these effects were reversed. This study further refined the role of different DA receptors in effort-based decision making. It showed that the same effect seen with ACC lesions was produced by systemically blocking D<sub>1</sub> and D<sub>2</sub> receptors.

While the existing literature on effort-reward decision making had mainly examined ACC lesions and NAcc DA contributions, a novel study by Floresco and Ghods-Sharifi (2007) investigated an additional part of the potential effort-reward network, the BLA. In this experiment, the authors dissociated the connections between BLA and contralateral ACC on the standard T-maze cost-benefit paradigm. Here again, rats had to choose between a HRA and LRA and climb a 30-cm barrier to obtain the HRA. A local anaesthetic (bupivacaine hydrochloride) was used for temporary inactivation of ACC and BLA. Bilateral inactivation of BLA alone impaired effort-based decision making in rats. Similar to ACC lesions, here a shift away from the preferred, effortful HRA towards the LRA was observed. Disconnection of BLA and ACC produced the same effect. When a second barrier was placed in the LRA to equate effort,

the effects were reversed, as has been previously observed with ACC lesions (Walton et al., 2003). Interestingly, the area of ACC that was inactivated only spanned about 1 mm in diameter at anteroposterior +2.0 mm, mediolateral  $\pm$  0.7 mm, and dorsoventral -1.2 mm. Typically, ACC is lesioned along its entire length only sparing the most posterior part (Walton et al., 2002; 2003). This study showed that BLA lesions produce the same bias towards the LRA as ACC lesions. Thus, a potential role for BLA in effort-reward decisions is likely.

Pharmacological studies have further tried to disentangle which other neurotransmitter systems may be involved in effort-based decision making. Briefly, Floresco, Tse, and Ghods-Sharifi (2008) discovered that in addition to DA, glutamatergic action on NMDA receptors might also play a role in delay-based decision making. Specifically, they found that NMDA receptors played a selective role in shifting choice preference in delay-based tasks, where the animal had to choose between a small, immediate, and a large, delayed reward. Precisely, NMDA receptor blockade biased rats towards impulsive choices. Denk et al. (2005) also investigated serotonin in effort- and delay-based decisions. Here again, serotonin depletion resulted in more impulsive choices on the delay-based task, but had no effect on the effort task. In conclusion, while DA seems to mediate decisions in which physical effort or instrumental responding is involved as well as delay-based decisions, other transmitters such as glutamate and serotonin may mediate delay-based decisions only.

To investigate if there are other PFC structures that play a role in cost-benefit decisions, Rudebeck, Walton, Smyth, Bannerman, and Rushworth (2006) tested both delay- and effort-based decision making on the T-maze and were able to dissociate the

effects of OFC and ACC. In the delay experiment, the HRA:LRA reward ratio was 10:1. Rats chose between a HRA and LRA, and a 15-s delay was introduced as the decision cost in the HRA. In the effort experiment, the HRA:LRA reward ratio was 4:2. A 30-cm barrier was introduced as the decision cost in the HRA. Rats received lesions to either OFC or ACC. Results indicated that lesions to OFC, but not ACC, led to impulsive choices on the delay task. On the other hand, lesions to ACC, and not OFC, led to a shift in preference towards the LRA on the effort task. This finding indicated that OFC might not be part of the same network of effort-reward decisions that ACC belongs to. Instead, OFC seems to specifically mediate delay-based decisions.

Because previous studies had largely focused on either ACC or NAcc, Schweimer and Hauber (2009) set out to test the relative contributions of NAcc core and ACC to effort-based decision making by investigating the connections between the two structures. On the standard T-maze ramp-climbing task, they found that bilateral, but not unilateral, NAcc core lesions led to a preference shift away from the high-reward, high-effort option. Further, when ACC and NAcc were disconnected contralaterally, but not ipsilaterally, the same deficit could be observed. Thus, it seems the case that the connections between ACC and NAcc are sufficient for impairing rats on this effort task. This indicates again that ACC and NAcc are part of a network that as a whole mediates effort-based decision making.

In an unprecedented positron emission tomography (PET) study in rats, Endepols et al. (2010) looked at ACC activity following an effort-based decision task using a fixed ratio (FR) lever pressing paradigm. Rats chose between a high-reward lever (HRL; 4 pellets) and a low-reward lever (LRL; 2 pellets) in either a different-effort or a same-

effort condition. In the different-effort condition, rats obtained the respective reward after eight presses on the HRL (FR8) or four presses on the LRL (FR4). In the same-effort condition, both HRL and LRL required the FR8 schedule. On the testing day, animals received injections of fluorodeoxyglucose (FDG) 5 min prior to commencing the task. Rats spent 30 min on the task. At 60 min following FDG injection, rats underwent PET scans for 30 min. This timeline was based on previous literature showing that FDG concentration in the blood is highest 5-35 min following injection, which here corresponded to the time period rats spent on the effort task, meaning that PET scans reflected the behavioural testing period. Rats preferred the HRL in both the different-effort and the same-effort conditions. Positron emission tomography images revealed differences between the conditions in metabolic activity in left OFC, PL, and ACC (Cg2). Increased activity in ACC is consistent with lesion studies that imply a role of ACC in effort-based decisions (Walton et al., 2003). While the authors conceded that this experiment alone could not corroborate a hemispheric specialization, a human effort study (Croxson et al., 2009) coincides with this activity being constrained to the left hemisphere. Two discrepancies between this experiment and similar lesion studies were found: (a) OFC and PL also showed an increase in activity, and (b) no activity increase was found in NAcc. The authors suggested that activation of OFC and PL may correspond to the representation of response options and may indeed contain effort-related information, while not being necessary for making effort-based decisions, whereas ACC is. Regarding the lack of increase in metabolic activity in NAcc, the authors conjectured that sometimes glucose demand is not evidenced by FDG uptake, and that therefore, potential NAcc activity may not have shown up, or that NAcc activity

changes were too small to be visible. Yet, this seems like a rather weak explanation, as many other areas may have shown some activity that simply did not reach threshold. Using this inclusion criterion is not specific enough to NAcc. At any rate, the involvement of ACC in effort-based decisions on an operant task received further confirmation in this study.

A recent trend in studying ACC has been the move towards electrophysiological recordings from rat ACC during effort-based decision making (Cowen et al., 2012; Hillman & Bilkey, 2010; 2012). Hillman and Bilkey (2010) investigated ACC activity during a dynamic cost-benefit analysis. Here, rats were trained on a continuous T-maze, which connected the arms of the T back to the stem to form a figure-eight shape. In this task, rats chose between a no-effort LRA with two food pellets and a high-effort HRA with six food pellets and a 30 cm high barrier. Rats were given 60 trials per session. Recordings were collected from ten animals over 124 sessions, and 54 neurons were analyzed. About two thirds of neurons displayed higher firing rates in the HRA trajectory. These cells were found in all animals tested, even though some rats did not show a behavioural preference for the HRA. A follow-up revealed that this firing rate increase was due to the anticipation of the effort involved to obtain the high reward, since these cells did not show more activity when the barrier was removed from the HRA. Further, when a second barrier was placed in the LRA, cell firing increased only to the HRA. Thus, the increased firing cannot be attributed solely to the difference in reward magnitude, or solely to the cost associated with obtaining the reward, but indeed reflects

a combination of cost-benefit evaluation<sup>1</sup>.

Hillman and Bilkey (2012) further recorded from ACC during a competitive-effort task. Rats were trained on the continuous T-maze to discriminate reward magnitude on a LRA and HRA as previously explained (Hillman & Bilkey, 2010). In this version, however, there was no barrier present. Instead, a side box was attached to each of the two reward arms. Reward was now inaccessible to the rat behind a food chute that was located between the maze and the side box. Rats were initially trained with a peer rat inhabiting the side box, but no direct contact was possible apart from sight and smell. During testing, on the LRA, the partition remained, and both the test rat and the peer rat received a small reward (2 pellets). On the HRA, there was no partition between the test rat and the peer rat. That is, once the food chute door was raised, rats had to compete for the high reward (12 pellets). Anterior cingulate firing was increased to trajectories that involved competitive effort. These neural responses were similar to what had been observed during physical effort (Hillman & Bilkey, 2010). Therefore, it might be the case that social effort, or competitiveness, is considered a decision cost on a neural level. Again, firing rates did not increase when the rat was presented with different reward magnitudes only (i.e., without effort present), but ACC cells fired preferentially on the trajectory with the optimal effort-reward ratio. Interestingly, at the beginning of a session, or at the beginning of a block of within-session changes, ACC activity was generally increased. Consecutively, over the course of the session, activity became biased towards the HRA with high competitive effort. The authors suggested

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<sup>1</sup> As a side note, the Hillman and Bilkey (2010) found no difference in running speed of rats for arm choices. Sometimes it is argued that obtaining the high reward takes longer because rats have to climb the ramp. That could indicate that rather than measuring effort, this paradigm might measure impulsivity, as ACC lesion rats choose the LRA, which they can access faster. However, this experiment supported that

that this initially high activation might resemble surprise or an expectation violation. These results showed that even when the decision cost is competitive, rather than physical, ACC activity is similar to that previously seen on the physical effort task.

In another recording study, Cowen et al. (2012) found that activity in ACC during reward anticipation was dynamic, and different neurons responded to effort or reward at different locations on the maze. In this study, rats chose between two paths, each with an associated level of effort and reward. Activity of ACC corresponded to both anticipated reward as well as anticipated effort. Specifically, a small proportion of cells encoded anticipated effort, another proportion encoded anticipated effort and reward, and the smallest proportion encoded anticipated reward. Further, ACC was active during path traversal regardless of the level of reward or effort associated with that path. This led the authors to suggest that ACC may be important in the maintenance of ongoing actions, rather than in decision making before a response is executed. In that sense, if ACC were lesioned or inactivated, rats would be unable to maintain the motivation to continue the action that was associated with a high-effort, high-reward option (Cowen et al., 2012). The authors proposed that, in ACC-lesioned animals, this would behaviourally lead to an initial selection of the HRA, followed by a consequent abandonment of the plan when the specific action leading to this choice (e.g., climbing the ramp) has been commenced.

Lastly, a recent study attempted to measure cognitive effort in rodents. Cocker, Hosking, Benoit, and Winstanley (2012) developed a novel rat cognitive effort task (rCET) based on the 5-choice serial reaction time task. In this task, rats had to choose between an easy or difficult visuospatial discrimination. On difficult trials, the reward was double that of easy trials. Rats turned out to be separable into *worker* rats and

*slacker* rats. The consecutive administration of stimulants to animals resulted in differential effects for workers and slackers. Specifically, worker rats began to slack after amphetamine and caffeine injection, whereas slacker rats started working harder, but only after amphetamine injection. The authors concluded that rats could indeed distinguish mental effort on this rCET, and preferred the effortful option for a larger reward.

### **2.3 Conclusions From The Existing Literature.**

It has been repeatedly shown that rats are both willing and able to exert effort to obtain a larger or preferred reward. This effort can be physical, such as climbing a barrier (Walton et al., 2003), instrumental, such as pressing a lever multiple times (Schweimer & Hauber, 2005), cognitive, such as increasing cognitive load (Cocker et al., 2012) or competitive when competing for food resources (Hillman & Bilkey, 2012). The research findings discussed above indicate that, while there are common trends for ACC function in cost-benefit decision making, there are some contradictory findings as to which conditions exactly recruit ACC during effort-based decision making.

Based on these results, it is conceivable that ACC mediates all effort unequivocally, or that it mediates only specific types of effort. While ramp-climbing effort is typically impaired in rats with ACC lesions (Walton et al., 2003), this is not the case for delay-effort (Rudebeck et al., 2006; Walton et al., 2003), and results are mixed for instrumental effort (Endepols et al., 2010; Schweimer & Hauber, 2005). These mixed results stem from the findings that a bias away from the HRA was not found in a PR lever pressing task after ACC lesions, but activity in ACC was found during a FR lever pressing task (Endepols et al., 2010; Schweimer & Hauber, 2005). Further, when DA

was depleted in ACC on the ramp-climbing task, one study showed deficits, and a second one did not (Schweimer & Hauber, 2005; Walton et al., 2005).

These results pose the question of why, if ACC mediates all effort-reward decisions, task discrepancies emerge. It could be the case that the tasks discussed above are not comparing the same spectrum of costs and benefits. For instance, it may be argued that operant paradigms simply require the animal to apply more work (i.e., multiple presses of a lever) on the high reward option, rather than making this option more effortful per se (i.e., a heavier lever). For that reason, the instrumental paradigm is not parallel to the physical effort paradigm used in the ramp-climbing task. On this task, the rat chooses two equal trajectories, but climbs a barrier to obtain the high reward. It turns out that climbing is a naturalistic<sup>2</sup> behaviour for the rat, whereas lever pressing is a learned behaviour. Climbing is an innate, species-typical behaviour that rats readily exhibit, alongside other behaviours such as swimming, hopping, running, and digging (Iwaniuk, 2005). Even though rats can engage in lever-pressing behaviour after one-time exposure, it is not something that is naturally required from wild rats in their environment, and is thus acquired in laboratory animals. However, it must be pointed out that both tasks are parallel in that they require an initial stage of reward contingency learning in which no effort is present. While both tasks thus are first and foremost decision making tasks in which a behaviour is associated with a response, the type of behaviour fundamentally differs (i.e., species-typical vs. learned).

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<sup>2</sup> When laboratory rats are housed in enriched environments, so called *condos*, they can be seen climbing the wire mesh doors over three storeys. Furthermore, wild rats are naturally good climbers (Barnett, 2005). This could be due to the fact that the rat is a prey animal and must often climb obstacles to escape predators or to seek food.

## **Chapter 3: Experiment 1 – Does ACC Mediate Learned Physical Effort And Courage Effort?**

### **3.1 Rationale**

The question asked in this thesis is twofold: (a) does ACC mediate other types of physical effort that are not ramp-climbing effort, but parallel to it, and (b) does ACC mediate emotional effort? In this chapter, the initial experiment will be discussed which included a) a modified version of the ramp-climbing task, b) a novel weight-lifting effort task, and c) a courage-effort task.

In the existing ramp-climbing lesion literature, no study has investigated how rats perform within a session. Rather, rats typically received ten trials in a session, and this performance was compared across several training and testing days. However, electrophysiological data have indicated that the weighing of effort and reward can continue over a much longer time span. In the present experiment, rats completed 60 trials per session on a modified, automated version of the standard T-maze ramp-climbing task (Walton et al., 2003). This extended timescale is particularly helpful in detecting in performance changes across a single session, rather than a single average point. Further, the present ramp-climbing effort experiment included an effort discounting session, in which rats were tested on a continuously incremented effort. In the past, effort testing in ACC lesion rats has solely been conducted in discrete sessions where effort was incremented across, but not within a session.

As discussed previously, the existing operant effort paradigms cannot be regarded as parallel to the ramp-climbing paradigm. Because no other physical effort paradigm apart from the one standard ramp-climbing task existed in the literature, a novel task was

devised. This was a weight-lifting task that involved lever presses requiring harder work, not just repeated presses as in instrumental paradigms. With this paradigm, a different but parallel type of physical effort could be directly compared to the effort that is tested on the ramp-climbing task. One major difference between paradigms included that, as stated earlier, ramp-climbing is a naturalistic behaviour, whereas lever-pressing is a learned behaviour as explained in section 2.3. On this weight-lifting task, rats had to choose between pressing one of two seesaw levers on a Y-maze. Analogous to the ramp-climbing task, one lever dispensed a high reward (high reward lever; HRL), and the other lever a low reward (low reward lever; LRL). After initial reward discrimination training, the HRL was weighted with a weight that was a percentage of the rat's body weight. Piloting revealed that healthy rats prefer pressing such a heavy, weighted lever for a high reward to pressing a light, unweighted lever for a small reward. Thus, healthy rats behaved identical on this weight-lifting paradigm as they did on the ramp-climbing paradigm. Also, as on the ramp-climbing task, lever weights were incrementally increased. Pilot data revealed that, on both tasks, when rats were presented with the maximum effort on the first day, they would not choose the HRA. Therefore, this weight-lifting paradigm was considered an appropriate task to (a) compare physical effort with an analogous task requiring more physical effort rather than repeated lever presses, and (b) compare an innate to a learned effort behaviour.

Lastly, emotional effort has not been tested in rats, but it has been examined in humans (Nili et al., 2010). In fact, in humans, ACC seems to be involved during courageous actions, such as choosing to bring a fearful stimulus closer. A recent study in rats has focused on cognitive effort (Cocker et al., 2012), which is the form of effort most

often tested in humans. However, this cognitive effort is mostly associated with cognitive load or cognitive demand, therefore also not parallel to what is seen in the physical effort rat literature. We therefore devised a spatial courage-effort paradigm. On this task, similar to the ramp-climbing task, the rat could choose between two trajectories on a Y-maze, one leading into a HRA, and the other into a LRA. During testing, the walls and floorboard of the HRA were taken off, and in a later version, the room lights were turned on as well, presenting the rat with an innately fearful situation. The LRA remained covered by high walls, dark, and thus safe for the rat. Now, to obtain the HRA, rats needed to cross an elevated wire mesh bridge. That is, rats needed to display courage, or emotional effort, to acquire the high reward, or they could choose the safe LRA which required no effort.

This chapter will report the initial methodology and results on the three tasks described above. Chapters 4 and 5 will report additional experiments (Experiments 2 and 3) to clarify results from Experiment 1 in the present chapter. In Experiment 2, both the weight-lifting and courage tasks were modified. In Experiment 3, an additional modification was made to the weight-lifting task.

### **3.2 Hypotheses**

First, it was predicted that after ACC lesions, rats would show a preference shift from the high-reward, high-effort option to the low-reward, low-effort option on both the ramp-climbing and the weight-lifting task. Precisely, it was expected that rats would behave the same way as on the standard ramp-climbing task (Walton et al., 2003). That is, on the first post-lesion testing day, it was expected that ACC-lesioned rats would drop in performance compared to their baseline performance, but control rats would maintain

their baseline performance. It follows that ACC lesion rats should perform significantly worse on the first testing day compared to controls. When the effort was equated on LRA and HRA after testing days had been completed, it was predicted that ACC-lesioned rats would choose the HRA (a) as often as controls, and (b) as often as they did during baseline performance. This equated effort session was included to rule out a motor impairment and demonstrate that reward contingency representation is still intact in lesioned animals. Because the weight-lifting task was designed parallel to the ramp-climbing task, identical results were expected.

Second, on the courage task, the prediction was that after ACC lesions, rats would show a preference shift from the high-reward, high-effort option to the low-reward, low-effort option. That included the expectation that controls would show a performance curve starting with few open-arm entries after initial exposure to the open arm, but increasing to many open-arm entries over the course of the session. That prediction was made because even healthy rats should initially hesitate when first faced with a fearful environment. It was further expected that ACC-lesion rats would (a) hesitate more than controls after initial exposure to the open arm, and (b) if they were to improve over the course of the session, this improvement would be slower than that of controls over the course of the session. In summary, we expected rats to show impairments in effort-based decision making of ACC-lesioned rats on all three effort tasks, where the impairment would be such as to bias the rats away from the high-reward, high-effort option.

Lastly, to rule out that any of the hypothesized impairments would be caused by motor deficits or differences in baseline anxiety between lesion and control groups, two control tasks were implemented. The *open field* and *activity box* tasks tested activity of

rats, as ACC lesions have been reported to cause hypoactivity in some cases (Kolb, 1974). Because ramp-climbing and weight-lifting are motor behaviours, any differences in baseline activity of animals must be ruled out. The open field task further tested anxiety levels, as animals that are fearful will spend more time along the walls of the open field compared to the centre. Also, ACC lesion rats were expected to show no difference from controls in fear behaviour on the open field task.

### **3.3 Method**

#### **3.3.1 Animals.**

Twenty-four male Long-Evans rats (Charles River Laboratories International Inc., Montreal, QC) were trained in two cohorts in this study. Cohort 1 was 82 and cohort 2 was 112 days of age at the start of training. Rats' weights were maintained at 85% of their free-feeding weight (350-450 g at start of training) one week prior to and for the duration of training and testing. For at least one day before and five days after surgery, rats were allowed *ad libitum* food. Rats were between 102-109 (cohort 1) and 146-151 (cohort 2) days old at the time of surgery, and between 114-123 (cohort 1) and 161-170 (cohort 2) days old at the time of weight-lifting and ramp-climbing testing. During courage testing, cohort 1 was 124-132 days of age, and cohort 2 between 171-174. Open field and activity box were conducted on day 138 (cohort 1), and days 196 and 200, respectively (cohort 2). Cohort 1 was perfused at 152 days, cohort 2 at 204 days of age. Three animals died from peri-surgery complications. Two animals in the control group were excluded from data analysis because of accidental brain damage to ACC or M2 from sham surgery. One animal did not complete control tasks due to the sudden appearance of seizures. Two animals did not reach criterion performance on the ramp-

climbing task and were thus excluded from the ramp-climbing analysis. All procedures in all experiments were in accordance with the University of Lethbridge institutional animal care and use committee and Canadian Council on Animal Care recommendations and guidelines.

### **3.3.2 Materials and apparatus.**

All tasks were videotaped via an in-ceiling video camera.

#### ***Ramp-climbing task.***

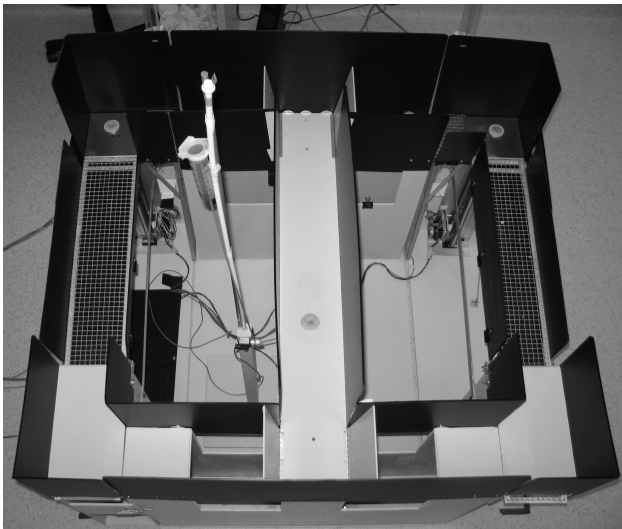
A 101.6 cm long by 114.3 cm wide figure-8 shaped maze was used for this task (Figure 3.1). This was an adaptation of the standard T-maze most often used in the literature for this task (Salamone et al., 1994; Walton et al., 2002) to form a continuous maze. The track was painted in light grey epoxy paint. Walls were made of corrugated black plastic of a height of 35.6 cm. The distance from the floor to the track of the maze was 60 cm, and the track was 1.9 cm thick. Rats were placed in the stem of the original T-figure, where two automated gates prevented them from entering the return arms backwardly. Rats then advanced to a base feeder at the centre of the T-stem. This triggered the beginning of a trial. Rats then further progressed to a decision region, where they could choose to enter either of the reward arms of the T. After one reward arm was chosen, an automated gate on the opposite arm would open, so that rats had access to the stem of the T, but not to the other reward arm. A feeder was located at the end of each reward arm. Rats then continued on a return arm, which closed the T-shape to a figure-8 shape. Once rats reached the corner that led back to the stem of the T, an automated gate opened at the opposite wall of the stem so rats were prevented from entering the other return arm and going backwards on the track. This provided a

continuous track for the rat once a reward arm was chosen and allowed for no interference from the experimenter. The experimenter observed the live video feed of the rat in an adjoining room.

Vertical wire mesh ramps of a height of 61.9 cm were attached to the 15.2 cm long piece on which the feeder was located. Wire mesh was made of .002 cm thick galvanized steel wire with a 0.2 cm square spacing. Stepper motors (Model 23Y9, Anaheim Automation, Anaheim, CA) controlled via a stepper motor controller (Model G251X, Gecko Drive, Tustin, CA) and a counterweight attached to the bottom of the floorboard allowed for raising of this corner piece, so that the rat had to climb the wire mesh ramp before obtaining the reward. The descending arm was also covered in wire mesh to allow for better grip. This piece was placed on two wheel pulleys (McMaster-Carr, Robbinsville, NJ) to allow for smooth retraction and extension of the descending arm. In the reward arms and in the base arm, a hole drilled through the floorboard allowed for feeding through a silicone feeder tube (91.44 cm long; inner diameter 1.98 mm, outer diameter 3.18 mm, wall thickness 0.61 mm; VWR International, Mississauga, ON) to connect from a feeder reservoir to a feeder well. Each feeder tube was fed through a pinch solenoid valve (Model SCH284B004-12/DC, ASCO Scientific, Florham Park, NJ) that was attached to the bottom of the floorboard, invisible and inaccessible to the animals.

The solenoid valve was controlled via a digital I/O board with on-board field-programmable gate arrays (FPGA; National Instruments, Vaudreuil-Dorion, QC) connected to a standard Windows-based computer. Custom-written software written in Microsoft Visual Basic and LabView (National Instruments, Vaudreuil-Dorion, QC) allowed for

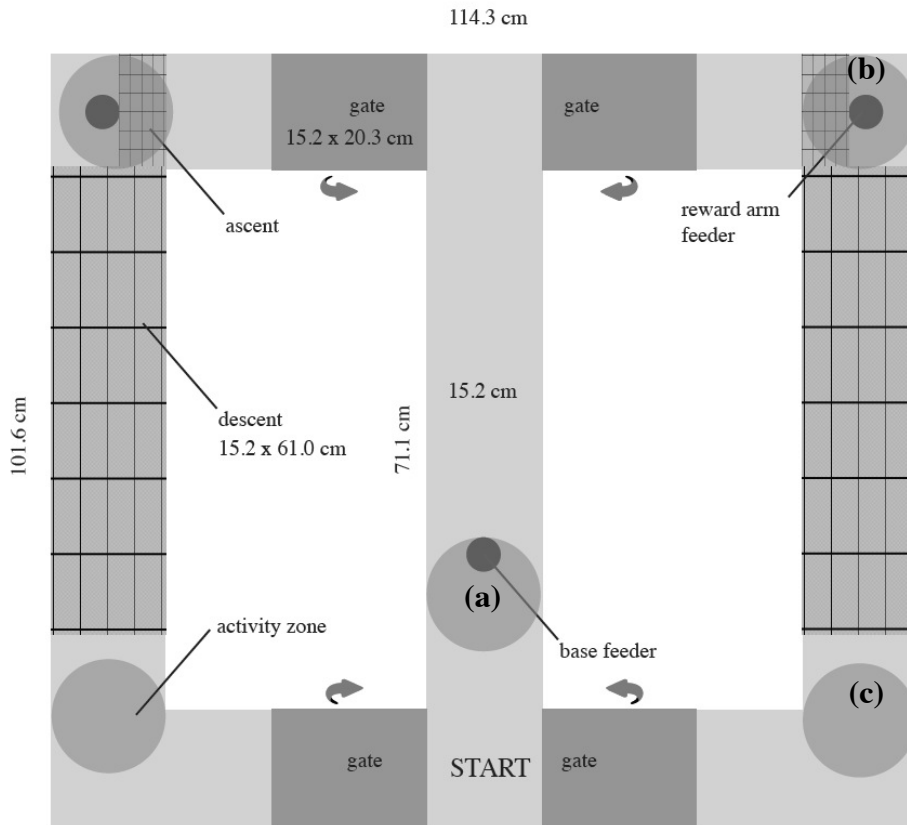
video-based monitoring of the animal, and automated control of all motors on the maze. Luer lock tips were placed at each end of the 76.2 cm long feeder tube. A syringe tip was glued into a 4.5 cm diameter washer to serve as a feeder well to which the feeder tube was connectable. Three 60 mL syringes served as food reservoirs and were hung at 114.3 cm from the floor (50.5 cm from the top of the track) from 76.2 cm long hollow plastic tubes affixed to the outside wall parallel to the feeder well. Liquid chocolate reward (Ensure®, Abbott Laboratories, Brockville, ON) was dispensed as the reinforcer on the task.



*Figure 3.1.* Picture of ramp-climbing task apparatus.

The task was conducted in a dimly lit room with the only light source stemming from twelve small LED lights mounted on the ceiling to allow for filming the experiment and monitoring the animal. The animal wore a flexible fabric belt to which red reflective tape was affixed for video tracking. Video tracking was provided via a standard video camera by Cheetah neurophysiology data acquisition software (Neuralynx, Bozeman, MT). The tracking feed was communicated to the Visual Basics program in real-time, so that activity zones could trigger responses based on the animal's location on the track.

This allowed for full automation of the task, rendering experimenter intervention unnecessary.



*Figure 3.2.* Schematic of ramp-climbing apparatus with dimensions and location of gates, feeders, ramps, and activity zones (a), (b), and (c); refer to text for details.

The activity zones and corresponding triggered events were as follows (see Figure 3.2 for activity zones): (a) base zone; base feeder was triggered, and both stem gates opened so that backward entry into return arms was prevented, (b) feeder zone; reward arm feeder was triggered in the arm of the rat's choice, the gate on the opposite reward arm closed to prevent entry into the discarded arm, and the stem gate on the return path of the feeder arm opened to allow entry into the base arm, and (c) return zone; when the rat reached the corner of the return arm, the reward arm gate opened to allow for free choice between reward arms, and the trial number incremented. After any given arm choice, the

rat could not enter the discarded arm, but had access to the stem of the T without obtaining further reward. Reward was only dispensed at the first traversal through each zone.

***Weight-lifting task.***

A novel weight-lifting effort-based decision-making task was developed. This task was conducted on a Y-shaped maze, painted in light grey epoxy paint, with 35.6 cm high black corrugated plastic walls. The base arm of the maze was a total of 76.2 cm long and 15.2 cm wide. Relative to the base arm, the two decision arms were shortened to 37 cm in length to minimize the distance to be covered by the rat. The maze was elevated 40 cm from the floor and the floorboards were 1.9 cm thick. A rectangular 4 cm high × 3.5 cm wide window allowed for a 30.5 cm long × 2.54 cm wide × 0.5 cm thick seesaw lever to protrude from the short walls, with the back and base of the lever obscured by the wall. A circuit board with Hall effect sensors was affixed to the lever stand to allow a magnet affixed to the lever to trigger the sensor at one of three stages (low, mid, high). Only the low (0 and high (2) stages were evaluated in the task, where *low* meant that for the food reward to trigger and the trial to increment, the rat had to barely press the lever, and *high* meant the rat had to press the lever all the way down.

A small LED cue light, positioned 24.1 cm from the floor, illuminated when a successful lever press was made. In the base arm, an emitter-sensor pair created an infrared beam which, upon being disrupted by the rat's passing, elicited food reward at the base feeder. In each of the three arms, a hole drilled through the floorboard at a 10.6 cm distance to the end wall allowed for feeding through a feeder tube (91.4 cm long) to

connect from a feeder reservoir to a feeder well. Feeder tube setup was identical to the ramp-climbing task.

Liquid chocolate reward was dispensed as the reinforcer on this task as well. The task was conducted in a dimly lit room with the main light source stemming from twelve small LED lights mounted on the ceiling to allow for filming the experiment and monitoring the animal. In-ceiling infrared lights were used for additional illumination. The experimenter was separated from the testing apparatus via a curtain that divided the testing room.



*Figure 3.3.* Picture of weight-lifting apparatus (left) and courage apparatus (right).

#### ***Courage task.***

A newly developed courage task was also conducted on a Y-maze, painted in light grey epoxy paint, with 35.6 cm high black corrugated plastic walls. All three arms of the maze were 76.2 cm long and 15.2 cm wide. The maze was elevated 60 cm from the ground, and the floorboards were 1.9 cm thick. Feeder tube setup was identical to that previously described. Here, infrared beams were used on all three arms to trigger reward delivery and increment trials. On one of the reward arms, the walls were clipped on, rather than screwed on, to allow for easy removal of walls, and a wooden floorboard

could be taken off to reveal a wire-mesh track. This open arm was presented to rats on testing day(s) only. As on all experimental tasks, liquid chocolate reward was used.

#### ***Open field and activity box.***

Rats were tested on two control tasks after completion of all experimental tasks, (a) the open field task, conducted on a 123 × 123 cm square field made of corrugated plastic with 50.8 cm high walls, and (b) the activity box (VersaMax, AccuScan Instruments Inc.). Rats were placed on the open field and allowed to explore for 5 min. The task was videotaped and an in-house program allowed for analysis of distance covered, time spent in periphery vs. centre, and running speed. In the activity box, 10 samples of 1-min duration were taken. For a full list of behaviours measured please refer to Table 3.9.

#### **3.3.3 Surgery and histology.**

Rats were matched according to task performance on the ramp-climbing task and then divided into sham and ACC lesion groups prior to surgery. Matching occurred by rank-ordering rats based on (a) how many days of training they required on the ramp task, and (b) how many days of training they required on the lever task. For the first pair of animals, the first rat was assigned to the lesion group. For the second pair, the first was assigned to the control group, and so on.

All rats underwent surgery. Thirty minutes prior to commencing isoflurane anaesthesia, rats were injected with 0.03mg/kg buprenorphine (concentration: 0.3mg/mL, 10x diluted). Isoflurane (Abbott Laboratories, Abbott Park, IL) was kept between 1-3% throughout the duration of the surgery depending on the rat's breath rate. All rats received a 3 mm wide × 5 mm long craniotomy. For sham rats, the craniotomy was then

covered with a thin film of *brain butter* (one part bone wax and two parts mineral oil). For lesion rats, dura was cut with a 30 G needle, and the bilateral injectors were lowered at the coordinates listed below across the central sinus. Bilateral, 45° bevelled, 28 G injectors were connected to thin-walled PE50 tubing (Fisher Scientific, Toronto, ON), which was connected to two Hamilton syringes (Hamilton, Reno, NV) in a syringe pump (Legato 100 Series, KD Scientific, Holliston, MA).

Injections were made at a rate of 0.15  $\mu\text{L}/\text{min}$  at 30% force and injectors were left in place for 5 min after injection to allow for diffusion. 300  $\mu\text{L}$  of N-Methyl-D-aspartic acid (NMDA, 15 mg/mL) were injected to create excitotoxic lesions along ACC at five bilateral injection sites after Walton et al., 2002 (anterio-posterior [AP]/dorso-ventral [DV]/medio-lateral [ML]): +3.0mm/-1.5mm/ $\pm$ 0.75mm, +2.3mm/-2.0mm/ $\pm$ 0.75mm, +1.6mm/-2.0mm/ $\pm$ 0.75mm, +0.9/-2.0mm/ $\pm$ 0.75mm, +0.2mm/-2.0mm/ $\pm$ 0.75mm from bregma. Dorso-ventral measurements were taken from brain surface. Following the final injection, the craniotomy was covered with a thin film of brain butter prior to suturing.

Post-surgery, rats were treated with 1mg/kg Metacam (meloxicam, concentration: 5mg/mL) for 3 days at 24 h intervals. Following completion of all experiments, rats were deeply anaesthetized with 100mg/kg sodium pentobarbital, then transcardially perfused with 1  $\times$  phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA). Brains were post-fixated in 4% PFA for at least 48 hours before they were transferred to a 30% sucrose solution with sodium azide. Brains were sectioned at 40  $\mu\text{m}$  on a cryostat (Leica), stained with 0.5% Cresyl violet, and mounted with Permount (Fisher Scientific) on superfrosted microscope slides. Sections were imaged using a nanozoomer (Hamamatsu) and lesion volumes were compiled using StereoInvestigator (MBF

Bioscience).

### 3.3.4 Task procedure.

Rats were trained on the ramp-climbing task and the weight-lifting task in conjunction. That is, rats usually underwent ramp-climbing training prior to the beginning of their dark cycle (0600-1200) and weight-lifting training afterwards in a staggered fashion (0900-1500 or 1200-1800). That way, after six out of twelve animals had completed the ramp-climbing task, they were trained on the weight-lifting task while the second half of animals began the ramp-climbing task. When staggering was impossible, all animals first completed the ramp-climbing task before starting the weight-lifting task. For a full timeline of training and testing according to experimental phases, see Table 3.1.

Table 3.1  
*Timeline According to Experimental Phase For All Experiments*

Phase	Experiment 1	Experiment 2	Experiment 3
1	Handling, Weighing	Handling, Weighing	Handling, Weighing
2	Food Restriction	Food Restriction	Food Restriction
3	Pre-Training – Ramp Climbing, Weight-Lifting	Pre-Training – Weight Lifting	Pre-Training – Weight Lifting
4	Training – Ramp Climbing, Weight Lifting	Training – Weight Lifting	Surgery
5	Surgery	Surgery	Recovery
6	Recovery	Recovery	Testing Day 1 (incremental) – Weight Lifting

Table 3.1 *Timeline According to Experimental Phase For All Experiments* (continued)

Phase	Experiment 1	Experiment 2	Experiment 3
7	Testing Days 1, 2, 3, Equate effort – Ramp Climbing, Weight Lifting	Testing Days 1, 2, 3, Equate effort –Weight Lifting	Activity Box, Open Field
8	Incremental Testing – Ramp Climbing	Incremental Testing – Weight Lifting	
9	Incremental Testing – Weight Lifting	Courage Training	
10	Courage Training	Courage Testing	
11	Courage Testing	Activity Box, Open Field	
12	Challenge Baseline – Weight Lifting		
13	Challenge Day – Weight Lifting		
14	Challenge Baseline – Ramp Climbing		
15	Challenge Day – Ramp Climbing		
16	Activity Box, Open Field		

***Ramp-climbing task.***

Rats underwent two stages of pre-training prior to effort training (Table 3.2). Each session lasted 20 min or 60 trials, whichever came first. After successful completion of effort training, rats underwent lesion or sham surgery and were allowed to recover for 10-14 days. Initially, rats first learned to complete trials on the maze without any effort. In this stage, all pulse widths (PW; the time the solenoid valve was open to let

fluid run through) were set at equal durations (600 ms), which produced a medium amount of food reward. Once rats reached >30 trials on the task, the base/centre PW was lowered to 300 ms, which produced a small amount of food reward. After rats successfully completed 60 trials in one session, they moved on to reward contingency learning.

Table 3.2  
*Ramp Task Schedule*

Objective	Stage	D	Height HRA <sup>T</sup> (cm)	Height LRA <sup>T</sup> (cm)	HRA PW (ms)	LRA PW (ms)	Base PW (ms)	Criterion to move on
Learn the maze	Pre-Training	1	0	0	600	600	600	30 total trials
	Pre-Training	2	0	0	600	600	300	60 total trials*
Learn reward contingencies	Pre-Training	3	0	0	1200	300	300	minimum 80% HRA trials (60 total trials)**
	Pre-Training	4	0	0	1200	300	300	minimum 80% HRA trials (60 total trials)
Attain effort stage 1	Training	5	15.2	0	1200	300	300	4 forced trials prior***; minimum 80% HRA trials (60 total trials)****
Attain effort stage 2	Training	6	25.4	0	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 total trials)
Attain effort stage 3	Training	7	30.5	0	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 total trials)

Table 3.2 *Ramp Task Schedule (continued)*

Objective	Stage	D	Height HRA <sup>†</sup> (cm)	Height LRA <sup>†</sup> (cm)	HRA PW (ms)	LRA PW (ms)	Base PW (ms)	Criterion to move on
Attain effort stage 4 (final)	Training	8	35.6	0	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 total trials)
	Training	9	35.6	0	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 total trials)
Surgery (ACC Lesion or Sham)	Testing	10	35.6	0	1200	300	300	4 forced trials prior; no criterion
	Testing	11	35.6	0	1200	300	300	4 forced trials prior; no criterion
	Testing	12	35.6	0	1200	300	300	4 forced trials prior; no criterion
	Control-Equate Effort	13	35.6	14	1200	300	300	4 forced trials prior; no criterion
Ramp additional task I	Incremental Effort	14	0-15.2-25.4-30.5-35.6-40.6-45.7-50.8	0	1200	300	300	no forced trials increment every 10 trials
Ramp additional task II	Challenge Baseline	15	0	0	1200	300	300	no forced trials
	Challenge	16	20	0	1200	300	300	4 forced trials prior

<sup>†</sup>HRA = high reward arm, LRA = low reward arm. \*This stage was repeated until 60 trials were achieved once. If it took more than five days, rats were moved on to the next stage on the following day. \*\*This stage was repeated until >80% HRA trials while performing 60 trials were achieved for two sessions. \*\*\*Forced trials: ramp height was 0 cm for the duration of four forced trials. Two trials were given to each arm in an alternating fashion. The starting arm alternated every day. \*\*\*\*Rats remained at each increment until reaching the criterion. Maximum training days = 14. Maximum testing days = 3. After 5 days of inadequate percentage in reward contingency learning and after 3 days in effort training (<80%), rats were moved on regardless. D = Day.

For each rat, a high reward arm (HRA) and a low reward arm (LRA) were determined. Arms were alternated in a pseudo-random way, so that even-numbered rats received arm 0 as their HRA, and odd-numbered rats received arm 1 as their HRA. However, if a rat exhibited an arm bias in the last stage of maze learning, the opposite arm to the biased one was assigned as HRA. Arm assignments stayed consistent for each rat and were never changed throughout the duration of the experiment. Based on the solenoid PW of reward delivery, the HRA:LRA reward ratio was 4:1. That is, the LRA PW was 300 ms and the HRA PW 1200 ms. The volume and calorie ratio, however, was 12.14:1. The volume at 300 ms was 0.02 mL (0.02 kcal), at 600 ms it was 0.1 mL (0.11 kcal), and at 1200 ms it was 0.24 mL (0.26 kcal). The lowest PW of 300 ms (0.02 mL) was the lowest measurable amount because a large fraction of the 300 ms PW was taken up by the response latency of the solenoid valve opening and closing, and the time it took for the liquid to travel down the tube. Rats had to reach a criterion of 80% or greater HRA trials for two non-consecutive days before moving on to effort training.

For all stages in effort training, rats received four forced trials without any effort present, two to each arm in alternation, prior to commencing the effort stage. These trials were not counted in any analysis and simply provided the rat with a chance to sample both arms prior to beginning the session. This is consistent with methodology used in previous studies (Walton et al., 2003). During testing, this allowed for exclusion of the possibility that lesion rats failed to choose the HRA because they did not remember the reward contingencies on the task. After the four forced trials, the rat was taken off the maze while the ramp of the HRA was raised to the specific height for the session. In effort stage 1, rats were trained to climb a 15.2 cm high ramp, followed by a 25.4 cm high

ramp in stage 2, 30.5 cm in stage 3, and 35.6 cm in the final effort stage 4. In stages 1-3, rats had to achieve a criterion of one day of 60 trials and 80% of HRA entries or greater. In stage 4, rats had to achieve a criterion of two non-consecutive days of criterion performance. After recovery from surgery, rats received 3 days of testing on the ramp-climbing task. Here, rats received four forced trials without effort as previously described. Then the ramp height of the HRA was raised to 35.6 cm.

Two additional tasks followed ramp-climbing testing. On the incremental task, rats received a number of height increments in the HRA within one single session, where heights were incremented every ten trials. Increments consisted of 0 cm, 15.2 cm, 25.4 cm, 30.5 cm, 35.6 cm, 40.6 cm, 45.7 cm, and 50.8 cm. On the challenge task, rats first received a zero effort control day to re-establish their reward learning. In the second session on the following day, rats received the four forced trials at 0 cm, followed by the remainder of the session at 50.8 cm, the highest height they had previously experienced.

#### ***Weight-lifting task.***

Rats underwent two stages of pre-training prior to effort training (Table 3.3). Each session lasted 20 min or 60 trials, whichever came first. After successful completion of effort training, rats underwent lesion or sham surgery and were allowed to recover for 10-14 days. Initially, rats first learned to press a seesaw lever for food reward at the low trigger stage (0), as well as to return to the base arm after each successful trial. In this stage, all pulse widths (PW) were set at equal durations (600 ms). This produced a medium amount of food reward. Once rats reached >30 trials on the task, the base PW was lowered to 300 ms, which produced a small amount of food reward, and the lever trigger stage was set to high (2), meaning that the rat now had to make a full lever press

to receive reward. The lever trigger stage remained at high for the remainder of the experiment. After rats reached 60 trials, they moved on to reward contingency learning and HRA/LRA were determined the same way as described above for the ramp-climbing task. After the four forced trials, the rat was blocked from access to the decision arms while a weight was affixed to the end of the seesaw lever. Weights were tailored to each individual rat's target weight and rounded up or down to the nearest 5 g. In effort stage 1, rats were trained on a weight of 10% of their body weight. Once a minimum of 80% HRA trials was reached in a session, rats moved on to the next stage with the same criterion. Throughout stages 2-4, rats were trained on 15%, 17.5%, and 20% of their

Table 3.3  
*Weight-Lifting Task Schedule*

Objective	Stage	Day	Lever weight	Lever mode	HRA <sup>F</sup> PW (ms)	LRA <sup>F</sup> PW (ms)	Base PW (ms)	Criterion to move on
Learn the maze, return-to-base mode	Pre-training I	1	0	0	600	600	600	30 total trials
	Pre-training I	2	0	2	600	600	300	60 total trials*
Learn reward contingencies	Pre-training II	3	0	2	1200	300	300	minimum 80% HRA trials (60 trials)**
	Pre-training II	4	0	2	1200	300	300	minimum 80% HRA trials (60 trials)
Attain effort stage 1	Training	5	10%	2	1200	300	300	4 forced trials prior***; minimum 80% HRA trials (60 trials)****
Attain effort stage 2	Training	7	15%	2	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 trials)

Table 3.3 *Weight-Lifting Task Schedule* (continued)

Objective	Stage	Day	Lever weight	Lever mode	HRA <sup>†</sup> PW (ms)	LRA <sup>†</sup> PW (ms)	Base PW (ms)	Criterion to move on
Attain effort stage 3	Training	8	17.5%	2	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 trials)
Attain effort stage 4 (final)	Training	9	20%	2	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 trials)
	Training	10	20%	2	1200	300	300	4 forced trials prior; minimum 80% HRA trials (60 trials)
Testing (post-surgery) after Walton et al. 2002	Testing	11	20%	2	1200	300	300	4 forced trials prior; no criterion
	Testing	12	20%	2	1200	300	300	4 forced trials prior; no criterion
	Equate effort	13	20% both	2	1200	300	300	4 forced trials prior; no criterion
Weight-lifting additional task I	Incremental effort	14	0-15-20-25-27.5-30-32.5-35-40%	2	1200	300	300	no forced trials increment every 10 trials
Weight-lifting additional task II	Challenge Baseline	15	0%	2	1200	300	300	no forced trials
	Challenge	16	40%	2	1200	300	300	4 forced trials prior

<sup>†</sup>HRA = high reward arm, LRA = low reward arm. \*This stage was repeated until 60 trials were achieved once. If it took more than five days, rats were moved on to the next stage on the following day. \*\*This stage was repeated until >80% HRA trials while performing 60 trials were achieved for two sessions. \*\*\*Forced trials: lever weight was 0% for the duration of four forced trials. Two trials were given to each arm in an alternating fashion. The starting arm alternated every day. \*\*\*\*Rats remained at each increment until reaching the criterion. Maximum training days = 14. Maximum testing days = 3. After 5 days of inadequate percentage in reward contingency learning and after 3 days in effort training (<80%), rats were moved on regardless.

Table 3.4  
*Courage Task Schedule*

Objective	Stage	Day	HRA <sup>†</sup>	HRA PW (ms)	LRA <sup>†</sup> PW (ms)	Base PW (ms)	Criteria to move on
Learn the maze, return-to-base mode	PreTraining	1	walls	600	600	300	60 total trials*
Learn Reward Contingencies	Training	2	walls	1200	300	300	minimum 80% HRA trials (60 trials)**
	Training	3	walls	1200	300	300	minimum 80% HRA trials (60 trials)
(Lesion)	Testing***	4	no walls, no floor	1200	300	300	minimum 80% HRA trials (60 trials)
	Testing	5	no walls, no floor	1200	300	300	minimum 80% HRA trials (60 trials)
Control task	Open Field Activity Box	6					5 min 10 samples of 1 min

<sup>†</sup>HRA = high reward arm, LRA = low reward arm. \*This stage was repeated until 60 trials were achieved once. If it took more than five days, rats were moved on to the next stage on the following day. \*\*This stage was repeated until >80% HRA trials while performing 60 trials were achieved for two sessions. \*\*\*Up to 7 days for testing were allowed, if criterion performance was not reached earlier. Two non-consecutive days of 60 trials and 80% or greater were required to terminate testing. A maximum of 14 days were allowed for reward contingency learning; however, if a rat did achieve high performance (>= 80%) for five non-consecutive days, even if 60 trials were not always reached.

body weight, respectively. In stage 4, rats had to achieve two non-consecutive sessions of criterion performance. In testing, rats were first presented with the four forced trials, and then directly with a weight of 20% of their body weight on the HRL. All animals received two testing days regardless of performance. On the third day, rats were tested for a motor impairment by placing a second 20% weight in the LRA.

Two additional lever tasks were conducted. In the incremental task, rats received a number of weight increments in the HRA within one single session, where weights were incremented every ten trials. Increments consisted of 0%, 15%, 20%, 25%, 27.5%, 30%, 32.5%, 35%, and 40% of the rats' body weights. In the challenge task, rats first

received a zero effort baseline day to re-establish their reward learning. In the second session on the following day, rats received the four forced trials at 0%, followed by the remainder of the session at 40%, the highest weight they had previously experienced.

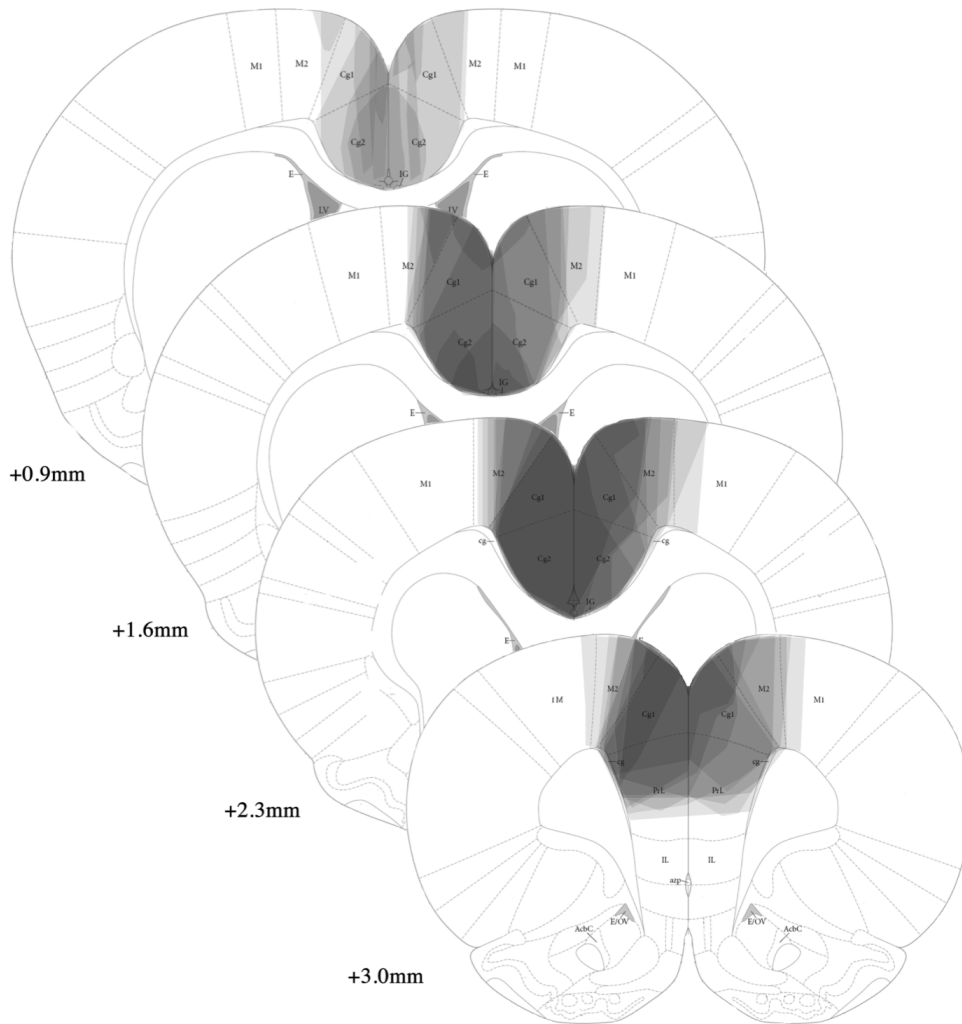
### ***Courage Task***

This task was conducted entirely post-surgery. Rats completed pre-training and reward contingency training (Table 3.4). Here, again, criteria to move on were the same as on the other experimental tasks. During these first two stages, all walls of the maze were left up. Once rats achieved two non-consecutive sessions of 60 trials and 80% HRA, walls and floorboard of the HRA were taken off. Rats were allowed up to 7 days of testing on the open arm maze. On this task, rats were started with a base PW of 300 ms immediately, since the task trajectories are highly similar to the weight-lifting task, which all rats had completed at that point.

## **3.4 Results**

For all experiments, StereoInvestigator (MBF Bioscience) was used to classify the intact tissue of the Cg1 and Cg2 fields of ACC along its entire length from +4.20 mm anterior to bregma to -1.56 mm posterior to bregma (Paxinos & Watson, 1998). All lesion brains and 70% of all control brains were examined. The total area and volume of ACC were noted for each brain and transformed into  $z$ -scores to determine outliers. A conservative  $z$  value of 3.09 (i.e.,  $p = .001$ ) was used to determine outliers. Based on this criterion, no rat was excluded from the study due to extreme or insufficient lesion size. For a comparative depiction of lesion sizes in Nissl stained brain sections, see Appendix A, and for a schematic tracing of lesion sizes in Experiment 1 see Figure 3.4. Analyses are reported with the following animal numbers: (a) ramp-climbing task: ACC lesion:  $n =$

9, sham: n = 8; (b) weight-lifting task: ACC lesion: n = 10, sham: n = 9; (c) courage task: ACC lesion n = 01, sham: n = 9; (d) activity box and open field: ACC lesion: n = 9, sham: n = 9.



*Figure 3.4.* Schematic of lesion extent from all animals in Experiment 1. Darkest gradient indicates the largest overlap of lesioned cortex in all animals. References are from bregma, anteroposterior (AP) axis, and correspond to four out of five injection sites (+0.2mm not shown).

### 3.4.1 Anterior cingulate mediates ramp-climbing effort.

Experiment 1 revealed that ACC lesions mediated ramp-climbing effort, but not weight-lifting or courage effort. First, because rats were run in separate cohorts, a

repeated measures analysis of variance (ANOVA) with cohort (cohort 1, cohort 2) as the between- and training time bin (trial bins 10, 20, 30, 40, 50, 60) as the within-subjects factor was conducted to determine whether rats in both cohorts were equal on the last day of training prior to being combined for further analyses. The division into groups was based on ramp-climbing performance. Therefore, this analysis was conducted with the scores of the last ramp-climbing training day. For all analyses, data were binned into trial bins of ten trials. Each bin represented an average of the previous ten trial scores. Seven animals were in cohort 1, and ten animals in cohort 2. The repeated measures ANOVA revealed that there was a significant Time  $\times$  Cohort interaction,  $F(5, 75) = 2.36, p = .048, \eta^2 = .14$ , observed power = .72. For post-hoc analysis of SME of time within group, a repeated measures ANOVA was executed with only time as the within-subjects factor. Alpha was adjusted via a Bonferroni correction for two tests, yielding a test parameter of 0.025. Post-hoc analyses revealed no significant simple main effects of either time within the cohort 1,  $F(5, 30) = 2.94, p = .029, \eta^2 = .34$ , observed power = .78, or within the cohort 2,  $F(5, 45) = 2.81, p = 0.27, \eta^2 = .24$ , observed power = .79. For post-hoc analysis of SME of cohort within time, six independent t-tests were run. Alpha was adjusted via the Bonferroni adjustment for six tests, yielding a test parameter of .008. There was no SME of time within cohort (for test statistics, see Table 3.5). Because follow-up tests revealed no significant differences, it was concluded that cohorts 1 and 2 were identical and thus were treated as such in the analyses.

Our results confirmed previous studies and showed that ACC lesions biased the rats' preference away from the high reward, high effort option (Rudebeck et al., 2006; Schweimer & Hauber, 2005; Walton et al., 2003). First, to answer the questions whether

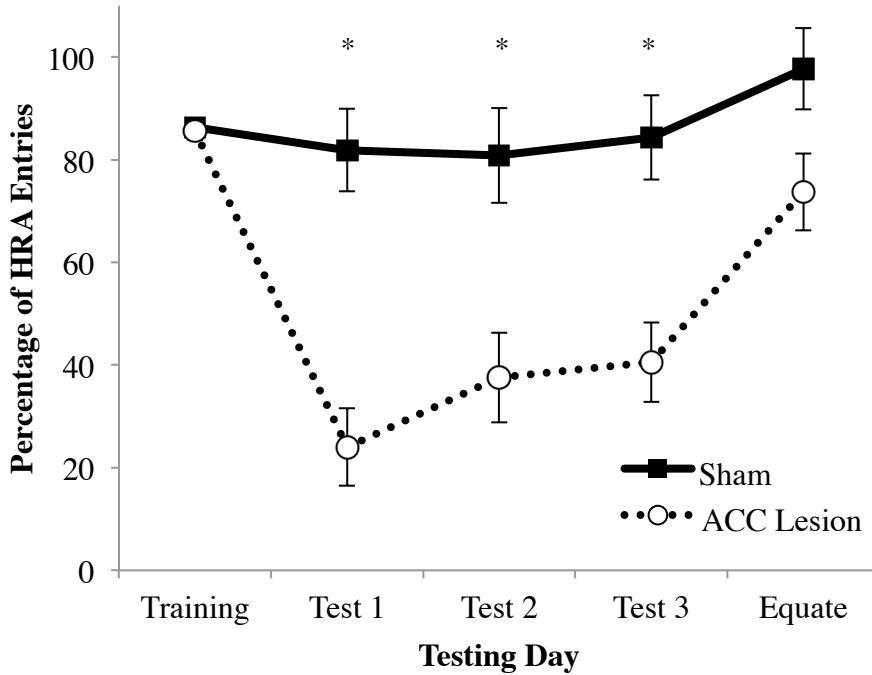
groups changed with time and differed from each other across time, a repeated measures ANOVA with testing day as the within-subjects factor (last day training, testing 1, testing 2, testing 3, equate effort) and group (control, ACC lesion) as the between-subjects factor was performed (see Figure 3.5).

Table 3.5  
*Test Statistics for Post-Hoc Analysis of SME of Cohort Within Time*

		<i>M</i>	<i>SD</i>	<i>t</i> (df)	<i>p</i> (two-tailed)
Training 10	Cohort 1	78.57	6.9	-.59(15)	.56
	Cohort 2	81	9.94		
Training 20	Cohort 1	90	10	2.03(10.43)	.069
	Cohort 2	81	7.38		
Training 30	Cohort 1	88.57	3.78	1.17(13.22)	.26
	Cohort 2	85	8.5		
Training 40	Cohort 1	85.71	7.87	-1.02(14.42)	.33
	Cohort 2	90	9.43		
Training 50	Cohort 1	82.86	9.51	-1.53(9)	.16
	Cohort 2	89	5.68		
Training 60	Cohort 1	91.43	12.15	.56(10.36)	.59
	Cohort 2	88.4	8.88		

Here, group averages for each testing stage (last day of training, first, second, and third days of testing, and equate effort day) were entered into the analyses. Because the sphericity assumption was violated,  $\chi^2(9) = 26.9, p = .002$ , the conservative Greenhouse-Geisser statistic was used ( $\epsilon = .71$ ). In general, if sphericity was violated and the

Greenhouse-Geisser statistic was equal to or greater than 0.7, then the latter was used. If the Greenhouse-Geisser statistic was less than 0.7, the more powerful multivariate analysis was used.



*Figure 3.5.* Performance of sham (solid line) and ACC lesion (dotted line) groups on the ramp-climbing task over the last day of training (Training), testing days 1, 2, and 3 (Test 1, Test 2, Test 3) and the equate-effort day (Equate). Asterisks indicate significant differences between groups on testing day 1, 2, and 3. See text for full details. Error bars represent standard error of the mean (SEM).

The ANOVA revealed a significant Testing Day  $\times$  Group interaction,  $F(2.82, 42.14) = 7.92, p < .001, \eta^2 = .35$ , observed power = .98. For post-hoc analysis of simple main effects (SME) of group within testing day, five independent t-tests were run. Alpha was adjusted via the Bonferroni adjustment for five tests, yielding a test parameter of .01. Post-hoc analyses identified an SME of group within testing day, which indicated that control rats performed significantly better on testing days 1, 2, and 3 compared to ACC lesion rats, see Table 3.6 for test statistics, means, and standard deviations.

Table 3.6  
*Test Statistics for Post-Hoc Analysis of SME of Group Within Testing Day*

	Group	<i>M</i>	<i>SD</i>	<i>t</i> ( <i>df</i> )	<i>p</i> (two-tailed)
Last training day	Sham	86.33	4.72	0.32(15)	.75
	Lesion	85.56	5.34		
Testing day 1	Sham	81.88	9.23	5.53(9.69)	.000
	Lesion	24	29.82		
Testing day 2	Sham	80.83	17.99	3.51(12.91)	.004
	Lesion	37.56	31.72		
Testing day 3	Sham	84.38	13.91	4.03(11.76)	.002
	Lesion	40.58	29.1		
Equate effort	Sham	97.71	2.81	2.34(8.15)	.047
	Lesion	73.7	30.63		

Further, the post-hoc repeated measures ANOVA testing for SME of testing day within the ACC lesion group, was significant,  $F(4, 32) = 14.86, p < .001, \eta^2 = .65$ , observed power = 1, with performance on testing day 1 ( $M = 24, SD = 29.82$ ) being significantly lower than during training ( $M = 85.56, SD = 5.34, p = .005$ ) or during the equate effort day ( $M = 73.7, SD = 30.63, p = 0.024$ ). Here, alpha was adjusted for two comparisons, with a new test parameter of .025. There was no SME of testing day within the control group,  $F(4, 4) = 8.42, p = .031, \eta^2 = .89$ , observed power = .75.

These post-hoc analyses indicated that there was indeed a change over time in the lesion group's performance. Specifically, ACC lesion rats performed well on the last day

of training, going to the HRA over 80% of the time, but then performance declined compared to that day, as well as compared to control rats on the first testing day. Lesion rats performed more poorly than controls on testing days 2 and 3 as well, meaning that they preferred the LRA. When the effort was equated, however, ACC lesion rats performed not significantly different from the last day of training, and also did not significantly differ from control rats, even though error bars did not overlap. However, caution must be exercised when eyeballing error bars (Lanzante, 2005; Payton, Greenstone, & Schenker, 2003).

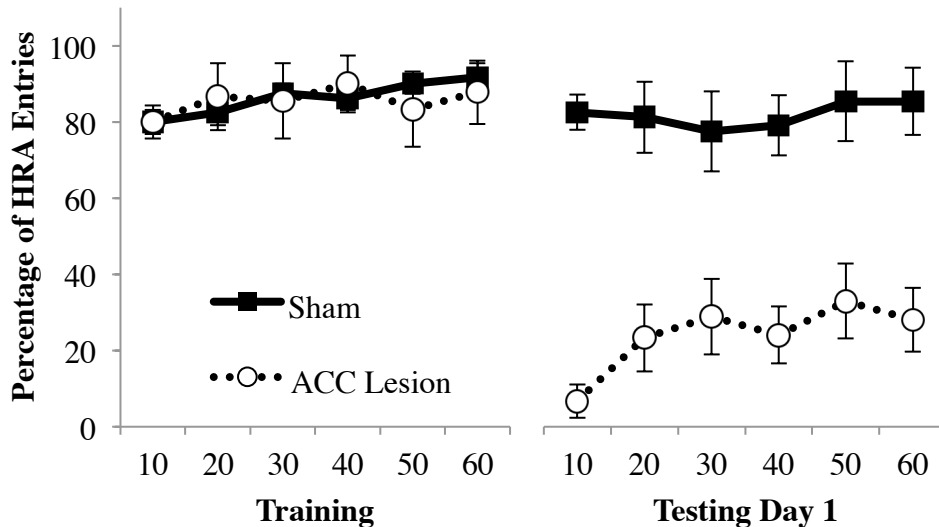
Table 3.7  
*Test Statistics for Post-Hoc Analysis of SME of Group Within Block*

	Group	<i>M</i>	<i>SD</i>	<i>t</i> (df)	<i>p</i>
Training	Sham	86.33	4.72	.32(15)	.75
	Lesion	85.56	5.34		
Testing Day 1	Sham	81.87	9.23	5.53(9.69)	< .001
	Lesion	24	29.81		

These results confirmed the hypotheses that lesion rats would prefer the LRA on testing days, at least for day 1, compared to their own baseline performance, and that on the equate effort day, they would return to baseline levels. The results further confirmed the prediction that lesion rats would do worse than controls on all testing days, but not on the equate effort day. Lastly, these results showed that lesion rats were not impaired on motor behaviour or reward contingency representation.

A second test was run to compare the within-session performance of control and ACC lesion rats. Here, trials in bins of ten within each block (training, testing 1) were

analyzed using a repeated measures ANOVA (see Figure 3.6). The within-subjects factors were block (training, testing) and time (trial bins 10, 20, 30, 40, 50, 60), and the between-subjects factor was group (control, ACC lesion). The analysis found a significant Block  $\times$  Group interaction,  $F(1, 15) = 22.86, p < .001, \eta^2 = .6$ , observed power = .99, but no other significant interactions involving time, all  $ps > .12$ .

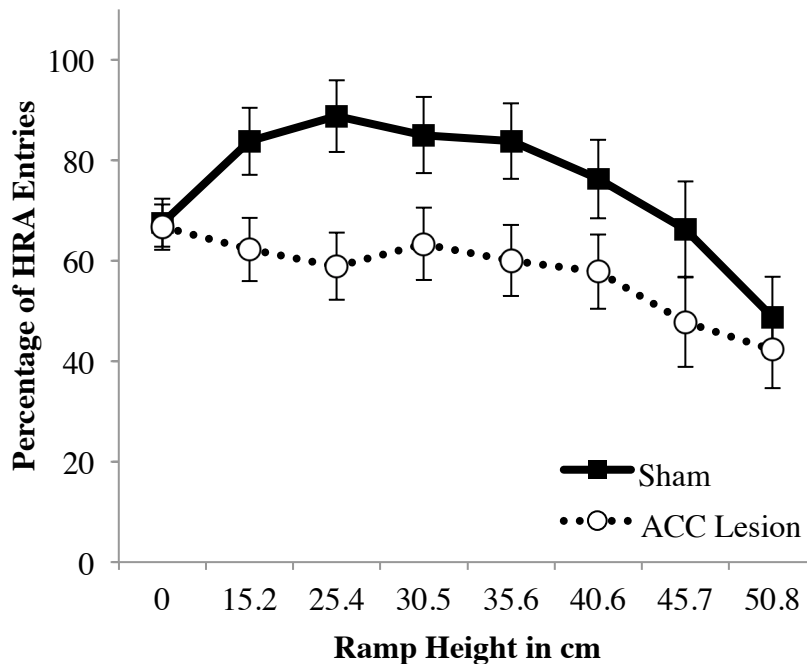


*Figure 3.6.* Within-session performance on the last day of training (Training) and the first testing day (Testing Day 1) on the ramp-climbing task split into trial bins. Control rats had significantly more HRA entries on testing day 1 compared to ACC lesion rats ( $p < .001$ ). Error bars represent SEM.

To test for an SME of block within group, a repeated measures ANOVA with block as the within-subjects factor was conducted. Due to multiple comparisons, alpha was adjusted via the Bonferroni correction to yield a test parameter of .025. This analysis revealed an SME of block within the ACC lesion group,  $F(1, 8) = 32.4, p < .001, \eta^2 = .8$ , observed power = 1, showing that the ACC lesion rats performed worse in testing 1 ( $M = 24, SD = 29.82$ ) than in training ( $M = 85.56, SD = 5.34$ ). The SME of block within the control group was not significant,  $F(1, 7) = 1.74, p = 2.28, \eta^2 = .2$ , observed power = .21. To test for a SME of group within block, two independent samples  $t$ -tests were conducted

with an adjusted alpha of .025. The control group performed significantly better than the ACC lesion group on testing day 1, but there was no group difference in the training session (see Table 3.7 for all statistics).

These results indicated that there was not a significant within-session change for either group, as neither interaction involving time was significant. Thus, they confirm what has already been established, namely that lesion and control rats differed on testing day 1, but not in training, and that lesion rats shifted away from their baseline HRA preference to a LRA preference in testing 1.



*Figure 3.7.* Performance on the ramp incremental test. Effort was incremented every 10 trials. Ramp height increments are plotted. Error bars represent SEM.

Two additional tests were run for the ramp-climbing task. One was an incremental task. On this task, the effort was incremented every ten trials within a single testing session (see Figure 3.7). It was hypothesized that ACC lesion animals (a) will reach a breaking point, where the amount of effort on the HRA is not worth the reward

and rats will go to the LRA more often instead, and (b) that this breaking point is at a lower effort level than it is for control animals. A repeated measures ANOVA with increment (0 cm, 15.2 cm, 25.4 cm, 30.5 cm, 35.6 cm, 40.6 cm, 45.7 cm, 50.8 cm) as the

Table 3.8  
*Test Statistics for Main Effect of Increment on the Ramp-Climbing Task*

	<i>M</i>	<i>SD</i>	<i>p</i> (two-tailed)
Increment 1 (0 cm)	67.08	13.12	
Increment 8 (50.8 cm)	45.49	22.39	.027
Increment 2 (15.2 cm)	72.99	21.37	
Increment 8 (50.8 cm)	45.49	22.39	.002
Increment 3 (25.4 cm)	73.82	24.94	
Increment 8 (50.8 cm)	45.49	22.39	< .001
Increment 4 (30.5 cm)	74.17	23.7	
Increment 8 (50.8 cm)	45.49	22.39	.001
Increment 5 (35.6 cm)	71.88	23.95	
Increment 8 (50.8 cm)	45.49	22.39	.002
Increment 6 (40.6 cm)	67.01	23.44	
Increment 8 (50.8 cm)	45.49	22.39	< .001
Increment 7 (45.7 cm)	57.01	27.6	
Increment 8 (50.8 cm)	45.49	22.39	.56

within-subjects factor and group (control, ACC lesion) as the between-subjects factor was performed. Sphericity could not be assumed,  $\chi^2(27) = 43.34, p = .03$ , Greenhouse-

Geisser  $\epsilon = .57$ , so the more powerful multivariate test was used, Wilks'  $\Lambda = .23$ . There was a significant main effect of increment,  $F(7, 9) = 4.23, p = .024, \eta^2 = .77$ , observed power = .81, but no significant Increment  $\times$  Group interaction,  $F(7, 9) = 2.14, p = .14, \eta^2 = .62$ , observed power = .49, and no main effect of group,  $F(1, 15) = 4.19, p = .059, \eta^2 = .22$ , observed power = .48. Pairwise comparisons showed that the following increments significantly differed from each other when collapsed across groups: all increments from 1-6 differed significantly from increment 8, but increment 7 and 8 did not differ (see Table 3.8 for all descriptive statistics). These results indicate that performance on the maximum increment (50.8 cm) was significantly worse than on any other effort level, except the one immediately preceding it. This also indicates that there is a drop-off, or effort-discounting curve. As the effort increases, performance drops down. Yet, it must be noted that this was an overall effect without group differences.

The second additional test was a challenge test. Here, rats first received a baseline session with simple reward discrimination without effort to re-establish a baseline post-surgery, termed the baseline stage. In the consecutive session on the following day, rats received the maximum increment they had so far experienced, (i.e., 50.8 cm) directly after the forced trials at 0 cm, termed the challenge stage (see Figure 3.8). A repeated measures ANOVA with the within-subject factors time (trial bins, 10, 20, 30, 40, 50, 60) and stage (baseline, challenge) and the between-subjects factor group (control, ACC lesion) was conducted. Sphericity could not be assumed,  $\chi^2(42.77) = .038, p < .001$ , Greenhouse-Geisser  $\epsilon = .41$ , so the more powerful multivariate test was used, Wilks'  $\Lambda = .19$ . Only a main effect of stage was found,  $F(1, 15) = 63.76, p < .001, \eta^2 = .81$ , observed power = 1, indicating that in the baseline stage, all rats (i.e., collapsing across group)

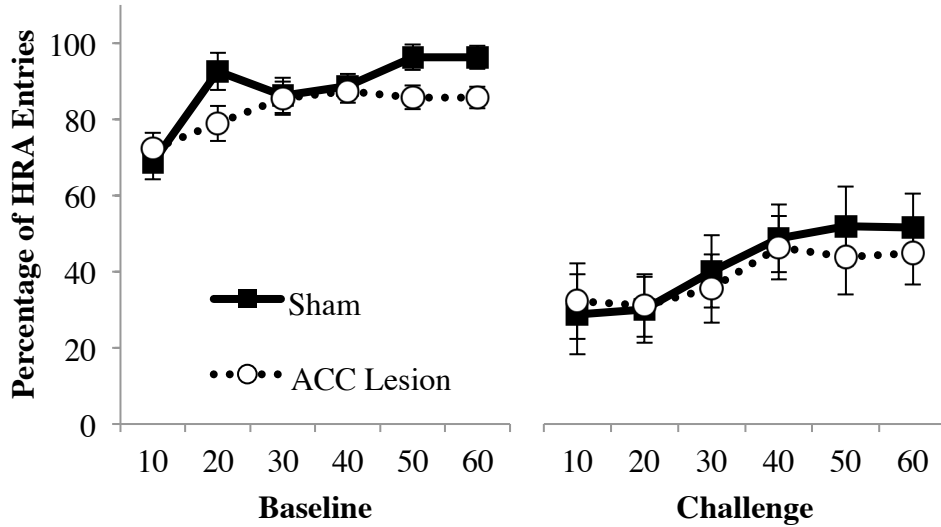
achieved significantly more HRA trials ( $M = 85.34, SD = 5.2$ ) than in the challenge stage ( $M = 40.44, SD = .89$ ). There was no significant interaction that involved stage or time, and no significant main effect of group. Results are reported in Table 3.9. Again, here the manipulation from baseline to challenge had an impact, but both groups showed the same trend, achieving over 80% HRA trials during baseline, but less than chance during the challenge day.

Table 3.9  
*Test Statistics for Ramp Challenge Task Repeated Measures ANOVA*

	$F(df)$	$p$	$\eta^2$	observed power
Time	3.02(5, 11)	.059	.58	.65
Time $\times$ Group	.86(5, 11)	.54	.28	.21
Stage	63.76(1, 15)	<.001	.81	1
Stage $\times$ Group	.059(1, 15)	.81	.004	.056
Time $\times$ Stage	1.62(5, 11)	.23	.42	.37
Time $\times$ Stage $\times$ Group	.55(5, 11)	.74	.2	.14
Group	.5(1, 15)	.49	.032	.1

Finally, the behaviour of rats on the ramp-climbing task during the first testing day was more closely examined. This was due to the experimenter noticing a distinct behaviour of lesion, but not control animals. Specifically, lesion animals would often go up to the ramp, sometimes place the forepaws on the wire mesh, but then turn around and enter the LRA. Some rats even went as far as clinging to the wire mesh with all four paws and subsequently still turning around to the LRA. Occasionally, once a lesion rat

entered the LRA, he would turn around and place his paws onto the gate which blocked the backward trajectory, and gaze towards the HRA option.

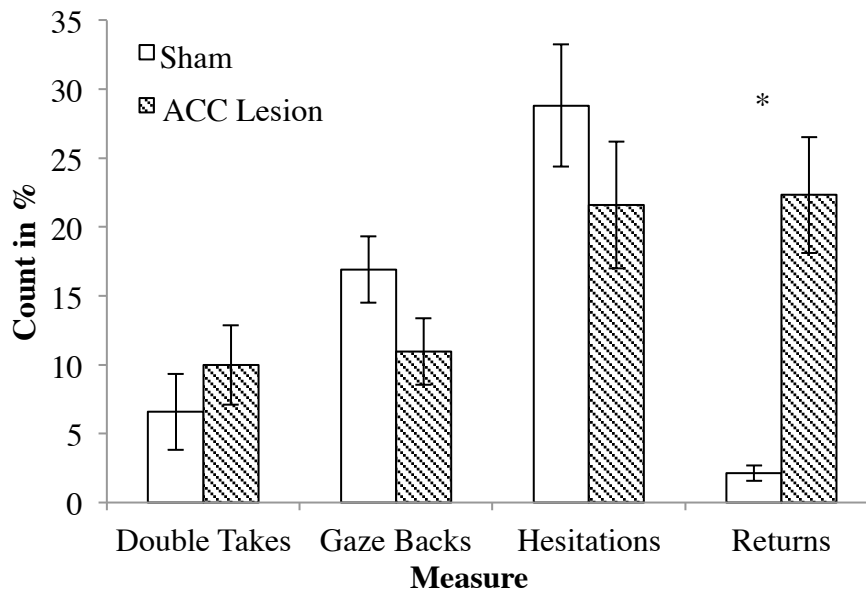


*Figure 3.8.* Performance on the ramp challenge task in baseline and challenge conditions. In the challenge session, effort was a ramp height of 50.8 cm. No group differences were observed. Error bars represent SEM.

In an analysis of the videos, these behaviours were scored. These behaviours included (a) double-take, which occurred when the rat came up to the decision region, and looked into a different arm than he entered first; (b) gaze back, which represented a gaze back at the other option that was not taken, involves turning around on the existing arm after reaching the feeder; (c) hesitation at the decision region or at the ramp; (d) return on turning arm before reaching the feeder; (e) ramp approach with return, where at least one paw was placed on the ramp before returning to the LRA. These behaviours were examined to potentially reveal more finely grained differences in behavioural strategies between groups. .

Four independent samples *t*-tests were conducted with these behaviours as independent variables, and group (control, ACC lesion) as the grouping variable. The counts were normalized based on the number of trials the rats completed in the first

testing session, as not all rats achieved 60 trials. Returns were collapsed into one variable and grouped together for HRA and LRA, because only two rats (one lesion, one control) made one return each on the LRA. A statistically significant group difference was found for returns, which corresponded with the observations, showing that lesion rats performed significantly more returns ( $M = 22.32, SD = 12.66$ ) than controls ( $M = 2.13, SD = 1.59$ ),  $t(8.29) = -4.74, p = .001$  (see Figure 3.9). This was still the case when returns were analyzed as a fraction of LRA entries. Moreover, when looking at HRA turns only, it



*Figure 3.9.* Behaviours identified in video analysis. ACC lesion rats performed significantly more returns than controls. The asterisk indicates that lesion animals performed significantly more returns than controls. Error bars represent SEM.

was observed that both ACC-lesioned and control rats turned into the HRA 80% of the time, but actual entries were restricted to 20% for ACC-lesioned rats and followed by returns and LRA entries the remainder of the time. This is an interesting finding that may support hypotheses postulating that ACC is responsible for action maintenance (Cowen et al., 2012). That is, lesion rats actually walk towards the HRA ramp, but just before the

ramp realize that they cannot obtain the high reward without exerting effort, and thus turn around to the LRA.

Lastly, data from these animals were compared on the control tasks: (a) the open field and (b) the activity box. As mentioned earlier, one rat from the lesion group had to be euthanized due to health complications prior to completing the control tasks. This left eight lesion rats and eight control rats for these remaining two analyses.

Open field data were analyzed using five independent *t*-tests for each of the following measures: (a) percentage of time spent in centre, (b) path length, (c) running speed in m/s, (d) path length in centre, and (e) path length in periphery. On this test, groups did not significantly differ on any of these measures.

Table 3.10  
*Measures and Test Statistics for Activity Box Data*

	Group	<i>M</i>	<i>SD</i>	<i>t</i> (df)	<i>p</i> two-tailed
Horizontal activity	Sham	516.09	144.017	-.091(9.95)	.93
	Lesion	521.21	67.74		
Total distance (cm)	Sham	303.13	109.65	.44 (11.52)	.67
	Lesion	283.23	66.35		
Number of Movements	Sham	11.18	1.61	-2.47(13.48)	<b>.028</b>
	Lesion	13.39	1.96		
Movement time (s)	Sham	26.89	6.76	-.055(10.8)	.96
	Lesion	26.99	3.67		
Rest time (s)	Sham	33.21	6.71	.009(10.81)	.99
	Lesion	33.19	3.65		

Table 3.10 *Measures and Test Statistics for Activity Box Data* (continued)

	Group	<i>M</i>	<i>SD</i>	<i>t</i> ( <i>df</i> )	<i>p</i> two-tailed
Vertical activity	Sham	44.6	14.22	-.023(12.04)	.98
	Lesion	44.74	9.28		
Number of vertical movements	Sham	5.65	1.69	-.61(11.58)	.56
	Lesion	6.08	1.03		
Vertical time (s)	Sham	15.2	5.15	.48(13.25)	.64
	Lesion	14.09	4.03		
Stereotypy counts	Sham	256.91	74.41	-.81(10.13)	.44
	Lesion	280.46	36.17		
Number of stereotypy	Sham	9.11	1.11	-2.49(11.18)	<b>.03</b>
	Lesion	10.24	.64		
Stereotypy time (s)	Sham	14.06	2.12	-2.57(13)	<b>.023</b>
	Lesion	16.48	1.59		
Clockwise revolutions	Sham	1	.62	.74(11.43)	.48
	Lesion	.81	.37		
Counter-clockwise revolutions	Sham	.95	.73	.69(10.37)	.51
	Lesion	.75	.37		
Margin distance (cm)	Sham	124.21	47.23	.69(13.97)	.5
	Lesion	108.33	45.02		
Margin time (s)	Sham	26.46	7.31	.33(13.57)	.75
	Lesion	25.13	8.76		

Table 3.10 *Measures and Test Statistics for Activity Box Data* (continued)

	Group	<i>M</i>	<i>SD</i>	<i>t</i> (df)	<i>p</i> two-tailed
Centre distance (cm)	Sham	178.93	71.27	.13(13.29)	.9
	Lesion	174.88	56.35		
Centre time (s)	Sham	33.68	7.3511	-.32(13.61)	.76
	Lesion	34.96	8.73		
Left front time (s)	Sham	.56	1.43	1.04(7.03)	.33
	Lesion	.033	.071		
Right front time (s)	Sham	.05	.11	-.59(12.84)	.59
	Lesion	.088	.15		
Left rear time (s)	Sham	.13	.32	1(7.18)	.35
	Lesion	.013	.035		
Right rear time (s)	Sham	.53	1.08	1.02(7.68)	.34
	Lesion	.13	.24		

These results indicated that rats in the lesion group did not differ in anxiety from rats in the control group. Further, groups did not differ from each other based on the motor variables investigated here, path length and running speed. To further qualify if any motor differences existed between groups, activity box data were analyzed using independent *t*-tests. Measures included all of the ones listed in Table 3.10.

Lesion rats spent significantly more time on stereotypy (i.e., the total amount of time stereotypy is exhibited) and also had significantly more accounts of stereotypic behaviours (i.e., if the animal breaks the same beam repeatedly; where stereotype counts are number of beam breaks, and number of stereotypy is the number of stereotypic counts

separated by at least 1 s). Stereotypic behaviours may include grooming behaviours. Finally, lesion rats also had significantly more overall movements (i.e., number of discrete horizontal movements with breaks in activity of 1 s between movements). This is interesting inasmuch that previously, ACC lesions have been hypothesized to cause anergia, or lethargy. Anergia however has been ruled out as an experimental confound for the impairment in effort-based decision making (Rudebeck et al., 2006; Salamone et al., 1994).

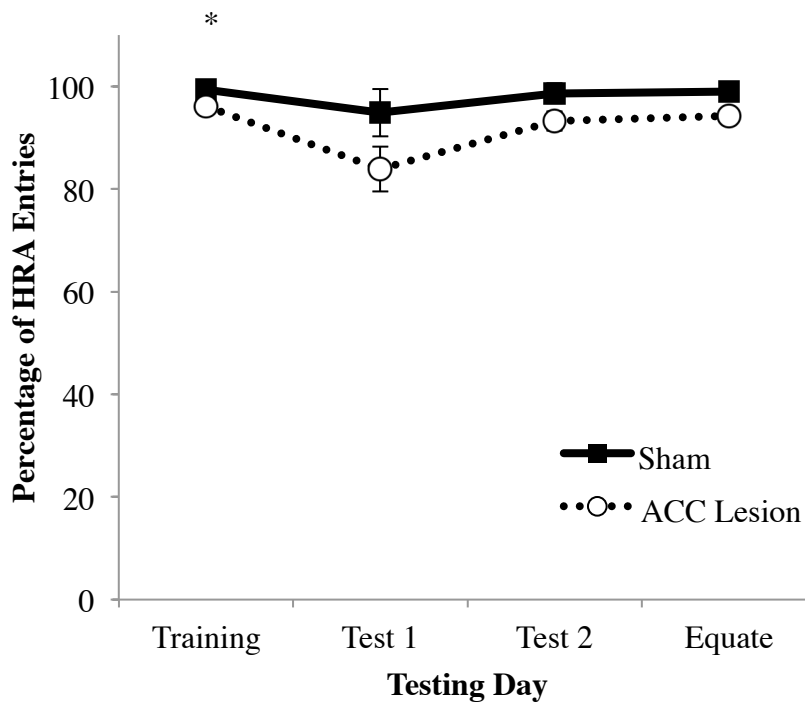
In summary, these results indicated that, as previously shown (Walton et al., 2003), rats with lesions to ACC prefer the low effort, low reward option, whereas sham animals continue to select the high effort, high reward option. This result corroborated that ACC mediates effort on the ramp-climbing task.

### **3.4.2 Anterior cingulate does not mediate weight-lifting effort.**

Analyses were conducted in parallel to the ramp-climbing analyses. First, a change over time was investigated. For this analyses, averages of the last training day, testing day 1, testing day 2, and equate effort day were used. Second, to see a change within session, the last training session and first testing session were analyzed according to trial bins. Lastly, incremental and challenge sessions were analyzed.

A repeated measures ANOVA was performed with testing days as the within-subjects factor (last training day, testing day 1, testing day 2, and equate effort) and group (control, ACC lesion) as the between-subjects factor (see Figure 3.10). Mauchly's test of sphericity showed that this assumption was violated,  $\chi^2(5) = 37.14, p < .001$ , Greenhouse-Geisser  $\epsilon = .46$ , so the more powerful multivariate test was used. Results showed that there was no significant Testing Day  $\times$  Group interaction,  $F(3, 15) = .52, p = .68, \eta^2 =$

.09, observed power = .13, Wilks'  $\Lambda$  = .91; and no significant main effect of testing day,  $F(3, 15) = 2.98, p = .065, \eta^2 = .37$ , observed power = .58, Wilks'  $\Lambda$  = .63. However, there was a significant main effect of group,  $F(1, 17) = 5.11, p = .037, \eta^2 = .23$ , observed power = .57, showing that when collapsing across time, control rats performed significantly better ( $M = 97.97, SD = 2.06$ ) than lesion rats ( $M = 91.85, SD = 5.44$ ) across all testing days. This finding was problematic insofar as it indicated that groups were different throughout the entire period analyzed here, including the last day of training.



*Figure 3.10.* Performance of sham (solid line) and ACC lesion (dotted line) groups on the weight-lifting task over the last day of training (Training), testing days 1 and 2 (Test 1, Test 2), and the equate-effort day (Equate). The asterisk represents a significant group difference on the training day. Post-hoc tests revealed a significant simple main effect of group within the training day, with the control group outperforming the lesion group (see text for details). Error bars represent standard error of the mean (SEM).

Yet, considering the mean values, it is noteworthy that values for both groups were well above the required 80% criterion, which was used to determine a strong preference to the HRA. That is, both groups continued to prefer the HRA nearly 100% of the time. In that sense, a group difference is rather meaningless. The highest discrepancy between groups was seen on testing day 1, where control rats performed at 94.89% and lesion rats at 83.9% (see Table 3.11). However, this difference was not significant. Again, it must be noted that lesion rats are still performing above the 80% criterion.

Table 3.11  
*Means and Standard Deviations for Weight-Lifting Task by Group*

	Group	<i>M</i>	<i>SD</i>
Last Day Training	Sham	99.33	1
	Lesion	96.1	3.03
	Total	97.63	2.79
Testing Day 1	Sham	94.89	5.75
	Lesion	83.9	18.27
	Total	89.11	14.61
Testing Day 2	Sham	98.67	2.18
	Lesion	93.2	7.9
	Total	95.79	6.42
Equate Effort	Sham	99	1.22
	Lesion	94.2	6.81
	Total	96.47	5.47

To further investigate these data, a repeated measures ANOVA was run to compare within session performance for the last training day and the first testing day. Time (trial bins 10, 20, 30, 40, 50, 60) and block (training, testing 1) were entered as the within-subjects factors, and group (control, lesion) as the between-subjects factor (Figure 3.11). Mauchly's test of sphericity revealed a violation,  $\chi^2(14) = 67.65, p < .001$ , Greenhouse-Geisser  $\epsilon = .44$ , so the more powerful multivariate test was used. The analysis revealed a significant Time  $\times$  Block  $\times$  Group interaction,  $F(5, 13) = 3.81, p = .024, \eta^2 = .6$ , observed power = .79, Wilks'  $\Lambda = .41$ .

A total of seven post-hoc tests were conducted to test for SMEs of time 1 (training trial bins) within group, time 2 (testing trial bins) within group, SMEs of block 1 (training) within group, block 2 (testing) within group, and SMEs of group within block (training, testing), time 1 (training trial bins), and time 2 (testing trial bins).

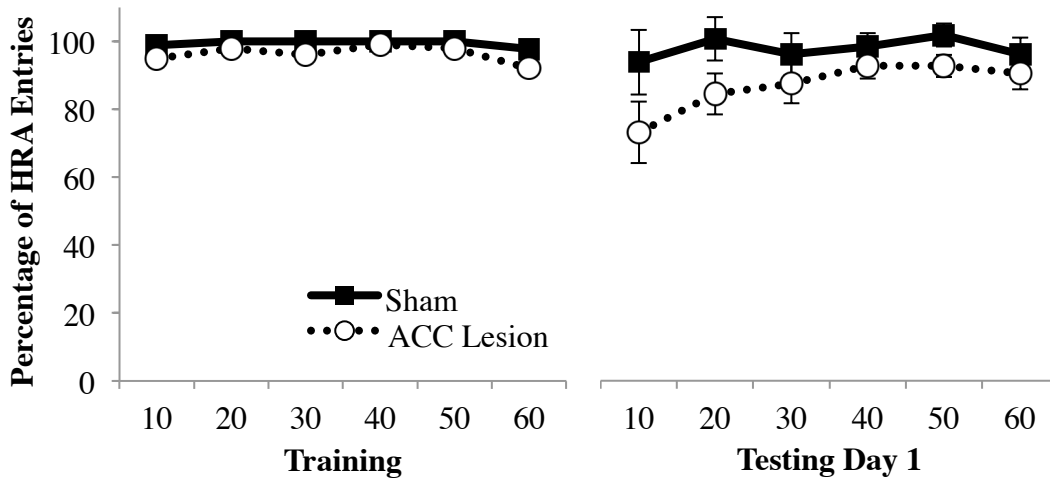


Figure 3.11. Within-session performance on the weight-lifting task on the last day of training (Training) and the first testing day (Testing Day 1) on the weight-lifting task split into trial bins. Control rats performed significantly better on the last training day compared to lesion rats ( $p = .009$ ). Error bars represent SEM.

To test for an SME of training time (trial bins 10, 20, 30, 40, 50, 60) within group, data were split according to the between-subjects factor group (control, ACC lesion), and

a repeated measures ANOVA was conducted with training time as the within-subjects factor. Alpha was adjusted for two comparisons using the Bonferroni adjustment, yielding a test parameter of .025. This test showed no significant SMEs of training trials within either the control,  $F(2, 7) = 1.75, p = .24, \eta^2 = .33$ , observed power = .25, Wilks'  $\Lambda = .67$ , or the lesion group,  $F(5, 45) = 2.57, p = .04, \eta^2 = .22$ , observed power = .74. For the first test, sphericity was not assumed and the multivariate test was used (not enough degrees of freedom to be calculated), but for the second test it was assumed,  $\chi^2(14) = 21.25, p = .11$ .

Next, to test for an SME of testing 1 time (trials 10, 20, 30, 40, 50, 60) within group, the same test was run but with testing 1 time as the within-subjects factor. No significant SMEs for testing 1 within the control or the lesion group were found; control, sphericity not assumed,  $\chi^2(14) = 30.39, p = .01$ , Greenhouse-Geisser  $\epsilon = .46, F(5, 4) = 1.6, p = .34, \eta^2 = .67$ , observed power = .21, Wilks'  $\Lambda = .33$ , and lesion, sphericity not assumed,  $\chi^2(14) = 39.1, p = .001$ , Greenhouse-Geisser  $\epsilon = .4, F(5, 5) = 2.94, p = .13, \eta^2 = .75$ , observed power = .42, Wilks'  $\Lambda = .25$ . In summary, there were no significant SMEs of time within either group.

To test for SME of block (training, testing 1) collapsed across time (trials) within group (control, lesion), data were split according to group, and a repeated measures ANOVA was run with block as the within-subjects factor. Alpha was adjusted for two tests to yield .025. For the control group, sphericity was not assumed (lack of degrees of freedom to be calculated), and the multivariate tests showed that  $F(1, 8) = 5.04, p = .055, \eta^2 = .39$ , observed power = .51, Wilks'  $\Lambda = .61$ . For the lesion group, sphericity was again not assumed (lack of degrees of freedom to be calculated), and the multivariate

tests showed that  $F(1, 9) = 5.04, p = .051, \eta^2 = .36$ , observed power = .52, Wilks'  $\Lambda = .64$ . In summary, there were no significant SMEs of block within either group.

Table 3.12

*Test Statistics for Post-Hoc Analysis of SME of Group Within Time on Weight-Lifting Task*

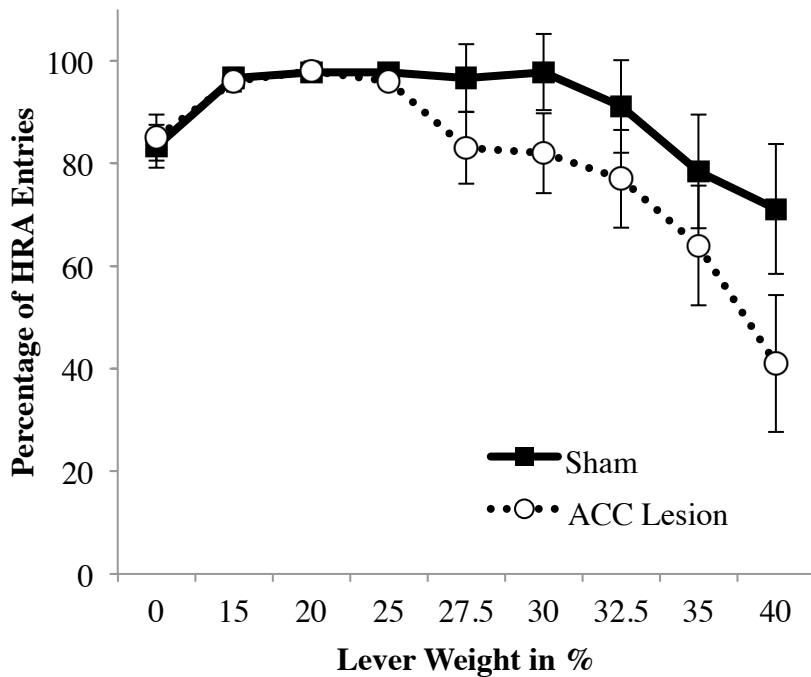
	Group	<i>M</i>	<i>SD</i>	<i>t</i> (df)	<i>P</i> (two-tailed)																																																												
Training trials – 10	Control	98.89	3.33	1.56(13.1)	.14																																																												
	Lesion	95	7.07			Training trials – 20	Control	100	0	1.5(9)	.17	Lesion	98	4.22	Training trials – 30	Control	100	0	1.81(9)	.1	Lesion	96	6.99	Training trials – 40	Control	100	0	1(9)	.34	Lesion	99	3.16	Training trials – 50	Control	100	0	1.5(9)	.17	Lesion	98	4.22	Training trials – 60	Control	97.78	4.41	2.33(16.07)	.033	Lesion	92	6.32	Testing 1 trials – 10	Control	91.11	16.91	1.63(13.32)	.13	Lesion	71	34.79	Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082
Training trials – 20	Control	100	0	1.5(9)	.17																																																												
	Lesion	98	4.22			Training trials – 30	Control	100	0	1.81(9)	.1	Lesion	96	6.99	Training trials – 40	Control	100	0	1(9)	.34	Lesion	99	3.16	Training trials – 50	Control	100	0	1.5(9)	.17	Lesion	98	4.22	Training trials – 60	Control	97.78	4.41	2.33(16.07)	.033	Lesion	92	6.32	Testing 1 trials – 10	Control	91.11	16.91	1.63(13.32)	.13	Lesion	71	34.79	Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082	Lesion	82	25.3						
Training trials – 30	Control	100	0	1.81(9)	.1																																																												
	Lesion	96	6.99			Training trials – 40	Control	100	0	1(9)	.34	Lesion	99	3.16	Training trials – 50	Control	100	0	1.5(9)	.17	Lesion	98	4.22	Training trials – 60	Control	97.78	4.41	2.33(16.07)	.033	Lesion	92	6.32	Testing 1 trials – 10	Control	91.11	16.91	1.63(13.32)	.13	Lesion	71	34.79	Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082	Lesion	82	25.3															
Training trials – 40	Control	100	0	1(9)	.34																																																												
	Lesion	99	3.16			Training trials – 50	Control	100	0	1.5(9)	.17	Lesion	98	4.22	Training trials – 60	Control	97.78	4.41	2.33(16.07)	.033	Lesion	92	6.32	Testing 1 trials – 10	Control	91.11	16.91	1.63(13.32)	.13	Lesion	71	34.79	Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082	Lesion	82	25.3																								
Training trials – 50	Control	100	0	1.5(9)	.17																																																												
	Lesion	98	4.22			Training trials – 60	Control	97.78	4.41	2.33(16.07)	.033	Lesion	92	6.32	Testing 1 trials – 10	Control	91.11	16.91	1.63(13.32)	.13	Lesion	71	34.79	Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082	Lesion	82	25.3																																	
Training trials – 60	Control	97.78	4.41	2.33(16.07)	.033																																																												
	Lesion	92	6.32			Testing 1 trials – 10	Control	91.11	16.91	1.63(13.32)	.13	Lesion	71	34.79	Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082	Lesion	82	25.3																																										
Testing 1 trials – 10	Control	91.11	16.91	1.63(13.32)	.13																																																												
	Lesion	71	34.79			Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082	Lesion	82	25.3																																																			
Testing 1 trials – 20	Control	97.78	4.41	1.94(9.61)	.082																																																												
	Lesion	82	25.3																																																														

Table 3.12 *Test Statistics for Post-Hoc Analysis of SME of Group Within Time on Weight-Lifting Task (continued)*

	Group	<i>M</i>	<i>SD</i>	<i>t</i> (df)	<i>p</i> (two-tailed)
Testing 1 trials – 30	Control	93.33	8.66	1.06(11.68)	.31
	Lesion	85	23.21		
Testing 1 trials – 40	Control	95.56	5.27	1.1(11.43)	.29
	Lesion	90	14.91		
Testing 1 trials – 50	Control	98.89	3.33	2.04(10.24)	.068
	Lesion	90	13.33		
Testing 1 trials – 60	Control	93.33	13.23	.83(16.97)	.42
	Lesion	87	15.48		

Subsequently, to test for SME of group within block (training, testing 1), two independent samples *t*-tests were performed. Alpha was adjusted for two comparisons with a new test parameter of .025. This analysis revealed a significant SME for the last day of training, indicating that the control group performed significantly better ( $M = 99.33, SD = 1$ ) than the lesion group ( $M = 96.1, SD = 18.27$ ),  $t(11.12) = 3.18, p = .009$ . However, there was no SME of group on testing day 1,  $t(10.94) = 1.81, p = .099$ . Results confirmed the initial analysis, namely that there was an overall group difference, and it seems now that this group difference was due to a difference on the last day of training. However, it must be emphasized that both groups performed exceedingly well, suggesting that the task may have been too easy for both groups and that the lack of difference was due to a ceiling effect.

Lastly, the two tests for SMEs of group within time (training trials, testing trials) were conducted. Six independent *t*-tests were run for each analysis. Alpha was adjusted to .008 for six comparisons. In the first analysis, the independent variable was training trials (10, 20, 30, 40, 50, 60). Here, there was no significant SME of group within any time point (see Table 3.12 for all statistics). In the second analysis, the independent variable was testing 1 trials (10, 20, 30, 40, 50, 60). Again, there was no significant SME of group within any time point (see Table 3.12).



*Figure 3.12.* Performance on the weight-lifting incremental test. Effort was incremented every 10 trials. Weight increments are plotted. No significant group differences were observed, but test of increments showed that effort was discounted over the course of the session. Error bars represent SEM.

Overall, results indicated that lesion rats did not behave differently from control animals on the testing days on the weight-lifting task. It is noteworthy to highlight that there was an overall group difference, with the lesion rats performing generally more

poorly, but both means were above 90% HRA entries. Further analyses revealed that this difference was caused by a group difference on the last day of training. Because rats were initially matched based on ramp-climbing performance, this was a possibility from the beginning.

Table 3.13  
*Test Statistics for Main Effect of Increment on Weight-Lifting Incremental Task*

	<i>M</i>	Standard error	<i>p</i> (two-tailed)
Increment 1 (0%)	84.17	3.08	.021
Increment 2 (15%)	96.33	1.41	
Increment 1 (0%)	84.17	3.08	.016
Increment 3 (20%)	97.89	1.27	
Increment 1 (0%)	84.17	3.08	.027
Increment 4 (25%)	96.89	1.11	
Increment 2 (15%)	96.33	1.41	.019
Increment 9 (40%)	56.06	9.19	
Increment 3 (20%)	97.89	1.27	.01
Increment 9 (40%)	56.06	9.19	
Increment 4 (25%)	96.89	1.11	.012
Increment 9 (40%)	56.06	9.19	

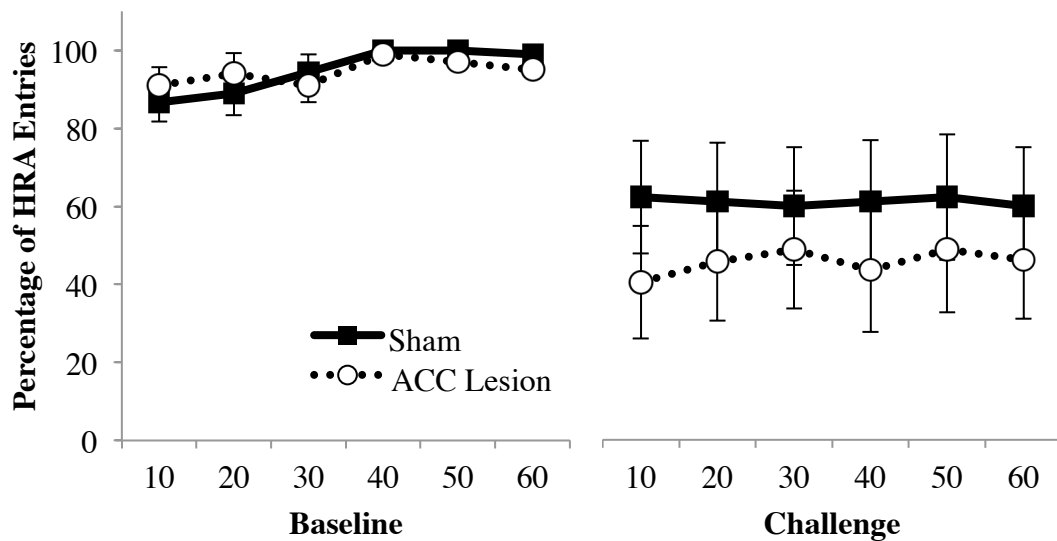
Since performance on the weight-lifting task was very high for both groups, we introduced an incremental test as well as a challenge test in parallel to what was tested on the ramp-climbing task. Briefly, on the incremental test, rats underwent a number of

increments (0%, 15%, 20%, 25%, 27.5%, 30%, 32.5%, 35%, 40%) within one single session (see Figure 3.12). For the incremental session, a repeated measures ANOVA was conducted with the within-subjects factor increment (0%, 15%, 20%, 25%, 27.5%, 30%, 32.5%, 35%, 40%) and the between-subjects factor group (control, lesion). Sphericity could not be assumed,  $\chi^2(35) = 138.88, p < .001$ , Greenhouse-Geisser  $\epsilon = .32$ , so multivariate tests are reported. There was no Increment  $\times$  Group interaction,  $F(8, 10) = .58, p = .78, \eta^2 = .32$ , observed power = .16, Wilks'  $\Lambda = .69$ , and no significant main effect of group,  $F(1, 17) = 2.13, \eta^2 = .11$ , observed power = .28, but a significant main effect of increment,  $F(8,10) = 3.48, p = .034, \eta^2 = .74$ , observed power = .77, Wilks'  $\Lambda = .26$ .

Pairwise comparisons revealed that increment 1 (0%) differed significantly from increments 2 (15%), 3 (20%), and 4 (25%), showing an increase in performance after the initial zero-effort phase to nearly 100%, and increments 2, 3, and 4 also differed significantly from increment 9 (40%), showing a significant drop by 40% to just above chance performance (see Table 3.13 for significant test results with means and standard errors). Overall, this analysis showed an effort-discounting curve similar to that seen on the ramp-climbing task. That is, rats went more often to HRA when the effort was easy, but as it got harder, there was a marked shift towards the LRA.

On the challenge test, a new baseline and a challenge day with the maximum weight experienced (40%) were conducted (see Figure 3.13). In the baseline stage, rats received a zero-effort reward discrimination session to re-establish a new baseline performance. In the challenge stage on the following day, rats received the highest weight they had so far lifted (i.e., 40%) immediately after the zero-effort forced trials. A

repeated measures ANOVA showed a significant main effect of stage (baseline, challenge) indicating that rats overall performed significantly better during the baseline stage ( $M = 94.66, SD = .17$ ) than during the challenge stage ( $M = 51.4, SD = 10.69$ ),  $F(1, 17) = 18.86, p < .001, \eta^2 = .53$ , observed power = .98. There was no significant interaction involving stage (all  $ps \geq .34$ ), and no other main effects of time ( $p = .4$ ) or group ( $p = .45$ ). For this test, the sphericity assumption was violated and multivariate statistics are reported,  $\chi^2(14) = 51.16, p < .001$ , Greenhouse-Geisser  $\epsilon = .45$ .



*Figure 3.13.* Performance on the weight-lifting challenge task in baseline and challenge conditions. In the challenge session, the weight was 40%. All rats performed significantly worse in the challenge stage compared to baseline ( $p < .001$ ). Error bars represent SEM.

These analyses showed that rats are indeed sensitive to weight-lifting effort when the effort is continuously increased, and when they are faced with a higher effort. We concluded that 20% of rats' body weight was not a sufficiently heavy effort. This was supported by the fact that both lesion and control rats performed nearly at 100% throughout the entirety of the experiment when faced with 20% or less effort. Because such high performance is not usually seen on the ramp-climbing task, this could indicate

a ceiling effect. In summary, the lack of effect on this task might be due to the fact that the effort was indeed too easy for animals.

For this group of rats, the open field test indicated no statistically significant differences on any of the five measures (percentage of time in centre, path length, running speed, path length in centre, and path length in periphery). Activity box data showed the same results as for the ramp-climbing group, even though two additional rats were included in this analysis. Briefly, lesion rats had a greater number of movements ( $M = 13.34$ ,  $SD = 1.83$ ) than controls ( $M = 11.32$ ,  $SD = 1.57$ ),  $t(15.61) = -2.51$ ,  $p = .024$ , lesion rats had a higher number of stereotypy ( $M = 10.18$ ,  $SD = .62$ ) than controls ( $M = 9.16$ ,  $SD = 1.04$ ),  $t(15.01) = -2.53$ ,  $p = .025$ , and, finally, lesion rats spent more time in stereotypy ( $M = 16.34$ ,  $SD = 1.53$ ) than controls ( $M = 14.01$ ,  $SD = 1.99$ ),  $t(15.01) = -2.78$ ,  $p = .014$ .

### **3.4.3 Anterior cingulate does not mediate courage effort.**

All rats that were included in the weight-lifting analysis were also included in the courage analysis. In the courage effort task, the focus was not on average performance per day, but only on performance within the testing 1 session and the last training session. Because the same rats were used here, open field and activity box data were already reported in section 3.3.2.

Data were analyzed using a repeated measures ANOVA with time (trial bins 10, 20, 30, 40, 50, 60) and block (training, testing day 1) as the within-subjects factors, and group (control, ACC lesion) as the between-subject factor (see Figure 3.14). Mauchly's test of sphericity showed a violation of this assumption,  $\chi^2(14) = 54.43$ ,  $p < .001$ , Greenhouse-Geisser  $\epsilon = .54$ , so the more powerful multivariate test was used. The

ANOVA discovered a main effect of time,  $F(5, 13) = 8.96, p = .001, \eta^2 = .78$ , observed power = .99, Wilks'  $\Lambda = .23$ , and a main effect of block,  $F(1, 17) = 11.08, p = .004, \eta^2 = .4$ , observed power = .88, Wilks'  $\Lambda = .61$ . There was no main effect of group,  $F(1, 17) = .34, p = .57, \eta^2 = .02$ , observed power = .086, and no significant interactions involving either time or block, all  $ps > .069$ . Pairwise comparisons showed that, overall, rats had significantly more HRA entries during the last day of training ( $M = 91.77, SD = 2.71$ ) than during the first testing day ( $M = 77.11, SD = 6.84$ ).

Table 3.14  
*Test Statistics for Main Effect of Time on Courage Task*

	<i>M</i>	<i>SD</i>	<i>p</i> (two-tailed)
Time – trials 10	67.08	20	.001
Time – trials 20	81.03	12.53	
Time – trials 10	67.08	20	.007
Time – trials 30	84.75	12.92	
Time – trials 10	67.08	20	.003
Time – trials 40	87.42	9.31	
Time – trials 10	67.08	20	.001
Time – trials 50	93.66	4.57	
Time – trials 10	67.08	20	.005
Time – trials 60	92.71	2.88	

Pairwise comparisons for time bins collapsed across both training and testing showed significant differences between time bin 10 and bins 20, 30, 40, 50, and 60.

There were no other significant differences between any other time bins (see Table 3.14 for all descriptive statistics). These results indicated that even though there were no group differences, all animals went to the HRA less often when the open arm was presented, and the performance on the first ten trials was significantly worse than all other time bins. When looking at the mean values, one could assume that this time bin difference was carried by the performance on the testing day, as, even when collapsed across groups, the first time bin (trials 10) on the training day had a mean of  $M = 81.22$ , whereas this first time bin on the testing day was only  $M = 52.94$ .

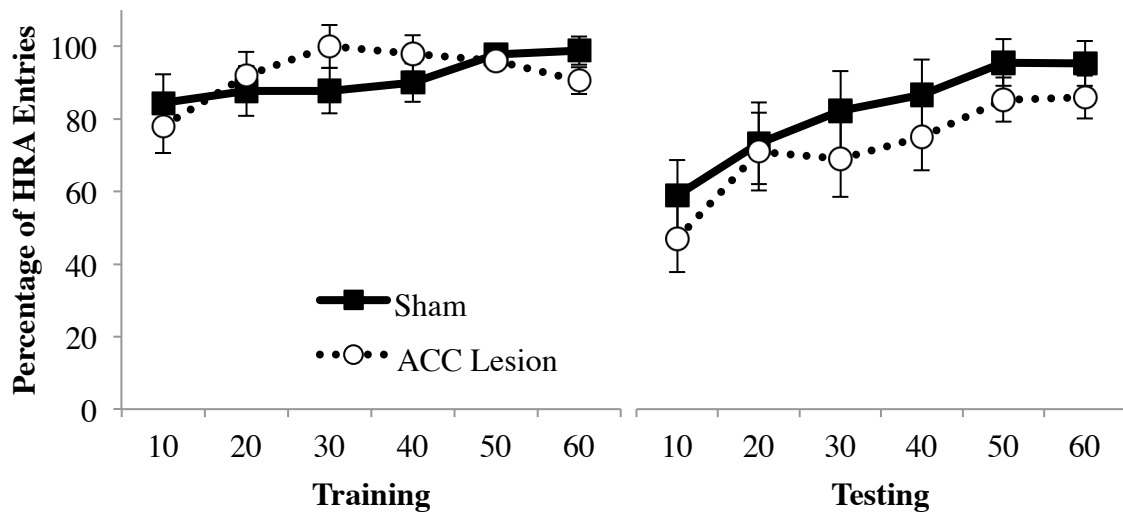


Figure 3.14. Performance on the courage task for the last day of training (Training) and the testing session (Testing). On the testing day, all rats went significantly less often to the open arm during the first ten trials compared to any other time within the session (all  $ps < .008$ ). Error bars represent SEM.

Overall, these results showed that this task was effortful, as performance on the testing day dropped down significantly, and performance in the first time bin was significantly worse than all the others. When visually examining the data, this notion was confirmed. However, animals did not drop below chance performance on the testing day, although there was a slight trend for lesion rats to initially perform worse ( $M = 47, SD =$

18.29) than controls ( $M = 58.89$ ,  $SD = 38$ ). Further, by the end of the session, both groups performed above criterion. This indicated that there might be a ceiling effect similar to that observed on the weight-lifting task. Thus, the effort used here might not have been sufficient to (a) deter animals appropriately at the start of the session, and (b) be considered difficult to overcome, as all animals readily regained baseline performance.

During the testing session, however, it was noticed that rats committed numerous sequence errors. A sequence error on the courage task was classified as an arm entry that was not rewarded following a rewarded arm entry. That is, a rat went from the base to a reward arm and received the reward, and then, instead of returning to the base, entered the other reward arm. This entry did not yield any reward and can thus be considered an error. Oftentimes, even though rats chose the LRA on the first attempt, they entered the open arm as a sequence error. This indicated that rats were not actually afraid of the open space, but for some reason did not initially enter it on the first outward trajectory after leaving the base arm. When combining all open arm entries, including first and second arm choices, it was discovered that all animals in both groups in fact entered the open arm over 80% of the time from the beginning of the session, and no group difference was observed. This indicated that indeed the effort was insufficient to deter animals from entering the arm.

### **3.5 Discussion**

Results from Experiment 1 supported previous findings for ramp-climbing effort (Rudebeck et al., 2006; Schweimer & Hauber, 2005; Walton et al., 2003). It was shown that rats with ACC lesions shifted their bias from the high-effort, high-reward to the low-effort, low-reward option. When the effort was equated, rats returned to the HRA at

levels similar to baseline performance. These findings confirmed the hypotheses. In addition, the results extend previous studies by showing that these findings hold true when rats receive longer sessions with more trials. This indicates that ACC lesions on the ramp-climbing task produce a robust effect that is not diminished by variations in the task.

Moreover, looking at the behavioural correlates of decisions in ACC lesion rats more closely, it was found that lesion rats approached the ramp, and sometimes placed paws on the ramp, before deciding to turn around and enter the LRA. To date, this is the first account of such behaviour. Studies have shown that in monkeys, a motor response can be chosen based on reward expectation (Matsumoto et al., 2003). It may be the case that in the present experiments, rats chose the motor response of turning into the HRA because of the intact reward expectation. That is, rats chose to turn into the HRA because they anticipated the high reward due to other inputs to the remaining structures of mPFC from areas such as amygdala that are known to be part of the network that governs reward-guided behaviour (Floresco & Ghods-Sharifi, 2007). Once faced with the effort, animals with ACC lesions might not have been able to carry out the action and weigh effort vs. reward. This might indicate that ACC is indeed critically needed to weigh costs and benefits when directly faced with such a situation (i.e., at the time when they stand in front of the ramp, rather than at the decision region), while prospective effort-reward decisions can be carried out without it (i.e., turning into the HRA). In this situation, rats may not be able to weigh effort and reward when directly presented with the options, but reward expectation alone might be intact.

Another possibility is that ACC mediates action maintenance, as previously

proposed by Cowen et al. (2012). Without ACC, while the initial action planning might have been carried out and the rat was steered towards the HRA option, once faced with the effort, the action plan could not be maintained and was abandoned. Here again, the rat would turn around when faced with the effort directly, because the action plan cannot be carried out without ACC.

For both weight-lifting and courage effort, the expected preference shift towards the LRA in ACC-lesioned animals did not occur. Even though both tests showed that they were effortful, judging by the discounting curve seen for the weight-lifting task when the effort was increased, and by the initial hesitation on the courage task, there was no difference between groups. For the weight-lifting task, it was likely the case that the effort presented to the animals was insufficient. The control group showed a ceiling effect, going to the HRA close to 100% of the time, whereas animals typically enter the HRA about 80% of the time on the ramp-climbing task. The incremental task results confirm this ceiling effect. All animals performed at 80% during this session and did not drop in performance until the effort was incremented to 35-40% of their body weight. It might be the case that the extensive training on the task resulted in this ceiling effect, but it could also be that the effort was not sufficient to deter lesion animals from pressing the HRL. This question was addressed in Experiment 2, where the experiment was repeated with heavier weights (see section 4.3.1).

On the courage task, both groups started out with open arm entries at about chance level. If this task was deterring at a similar level as the ramp-climbing task, we expected lesion animals to enter the open arm at about 20% at the beginning of the session. Here again it was hypothesized that the effort used was not sufficient, and

therefore group differences may not have been seen. When developing a novel task, incidents like this are quite possible. In Experiment 2, the effort was increased by turning on the room lights during testing (see section 4.3.2). This was indeed more in line with the elevated plus maze, after which our task was modelled (Walf & Frye, 2007).

## **Chapter 4: Experiment 2 – Does ACC Mediate Weight-Lifting And Courage Effort When the Effort is Increased?**

### **4.1 Hypotheses**

Experiment 1 revealed that 20% of rats' body weight was not sufficient to deter either ACC-lesioned or control rats from choosing the HRA. This raised the possibility that a lack of group difference may have been caused by a ceiling effect. Judging by the performance curve from the incremental session, the present experiment was conducted with a weight of 40% of the rats' body weight, as this was the increment at which performance started to drop for both lesion and control rats in Experiment 1. Rats with ACC lesions were expected to perform significantly worse on the post-lesion testing days compared to (a) control animals, and (b) their own baseline. Further, it was predicted that ACC lesion rats would regain baseline performance when effort was equated and weights were attached to both the HRL and the LRL. In this last condition, it was also predicted that lesion rats would not differ from controls.

Moreover, Experiment 1 showed that effort on the courage task might have been insufficient as well. A separate analysis of sequence errors on this task showed that all rats readily entered the open arm from the beginning of the testing session. To increase the effort on this task, testing room lights were left on during the testing session. This was more in line with the elevated plus maze, a commonly used task for anxiety, in which rats show hesitation to enter a bright, open space (Walf & Frye, 2007). Here, it was expected that (a) even control rats would hesitate to enter the open arm due to their innate fear of bright, open spaces, but (b) control rats would improve fast (i.e., by 30 trials) and reach baseline performance over the course of the session, whereas (c) lesion rats would

start entering the open arm later in the session and (d) would require more trials to show improvement.

## **4.2 Method**

### **4.2.1 Animals.**

Twenty-six male Long-Evans rats (Charles River Laboratories International Inc., Montreal, QC) were trained in two cohorts in this study. Cohort 3 was 109 and cohort 4 was 108 days of age at the start of weight-lifting training. Rats' weights were maintained at 85% of their free feeding weight (350-450 g at start of training) one week prior to and for the duration of training and testing. For at least one day before and five days after surgery, rats were allowed *ad libitum* food. Rats were between 135-140 (cohort 3) and 141-146 (cohort 4) days old at the time of surgery, between 151-155 (cohort 3) and 155-160 (cohort 4) days old at the time of weight-lifting testing, and between 161-172 (cohort 3) and 161-168 (cohort 4) during courage testing. Open-field testing was conducted on day 177 (cohort 3) and 170 (cohort 4) and activity-box testing on day 182 (cohort 3) and 169 (cohort 4). Cohort 3 was perfused at 188 days, cohort 4 at 176 days of age. Three animals died from peri-surgery complications. Two additional animals were removed from the study due to health problems. Eight animals were excluded from weight-lifting data analysis due to failure to achieve criterion performance. This failure to achieve criterion will be discussed in more detail in sections 4.3 and 4.4. One animal in the control group was excluded from all analyses because of accidental brain damage to ACC or M2.

### **4.2.2 Materials and apparatus.**

All tasks were videotaped via an in-ceiling video camera.

### ***Weight-lifting task.***

Materials on the weight-lifting task apparatus remained the same as in Experiment 1 (see section 3.3.2), except that wall height was increased to 50.8 cm, and additional weights were used to achieve effort levels up to 40% of the rats' body weights and up to 50% in the incremental session.

### ***Courage task.***

Materials on the courage task apparatus remained the same as in Experiment 1 (see section 3.3.2).

### ***Open field and activity box.***

All testing remained the same as in Experiment 1 (see section 3.3.2).

## **4.2.3 Surgery and histology.**

Procedures were the same as in Experiment 1 (see section 3.3.3). In Experiment 2, rats were again matched according to task performance on the weight-lifting task and then divided into sham and ACC lesion groups prior to surgery. Matching occurred by rank-ordering rats based on (a) the number of effort training days from low to high, (b) their performance percentage on the last day from high to low, and (c) the number of pre-training days from low to high. For every first pair, the first rat was allocated to the lesion group. For every second pair, the first rat was allocated to the control group.

## **4.2.4 Task procedure.**

### ***Weight-lifting task.***

New effort training parameters were incremented up to 40% of rats' body weight. Session criteria remained the same (60 trials, minimum of 80% HRA). Instead of four effort stages, rats now received eight effort stages: 15%, 20%, 25%, 30%, 32.5%, 35%,

37.5%, and 40%. In the final effort stage, two non-consecutive days of criterion performance were again required.

### *Courage task.*

All training and testing on the courage task took place post-surgery. While all main parameters remained the same, now during testing, room lights were left on to make the task more effortful. This was more in line with similar tasks used for anxiety, such as the elevated plus maze. Further, the maximum amount of reward contingency learning days was reduced to seven, and all rats received exactly three days of testing regardless of performance.

## **4.3 Results**

Analyses are reported with the following animal numbers: (a) weight-lifting task: ACC lesion  $n = 6$ , sham:  $n = 6$ ; (b) courage task: ACC lesion  $n = 11$ , sham:  $n = 9$ ; (c) activity box and open field: ACC lesion:  $n = 11$ , sham:  $n = 9$ .

As in Experiment 1, to determine whether cohorts were equal, those data from rats included in the weight-lifting analyses on the last training day, were subjected to a separate analysis prior to test analyses. Here, a repeated measures ANOVA with the within-subjects factor time (weight-lifting training trial bins 10, 20, 30, 40, 50, 60) and the between-subjects factor cohort (cohort 3, cohort 4) was conducted. This analysis revealed that cohorts did not significantly differ from each other,  $F(1, 10) = 3.36, p = .097, \eta^2 = .25$ , observed power = .38, and there was no significant Time  $\times$  Cohort interaction,  $F(5, 6) = .84, p = .57, \eta^2 = .41$ , observed power = .16. Thus, cohorts were treated as equal and combined in all further analyses.

### 4.3.1 Anterior cingulate does not mediate weight-lifting effort, even when effort was increased.

In this experiment, rats were trained with weights in increments up to 40% of their body weight. Surprisingly, eight out of 20 animals did not achieve criterion performance by the end of training. Thus, they were excluded from the analysis, reasons for which will be discussed towards the end of this section. Analyses were conducted in parallel to Experiment 1. First, averages of the last training day, three testing days, and the equate effort day were compared to gauge a change over time. Second, the within-session performances on the last training day and the first testing day were compared.

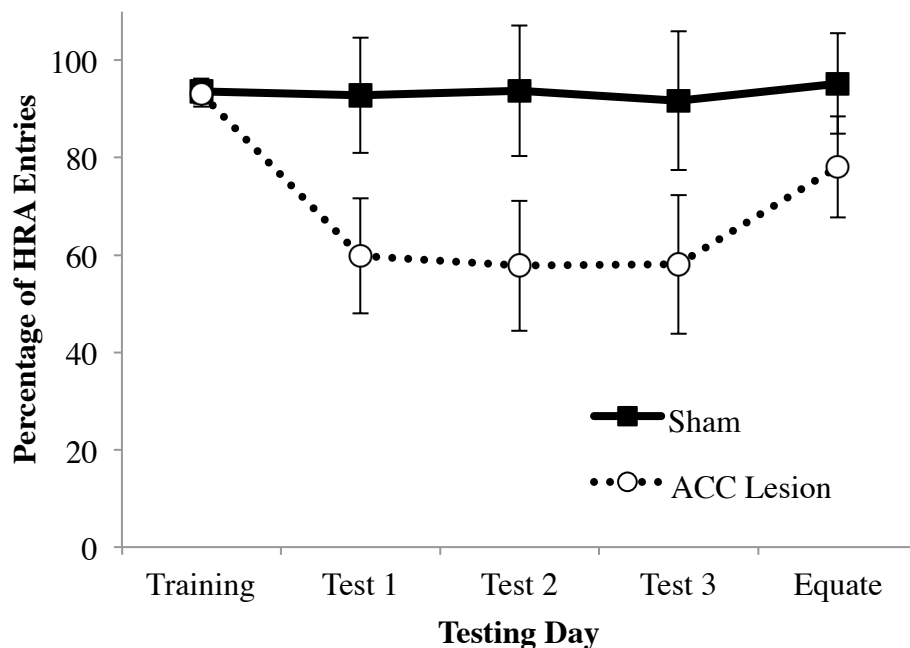


Figure 4.1. Performance on weight-lifting task over last day of training, testing days, and equate effort day. No significant differences were observed. Error bars represent SEM.

To determine how each group changed over time compared to their own baseline and to each other, a repeated measures ANOVA was conducted with testing day (last day

of training, testing day 1, 2, 3, equate effort) as the within-subjects factor and with group (control, ACC lesion) as the between-subjects factor (see Figure 4.1). Mauchly's test of sphericity found that sphericity was violated,  $\chi^2(9) = 28.53, p = .001$ , Greenhouse-Geisser  $\epsilon = .47$ , so the more powerful multivariate test is reported. This test revealed no significant main effects of testing day,  $F(4, 7) = 1.07, p = .44, \eta^2 = .38$ , observed power = .2, Wilks'  $\Lambda = .62$ ; none of group,  $F(1, 10) = 3.21, p = .1, \eta^2 = .24$ , observed power = .37, and no significant Testing Day  $\times$  Group interaction,  $F(4, 7) = .88, p = .52, \eta^2 = .33$ , observed power = .17, Wilks'  $\Lambda = .67$ .

Table 4.1  
*Test Statistics for Repeated Measures ANOVA for Weight-Lifting Task*

	<i>F(df)</i>	<i>p</i>	$\eta^2$	observed power
Time	.87(5, 6)	.55	.42	.16
Time $\times$ Group	1(5, 6)	.49	.45	.18
Block	4.19(1, 10)	.068	.3	.46
Block $\times$ Group	4.02(1, 10)	.073	.29	.44
Time $\times$ Block	1.39(5, 6)	.35	.54	.24
Time $\times$ Block $\times$ Group	2.99(5, 6)	.11	.71	.48
Group	2.83(1, 10)	.12	.22	.33

Next, to compare within-session performance on the last training and first testing days, a repeated measures ANOVA with the within-subject factors of time (trial bins 10, 20, 30, 40, 50, 60) and block (training, testing 1) and the between-subjects factor of group (control, ACC lesion) was executed (see Figure 4.2). Sphericity could not be assumed

for at least one comparison,  $\chi^2(14) = 33.09, p = .004$ , Greenhouse-Geisser  $\epsilon = .45$ , so the more powerful multivariate test was used. The ANOVA also did not reveal any significant interactions or main effects (Tables 4.1, 4.2).

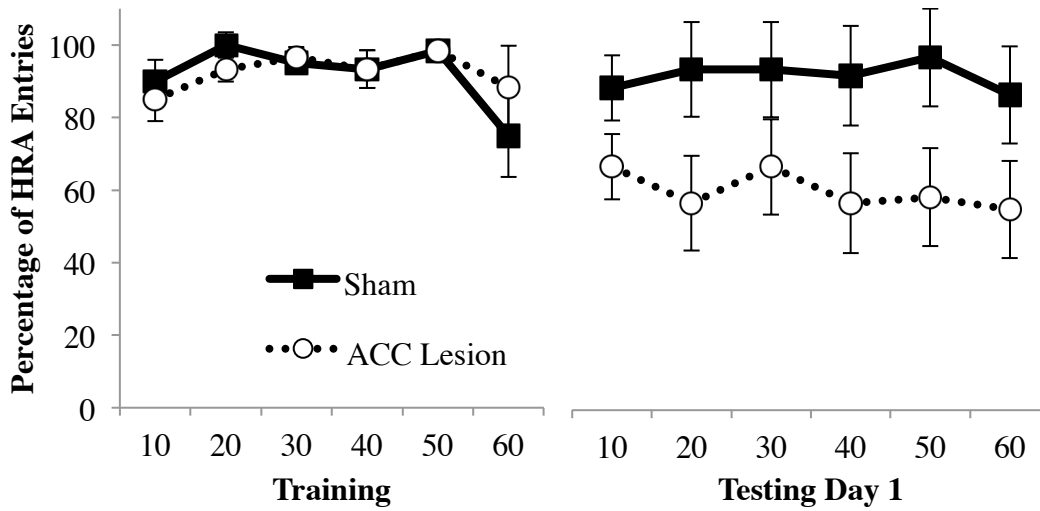


Figure 4.2. Performance on weight-lifting task within the last training session (Training) and the first testing session (Testing Day 1). No significant group differences were observed. Error bars represent SEM.

Table 4.2  
Means and Standard Deviations for Weight-Lifting Task by Group

	Group	<i>M</i>	<i>SD</i>
Training – 10 trials	Sham	90	8.94
	Lesion	85	18.71
	Total	87.5	14.22
Testing 1 – 10 trials	Sham	88.33	13.29
	Lesion	66.67	28.05
	Total	77.5	23.79

Table 4.2 *Means and Standard Deviations for Weight-Lifting Task by Group (continued)*

	Group	<i>M</i>	<i>SD</i>
Training – 20 trials	Sham	100	0
	Lesion	93.33	12.11
	Total	96.67	8.88
Testing 1 - 20 trials	Sham	93.33	8.16
	Lesion	56.67	44.12
	Total	75	35.8
Training – 30 trials	Sham	95	8.37
	Lesion	96.67	5.16
	Total	95.83	6.69
Testing 1 – 30 trials	Sham	93.33	8.16
	Lesion	66.67	44.57
	Total	80	33.57
Training – 40 trials	Sham	93.33	8.16
	Lesion	93.33	16.33
	Total	93.33	12.31
Testing 1 - 40 trials	Sham	91.67	16.02
	Lesion	56.67	44.57
	Total	74.17	36.79
Training – 50 trials	Sham	98.33	4.08
	Lesion	98.33	4.08
	Total	98.33	3.89

Table 4.2 *Means and Standard Deviations for Weight-Lifting Task by Group (continued)*

	Group	<i>M</i>	<i>SD</i>
Testing 1 – 50 trials	Sham	96.67	8.16
	Lesion	58.33	45.79
	Total	77.5	37.2
Training – 60 trials	Sham	75	36.74
	Lesion	88.33	14.72
	Total	81.67	27.58
Testing 1 – 60 trials	Sham	86.39	13.35
	Lesion	55	44.16
	Total	70.69	35.16

Further, an incremental effort session was run as in Experiment 1 to see if a similar effort-discounting curve could be observed. Here, the increments were 0%, 27.5%, 30%, 32.5%, 35%, 37.5%, 40%, 45%, and 50% (see Figure 4.3). A repeated measures ANOVA with increment (as listed) as the within-subjects factor and group (control, ACC lesion) as the between-subjects factor was conducted. Sphericity for this test could not be assumed,  $\chi^2(35) = 113.32, p < .001$ , Greenhouse-Geisser  $\epsilon = .23$ , so the more powerful multivariate test is referred to. Results indicated that there was no significant main effect of increment,  $F(8, 3) = 2.05, p = .3, \eta^2 = .85$ , observed power = .21, Wilks'  $\Lambda = .15$ , or of group,  $F(1, 10) = .36, p = .56, \eta^2 = .035$ , observed power = .085, and no significant Increment  $\times$  Group interaction,  $F(8, 3) = 1.12, p = .52, \eta^2 = .75$  observed power = .13, Wilks'  $\Lambda = .25$ .

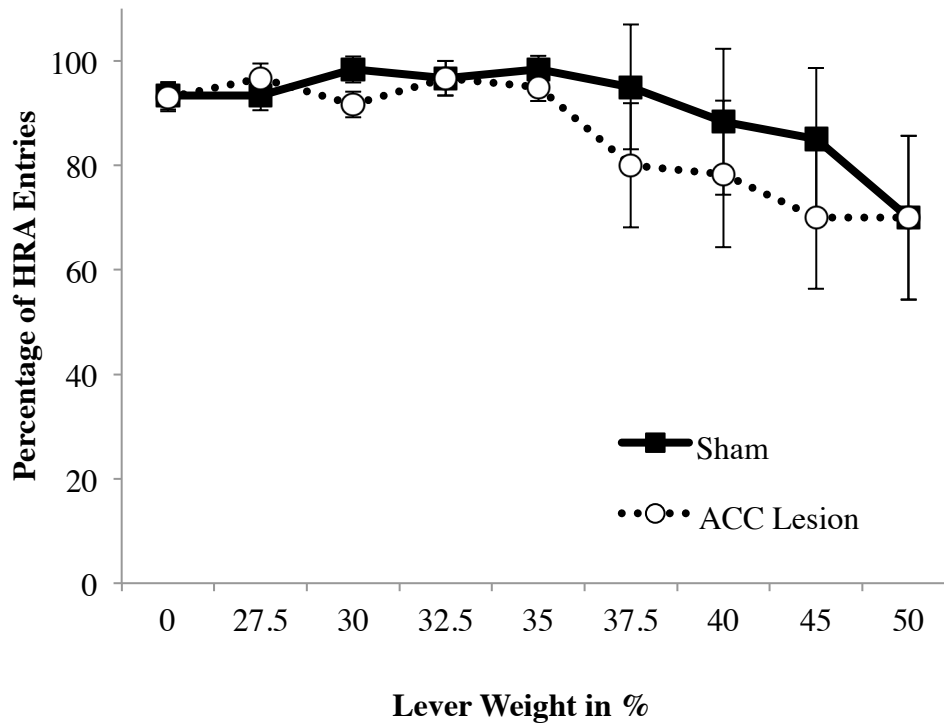


Figure 4.3. Performance on the weight-lifting incremental test. Effort was incremented every 10 trials. No group differences were observed. Weight increments are plotted. Error bars represent SEM.

Next, the open field and activity box analyses from this group of rats were examined. On the open field, rats did not show any statistically significant differences in percentage of time spent in the centre, path length, running speed, path length in centre, or path length in the periphery (all  $ps > .37$ ) based on independent samples  $t$ -tests. These results indicated that animals did not differ based on anxiety or motor behaviour. To further investigate motor behaviour, results from the activity box were analyzed using independent samples  $t$ -tests. One difference was observed (refer to Table 3.9 for a full list of measures analyzed); ACC lesion rats performed significantly more vertical movements ( $M = 7.17, SD = 2.09$ ) compared to controls ( $M = 4.7, SD = 1.41$ ),  $t(8.78) = 2.39, p = .041$ . A vertical movement was counted every time the animal reared up. To separate movements in this count, the animal must go below the level of the vertical

sensor for a new vertical movement to be counted.

Because eight animals did not achieve criterion performance on the weight-lifting task, those rats that reached criterion performance were compared to those who did not and were thus excluded from the analyses. This was done to compare whether these animals differed in a systematic way that could explain performance differences on the weight-lifting task. These two groups were compared on the control tasks (i.e., activity box, open field) as well using independent samples *t*-tests for the five open field variables, and for all activity box variables. Rats that achieved criterion in training did not significantly differ from rats that did not on the open field, all *ps* > .81. In the activity box, rats that reached criterion performed significantly more clockwise revolutions ( $M = 1.35, SD = .62$ ) compared to rats that did not make it ( $M = .81, SD = .28$ ),  $t(16.4) = -2.64$ ,  $p = .017$ . It is unclear how this finding relates to weight-lifting or lever pressing, or how clockwise revolutions predict performance on the weight-lifting task. It was thus considered an artefact and groups were considered equal. Further, it could have also been the case that better performance on the weight-lifting task led rats to exhibit more clockwise revolutions.

Overall, these results showed that weight-lifting effort is not mediated by ACC. Even though the average for lesion rats indicated that they performed worse, this was not statistically significant. When looking at individual performances, it turned out that four out of six lesion animals performed at 80% and were indistinguishable from controls, and only two lesion animals dropped down to 10% or lower (see Appendix B for individual performance traces). Overall, all rats still performed well during testing on the task (> 60%), even when effort was increased from 20% to 40% of the rats' body weights. It

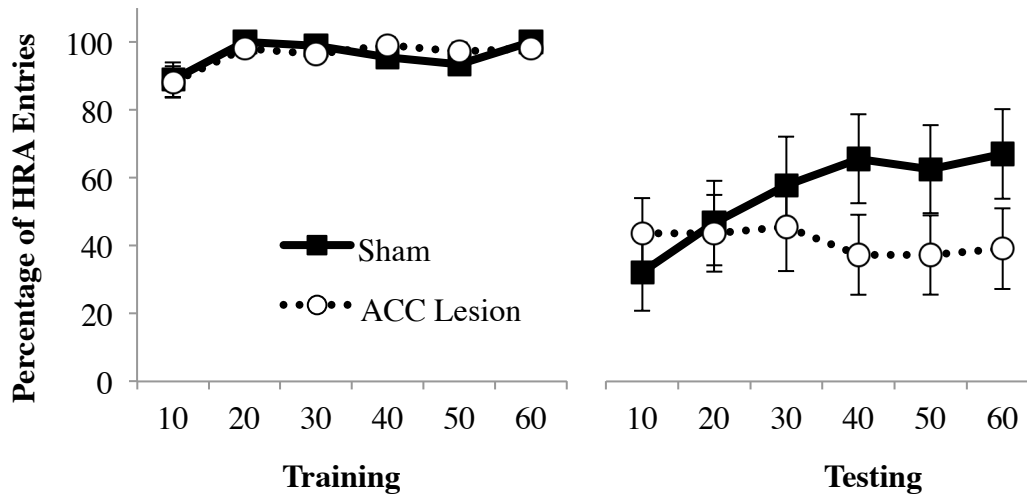
may be argued that even 40% constitutes insufficient effort, but the fact that initially eight out of 20 rats were unable to reach criterion performance (i.e., 80% or greater HRA entries) during training indicated that this level is effortful for rats even pre-surgery. It is with confidence then that the claim can be made that ACC does not mediate this type of effort.

#### **4.3.2 Anterior cingulate may mediate courage effort in an unexpected way.**

The eight rats excluded from the weight-lifting analysis were re-included in the courage analysis, as no systemic differences on control tasks (activity box, open field) were observed between those rats who achieved weight-lifting criterion performance and those who did not. For courage data, a repeated measures ANOVA with time (trial bins 10, 20, 30, 40, 50, 60) and block (last training day, testing 1) as the within-subjects factors and group (control, ACC lesion) as the between-subjects factor was performed. Here, again, the sphericity assumption was violated,  $\chi^2(14) = 30.35, p = .007$ , Greenhouse-Geisser  $\epsilon = .64$ , and thus the more powerful multivariate test was used. Results showed a significant Time  $\times$  Block  $\times$  Group interaction,  $F(5, 14) = 5.11, p = .007, \eta^2 = .65$ , observed power = .92, Wilks'  $\Lambda = .35$ . Six post-hoc tests were conducted to test for SME of: (a) time 1 (training) within group, (b) time 2 (testing 1) within group, (c) block (collapsed across time) within group, (d) group within block, (e) group within time 1 (training), and (f) group within time 2 (testing).

To test for SME of group within training (trials 10, 20, 30, 40, 50, 60), the data file was split according to group and a repeated measures ANOVA with training as the within-subjects factor was conducted. Alpha was adjusted for two comparisons to .025. No SME of training was found within either the control group,  $F(4, 5) = 1.91, p = .25, \eta^2$

= .6, observed power = .27, or the lesion group,  $F(5, 6) = 1.83, p = .24, \eta^2 = .6$ , observed power = .31. To test for SME of group within testing 1 (trials 10, 20, 30, 40, 50, 60), the same procedure was conducted. Here, a SME of testing within the control group was found,  $F(5, 40) = 5.93, p < .001, \eta^2 = .43$ , observed power = .99.



*Figure 4.4.* Training and testing performance for the courage task. Control animals improved within the testing session, whereas lesion animals did not. Error bars represent SEM.

However, pairwise comparisons revealed no significant differences between time points at the .025 alpha level, but there was a trend towards time point 1 (10 trials;  $M = 32.22, SD = 34.56$ ) to be significantly lower than time point 6 (60 trials;  $M = 66.94, SD = 36.95$ ),  $p = .05$ . In the lesion group, there was no SME of time,  $F(2.64, 50) = 1.1, p = .36, \eta^2 = .099$ , observed power = .25. Subsequently, to test for SME of block within group, the data file was again split according to group and a repeated measures ANOVA with the within-subjects factor of block (training, testing 1), collapsed across time, was performed. Alpha was adjusted to .025 for two comparisons. This test revealed a significant SME of block within the control group,  $F(1, 8) = 12.06, p = .008, \eta^2 = .6$ , observed power = .86, and showed that control rats had significantly more HRA entries

during training ( $M = 96.11$ ,  $SD = 4.41$ ) than during testing on the open arm ( $M = 55.28$ ,  $SD = 34.26$ ). For the lesion rats, the same was true,  $F(1, 10) = 24.27$ ,  $p = .001$ ,  $\eta^2 = .71$ , observed power = .99, with significantly more HRA entries during training ( $M = 96.21$ ,  $SD = 3.26$ ) than during testing ( $M = 41.06$ ,  $SD = 38.55$ ).

Table 4.3  
*Means and Standard Deviations for Courage Task by Group*

	Group	<i>M</i>	<i>SD</i>
Testing day 1 – 10 trials	Sham	32.22	34.56
	Lesion	43.64	34.14
Testing day 1 – 20 trials	Sham	46.67	34.28
	Lesion	43.64	39.82
Testing day 1 – 30 trials	Sham	57.78	42.95
	Lesion	45.45	43.44
Testing day 1 – 40 trials	Sham	65.56	37.79
	Lesion	37.27	40.27
Testing day 1 – 50 trials	Sham	62.5	37.58
	Lesion	37.27	39.77
Testing day 1 – 60 trials	Sham	66.94	36.95
	Lesion	39.09	41.58

To test for SME of group within block (training, testing), two independent *t*-tests were conducted. Alpha was adjusted for two comparisons, yielding a test parameter of .025. There was no SME of group within training,  $t(14.43) = -.057$ ,  $p = .96$ , and no significant SME of testing,  $t(17.84) = .87$ ,  $p = .4$ , indicating that groups did not differ

from each other on either day.

Six independent *t*-tests were conducted to test for an SME of group within time 1 (training trial bins 10, 20, 30, 40, 50, 60). Alpha was adjusted for six comparisons, yielding a test parameter of .008. No significant group differences were found for any of the six time levels, all *ps* > .16. The same procedure was followed for testing for an SME of group within time 2 (testing trial bins 10, 20, 30, 40, 50, 60). Here, again, no significant group differences at any time point were found, all *ps* > .13).

Upon visual examination of the data, we found that during the testing session, lesion rats *flat-lined* in their performance, meaning that their performance did not change over the course of the session. For control rats, however, even though it was not statistically significant, the graph indicated that performance increased slightly over the course of the session, as initially predicted and as observed in Experiment 1.

Looking at the standard deviations of the test for SME of block within groups, very large standard deviations for both the control group ( $M = 55.28, SD = 34.26$ ) and the lesion group stood out on the testing day ( $M = 41.06, SD = 38.55$ ). Thus, individual group data for each time point within the testing session are presented in Table 4.3. These high standard deviations raised concern that rats were not actually behaving as a homogenous group. Therefore, individual data points for both the lesion and the control group were plotted (see Appendix C for individual performance traces). These graphs revealed that rats started out anywhere from 10% to 90% on the open arm during testing – in both the control and the lesion group. However, the general trend that can be observed in the mean values seemed to hold true for individual performance traces as well. That is, while control rats tended to improve slightly between the first ten trials and

the last ten trials of the session, lesion rats remained at about the same level, and, if anything, decreased slightly in performance (see Figure 4.5).

To test whether there was a statistical difference between this change at the starting point (10 trials) and the end point (60 trials), these two values were subjected to a separate analysis. Two paired-samples *t*-test were conducted; one examined the difference of starting point to end point within the control group, and one examined this difference within the lesion group. For the control group, the change between the starting point ( $M = 32.33$ ,  $SD = 34.56$ ) and the end point ( $M = 72.22$ ,  $SD = 38$ ) was significant,  $t(8) = -3.62$ ,  $p = .007$ . Please note here that the end point mean value is slightly different from that previously reported. This is due to one animal that did not achieve 60 trials in the testing session. Normally, missing values are replaced with the average for that rat for that session, which is what occurred in the initial analysis. If values were not replaced, the animal would be excluded from the analysis altogether. Here, however, the specific end points were important, even if the animal did not achieve 60 trials. Therefore, the animal's actual end point was used (at trial bin 40), rather than its session average (at trial bin 60). For the lesion group, the change between the starting point ( $M = 43.64$ ,  $SD = 34.14$ ) and the end point was not significant ( $M = 39.09$ ,  $SD = 41.58$ ),  $t(10) = .79$ ,  $p = .45$ . This finding confirmed the visually evident effect and the trend found in the first analysis (where  $p = .05$ ) that control animals improved throughout the session, whereas lesion rats stayed at their initial level. This latter finding could indicate that lesion rats were unable to adapt their course of action once they have chosen one. It seemed to be the case that no matter where between 0% and 100% HRA entries they started out, they remained at that very same level over the course of the session. Rather

than weighing effort and reward, these rats may have simply chosen a response level and persevered on it. Alternatively, these rats may have failed to learn to adapt to the novel situation (i.e., failed to habituate to the open arm).

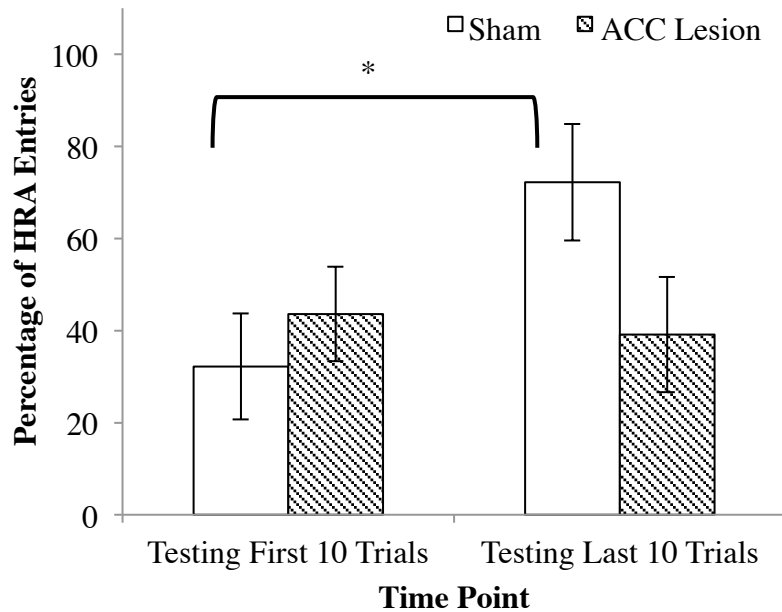


Figure 4.5. Difference scores between the first ten and the last ten trials on the courage task. Asterisk indicates a significant difference. Control rats improved significantly throughout the course of the session, whereas lesion rats did not ( $p = .007$ ). Error bars represent SEM.

Lastly, rats were again compared on the open field and activity box tasks, as there were additional rats that were not included in the weight-lifting analysis included in the courage analysis. On all measures of open field activity, groups did not statistically differ, all  $ps > .18$ . For the activity box task, the lesion rats had higher scores on vertical activity, number of vertical movements, and centre time, compared to controls, and a lower score on margin time. For all variables examined and corresponding statistics, see Table 4.4.

Table 4.4  
*Test Statistics for Significant Measures on the Activity Box Task*

		<i>M</i>	<i>SD</i>	<i>t</i> (df)	<i>p</i> (two-tailed)
Vertical activity	Sham	40.82	10.45	-2.14(17.75)	.047
	Lesion	53.33	15.55		
Number of vertical movements	Sham	4.98	1.2	-2.79(17.37)	.012
	Lesion	6.86	1.81		
Margin time (s)	Sham	34.64	10.03	2.95(11.54)	.013
	Lesion	23.74	5.26		
Centre time (s)	Sham	35.4	10.05	-2.97(11.52)	.012
	Lesion	36.39	5.25		

Here again it was observed lesion rats spent more time in vertical activity, and produced more vertical movements. Interestingly, ACC lesion rats also spent less time in the margin, and more time in the centre of the box than controls. As time spent along walls is usually an indication for anxiety, one might conclude from this finding that indeed lesion rats were less anxious. This would find support in the data reported here when considering the initial trial bin of the courage testing day, where lesion rats started at 43%, while controls started at 32%. It could be that lesion rats were overall less anxious, and thus entered the open arm more often initially. Yet, looking at the individual traces, one could also observe some high-performing control rats. It thus remains an open question whether this was indeed an issue related to baseline anxiety. Further, baseline anxiety does not explain why control rats adapted throughout the course

of the session, while lesion rats did not.

#### **4.4 Discussion**

In Experiment 2, the effort level on the weight-lifting task and the courage task was increased. That is, on the weight-lifting task, lever weights were increased to 40% of the rats' body weights. This level was chosen based on the discounting curve seen in the incremental test in Experiment 1. On the courage task, the room lights were turned on during testing. This was done because, in Experiment 1, rats readily entered the open arm even when they did not receive reward.

On the weight-lifting task, Experiment 2 showed that ACC did not mediate effort as expected. Thus, differences between the two tasks are worth discussing here. One major difference between the weight-lifting task and the ramp-climbing task is that the former investigates an acquired behaviour, and the latter investigates a species-typical behaviour. It turns out that species-typical behaviours do not recover following PFC damage in adulthood, whereas learned behaviours do (Nonneman & Kolb, 1974). It could be the case that the impairment observed on the ramp-climbing task is purely an impairment of a species-typical behaviour. It follows thus, because weight-lifting is a learned behaviour, this effect is not observed. Further support for the latter comes from the fact that, as McKee et al. (2010) demonstrated, the *acquisition* of learned behaviours, such as operant lever pressing, depended on ACC, but not the *performance* of lever pressing once it was learned. Experiment 3 will address the question of whether training played a role in weight-lifting effort and subject rats to a situation in which the effort learning did not have to be acquired prior to testing. Thus, effects of training as well as of the distinction between acquisition and performance can be separated.

A second difference between the tasks is that ramp-climbing effort is visible to the animals, whereas weight-lifting effort is not. That is, the effort magnitude of each lever must be held in recency memory at any given time, as no cues indicate which lever constitutes higher effort. This, the task is memory-dependent from trial to trial. Even though pilot experiments have indicated that, similar to the ramp-climbing task, rats will remember the HRL up to four weeks after last exposure, a short-term memory disruption could have existed on this task. Because the effort is not visible, animals have to press the lever to experience the effort, or remember it from previous exposure. It might thus be the case that the weight-lifting task is primarily a task of rule-learning, and that effort is a secondary factor. Indeed, in rule-learning scenarios, ACC is not required for decisions (Walton et al., 2007).

On the courage task, it was expected that lesion rats would start entering the open arm more slowly, and improve more slowly over the course of the session. However, that was not the case. Lesion rats initially entered the open arm more readily than control animals, and remained at that level throughout the course of the session, whereas control rats improved. The improvement seen in control rats could indicate that ACC is needed to overcome fear when exposed to a fearful situation for a period of time. It has been shown that ACC is active when humans choose to overcome fear (Nili et al., 2010). However, the present experiment revealed large individual differences within both the control and the lesion group. Thus, it may not be surprising that differences after ACC lesions in rats conflict with the human literature. Furthermore, the subregion of human ACC that plays a role in courage, subgenual ACC, is functionally most closely related to rat IL (Slattery et al., 2011). It could be the case that, in rats, a failure to enter the open

arm is only observed after IL lesions.

The fact that control rats adapted their responses over the course of the session, whereas lesion rats did not, deserves further illumination. This adaptation could be a habituation to (a) the novelty of the open arm, and (b) the fearful environment itself. Sequence errors in which the animal entered the open arm after receiving reward in the LRA were not observed in this experiment; rats in Experiment 1 committed on average 10-13 sequence errors to the open arm, whereas rats in this experiment committed only 2-3. Thus, the desired deterrent effect was achieved successfully by turning on the room lights, showing that rats generally did not enter the open arm unless it was for reward. The novelty explanation is further supported by data from the activity box. Rats with ACC lesions that also underwent the courage task showed significantly more vertical movements and higher vertical activity. They also showed more overall movements. This supports the explanation that ACC-lesioned rats failed to adapt to a novel environment, as they showed more time related to exploring the activity box.

It could be the case that the open arm presented each rat with a baseline starting point based on each individual's general anxiety. That is, individual rats that were generally less anxious may have entered the open arm more readily (higher percentages), whereas rats that were generally more anxious may have stayed away from the open arm more (lower percentages). Then, given this baseline, those rats with ACC lesions did not habituate to the open arm and did not enter it more frequently. This habituation could be to either (a) the novelty of the open arm, or (b) the fearfulness of the open arm, or (c) both. The explanation that has evidence in the rodent literature is the one of failure to adapt to novelty. Out of the three tasks employed in this study, the courage task is the

only task in which animals are presented with an entirely new situation during testing. Whereas lever weights and ramp heights had been experienced before, the open arm had not. This made the courage task fundamentally different from the physical effort tasks. A recent study showed that mPFC cells respond to novelty recognition (Weible et al., 2009). Even though novelty recognition was limited to objects in this study, the object location was also neurally coded by ACC, and ACC activity was different for familiar and novel objects. It could be the case that the novelty of the open arm could not be properly integrated in rats without ACC, and therefore, rats did not habituate to the novelty of the open arm and consequently did not improve throughout the session. Support for this explanation comes from the present experiments' activity box data: ACC-lesioned rats that participated in the courage task showed more vertical movements (i.e., exploring the vertical perimeter of the environment) in the activity box as well as more overall movement. Thus, one could argue that they were also unable to habituate to the novelty of this environment.

## **Chapter 5: Experiment 3 – Does ACC Mediate Weight-Lifting Effort When Effort Training is Eliminated?**

### **5.1 Hypotheses**

Results from the weight-lifting task in Experiment 2 showed no differences between ACC-lesioned and sham animals. In addition, both groups had a similar breaking point on the effort-discounting curve that was established in the incremental weight-lifting session. Interestingly, comparing these discounting curves from Experiments 1 and 2, one can see that rats perform well up to whichever increment they had been previously trained on. Thus, the question remained whether there would be a difference between ACC-lesioned and control rats if the training component were eliminated. Since training was extensive in both Experiments 1 and 2, it could be the case that a possible group difference was eliminated. There is some evidence in the literature that ACC lesions disrupt the acquisition, but not performance once learned, of operant behaviours (McKee et al., 2010). Therefore, we hypothesized that without training, there would be a group difference between lesion and control rats during an incremental session, where lesion rats would show a sharper discounting curve with a performance drop off significantly sooner than controls.

### **5.2 Method**

#### **5.2.1 Animals.**

Twenty-four male Long-Evans rats (Charles River Laboratories International Inc., Montreal, QC) were trained in two cohorts in this study. Cohort 5 was 99 and cohort 6 was 95 days of age at the start of training. Rats' weights were maintained at 85% of their free feeding weight (350-450 g at start of training) one week prior to and for the duration

of training and testing. For at least one day before and five days after surgery, rats were allowed *ad libitum* food. Rats were between 113-118 (cohort 5) and 113-116 (cohort 6) days old at the time of surgery, and between 125-130 (cohort 1) and 122-125 (cohort 6) days old at the time of weight-lifting testing. Open field and activity box were conducted at 153 (cohort 5) and 126 (cohort 6) days. Cohort 5 was perfused at 161 days, cohort 6 at 129 days of age. No animals died or had to be excluded from the analyses.

### **5.2.2 Materials and apparatus.**

The task was videotaped via an in-ceiling video camera.

#### ***Weight-lifting task.***

Materials on the weight-lifting task apparatus remained the same as in Experiment 2 (see section 4.2.2).

#### ***Open field and activity box.***

All testing remained the same (see section 3.2.2).

### **5.2.3 Surgery and histology.**

Procedures were the same as in Experiment 1 (see section 3.3.3). In Experiment 3, rats were again matched according to task performance on the weight-lifting task and then divided into sham and ACC lesion groups prior to surgery. Matching occurred by rank-ordering rats based on (a) their performance percentage on the last day from high to low, (b) the number of days it took for HRA pre-training from low to high, and (c) the initial number of pre-training days from low to high. For every first pair, the first rat was allocated to the lesion group. For every second pair, the first rat was allocated to the control group.

#### **5.2.4 Task procedure.**

##### ***Weight-lifting task.***

After criterion performance in the reward contingency learning stage was reached for two non-consecutive days, animals underwent lesion or sham surgery. Each rat received one testing session, in which lever weights in the HRA were incremented every 10 trials. Increments were identical to the ones used in Experiment 2, plus two additional stages at 45% and 50% for a total of 10 stages. For the 0% stage, rats completed 15 trials, followed by 10 trials for every effort stage. While weights were affixed to the HRA lever, rats were blocked from entry into the decision arms and remained in the base arm.

#### **5.3 Results: Anterior Cingulate Does Not Mediate Weight-Lifting Effort, Even When Effort Training Was Eliminated.**

Final animal numbers in Experiment 3 were: (a) weight-lifting task: ACC lesion:  $n = 12$ , sham:  $n = 12$ ; (b) open field and activity box tasks: ACC lesion:  $n = 12$ , sham:  $n = 12$ . In Experiment 3, the question was whether training affected performance on the weight-lifting task. Since it is a learned-effort task, it could be the case that the amount of training provided obscured potential group differences. Thus, rats were only trained on reward contingency learning, but not effort. All 24 animals initially trained were included in the analyses. To determine whether the two cohorts had systematic differences, a repeated measures ANOVA compared cohorts on the last day of pre-training. There was no main effect of cohort,  $F(1, 22) = .47, p = .5, \eta^2 = .021$ , observed power = .1, and no Cohort  $\times$  Time (trial bins 10, 20, 30, 40, 50, 60) interaction,  $F(5, 18) = .98, p = .46, \eta^2 = .21$ , observed power = .27. However, there was a main effect of time,

$F(5, 18) = 4.18, p = .011, \eta^2 = .54$ , observed power = .88. Pairwise comparisons showed differences between time 1 (10 trials) and times 3 (30 trials), 4 (40 trials), and 5 (50 trials) when collapsed across group. Descriptive statistics are reported in Table 5.1.

Table 5.1  
*Test Statistics for Main Effect of Pre-Training  
 Collapsed Across Cohort and Group on the Weight-  
 Lifting Task*

	<i>M</i>	<i>SD</i>	<i>p</i>
Pre-training – 10 trials	84.17	4.71	.054
Pre-training – 20 trials	93.75	.59	
Pre-training – 10 trials	84.17	4.71	<b>.01</b>
Pre-training – 30 trials	95	2.36	
Pre-training – 10 trials	84.17	4.71	<b>.009</b>
Pre-training – 40 trials	95.42	.59	
Pre-training – 10 trials	84.17	4.71	<b>.009</b>
Pre-training – 50 trials	95.71	1.05	
Pre-training – 10 trials	84.17	4.71	1
Pre-training – 60 trials	92.5	3.54	

This finding merely indicated that throughout the last day of pre-training, rats did improve slightly within the session, but it was inconsequential for any cohort differences. Therefore, cohorts were considered equal and treated as such throughout the weight-lifting analysis. A repeated measures ANOVA with the within-subjects factor of increment (0%, 15%, 20%, 25%, 30%, 32.5%, 35%, 37.5%, 40%, 45%, 50%) and the between-subjects factor of group (control, lesion) was performed (see Table 5.2 for all

means and standard deviations). Mauchly's test of sphericity showed a violation of this assumption,  $\chi^2(54) = 130.54, p < .001$ , Greenhouse-Geisser  $\epsilon = .41$ . Thus, the more powerful multivariate test was used. The ANOVA revealed no main effect of group,  $F(1, 22) = .29, p = .6, \eta^2 = .013$ , observed power = .08, and no significant Increment  $\times$  Group interaction,  $F(10, 13) = .83, p = .61, \eta^2 = .39$ , observed power = .25, Wilks'  $\Lambda = .61$ . However, there was a main effect of increment,  $F(10, 13) = 57.68, p < .001, \eta^2 = .98$ , observed power = 1., Wilks'  $\Lambda = .02$ .

Table 5.2  
*Means and Standard Deviations for  
 Incremental Weight-Lifting Task*

	<i>M</i>	<i>SD</i>
Increment 1 – 0%	83.33	14.82
Increment 2 – 15%	67.92	19.33
Increment 3 – 20%	47.92	29.78
Increment 4 – 25%	39.58	27.26
Increment 5 – 30%	34.17	29.62
Increment 6 – 32.5%	27.92	33.1
Increment 7 – 35%	25.42	35.51
Increment 8 – 37.5%	17.5	27.39
Increment 9 – 40%	12.92	18.67
Increment 10 – 45%	7.92	16.15
Increment 11 – 50%	3.75	11.73

Pairwise comparisons again revealed the familiar effort-discounting curve (see Figure 5.1), with rats performing significantly better on increment 1 (0%;  $M = 83.33, SD$

= 14.82) than on any other increment, all  $ps < .004$ . Rats performed significantly worse on the 11<sup>th</sup> increment (50%;  $M = 3.75$ ,  $SD = 11.73$ ) than on increments 1 (0%), 2 (15%), 3, (20%), 4 (25%), 5 (30%), and 6 (32.5%). This finding showed the effort-discounting curve again that was observed in Experiments 1 and 2 (see sections 3.3.2 and 4.4.1). However, even when training was eliminated as a factor, there was still no group difference in effort-based decision making on the weight-lifting task.

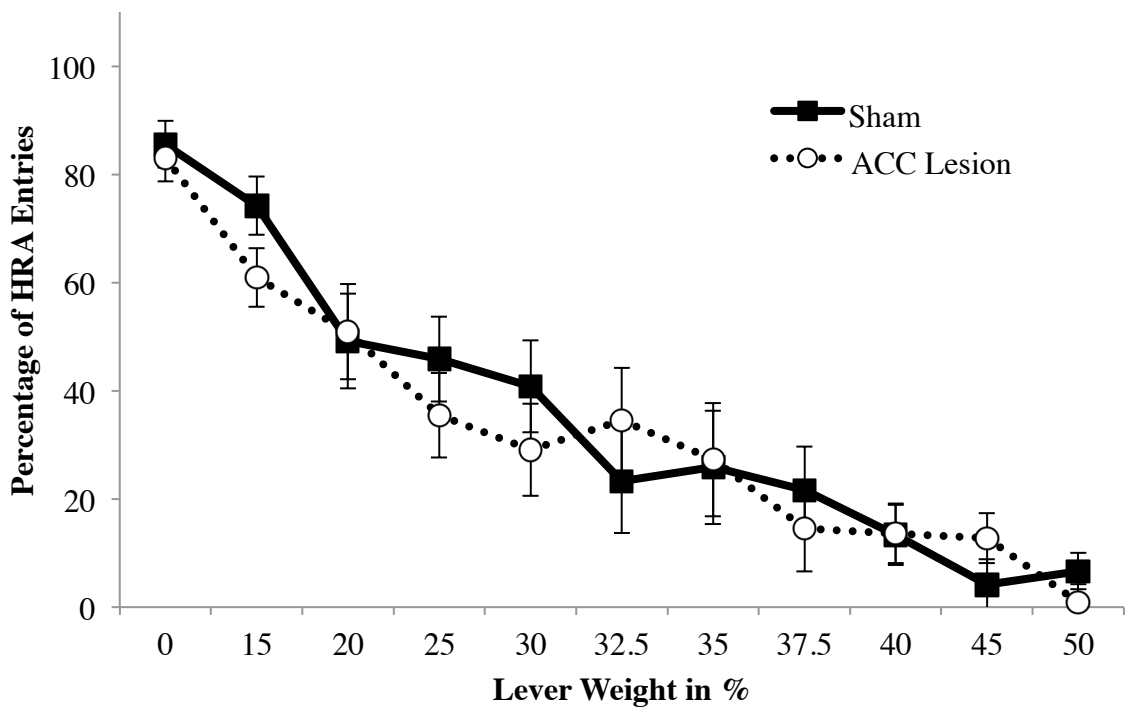


Figure 5.1. Performance on the weight-lifting incremental task. No group differences were observed even when training was eliminated. Error bars represent SEM.

Analysis for the open field test did not reveal any differences between groups on any of the five measures analyzed, all  $ps > .11$ . Analysis for the activity box test revealed two differences; lesion rats had fewer vertical time ( $M = 12.49$ ,  $SD = 3.22$ ) compared to controls ( $M = 17.02$ ,  $SD = 4.1$ ),  $t(20.84) = 3.01$ ,  $p = .007$ , and a smaller number of stereotypy ( $M = 9.36$ ,  $SD = .88$ ) compared to controls ( $M = 10.15$ ,  $SD = .7$ ),  $t(20.91) = 2.45$ ,  $p = .023$ .

## 5.4 Discussion

Experiment 3 revealed that even when training is eliminated, ACC did not mediate weight-lifting effort-reward decision making. However, without prior training on this task, rats showed a steep and almost linear discounting curve. That is, as effort increased, performance on the task decreased. Thus, the task itself was shown to be effortful, but no group difference was found. This raised the question about what distinguished this task from tasks that require multiple operant lever presses and from the ramp-climbing task. Interestingly, results on operant tasks are also mixed. Schweimer & Hauber (2005) found that ACC lesions did not impact rats' performance on a PR operant lever-pressing task, whereas a more recent study did (Walton et al., 2009) and activity in ACC is found in such a task (Endepols et al., 2010). This task has some commonalities to operant lever-pressing tasks. It uses a lever whose reward dispense schedule and amount can only be inferred by trial and error, and the current choice must be guided by the memory of past experience with each lever. It could be the case that the nature of this task (i.e., being a learned, memory-guided vs. an innate behaviour), leads to differences in the way it is processed neurally. However, there is evidence to the contrary. Dopamine depletions in NAcc affect lever pressing on an operant task, biasing the rats' choices away from the HRA and towards the LRA (Cousins et al., 1996; but see Walton et al., 2009). Future research may test DA depletions on the weight-lifting task to see if a similar effect can be found.

It was observed that animals used different strategies for lever pressing throughout all experiments. In the initial stages, when there was no effort and the trigger was low, some animals learned that a simple *flick* of the lever with one or two paws

elicited the desired result. That is, the animal could flick the lever and immediately turn to the reward well. It did not need to fully press the lever down (i.e., stay in touch with the lever until the lever reached a trigger and the cue light came on). However, as the trigger moved to the highest stage, the animal now needed to fully press the lever. Some rats that initially learned to flick the lever flicked with such inertia that it was sufficient for the high trigger to go off. However, it was observed that as the effort got higher, flicking was not a successful strategy. In fact, in addition to flicking, even using only one paw for pressing was not a successful strategy anymore when the effort neared 40%. It could be the case that animals that did not achieve criterion performance in Experiment 2 were *flickers*, rather than *pressers*. However, because of the low quality of experimental video and the fact that the animals' upper bodies obscured their front paws half of the time, detailed video analysis of strategies (i.e., flicking vs. pressing) was impossible. Further, an additional analysis on attempted lever presses conducted in Experiment 2 provides evidence against the idea that a differing strategy deterred rats from lever presses, as flicks would have shown up as attempts in that case. Specifically, this analysis showed that ACC-lesioned rats did not differ from sham animals in the number of attempted, but not full, lever presses. Thus, lesion rats did not *attempt* to press the HRL and fail, which could have been caused by differing strategies in lever pressing.

Yet, the idea that rats used different strategies does not explain why the weight-lifting task was not affected by ACC lesions. Initially it was thought that the effect in Experiment 2 was not found due to the low animal numbers and ensuing low power for statistical tests. However, Experiment 3 did eliminate the concern about group sizes and still did not find a group difference on the weight-lifting task.

## **Chapter 6: General Discussion And Conclusion**

In this chapter, potential explanations for the present findings will be discussed, and limitations of the current research and directions for future research will be addressed. The purpose of this thesis was to explore the role of ACC in effort- and courage-based decision making. Two novel effort-based decision making tasks were developed to determine if ACC mediates other types of effort that are (a) physical and learned, and (b) emotional. The first was a weight-lifting task, on which rats had to choose between a heavy, weighted lever to obtain a high reward, or an easy, unweighted lever to obtain a low reward. The second was a courage task, on which rats had to choose between an open, scary arm to obtain a high reward, and an enclosed, safe arm to obtain a low reward.

### **6.1 Conclusions From Ramp-Climbing Task**

There is one main task that is used in the rodent literature to test effort-based decision making, the ramp-climbing task. On this task, rats choose between climbing a ramp to obtain a high reward, and between freely obtaining a low reward. Healthy rats will choose the high effort, high reward option about 80% of the time. Rats with ACC lesions are impaired on this task and show a marked preference shift towards the low-effort, low-reward option (Rudebeck et al., 2006; Schweimer & Hauber, 2005; Walton et al., 2003). This effect was replicated on a modified, automated version of the ramp-climbing task. In the present study, rats received many more trials (60 vs. 10), which was useful to determine within-session performance rather than only across-session performance. Behaviours of rats on the first testing day were investigated in more detail. Rats with ACC lesions oftentimes turned into the HRA, even placed their paws on the

ramp, but then turned and ultimately chose the LRA. The approaching behaviour might fit with the explanation that ACC is responsible for action maintenance (Cowen et al., 2012): lesion animals had an intact reward representation, probably due to an intact reward signal from the DA system, but were unable to carry out the action once faced directly with the effort.

Questions could arise as to how these results fit with what is known from the human effort literature. Naccache et al. (2005) showed that cognitive effort signals could not be translated to bodily responses after extensive unilateral ACC lesion in a human patient. This patient did not have consciousness of mental effort and also lacked the physiological responses related to cognitive effort. However, she had an intact ability to consciously experience emotional feelings. First, if ACC lesions on the ramp-climbing task resulted in a similar inability to translate effort signals, rats would have shown insensitivity to the effort. This would have resulted in ACC-lesioned rats entering the HRA only. However, that was not the case, as ACC-lesioned rats showed a significant decrease in HRA entries compared to control rats on the first testing day. Further, ACC-lesioned rats also showed hesitation before deciding to climb the ramp or turn around, which is inconsistent with the insensitivity explanation. Second, it could be the case that damage to ACC prevents a translation of the effort response signal to other structures. This would have resulted in the opposite effect, where lesion rats should have avoided the HRA completely. However, behaviourally, lesion rats showed turns into the HRA about 80% of the time, even though they turned around to the LRA the majority of the time. Thus, this explanation is also unlikely. It must be kept in mind that the lesion in Naccache et al.'s (2005) patient was rather extensive and spanned PL and IL as well as

some OFC areas. It is therefore not paramount to translate this effect, likely produced by a combination of functional deficits due to the array of structural impairments in this case study to the ACC lesion produced in the present animals. Further, this patient was subjected to a mental/cognitive effort task, which is not parallel to anything seen in the physical effort rat literature. Therefore, it is unlikely that parallel results would be produced.

## **6.2 Conclusions From Weight-Lifting Task**

On the weight-lifting task, there were no group effects in any of the three experiments. Lesion rats did not significantly differ from controls in the amount of HRA entries in any of Experiment 1, 2 or 3. Initially, it was thought the effort used was insufficient, as a ceiling effect was observed in Experiment 1, where all rats remained between 80-100% of HRA entries during testing. However, when the effort was increased in Experiment 2, 67% of rats did not learn the task to criterion performance prior to surgery, indicating that the effort was indeed sufficient. Because there is evidence that some mPFC structures mediate the acquisition of a behaviour and the performance of the behaviour once acquired differently (McKee et al., 2010), it was decided to eliminate training/learning of the task in Experiment 3. Despite this elimination, no group difference in HRA entries was found. Yet, data from Experiment 3 showed evidence that the task was indeed effortful, as both groups showed a similar, rather steep and nearly linear discounting curve, with performance nearing 0% HRA entries when the effort was highest. That is, as the effort was increased, performance dropped.

The most parsimonious explanation is that this task simply does not require ACC.

Some evidence for that comes from the finding that ACC lesions did not produce the same effect as previously seen with DA depletions on an operant lever-pressing task (Cousins et al., 1996; Schweimer & Hauber, 2005), whereas such depletions produce the same effect as ACC lesions on the ramp-climbing task. For instance, DA depletions in NAcc might yield a different result, as these DA depletions often demonstrate different effects than ACC lesions, although not without contradictions either (Salamone et al., 1996; Schweimer & Hauber, 2005; Schweimer et al., 2005; Walton et al., 2005). It could also be the case that learned effort is not mediated by ACC, but more research is needed to establish that. For example, this type of effort might be mediated by the larger effort network, which likely includes NAcc and BLA as well. Future research could focus on dissociating potential roles of these structures on the weight-lifting task.

In fact, it has been proposed that together, the striatum, amygdala, ventral hippocampus, and ventromedial PFC form a network to guide behavioural responses to stimuli based on the associated affective outcomes (Gruber & McDonald, 2012). According to this model, the dorsomedial striatum (medial CPu) guides goal-directed behaviours, and the dorsolateral striatum (lateral CPu) guides habitual responses. When the environment is dynamic, goal-directed responses adapt quickly, whereas habitual responses adapt slowly. It might be the case that, when ACC is lesioned, the striatum takes over to provide information about reward (NAcc), as well as habit and goal-oriented behaviour (CPu). Whereas ACC now cannot send information about goal-directed behaviour to the dorsomedial striatum any longer, M2 can still send information about habit to the dorsolateral striatum. It could be the case that the lack of impairment seen after ACC lesions on the weight-lifting task is due to the fact that the learned lever-

pressing behaviour has transferred to the habit system. This system is still fully intact, and thus the task can be carried out as normally. It might be the case that ramp-climbing is more of a goal-directed behaviour, which due to lack of ACC input to dorsomedial striatum can no longer be processed.

The two research groups who have extensively studied ACC in effort decisions over the past ten years, the group of Wolfgang Hauber in Germany and that of Mark Walton in England, along with others who have studied ACC in effort-based decisions, have suggested that ACC is not involved in all cost-benefit decisions or may not mediate cost-benefit decisions uniformly (Cocker et al., 2012; Hauber & Sommer, 2009; Hillman & Bilkey, 2010; Schweimer & Hauber, 2005; Walton et al., 2007). The following will discuss some scenarios in which ACC does not seem to be involved and how the present tasks might relate to those situations.

First, Walton et al. (2007) suggested that where rule-learning is concerned, such as in delay-matching tasks, ACC lesion rats perform as well as control animals. It could be the case that the weight-lifting task is more dependent on rule learning than effort-reward decision making, as the effort is not easily accessible unless a rat actually attempts to press the lever. The effort itself might be a secondary consideration, which initially does not factor into the rat's decision. As previously explained, the weight-lifting task relies on recency memory, whereas the ramp-climbing task relies on visual information about the effort. Thus, on the ramp-climbing task, the effort is easily accessible, as rats can see and touch the ramp without having to commit to a decision.

A related explanation is that these visual cues present on the ramp-climbing task could have been the main cause behind the effect. The visual discrimination of reward

arms is fundamentally different on both tasks. In an environment void of visual cues, animals rely on internal spatial navigation based on the point of departure (Etienne, Maurer, & Séguinot, 1996). It could be the case that visual, externally guided, but not spatial, internally guided navigation of the mazes caused the task differences.

Specifically, when the rat had to rely on internal mechanisms of spatial navigation in a cue-less environment, such as on the weight-lifting task, the effects of ACC lesions may have been negated. Here, again, the effort invested might be a secondary parameter that becomes irrelevant once the initial reward-arm decision has been made.

Second, some researchers have proposed that ACC guides action according to reward expectation (Matsumoto et al., 2003; Schweimer & Hauber, 2005). Thus, on effort-reward decision tasks, the primary decision might be about reward, and the decision about effort is secondary. On the ramp-climbing task, it was found that lesion rats often turned into the HRA first, but only once they reached the effort did they turn around. This indicates that rats' reward expectation was intact, and that they initially decided where to turn based on that reward. However, once they were faced with the effort, they decided to turn around and enter the LRA. Again, because on the weight-lifting task the effort is not tangible unless a rat actually tries to press the lever, it could be the case that the rat was already in the midst of the pressing action before making a secondary decision about effort. Because the rat already committed to a lever at that point, and indeed rats are physically able to carry out the effort on both tasks once committed to it, the decision about effort became negligible here.

### **6.3 Conclusions From Courage Task**

On the courage task, initially no effect of ACC lesions was found in Experiment

1. However, it was discovered that the effort used was insufficient, as the performance curve of both groups started at about 50% HRA entries, which is not different from chance. It was determined that, for the task to be effortful, the effort should be similarly deterring as on the ramp-climbing task. This was the case in Experiment 2, where even control rats, on average, started at about 30% HRA entries at the beginning of the session. In addition, rats made many more sequence errors on the task in Experiment 1, meaning that they entered the open arm without receiving reward. Thus, it was determined that the task was not scary enough. In Experiment 2 it was found that control rats improved over the course of the session, whereas lesion rats remained at their initial level of performance. Interestingly, rats in both the control and the lesion group showed large variation in performance on the courage task. In either group, rats displayed performances along the entire range of possibilities, with no marked trend emerging.

It was predicted that lesion rats would exhibit a shift in arm preference parallel to that observed on the ramp-climbing task. Precisely, lesion rats were expected to enter the open arm later and improve more slowly within the session compared to controls. However, ACC lesions did not produce this behaviour. Rats with ACC lesions were not exceptionally deterred from the open arm, and in the initial ten trials, where the largest effect was expected, even less so than controls. Despite this prediction not being confirmed, a change in behaviour in lesion rats from their baseline performance on the testing day was observed. This change was different from that seen in control animals. During baseline performance, all rats entered the HRA nearly 100% of the time. During testing, lesion rats dropped down to about 40% HRA entries and remained at that level throughout the entire course of the session. Control animals, however, started at about

20% HRA entries in testing and improved to about 60% by the end of the testing session. Thus, it might be possible that ACC mediated some aspect of this task. Which aspect this might be exactly remains subject of further investigation, but possible explanations are (a) poor novelty recognition, meaning that lesion rats failed to habituate to the open arm, as they continued to be impaired by the novelty over the course of the session; or (b) poor fear habituation, again rendering rats unable to habituate to the open arm. Support for the novelty explanation comes from the present activity box data in Experiments 1 and 2 for rats that were run on the courage task. Here, lesion animals showed higher overall movement as well as more vertical movements and more time in vertical activity. Those behaviours can be classified as exploratory behaviours. Thus, it might be the case that ACC-lesion rats failed to habituate to another novel environment, the activity box, as well. This supports the idea that ACC-lesion rats show a failure of novelty habituation.

Since the present experiment was designed to address emotional effort, it should be noted that it is unlikely, that the observed effect can be classified as impairment in exerting emotional effort. That is because some rats very readily entered the open arm early on. The common feature observed in lesion rats was an impairment in adaptation to the open arm on the testing day. The most likely explanation for the present results is that rats were incapable of adjusting to the novelty of the open arm situation and thus simply flat-lined, as ACC is said to play a role in novelty recognition for locations (Weible et al., 2009).

#### **6.4 Limitations And Directions For Future Research**

Both tasks developed for this study had little precedent in the existing literature. However, part of the rationale for this thesis was to develop novel tasks to test an existing

concept. This proved very difficult in investigating ACC, which does not behave uniformly in cost-benefit decision-making paradigms and is in fact not necessary for all such decisions as evidenced by existing literature, and the current data (Cocker et al., 2012; Hauber & Sommer, 2009; Hillman & Bilkey, 2010; Schweimer & Hauber, 2005; Walton et al., 2007). To further elucidate whether the tasks work and validate them for the concept that was expected to be tested, NAcc DA depletions would have been able to shed insight. It seems that these DA depletions are reliable and give consistent results on cost-benefit tasks. It might be worth examining these novel tasks with NAcc DA depletions. Since ACC is hypothesized to belong to a network of structures that mediates cost-benefit decisions, it might be the case that ACC and NAcc work together to mediate effort-based decisions (Floresco & Ghods-Sharifi, 2007; Hauber & Sommer, 2009).

Concerns might be raised about the extensive training in our experiments. Past studies, in which rats were trained for up to eight weeks, have used very few trials within a session, whereas we used 60 trials within a single session to allow for observation of within-session changes (Rudebeck et al., 2006; Salamone et al., 1994; Schweimer & Hauber, 2005; Walton et al., 2002; 2003). However, we have successfully demonstrated that the number of trials and number of sessions did not impact replication on the existing ramp-climbing task. In fact, despite extensive training, we were able to robustly demonstrate that rats remained at low performance for the entire three testing days. Further, in the weight-lifting task, when training was eliminated, we did not see a group difference in performance.

Moreover, more behavioural analyses might be necessary to firmly link behaviour to existing theories about ACC. Unfortunately, the video data we collected from the

weight-lifting task is of poor quality and rats' behaviour cannot be properly detected, at least not on the level of paw-lever contact, which is what needs to be analyzed.

Furthermore, due to a persistent time stamp-logging problem on all three tasks, it was impossible to analyze reaction time data for all rats in all sessions. However, previous studies that have recorded reaction times to both HRA and LRA on the ramp-climbing task found that any delays imposed by climbing the ramp are negligible (Floresco et al., 2007; Hillman & Bilkey, 2010). In fact, for working analyses, we found that pressing the HRA lever and climbing (or jumping) the ramp did often take *less* time than going to the LRA.

A concern might be raised about animals' performances on both the weight-lifting task and the courage task in Experiment 2. In fact, looking at the averages alone, the question arises of whether these rats performed at the chance level, or responded randomly. After all, it could be the case that impairments we see after ACC lesions are due to random-choice behaviour, rather than a preference shift. However, as the experiment has revealed after more detailed examination of the data, an average judgment is very misleading. Rats with ACC lesions did not behave in a uniform fashion in both tasks (Appendices B, C). This rules out the idea that rats went into the arms randomly. Specifically, on the weight-lifting task, we see a bimodal distribution of performance of lesion animals, where four out of six animals average 85.6% of HRA entries, and two out of six animals average 8.33% of HRA entries. Both the values are not near the 50% mark. On the courage task, performance of lesion animals spanned the entire spectrum of performances. Averages values on Testing Day 1 in the ACC lesion group were 0%, 1.67%, 3.33%, 5%, 13.33%, 35%, 58.33%, 66.67%, 84.79%, 85%, and

98.33%. Thus, three out of eleven animals performed in the range between 30%-70%, which could be considered close to 50%, and which might turn out not statistically different from 50% when tested. However, the important point here is that not all animals behaved the same way; therefore, an explanation of random responding following ACC lesion does not apply.

Lastly, at the weight-lifting task in Experiment 2 had a rather high attrition rate. Therefore, one could assume that with a greater number of animals, a desired effect might be achieved. Yet, when looking at the individual data in Experiment 2 on the weight-lifting task, it turned out that out of the six lesion animals remaining, four behaved identical to controls, and two dropped in performance. It would be interesting to investigate if there were differences like those discovered on a cognitive effort task, where some rats turned out to be lazy, and *slackers*, and other rats motivated, and *workers* (Cocker et al., 2012). However, the current experiments did not find a difference in motivation based on the number of trials achieved to either arm.

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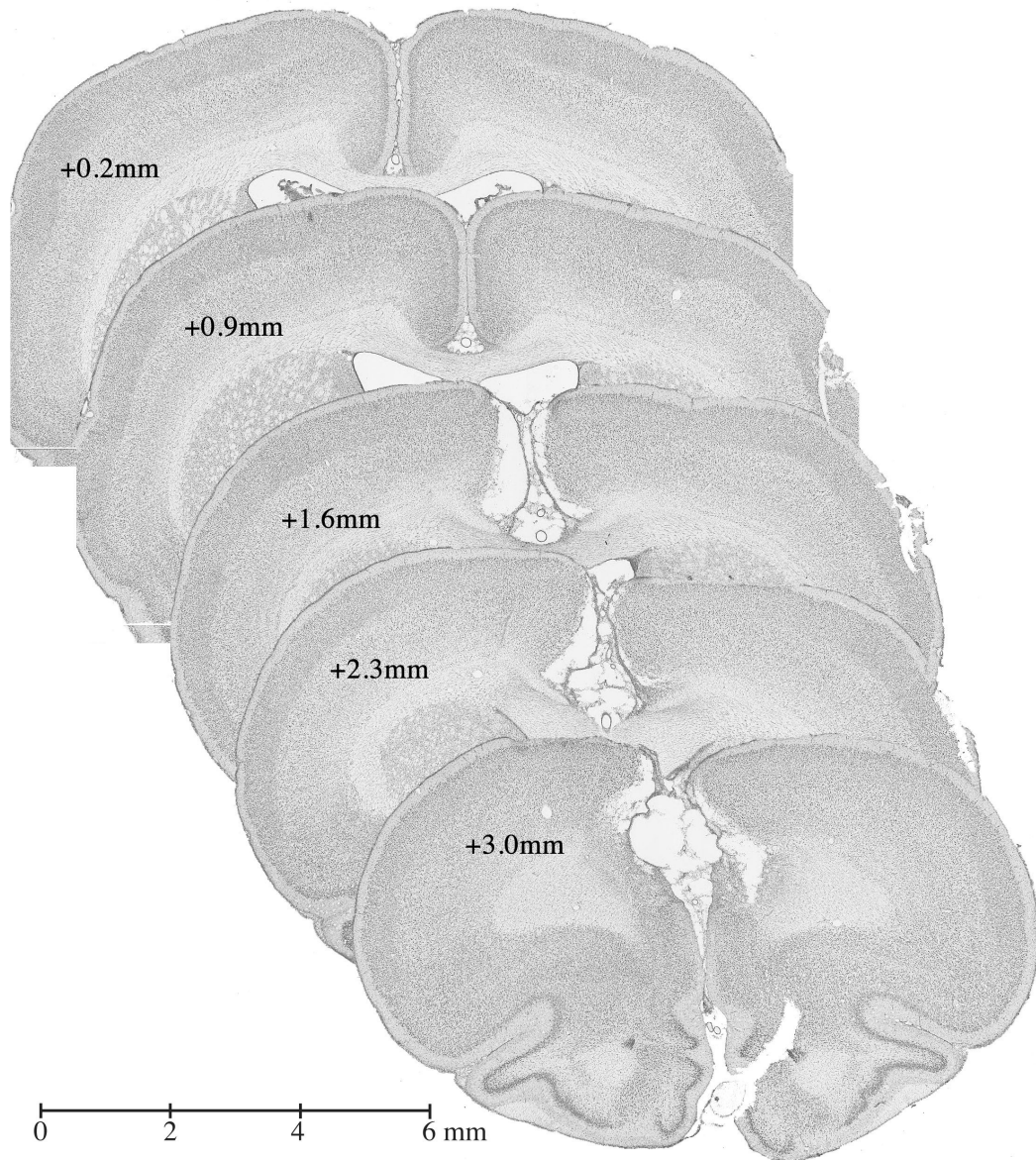
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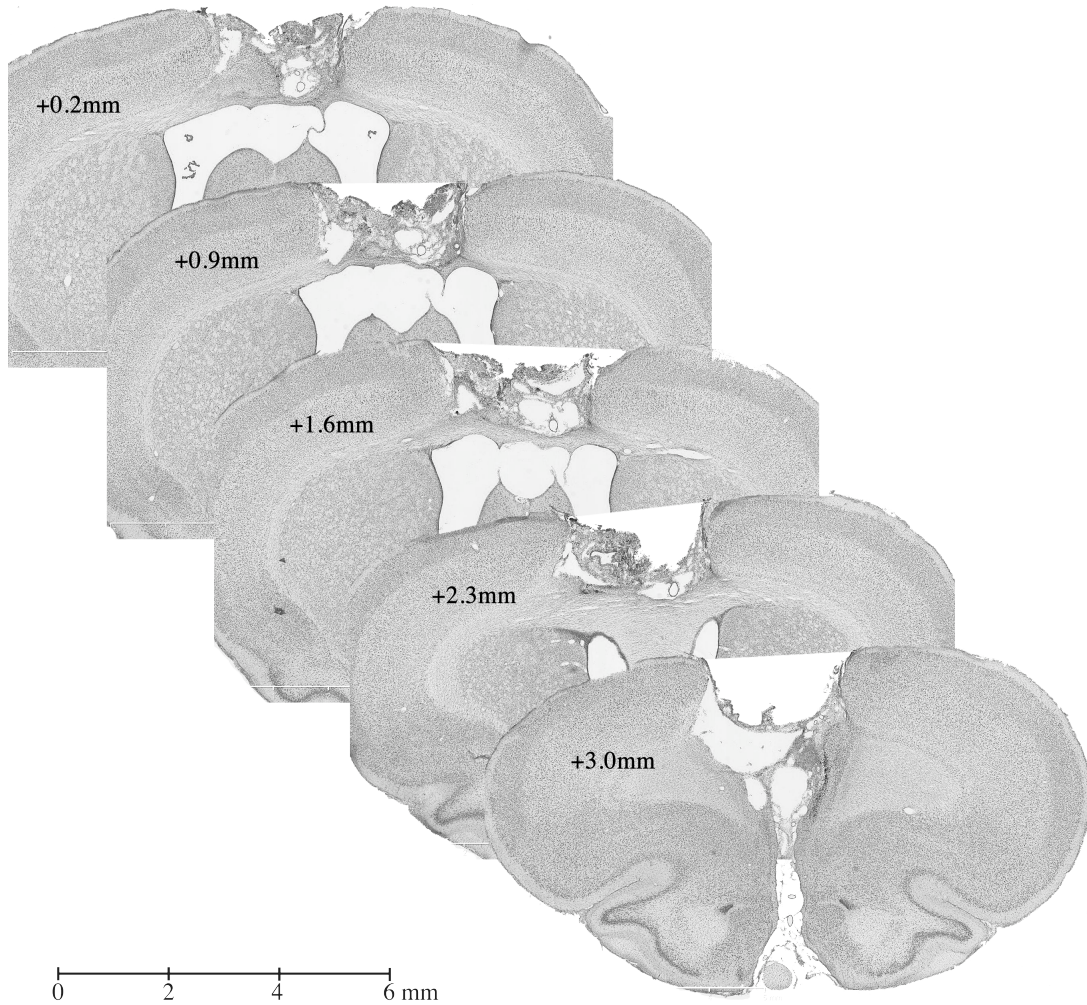
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Appendix A

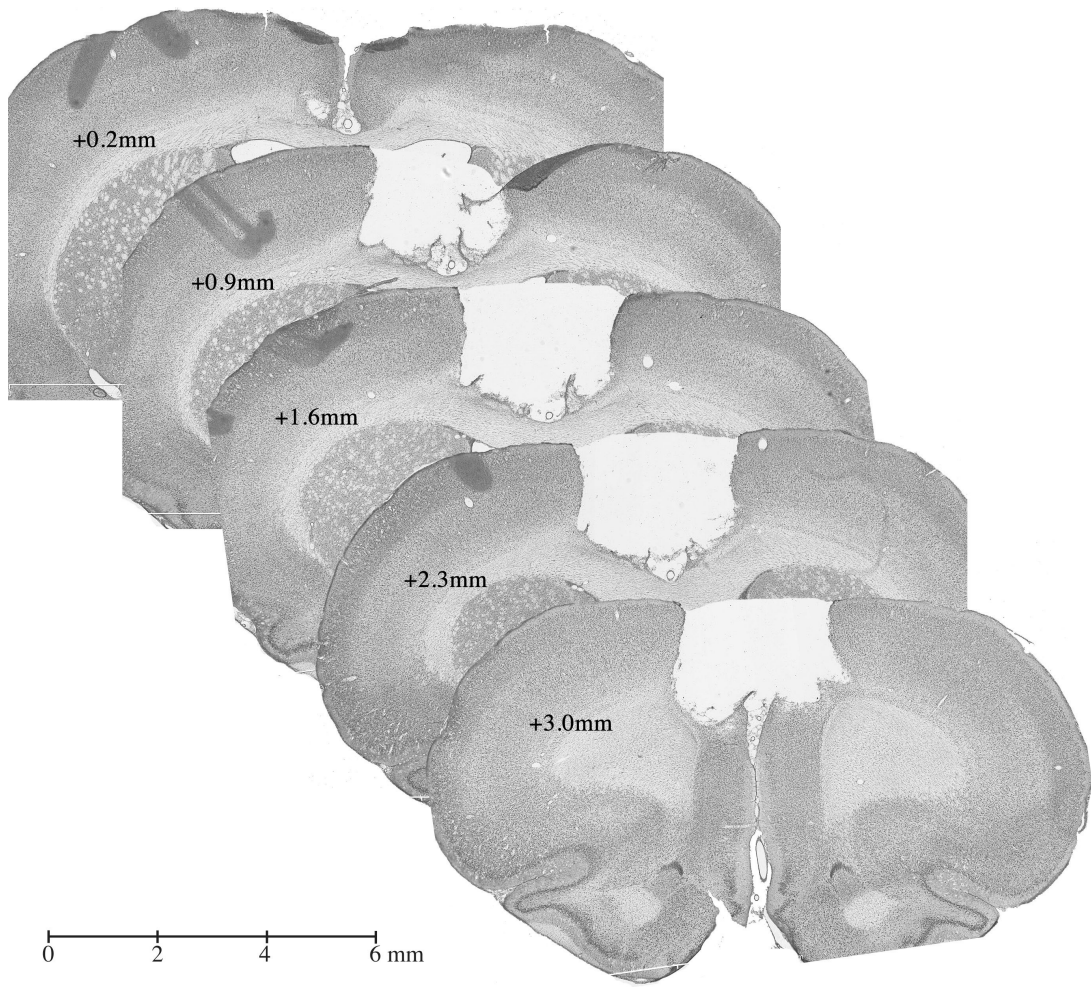
Nissl Stained Sections of Representative Lesion Sizes (Smallest And Largest) From  
Experiments 1 And 2



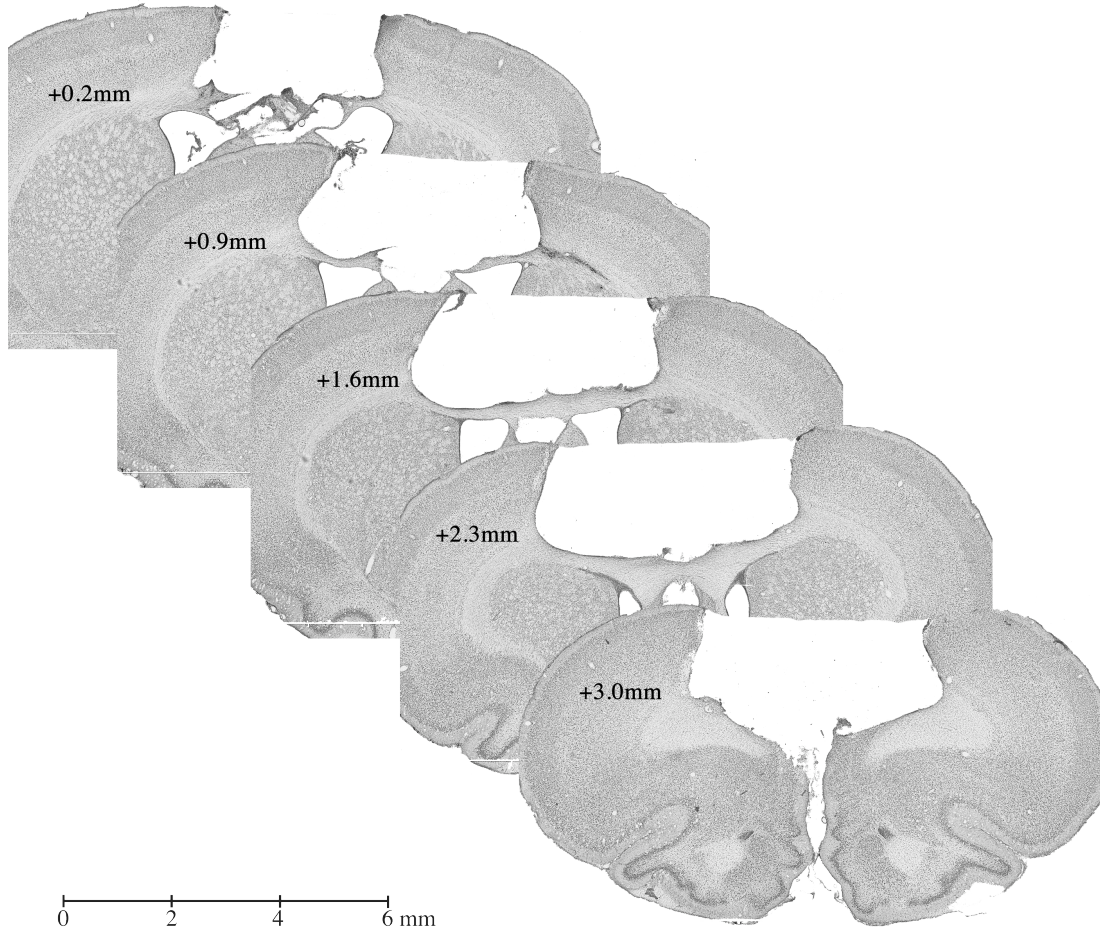
*Figure A1.* Brain sections from smallest lesion (ACC16) of animals in Experiment 1. References are AP from bregma and correspond to injection sites.



*Figure A2.* Brain sections from largest lesion (ACC05) of animals in Experiment 1. References are AP from bregma and correspond to injection sites.



*Figure A3.* Brain sections from smallest lesion (ACC48) of animals in Experiment 2. References are AP from bregma and correspond to injection sites.



*Figure A4.* Brain sections from largest lesion (ACC27) of animals in Experiment 2. References are AP from bregma and correspond to injection sites.

Appendix B

Individual Performances on Weight-Lifting Task in Experiment 2.

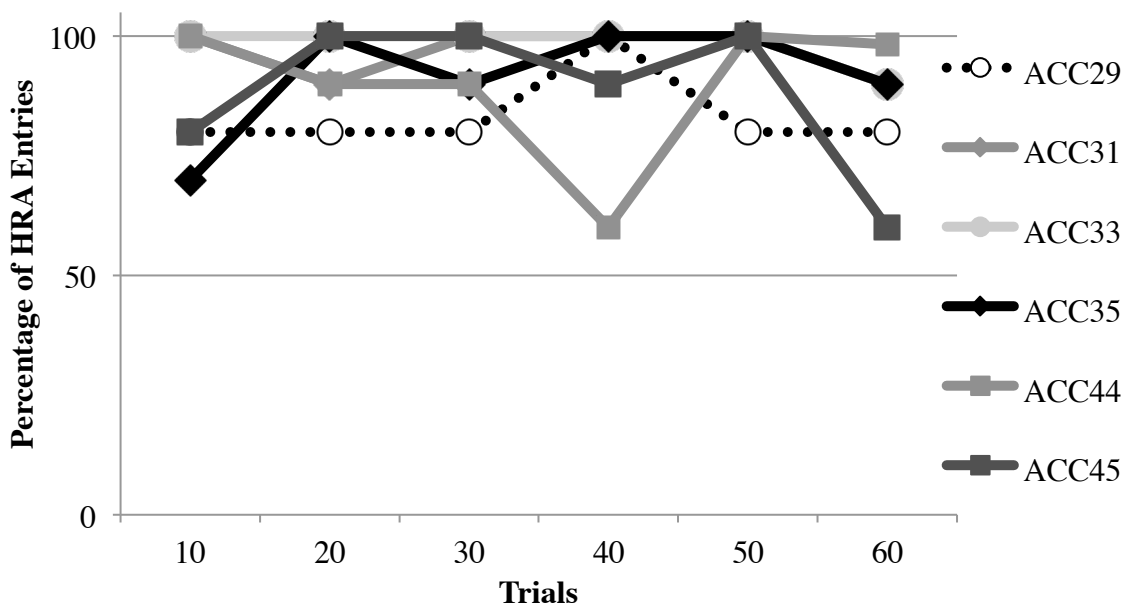


Figure B1. Individual performance curves for weight-lifting task Testing Day 1 in Experiment 2, cohorts 3 and 4, control group.

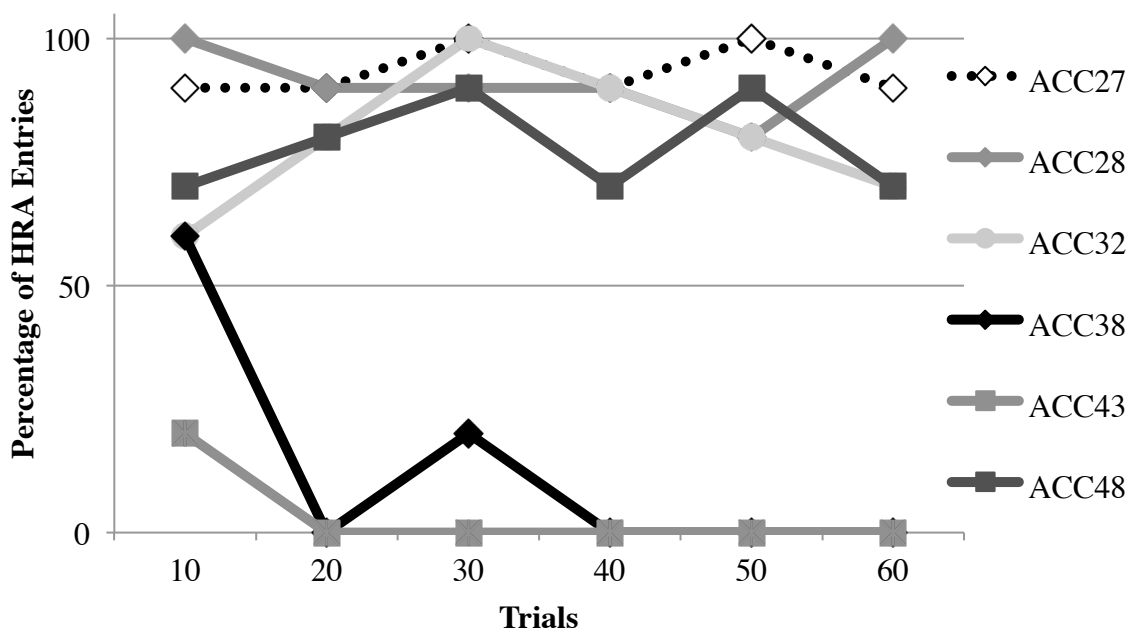


Figure B2. Individual performance curves for weight-lifting task Testing Day 1 in Experiment 2, cohorts 3 and 4, lesion group.

Appendix C

Individual Performances on Courage Task in Experiment 2.

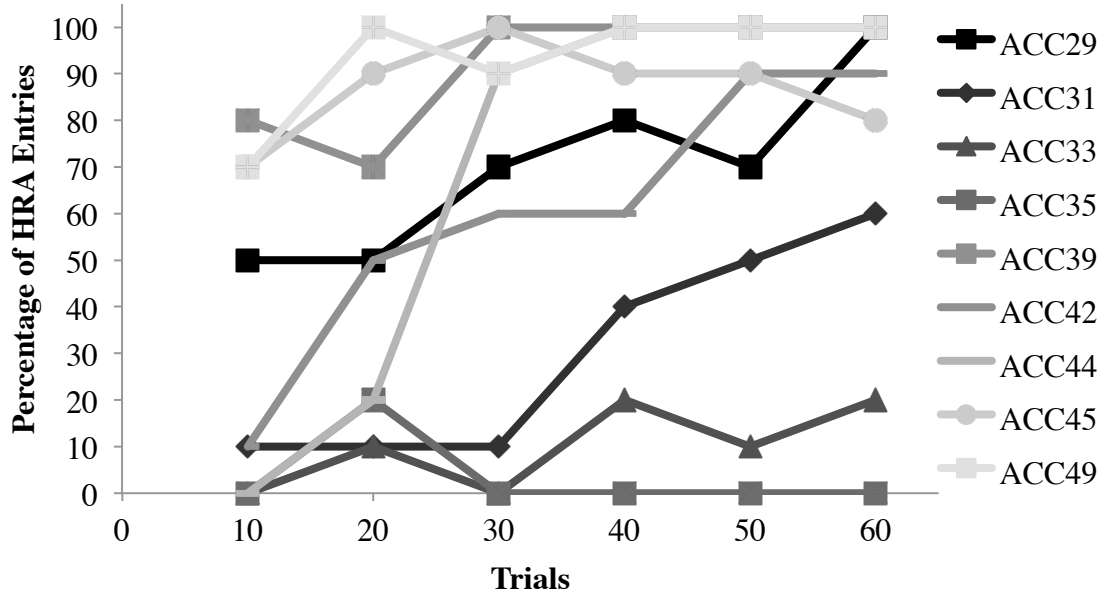


Figure C1. Individual performance curves for courage task in Experiment 2, cohorts 3 and 4, control group.

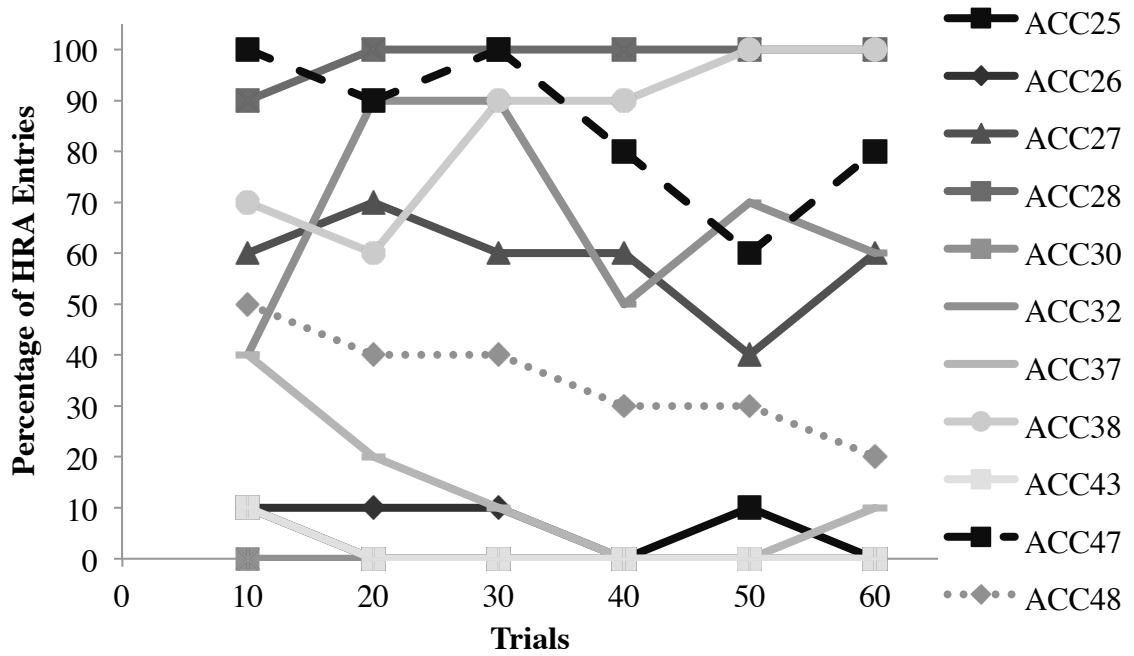


Figure C2. Individual performance curves for courage task in Experiment 2, cohorts 3 and 4, lesion group.