# THE INFLUENCE OF INTER-TRIAL BEHAVIOUR ON DECISION MAKING

**SIENNA RANDOLPH**
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SIENNA RANDOLPH

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|---|---|---|
| Dr. A. Gruber<br>Supervisor | Associate Professor | Ph.D. |
| Dr. M. Mohajerani<br>Thesis Examination Committee Member | Associate Professor | Ph.D. |
| Dr. D. Euston<br>Thesis Examination Committee Member | Associate Professor | Ph.D. |
| Dr. M. Tatsuno<br>Chair, Thesis Examination Committee | Associate Professor | Ph.D. |

**Dedication**

To my cat Aston: Thank you for licking the tears of my face during my breakdowns and staring at me judgementally when I had to pull an all-nighter.

**Abstract**

Animals quickly learn to approach sources of food. Here, we characterize a form of behaviour in which rats made volitional orofacial contact with inactive feeders between trials of a self-paced operant task. This extraneous feeder sampling (EFS) was never reinforced and therefore imposed opportunity and effort costs. EFS decreased during initial training but persisted thereafter. The relative rate of EFS to operant responding increased with either novel changes to the operant chamber, or reward devaluation by pre-feeding. We speculate that this may function to increase exploration when the task is uncertain (early in learning or introduction of novel apparatus components), when the opportunity cost is low, or when the learned sensorimotor solution is compromised. Analysis of sex differences revealed females have a higher propensity for EFS than their male counterparts, further supporting our speculations that EFS is rooted in exploratory systems. Preliminary results suggest the anterior cingulate cortex (ACC) is involved in the expression of this behaviour.

**Acknowledgements**

I would firstly like to thank my amazing supervisor, Dr. Aaron Gruber, for his patience and support during this graduate degree. I would also like to thank the members of my supervisory committee, Dr. Majid Mohajerani and Dr. David Euston, for contributing their valuable expertise and time.

I would also like to thank the members of the Gruber lab. We've stuck together through late nights and early mornings, had lots of great laughs, and consumed countless cups of coffee.

Finally, thank you to my friends and family who have supported me and put up with me throughout this experience. Thank you also to my lovely Rotary family who have always been interested in my work and encouraged me through hard times.

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**List of Abbreviations**

| | |
|---|---|
| ACC | anterior cingulate cortex |
| ANOVA | analysis of variance |
| EFS | extraneous feeder sampling |
| IGT | Iowa Gambling Task |
| ITI | inter-trial interval |
| LE | Long-Evans |
| LS | lose-shift |
| MP | Matching Pennies |
| PBS | phosphate-buffered saline |
| PFA | paraformaldehyde |
| RDT | risky decision-making task |
| RM-ANOVA | repeated-measures analysis of variance |
| R-O | response-outcome |
| SEM | standard error of the mean |
| S-O | stimulus-outcome |
| S-R | stimulus-response |
| TH:Cre | cre-recombinase expressed under the tyrosine hydroxylase promotor |
| UCS | unconditioned stimulus |
| WS | win-stay |
| WSLS | win-stay lose-shift |

## 1. General Introduction

The neural mechanisms underlying flexible decision-making have been debated for more than a century. It has been theorized that the brain operates to optimize collection of resources. Optimal reward collection requires the ability to adjust behaviour based on past reinforcements and to inhibit unproductive actions (Thorndike, 1927). In practice however, both humans and animals produce a variety of non-optimal actions in laboratory tasks (Breland & Breland, 1961; Gruber & Thapa, 2016; Kahneman & Tversky, 1979; Sugrue, Corrado, & Newsome, 2004).

The predominant hypothetical mechanisms underlining how behaviours are acquired are founded in Reinforcement Learning Theory. Reinforcement learning suggests a choice bias that evolves over many trials and encourages choices towards actions that, on average, have yielded more favourable outcomes (Herrnstein, 1961; Rescorla & Wagner, 1972). Work from the Gruber lab among others has challenged this notion, noting that behaviour often does not follow this algorithm.

One important characteristic that Reinforcement Learning Theory does not account for is that decisions are often disproportionately influenced by more recent reinforcements (Ito & Doya, 2009; Skelin et al., 2014). This recency effect is particularly apparent in tasks where reward presentation varies from trial to trial. In these tasks, a win-stay lose-switch (WSLS) response strategy is commonly observed. This strategy encourages staying with the same response following a reinforcement (e.g win-stay, WS), and to shift responses following unexpected reward omission (lose-shift; LS) (Evenden & Robbins, 1984; Kravitz, Tye, & Kreitzer, 2012). WSLS responding has been observed across a variety of tasks and species (Barraclough, Conroy, & Lee, 2004; Frank, Moustafa, Haughey, Curran,

& Hutchison, 2007; Komischke, Giurfa, Lachnit, & Malun, 2002; Rayburn-Reeves, Laude, & Zentall, 2013). Previous research from our lab has further characterized WS and LS responses. We have reported that these two responses have different properties, originate in different areas of the brain, and appear to be in competition with one another (Gruber, Thapa, & Randolph, 2017; Skelin et al., 2014; Wong et al., 2017). Clearly, the Reinforcement Learning framework does not capture the full gambit of animal behaviour.

This thesis focuses on another non-optimal behaviour which does not appear to have been previously reported. Within a competitive binary choice task, rats occasionally ignore task contingencies and shuttle from one reward feeder to the other rather than initiate the next trial. We have termed this behaviour extraneous feeder sampling (EFS). EFS is never reinforced yet this behaviour never fully extinguishes. EFS also strongly affects subsequent choices. Particularly, it triggers a LS response away from the last sampled feeder (Gruber et al., 2017).

This thesis aims to identify the properties and neural mechanisms of EFS. I hypothesized that EFS could arise either from impulsive actions, Pavlovian approach, or intentional exploration. I conducted experiments to test each of these. I also conducted experiments to test the hypothesis that this behaviour will be sexually dimorphic, and that it involves the medial prefrontal cortex. My experiments lead me to conclude that EFS is primarily related to task uncertainty, and that females express it more than do males. The experiments on the medial prefrontal cortex are insufficient to make strong claims as to its role in EFS, although lesions of this brain structure do affect relative EFS rates in some conditions.

This thesis is organized so as to first present the methodologies used in the subsequent set of experiments. I then devote independent chapters showing the effects of devaluation (chapter 3), novelty (chapter 4), and biological sex (chapter 5). I then include a chapter on preliminary findings of the effect of ACC lesions (chapter 6). Each of these data chapters has a focused introduction and discussion, and a general discussion follows in Chapter 7.

## 2. General Methods

### 2.1 Rat Housing Conditions

Housing conditions, training, and testing methods were common to animals from all experiments. Rats were housed in pairs in a transparent plastic cage with corncob bedding and a section of PVC pipe for enrichment. Access to water was restricted to one hour per day during behavioural training and testing but was unrestricted otherwise. The vivarium was maintained at 21°C and 12-h light/dark cycle (lights off at 7:30 pm). Experimenters handled the rats daily for one week before the beginning of training. All experimental procedures were approved by the University of Lethbridge Animal Welfare Committee and adhere to the guidelines of the Canadian Council on Animal Care.

### 2.2 Matching Pennies Task

To assess reward-based choice behaviour, the Gruber lab utilizes a binary choice task with unpredictable rewards (Skelin et al., 2014). With this task, called Matching Pennies (MP), we are able to assess sensitivity to wins or losses, motivation, and feeder approach behaviour.

MP training and testing took place in six identical custom-built aluminum boxes (26 X 26 cm). Each box contained two cue lights mounted proximally above a nose-poke port and two liquid delivery feeders on either side (separated from each other by 14 cm) (Fig. 1A). Infrared emitters and sensors in the feeders and central port detected animal entry. Following the illumination of the cue lights, the rats poked their snout into the central port to initiate a trial and then responded by locomoting to one of the two feeders (Fig. 1B). A 13-cm-long aluminum barrier orthogonal to the wall separated each feeder from the

central port. This added a choice cost and reduced choice bias originating from body orientation. Control of the behavioural task was automated with a microcontroller (Arduino Mega, Italy) receiving commands via serial communication from custom software on a host computer. We reduced acoustic startle from sounds outside of the testing chamber by presenting constant background audio stimuli (local radio station).

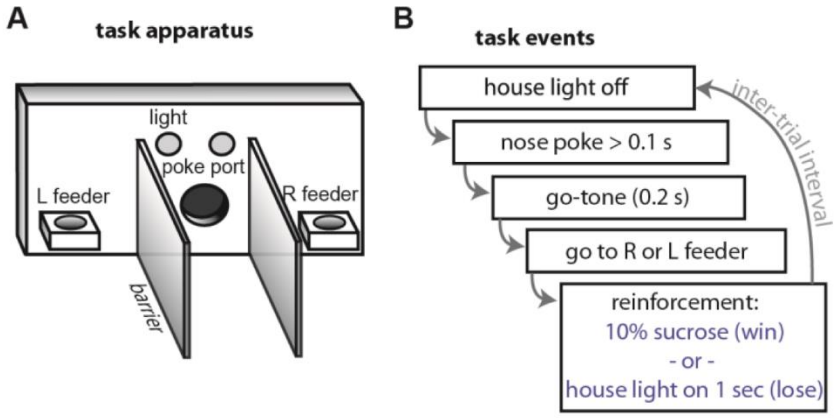


Figure 1. Matching Pennies task apparatus and operant response sequence. (A) Schematic representation of the operant chamber. (B) Flow chart of a valid operant response sequence. A nose-poke into the poke port initiates a trial and is confirmed by an auditory go-tone. The rat then locomotes to one of the two feeders and received reinforcement on the choice: the presentation of 10% sucrose in the feeder for a win or the illumination of the house light to signify a loss.

All animals were trained on MP by gradually shaping components of the task. Initially, there were no barriers between the central port and feeders. Each trial of the task began with the illumination of the two cue lights. At this stage, the animals discovered that every poke port entry and a subsequent entry to either feeder within 15 s resulted in a reward of 60 µL of 10% sucrose solution. Once rats performed 150 trials (typically in the first session), the stage was considered complete. In the following stage, feeder entry (following a nose-poke) was rewarded with a probability of 0.5 regardless of previous responses. Subsequent stages used the competitive algorithm (described below). A barrier separating

the nose-poke port and feeders was increased in discrete lengths (4, 8, and 13-cm) over several sessions (typically 4-5). The training was complete when the animals performed at least 150 trials with the 13-cm barrier within the 45-min session over two consecutive days (typically 7-10 training sessions in total).

A computer program served as a competitor for the rats and was implemented as in previous studies (Barraclough et al., 2004; Gruber & Thapa, 2016; Lee, Conroy, McGreevy, & Barraclough, 2004; Skelin et al., 2014). The algorithm attempts to predict the rat's next choice by comparing the pattern of choice sequences in the preceding trials (1-4 back) with the choice history of the current session. If any of the patterns occurred more likely than chance (computed by the binomial test), the algorithm baited the least likely feeder to be selected on the current trial. If no pattern was detected, the rewarded side was picked randomly. The optimal response policy of the rat is to choose randomly on each trial and disregard reinforcements. The statistical power of the algorithm to detect patterns is initially very weak, and so the rewarded feeder is selected randomly for the first several trials of each session.

### 2.3 Behavioural Analysis

We quantify several behavioural measures in MP. Of importance for this thesis, EFS was defined as the trials where the animals sampled both feeders after making an entry into the poke port (Fig. 2).

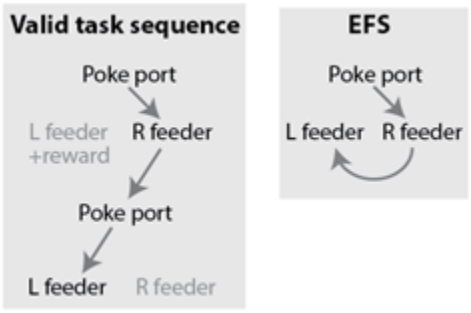


Figure 2. Schematic representation of a valid task sequence vs EFS. Rats sometimes chose to locomote from one feeder to the other without committing a nose poke; we term this extraneous feeder sampling (EFS).

The probability of lose-shift was calculated as the probability that the rat would shift feeder choice in the consecutive trial following reward omission. Likewise, the probability of win-stay was calculated as the probability that the rat would repeat the selection of the same feeder on trials immediately following rewarded trials. The number of trials represents the total number of complete operant trials within a session. Only sessions with more than 100 trials were included in the analysis. The calculation of the percent of rewarded trials represents the percentage of all complete trials in which the rat was reinforced with sucrose. Response time measures the time taken to reach the feeder after the exit of poke port, whereas inter-trial interval (ITI) was defined as the time between the first exit of the reward feeder and the next entry into the poke port. Data were analyzed with MATLAB (version R2013a; MathWorks, MA, USA) and SPSS (version 21.0; IBM, NY, USA).

## 3. Devaluation of Task Reward

### 3.1 Introduction

In Pavlovian conditioning paradigms, individual differences arise in how learned associations are expressed. Some individuals preferably interact with a signal of reward in what is called a sign-tracking response, whereas others interact with the location of the reinforcer, such as a feeder, in a goal-tracking response (Boakes, 1977; Farwell & Ayres, 1979; Patitucci, Nelson, Dwyer, & Honey, 2016; Robinson & Flagel, 2009). In 1977, R.A. Bloakes introduced reward omission conditions in a study of goal-tracking behaviours. His omission contingencies were effective in reducing the frequency of the goal-tracking response, although it rarely eliminated them (Boakes, 1977). We have previously reported a similar pattern in EFS behaviour. EFS behaviour never fully diminished despite the lack of any positive reinforcement, and occurred in control animals about a quarter of their trials even after extended training (Gruber et al., 2017). While this supports the hypothesis that EFS is a form of Pavlovian conditioned approach, we sought to evaluate this possibility more thoroughly.

Goal-tracking behaviours are guided by endogenous information such as motivational states and are sensitive to changes in the valuation of reward (Ernst, Romeo, & Andersen, 2009). As such, goal-tracking is reduced by outcome devaluation since this decreases the motivational power of the reward (Hammerslag & Gulley, 2014; Morrison, Bamkole, & Nicola, 2015). We would expect our EFS phenomena to be comparably sensitive to devaluation if it involves a Pavlovian goal-tracking component.

## 3.2 Methods

### 3.2.1 Subjects

This study involved 2 cohorts of Long-Evans (LE) rats ($n$ = 78 total animals). Cohort 1 consisted of 30 male LE rats (Charles River, Saint-Constant, QC, Canada) weighing between 350 and 450 g (postnatal day 88-106) at the beginning of behavioural testing. Cohort 2 consisted of 14 male and 5 female wild-types LE rats, and 14 males and 15 female LE rats expressing cre-recombinase under the tyrosine hydroxylase (*TH:cre*) born on site and weighing between 200 and 600 g (postnatal day 75-116) at the time of behavioural testing.

### 3.2.2 Devaluation Procedure

Rats were trained on MP was as described above. Once all subjects met the training criterion, rats were divided into three groups. Individuals of each group received free access to a limited amount of the reward (sucrose solution) 20 minutes prior to the start of MP. The amount of pre-feeding was counterbalanced among rats so that an approximately equal number of rats received each of the three pre-feeding volumes (0, 5, 10 ml) each testing day. The volume given to each group rotated each of three consecutive days so that each rat had received one of the three levels prior to behavioural testing.

### 3.2.3 Statistical Analysis

Repeated-measures analysis of variance (RM-ANOVA) was used to assess the effect of reward devaluation on behavioural measures ($p < 0.05$). Where the assumption of sphericity was violated, a Greenhouse-Geisser correction was applied.

### 3.3 Results

In order to discern if EFS is promoted by the motivation for the reward, as would be expected by phenomena driven by Pavlovian systems, we conducted a devaluation experiment in Cohort 1 after 12 sessions of training. Animals were allowed to drink a fixed amount of liquid sucrose prior to the task, in a counterbalanced design. This should decrease EFS if it is promoted by the motivation for the outcome. Pre-feeding decreased the number of trials completed in a volume-dependent manner (RM-ANOVA, main effect: $F_{2,46} = 35$, $p = 1.00E\text{-}10$; Fig. 3A), but had no effect on the number of trials with EFS ($F_{2,46} = 2.4$, $p = 0.10$; Fig. 3B). Thus, the relative rate of EFS to operant responses *increased* with devaluation (RM-ANOVA with Greenhouse-Geisser correction: $F_{1.9,43} = 6.7$, $p = 0.003$; Fig. 3C). This was unexpected, and we wanted to test whether this could be an artifact of an unplanned factor within our control. We, therefore, replicated the experiment under conditions of the increased variance of originally unplanned factors. The replication was conducted by new investigators (female instead of male), at a different time of year, and with a new heterogeneous group of rats (Cohort 2; $n = 48$) that included male LE ($n = 14$), female LE ($n = 5$), transgenic female LE ($n = 15$) and transgenic male LE ($n = 14$) with an inert transgene. This cohort was bred in our facility, whereas Cohort 1 was shipped from a commercial breeder. Despite these changes, the results were remarkably similar to the first devaluation experiment. Devaluation again decreased trial completion ($F_{1.6,43.8} = 51.0$, $p = 1.00E\text{-}6$; Fig. 3D) but not EFS ($F_{2,50} = 1.0$, $p = 0.36$; Fig. 3E), yielding an increased relative rate of EFS ($F_{1.64,41.0} = 8.0$, $p = 0.002$; Fig. 3F). Note that the rate of EFS is higher in this group (Cohort 2) as compared to that in Cohort 1 because they had fewer training sessions

prior to the devaluation. These data provide strong evidence that EFS is a robust phenomenon independent of outcome valuation.

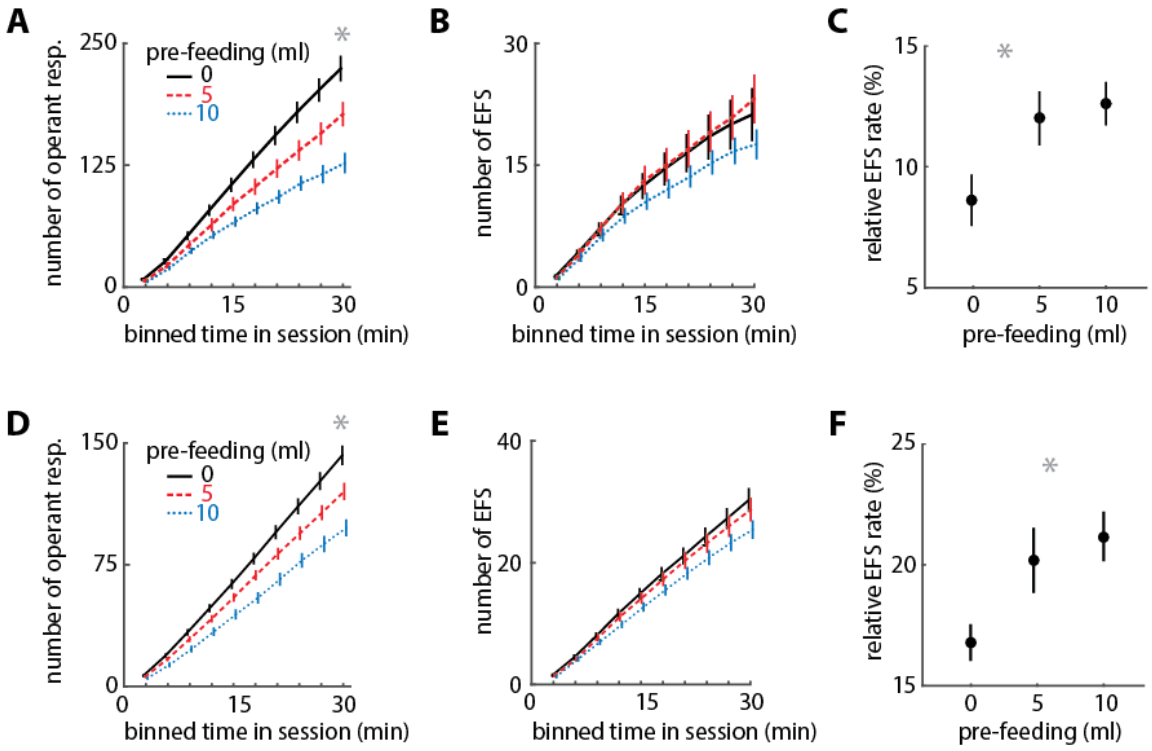


Figure 3. Effect of devaluation on task performance. (A) Mean cumulative sum of operant responses (nose-poke to feeder) in bins of time within a session (Cohort 1: n = 30 rats in panels A-C). Pre-feeding rats 20 minutes before the task reduced the number of trials performed. (B) The mean cumulative sum of EFS events in the same sessions, which was not reduced by pre-feeding. (C) The mean relative rate of EFS/trials for each pre-feeding level, showing an increase with devaluation. (D-F) Same plots as above for a new heterogeneous cohort collected by different experimenters (Cohort 2: n = 48 in panels D-F), showing replication of the devaluation effects. Error bars indicate standard error of the mean, and asterisks '*' indicate group means that were significantly different from the comparison group ($p < 0.003$). Adapted from "Feeder approach between trials is increased by uncertainty and affects subsequent choices" by A.J. Gruber, R. Thapa, & S.H. Randolph, 2017, eNeuro, 4(6).

### 3.4 Discussion

In Pavlovian conditioned approach, animals learn to approach sites of reward administration despite not being required for reward delivery. (Boakes, 1977; Farwell & Ayres, 1979; Robinson & Flagel, 2009). Pavlovian-driven behaviours have resulted in unproductive behaviours. For instance, pigeons will peck at a stimulus (a Pavlovian sign-tracking-driven action) rather than collect reward via instrumental responding (Williams & Williams, 1969). Expression of goal-tracking behaviours can be reduced by reward devaluation and also have been shown to decrease, but rarely eliminate, following omission contingencies (Boakes, 1977; Morrison et al., 2015). While EFS does persist despite never being reinforced, we repeatedly demonstrated that EFS is insensitive to devaluation. We speculate that these inconsistencies may be an indication that the goal-tracking and sign-tracking responses are in competition for behavioural control in EFS.

Although there are no explicit discriminative stimuli predicting reward delivery in our task, we cannot rule out the formation of associative learning involving implicit stimuli. These could involve stimulus-outcome (S-O) or response-outcome (R-O) contingencies when the rat is reinforced at the feeder. Indeed, the use of multiple outcomes and lack of discriminative stimuli promote R-O and/or S-O control (Holland, 2004). It is possible that rats break the operant response into multiple components. If one of these represents entry of the lane to the feeder, it is possible that the R-O of this portion gains strength during training. However, this suggests the EFS should increase with training, whereas the data reveal that it decreases. Alternately, the feeder could have gained incentive salience because it is the most proximal conditioned stimuli (CS) to the unconditioned stimuli (UCS, i.e., sucrose). Rats, therefore, may be motivated to make an EFS response due to Pavlovian

(S-O) attraction to stimuli proximal to the UCS. The main problem with such an interpretation is the fact that the absolute rate of EFS trials was not reduced by the devaluation of the outcome via pre-feeding in either of two distinct cohorts. These data suggest that EFS is driven by associations other than R-O or S-O and is unlikely to be primarily governed by Pavlovian associations.

## 4. Introduction of Task Novelty

### 4.1 Introduction

When there is uncertainty in a task, optimal choice theory dictates that exploitative actions should be interspersed with some exploratory actions to gain information (Daw, O'Doherty, Dayan, Seymour, & Dolan, 2006; Kakade & Dayan, 2002; Staddon & Motheral, 1978). Exploration allows for discovery of better reward sources or shortcuts to obtain known sources. Novel objects are often approached and explored in many species including rodents, humans, primates, and birds (Bronson, 1972; Menzel, 1968; Stryjek, Modlińska, & Pisula, 2012; Verbeek, Drent, & Wiepkema, 1994).

Rat behaviour is sensitive to even minute changes in laboratory tasks. Different scents, lighting or other sensory stimuli can affect behaviour (Alstott & Timberlake, 2009). To induce uncertainty about task contingencies, the MP task apparatus was manipulated by inserting novel hallway lengths mid-session. If EFS is an exploratory behaviour, we expect that introduction of novelty and uncertainty will promote exploration and increase EFS propensity.

### 4.2 Methods

#### 4.2.1 Subjects

This study involved male LE rats ($n$ = 16) born on site and weighing between 400 and 600 g (postnatal day 75-93) at the time of behavioural testing.

### 4.2.2 Mid-Session Hallway Switch

Training on MP occurred as described above. We then allowed the rats to perform the task for 100 trials with their customary 13 cm barrier separating the nose-poke from the feeders. We then took the rats out of the box and replaced the barrier with a longer one (21 cm), a shorter one (8 cm), or one the same length. Rats were then placed back in the box and allowed to perform an additional 100 trials. The order of the novel hallways was counterbalanced among rats so that an approximately equal number of rats received each length of novel hallways (8, 13, or 21 cm) each testing day. The novel hallway length given to each group rotated each of three consecutive days so that each rat had received all three possible hallway manipulations.

### 4.2.3 Statistical Analysis

To analyze the effect of the mid-session hallway shift, data was binned into eight bins of 20 trials each to account for 160 trials (100 pre-switch and 60 post-switch). The bins were then plotted against each of the task variables using MATLAB. RM-ANOVA was used to assess the significance of a mid-session hallway change on behavioural measures ($p < 0.05$).

## 4.3 Results

We tested if uncertainty would affect the relative EFS rate. We allowed rats to perform the task for 100 trials with their customary 13 cm barrier separating the nose-poke from the feeders. We then replaced the barrier with a longer one, a shorter one, or one the same length and allowed them to perform an additional 100 trials. The relative EFS rate

increased for either novel barrier length as compared to the familiar one (RM-ANOVA, time*barrier: $F_{14,294} = 3.34$, $p = 1.00E\text{-}5$; Fig. 4). These data indicate that EFS is not related to the effort of circumnavigating the barriers because we would then expect a monotonic length-EFS relationship rather than a parabolic one. These results indicate that a change in the apparatus is sufficient to transiently increase EFS, suggesting that EFS is promoted by uncertainty about the task or apparatus.

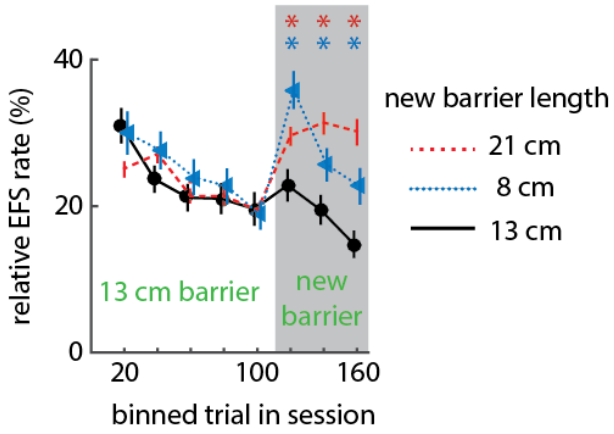


Figure 4. Effect of mid-session change in the barrier on EFS rate. Mean relative EFS rate within a session before and after the barrier was replaced at trial 101 ($n$ =16). Replacing the barrier with either a longer (red dashed line) or shorter (blue dotted line) barrier increased EFS as compared to replacing with the same length barrier (black solid line). Asterisks '*' indicate a significant difference of means by post-hoc analysis ($P$< 0.04). Adapted from "Feeder approach between trials is increased by uncertainty and affects subsequent choices" by A.J. Gruber, R. Thapa, & S.H. Randolph, 2017, eNeuro, 4(6).

### 4.4 Discussion

Our results show that EFS appears to increase at time of less certainty of the task: initial training, the beginning of sessions, and following a switch of barriers. We argue that the natural environment involves sufficient variability in such a large state space that animals will always face some level of uncertainty about features pertinent to survival. We

speculate that the rodent brain may, therefore, have evolved a system that promotes exploration for foraging, particularly at times of uncertainty or when opportunity costs are low. Moreover, the neural systems promoting exploration may be inhibited as those that promote exploitative actions gain associative strength. This would account for the reduction of EFS with training.

## 5. Sex Differences in Exploratory Behaviour

### 5.1. Introduction

Men and women sometimes differ in the way they use past rewards to guide future choices. Both rodent and human studies of decision-making have revealed many task-specific disparities due to sex (Becker, Perry, & Westenbroek, 2012; Byrnes, Miller, & Schafer, 1999; Cross, Copping, & Campbell, 2011; Jentsch & Taylor, 2003; Orsini, Willis, Gilbert, Bizon, & Setlow, 2016). Sex differences have also been reported in Pavlovian approach behaviour. Research has shown that females are quicker to acquire Pavlovian approach tasks and are also slower to extinguish this behaviour (Hammerslag & Gulley, 2014; Pitchers et al., 2015). Additionally, females are less sensitive to reward devaluation than their male counterparts (Hammerslag & Gulley, 2014).

Past research utilizing a variety of tasks has consistently demonstrated female rats display more exploratory behaviour than males (Alstott & Timberlake, 2009; Johnston & File, 1991; Lynn & Brown, 2009; Nasello, MacHado, Bastos, & Felicio, 1998; Ray & Hansen, 2004). Female rats also show more exploratory behaviour in the novel object recognition task (Sutcliffe, Marshall, & Neill, 2007). We have reported a non-significant trend for females to traverse from the nose-poke port to the feeder well faster than their male counterparts (Donovan et al., 2018). We would expect the sex disparities of EFS to parallel its underlying control system – be it Pavlovian approach or an exploratory behaviour in reaction to novelty.

## 5.2 Methods

### 5.2.1 Subjects

We collected behavioural data from 106 rats in three separate cohorts. Each cohort contained both male and female animals. Animals that did not complete at least 150 trials in the testing session were removed from analysis. This exclusion criteria left us with data from three cohorts consisting of: Cohort 1: 28 Long Evans (15 male, 13 female, 71-117 days old); Cohort 2: 23 Long Evans rats (17 male, 6 female, 80-103 days old); and Cohort 3: 28 Long Evans rats expressing a transgene in some cells (Cre+; 13 male, 15 female; 71-112 days old). The animals from Cohort 3 expressed a transgene (Cre-recombinase) under the control of the Tyrosine Hydroxylase promoter but had no other manipulations. This transgenic cohort was not statistically different from the others, so their data were pooled with the other cohorts, giving a total of 79 animals (45 male, 34 female) in the study.

### 5.2.2 Behavioural Procedure

Training and testing on MP was as described above.

### 5.2.3 Session-Averaged Analysis

The potential effects of the transgene on measures in our task were analyzed using a one-way ANOVA. Finding no significant differences, the data was then pooled across all the animals and two-tailed t-tests ($\alpha = 0.05$) were performed to compare sex differences in session-averaged behavioural measures. When measures exhibited unequal variance according to Levene's test, ($\alpha = 0.05$), Welch's t-test was used with the Welch-Satterthwaite equation to approximate the degrees of freedom (Hall & Willink, 2001).

### 5.2.4 Correlation Analysis

Correlation analysis was performed to study the relationship between session-averaged EFS propensity, grouped by sex, with weight and age. EFS propensity grouped by sex was plotted against weight and age, and linear regression was performed using MATLAB on rats in Cohort 3. Pearson correlation coefficients were calculated and checked for significance at $\alpha = 0.05$ using MATLAB. A two-way ANOVA was used in SPSS to test for sex*postnatal age interaction effects after data was binned into two groups according to age.

### 5.2.5 Within-Session Analysis

To compare the change in behaviour within a session, data was binned into eight bins of five minutes each to account for 40 minutes of the 45 minute session. The final five minutes of each session were not included to avoid the potential confound of animals being distracted by the experimenter returning to the room. Of the 79 animals included in the study, two did not complete at least a 40 minute session and were excluded from this within-session analysis. The bins were then plotted against each of the task variables using MATLAB. A mixed model ANOVA was performed to compare within group (time bin) and between group (sex) variables. The Greenhouse-Geisser correction was applied to all the measures on the mixed model ANOVA, as they violated the assumption of sphericity.

### 5.3 Results

We found that female rats engaged in EFS more often than males ($t_{52.6} = 2.60$, $p = 0.012$; Fig 5A). Moreover, EFS was higher in females regardless if rats received a reward in the trial ($t_{53.1} = 2.55$, $p = 0.014$; Fig. 5B) or not ($t_{77} = 2.37$, $p = 0.021$; Fig. 5C). Therefore, female rats approach the feeder outside of the task sequence more than males, regardless of reward outcome.

Analysis of lose-shift across all trials (including those after EFS) showed that males were more likely to lose-shift than females (lose-shift: $t_{77} = 2.16$, $p = 0.034$; Fig. 5D). There was no significant difference between the sexes in their probability to win-stay ($t_{77} = 0.103$, $p = 0.918$). However, to further test for a confounding role of EFS, we tested for sex-based differences exclusive of EFS by analyzing only those trials that did not follow EFS. This exclusion resulted in rejection of 4,669 out of 19,073 trials. By eliminating these EFS-preceded trials, we found no significant sex difference in lose-shift ($t_{77} = 1.77$, $p = 0.081$; Fig. 2E) or win-stay ($t_{77} = 0.302$, $p = 0.764$; Fig. 5F). Thus, the observed increased EFS in females was exerting a confounding effect and causing an apparent decrease in lose-shift responding.

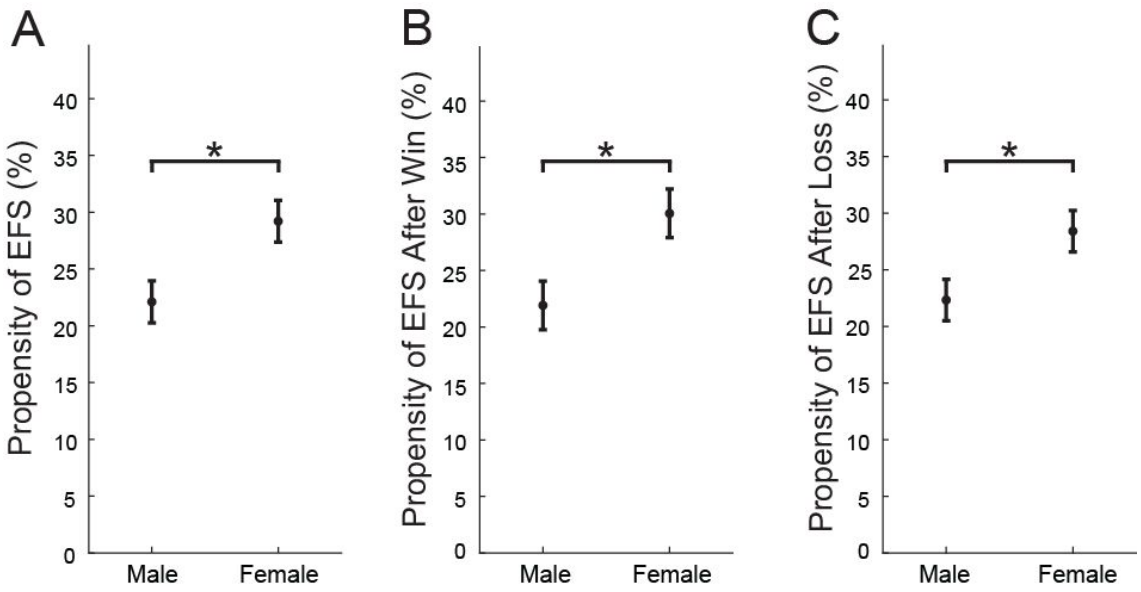


Figure 5. Sex differences in behaviour. (A) The propensity of rats to sample both wells within one trial (EFS) was significantly greater in females. The propensity of rats to sample both wells within one trial following (B) reward and (C) reward omission were both significantly greater in females. Error bars indicate standard error of the mean and asterisks '*' indicate statistically different means as determined by the two-tailed t-test ($p < 0.05$). Adapted from "Sex differences in rat decision-making: The confounding role of extraneous feeder sampling between trials" by C.H Donovan, S.A. Wong, S.H. Randolph, R.A. Stark, R.L Gibb, & A.J. Gruber, 2018, Behavioural Brain Research, 342.

The probability of lose-shift and win-stay responding on this task depend on the inter-trial interval (ITI, the duration between reinforcement and the subsequent trial). Specifically, we have previously shown probability to lose-shift follows a log-linear negative relationship with increasing ITI, reaching chance levels beyond 7 seconds. Conversely, win-stay follows a log-parabolic relationship with ITI, with the highest probability to lose shift at approximately an 8 second ITI (Gruber & Thapa, 2016). This indicates that the speed of the animal to complete trials should have an effect on the likelihood of shifting choice after a loss or a win. Excluding trials following EFS, the mean ITI following wins ($t_{77} = 1.62$, $p = 0.101$; Fig. 6A) or losses ($t_{77} = 0.849$, $p = 0.398$; Fig.

6B) was not different between sexes. There was however, a non-significant trend for the male rats to have a slower response time (the time in going from the nose-poke to the reward feeders) ($t_{77} = 1.83$, $p = 0.070$; Fig. 6C). These data indicate that there may be differences in movement speed on the task, which could affect choice. We suspect this difference is likely due to differences in body size and weight, rather than a difference in motivational drive.

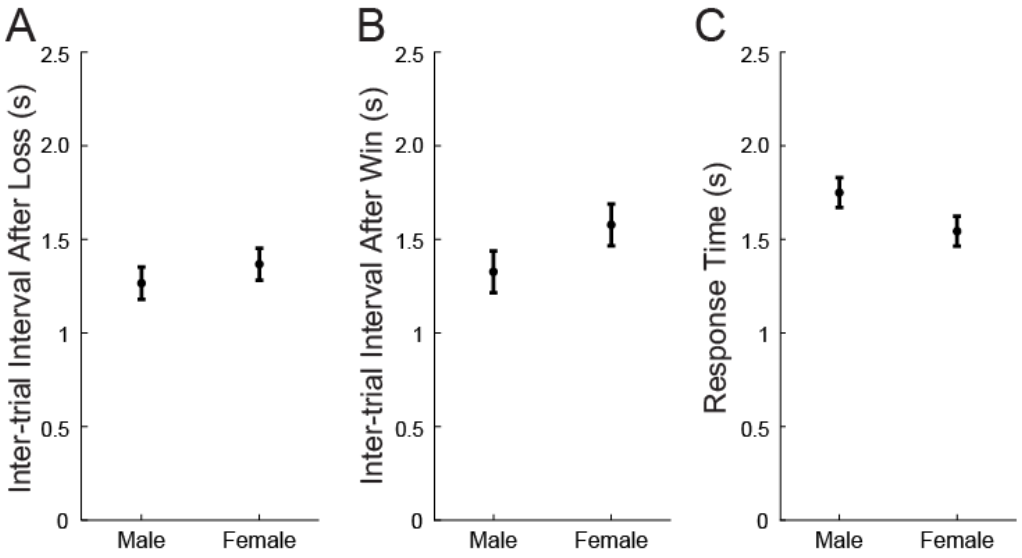


Figure 6. Sex differences in motivation and motoric speed on the choice task. (A) The time interval from loss reinforcement to the next nose-poke did not differ between sexes. (B) The time to start a new trial following a win did not vary between sexes. (C) There was a non-significant trend for female rats to be faster than males in their locomotion to the feeder well following trial initiation. None of the tested means were significantly different, as determined by the two-tailed t-test ($p < 0.05$). Error bars indicate standard error of the mean. Adapted from "Sex differences in rat decision-making: The confounding role of extraneous feeder sampling between trials" by C.H Donovan, S.A. Wong, S.H. Randolph, R.A. Stark, R.L Gibb, & A.J. Gruber, 2018, Behavioural Brain Research, 342.

To determine if weight or age were confounding factors in session-averaged EFS propensity, correlational analysis was performed. There was no significant correlation between EFS propensity and postnatal age for either males ($r = 0.470$, $p = 0.077$; Fig.7A) or females ($r = -0.061$, $p = 0.830$; Fig. 7A). There was no significant interaction effect between sex and postnatal age ($F_{1,26} = 0.949$, $p = 0.339$). There was also no significant correlation between EFS propensity and weight at the time of data collection for females ($r = -0.260$, $p = 0.297$; Fig. 7B). While not significant, there is a trend for EFS propensity to increase with increasing body-weight in male rats ($r = 0.462$, $p = 0.083$; Fig. 7B). This relationship among weight and EFS propensity does not account for significantly higher levels found in females (because of their *lower* weight) and suggests the EFS is a result of other sexually dimorphic factors. However, this analysis has low statistical power and would benefit from further consideration with a larger sample size.

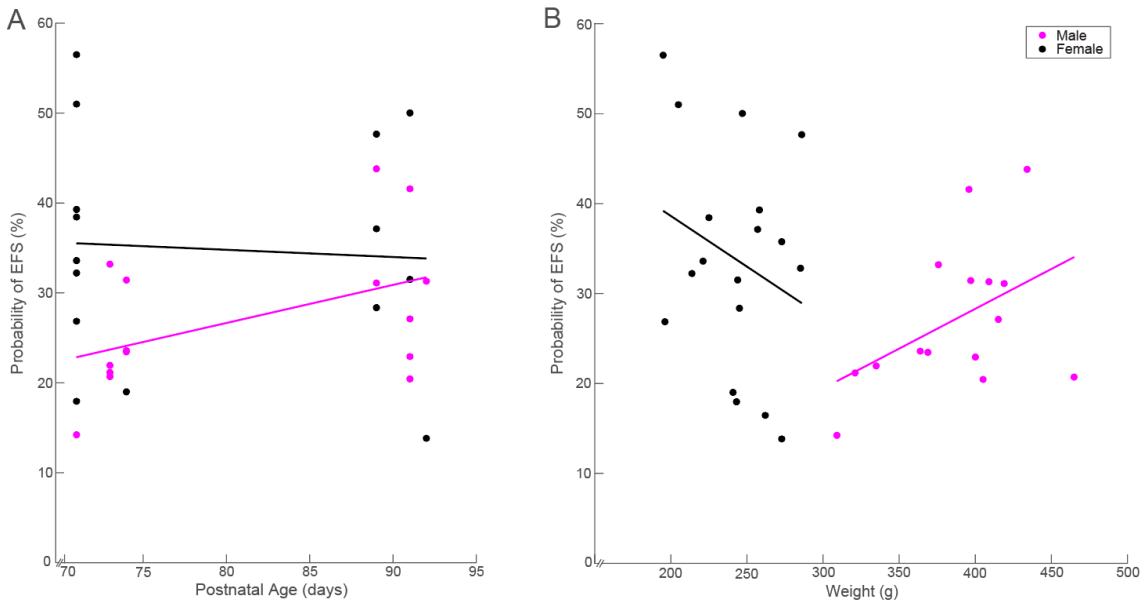


Figure 7. EFS correlations with age and weight. (A) The probability of sampling both feeders between trials showed no significant correlation ($\alpha = 0.05$) with postnatal age for either sexes. No significant postnatal age*EFS propensity interaction effect was found using a two-way ANOVA. (B) The probability to sample both wells between trials also showed no significant correlation ($\alpha = 0.05$) with weight for either sexes.

These sex-based disparities appear to be primary differences in decision-making and not artifacts of performance or motivation. However, motivation does change within the session as animals become sated. Because males and females differ in weight and calorie consumption (Wade, 1972), it could be that their motivation level changes differently during the session. For instance, females could become sated more quickly and therefore become less sensitive to reward omission as the session progresses. We investigated this by quantifying the dependent response variables in bins of time during the session. The session was broken into eight time bins of five minutes each and a RM-ANOVA was used to test for statistical significance of the means. We found that females performed significantly more EFS throughout the session (Main effect: $F_{1,75} = 7.83$, $p =$

0.007; Fig. 8A). In order to eliminate the possible confounding role of EFS on other response variables, we eliminated the trials following EFS for subsequent analysis. There was no significant main effect due to sex on the number of trials completed ($F_{1, 75} = 1.342$, $p = 0.250$; Fig. 8B), or the number of rewarded trials over the time bins ($F_{1, 75} = 3.01$, $p = 0.087$; Fig. 8C). These data suggest that motivation is not different between sexes within a session. There was no significant interaction effect between sex and time bins on any of the dependent response variables (Probability of EFS: ($F_{4.50, 338} = 1.342$, $p = 0.250$), Number of Trials ($F_{4.86, 365} = 0.970$, $p = 0.435$), Rewarded Trials ($F_{5.79, 434} = 0.562$, $p = 0.755$)). The response trends are stable within sessions and are consistent with the univariate analysis on this data collapsed over the session presented above.

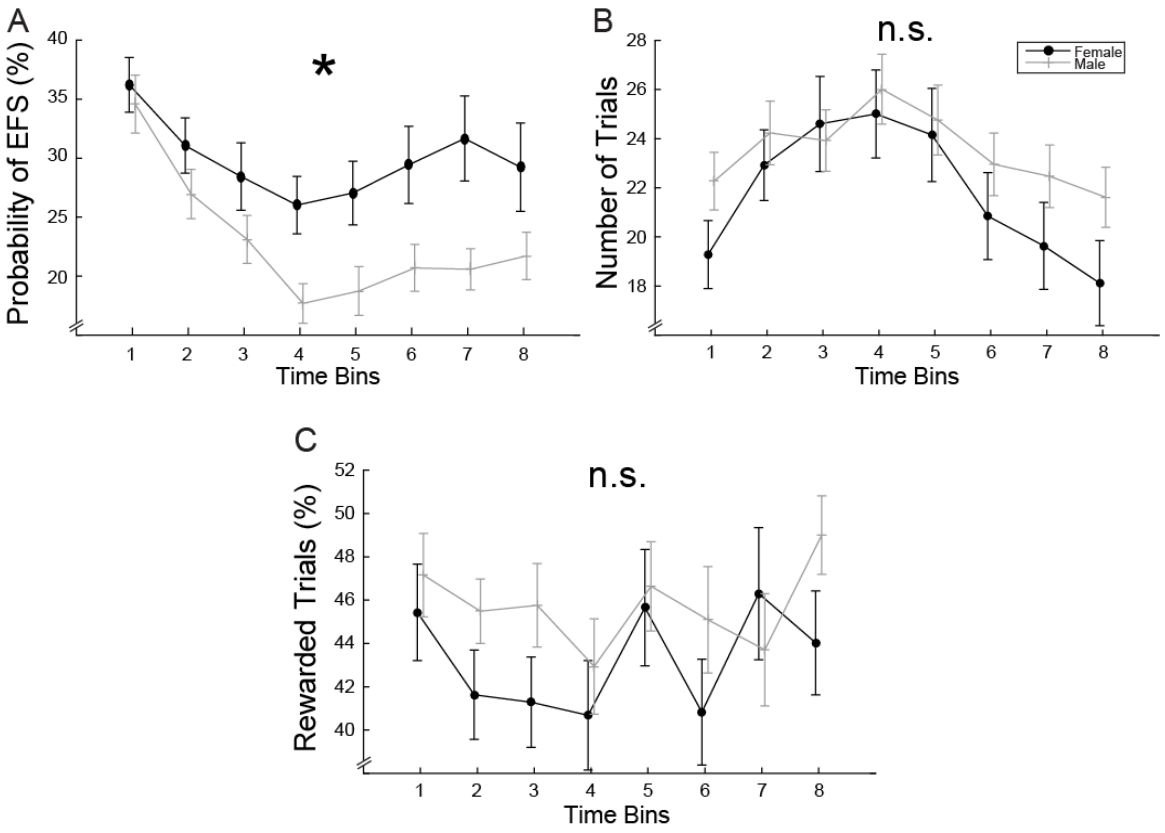


Figure 8. Sex differences in within-session task performance. (A) The probability to sample both feeders between trials decreased as sessions progressed, but females exhibited a higher rate of this behaviour throughout the session. There was no significant difference between the sexes in (B) the number of trials completed over the time bins or (C) on the rats' percentage of rewarded trials during the session. Trials following the rats sampling both wells were excluded in panels B and C. '*' indicates a significant main effect of sex by RM-ANOVA ($p < 0.05$); 'n.s.' indicates no significance. There were no significant interaction effects between sex and time bins for any of the dependent response variables. Error bars indicate standard error of the mean. Adapted from "Sex differences in rat decision-making: The confounding role of extraneous feeder sampling between trials" by C.H Donovan, S.A. Wong, S.H. Randolph, R.A. Stark, R.L Gibb, & A.J. Gruber, 2018, Behavioural Brain Research, 342.

### 5.4 Discussion

Previous rat research has produced inconsistent evidence of sex differences in choice behaviour. The females' increased propensity of EFS in the present data may be interpreted as a result of females seeking reward following losses more often than males. However, we found this increase to be independent of whether the animal was rewarded or not, and we did not find females to have an increased probability of lose-shift responding. This leads us to suggest that EFS is not an immediate result of reward omission in female rats. It is also possible that their increased EFS propensity is more indicative of a lack of effortful control. In humans, however, a substantial meta-analysis examining sex differences in impulsivity (Cross et al., 2011) found no differences in effortful control between men and women. Furthermore, past rodent studies utilizing the delay discounting task, a typical measure of effortful control, have found no baseline sex differences on the task (Eubig, Noe, Floresco, Sable, & Schantz, 2014; Lukkes, Thompson, Freund, & Andersen, 2016; Smethells, Swalve, Eberly, & Carroll, 2016). We also altered the length of the barriers separating the reward feeders from the nose-poke port; if EFS was related to motoric effort, we would expect its propensity to decrease with increasing barrier length. However, rate of EFS increased regardless of an increase or decrease in barrier length (Gruber et al., 2017). Thus, these data suggest this sex difference in EFS is not due to sex-based differences in choice behaviour following reward omission, differences in effortful control, or differences in motoric effort.

We believe that EFS is most indicative of exploration; the sampling of the opposing feeder outside of the task context may be the rodent's attempt to gain more information and explore the environment. Past research utilizing a variety of tasks has consistently

demonstrated female rats display more exploratory behaviour than males (Alstott & Timberlake, 2009; Johnston & File, 1991; Lynn & Brown, 2009; Nasello et al., 1998; Ray & Hansen, 2004). Although these exploration tasks are confounded by increased anxiety in the rat, female rats also show more exploratory behaviour in the novel object recognition task: likely a more valid measure of the animal's drive to gather information (Sutcliffe et al., 2007). Furthermore, we have also reported a non-significant trend for females to traverse from the nose-poke port to the feeder well faster than their male counterparts. Although this difference in response time may be due to disparities in body size and weight, it may also be indicative of an increased exploratory drive in the female animals.

Female rats and humans also engage in sub-optimal choice strategies longer than males before ultimately maintaining the optimal choice (Van den Bos, Homberg, & de Visser, 2013; Van den Bos, Lasthuis, den Heijer, Van der Harst, & Spruijt, 2006). Although this difference is commonly attributed to disparities in loss-sensitivity, it may be more indicative of differential exploratory behaviour in which females may explore more (i.e. require more information) than males to ultimately converge on the optimal choice (Van den Bos et al., 2013). Ethologically, rats face uncertainty in their food source, so there is likely an intrinsic, inextinguishable drive to explore (Gruber et al., 2017). We speculate this drive to reduce uncertainty of food availability may be stronger in females as food deprivation may hinder their reproductive success (Hussain, Tassabehji, Ashton, & Glazier, 2017; Wade, Schneider, & Li, 1996).

## 6. Lesions of the Anterior Cingulate Cortex

### 6.1. Introduction

We have found that EFS transiently increases when novelty is introduced into our task and speculated that the rodent brain may have evolved a system that promotes exploration for foraging, particularly at times of uncertainty or when opportunity costs are low (Gruber et al., 2017). The anterior cingulate cortex (ACC) has been strongly implicated in detecting novelty and governing foraging behaviour (Downar, Crawley, Mikulis, & Davis, 2000; Hayden, Pearson, & Platt, 2011; Holroyd & Yeung, 2012; Kolling, Behrens, Mars, & Rushworth, 2011; Shenhav, Cohen, & Botvinick, 2016). It has been proposed that the ACC evaluates the costs and benefits of decisions, and is then responsible for selecting and maintaining actions that pursue a particular goal (Hosking, Cocker, & Winstanley, 2014; Kolling et al., 2011; Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006). Activity of ACC is especially pronounced when cognitive control must be exerted over behaviour; when distractions are present or strong, or habitual competing responses must be overcome (Procyk, Tanaka, & Joseph, 2000; Shenhav, Botvinick, & Cohen, 2013).

Inactivation of the ACC has been shown to reduce willingness to expend both cognitive and physical effort to reach rewards in rats (Floresco & Ghods-Sharifi, 2007; Hosking et al., 2014; Walton, Bannerman, Alterescu, & Rushworth, 2003). Macaques with ACC lesions use only the outcome of the most recent trial to guide their next decision, and also fail to gauge risk and payoff in a dynamic foraging task (Kennerley, Walton, Behrens, Buckley, & Rushworth, 2006). These data suggest that the ACC is essential for learning the value of actions and performing cost/benefit analysis to guide behaviour and reach goals. Since EFS imposes a physical effort cost and we have speculated that EFS behaviour

arises from an innate foraging system, we expect that lesions to ACC will reduce EFS propensity.

## 6.2 Methods

### 6.2.1 Subjects

This study involved male LE rats ($n$ = 18). Rats were born on site and weighed between 300 and 500 grams at the start of behavioural testing (postnatal day 160-163).

### 6.2.2 Surgery

Surgeries were performed after MP training was complete as described above. Rats were then randomly assigned to either ACC lesion ($n$ = 10) or sham ($n$ = 8) groups. All rats received Buprenorphine (Alstoe Ltd., UK) via subcutaneous injection to mitigate pain 30 min prior incision. The animals were anesthetized using 4% isoflurane gas (Benson Medical Industries Inc., Ontario, Canada) in oxygen flowing at 1.0 L/min and the surgical plane was maintained with 2% isoflurane throughout the surgery. The animals were mounted on a stereotaxic frame (Kopf Instruments, Tujunga, CA, USA) and a midline incision was made to expose the skull. Burr holes were drilled through the skull to allow lowering of infusion cannulas at the following coordinates from bregma [in mm (AP, ML, DV)]: site 1 (2.2 0.7, -2.5), site 2 (2.7, 0.7, -2.7), site 3 (3.2, 0.7, -2.6). Bilateral lesions were achieved by microinfusion of 0.09 M quinolinic acid (Sigma-Aldrich Canada Co., Oakville, Ontario, Canada) dissolved in a phosphate buffer (pH 7.2). A total volume of 0.30 µl of quinolinic acid was infused at the rate of 0.20 µl per min in each site using a 30-gauge injection cannula attached to a 10 µl Hamilton syringe via polyethylene tubing (PE-50).

The injection needle was left in place for 5 min following the injection to allow diffusion of the drug. The scalp incision was then closed with sutures. Rats were given subcutaneous injections (0.02 mg/kg) of meloxicam (Boehringer Ingelheim, Germany) and monitored for 24 hr before returning them to the vivarium. The animals recovered in their home cages (pair housed) for one week before resuming behavioural testing.

### 6.2.3 Behavioural Procedure

Testing began on day 68 after surgery because of electrical and software issues with the test apparatus. Because of these delays, we consider these results to be preliminary and in need of replication. On post-surgical day 65, water restriction was resumed and animals were given one day of MP retraining. After retraining, animals were tested on the standard, competitive MP task for 4 consecutive days. During standard behavioural testing, one control rat had to be unexpectedly euthanized and was not included in any statistical analysis.

### 6.2.4 Hallway Novelty

Animals ($n$ = 17) underwent the hallway novelty test as described above.

### 6.2.5 Novel Apparatus Environment

To asses sensitivity to large environmental changes of testing conditions, animals underwent testing in a new behavioural box in an adjacent room. This new box was analogous to the original boxes with the following adaptatations. The box was custom-built from black plexiglass and larger (50 X 50 cm). The two liquid delivery feeders on

either side were separated from each other by 32 cm. A 10-cm-long plexiglass barrier orthogonal to the wall separated each feeder from the central port. All other testing conditions remained the same.

### 6.2.6 Histology

At the end of behavioural testing, all subjects received lethal, intraparitoneal injections of sodium pentobarbital (100 mg/kg) and were perfused with phosphate buffered saline (PBS) and 4% paraformaldehyde (PFA). The brains were post-fixed for 24 h in PFA and then transferred and stored in 30% sucrose with PBS for a minimum of 48 h before sectioning. The brains were sectioned in the coronal plane at 55 μm thickness using an SM2010R freezing microtome (Leica, Germany). Every section through the region of interest was mounted on glass microscope slides and stained with cresyl violet. Images of sections were digitized using a NanoZoomer (Hamamatsu, Japan) and evaluated for lesion quality.

### 6.2.7 Statistical Analysis

The effects of ACC lesions on measures in our MP task were analyzed using an ANOVA. To analyze the effect of the mid-session hallway shift on each group, data was binned into eight bins of 20 trials each to account for 160 trials (100 pre-switch and 60 post-switch). The bins were then plotted against each of the task variables using MATLAB. A two-tailed t-test ($\alpha = 0.05$) were performed to compare differences in to the reaction to the hallways switch of the ACC lesion and sham groups for trials 101-120. To compare the change in behaviour within a session for the novel apparatus environment manipulation,

data was binned into eight bins of five minutes each to account for 40 minutes of the 45 minute session. The bins were then plotted against each of the task variables using MATLAB. A RM-ANOVA was performed to compare within group (time bin) and between treatment group (lesion) variables.

### 6.3 Results

We sought to determine if the ACC is responsible for the expression of EFS. We first assessed the potential effects of ACC lesions on motivation and motor output. No effect of ACC lesions was found in the number of operant trials completed in a 45-min session ($F_{1,15} = 0.198$, $p = 0.663$; Fig. 9B), percent of choice trials correct ($F_{1,15} = 3.347$, $p = 0.087$; Fig. 9C), ITI ($F_{1,15} = 0.270$, $p = 0.611$; Fig. 9D), or response time ($F_{1,15} = 0.003$, $p = 0.961$; Fig. 9E). These results suggest that lesions of the ACC do not affect motor output and motivation. ACC lesions also have no effect on measures of sensitivity to reward and reward omission as there was no significant difference between groups on both lose-switch responding ($F_{1,15} = 0.278$, $p = 0.605$) or win-stay responding ($F_{1,15} = 1.329$, $p = 0.267$). There was also no effect of ACC lesions on EFS propensity ($F_{1,15} = 0.584$, $p = 0.457$; Fig. 9F).

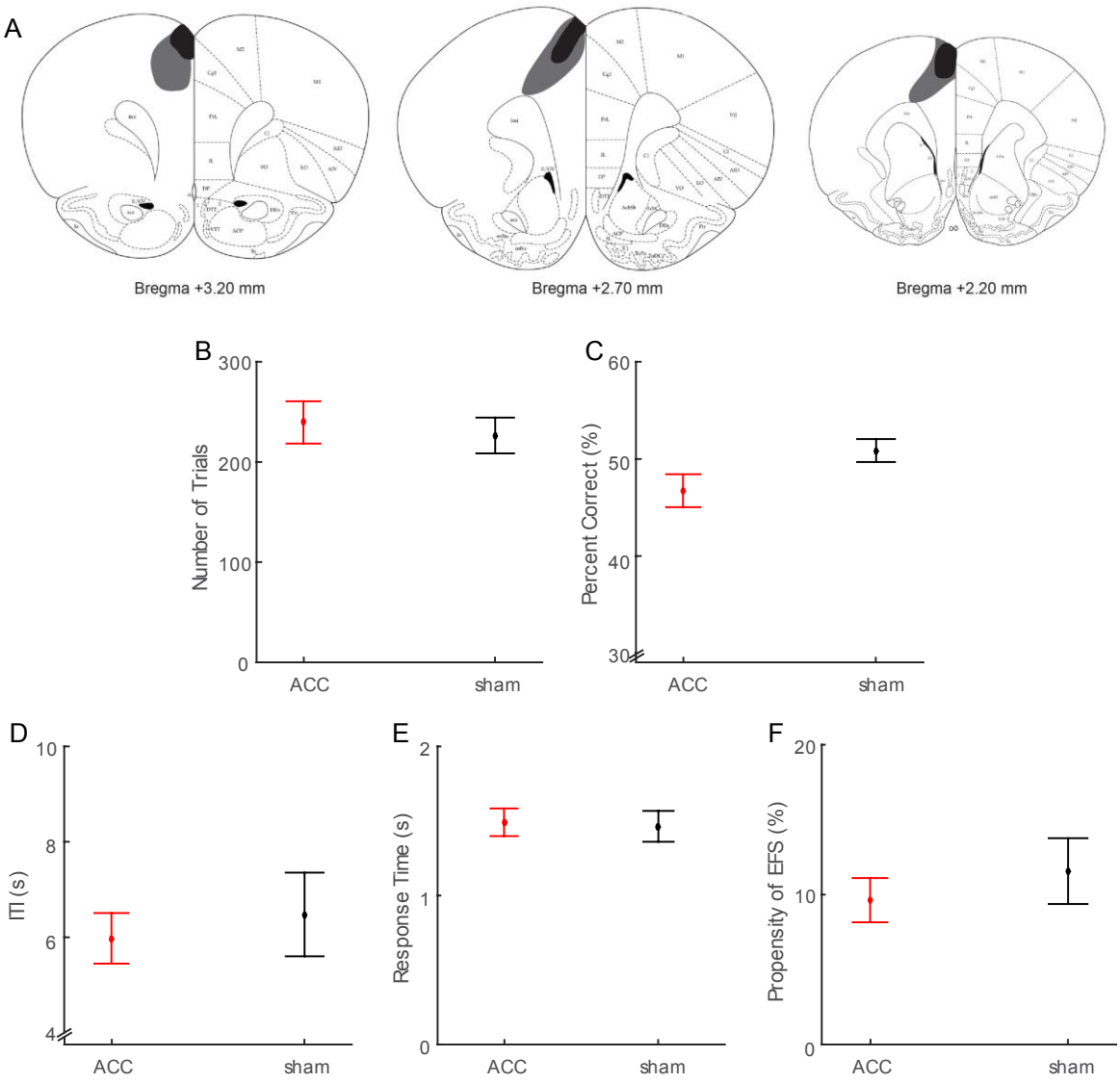


Figure 9. Effects of anterior cingulate cortex (ACC) lesions on behavioural measures in the MP task. (A) Quantification of ACC lesions. Lesions were bilateral and lesion extents were collapsed to one side. Gray shading shows maximal extent of lesions, and black shading shows minimum extent of lesions. ACC lesions had no effect on (B) the number of trials performed or (C) the percent of trials where the correct choice was made. (D) Intertrial interval (ITI) time was also unaffected by lesions of the ACC. (E) There was no significant difference in response time, the locomotion to the feeder well following trial initiation, between the two groups. (F) ACC lesions had no significant effect on the propensity to perform EFS trials. None of the tested means were significantly different, as determined by an ANOVA ($p < 0.05$). Error bars indicate standard error of the mean.

We next tested if uncertainty would affect the relative EFS rate by implementing the mid-session hallway switch protocol described above. The relative EFS rate in the trial bin immediately after the hallway switch was not significantly different between control and ACC-lesion groups for either novel barrier: the 21 cm novel barrier length as compared to the familiar, 13 cm, one ($t_{15}$ = -0.293, $p$ = 0.814; Fig. 10) or the 8 cm novel barrier length as compared to the familiar one ($t_{15}$ = 1.470, $p$ = 0.162). These results indicate that this change in the task environment is not sufficient to transiently produce significant between group effects, suggesting that the ACC is not involved in detecting minute changes in the task or apparatus.

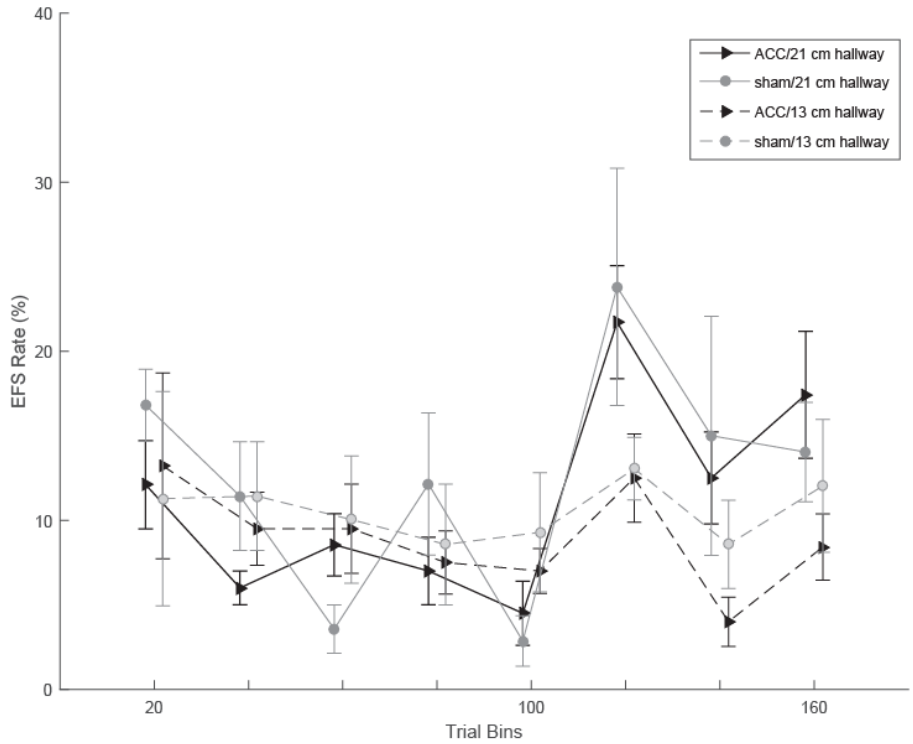


Figure 10. Effects of anterior cingulate cortex (ACC) lesions on the mid-session hallway switch task to assess sensitivity to uncertainty. Immediately following the switch to the 21-cm hallway, there were no significant differences in EFS rate between the ACC-lesion

and sham groups as determined by the two-tailed t-test ($p < 0.05$). Error bars indicate standard error of the mean.

To assess the effects of ACC lesions on the sensitivity to more extensive environmental changes of testing conditions, animals underwent testing in a new behavioural box in an adjacent room. We found that ACC-lesioned rats performed significantly more operant trials in a 45-min session than sham rats ($F_{1,15} = 17.90$, $p = 7.3E-4$; Fig. 11A). ACC-lesioned rats had a significantly lower session-averaged response time ($F_{1,15} = 8.14$, $p = 0.012$; Fig. 11B). ACC lesions also significantly reduced the propensity of EFS ($F_{1,15} = 9.980$, $p = 0.006$; Fig. 11C).

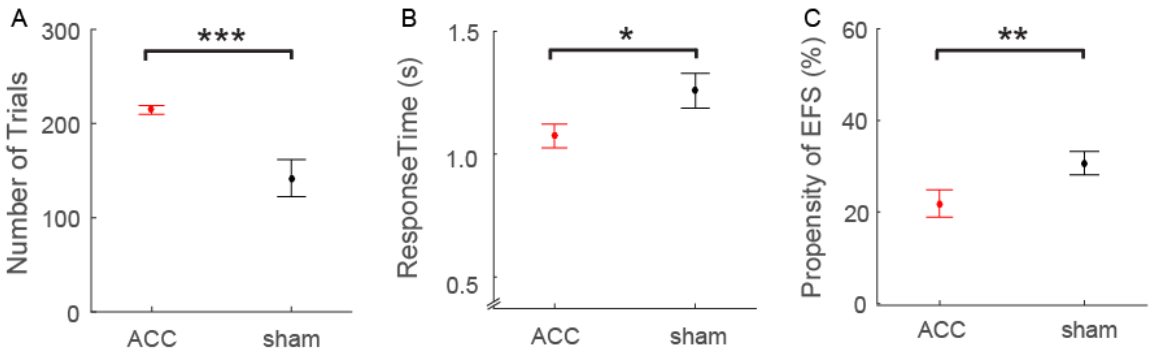


Figure 11. Session-averaged effects of anterior cingulate cortex (ACC) lesions on the novel apparatus environment test to assess sensitivity to uncertainty. ACC lesions had a significant effect on (A) the number of trials performed in a session, (B) response time, and (C) the propensity to perform EFS trials as determined by an ANOVA. Statistical significance for ANOVAs are indicated by '*" for $p < 0.05$, '**' for $p < 0.01$, and '***' for $p < 0.005$.

Significant within-session effects were also found. Effects of time bin were found in the number of trials ($F_{7,21} = 2.681$, $p = 0.038$; Fig.12A), response time ($F_{7,21} = 4.898$, $p = 0.002$; Fig.12B), and propensity of EFS ($F_{7,21} = 12.869$, $p = 2.0E-6$; Fig.12C). There was also a significant time*treatment group interaction for EFS propensity (RM-ANOVA, time*treatment group: $F_{7,21} = 2.621$, $p = 0.041$; Fig. 12C) though no interaction effects were

significant for either the number of trials ($F_{7,21} = 1.795$, $p = 0.141$) or response time ($F_{7,21} = 1.663$, $p = 0.173$).

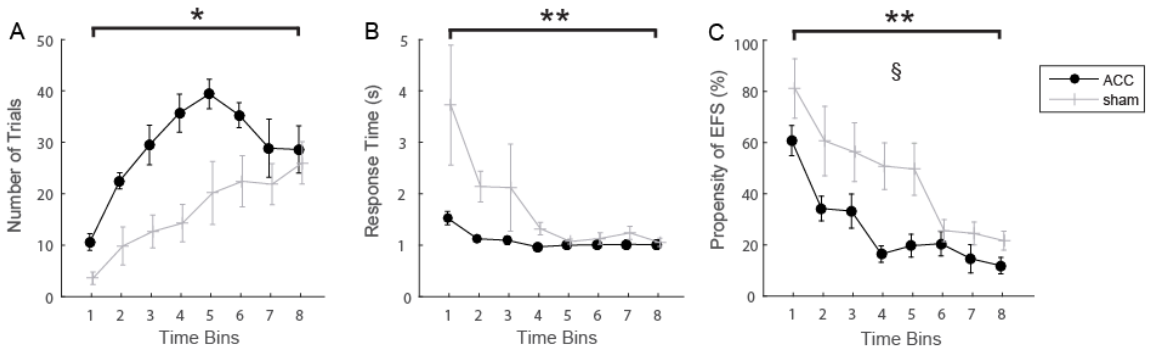


Figure 12. Within-session effects of anterior cingulate cortex (ACC) lesions on the novel apparatus environment test to assess sensitivity to uncertainty. (A) The number of trials performed in each time bin increased over a session, but rats with ACC-lesions exhibited a higher rate of this behaviour throughout the first 30-minutes of session. (B) There was a significant decrease in response time over the time bins with ACC-lesioned rats starting closer to the steady-state level at the beginning of the session than did sham rats. (C) The rats' propensity of EFS decreased throughout the session. Additionally, there was a time*treatment group interaction effect on the propensity of EFS. '*' indicates a significant main effect of sex by repeated-measures ANOVA ($p < 0.05$); Statistical significance for repeated-measures ANOVAs are indicated by '*" for $p < 0.05$, '**' for $p < 0.01$ for the effects of time bin on the behavioural measure while '§' denotes a significant time*treatment group interaction effect for $p < 0.05$.

### 6.4 Discussion

Previous research has implicated the ACC in a wide range of foraging, decision-making and reward sensitive behaviours. We demonstrated that lesions of the ACC do not induce any significant differences in motivation, motor function, or sensitivity to reward or reward omission in our MP task. Additionally, we see no significant reduction in EFS propensity on an already learned task. The ACC has also been implicated in the detection of novelty and salient features in a task (Weible, Rowland, Pang, & Kentros, 2009). ACC

lesions resulted in no significant differences when changes to the task apparatus were introduced by switching hallway lengths. However, we demonstrated that the ACC is sensitive to major environmental changes and that this can affect EFS propensity.

While this study implicates the ACC in EFS expression in novel environments, there were complications in this study. Firstly, the extent of the ACC lesions was minimal due to issues with drug diffusion (see Fig. 9A). Additionally, several interruptions in the testing schedule occurred due to technical issues. As such, a replication is currently being conducted. Histology revealed that second cohort sustained more complete ACC lesions than did this original cohort. This may account for differences in results observed but further analysis is required to truly understand the role of the ACC in EFS.

## 7. General Discussion

Decision-making is a complex process influenced not only by the drive to maximize cumulative reward but also by proximate influences such as the drive to approach feeders, outcome-related cues, and 'choice reflex' tendencies like lose-shift and win-stay responses. These influences likely involve interactions among multiple brain circuits with unique information processing capacities (Daw et al., 2005; Balleine and O'doherty, 2010; Gruber and McDonald, 2012). Here we investigated a form of unproductive behavior that we refer to as extraneous feeder sampling (EFS); this occurs when animals ignore task contingencies and choose to make contact with feeders rather than perform operant trials. This behavior (EFS) was insensitive to reinforcements, but it strongly affected subsequent choice in the task; rats lose-shifted away from the last feeder sampled prior to the subsequent nose-poke, regardless if feeder entry was from a choice within the operant task or if it was a consequence of EFS (Gruber et al., 2017). I show here that EFS is insensitive to devaluation (chapter 3), sexually dimorphic (chapter 5), and my preliminary experiment suggests that rats with lesions of ACC showed significantly less EFS than controls in a novel testing chamber (chapter 6). Whatever its neural basis, the effect of EFS on subsequent choice highlights the need to consider actions prior to trial initialization when analyzing the effects of treatments on decision-making.

EFS may involve Pavlovian attraction to the feeder wells. Sex differences have been previously reported in Pavlovian approach. Females have been shown to acquire Pavlovian approach tasks quicker than their male counterparts (Hammerslag & Gulley, 2014; Pitchers et al., 2015). Our reported increase in EFS by females is consistent with the findings that female rats made more responses in a Pavlovian conditioning task (Pitchers et al., 2015).

Indeed, we first assumed Pavlovian approach was the primary cause of EFS behavior. However, goal-tracking behaviors are sensitive to changes in the valuation of reward and, as such, are reduced by outcome devaluation since this decreases the motivational power of the reward (Ernst et al., 2009). We devalued the reward by giving the rats free access to the sucrose solution prior to starting our MP task. However, this devaluation did not have an effect on the rate of EFS. Although these data do not eliminate a potential role for Pavlovian associations in EFS, it is likely other mechanisms are responsible for this behaviour.

Is the shuttling between feeders (EFS) simply an error reflecting incomplete mastery of the task contingencies, or does it reveal something about ingrained foraging behaviours in rats? We argue that it is the latter. EFS does not fully extinguish after extensive training and appears to increase at times of less certainty of the task: initial training; the beginning of sessions; and following a switch of the barriers. Its insensitivity to both devaluation and to reward outcome (wins/losses) indicates that EFS is not driven by motivation, frustration, or outcome expectation. We also see higher rates of EFS in female rats as opposed to males which fits with previous research investigating sex differences in exploratory behaviour; female rats show more exploratory behaviour in the novel object recognition task (Sutcliffe et al., 2007). We, therefore, speculate that EFS may serve a role in ethological contexts to increase explorative actions. Reinforcement theory indicates this is a good policy in environments with uncertainty (Daw et al., 2006; Sugrue et al., 2004). We argue that the natural environment involves sufficient variability in such a large state space that animals will always face some level of uncertainty about features pertinent to survival. We speculate that the rodent brain may, therefore, have evolved a

system that promotes exploration for foraging, particularly at times of uncertainty or when opportunity costs are low. Moreover, the neural systems promoting exploration may be inhibited as those that promote exploitative actions gain associative strength. This would account for the reduction of EFS with training.

The ACC has been implicated in a wide range of foraging, reward sensitive behaviours, and the detection of novelty and salient features in a task (Holroyd & Yeung, 2012; Kolling et al., 2011; Shenhav et al., 2016; Weible et al., 2009). ACC lesions resulted in no significant differences in any behavioural measures in our MP task when minute changes to the task apparatus were introduced by switching hallway lengths. However, we demonstrated that the ACC is sensitive to major environmental changes and that this can affect EFS propensity. Different brain regions have been shown to activate preferentially to either novel objects or a novel environment (Misslin & Ropartz, 1981; Zhu, McCabe, Aggleton, & Brown, 1997). Perhaps a similar dual system underlies EFS with the ACC only activated in response to environmental changes that are more relevant to foraging decisions.

This thesis has investigated a form of inter-trial behaviour in an operant decision-making task. We have described an exploratory behaviour which appears to arise from neural foraging systems and may involve in the ACC. In humans, the ACC is similarly implicated in promoting a switch towards exploratory behaviours (Daw et al., 2006). Pathology of the ACC has resulted in cognitive, emotional, and behavioral perseveration, which present in many forms of mental illness including anxiety, depression, post-traumatic stress disorder, and obsessive-compulsive disorder (Weisholtz, Sullivan, Nelson, Daffner, & Silbersweig, 2017). While these are preliminary results, we speculate that a properly

functioning ACC is required to explore options and shift behaviour towards optimal strategies, and that disruption of this function in such mental illnesses is one factor that contributes to a reduced ability to shift away (i.e. perseveration) from cognitive, emotional, and behavioural responses when they are inappropriate.

## 8. References


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