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**The Breakfast Effect Revisited: Evaluating the Influence of a Recent Meal on Canine  
(Canis familiaris) Performance in a Scent Detection Task.**

A Thesis

Submitted in fulfillment

of the requirements for the degree of

**Master of Applied Psychology in Behaviour Analysis**

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**Journie Yee**



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## Abstract

Domestic dogs (*Canis familiaris*) are known for their excellent sense of smell, which is widely used to assist humans with important tasks. Despite this, there is limited research on how a dog's hunger state might influence their performance in scent-detection tasks. Previous research suggests that dogs demonstrate higher accuracy in search tasks when tested within 30 minutes of breakfast consumption. However, the underlying mechanisms are not clear, and whether similar effects might also occur in scent-detection tasks is unknown. One possible explanation is the glucose effect, which suggests that a recent meal improves cognitive performance. Alternatively, motivating operations (MO) theory suggests that feeding state modifies the dogs' behaviour by changing the reinforcing effectiveness of food. Under this framework, recent food consumption is an abolishing operation (AO) that decreases the value of reinforcement and narrows stimulus control, while not eating recently is an establishing operation (EO), that increases the value of reinforcement and broadens response bias. This study evaluated scent-detection performance using an alternating-treatments design across breakfast (AO) and non-breakfast EO conditions. To prevent ceiling and floor effects, task difficulty was adjusted by increasing the formal similarity between target (discriminative stimulus;  $S^D$ ) and non-target odours across experimental phases. We hypothesised that the dogs would perform more accurately on the scent-detection task on breakfast days, as the absence of breakfast may increase the likelihood of false alarm indications, thereby reducing accuracy. Results showed that hit rates remained high, 89% across all phases in both conditions, and across individual sessions, ranging from 87%-93% for breakfast and 82% 94% for non-breakfast conditions. While correct-rejection rates were significantly higher on breakfast days ( $p = .016$ ). This suggests that the AO narrowed stimulus control, whereas the EO broadened generalisation. A measure of log  $d$  and correct rejection rates improved significantly over time ( $p = .025$ ;  $p = .014$ ), suggesting that, cumulative food reinforcement

(AO) throughout the day refined discrimination. These findings suggest that a recent meals functions as a MO which influence the precision of stimulus control, instead of, or possibly in addition to, a glucose effect.

**Keywords:** motivating operations, stimulus control, stimulus generalisation, canine scent detection, pre/post feeding

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## Introduction

### *1.1 Background.*

It has been well-established that domestic dogs have exceptional scent capabilities. When these capabilities are combined with their responsiveness to operant conditioning, dogs become invaluable across a broad spectrum of service roles. Dogs are frequently employed to assist humans with scent-detection tasks such as crime scene detection, locating missing people, and biological material (Rust et al., 2018); detecting drugs and explosives (Furton & Myers, 2001); and identifying illnesses like COVID-19 and cancer (Moser et al., 2019; Jendry et al., 2021). Dogs are highly trainable and respond effectively to positive reinforcement such as treats and praise and often interact successfully with people in various settings (Riemer et al., 2018). Dogs' scent detection abilities are frequently described as both accurate and versatile, and they are often considered one of the most cost-effective scent detection methods (Furton & Myers, 2001; Lorenzo et al., 2003; Settle et al., 1994).

Despite their importance and the substantial amount of research in scent detection, relatively little research has examined how specific procedures might enhance a dog's performance. In particular, questions remain about whether a dog's hunger level influences accuracy and whether providing breakfast before a task could enhance their performance.

### *1.2 The Effects of Feeding in Dogs' Performance*

Research has shown that feeding state can influence cognitive performance, with recent food intake. Miller and Bender (2012) investigated whether dogs would demonstrate greater accuracy in a memory-based search task after consuming breakfast compared to when they were fasted. The authors hypothesised that search and memory-task accuracy would be higher following breakfast than when fasted, and that performance differences would vary between early and later testing sessions.

The study employed a within-subjects design where all dogs participated in two main conditions. Condition one involved testing 30 minutes after breakfast or 30 minutes after no breakfast. Condition two involved testing 90 minutes after breakfast or 90 minutes after no breakfast. In each task, the dogs were cued to sit and stay on the mat while the researcher placed a treat in one of five bowls lined up in front of them. The researcher returned to their position in the room and cued the dog to search. A correct response was recorded if the dog indicated the bowl containing the hidden treat.

The study's findings indicated that dogs were more accurate when tested 30 minutes after breakfast compared to when fasted. However, this performance advantage vanished when they were tested 90 minutes after breakfast, suggesting that the positive effect of feeding is short-lived. The researchers proposed that a recent meal improves cognitive performance by providing glucose, which fuels attention and working memory (Scholey et al., 2001).

This study is interesting, as one might expect dogs to perform best when hungry; one possibility is that breakfast consumption can temporarily improve accuracy by providing energy from glucose in food, which supports executive-functioning mechanisms such as attention and memory.

Nutrition, specifically feeding state and glucose availability, has been shown to play an important role in cognitive performance, with research across species examining how pre- and post-feeding conditions influence behaviour and executive functioning (Benton & Sargent, 1992). Better performance is sometimes observed when the animal has not eaten, for example, Kirkby et al. (1995), examined the influence of pre-feeding on rats' performance in a delayed matching-to-position task. The rats that were tested before they received their meal demonstrated higher accuracy on the task when compared to rats that had unlimited access to food. These results suggest that pre-feeding can affect task outcomes, by changing either

motivational states or the cognitive processes required for the task. Specifically, as the rat became more satiated, their response accuracy decreased. While this research provides important insight into how pre-feeding can influence cognition and behaviour, these effects have primarily demonstrated in rodents.

Extending this line of research, Chan et al. (2005) investigated how hunger affects dogs' accuracy on visuospatial memory tasks using a three-component delayed-non-matching-to-position task with two pre-feeding conditions (one meal portion and two meal portions before the task). They observed that giving dogs a double portion of food prior to testing resulted in longer response times for both young and old dogs. While pre-feeding lowered the dog's response speed, it did not affect their performance accuracy, suggesting that the dog's spatial working memory was not influenced by satiety, but that motivation levels primarily affected response speed.

### *1.3 Nutrition – Pre/Post Feeding*

A possible explanation for the results found by Miller and Bender (2012) is that glucose acts as the primary energy source for demanding cognitive processes, including executive functioning, memory, and attention (Benton et al., 1994). The brain relies exclusively on glucose for energy, meaning that maintaining adequate levels is crucial for optimal cognitive performance. Fasting or waiting before completing a task can temporarily lower the available glucose in the bloodstream, which may subsequently impair cognitive functions such as working memory and attention. These cognitive processes are especially susceptible to variations in glucose concentration, as glucose supplies the energy needed to sustain neural activity and efficient information processing (Parrish et al., 2016).

Consuming a meal before a memory-based search task provides an immediate surge of glucose, which can temporarily improve performance on tasks reliant on attention and executive functioning (Benton & Owens, 1993). However, this boost in performance is likely

to be transient. In dogs, this mechanism could explain why cognitive performance is higher immediately following breakfast but tends to decrease as time passes and blood glucose levels stabilise back to baseline (Miller & Bender, 2012). As circulating glucose normalises, the initial increase in cognitive efficiency may fade, demonstrating the dynamic interplay between energy and cognitive demand (Benton & Owens, 1993; Miller & Bender, 2012). This suggests the fluctuations in energy availability could influence both the sensory and cognitive components of detection performance.

While the Miller and Bender (2012) study acknowledged the differences in performance depending on feeding state and theorised that attention and memory are enhanced after a recent meal due to an increase in energy from blood glucose. The authors framed their findings as supportive of the glucose hypothesis, but they also acknowledge an alternative explanation; changes in feeding state may alter a dog's motivational state (Baldi & Bucherelli, 2005; Broadhurst, 1959; Yerkes & Dodson, 1908) rather than directly influencing cognitive capacity. This perspective can be understood through the framework of motivating operations (MO). Feeding may not only influence cognitive processes through glucose availability but also modify the dogs' behaviour due to changes in the reinforcing effectiveness of food, providing a different interpretation of feeding effects on scent detection performance. To understand MO theory, we may first review the fundamental behavioural concepts that are relevant to this study.

#### *1.4 Key Behavioural Concept*

B. F Skinner (1958) defined reinforcement as any stimulus or event that increases the future probability of the behaviour it follows. Operant conditioning, as described by Skinner (1938) is learning shaped by the consequences of behaviour. Within this framework, reinforcement, whether positive (addition of a stimulus) or negative (removal of a stimulus), increases the future probability of the behaviour it follows. In animal training, positive

reinforcement is used to teach new and reliable behaviours. This concept was demonstrated by China et al. (2020), whose research compared the effectiveness of three training methods: one using positive reinforcement (food reward) and two using negative reinforcement (e-collars that deliver a static electronic stimulus to the dog's neck, and lead pressure). The research focused on dogs exhibiting problematic off-lead behaviour and poor recall skills. The authors concluded that the group trained with positive reinforcement was most effective in improving general obedience and addressing the target behaviours.

Discriminative stimuli ( $S^D$ ) can set the occasion for multiple responses, which occurs when distinct antecedent stimuli signal different operant behaviours. This mechanism is crucial in training, as it leads to context specific behaviour. For example, in the presence of the target odour ( $S^{D1}$ ), a dog performs one response, such as maintaining a nose hold in a nose port (R1), while in the presence of the non-target odour ( $S^{D2}$ ), the dog performs a different response, such as pressing a lever (R2). Each  $S^D$  signals the occasion for a specific behaviour, demonstrating that stimulus control can govern multiple responses. This concept has been demonstrated in a study by Turner and Balleine (2024) where they trained rats with two different  $S^D$ s that set the occasion for two different responses. Specifically, when background noise ( $S^{D1}$ ) was present, the rat pressed lever A (R1), but when the houselights were flashing ( $S^{D2}$ ), the rat pressed lever B (R2). Each  $S^D$  set the occasion for a specific behaviour, demonstrating stimulus control of multiple responses.

Another key aspect of operant conditioning is the three-term contingency, comprising of antecedent ( $S^D$ ), behaviour (response), and consequence. It describes the process by which a consequence (reinforcer or punisher) determines the future likelihood of a response (McSweeney & Murphy, 2017). Within this framework, a consequence is delivered when a specific behaviour occurs in the presence of a  $S^D$ . In the absence of the  $S^D$ , the behaviour is unlikely to be evoked, and the corresponding consequences is withheld (Skinner, 1953). This

contingency explains how environmental conditions maintain and shape behaviour through punishment and reinforcement. An example of the three-term contingency is the presence of a target scent mixture (antecedent), the dog sits (response), which is then followed by the delivery of kibble (consequence), functioning as positive reinforcement. Without the presence of the target scent mixture (S) the dog's sitting behaviour is unlikely to occur. This concept is demonstrated in a study conducted by Terrace (1963), where pigeons were trained to peck a key only when a specific colour was present and not to peck when another colour was present. When the red light was illuminated ( $S^D$ ), the pigeon pecked the key (R), and food was delivered ( $S^R$ ). When the green light ( $S$ -delta;  $S^A$ ) was illuminated, the pigeon learned to stop pecking the key, as no reinforcer was delivered. The pecking response was subsequently unlikely to occur when the green light was present.

### *1.5 Motivating Operations (MO)*

To understand the performance differences observed by Miller and Bender (2012), it is important to consider how pre-feeding or post-feeding functions as a MO by modifying the reinforcing effectiveness of reinforcers relevant to the MO. An MO is an environmental event or condition that temporarily influences behaviour by altering both the value of a consequence and the likelihood of behaviours that have previously produced that consequence (Michael, 1982, 1988, 1993). However, Edwards et al. (2019) proposed a conceptual shift, arguing that the effects of MOs are better understood as changes in stimulus control rather than a direct “evocative push”. From this perspective, the  $S^D$  signals the strength or effectiveness of the reinforcement and MOS alter the evocative strength of  $S^D$ s.

The MO construct can be divided into establishing operations (EOs) and abolishing operations (AOs). EOs increase the value of a reinforcer and strengthen behaviours that have historically produced that reinforcer. Food deprivation, for example, increases both the value of food and the control of behaviour by food-related  $S^D$ s. Prior to feeding (EO) a dog is more

likely to respond to the verbal cue “sit” ( $S^D$ ), as this response has historically been reinforced with food in the presence of the  $S^D$ . In contrast, AOs decrease the value of a reinforcer and reduce the likelihood of related behaviours maintained by that reinforcer. Following feeding (AO), when food value is reduced, the same  $S^D$  (verbal cue “sit”) is less likely to evoke food-reinforced behaviour. Importantly, MOs differ from  $S^D$ s, while an  $S^D$  signals the availability of reinforcement, the MO is an environmental variable that modifies the effectiveness of that reinforcer. When the efficacy of a reinforcer is altered, the influence of antecedent stimuli on behaviour also changes. Consequently, MOs affect both reinforcer value and stimulus control, including altering the evocative strength of the  $S^D$ s (Edwards et al., 2019).

Research and MO theories indicate that high-intensity MOs can lead to less precise stimulus control. Lotfizadeh et al. (2012) demonstrated that MOs influence stimulus generalisation by modifying the degree to which a behaviour occurs in the presence of stimuli that share features of the  $S^D$ . Under high EO conditions, such as prolonged food deprivation, the value of the reinforcer is high, and the organism is more likely to respond both to the  $S^D$  and similar stimuli, producing broader responding across a wider set of stimuli. Under AO conditions, such as, recent feeding, the value of food reinforcement decreases, and responding becomes restricted primarily to the  $S^D$ , resulting in the dog being less likely to respond to similar stimuli, demonstrating tighter stimulus control and reduced generalisation. This effect was demonstrated in a study conducted by Thomas and King (1959), which examined pigeons under four different levels of food deprivation. The pigeons in the most food-deprived group exhibited a wider generalisation gradient, responding more frequently across all tested stimuli values. Under extreme deprivation, stimulus control was degraded, meaning the  $S^D$  became less precise, and the pigeons responded to more non-target stimuli. In contrast, moderately hungry group showed a sharp, narrow gradient, responding mainly to the  $S^D$  and demonstrating stronger stimulus control and discrimination. The least deprived group

displayed a moderate gradient with fewer total responses, reflecting lower reinforcement value. For example, when a dog has not recently eaten, food becomes more reinforcing, and behaviours previously reinforced with food, such as sitting by the food bowl in the presence of other stimuli (unfamiliar person), are more likely to occur.

Understanding how MOs influence the value of food reinforcement is essential, as it could affect a dog's performance in scent-detection tasks. On non-breakfast days (EO), when the dog has not eaten, food functions as a highly effective reinforcer. As a result, when the target odour ( $S^D$ ) is present, the dog is more likely to indicate the target as present, producing a hit, however, the same EO increases responding in the presence of the non-target ( $S^A$ ), producing false alarms, as the dog is more likely to increase responding, even in the presence of stimuli that differ from the  $S^D$ . When the dog has recently eaten (AO), and the value of food reinforcement is reduced, the dog is less likely to indicate the target as present in the presence of the target odour ( $S^D$ ), resulting in misses. In the presence of non-target ( $S^A$ ), the dog is more likely to correct reject the non-target as present, producing a correct rejection. Under AO conditions, dogs may also progress through the task slower, as the reinforcer for engaging with the task is not effective. MO theory predicts that changes in reinforcer value produce directional shifts in response bias, such that EO conditions increase the likelihood of responding to both target and non-target stimuli, whereas AO conditions reduce the likelihood of responding and narrow responses to the target stimulus. In contrast, glucose theory predicts changes in overall performance without specifying sensitivity or response bias. Signal Detection Theory (SDT) therefore provides the framework for determining whether observed performance differences reflect changes in response bias instead of sensitivity.

### *1.6 Signal Detection Theory (SDT)*

Signal Detection Theory (SDT) is a framework that examines how an organism successfully identifies a stimulus as present or absent amidst uncertainty. It enables the

separation of true discriminative control from any response bias by assessing four possible outcomes: hits, misses, false alarms and correct rejections. This structure allows researchers to evaluate both the precision of stimulus control and the environmental factors that impact detection performance (Swets & Green, 1978). For example, if the signal is present and the organism correctly identifies its presence, this is called a “hit”. If the signal is present but the organism fails to detect it, this is a “miss”. If the signal is absent but the organism incorrectly signals its presence, this is a “false alarm”. Finally, if the signal is absent and correctly identified as absent, this is a “correct rejection” (Nevin, 1969); (illustrated in Table 1).

Signal detection theory also has practical applications in real-world settings, such as medical imaging. For example, Swensson (1996) applied SDT to measure radiologist performance in detecting tumours in X-ray images. In this study, a hit was recorded when the radiologist correctly identified a tumour, a miss occurred if a tumour was present but was not reported, a false alarm was recorded when a tumour was incorrectly reported as being present, and a correct rejection occurred when no tumour was present, and none was reported. By applying SDT, the study was able to separate radiologists’ true discriminative ability to detect tumours from their decision-making biases, providing a clearer understanding of factors that influence diagnostic accuracy.

**Table 1**

*Yes/No Response when Signal is Present/Absent.*

	<b>“Yes Response”</b>	<b>“No Response”</b>
<b>Signal Present (S+)</b>	Hit	Miss
<b>Signal Absent (S-)</b>	False Alarm	Correct Rejection

### *1.7 Aim of Study*

The primary aim of this project was to explore how dogs performed in a scent-detection task before and after feeding. In this task, dogs were trained to indicate the presence of a fixed target odour mixture (Amyl Acetate, Benzaldehyde, Hexanol and Benzyl Acetate) by holding their nose in the port for the required observation threshold. For any other odour mixture presentation (Butyl Acetate and/or Ethyl Butyrate, combined with an increasing overlap of any combination of components of the target mixture), the dogs were required to press a lever after sniffing the sample briefly. Across the experimental phases, the degree of overlap between the target and non-target odours increased, progressively challenging the dog's ability to discriminate between them.

It was hypothesised that the dogs would perform more accurately on the scent-detection task on breakfast days. According to MO theory, not having breakfast may increase the probability of false alarm indications, reducing accuracy. Specifically, a liberal bias shift was expected, in which dogs who had not eaten would treat a greater number of samples as S<sup>D</sup>s leading to a higher number of indication responses in both hit rates and false alarm rates. In contrast, dogs who had eaten would elicit fewer indication responses resulting in more non-target samples being correctly rejected.

This study builds on previous research suggesting that an increase in energy from blood glucose and feeding state can influence attention, memory, and response motivation (Miller & Bender, 2012; Benton & Owens, 1993). By assessing whether recent feeding affects dogs' accuracy in a scent detection task, the study aims to clarify whether these effects can also be observed in a scent-detection tasks and whether a hypothesis based on MO theory can help explain such effects. The independent variable was the feeding condition, the difference between breakfast days and non-breakfast days, and the dependent variables included task accuracy and session duration.

## **Methods**

### *2.1 Recruitment*

Dogs from the Waikato community were recruited through flyers, social media platforms, word of mouth and the current research database of dogs who had participated in recent scent-detection projects and whose owners had indicated a desire to take part in additional studies. Interested owners were sent an initial screening form to assess their dog's food motivation, current feeding routine, potential aggression toward other dogs, and signs of separation anxiety. If no concerns were identified, an induction session was conducted with the owner. During this session, detailed information about the study was provided, including its purpose, a summary of the training procedures, and time allowed for questions. Each dog was then observed to assess its response to being crated, remaining in the experimental room, and being briefly separated from its owner. Owners were asked to sign written consent for dogs that met the required criteria, and they proceeded to the training phase.

### *2.2 Participants.*

A total of seven dogs met the initial criteria and were recruited for this study, as shown in Table 2. One dog (Ernie) was excluded during the initial training sessions due to an aversion to the automatic feeder.

**Table 2***Participant Information*

<b>Participant</b>	<b>Breed</b>	<b>Age (years)</b>	<b>Sex</b>
Mika	Border Collie X Springer Spaniel	7	NF
Darla	Schnauzer, Fox Terrier, Heading Dog mix	7	NF
Ludo	Whippet Cross	2	NM
Ora	Labrador Retriever	2	NF
Buddy	Cocker Spaniel	4	NM
Ernie	Golden Retriever	1	F
Kal	Mixed	1	NM

*Note.* NF represents neutered female, NM represents neutered male

*2.3 Ethics*

The study was approved by the University of Waikato Animal Ethics Committee (Protocol 1227). Prior to the start of training, written consent was obtained, all dog owners were informed that they could withdraw their dog from the study at any time. Throughout the study, dogs had constant access to water and were taken outside every two hours.

*2.4 Study Location*

The study took place at the University of Waikato's Scent Detection Research Group facility. Dogs attended two training sessions per week, held between 9:00 a.m. and 12:00 p.m. The experimental room measured approximately 3.1 by 3 m. A separate crate room housed five individual crates, each equipped with water, a mat, and a blanket specific to each dog.

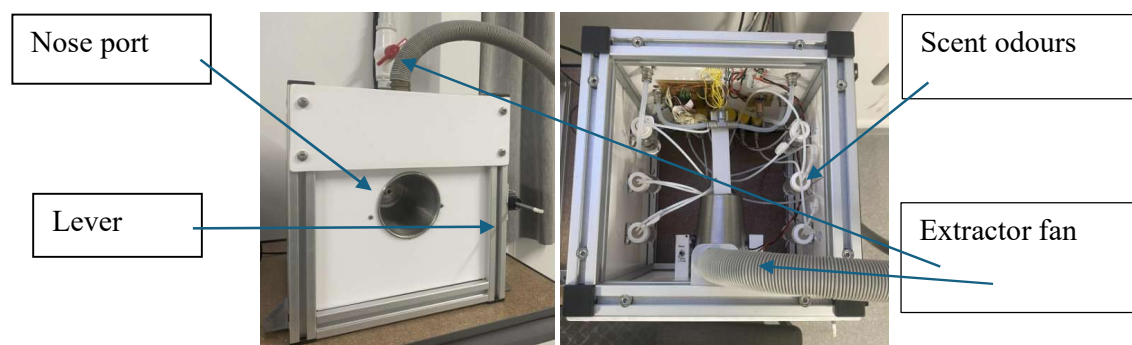
*2.5 Apparatus*

The airflow olfactometer apparatus was based on the design by Aviles-Rosa et al. (2021). The apparatus was a 12" cube with a nose port on the front and a lever on the side. The unit housed six odour channels with valves controlled by a central computer. The valves led to a central manifold that allowed any individual odour or combination of the six odours to be presented to the dogs via constant stream of airflow through the port. The rotameters

were set to 0.3 mL/min for the odours and 3.5 L/min for the air. All material after the odour vials in the apparatus was composed of PTFE to reduce odour contamination. The odours were vented outside the building. An infrared beam spanned the opening and was used to measure the dogs' access to and indications of samples (holding the nose in a port for a specified duration or pressing the lever). The computer controlled the apparatus, presenting the experimenter-specified sequence of trials with specific odour mixtures. In addition to automatically recording all the data associated with dogs' interaction with the apparatus including beam break timing, the occurrence of lever press and the duration of each response).

**Figure 1**

*Scent Delivery Apparatus*



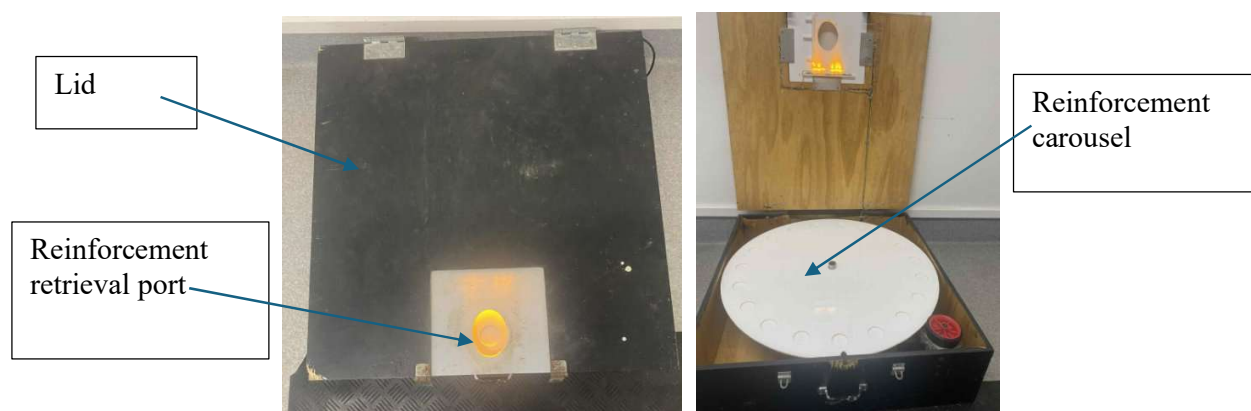
Reinforcement was either automatically delivered by the computer programme or manually triggered by the trainer using a remote. An auditory cue signalled the subsequent delivery of food in an automated apparatus. Either a commercially available apparatus, the Treat and Train kibble dispenser, or a custom apparatus that could present any type of food via a revolving carousel was used. The automatic feeders were positioned 1.5 m directly opposite the airflow olfactometer. Three types of food were used as reinforcement: dry laboratory kibble, Possyum and Awesome Pawsome peanut butter and cranberry treats.

**Figure 2**

*Automatic Feeder*

**Figure 3**

*Automatic Carousel Feeder.*



### *2.6 Sample Preparation*

Two samples of each chemical were mixed in a chemistry laboratory at a ratio of chemical to mineral oil of 1:2000 (see Appendix A). One batch was kept in the refrigerator at 7°C in the scent detection facility for no longer than four weeks, and the other batch was stored in the apparatus for no longer than two weeks. New glass vials or previously used vials

cleaned with nitric acid were used for each sample preparation (see Appendix A). The sample vials were placed in the same location in the apparatus; the locations and all the corresponding valve lines were numbered. The vial lid and lid seals were checked and tested each week for damage and leaks and replaced as needed. Disposable nitrile gloves were worn throughout the handling and replacement of sample types to prevent cross-contamination.

### *2.7 Odour Source*

Six odours were used in this study: amyl acetate, benzaldehyde, hexanol, benzyl acetate, butyl acetate, and ethyl butyrate. All the odours were standard laboratory chemicals with vapour pressures at 25 °C ranging between 0.928 and 12.8 mmHg (Table 3).

**Table 3**

#### *Odour Vapour Pressures and Odour Key*

<b>Odours</b>	<b>Vapour pressures</b>	<b>Odour key</b>
Amyl Acetate	5.17	1
Benzaldehyde	5	2
Hexanol	0.928	3
Benzyl Acetate	1.425	4
Butyl Acetate	11.5	5
Ethyl Butyrate	12.8	6

The target odour was a fixed combination of four compounds (Amyl Acetate, Benzaldehyde, Hexanol, and Benzyl Acetate). Non-target odours consisted of either Butyl Acetate and/or Ethyl Butyrate, with increasing overlap from the target mixture systematically introduced across phases to increase the task complexity. There were 46 unique non-target combinations distributed across five phases: NT0 (1), NT1 (12), NT2 (12), NT3 (18), and

NT4 (3) (see Table 4). NT level indicates the number of target odour chemicals present in the non-target mixture (NT0 = 0 overlap; NT1-NT4 = 1-4 overlapping components; see Table 4).

**Table 4**

*Non-Target Odour Key Combination Options by Phase*

<i>NT0</i>	<i>NT1</i>	<i>NT2</i>	<i>NT3</i>	<i>NT4</i>
6	5,1	5,1,2	5,1,2,3	1,2,3,4,5
	5,2	5,1,3	5,1,3,4	1,2,3,4,6
	5,3	5,1,4	5,2,3,4	1,2,3,4,5,6
	5,4	5,2,3	5,1,2,4	
	6,1	5,2,4	6,1,2,3	
	6,2	5,3,4	6,1,3,4	
	6,3	6,1,2	6,2,3,4	
	6,4	6,1,3	6,1,2,4	
	5,6,1	6,1,4	5,6,1,2,3	
	5,6,2	6,2,3	5,6,1,3,4	
	5,6,3	6,2,4	5,6,2,3,4	
	5,6,4	6,3,4	5,6,1,2,4	
		5,6,1,2		
		5,6,1,3		
		5,6,1,4		
		5,6,2,3		
		5,6,2,4		
		5,6,3,4		

*Note.* Target odour was composed of odours 1,2,3 and 4

### *2.8 Experimental Design*

This study was a single-subject alternating treatments design, with two randomised conditions, breakfast and non-breakfast days (as shown in Table 4). There were three training components and five phases. All dogs progressed through the phases dependent on their

performance. This study was designed to gradually increase task complexity, requiring the dogs to maintain accurate discrimination under progressively challenging conditions. The approach minimised ceiling and floor effects by ensuring the task was not too easy as the dogs progressed through the phases, nor too difficult such that discrimination between the target and non-target mixtures and performance could be maintained.

### *2.9 Experiment*

There were two research conditions, breakfast, and non-breakfast. In the breakfast condition, the dogs would receive their normal breakfast quantity before the first session of the day commenced. In the non-breakfast condition, the dogs would receive their normal breakfast quantity after the last session of the day ended. Each dog participated over two consecutive days each week, experiencing both conditions in a random order. The dogs' owners were asked to supply or advise their dog's regular food brand and quantity for their morning feed. The dog's weight and health were monitored by conducting weekly weigh-ins.

Blind trials were introduced in week 19 of the 32-week research programme to reduce unintentional behavioural cues from the researcher and to minimise potential bias. A research assistant managed the dogs' randomised breakfast schedule and feeding each week. The researcher left the laboratory for 10 minutes before each session started and after all sessions were finished each day to ensure that they were blind to the daily conditions. The dogs' breakfast schedule was kept on a shared folder that the researcher could not access. The research assistant advised the researcher of the feeding conditions at the end of each week. A blind trial standard operating procedure (SOP) was developed and followed for the remainder of the research (see Appendix E).

A minimum observation threshold of 500 ms was required for the dog to sample the odour prior to indicating the sample as target or non-target. Across each experimental phase (shaping, alternating target and non-target and phase training) a standard indication threshold

ranging between 500 ms and 6000 ms for each dog was established to indicate the presence of the target mixtures. The dog had to hold its nose in the sample port, breaking the infrared beam for at least this duration on positive trials to receive reinforcer. Standard proportions of target and non-target trials was set for all experimental phases (see Table 5).

#### *Training – Conditioned Reinforcer*

Firstly, dogs were habituated to the training environment. Once habituated, a conditioned reinforcer was established with the automatic feeder. Once the dog approached the feeder three times, they progressed to the next step.

#### *Training – Nose hold*

The dogs were then trained to insert their nose into the port, sample the odour and indicate the target mixture by maintaining a nose hold in the port at the indication threshold (Table 5). The nose hold was shaped using successive approximations to the sample port. Food reinforcement was initially delivered manually by the researcher and later automatically by the apparatus once the dogs-maintained nose holds at the indication thresholds (500 ms, 1000 ms, & 1500 ms). Progression to the subsequent indication threshold required the dog to successfully maintain a nose hold for the specific duration across all trials for one session. Once the dog had successfully maintained nose hold at all three indication thresholds, they moved to lever training.

**Table 5***Standardised Proportions and Response Thresholds*

	<b>Shaping Nose Hold</b>	<b>Shaping Lever Press</b>	<b>Alternating Target and Non-target</b>	<b>Phase Training</b>
<b>Standard Trial Proportions</b>	10 targets	10 non- targets	6 targets and 5 non- targets	10 target trials and 10 non- targets
<b>Standard Indication Thresholds</b>	500-1500 ms		1500 ms	2000-6000 ms
<b>Standard Observational Thresholds</b>	500 ms	500 ms	500 ms	500 ms

*Training Lever*

The dogs were trained to indicate non-target mixtures by pressing the lever. The lever press was shaped using successive approximation. Food reinforcement was initially delivered manually by the researcher. Progression to alternating target and non-target of alternate nose hold and lever press was dependent on the dog pressing the lever 100% of the time for three consecutive sessions.

*Training – Alternating target and non-target training*

Training alternating target and non-target involved the dogs alternating between nose hold indicating the target mixture and a lever press indicating the non-target odour. Food reinforcement was provided only for a correct nose hold response when the target mixture was present. Advancement to phase NT0 required the dog to perform a nose hold 100% of the time for a single session at the indication thresholds, or by completing three full sessions without prompting.

*Phase – NT0*

This phase involved randomised trials of the target mixture and a single non-target odour, while simultaneously establishing each dog's indication threshold. Thresholds began

in 2000 ms and were increased in 500 ms increments until a minimum of 4500 ms and a maximum of 6000 ms was reached. Progression to each subsequent threshold required a 100% hit rate within a single session. The dog's final indication threshold was defined by either achieving greater than 80% for both hit rate and correct rejection rate for three consecutive sessions or reaching the maximum indication threshold of 6000 ms. Progression to phase NT1 required the dog to achieve greater than 80% for both hit rate and correct rejection across three consecutive sessions.

#### *Phase – NT1- NT4*

Phases NT1 to NT4 involved randomised trials of the target mixture and non-target odour mixtures with progressively increasing overlap of the target odours. This gradual increase ensured that dogs were required to discriminate between the target mixture under increasingly challenging conditions. The dogs progressed through each phase based on their individual performance. In NT1: one target overlap was introduced (12 non-target combinations). NT2: two target overlaps (18 non-target combinations). NT3: three target overlaps (12 non-target combinations), and NT4: four target overlaps (3 non-target combinations) as shown in Table 4. Progression between phases required each dog to achieve greater than 80% for both hit rate and correct rejection across three consecutive sessions.

A training standard operating procedure (SOP) was developed and followed throughout the training procedures (see Appendix B).

#### *2.10 Data Analysis*

Data was collected each session for each dog, both automatically through the apparatus software and manually using electronic data sheets (one for each dog/each day). The measures collected included correct and incorrect hit rates and correct rejection rates, sample type, proportions of target and non-target samples for each trial. Additionally, for each session, the hit rate percentage, correct rejection rate percentage, start time, duration and time

since breakfast or no breakfast were recorded. The data were sorted by conditions and testing periods (breakfast and non-breakfast days). The manually collected electronic data was then graphed regularly to monitor progress in the form of scatter plots. The final data for each part of the research were graphed as bar graphs using Excel. The hit rates and correct rejection rates were calculated by the number of hits/correct rejection trials divided by the total number of correct hits/correct rejection trials multiplied by 100. The formulas for  $\log d = 0.5 * \log(H*CR/[M*FA])$  and  $\log B = 0.5 * \log(H*FA/[M*CR])$ , where H, M, FA and CR are as defined in Table 1. The 95% confidence intervals for hit rate and correct rejection rate were calculated using logit-transformed data; for all other metrics (session duration,  $\log d$  and  $\log B$ ) 95% confidence intervals were calculated using standard calculations.

Statistical analysis was conducted using the lme4 package (version 1.1-37) in R (version 4.5.1); mixed-effects general linear models were used to compare the dogs' performance in breakfast and no breakfast conditions. For all analyses, dog was set as a random factor, and breakfast condition was set as a fixed factor. Additionally, to evaluate the influence of the passage of time since 9 a.m. (when breakfast was given on breakfast days), the number of minutes since breakfast was included as a continuous factor, and the interaction between this factor and the breakfast condition was also included in the model. To improve stability, a z-score conversion was carried out on the time-since-9 a.m. measure prior to analysis. For hit rate and correct rejection rate, a binomial (logit) function was used; for  $\log d$  and  $\log B$  analyses, a Gaussian function was used, and for time since breakfast, a Gamma function was used. To visualise any potential interactions between time since breakfast and the breakfast condition, interaction plots were generated using the ggplot2 package (version 4.0.0), with 95% confidence intervals based on the relevant model's variance-covariance matrix.

When producing  $\log d$  and  $\log B$  values at the session level for statistical analysis, to prevent issues associated with zeros in any of the signal detection quadrants (e.g., no misses), a standard constant of 0.5 was added to each of the terms in the  $\log d$  and  $\log B$  equations, as recommended by Voss et al. (1993).

## Results

### *3.1 Hit Rate*

Each dog completed between three and ten sessions each day, and a total of 22 to 87 breakfast sessions and 18 to 90 no breakfast sessions. There were some variabilities in the phases that each dog reached and in how long it took each dog to meet the criterion for advancing to the next phase, defined as an 80% or higher hit rate and an 80% or higher correct-rejection rate across three consecutive sessions.

All dogs experienced phases NT0 and NT1. Three dogs met criteria to progress to phase NT2, and one dog met criteria to progress to phase NT3. Ora progressed the furthest across all phases. Kal completed the highest number of total sessions (177), with 87 sessions in the breakfast condition and 90 sessions in the non-breakfast condition. Mika completed the fewest total sessions (40), with 22 in the breakfast condition and 18 in the non-breakfast condition. The variation in the number of sessions across conditions was due to factors such as dog absences, dogs not exiting the crate, or sessions being cut short due to time constraints. Table 6 shows the number of sessions required to reach criteria in each phase. Mika achieved criteria for two consecutive sessions (out of the required three) once during NT2 phase, while Ora achieved criteria for two consecutive sessions (out of the required three) once during NT3.

**Table 6**

*Number of Session to Reach Criteria Across Phases.*

	<b>NT0</b>	<b>NT1</b>	<b>NT2</b>	<b>NT3</b>
<b>Ora</b>	25 sessions (12 breakfast and 13 non-breakfast)	12 sessions (8 breakfast and 4 non-breakfast)	6 sessions (4 breakfast and 2 non-breakfast)	**27 sessions (11 breakfast and 16 non-breakfast)
<b>Ludo</b>	89 sessions (43 breakfast and 46 non-breakfast)	**59 sessions (32 breakfast and 27 non-breakfast)		
<b>Kal</b>	79 sessions (33 breakfast and 46 non-breakfast)	56 sessions (26 breakfast and 30 non-breakfast)	**42 sessions (28 breakfast and 14 non-breakfast)	
<b>Buddy</b>	70 sessions (31 breakfast and 39 non-breakfast)	**22 sessions (10 breakfast and 12 non-breakfast)		
<b>Darla</b>	45 sessions (30 breakfast and 15 non-breakfast)	**28 sessions (18 breakfast and 10 non-breakfast)		
<b>Mika</b>	13 sessions (7 breakfast and 4 non-breakfast)	15 sessions (6 breakfast and 9 non-breakfast).	**20 sessions (9 breakfast and 11 non-breakfast)	

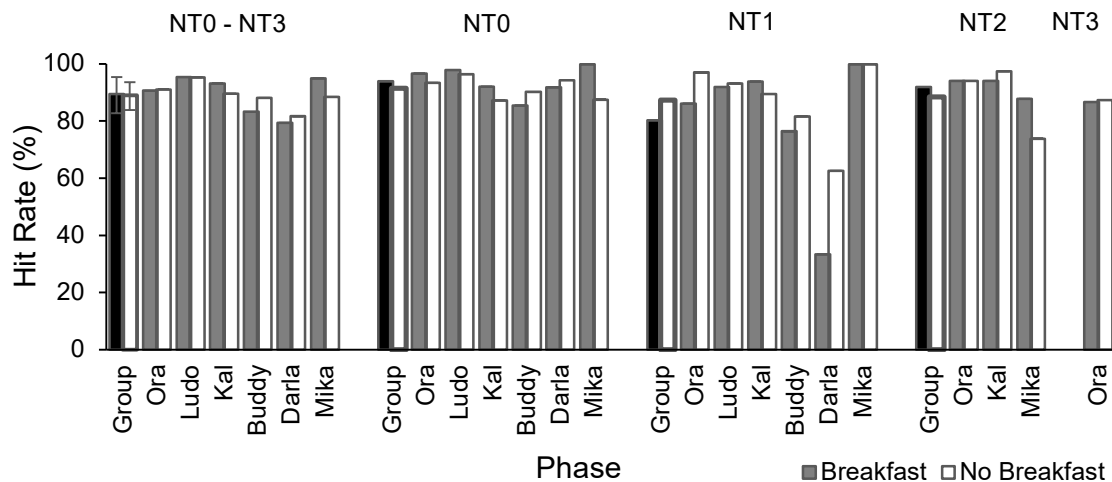
*Note.* \*\* indicates incomplete phases

Figure 4 displays the hit-rate accuracy for all dogs across all phases NT0 through to NT3, including overall performance and performance within each individual phase. Hit rate refers to the proportion of target trials in which the dog correctly indicates the target mixture.

The overall hit rate across all dogs and phases in the breakfast condition ( $M = 0.89$ , 95% CI [0.827, 0.954]) was comparable to that of the non-breakfast condition ( $M = 0.89$ , 95% CI [0.839, 0.936]). While the overall means across phases were identical, slight variations were observed within specific phases. Hit-rate accuracy was marginally higher on breakfast days during phases NT0 and NT2, whereas phases NT1 showed slightly higher accuracy on non-breakfast days. While performance during NT3 was equal across both conditions. Overall, Ludo, Kal, and Mika demonstrated higher accuracy on breakfast days, while Ora, Buddy, and Darla showed higher accuracy on non-breakfast days. Darla showed the lowest accuracy in NT1 on both conditions. Overall, with a few exceptions, accuracy was very high across phases and across individual dogs.

**Figure 4**

*All Dogs Hit Rate (%) by Phases Data*



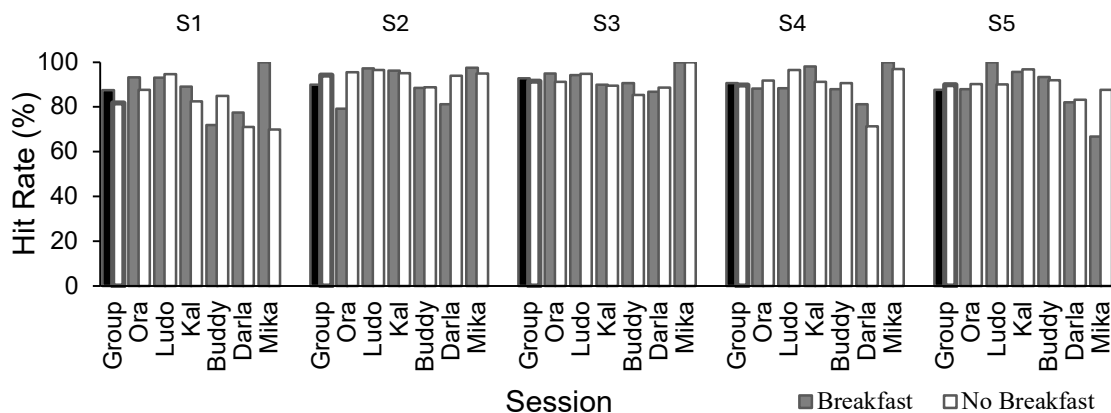
*Note:* Error bars on Group columns in all phases represent 95% confidence intervals (CI).

Figure 5 displays the hit rate accuracy for all dogs across sessions 1 to 5 (an analysis of order effects across sessions over the course of the day). There were some variations in how many sessions were completed each day across dogs. Overall, hit-rate accuracy was

slightly higher on breakfast days in session 1, 3, and 4, whereas session 2 and session 5 showed slightly higher accuracy on non-breakfast days. Session 1 showed the lowest group hit-rate accuracy for both conditions, while session 3 showed the highest accuracy for breakfast days, and session 2 showed the highest accuracy for non-breakfast. Across all sessions group hit-rate accuracy ranged from 87% to 93% on breakfast days and from 82% to 94% on non-breakfast days, indicating consistently high accuracy across dogs, sessions, and conditions. Session 3 showed the most consistent performance, with breakfast and non-breakfast hit rates closely aligned. Mika had the highest hit-rate accuracy on breakfast days across session 1 to 4 and showed the lowest breakfast accuracy in session 5. In the non-breakfast condition, she showed the lowest accuracy in session 1 and the highest accuracy in session 3. Darla showed lower accuracy than the group across all sessions in both conditions, with session 1 showing her lowest hit rate in both breakfast and non-breakfast condition. Ludo showed higher accuracy in session 1, session 3 and session 4 on non-breakfast days, and Buddy also showed higher accuracy under non-breakfast condition in sessions 1, session 2, and session 4.

**Figure 5**

*All Dogs Session 1-5 Hit Rate (%) Data*

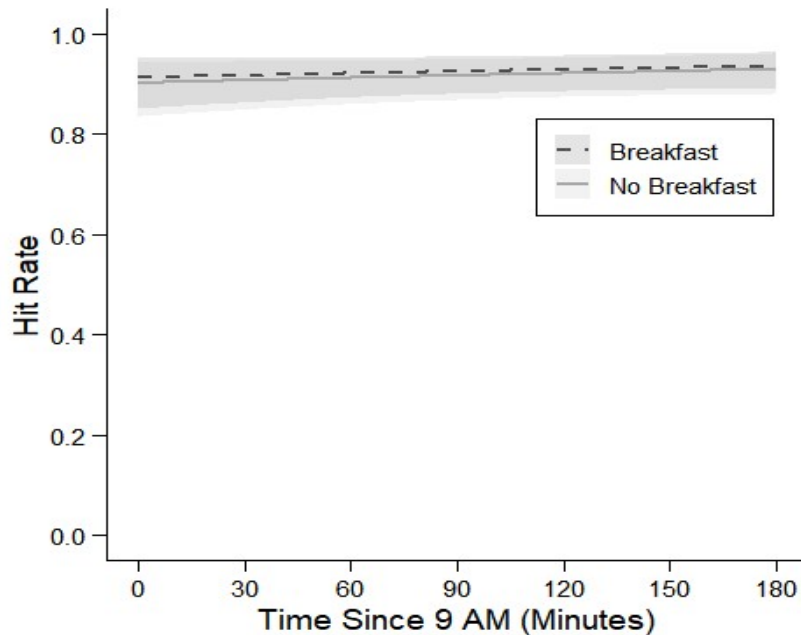


*Note:* Only the first five sessions were analysed because later sessions varied in number and consistency.

Figure 6 displays an interaction plot showing model-predicted hit rate across time for breakfast and no-breakfast conditions, the influence of breakfast was not statistically significant ( $p = .360$ ); time since 9a.m. was not a significant predictor of performance ( $p = .238$ ), and there was no statistically significant interaction between these two factors ( $p = .935$ ).

**Figure 6**

*Interaction Plot Showing Model-Predicted Hit Rate Across Time for Breakfast and No-Breakfast Conditions. Shaded Regions are 95% Confidence Intervals.*



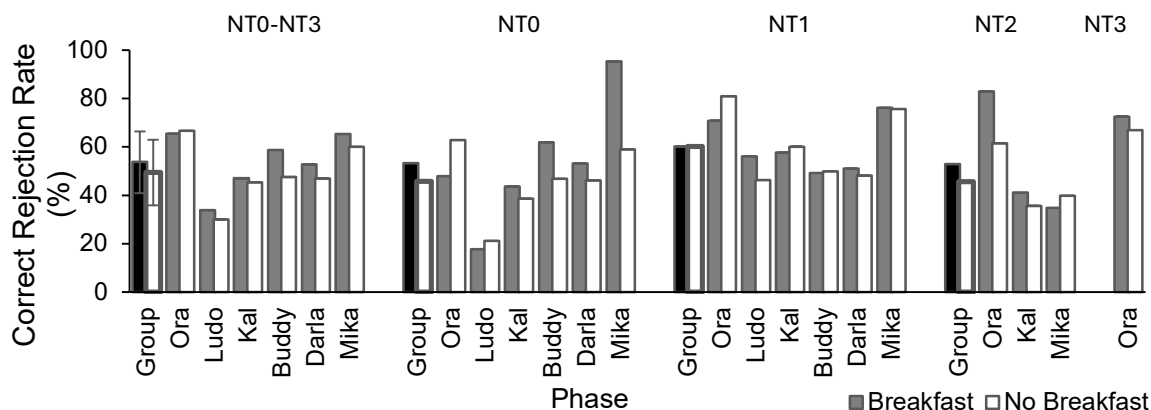
### 3.2 Correct Rejection Rate

Figure 7 displays the correct rejection accuracy rates for all dogs across phases NT0 through to NT3, including overall performance and performance within each individual phase. Correct rejection rate refers to the proportion of non-target trials in which the dog correctly rejects a non-target mixture. The overall correct rejection rate across all dogs and

phases in the breakfast condition ( $M = 0.54$ , 95% CI [0.409, 0.665]) was comparable to the non-breakfast condition ( $M = 0.49$ , 95% CI [0.358, 0.631]). Overall, correct rejection rate was higher on breakfast days across all dogs and phases. Ora demonstrated higher correct rejections rate on non-breakfast days in phases NT0, and NT1. Ludo showed higher correct rejections rate on breakfast days overall, with non-breakfast days higher only in phase NT0. Buddy showed higher correct rejection rates on breakfast days overall, with non-breakfast days correct rejection rates higher only in phase NT1. Mika showed higher correct rejection rates on breakfast days overall, and higher correct rejection rates in phase NT2 on non-breakfast days. Mika also showed the largest difference between breakfast days and non-breakfast days in phase NT0.

**Figure 7**

*All Dogs Correct Rejection Rate (%) by Phases Data*



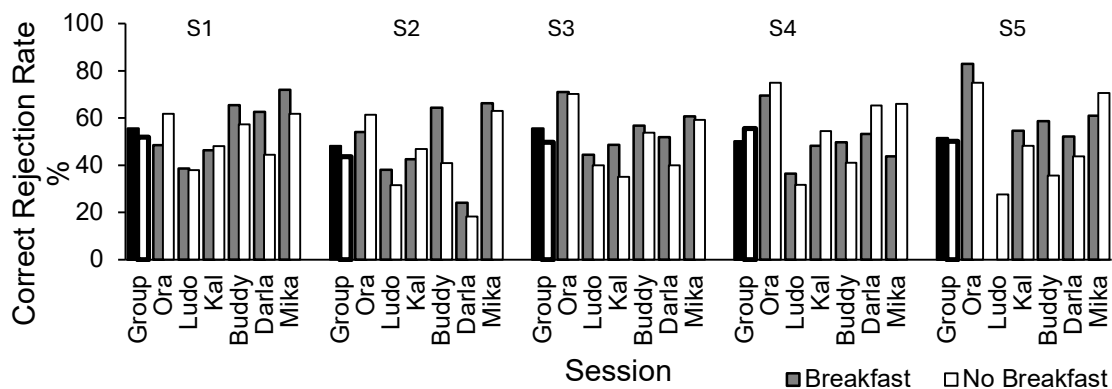
*Note:* Error bars on Group columns in all phases represent 95% confidence intervals (CI).

Figure 8 displays the correct rejection accuracy rates for all dogs across sessions 1 to 5. There was some variability in how many sessions each dog participated in each day and over the data collection period. Overall, correct rejection accuracy rates were higher on breakfast days for all sessions except session 4, where non-breakfast days were higher. The

highest group correct rejection accuracy rate occurred in session 1 and 3 on breakfast days, and in session 4 on non-breakfast days, while session 2 showed the lowest accuracy across both conditions. Across all sessions, group correct rejection rate accuracy ranged from 48% to 56% on breakfast days and from 44% to 56% on non-breakfast days. Mika generally showed higher correct rejection rate accuracy than the group across all sessions and conditions, except in session 4 on breakfast days. Ludo's results showed higher accuracy on non-breakfast days except for session 1 and session 2. Ludo also had the lowest session accuracy in session 1, 3, 4, and 5 across both conditions, except session 3 on non-breakfast days. Darla showed the lowest correct rejection accuracy rate for both conditions' session 2.

**Figure 8**

*All Dogs Session 1-5 Correct Rejection (%) Rate Data*



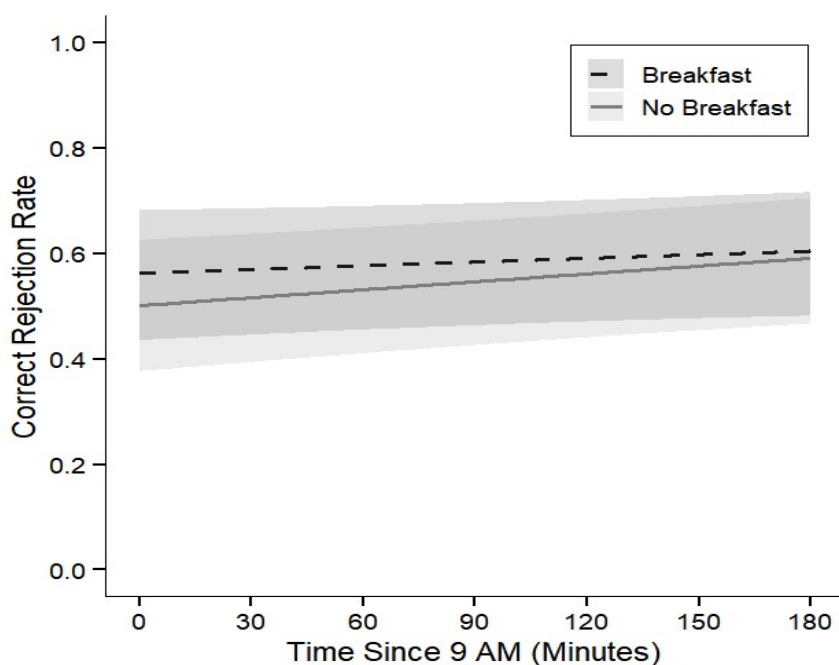
*Note:* Only the first five sessions were analysed because later sessions varied in number and consistency.

Figure 9 displays an interaction plot showing model-predicted correct rejection rate across time for breakfast and no-breakfast conditions, the influence of breakfast was statistically significant ( $p = .016$ ). Additionally, 9a.m. was a statistically significant predictor of the dog's performance with correct rejection rates improving over the course of the day ( $p$

= .014), but the interaction between these two factors was not statistically significant ( $p = .366$ ).

**Figure 9**

*Interaction Plot Showing Model-Predicted Correct Rejection Rate Across Time for Breakfast and No-Breakfast Conditions. Shaded Regions are 95% Confidence Intervals.*



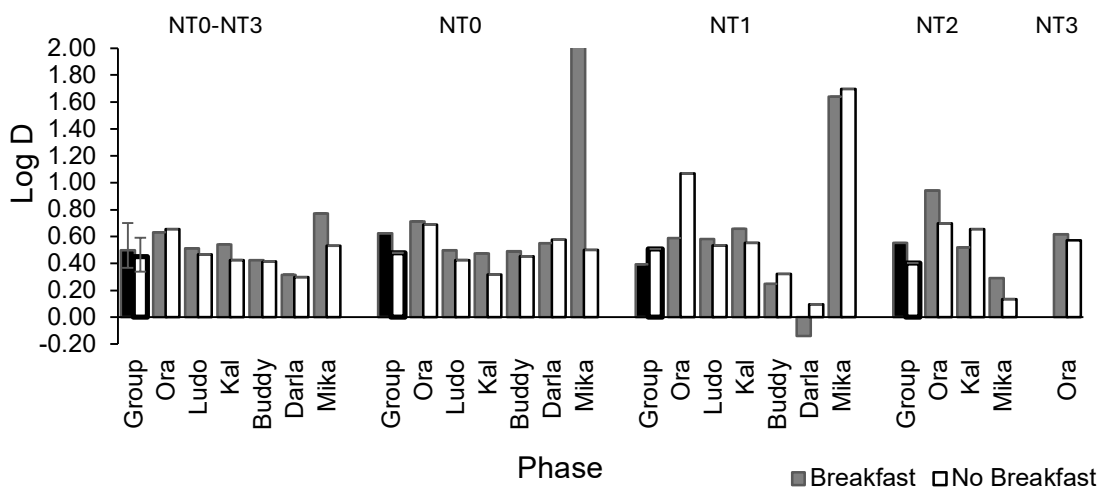
### 3.3 Log $d$

Figure 10 displays the log  $d$  discrimination performance for all dogs across all phases NT0 through to NT3, including overall performance and performance within each individual phase. Log  $d$  measures how accurately dogs discriminate the target mixture from the non-target mixture, with higher values indicating stronger discrimination. The overall log  $d$  across all dogs and phases in the breakfast condition ( $M = 0.50$ , 95% CI [0.37, 0.70]) was comparable to the no breakfast condition ( $M = 0.45$ , 95% CI [0.34, 0.59]). Overall, the dogs

showed lower discrimination on breakfast days, except in phase NT1, where discrimination was lower on non-breakfast days. On non-breakfast days phases NT0 and NT2 showed very low discrimination, while phases NT1 and NT3 showed low discrimination. Mika demonstrated the highest overall  $\log d$  values on breakfast days. Her NT0 phase discrimination on breakfast days was higher, with performance more than double that observed on non-breakfast days. Similarly, during the NT1 phase, Mika showed higher discrimination than the other dogs across both conditions, although discrimination was slightly higher on non-breakfast days. Ora showed the highest overall values on non-breakfast days, with most phases showing low discrimination, and NT2 showing higher discrimination on non-breakfast days. Darla had the lowest overall discrimination in both breakfast and non-breakfast conditions, with NT1 phase on breakfast days showing a negative  $\log d$  value, indicating no discrimination between target and non-target samples.

**Figure 10**

*All Dogs Log d by Phases Data*

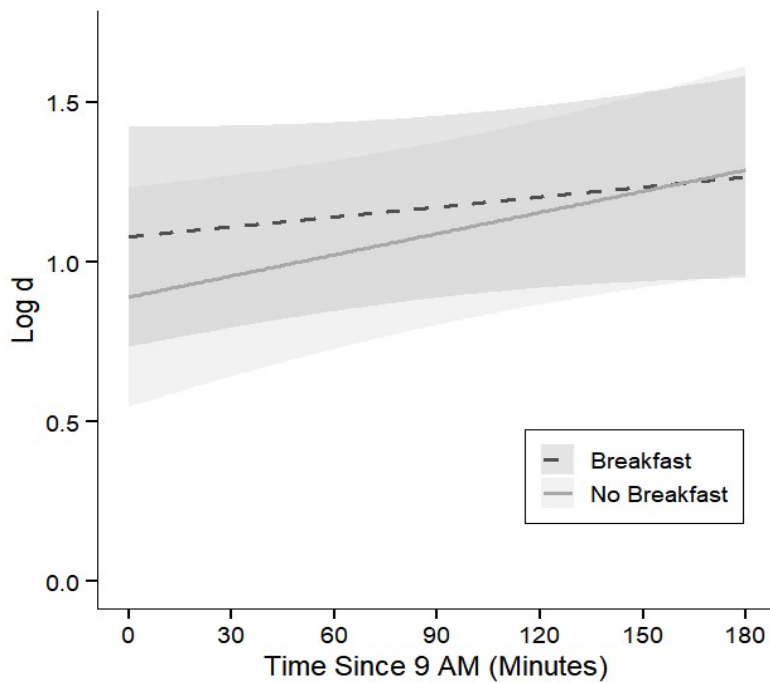


*Note:* Error bars on Group columns in all phases represent 95% confidence intervals (CI).

Figure 11 displays an interaction plot showing model-predicted  $\log d$  across time for breakfast and no-breakfast conditions, there was no significant difference between performance in the two breakfast conditions ( $p = .318$ ), but there was a statistically significant influence of time since 9a.m. on  $\log d$  ( $p = .026$ ), with accuracy improving across the day, but no significant interaction between these two factors ( $p = .395$ ).

**Figure 11**

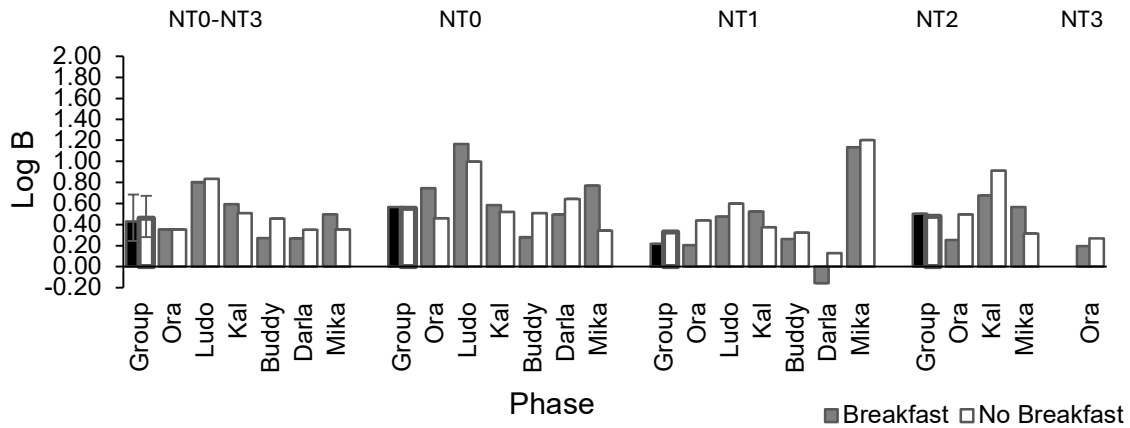
*Interaction Plot Showing Model-Predicted Log  $d$  Across Time for Breakfast and No-Breakfast Conditions. Shaded Regions are 95% Confidence Intervals.*



### 3.4 Log B

Figure 12 displays the log B response bias performance for all dogs across all phases NT0 through to NT3, including overall performance and performance within each individual

phase. Log B measures response bias toward indicating the target mixture versus the non-target mixture. Positive values indicate the dog has a bias toward responding to target mixtures, and negative values indicate the dog has a bias toward responding to non-target mixtures. The overall log B across all dogs and phases in the breakfast condition ( $M = 0.43$ , 95% CI [0.24, 0.68]) was comparable to the no breakfast condition ( $M = 0.46$ , 95% CI [0.28, 0.67]). Overall, the dogs showed a positive bias toward responding to the target mixture throughout (with one exception). This bias was slightly stronger on non-breakfast days. Across phases and all dogs NT0 and NT2 showed stronger target response bias on breakfast days, whereas NT1 and NT3 showed lower target response bias on non-breakfast days. At the individual-dog level, Darla showed the lowest log B values across both breakfast and non-breakfast conditions, reflecting a low target bias, which was slightly lower on breakfast days. She also showed a negative value in NT1 on breakfast days, indicating a bias toward non-targets in that phase. Ludo showed the highest log B values, exceeding the group average on both breakfast and non-breakfast days, indicating a higher bias toward responding to the target. This bias was slightly higher on non-breakfast days. In phase NT0, Ludo also showed the highest bias toward the target mixture on breakfast days. Mika displayed the highest target response bias in NT1 on both breakfast and non-breakfast days, slightly higher on non-breakfast days. Kal showed highest target response bias in phase NT2 on both breakfast and non-breakfast days, again, slightly higher on non-breakfast days.

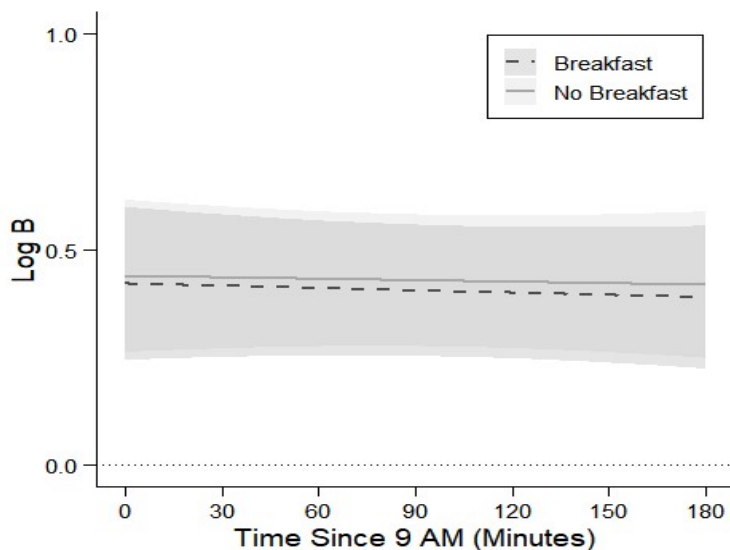
**Figure 12***All Dogs Log B by Phases Data*

Note: Error bars on Group columns in all phases represent 95% confidence intervals (CI).

Figure 13 displays an interaction plot showing model-predicted log B across time for breakfast and no-breakfast conditions, there were no statistically significant main effects ( $p = .467$ ), time effects ( $p = .807$ ), or interaction effects ( $p = .922$ ).

**Figure 13**

*Interaction Plot Showing Model-Predicted Log B Across Time for Breakfast and No-Breakfast Conditions. Shaded Regions are 95% Confidence Intervals.*



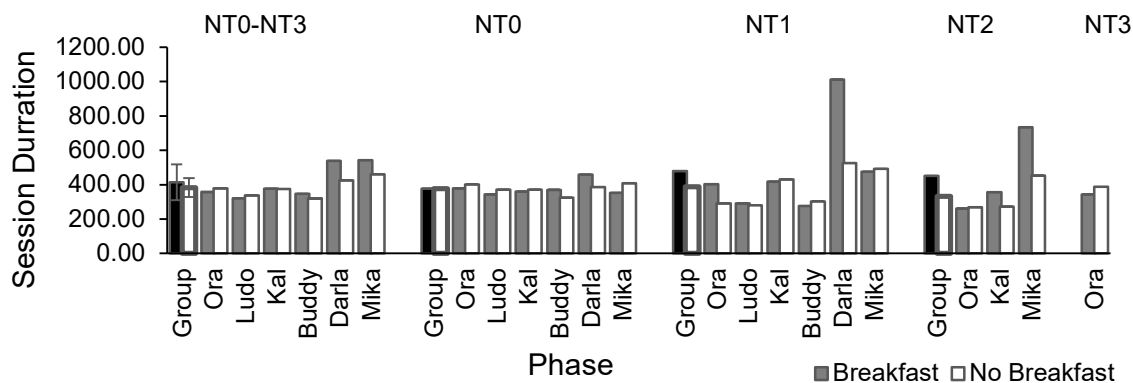
### 3.5 Session Duration

Figure 14 displays the session duration performance for all dogs across all phases NT0 through to NT3, including overall performance and performance within each individual phase. Session duration measures the total time from the dog's first interaction with the apparatus to their final interaction for each session. The overall session duration across all dogs and phases in the breakfast condition ( $M = 414.00$  s, 95% CI [308.93, 519.06]) was comparable to the no breakfast condition ( $M = 382.96$  s, 95% CI [327.79, 438.14]). Overall, there was only a slight difference in average session duration between breakfast and no breakfast days, with breakfast days showing marginally longer average durations across all phases. Phases NT0 and NT3 showed slightly longer session durations on non-breakfast days,

whereas phases NT1 and NT2 showed longer session times on breakfast days. Darla consistently had longer average session times than the group on breakfast days across all phases. Her session duration in NT1 on breakfast days was the longest observed, more than double the group average. Mika also showed longer-than-average session durations on breakfast days in phases NT1 and NT2, with her longest session duration occurring in NT2 during breakfast. Overall, both Darla and Mika had longer average session times on breakfast days compared to the group. In contrast, Ora's session durations were generally longer on non-breakfast days, except for NT1. Buddy's longest session durations were in NT0 during breakfast days, Kals longest session duration was in NT1 during non-breakfast days, while Ludos longest session duration was in NT0 on non- breakfast days.

**Figure 14**

*All Dogs Session Duration by Phases Data*



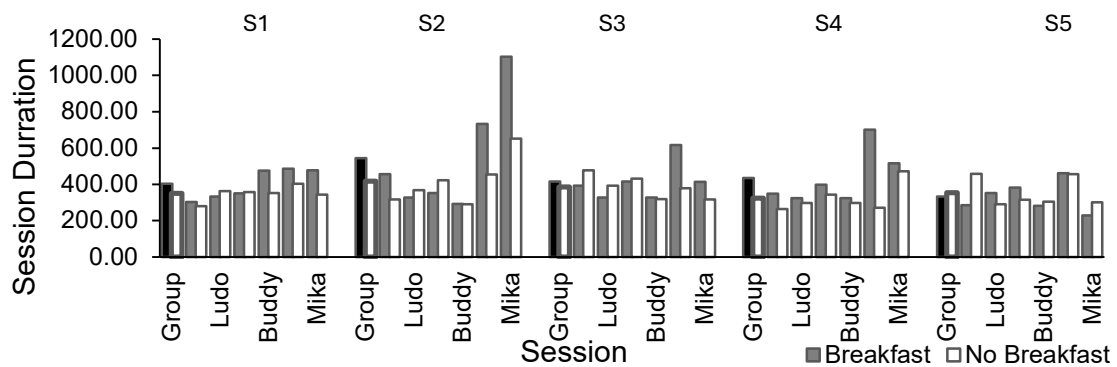
*Note:* Error bars on Group columns in all phases represent 95% confidence intervals (CI).

Figure 15 displays session duration performance for all dogs across sessions 1 to 5. There was some variability in how many sessions each dog participated in each day and over the data collection period. Overall, session duration was fastest on breakfast days for all sessions except session 5, where non-breakfast days were slightly faster. The fastest session

during breakfast was session 5, while the fastest non-breakfast session was session 4. The slowest session was session 2 for both conditions. Darla had the longest session duration across all breakfast session except session 2 and in non-breakfast session 1. Mika had the fastest session across the group during the breakfast condition in session 5. Mika had the longest session across both conditions in session 2, more than double the group average.

**Figure 15**

*All Dogs Session 1-5 Session Duration Data*

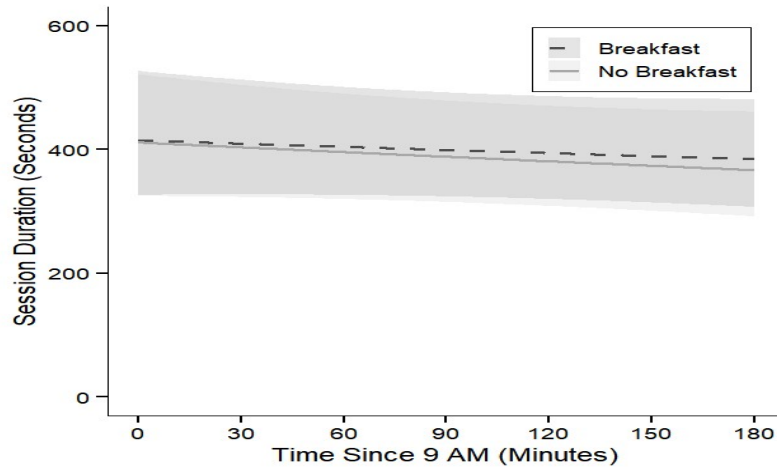


*Note:* Only the first five sessions were analysed because later sessions varied in number and consistency.

For session duration, there were no statistically significant main effects ( $p = .459$ ), time effects ( $p = .266$ ), or interaction effects ( $p = .788$ ).

**Figure 16**

*Interaction Plot Showing Model-Predicted Session Duration Across Time for Breakfast and No-Breakfast Conditions. Shaded Regions are 95% Confidence Intervals.*



## Discussion

In the present study, we hypothesised that the dogs would perform more accurately on the scent-detection task after eating breakfast, as the absence of breakfast may increase the likelihood of false-alarm indications due to poorer stimulus discrimination. Specifically, we predicted a liberal bias shift; dogs who had not eaten were expected to treat a greater number of samples as S<sup>D</sup>s, whereas, dogs who had eaten were expected to elicit fewer indication responses, resulting in more non-target samples being correctly rejected.

### *4.1 Summary of Findings*

Overall, the dogs in this study demonstrated consistently high hit rate accuracy, 89% across all phases in both conditions, and across individual sessions, ranging from 87%-93% for breakfast and 82% 94% for non-breakfast conditions. While these observed differences followed the predicted direction, they were not statistically significant based on feeding status. However, the correct rejection rate was significantly influenced by feeding conditions, with higher correct rejection rates observed on breakfast days. This finding supports the hypothesis that satiety leads to higher correct rejection rates and fewer false alarms. This aligns with the prediction that food deprivation leads to a liberal response bias, wherein dogs are more likely to indicate on non-target samples (Voss et al., 1993). This suggests that stimulus control remains strong for the target scent; however, the presence of non-target scent under deprivation leads to a broadened generalisation gradient, resulting in an increased probability of false-alarm indications (Terrace, 1963).

Analysis of log *d* values generally indicated stronger discrimination on breakfast day; however, the effect was non-significant. Notably, sensitivity significantly improved as a function of time of day. Phase-specific variations were observed, particularly in NT1, where performance on non-breakfast days exceeded that of breakfast days. While these

observed differences followed the predicted direction. As hypothesised, session duration was longer on breakfast days, particularly for Darla and Mika. This likely reflects an AO effect, where the reduced reinforcing value of food reward under these conditions resulted in decreased task engagement and slower response speeds (Laraway et al., 2003; Michael, 1993).

A notable finding was the significant influence of time (minutes elapsed from 9a.m. since breakfast or no breakfast) on both correct rejection rates and  $\log d$ . As the day progressed, dogs demonstrated a significant increase in discrimination between target and non-target odours and a greater ability to correctly withhold responses to non-target stimuli. This improvement suggests that food reinforcement provided during the session may have functioned as an AO. Specifically, the cumulative consumption of within-session reinforcers may shift the dogs toward a post-breakfast state (Skinner, 1953), illustrating the influence of MOs. While the value of food reinforcement was initially enhanced by an EO of food deprivation (Michael, 1993). This process likely facilitates a refinement of stimulus control from a broad stimulus generalisation to narrowed stimulus control (Terrace, 1963). Consequently, the higher  $\log d$  values in later sessions suggest that the dogs' discrimination became more refined as the evocative effect of the  $S^D$  increased (Chan et al., 2005; Turner & Balleine, 2024). However, other factors may have been responsible for or contributed to this finding.

Significant individual differences were evident across participants, with dogs such as Ora, Ludo, and Mika demonstrating notable variability in performance across phases and sessions. These individual variations impacted group means and the statistical analysis. For instance, Ora progressed to phase NT3 and showed higher accuracy on non-breakfast days. In contrast, Ludo displayed a consistently high target bias, while Mika achieved the highest  $\log d$  on breakfast days. These variations suggest that the influence of MOs is modulated by each

dog's history of reinforcement (Skinner, 1953). Consequently, these findings highlight that the ideal feeding protocol is dog dependent, while deprivation may sharpen discrimination for some, it may weaken stimulus control for others, increasing the frequency of false-alarm indications.

The present findings partially support the initial hypothesis that breakfast enhances overall task accuracy by reducing false alarms and increasing correct rejection rates. While correct rejection rates improved under breakfast conditions, hit rate and log  $d$  values showed only marginally or inconsistent differences between feeding conditions. The expected directional bias shift (higher false alarms in non-breakfast conditions and fewer in breakfast conditions) was only partially observed. Specifically, this indicates that the dogs demonstrated a bias— indicating non-target stimuli more frequently in the non-breakfast condition.

#### *4.2 Implications*

Currently, research on dogs' performance relative to feeding status is limited, and to date, both Miller and Bender (2012) and Chan et al. (2005) investigating this phenomenon, albeit through a different methodology. The present study extends and refines their findings by examining the effects of feeding state within a scent-detection task and utilising SDT allowed us to explore the role of motivation-relevant processes on their performance. Our findings suggest that while sensory sensitivity remained stable, variations in accuracy were functionally related to MOs that altered the evocative effect of the target and non-target stimuli. Miller and Bender (2012) tested dogs on a memory-search task at two time points (30- and 90-minutes non-breakfast and no breakfast) and reported significantly higher accuracy 30 minutes after feeding. They attributed this effect primarily to glucose-based cognitive enhancement, while noting that changes in motivational mechanisms consistent with AO processes may also have contributed to performance differences. However, their

design did not allow for direct testing of these motivational mechanisms. Chan et al. (2005) tested dogs' accuracy on visuospatial memory tasks using a three-component delayed-non-matching-to-position task with two pre-feeding conditions (one meal portion and two meal portions before the task). They reported that while a double portion (AO) significantly increased response latency, it had no significant effect on accuracy across the dog groups. These findings led Chan et al. (2005) to conclude that satiety influences a dog's motivation to perform rather than their cognitive capacity or memory.

In the present study, we assessed scent-detection performance across extended testing periods (3-5 sessions over 2.5 hours) under both breakfast and no-breakfast conditions. The task required dogs to discriminate between target and non-target stimuli. To reduce the potential for ceiling or floor effect, the stimulus overlap between the target and non-target odours was increased across experimental phases. This progression required stronger discriminative control to maintain accuracy as the target and non-target odours became more formally similar. Rather than relying on accuracy measures, which can mask the underlying behavioural processes, SDT was used to distinguish sensory sensitivity from response bias (Swets & Green, 1978; Nevin, 1969). Results revealed that breakfast (AO) primarily increased correct rejection rate of non-target stimuli. Under these conditions, dogs demonstrated narrower stimulus control, with higher probability of withholding the response in the presence of non-targets, thereby increasing correct rejection rates. Conversely, withholding breakfast (EO) lowered session duration and increased responding to both target and non-target stimuli, consistent with a more liberal bias. These findings support a MO interpretation, which predicts a directional shift in response bias depending on reinforcer value, whereas a glucose-based explanation would predict changes in overall performance (accuracy) without specifying bias (Michael, 1982, 1993; Edwards et al., 2019).

Importantly, while Miller and Bender (2012) observed a transient feeding effect that diminished over time, and Chan et al. (2005) observed that the AOs primarily influenced the response latency without altering accuracy, our data demonstrates that in scent detection, AOs modulate the precision of stimulus control. The present study demonstrated consistent AO/EO effects on correct rejection rates across all phases. This occurred alongside significant time-dependent improvements in sensory sensitivity ( $\log d$ ). The findings suggest that motivational variables likely account for feeding-related performance differences, or possibly in addition to glucose-mediated cognitive enhancement. This aligns with findings by Chan et al. (2005) and China et al. (2020), who suggest that internal states and reward expectancy significantly modulate the precision of stimulus control. Taken together, the present findings provide empirical support for the motivational mechanisms that Miller and Bender (2012) acknowledge but were unable to evaluate directly, while also demonstrating that these effects depend on the functional interaction of MOs and the strength of stimulus control.

The functional interaction between MOs and stimulus control observed here suggests that scent-detection performance is not a static measure of sensory capacity, but a dynamic behavioural process. A critical contribution of the present methodology was the use of overlapping target and non-target stimuli. While standard scent detection tasks typically utilise distinct, easily discriminable stimuli, the present study intentionally increased the formal similarity between  $S^D$  and  $S^A$ , challenging the dogs' discrimination learning (Terrace, 1963; Williams & Johnston, 2002). Specifically, while an EO may lead to increased generalised responding, the AO serves to confine the gradient. This facilitates a transition from broad stimulus generalisation to narrowed discriminative control, ensuring that the dog's response is evoked by the target  $S^D$  rather than formally similar non-target stimuli.

In an applied sense, this implies that managing a dog's feeding state is a strategic tool to maintain the integrity of discrimination when target and non-target stimuli are similar.

These findings reveal that accuracy in complex environments is not only a matter of olfactory threshold but is heavily dependent on how MOs narrow or broaden the dogs' response range. In instances where the cost of a miss is high such as explosive detection or forensic searching, having an EO in effect (no breakfast) might improve hit rates at cost of reduced correct rejection rate (or increased false alarm rate). In these high stake scenarios, maintaining an EO state may be preferable to ensure the dog remains under tight stimulus control. Moreover, in scenarios where the target is rare and the cost of a miss is significantly higher than false alarm, an EO state might be strategically employed to ensure the dog maintains a liberal response criterion and a high level of vigilance.

The findings of this study both support and extend the work of Miller and Bender (2012) and Chan et al. (2005) regarding the role of MOs in canine scent detection. While these researchers demonstrated that the EO of food deprivation increased search persistence, response frequency, and speed, a behavioural effect further corroborated by Parrish et al. (2016), who examined whether self-control was improved in capuchin monkeys after eating breakfast following a 12-hour fast, finding that while glucose ingestion was a factor, it did not yield a consistent performance boost. The results of the present study suggest that MOs are an important contributor to the underlying mechanisms of this effect.

#### *4.3 Limitations and Recommendations for Future Research*

There were several limitations in the current study. Firstly, the lack of standardisation in the type and amount of food provided to each of the dogs; owners followed their usual feeding routine, leading to differences in meal sizes, food types, and calorie content across dogs and sessions. This variability could have affected the dogs' level of satiety, thereby influencing task performance. To address this in future research, studies could implement a controlled feeding protocol that standardises meal type and portion size based on nutritional recommendations. Providing standard meals could minimise variability in group data and

ensure that observed behavioural differences are attributed to the experimental conditions and not dietary factors.

Secondly, researcher bias may have influenced breakfast and no breakfast conditions. Prior to the blind breakfast being introduced, the research was aware of the dogs' breakfast and no-breakfast status, creating the potential for unconscious bias in handling or interpreting behaviour. Subtle differences in interactions, pacing or prompting may have influenced performance and introduced expectancy effects. Future research should implement blinding from the outset of all phases and training to reduce the possibility of researcher influence.

Thirdly, the variation in time since breakfast during sessions. Although all dogs were fed at the same time, the time that sessions were conducted and the order in which the sessions were conducted varied across the days and throughout the data collection phase. Additionally, individual differences in speed of task completion meant that some dogs took longer to work, pushing subsequent sessions later, while others completed sessions quickly. This resulted in inconsistent intervals between feeding and testing, which may have influenced task performance. Although the analytical approach corrected for this by looking at time since breakfast as a continuous factor to address this in future research, session scheduling should be standardised, either by fixing start times for each session and each dog or by accounting for individual differences in task duration when planning the order of testing. Consistently controlling the time since feeding would reduce variability and allow for a clearer assessment of the effects of breakfast and no breakfast on performance.

Building on the current findings, future research could further extend these insights by exploring the mechanisms of stimulus control through a segmented exposure structure. In the present study, dogs were exposed to both  $S^D$  and  $S^A$  stimuli from the outset (shaping, discrimination training and all phases). While this approach reflects applied training practice, such a design can obscure the distinction between acquisition of stimulus control and

stimulus generalisation. To refine this, a phased mastery could be implemented prior to advancement between phases. This would begin with  $S^D$  and  $S^A$  stimuli with zero overlap to establish a clear baseline of accuracy. Once mastery criteria are met for a given phase, test probes could be introduced to assess learning without reinforcement. These probes would feature overlapping non-targets stimuli from both the achieved phase and the subsequent phase. Including stimuli from both phases in a test probe serves to determine if the dog can identify the target when faced with competing distractors. This would allow for a more precise assessment of generalisation gradients, clarifying whether performance aligns with expected patterns under conditions of progressive similarity.

Finally, examining these effects using a line-up arrangement would enhance the ecological validity of the findings. Evaluating performance in a line-up would clarify whether the effects of feeding status on response bias, as identified in this study, persist in common operational scenarios where a dog must reject multiple distractors, thereby bridging the gap between laboratory findings and real-world scent detection contexts.

#### *4.4 Conclusion*

The present study demonstrates that MOs influence dogs' scent-detection performance. Specifically, dog's feeding state functions as the MO that modulates the strength of stimulus control by odours. While hit rates remained consistently high across both feeding conditions, when dogs did have breakfast (an AO), they demonstrated significantly higher correct rejection rates and a more conservative response bias. The improved correct rejection rates indicated enhanced stimulus control under satiation. In contrast, when dogs did not have breakfast (an EO), the increased reinforcing value of food evoked a liberal response bias, resulting in higher hit rates and lower correct rejection rates, this shift is consistent with MO theory. These findings highlight that operational accuracy is not merely a matter of sensory acuity, but a dynamic interaction between MOs and the precision of stimulus control.

Specifically, the results demonstrate how EOs of food deprivation enhance the value of reinforcement, which in turn alters stimulus generalisation gradients, shifting the subject's the behaviour from narrow stimulus control to broad generalisation This has important theoretical implications and implications for dog trainers and handlers.

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## *Appendices*

### **Appendix A: Standard Operating Procedure for Chemical Preparation and Acid Washing.**

Important: When entering the chemistry laboratory, a MAF-approved lab coat, safety glasses, covered shoes and clean latex/nitrile gloves must be always worn.

#### **Solution Preparation**

*Equipment needed for chemical mixing:*

- Gloves
- 5 mL pipette tip
- Purple autopipette set to 5ml
- Yellow pipette tip
- Yellow autopipette set to 5 $\mu$ L
- 50 mL beaker
- 5 mL breaker
- 40 mL Vials
- Lid
- Lid seal
- Chemical
- Paper towels
- Bucket of water
- Vivid

#### ***Mixing chemicals***

1. The ratio of water to chemical concentrate is 2000:1. For 10 mL of oil, use 5 $\mu$ L chemical concentrate.

2. Wipe down all surfaces where samples will be prepared (including in the hood) with isopropyl or ethyl alcohol.
3. First, ensure you have clean gloves on.
4. Grab a grey tray from under the sink to put the equipment in.
5. Label the bottle with the chemical name and date and move it away.
6. Collect 40 mL of mineral oil into the 50 mL beaker.
7. Using the purple pipette with attached tip, press down the pipette button to the first stop and place it into the oil then release the button to collect 5 mL sample of oil.
8. Place the pipette into the vial and press the button down all the way to release the oil.
9. Repeat previous two steps to collect a total of 10 mL of oil.
10. Under the hood, carefully pour a small amount of the chemical into the small beaker (concentrated chemicals are only processed under the hood)
11. Using the yellow pipette with attached tip, press down the pipette button to the first stop and place it into the chemical then release the button to collect 5  $\mu$ L.
12. Place the pipette into the Vial and press the button down all the way to release the chemical.
13. Dispose of the tip by wrapping it in a paper towel and placing it into the rubbish bin.
14. Place the clear side of the lid seal into the lid and the brown side onto the vial, place the vial and screw until it is tight.
15. Dispose of any unused chemical into the non-chlorinated and non-halogenide solvents only waste jar.
16. Place all glassware into the dirty container to be acid washed.
17. Wipe down the grey tray, the hood and workspace with isopropyl, and dispose of the gloves and paper towels.

For subsequent samples, change gloves and glassware between samples to avoid cross-contamination. When finished, wipe down all surfaces with isopropyl or ethyl alcohol.

### **Acid Washing**

*Equipment you will need:*

- Gloves
- Acid washing gloves
- Apron
- 10 mL Bucket of tap water (for removing glassware)
- Small container of tap water (for tongs after use)
- Tongs
- Paper towels for any spills

### ***Placing glassware into the bath***

Ensure all liquids in the glassware have been disposed of appropriately and remove any marker or label adhesive using ethyl alcohol.

1. All glassware must be thoroughly washed using detergent and water at the sink.
2. Glassware must be rinsed with distilled water before placing it in the acid
3. Place glassware in a 10 L bucket and take it to the fumehood
4. Fill a small container with tap water and place it in the fumehood so that the tongs covered in acid can be placed in at the end.
5. Put on the apron and green acid-proof gloves
6. Remove the lid of the acid bath and set it aside.
7. Using the tongs, pick up a piece of glassware and slowly place it in the acid bath with the bottom going in first. This ensures the water slowly fills the glassware and all air bubbles are removed.
8. If there are air bubbles, take the glassware back out and try again.

9. Once all the glassware is in the acid bath, put the lid back on the acid bath.
10. Place the tongs into a small container of water. Take this to the sink and turn the water on so it overflows refresh the water and dilute the acid. IMPORTANT: ensure that the container is not blocking the drain.
11. Wipe any residue in the surrounding area (especially where the lid was sitting) with cold water and paper towels. Ensure the paper towels have been rinsed before disposing of them in the bin under the sink.
12. Rinse the green gloves under running water to remove any potential acid.
13. Turn off the tap, remove and dry the tongs.
14. Leave the glassware in the acid bath overnight.

***Removing the glassware from the bath***

1. Put on the apron and gloves and grab a pair of tongs.
2. Fill the bucket half full of cold tap water. Depending on the amount and size of glassware, you may need more water to ensure that everything from the acid bath is submerged.
3. Remove the lid from the acid bath and place it aside.
4. Carefully remove glassware one by one using the tongs, ensuring all the acid is tipped out before placing it in the bucket. The glassware needs to be submerged with no air bubbles.
5. Once all the glassware is collected in the bucket, place tongs into the bucket then place the bucket in the sink (avoiding covering the drain) and run cold water into the bucket for 5-10 minutes so that the water overflows and dilutes the acid.  
IMPORTANT: ensure that the container is not blocking the drain.
6. Rinse each piece of glassware individually with running tap water, then 3 times with distilled water to ensure it is rinsed thoroughly.

7. Tip the remaining water out of the glassware and put it in a metal wire basket.
8. Place the basket in the drying oven until glassware is dried.

***Removing glassware from the oven***

1. Wearing a pair of oven gloves, unlock the oven door and take your tray out and place it on the scent detection bench.
2. Ensure the oven door is shut and locked properly.
3. Once cooled down, remove the glassware from the tray and return it to its appropriate places.

To avoid occupying acid bath and oven space, remove the glassware from the acid and oven within 24 hours, and no longer than a 48-hour period, except over the weekend. Always place general laboratory glassware back in the appropriate place after it has been removed from the oven. When exiting, ensure that you remove your lab coat and wash your hands.

## **Appendix B: Standard Operating Procedure for Training Dogs to Use Six-channel Olfactory Stimulator**

### **1. Purpose**

This standard operating procedure (SOP) provides guidelines and standard procedures to be adopted during all phases of this experiment.

### **2. Apparatus Setup**

This section outlines the requirements for apparatus setup.

- 2.1.1. Start by positioning the apparatus in the notches on the shelf in the laboratory room, ensuring the nose port and lever face the front and are accessible to the dogs.
- 2.1.2. Remove any objects in the room that could cause distraction.
- 2.1.3. Connect the airline, power cable, serial connector, feeder control, and extractor fan to the corresponding ports on the back of the apparatus.
- 2.1.4. Set the rotameters to 0.3 mL/min for odour and 3.5 L/min for air.
- 2.1.5. Place the feeder 3 m in front of the apparatus.
- 2.1.6. Ensure the laboratory room light is on, the exterior door is securely closed and latched, and the room is appropriately lit for clear observation through the cameras.
- 2.1.7. Load odours into predetermined channels. Channel 1 Amyl Acetate, channel 2 Benzaldehyde, channel 3 Hexanol, channel 4 Benzyl Acetate, channel 5 Butyl Acetate and channel 6 Ethyl Butyrate.
- 2.1.8. Switch on the airline and air compressor. Ensure the main air pressure set to 2 bar.
- 2.1.9. Ensure there are no air leaks around the odours and air hoses. Test each odour sample by sniffing the port with the correct odour channel active. The odour should be just noticeable.

### 3. Software Setup

This section outlines the software used and the procedures for setup. Ensure that the control systems and observation station in the adjacent room are fully prepared and operational for monitoring and managing the apparatus during use.

“Six scents” is the software used to manage the apparatus. The threshold is set in file *Dognameconfig.txt*. The trials odour code and whether reinforcement will be given are loaded into file *Dognametrialorder.csv*. The data is automatically collected for each session and loaded into file *Dognamedata.csv*. An Excel randomising tool is used to randomise each session during the phases.

iSpy is the CCTV software used to record all working sessions with the dogs. There are two cameras set up capture every angle of the room including left and right angle of the apparatus. iSpy software is loaded onto the laboratory computer, and each session is recorded prior to the dog entering the room and terminated when the session is finished.

- 3.1. Switch on both computers and load Six scents software and iSpy
- 3.2. Open six scents 2.2 document folder and copy and paste files *dognametrialorder.csv*, *dognamedata.csv* and *dognameconfig.txt*, and rename *dogname* with the dog's name.
- 3.3. Open *dognametrialorder.csv*. and load the trial odour and reinforcement code according to the phase. Refer to *Table 4 - Non-Target odour key Combination Options by phase*. Reinforcement (1) is given for target trials and no reinforcement (0) for non-target trials.
- 3.4. The Randomising tool is used to shuffle the trial sequence for each session in the training phases. Load the non-target phase code (listed in table 4 *Non-Target odour key Combination Options by phase*) into the randomised tool and shuffle.
- 3.5. Standard proportions are set at 10 targets during nose hold shaping, 10 non-targets during lever press shaping, 6 target and 5 non-targets during alternating target and

non-target training and 10 target trials and 10 non-target trials during phase training.

Target and non-target proportion adjustment rules are based on the data.

3.6. Open *dognameconfig.txt*. And load "*minsif*" and "*possif*" according to the phase.

indication threshold is increased in 500 ms increments, to a minimum of 4500 ms and a maximum of 6000 ms.

Standard threshold:

- Shaping (nose hold & lever press): 500 ms observation, 500 - 1500 ms indication.
- Alternating target and non-target: 500 ms observation, 1500 ms indication".
- Phase training: 500 ms observation, 1500-6000 ms indication.

The threshold adjustment rules based on the data.

#### **4. Initial Training phase**

This section outlines the requirements for basic training.

##### **4.1. Introduction and Habituation**

The dog explores the room and facility and becomes habituated to the environment and researchers prior to the commencement of training sessions. During the early stages, sessions should be terminated at the first sign of fatigue or disinterest. Early sessions should not exceed 10 minutes.

##### **4.2. Conditioned Reinforcer Establishment**

Use the wireless remote to dispense food from the automatic feeder until the dog immediately approaches upon activation. The Dog should approach the feeder upon hearing activation and consume food within three seconds three times in a row before continuing training.

##### **4.3. Shaping of Nose to Sample Port**

Once the sound of the feeder has been established as a conditioned reinforcer, the remote is used to train the dog to put its nose into the sample port. Use differential reinforcement of successive approximations for this target behaviour. Prompting may be used, but the prompt must be faded and removed before proceeding to the next steps. Set target trial order to standard proportions (refer to 3.3). Set positive and minimum sniff threshold to 500 ms. Begin training by allowing the dog to sample the nose port, using shaping techniques as needed until it reliably triggers the feeder automatically. Once the dog successfully completes ten trials per session at the 500 ms indication threshold, gradually increase the threshold in 500 ms increments. Continue this process until the dog consistently completes ten trials in one session at the 1500 ms threshold. Upon successful completion of the 1500 ms session, proceed to the next steps.

#### **4.4. Shaping: Lever Activation**

Once shaping of the nose port has been completed at 1500 ms indication threshold, the remote is used to train the dog to press the lever. Use the method of differential reinforcement of successive approximations for this target behaviour. Prompting may be used and the prompt must be faded and removed before proceeding to the next steps. Set non-target trial order to standard proportions (refer to 3.3).

Depending on the specific dog, appropriate topography should be selected for shaping. Once the lever has been activated ten times without prompts (and reinforced through activation of the feeder), proceed to the next steps.

#### **4.5. Alternating target and non-target training**

This next step requires the dog to complete the entire sequence of behaviours, attending to the odour port for the target odour and pressing the lever for non-target odour to begin and if correct, the dog goes to the feeder for reinforcement. Set the

trial order to standard proportions (refer to 3.3) starting and ending with the target trial. Set positive sniff threshold to 1500 ms and minimum sniff threshold to 500 ms. Allow the dog to sample the port. If no nose hold is performed for more than 2 positive trials, use prompting but fade as soon as possible. The same should be done for lever presses with non-target trials. Once the dog is performing both behaviours independently, fade yourself out of the experiment room. Continue the procedure until the dog achieves a 100% hit rate in a single session or completes a maximum of three full sessions and proceed to the next steps.

## **5. Phase Training Increase of Target Overlap**

This section outlines the standard procedures for randomisation of the non-target sample through all phases.

### **5.1. Randomisation of Target Vs 1 Non-Target (NT0)**

This phase has one non-target, refer to table 4 for the NT0 non-target list. Start training by setting target and non-target trial order to standard proportions (refer to 3.3). Randomise each session (as stated in Section 3.5). Set positive sniff threshold to 1500 ms and minimum sniff threshold to 500 ms. Once the dog achieves a 100% hit rate in one session, increase the indication threshold by 500 ms. Continue increasing the threshold in 500 ms increments, to a minimum of 4500 ms and a maximum of 6000 ms. Maintain this progression until both the hit rate and correct rejection rate are above 80% for three consecutive sessions. Once these criteria are met, proceed to the next steps.

### **5.2. Target vs 1 Target Overlap and 1 or 2 Non-Targets NT1**

This phase includes one target overlap of the non-target resulting in twelve non-target combinations, refer to table 4 for the NT1 non-target list. Randomise each session (as stated in Section 3.5). The indication threshold, target and non-target trial proportions

are defined in the previous phase. Continue training until both the hit rate and correct rejection rate exceed 80% for three consecutive sessions. Once this criterion is met, proceed to the next phase.

### **5.3. Target vs 2 Target Overlaps and 1 or 2 Non-Targets NT2**

This phase includes two-target overlap of the non-target resulting in twenty-three non-target combinations, refer to table 4 for the NT2 non-target list. Randomise each session (as stated in Section 3.5). The indication threshold, target and non-target trial proportions are defined in the previous phase. Continue training until both the hit rate and correct rejection rate exceed 80% for three consecutive sessions. Once this criterion is met, proceed to the next phase.

### **5.4. Target vs 3 Target Overlaps and 1 or 2 Non-Targets NT3**

This phase includes three-target overlap of the non-target resulting in fifteen non-target combinations, refer to table 4 for the NT3 non-target list. Randomise each session (as stated in Section 3.5). The indication threshold, target and non-target trial proportions are defined in the previous phase. Continue training until both the hit rate and correct rejection rate exceed 80% for three consecutive sessions. Once this criterion is met, proceed to the next phase.

### **5.5. Target vs 4 Target Overlaps and 1 or 2 Non-Targets NT4**

This phase includes four-target overlap of the non-target resulting in three non-target combinations, refer to table 4 for the NT4 non-target list. Randomise each session (as stated in Section 3.5). The indication threshold, target and non-target trial proportions are defined in the previous phase. Continue training until both the hit rate and correct rejection rate exceed 80% for three consecutive sessions. Once this criterion is met, the experiment has ended.

**Table 4***Non-Target odour key Combination Options by phase*

<i>NT0</i>	<i>NT1</i>	<i>NT2</i>	<i>NT3</i>	<i>NT4</i>
6	5,1	5,1,2	5,1,2,3	1,2,3,4,5
	5,2	5,1,3	5,1,3,4	1,2,3,4,6
	5,3	5,1,4	5,2,3,4	1,2,3,4,5,6
	5,4	5,2,3	5,1,2,4	
	6,1	5,2,4	6,1,2,3	
	6,2	5,3,4	6,1,3,4	
	6,3	6,1,2	6,2,3,4	
	6,4	6,1,3	6,1,2,4	
	5,6,1	6,1,4	5,6,1,2,3	
	5,6,2	6,2,3	5,6,1,3,4	
	5,6,3	6,2,4	5,6,2,3,4	
	5,6,4	6,3,4	5,6,1,2,4	
		5,6,1,2		
		5,6,1,3		
		5,6,1,4		
		5,6,2,3		
		5,6,2,4		
		5,6,3,4		

## **Appendix C: Guidelines for Shaping**

### **1. Purpose**

This document outlines the basic training hierarchy for shaping by successive approximations. Generally, each step must be completed three times in a row before progressing to the next stage of training. Some dogs, however, may require additional learning trials before progressing. Keep sessions short (5-10 mins) and finish on a positive note, when possible, to ensure that the process is enjoyable for the dog.

### **2. Procedure**

2.1. Researcher should position themselves near the apparatus, ideally near the door, avoiding the dog's gaze to reduce unintentional cueing. This will facilitate fading of the researcher's presence during later trials when the dog is required to be in the experimental room alone. Gestural prompts may be used to facilitate training, but these should be used only as needed as they must be faded out before training is complete.

2.2. Shaping of sample port entry

2.2.1. Turn apparatus on and start a session so that the sample port beam breaks will produce a "beep."

2.2.2. Reinforce moving further

2.2.3. and further away from the feeder until the dog is reliably approaching the other side of the room (near apparatus).

2.2.4. Reinforce attending to the apparatus (putting nose near any part of the front panel).

2.2.5. Reinforce placing nose near port.

2.2.6. Reinforce placing nose in port.

2.2.7. Reinforce breaking the beam for any length of time (indicated by “beeping” sound).

2.3. Shaping of lever press

2.3.1. Turn apparatus off.

2.3.2. Reinforce any movement toward lever.

2.3.3. Reinforce movement of nose or paw toward lever (as appropriate).

2.3.4. Reinforce any contact with the lever (nose or paw, as appropriate).

2.3.5. Reinforce any movement of the lever.

2.3.6. Reinforce movement of the lever that produces the “click” (microswitch closure).

## **Appendix D: Troubleshooting for training and experimental phases.**

### **Introduction**

This troubleshooting guide provides guidelines and standard procedures to be adopted when issues arise during the training and experimental phases.

#### **1. Barking in Cage**

1.1. The dog barks excessively while in the cage and all their other needs have been met.

1.1.1. Give kibble when they are quiet and not barking. You may recruit an assistant. Train the assistant not to respond when the dog barks.

#### **2. Nose Hold Issues During Shaping**

2.1. The dog is not nose hold for observation threshold of 500 ms 100% of the time for four consecutive sessions.

2.1.1. Reduce indication threshold to 100 ms

2.1.2. Increase indication threshold by 100 ms each time the dog breaks the beam and automatically receives food 10 times in a row until 500 ms is reached.

2.1.3. Return to Standard Operating Procedure (SOP).

#### **3. Lever Press Issues During Alternating Target and Non-Target**

3.1. The dog is not nose holding for observation threshold of 500 ms required to activate the lever 100% of the time for four consecutive sessions.

3.1.1. Reduce observation threshold to 300 ms

3.1.2. Increase observation threshold by 100 ms each time the dog breaks the beam and automatically receives food 5 times in a row until 500 ms is reached.

3.1.3. Return to (SOP).

#### **4. Nose Hold Issues During *DSI***

4.1. The dog is not nose holding for their current observation threshold 100% of the time for four consecutive sessions.

4.1.1. Reduce indication threshold to 500 ms

4.1.2. Increase indication threshold by 500 ms each time they break the beam and automatically receive food 10 times in a row until 1500 ms is reached.

4.1.3. Return to SOP.

#### **5. Discrimination Rate**

5.1. If The dogs' hit rate is above 80% and the dogs correct rejection rate is less than 40% for twelve sessions.

5.1.1. Decrease the target proportion by two and increase the non-target proportions by two. Adjust proportions every four consecutive sessions or until both the hit rate and correct rejection are above 80% for three consecutive sessions. Maximum proportion adjust is four target and sixteen non-targets.

5.1.2. Return to SOP.

5.2. If the above step does not work, move to the next step:

5.2.1. Increase indication threshold in 500 ms increments, adjust the indication threshold after four consecutive sessions or until both the hit rate and correct rejection are above 80% for three consecutive sessions.

5.2.2. Return to SOP.

5.3. If the above steps do not work, move to the next step:

5.3.1. The dogs typical nose position is shallow, and the hit rate is 100%, and the correct rejection rate is less than 40% for twelve sessions.

5.3.2. Shape inner nose hold, reduce indication threshold indication threshold to 500 ms

5.3.3. Return to shaping nose in sample port in your Standard Operating Procedure (SOP).

5.4. If these steps do not lead to an increase in discrimination rate of above 50% for three consecutive sessions move to termination from the study.

## **6. Session Completion Issues**

6.1. The dog is not completing 100% of the trial set per session for four consecutive sessions.

6.1.1. Decrease the number of trials to the current average of completed trials per session.

6.1.2. Once the dog is completing 100% of the trial set per session for three consecutive sessions, increase the trials by two trials up to 20 trials.

6.2. If the above step does not work, move to the next step:

6.2.1. Decrease the indication threshold to the dog's current average nose hold time for positive trials.

6.2.2. Increase indication threshold in 500 ms increments, to a minimum of 4500 ms and a maximum of 6000 ms. Maintain this progression until both the hit rate and correct rejection rate are above 80% for three consecutive sessions.

6.3. If the above step does not work, move to the next step:

6.3.1. Conduct a paired stimuli preference assessment using Possum, kibble and other dog treats if they are available or recommended by the owner.

6.3.2. Switch to the identified highly preferred item as the reinforcer.

6.4. If the above step does not work, move to the next step:

6.4.1. Move the dog back to the previous successful phase e.g., *NTI* back to *NT0*.

6.4.2. Once the dog is completing 100% of session for three consecutive sessions, alternate between increasing the trials (4.1.2.) and increasing the indication threshold (4.2.2.) until both the hit rate and correct rejection are above 80% for three consecutive sessions.

6.4.3. Return to SOP.

6.4.4. If these steps do not lead the dog is completing 100% of session for three consecutive sessions move to termination from the study.

## **7. Study Termination Criteria**

7.1. The dog's session completions have not improved after four consecutive days, even after implementing all prior troubleshooting steps (e.g., adjusting trials, proportion of trials, indication threshold reinforcers, or phase regression).

7.1.1. The dog will be retired from the study.

## **Appendix E: Blind Breakfast Procedure**

### **1. Purpose**

This procedure ensures dogs receive breakfast in a consistent and unbiased manner while maintaining the blind conditions of the study.

### **2. Dog Arrival and Initial Setup**

2.1. Dog arrival and breakfast storage: Dogs will arrive at the lab, each with their breakfast. The researcher will place each dog's breakfast in a named, designated bowl, storing it in the sample preparation room cupboard.

2.2. Randomised breakfast schedule will be saved and accessed from a shared folder accessible only by the research assistants and not the researcher.

### **3. Breakfast Schedule**

3.1. Schedule Creation (Tuesdays & Thursdays): Using the randomised breakfast tool, the breakfast assistant will generate a feeding schedule for two consecutive days. All dogs present on Tuesdays and Thursdays will follow this same feeding schedule. The breakfast schedule will be saved to the private shared drive folder.

### **4. Early Morning Session (9:00 a.m)**

4.1. Notification and Researcher Exit: At 9:00 a.m., or as soon as the final dog has arrived, the researcher will inform the research assistant that the dogs are ready. The researcher will then leave the lab for 5 minutes.

4.2. Research Assistant's Actions:

4.2.1. The research assistant will refer to the feeding schedule.

4.2.2. On scheduled breakfast days: The assistant will remove the dogs' breakfast and plates from the cupboards, feed the dogs, remove any trace of food, and return the empty bowls to the cupboard.

4.2.3. On non-breakfast days: Each dog will be given 1-5 pieces of standard laboratory kibble.

4.2.4. Researcher returns.

## **5. Late Morning Session (11:50 a.m.)**

5.1. Notification and Researcher Exit: At 11:50 a.m., the researcher will again inform the research assistant that the dogs are ready. The researcher will then leave the lab for 10 minutes.

5.2. Research Assistant's Actions:

5.2.1. The research assistant will refer to the feeding schedule.

5.2.2. On scheduled post-feeding days: The assistant will remove the dogs' breakfast and plates from the cupboards, feed the dogs, remove any trace of food, and return the empty bowls to the cupboard.

5.2.3. On breakfast days: Each dog will be given 1-5 pieces of standard laboratory kibble.

5.2.4. Place all breakfast bowls in the sink and fill them with water.

5.2.5. Researcher returns.