Dogs' Scent Detection Performance with Rapidly Changing Targets

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Abstract

Scent detection dogs trained to detect one target scent are sometimes needed to detect a different target. Recent studies have demonstrated that scent detection animals can be trained to detect multiple scents simultaneously. However, in some cases, it may be problematic if the dogs indicate on previous targets. Therefore, it would be useful to know how quickly they can learn to detect new target scents, and not to indicate on previously trained target scents. The aim of this study was to evaluate dogs' ability to learn to detect new target scents while simultaneously rejecting all previously trained target scents. Firstly, five pet dogs were trained to detect a training scent (amyl acetate) using an automated apparatus. The dogs were then required to detect a four new target scents within four phases. Target scents were treated as non-target scents in all subsequent phases; indications on previously trained target scents were not reinforced. Non-target scents in each phase consisted of three randomly selected chemicals that were presented in a randomised rotation. The dogs were required to complete a phase with one target and meet the criteria of more than 80% for correct indication and correct rejection responses, without performing an indication response more than once on the previous target scent for four out of five sessions. The results of the experiment demonstrated that (1) the dogs were able to successfully discriminate between the specified target scent and all non-target scents within each phase; (2) the persistence of indication behaviour on previously trained target scents decreased more rapidly with each phase; and (3) there was a re-emergence of indication responses when each previously trained target scent was presented after extinction conditions. In summary, the present study demonstrates that dogs can be retrained to indicate on new targets and reject previous targets. The learning processes associated with the dogs learning to indicate on new targets, and learning not to indicate on previous target scents must both be considered. These results have significant practical

implications and it is hoped that the they will improve our ability to employ individual scent-detection animals to find multiple targets.

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TL;DR: Dogs are super cool.

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Introduction

1.1 Scent Detection Dogs

1.1.1 Working scent-detection dogs.

Dogs have an excellent sense of smell and are one of the most widely used biological organisms that are used to detect volatile organic compounds (henceforth referred to as VOCs) (Jezierski et al., 2014). VOCs are odour molecules that have significant vapour pressures at room temperature and result in evaporation of the molecules into the air (Hanks & Louglin, 2011). A dog's keen sense of smell is highly developed as compared to humans, mainly due to the difference in the number of neurons present in the olfactory epithelium (Horowitz, Hecht, & Dedrick, 2013). Dogs are estimated to have between 220 million to 2 billion, whereas humans have 2 to 5 million olfactory neurons (Szetei, Miklosi, Topal, & Csanyi, 2003).

Stejskal (2012) detailed how dogs' olfactory systems are influenced by breathing and sniffing patterns. The dog's nose flares and moves as it inhales and exhales. The movement of their nostrils during exhalation pushes the exhaled breath to the side. As such, the dogs do not inhale much of the same air it exhaled, ensuring a fresh supply of air with each inhalation. A dog's sniffing behaviour, comprising of rapid series of inhalation and exhalation, is advantageous in comparison to their normal breathing behaviour. Sniffing behaviour humidifies the odorant allowing increased absorption by the olfactory mucosa (Helton, 2009). More turbulent air flows through the nasal passages and enhances the perception of odours (Gazit & Terkel, 2003). With each inhalation, odour-containing air is trapped in the nasal pockets and passes through the dog's nasal turbinates, which are large bones that create a coiled pathway covered by the olfactory epithelium (OE) (Galibert, Azzouzi, Quignon, &

Chaudieu, 2016). The surface of the OE varies across dog breeds. It covers 200 cm^2 in a German shepherd as compared to 5 cm^2 of human's OE. The VOCs present in the inhaled air dissolves in the nasal mucus layer - and comes into contact with the cilia on specific olfactory receptors. The interaction between the odours and the olfactory receptors result in electric impulses that travel to the olfactory bulb and are processed in the specific centres of the brain. The development of the olfactory bulb and the olfactory cortex has been proposed to contribute to the sensitivity of the dog's olfactory system (Mesloh, Wolf, & Henych, 2002).

1.1.2 Applications of scent-detection dogs.

Both wild and domesticated dogs can be trained to detect a specific target scent (Nizio, Ueland, Stuart, & Forbes, 2017). Dogs have been used in many areas that utilize their keen sense of smell, and play important roles within military and civilian settings (Horowitz et al., 2013). They have been vital in maintaining safety and security around the world as they detect a wide variety of odours. Scent detection dogs can be trained to detect and locate either one specific scent (e.g. animal scat) or multiple scents (e.g. drugs and guns) (Helton, 2009). Research has shown that the dog's superior ability to recognize and localize chemical mixtures can be used to track the original scent source (Pickel, Manucy, Walker, Hall, & Walker, 2004).

Dogs are commonly used in scent detection tasks as they currently represent the fastest - and most versatile and reliable device available for use in applied settings (Furton & Myers, 2001). Most common encounters with scent detection dogs occur within airports, as dogs sniff passengers' luggage for illegal drugs, such as cocaine and methamphetamine (Browne, Stafford, & Fordham, 2006). The police also use drug-detection dogs in civilian settings, such as schools and workplaces, to detect and deter the use of illegal drugs (Browne et al., 2006). However, dogs have also been employed in many other roles within the airport,

from the initial bag check to the final screening before passing through customs. Known most commonly as “sniffer dogs”, these dogs are trained to alert on the presence of drugs by performing an indication response when the object of interest is detected. (Davidson, 2008).

The military also utilizes dogs in search-and-rescue (SAR) missions to find missing people (Barforoush, 2013), and human bodies after disasters (Riezzo et al., 2014). Dogs are also deployed in the detection of explosives and land mines (Corcelli et al., 2010). These trained explosives detection dogs are one of the largest groups of scent detection dogs in the world and are trained to detect chemicals present in landmines (Browne et al., 2006; Gazit & Terkel, 2003; Kranz, Strange, & Goodpaster, 2014). These dogs can detect explosives and improvised explosive devices (IEDs), enabling trained personnel to remove undetonated explosive materials that pose a risk to military and civilian populations (Lazarowski & Dorman, 2014). Dogs are used as a scent detection tool as it is a simple and flexible option compared to automated (machines) or manual (human) systems of detection (Goeth, McLean, & Trevelyan, 2003). Researchers are investigating dogs’ ability as a scent detection tool within civilian medical settings to detect various types of cancers, such as melanoma and ovarian cancer (Burnett, Stone, & Waugh, 2014; Cornu, Cancel-Tassin, Ondet, Girardet, & Cussenot, 2011; Edwards, Browne, Schoon, Cox, & Poling, 2017; Kitiyakara et al., 2017)

Research and conservation teams have used scent detection dogs to detect and localize objects in the field. Previous detection methods utilized human-only teams to conduct surveys and collect data. This method of manual data collection is expensive, and time-intensive (Cristescu et al., 2015). Manual data collection is very laborious as it requires researchers to use their eyesight to search for scat which is often concealed by ground cover. The use of scent detection dogs has shown to result in an increase of detection accuracy and a decrease in time spent surveying (DeMatteo, Davenport, & Wilson, 2019). Cristescu et al.

(2015) found that scent detection dogs trained to detect koala scat, were able to perform the task off-lead. The dogs were also able to consistently out-perform human-only teams, finding koala scat when human-only teams did not. Ballouard et al. (2019) trained scent detection dogs to detect Hermann tortoises, and compared the efficiency of scent detection dogs to conventional human survey methods. They also found that the scent detection dogs outperformed the human-only teams, finding three times more tortoises than humans.

Scent detection dogs have also been used in conservation studies. They have been trained to detect invasive species and contribute to the protection of endangered wild animals through detection of their faeces, or their nesting sites (Johnen, Heuwiser, & Fischer-Tenhagen, 2013). They have also been trained to detect illegal wildlife products, such as ivory and rhino horn in an effort to eliminate illegal wildlife trafficking (Mills, 2018). Dogs are often used as a conservation tool as they can detect samples independent of appearance or visibility (DeMatteo, Blake, Young, & Davenport, 2018). Scent detection dogs are also trained to make an indication response when they have detected the target species without harming the target species (Cablk & Heaton, 2006).

1.1.3 Methodological limitations.

However, working with scent detection dogs does present some challenges (Mochalski, Unterkofler, Teschl, & Aann, 2015). To determine if dogs can be effectively used in applied scent detection settings, it is important to first examine and understand the challenges associated with current methods. One area that requires examination is the relationship and interaction between the handler and scent-detection dog, which is a significant factor in the detection dog-handler dyad (Zubedat et al., 2014). One of the key confounding effects that may occur within the dyad is unintentional cueing and human error in data collection.

Unintentional cueing is a confounding factor that is present within scent detection research methods that require human interaction. Dogs are highly responsive to human communication signals and respond to gestures such as pointing to locate hidden objects (Lazarowski, Rogers, Waggoner, & Katz, 2019). While pets and assistant dogs rely mostly on human cues, working scent detection dogs must be less dependent on cueing from their handlers (Troisi, Mills, Wilkinson, & Zulch, 2019). The case of Clever Hans is one of the most notorious cases in animal psychology. Hans was a horse who – at that time – mystified researchers with displays of mathematical and linguistic abilities (Despret, 2015). Hans was able to answer questions by tapping his hoof on numbers and letters (Samhita & Gross, 2013). However, it was found that the horse 'solved' the tasks by reacting to subtle cues given by his trainer and the audience. The horse observed head jerks, breathing patterns and the audiences' body orientation to answer questions. This effect has been called the "Clever Hans" effect. This example has, rightly, made researchers cautious in interpretations of animal behaviour in scientific experiments involving human interaction (Schmidjell, Range, Huber, & Viranyi, 2012).

Working scent detection dogs are required to correctly detect and indicate the presence of a target odour to their handlers, and ignore any irrelevant or incorrect sources of information. The implications of dogs' sensitivity to cueing from their handlers were demonstrated by Lit, Schweitzer, and Oberbauer (2011). They found that handlers' beliefs regarding locations of the target scent were found to affect the performance of scent detection dogs. Handlers were given false information - and were misled into believing that a specific paper marker indicated the presence of explosives (target scent). However, no explosives were presented in the experiment. As such, any indication response would be considered a false alert. Handlers reported more indication alerts at marked locations as compared to unmarked locations, confirming that handlers' beliefs regarding the location of the target

scent influenced the performance of the dog in this task. Johnston, Huang, and Santos (2018) posited that dogs may incorrectly perceive a cue as a command, which may increase false indications. This demonstrated the need for scent detection dogs to maintain a degree of independence from their handlers in the field - and for researchers to take precautions to avoid such false positives in experiments.

Scent identification line ups are commonly used in scent detection research (Schoon, 2005). The dogs are presented with a target scent and are required to compare and match the target scent to a few scents (usually five to seven) placed in a row. The handler walks the dogs down the row, and the dogs are expected to perform a trained indication response when the target scent is detected (e.g. sit or lie down beside the presented scent). If the dog does not detect the target scent, it will proceed to the next scent in the row. However, research conducted using the scent identification line up is laborious and can be met with a few difficulties (Schoon, 2005). There exists a general lack of standardization of scent identification methods used within and between countries, as methods are usually developed based on the handler's personal experience (Ferry et al., 2019). This affects the reliability and efficacy of the scent line up method. Scent identification line ups are conducted manually, leaving room for human error in data collection. The handler may misinterpret the animal's behaviour - and record incorrect data which may result in a higher number of indication responses where there was none or a higher number of incorrect responses. The apparatus used in this present experiment is fully automated and requires dogs to work independently with the researcher out of the room. This eliminates any potential unintentional cueing from the researcher and also eliminates other issues associated with manual presentation methods, such as human error (Edwards, 2019).

1.2 Acquisition and Operant Extinction

Initial scent detection dog training allows the dogs to adapt to the training environment and methods (Johnen, Heuwiser, & Fischer-Tenhagen, 2015). Dogs learn observation (sniffing) behaviours and indication behaviours, such as lying down or sitting when encountering the target scent. During odour discrimination training, dogs are then required to identify and discriminate the target odour from other non-targets that are presented (Marchal, Bregeras, Puaux, Gervais, & Ferry, 2016). The acquisition of a new target scent requires extensive training, depending on the complexity and concentration of the target odour. As a result, training time for dogs to learn to identify a new target odour and discrimination between other non-targets will vary. Prior research training dogs to perform a specific scent discrimination task varied from two to three weeks (McCulloch et al., 2006), to eight months (Sonoda et al., 2011), to one and a half years (Koivusalo et al., 2017). The large difference in training times is largely unaccounted for as the number of sessions conducted over the timeframe of the study is often unreported. The differences could also be due to the level of difficulty of the scent discrimination task (Johnen et al., 2015).

The study of operant conditioning is essential in understanding the variables that influence voluntary or goal-directed behaviour (Bernal-Gamboa, Nieto, & Uengoer, 2017). Operant conditioning is a type of associative learning in which the strength of a behaviour is influenced by its consequences (Staddon & Cerutti, 2003). When a behaviour results in the delivery of reinforcement, the likelihood of the organism performing the behaviour increases. Likewise, when the behaviour no longer produces a reinforcer, the likelihood of the organism performing previously reinforced behaviour decreases, demonstrating a process called extinction (Berry, Sweeney, & Odum, 2014).

Traditional extinction procedures are often associated with undesirable side effects. One of the most common side effects is an extinction burst – a temporary but sudden increase in frequency, duration or intensity of the response (Lerman & Iwata, 1995). Following the initial increase in responding, responding rates will begin to decrease and reach eventual cessation. Extinction bursts occur when reinforcement is removed in the presence of a stimulus that was previously associated with reinforcement availability (van Haaren, 2020).

Behavioural momentum theory (BMT) is a quantitative account that evaluates the strength of a response when a disruptor, such as extinction is introduced (Bai, Chan, Elliffe, & Podlesnik, 2016). BMT posits that a stimulus that signals a higher overall magnitude of reinforcement increases the strength of the response, establishing greater resistance to change due to stronger stimulus-reinforcer relations (Nevin & Shahan, 2011). The strength of the response, however, is not measured by the rate or frequency of the response. Instead, it is measured as the response's resistance to change. The change in the strength of the response depends on the co-occurrence of the reinforcer and the response in the same context, regardless of the dependence of the reinforcer on the reinforcer. BMT is directly relevant to the extinction of a target behaviour, and the acquisition of an alternate behaviour as it provides a quantitative account of how the reinforcers influence behaviour within the discriminative stimulus context and thus influencing the strength of the response.

1.3 Re-emergence of Behaviour

Initial theories argued that the extinction process involved destroying all original learning (McClelland & Rumelhart, 1985). However, more recent studies have shown that the re-emergence of responses after extinction demonstrated that extinction does not destroy original learned behaviour, and proposes new theories on the processes of extinction.

One theory suggests that the removal of reinforcement for the target response violates the organism's expectation of reinforcement delivery that was previously contingent on the target response. As a result, new learning is initiated and the organism adapts to inhibit a previously trained response (Bouton, 2004). Although a decline in the strength of the behaviour is observed, several phenomena indicate that behaviour change is difficult to maintain - and can reoccur under certain conditions. Each type of relapse phenomena differs in the conditions under which they occur (Liddon, Kelley, & Podlesnik, 2017), as detailed in Table 1.

Table 1
Summary of typical procedures that assess renewal and resurgence

	Phase 1	Phase 2	Phase 3
Renewal*	Target behaviour reinforced in context A	Target behaviour extinguished in context B	Target behaviour extinguished in context C
Resurgence	Target behaviour reinforced Alternative behaviour not reinforced	Target behaviour extinguished Alternative behaviour reinforced	Target behaviour extinguished Alternative behaviour extinguished

*Refers to ABC renewal conditions (Other renewal conditions are AAB and ABB)

1.3.1 Renewal effect.

One phenomenon involving the re-emergence of behaviour is the renewal effect (Bernal-Gamboa et al., 2017). Renewal occurs when there is a change in the contextual stimuli present during the extinction of the operant response. Several versions of the renewal effect have been studied, namely "ABA", "ABC", and "AAB" renewal conditions, where the sequential letters represent the acquisition, extinction and testing contexts, respectively. In ABA renewal conditions, conditioning of a target response is conducted in context "A", and responding is extinguished in context "B". However, when tested in the initial training

context “A”, the previously extinguished response may reoccur (Bouton, 2004). The re-emergence of previously extinguished responses may also occur when acquisition, extinction and testing occur within three separate contexts (ABC renewal), or when acquisition and extinction occur in the same context but the behaviour is tested in a separate context (AAB renewal) (Bernal-Gamboa et al., 2017).

Bouton and Todd (2014) argue that context plays a central role in the expression of new learning. They further postulate that an organism learns to perform a specific response within a certain context - and learns to inhibit a specific response in another specific context. Various animal studies have been conducted to investigate the renewal effect, and these experiments postulate that contextual stimuli can refer to the physical stimuli present within a particular study, such as the illumination of the operant chamber with flashing or steady lights, or the stripes on the walls of the operant chamber (Sweeney & Shahan, 2015).

Bouton, Todd, Vurbic, and Winterbauer (2011) examined the renewal effects of operant behaviour. Reinforcement was delivered according to a VI-30 schedule of reinforcement when the rats performed the lever-pressing response in Context A. Responding was then extinguished in either the same context (AAB renewal) or a different context (ABA and ABC renewal). The results of the study showed that the magnitude of ABA renewal was larger than AAB renewal. The examination of both AAB and ABA renewal conditions show that the re-emergence of operant behaviour was possible when either extinction or testing was conducted outside the original acquisition context. This was consistent with the theory that the acquisition context set the occasion for high levels of responding due to the stronger context-reinforcer relations that the animal was exposed to during the acquisition context.

Bouton et al. (2011) then conducted an experiment that assessed the strength of the renewal effect. Previous research had found that increased responding due to the renewal

effect could be eliminated following extended extinction conditions (Tamai & Nakajima, 2000). The rats in the study were divided into two groups where one group received three times more extinction sessions (twelve sessions) as compared to the second group (four sessions). However, they found that the effects of renewal were not weakened by extended exposure to extinction conditions, demonstrating that extinction is specific to the context in which acquisition and conditioning took place. The abovementioned study demonstrates the context specificity of an organism's behaviour. When the scent detection dog encounters the target scent outside the extinction context, it is predicted that it would lead to the re-emergence of indication behaviours of that particular scent.

If the individual chemicals presented in this study were to be considered separate stimulus contexts, it would not directly map on to typical renewal procedure as all of the stimuli would be presented repeatedly in all contexts. Instead, the context may be more broadly construed as the experimental arrangement in which reinforcement is delivered for indication responses to one specific chemical (context A target scent) but indication responses to other chemicals are not reinforced. The contexts in this present study can be defined in relation to the target scent in question. If conceptualized in this way, the dogs are being exposed to ABC renewal procedures, as acquisition, extinction and testing occur in separate contexts. A change in experimental conditions occurs when reinforcement is delivered for indication responses on a new target introduced in the subsequent phase, and the dogs are required to learn to reject all previous targets. Therefore, the context in which reinforcement is delivered for responding to the target scent (2-phenylethanol) is the acquisition context, context A. The extinction procedure that would show the discontinuation of reinforcement for the previously trained target scent (2-phenylethanol). The extinction conditions in Context B were designed to demonstrate the discriminability of transitions between the baseline and extinction of each target scent. Context C would denote the testing

context where the previously trained target scent would be presented but reinforcement would still not be delivered. It is expected that when the animals are exposed to context C, the indication behaviour would re-emerge.

1.3.2 Resurgence.

Another phenomenon that details the re-emergence of previously extinguished behaviour is resurgence. The term resurgence refers to a behavioural effect, a process and a procedure (Lattal & Pipkin, 2009). It describes to the re-emergence of a previously reinforced response when reinforcement for a current response is discontinued (Bloom & Lambert, 2015). Resurgence effects are described using a three-phase procedure; (1) Training, (2) Response-elimination, and (3) Resurgence (Cancado & Lattal, 2013). In a typical resurgence study, reinforcement is delivered for the target response (R1) in the first phase, simulating a pre-treatment baseline condition where R1 first contacts reinforcement (Liddon et al., 2017). The second phase of response-elimination discontinues reinforcement for R1, and reinforcement is delivered for a second response (R2). This phase represents the condition where R1 contacts extinction, and R2 first contacts reinforcement. As a result, the response rates for R1 decrease, whereas the response rates for R2 increase (Sweeney & Shahan, 2015). The last phase of resurgence discontinues reinforcement for both R1 and R2, and both responses contact extinction. Operationally, resurgence effects are demonstrated by an increase of R1 response rates in the third phase as compared to previous sessions in the response-elimination phase. Sweeney and Shahan (2015) suggest that resurgence is a type of ABC renewal, whereby the initial reinforcement of the target response would be the acquisition context (Context A), and the simultaneous reinforcement of the alternative response and extinction of the target response would be the extinction context (Context B). Context C would refer to the removal of reinforcement for the alternative response.

Resurgence is a consistent effect that occurs across species and conditions. Many factors affect resurgence in each phase of the resurgence process. Leitenberg, Lawson, and Bath (1970) posited that the longer the R1 is in extinction, before the commencement of reinforcement of R2, the resurgence of R1 is less likely to occur when reinforcement of R2 is discontinued. Sweeney and Shahan (2013) further examined whether the length of time spent under extinction conditions influenced resurgence effects. Three response keys were placed on the front panel of the chamber. The centre key was illuminated during the ten baseline sessions that were conducted. Reinforcement (VI 60-s) was delivered when the pigeons pecked the centre key (target response). During extinction conditions, reinforcement was no longer delivered for pecks to the centre key, while pecks to the key on the right produced reinforcement (VI 30-s). The centre key and right key were both illuminated during the test phase. However, pecks to either key did not produce reinforcement. The pigeons then underwent extinction and test phases again (EXT 2, Test 2) without returning to the baseline phase. Reduced resurgence was observed in Test 2 as compared to Test 1, demonstrating that resurgence decreases across repeated extinction conditions. They concluded that increased exposure to extinction conditions may result in reduced resurgence effects.

Lambert, Bloom, Samaha, Dayton, and Rodewald (2015) also investigated the effect of serial alternative training on the resurgence of a target behaviour. Reinforcement was delivered for an arbitrary alternate behaviour in the first phase. In the second phase, three different arbitrary alternate behaviours were sequentially introduced. Responses to each alternate behaviour were extinguished prior to introducing the next alternate behaviour. Each alternate behaviour remained available and accessible after extinction. Resurgence was tested when the third alternate behaviour was placed under extinction conditions. The use of serial alternate behaviour training resulted in a decrease in overall responding allocated to the resurgence of the target behaviour.

Silva, Maxwell, and Lattal (2008) conducted an experiment to examine the effects of complete extinction of the first response on resurgence. Key pecking behaviour on two response keys was established on a concurrent tandem schedule (VI 27-s FR 5 and VI 27-s DRL 3s) in the reinforcement condition. Responses on both operanda were then eliminated by introducing a differential reinforcement of other behaviour (DRO) schedule. Reinforcements were then delivered when no key pecking occurred on either key for 20 seconds. After complete elimination of key peck responding, reinforcement was discontinued. They observed an increase in responding on both keys but with more responding on the key that correlated with higher response rates in the reinforcement condition. The results of this experiment suggest that reinforcement conditions are a factor that influenced resurgence and demonstrate that resurgence effects occur despite the complete extinction of the first response.

While typical resurgence procedures follow similar schedules of reinforcement to this present study, the difference between this present study and typical resurgence procedures lies in the required behaviours. A typical resurgence study evaluates the re-emergence of two or more behaviours (e.g., lever pull or key peck) in response to being presented with a specific S^D. However, the behaviour in this present study has the same topography but is emitted in the presence of a different scent in the different phases. As such, the procedures and layout of a typical resurgence study may not map on directly to the present study. In this study, the response that is reinforced is the response that indicates the presence of the new target. Conceptualized in this way, in each phase, a new response is reinforced while the previous response is extinguished.

1.3.3 Conclusion.

Renewal and resurgence appear to provide some basis for making predictions about how the dogs might respond to changing targets. The key difference between the two phenomena is that renewal is defined by context change without the presentation of alternative reinforcement, whereas resurgence is defined by the presentation and removal of alternative reinforcement. Although it seems that renewal and resurgence may be following different methodological procedures, one theoretical perspective posits that both effects may still be examined by using the same mechanism (Bernal-Gamboa, Gamez, & Nieto, 2018).

The re-emergence of responses demonstrates that extinction does not destroy original learned behaviour. Instead, the relapse of responses demonstrate the impermanence of extinction and indicates the initiation of new learning involving the extinction contingency (Bouton, 2004). This can be viewed as context-specific learning that results in the loss of extinction performance – extinction behaviour is restricted to the extinction context. Following this line of reasoning, resurgence may be characterized as an ABC renewal effort. While the removal of reinforcement for the alternative response may suggest a return to the original context (Context A), reinforcement was delivered for a new target scent in phase 3. This moves the animal to a new context (Context C), instead of returning to the original acquisition context (Context A).

In this present study, the resurgence of target responding could be observed when the reinforcement for the previous target response is discontinued, and an alternate response produced reinforcement. It could also be expected that the dogs who were exposed to extinction conditions for longer periods would have a less pronounced resurgence of responding to the target response. The aforementioned effects imply that the elimination of

responses through extinction is not permanent and the responses may re-emerge when the animal is exposed to similar or different conditions.

Few studies have investigated the extinction of indication responses to a target scent. Mahoney, Durgin, Poling, Weetjens, and Cox (2012) examined the effect of extinction conditions on detection performance, utilizing Giant African Pouched Rats (*Cricetomys gambianus*) that have been used for landmine detection in Mozambique. When the rats work in the field setting, they work under extinction conditions where reinforcers are not delivered immediately when an indication response is performed. This is due to the fact that the location of the mines is unknown to the handler. Therefore, reinforcers are not delivered immediately in operational field settings to avoid reinforcing inaccurate responses.

Mahoney and colleagues utilized multiple baselines with reversal design, exposing the rats to alternate reinforcement and extinction conditions twice. In the first reinforcement condition, the trainer would use a handheld clicker to produce a clicking noise when the rat was within 1 m of a landmine. The rats would progress to the extinction condition when detection accuracy was at 100% for four consecutive days in the first reinforcement condition. Indication responses to the target scent decreased abruptly within the first three sessions of the extinction condition. When reinforcement delivery was arranged again, accuracy remained at relatively low and inconsistent levels before eventually increasing to higher levels in later sessions. Accuracy rates were affected more drastically in the second extinction condition and decreased more rapidly as compared to the first extinction condition. This shows that extinction can influence scent-detection accuracy when placed in extinction conditions, and may be able to quickly learn to ignore the old target.

Kaulfuß and Mills (2008) found that dogs displayed a preference for novelty (neophilia), which is associated with an increased likelihood of approaching novel

environments and stimuli. The one-trial novel object recognition task (NOR) is a popular method that has been used to assess an animal's behaviour when it is simultaneously exposed to a familiar stimulus and a novel stimulus (Antunes & Biala, 2012). In NOR tasks, the animals explore the objects presented with their natural propensity, which allows the evaluation of stimulus recognition. Responses to novel stimuli are beneficial during early training sessions as the animals' wide behavioural repertoire provides the opportunity to deliver reinforcement when the animal approaches the novel stimuli. The animal's interest in novel stimuli may also lead to undesirable outcomes, such as an increase in false alarms, incorrectly indicating the presence of an S+ sample, especially in a work operational environment. In an experiment conducted by Gadbois and Reeve (2014), dogs were trained to detect a species-at-risk snake. However, another species of the same genus was common in that same habitat. They found that the dogs sniffed for a significantly longer period when they encountered the novel species, thus demonstrating a strong novelty effect.

However, Webb, Saccardo, Poling, Cox, and Fast (2020) examined rates of responding under extinction conditions in African pouched rats (*Cricetomys ansorgei)*. Two odours (Odours X and Y) were presented to four of the rats in a previous experiment. Responding to odour X was extinguished in the previous experiment, whereas responses to odour Y were never explicitly extinguished. The four rats displayed low responding rates to Odour Y, demonstrating almost complete forgetting of odour Y when it was presented five months after the previous experiment. They also found a minimal influence of the novelty effect for the remaining rats that were not previously introduced to Odour Y, as these rats also displayed low responding rates towards Odour Y.

1.4 Assessment Methods

Discrete trial testing (DTT) and free operant procedures have been traditionally employed in the study of learning. However, a majority of literature on extinction theory utilizes free operant assessment methods as opposed to DTT procedures which were used in this present study. The free operant and DTT assessment methods differ in the presentation of the S^D. In a typical free-operant framework, the enabling S^D is presented and freely available throughout the session. The opportunity to perform the specific response is made freely available to the subject (Hachiya & Ito, 1991). For example, the subjects may receive training for a lever press response. Reinforcement is then delivered on a predetermined schedule (e.g. VI-30 schedule). In that situation, the lever is made available to the subject throughout the session. However, in a DTT framework, the S^D signals the opportunity for the desired response to be performed. Although there is evidence that relapse effects may occur following the extinction of instrumental learning, there exists a dearth in the literature using a DTT approach.

This present study uses a discrete trial training (DTT) format for training and evaluation. During DTT, an externally controlled discriminative stimulus (S^D) is repeatedly and systematically presented for the subject to make the designated response, followed by a predetermined consequence (Hachiya & Ito, 1991). DTT is an instructional method that has been used to teach a variety of skills in different settings (Thomas, 2013). Based on the three term-contingency, DTT incorporates the following procedures: (1) the antecedent stimulus (or S^D) that signals an opportunity to respond, (2) a prompt, (3) a response (or lack thereof), (4) a consequence, and (5) an intertrial interval (Smith, 2001).

The antecedent stimulus in this present study would refer to the target scent that is meant to evoke an indication response. When the dog performs an indication response to the stimulus and reinforcement is delivered for doing so, the antecedent stimulus then becomes

the S^D. The S^D signals the availability of reinforcement following the behaviour, which sets the occasion for the occurrence of this response. This in turn produces an effect that will increase (or decrease) the frequency of a response in that specific situation (Pierce & Cheney, 2013). In this study, all non-target samples are stimuli in the presence of which reinforcement are not delivered (S^Δ). When discrimination training is consistently applied, the subject will reliably respond in the presence of the S^D, but will not respond in the presence of the S^Δ. The S^D and S^Δ will henceforth be referred to as S+ and S- respectively, to indicate samples for which reinforcement will be delivered.

The prompt is a supplementary stimulus that is meant to facilitate a correct response. An example of a prompt within this study would be the gestural prompts provided to the dog to press a lever to gain access to the next trial. There were no programmed consequences for the incorrect responses in this study. The goal when using prompts is to transfer stimulus control from the prompt to the S^D. This can be achieved through prompt fading. The intertrial interval occurs after consequences are provided, and lasts a few seconds before the start of the next trial in this study. Discrete trials are "discrete" as they have a definitive beginning and ending to each trial.

This present study employs the schedule of continuous reinforcement. This describes a typical reinforcement condition where reinforcement is delivered each time the indication response is made in the presence of the S^D. With two conditions of stimulus presence and two types of possible responses, there exist four possible outcomes (Table 2).

Table 2

2 x 2 response matrix of the stimulus-response condition

	"Yes" response	"No" response
Target present	Hit	Miss
Target absent	False alarm	Correct rejection

"Hits" refer to correct "yes" responses in trials where the target is present. However, "yes" responses in trials where the target is absent are termed "false alarms". "No" responses in trials where the target is present are incorrect responses and are termed "misses". "Correct rejections" are correct "no" responses in trials where the target is absent (Stanislaw & Todorov, 1999).

As the majority of the literature utilizes free-operant assessment methods, the principles related to extinction under free operant conditions will be reviewed as a starting point. The purpose of this present study is to review research on relapse phenomena that occur after the extinction of operant learning, such as renewal and resurgence.

1.5 Signal Detection Theory

Signal detection theory (SDT) is a mathematical decision-making framework that is applied to psychophysical experiments and serves as a way of quantifying responses in discrimination tasks (Alves-Pinto, Sollini, & Sumner, 2012). It may be applied to any experiment in which subjects are required to discriminate between two different types of stimuli, such as recognition memory studies and decision-making studies (Wixted, 2007).

The aim of the SDT framework is to discriminate between two mutually exclusive states - absence or presence of a target signals, and categorizes responses to determine if they are obtained by a known process (signal), or if they were obtained by chance (noise) (Lerman

et al., 2013). Therefore, the target odour is referred to as a "signal" (positive stimulus, S+ condition), and other non-target odours present are referred to as "noise" (negative stimulus, S- condition). The statistical and graphical representations derived from SDT provide a useful framework to assess the dogs' detection performance. SDT proposes that performance on a task is influenced by two factors, (1) sensitivity (or accuracy) of the subject's perception, and (2) their motivational state (bias) (Steckler, 2001). The SDT framework also outlines the decision maker's observation of events (defined as trials) and addresses the possibility that various factors, such as the subject's motivation, may affect their performance on the task (Steckler, 2001). The use of SDT also provides information regarding the type of errors (misses and false alarms) during the subject's performance (Gadbois & Reeve, 2016). SDT assumes that the observer's performance is influenced by the variability that exists within their perception of stimulus events (Macmillan, 2001). The use of the SDT framework allows researchers to separate the variability within the data using independent perceptual and decisional components. The perceptual component measures diagnostic accuracy and quantitatively represents the discrimination between two mutually exclusive states.

The decisional component quantitatively represents the observer's bias that was used to decide on discriminations (McFall & Treat, 1999). Within SDT literature, the term 'signal' refers to a specific target or meaningful information which requires a response upon presentation. The term "noise" refers to random and varied signals produced by natural processes, internal and external to the system (Wichchukit & O'Mahony, 2010). The presence of noise may corrupt and distort the information-bearing signal.

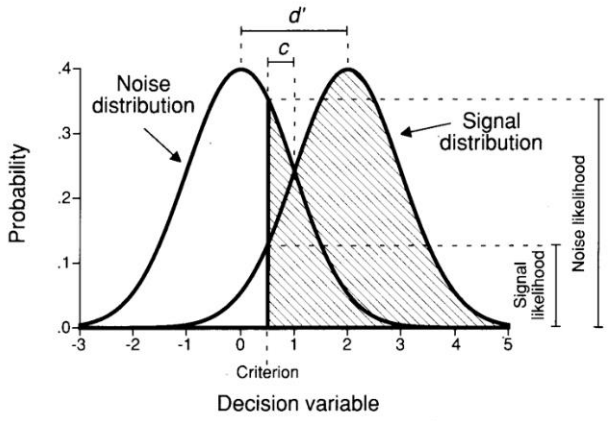

Figure 1. The distribution of the decision variable across signal and noise trials (Stanislaw & Todorov, 1999)

The criterion value, denoted by *c*, predicts the observer's bias towards a particular response (Stanislaw & Todorov, 1999). The criterion value is referred to as the 'neutral point', where *c* is equal to 0. At this point, the observer favours neither response, and it is unaffected by d'. Deviations from this criterion threshold are measured in standard deviation units. SDT assumes that if the criterion values are negative (less than the observer's criterion threshold), the observer displays a bias towards the "no" response. Likewise, if the criterion values are positive (stronger than the criterion threshold), the observer displays a bias towards a "yes" response (Stanislaw & Todorov, 1999). The observer's pattern of responding should also be considered. A liberal responder refers to a subject who always tends to give a "yes" response. While this results in a higher number of hits, it also results in a higher number of false alarms (Abdi, 2007). A conservative responder, on the other hand, reveals a tendency to respond "no", which results in a lower false alarm rate but also presents a low hit rate (McFall & Treat, 1999).

Every response leads to different consequences. Correct detections and correct rejections are beneficial to the perceiver, whereas false alarms and missed detections may result in varying costs depending on the task (Lynn & Barrett, 2014). For example, hits and

correct rejections are beneficial in airport settings, as efficiency and accuracy are optimal when dealing with the large number of people going through airport security. However, missed detections in explosives or drug detection searchers may incur heavy costs, as the danger (drugs or explosives) would be undetected and continue to pose a danger to the population. High false alarm rates or missed detection rates are undesirable as these outcomes reduce confidence in the reliability of the detection tool (Sargisson & McLean, 2010).

Confirmation of the reliability and validity of the dogs' ability in scent detection roles utilizes some form of odour discrimination task by measuring the sensitivity (detection of target samples) and specificity (displaying discrimination and correct rejection of non-target samples) (Porritt et al., 2015).

From the results, the hit rates also referred to as sensitivity (probability of subject responding "yes" on trials where target is present) and correct rejection rates (probability of subject responding "no" on trials where the target is absent) can be calculated to summarize the subject's performance of the discrimination task (Helton, 2009). Hit rates (HR) are calculated by dividing the number of hits by the total number of hits and misses

$$Hit\ rate = Hits\ \div (Hits + Misses)$$

False alarm rates (FA) also known as specificity - are calculated by dividing the number of correct rejections by the total number of false alarms and correct rejections.

$$False\ Alarm\ rate\ = False\ alarm\ \div (False\ alarms\ + Correct\ rejection\ )$$

The HR and FA were used as dependent variables in the present study, and are indicative of the dogs' ability to correctly identify positive samples (S+), and correctly reject negative samples (S-).

1.6 The Present Study

This present study investigated dogs' ability to learn to detect a new target while indications of previous targets are extinguished. The results of this study may be relevant to the re-training of scent detection dogs to find new target scents in the field. The re-emergence of previously reinforced behaviour, in addition to the acquisition of a new target scent, may affect the accuracy and reliability of the dog's detection performance. This research utilised a similar procedure that was used in Williams and Johnston (2002) and Webb et al. (2020), where animals were sequentially trained to detect an increasing number of target scents. With the conclusion of a project, scent detection dogs may benefit from being able to be retrained to accurately and reliably detect a new target scent.

Based on relevant research, three hypotheses were developed.

Hypothesis 1: The hit rate would improve more rapidly with each target phase. However, the false alarm rate would not decrease as quickly as the hit rate across phases.

Hypothesis 2: The dogs would demonstrate a re-emergence of indication behaviour when each previously trained target scent was presented again, after responding to each target scent was extinguished.

Hypothesis 3: The dogs would demonstrate a re-emergence of indication behaviour when each non-target scent was presented under extinction conditions across each phase.

Chapter 2
Methodology

2.1 Subjects

Dogs were recruited through flyers, posts on social media platforms and word of mouth. Recruitment procedures are outlined in standard operating procedures (SOP) (Appendix A and B). Dogs were brought into the scent detection facility on the University of Waikato campus for an initial meeting to determine the dogs' eligibility to participate in the experiment. The researcher observed how the dogs behaved in a crate, and if the dogs experienced separation anxiety when they were separated from their owners. Any dogs who displayed signs of distress in any of these situations were not eligible to participate. Dog owners were provided information about the experiment and were given the opportunity to ask questions before providing written consent (Appendix C) for their dog to participate in the research.

Dogs were then tested in probationary sessions in their owners' absence. Owners were asked not to feed their dog two hours prior to the sessions to promote in-test motivation and prevent food satiation. The dogs were trained with an automated feeder that would dispense kibble when it was manually activated using a wireless clicker. Dogs were eligible to proceed with training if they were able to approach the feeder and consume the dispensed kibble with three seconds.

Thirteen dogs were recruited to participate in the experiment over eight months (August to March). Seven dogs did not pass recruitment or training phases due to various reasons. One dog (Bella) withdrew from the study due to scheduling conflict; three dogs (Loki, Asti, and Deja) were excluded as they did not consume the kibble presented, and another dog (Storm) displayed anxiety when separated from her owners. As a result, only six

dogs proceeded to participate in the preliminary stage of the experiment. One dog (Rusty) was withdrawn at his owner's request before the start of the experimental stage. As a result, only five dogs participated in the final experimental stage.

Table 3 provides information on the dogs that participated in the experiment. The mean age of the dogs was 3 years (1 to 5 years). None of the subjects that participated in the experiment had any prior experience with scent detection training, or the apparatus used in this study. The researcher recorded the weight of each dog at the start of every experimental day and monitored their weight for the extent of the experiment (8 months). Regular weigh-ins were conducted to ensure that the dogs maintained their weight and were not given too much kibble via reinforcement. As two dogs were named Hollie, nominal labels "Hollie1" and "Hollie2" were used.

Table 3

Subject Information; NM indicates neutered male; NF indicates neutered female

Subject	Breed	Age (years)	Weight	Sex
Mika	Border collie x spaniel	1	19 kg	NF
Scout	Mixed*	~ 5	25 kg	NM
Hollie1	Mixed*	~ 3	25 kg	NF
Rusty	Mixed*	~ 3	32 kg	NM
Hollie2	Border collie x golden retriever	4	22 kg	NF
Jasper	Border collie x golden retriever	2	22 kg	NM

~ indicates the adoption agency estimated the age of the dog and is not known exactly.

* indicates the breed was estimated by the adoption agency.

2.2 Project Stages

This experiment was conducted in three stages (Table 4). Firstly, dogs underwent a probationary training stage to assess their behaviour and eligibility to participate in the project in their owners' absence. The dogs were assessed on whether they would approach the automatic feeder and apparatus, and whether they would consume the kibble

reinforcement provided. Dogs that were able to do so within the first few training sessions were considered eligible to proceed on to preliminary training sessions. The dogs (N = 6) were first trained to indicate on amyl acetate as a target scent (S+), and were then trained to perform a rejection response in the absence of the target scent (or in the presence of S- samples). Deionized water was introduced in discrimination training as a negative (S-) sample. Once the dogs met criteria, they proceeded to the second odour discrimination stage that examined their performance when a novel non-target scent (hexanol) was introduced. Finally, the experimental phase assessed their performance when the target scents were changed in each phase. Ethics approval for this research was obtained from the University of Waikato Animal Ethics Committee (AEC; Protocol number 1074).

Table 4

Experimental design

Phase	Task	Target Scent (S+)
Preliminary Training Stage		
	Initial acquisition of training scent Deionized water used as negative sample	Amyl acetate
Odour Discrimination Stage		
	Novel scent (Hexanol) introduced	Amyl acetate
Experimental Stage		
1	Initial olfactory discrimination Acquisition of target scent	2-phenylethanol
2	Extinction of previous target scent (2-phenylethanol) Acquisition of new target scent	Cinnamaldehyde
3	Extinction of previous target scent (Cinnamaldehyde) Acquisition of new target scent	Linalool
4	Extinction of previous target scent (Linalool) Acquisition of new target scent	Ethyl butyrate

2.3 Study Location

All experiments were conducted in the scent detection facility at the University of Waikato, Hamilton, New Zealand. Experiments were conducted three times a week (Mondays, Tuesdays, and Fridays), and were conducted in either morning (8:00 am - 12:00 pm) or afternoon sessions (1:00 pm - 4:00 pm). Dogs were dropped off by their owners at the facility, and picked up when the sessions were completed. There was an experimental room in the facility and an office where the dogs were kept in large crates (1.8 m high x 1.2 m wide). The dogs were housed in the kennel room (room G.02), and room G.01 was used as the experiment room (Figure 2). The dogs were given a mat, a blanket and water *ad libitum*. All dogs were cared for following a handling and care SOP that was approved by the University of Waikato Animal Ethics Committee (SOP #20). Dogs were walked outside the facility by the researcher or an assistant every two hours.

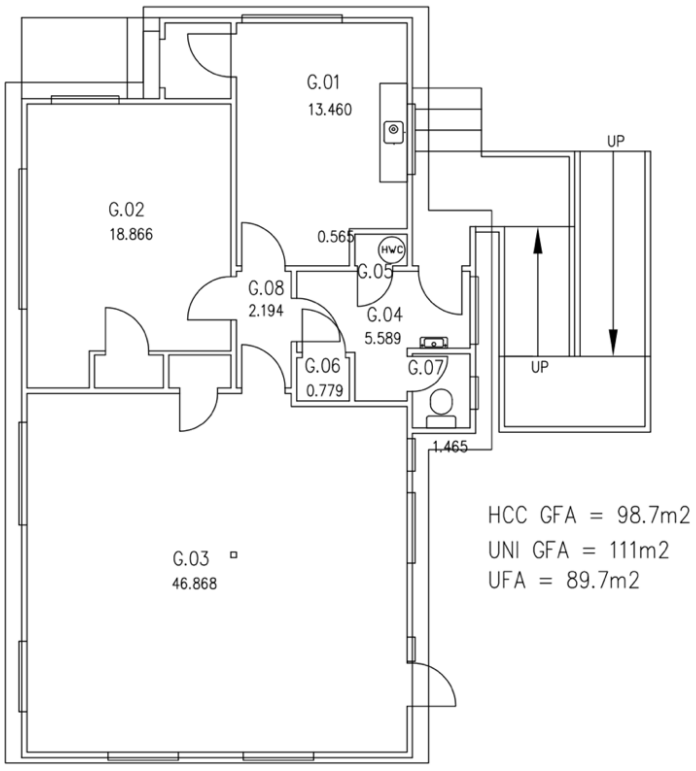

Figure 2. Blueprint of scent detection facility

2.4 Equipment

The scent detection apparatus was in the experiment room in the scent detection facility. The custom-built 1 m^3 apparatus had clear front and side plexiglass panels and held a 760 mm diameter carousel (Edwards, 2019). The carousel fits 17 removable metal segments (280 mm x 135 mm x 80 mm, volume 3.57 L), which had a metal tube affixed to the back panel of the segment that held a glass vial securely. The carousel of the apparatus rotated to present each individual scent sample to the dogs. There was a 10 cm by 10 cm square opening at the front of each segment, covered by a stainless-steel flap. The flap was fastened by an L-bracket, which allowed the flap to open to a 28^o angle. This prevented any contact between the samples and the dogs. A round metal lid was placed over the segments to prevent cross-contamination between samples which were randomly arranged (Figure 3).

The dogs gained access to the scent sample by placing their nose through a 10 cm port in the front panel and pushed through a flap at the front of the segment (Figure 3 and 4). The clear front panel provided visual feedback for the dog - and enabled the dog to see when the carousel had stopped turning. An omnidirectional lever was attached to the frame of the apparatus, to the right of the port. When activated, the carousel rotated counter-clockwise to present the next sample in front of the port. A buzzing sound was made to provide auditory feedback for the dog as the carousel rotated. While the carousel rotated, responses had no programmed consequences. The intertrial interval lasted approximately two seconds. When the segments were in position, the apparatus produced two short “beep” sounds, which indicated the opportunity for the dog to respond.

The computer used for the experiment was a Dell™ Optiplex 780 computer running on Windows Vista™. Each experimental session was recorded using a video camera (Logitech® 2 MP HD Webcam C600) with built-in microphones. This allowed the dogs to

work independently in the experiment room without the researcher present, and the researcher was able to record and monitor video footage from the office. All video footage of experiment sessions was recorded and saved. The computer was placed in room G.02, next to the door and facing the experiment room. A custom-designed software controlled the apparatus - and displayed and recorded real-time data from the apparatus. Each dog had its own configuration file created on the software, and each value in the file could be modified if needed. Information for each experimental session was detailed in the configuration file, such as the status of the sample (positive or negative), the minimum amount of time required to detect and record the observation of a scent sample (e.g., 500 ms), and the duration of time required for an indication response.

When the dog placed their nose into the port of the apparatus to evaluate a scent sample, it broke an infrared beam which produced a continuous beeping sound. This provided auditory feedback for the dog - and signalled that the computer software had detected and recorded the beam break. The software recorded the duration of the beam break, and the indication threshold was set to a value specified by the researcher (5000 ms). When the indication threshold was met, it represented an 'indication' by the dog that a positive sample had been detected.

Correct indications of the presence of a positive sample activated an automated feeder, which dispensed kibble. When an observation response was recorded, the lever was activated. This meant that a lever press would then turn the carousel to present the next sample. The carousel would not turn if the lever was pressed before meeting the required observation response duration.

The first feeder that was used for this experiment was the Treat and Train Remote Reward Dog Trainer™, which delivered Pedigree beef flavoured kibble. A second type of

feeder, the Pet Tutor Pro, which is capable of delivering a wider variety of food was used for dogs that demonstrated a lack of interest in the dry food. With this second feeder, the dogs were fed “possyum” (a meat paste). However, this feeder did not deliver food as reliably as the dry feeder did. Reinforcement delivery was programmed through the software or manually activated by a wireless handheld remote. A distinctive auditory stimulus was produced when the feeder dispensed a piece of kibble into the feeder tray. The feeder was placed approximately 3 metres diagonally from the apparatus.

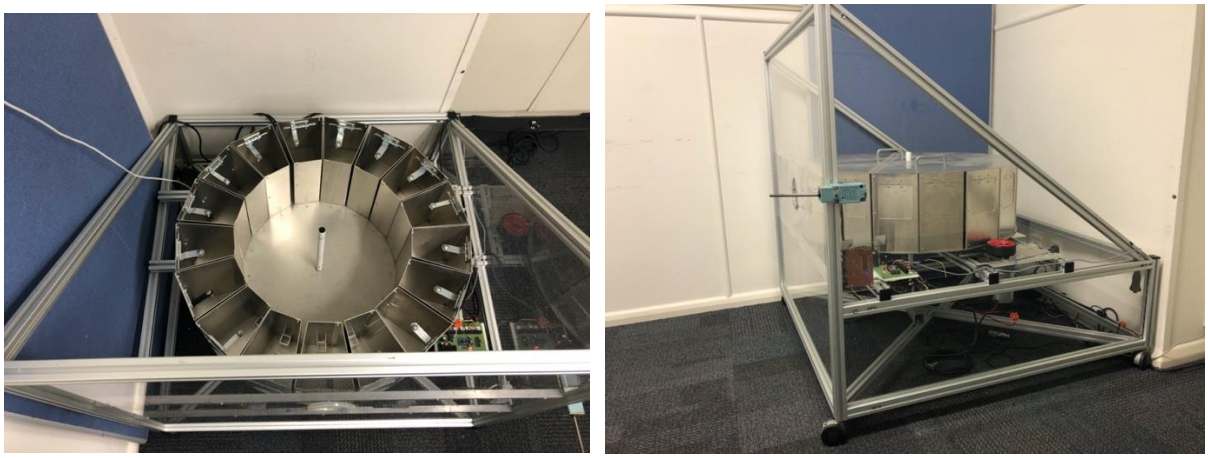

Figure 3. Top and side view of the apparatus with 17 segments placed on the carousel.

Figure 4. Rusty sitting in front of the port of the apparatus (lined with grey tape). The lever is attached to the frame of the apparatus (right).

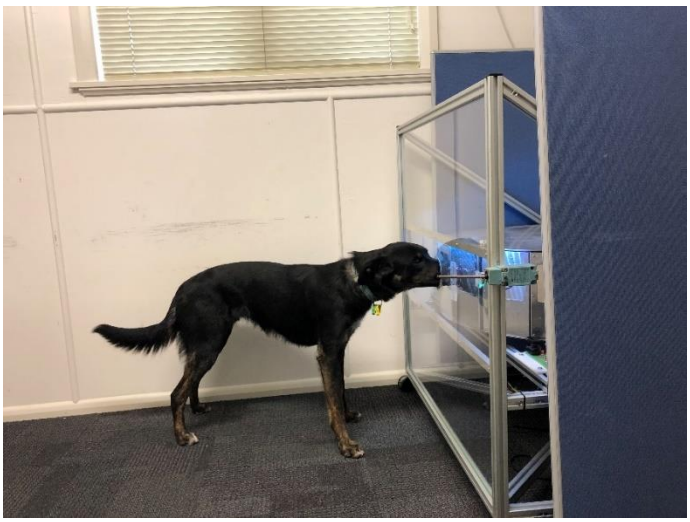

Figure 5. Jasper with nose in port to evaluate a sample. The automated feeder is placed diagonally from the apparatus, out of sight in this image

2.5 Chemical Solutions Used in Experiment

2.5.1 Vapour pressure of chemical solutions.

The 15 chemicals used in this experiment were selected based on the following criteria: (1) they were known to produce distinct odours, (2) the chemicals were not aversive or toxic to humans or dogs at the concentrations that were used in the study, and (3) they had previously been used in other scent detection research studies. Chemicals that had clear biological relevance (e.g., androstenone, a steroidal pheromone) were avoided.

The vapour pressure of each chemical solution was calculated to ensure that the concentration of VOCs within the headspace of the segment was similar across all chemical solutions. Raoult's law demonstrates the thermodynamic behaviour of liquid solutions - and states that the vapour pressure of a solution is the product of the mole fraction of the solvent (x) and the vapour pressure of the pure solvent (p^*) at a given temperature (Jenkins, 2008). The mole refers to the amount of substance within an element, such as a molecule or an atom (Giunta, 2015). The magnitude of a mole is 6.02252×10^{23}, which is also known as Avogadro's number (Tro, 2013). Therefore, the mole fraction is a unit of concentration - and refers to the number of moles in a component in comparison to the total number of moles in a solution. The mole fraction of a solution will equal 1, which is the sum of all moles of the component present in the solution (Foulkes, 2013). Vapour pressure, P, emerges from a pure solvent in a containing vessel when the volatile molecules within the solvent leave the surface of the liquid. This results in pressure in the vapour above the liquid solution. However, when a second component is added to the pure solvent, a decrease in vapour pressure is observed (Jenkins, 2008).

The following equations demonstrate the calculations performed to determine the vapour pressure of the amyl acetate solution, which was used during preliminary training

phases. Similar calculations were performed to obtain the vapour pressure of all chemical solutions used in the experiment.

Firstly, the mass of the chemical (amyl acetate) was calculated by converting the density from g/cm^3 to g/microlitre, and then multiplying the result by the number of microlitres of chemical (25). This yielded a mass of 0.0219 g of amyl acetate, as 25 µL of amyl acetate was dissolved in 99.975 g of deionized water. Next, to obtain the total number of moles of the solvent and the solute, the mass of the chemical was divided by the molar mass of the chemical. The equation was as follows:

$$Mass\ of\ chemical\ \div molar\ mass\ of\ chemical$$

$$0.0219\ g\ \div 130.19\ g/mol$$

This calculation yielded 0.00016821568 moles in the amyl acetate solute. The same calculations were performed to obtain the number of moles in water:

$$mass\ of\ water\ \div molar\ mass\ of\ water$$

$$99.975\ g \div 18.016\ g/mol$$

This calculation yielded 5.54923401421 moles in 99.975 g of deionized water. Therefore, the total number of moles in the amyl acetate solution was 5.54940222989 moles. This led to the calculation of the mole fraction of amyl acetate in the solution. This was achieved by dividing the number of moles in amyl acetate by the total number of moles in the solution. The equation was as follows:

$$moles\ in\ chemical\ \div total\ number\ of\ moles\ in\ solution$$

$$0.00016821568\ moles\ \div 5.54940222989\ moles$$

This calculation yielded a mole fraction of 0.00003031239. The mole fraction of deionized water was calculated following the same equation:

$$5.54823401421\ moles\ \div 5.54940222989\ moles$$

This calculation yielded a mole fraction of 0.9999696876. Raoult's law was demonstrated using the following equations:

$$P_1 = x_1 p_1^* \quad (1)$$

$$P_2 = x_2 p_2^* \quad (2)$$

"P_1" and "P_2" denote the vapour pressures of component 1 and component 2 separately, "x_1" and "x_2" refer to the mole fractions of component 1 and 2 respectively, and lastly, "P_1*" and "P_2*" refer to the pure vapour pressures of component 1 and component 2.

$$P_{total} = P_1 + P_2 \quad (3)$$

P_{total} refers to the total vapour pressure of the liquid solution. These figures were applied to equation (1) to obtain the new vapour pressure of the first component, P_1 (deionized water), when the vapour pressure of the pure solvent is 23.8 mmHg:

$$(0.9999696876 \times 23.8)$$

This calculation yielded a vapour pressure of 23.7992785649 mmHg. The new vapour pressure of the second component, P_2 (amyl acetate), was calculated using equation (2). The vapour pressure of the pure solute is 4 mmHg.

$$(0.00003031239 \times 4)$$

This calculation yielded a vapour pressure of 0.0012124956 mmHg. The vapour pressure of the amyl acetate solution was calculated using equation (3):

$$(23.79498981 + 0.000121248)$$

Therefore, the vapour pressure of the amyl acetate solution was 23.8004910605 mmHg.

Twenty-five microlitres (µL) of each chemical were added to deionized water to create a volume of 100 mL of chemical solution. The vapour pressure of all the chemicals used in this experiment are detailed in Table 5 and demonstrates similar vapour pressures across all chemical solutions (M = 23.803, SD = 0.01).

Table 5
Information of Chemicals used in Experiment

Status	Name	Molar Mass (g/mol)	Density (g/cm^3)	Vapour Pressure of Chemical (mmHg)	Vapour Pressure of Solution (mmHg)
Training samples					
	Deionized water	18.153	0.997	23.800	23.800
	Amyl acetate	130.190	0.876	4.000	23.800
	Hexanol	102.170	0.814	0.930	23.799
Target and non-current target samples					
	2-phenylethanol	122.160	1.020	1.000	23.799
	Propionic acid	77.079	0.990	3.530	23.801
	Cyclohexanone	98.150	0.948	5.000	23.799
	Ethyl acetate	88.110	0.902	93.200	23.803
	Benzyl acetate	150.180	1.040	0.177	23.799
	Ethyl butyrate	116.160	0.874	11.300	23.799
	Benzaldehyde	105.124	1.040	1.270	23.800
	Cinnamaldehyde	132.160	1.050	1.000	23.799
	Linalool	154.250	0.865	0.160	23.799
	Methyl Acetate	74.079	0.934	216.200	23.811
Distractor samples					
	Methyl Propionate	88.106	0.975	84.040	23.799
	Butyl Acetate	116.160	0.880	10.000	23.836
				Mean VP	23.803
				SD	0.01

2.5.2 Storage of chemical solutions.

Pure chemical concentrates were stored in a chemistry lab on the University of Waikato campus, inside a secure metal cabinet according to laboratory protocols. Diluted chemical solutions were stored in a container in the scent detection facility. Each bottle of the diluted chemical solution was stored in a separate plastic resealable bag to prevent cross-contamination of vapours, and reduce the chance of contamination if spillage occurred. After their use, chemicals were disposed of according to standard health and safety guidelines in the chemistry lab.

2.6 Solution Preparation

2.6.1 Solution preparation during preliminary training stage.

During the preliminary training stage, deionized water was presented as a negative (S-) sample and amyl acetate was presented as a positive (S+) sample. Preparation procedures are outlined in Appendix E. Negative samples were prepared by using a volumetric flask to measure out 100 ml of deionized water, using a beaker and a syringe. This solution was poured into a 250 ml Schott glass bottle. This procedure was done twice to create a 200 ml solution of deionized water.

Positive samples were prepared following a similar procedure. Firstly, approximately 50 ml of deionized water was poured into the volumetric flask, and 50 µL of amyl acetate concentrate was then added. The flask was then shaken to ensure the solution was mixed well. More deionized water was poured into the volumetric flask to create a 100 ml solution, and this solution was poured into a 250 ml Schott glass bottle. 100 ml of deionized water was poured into the glass bottle to create 200 ml of amyl acetate solution. Both bottles containing negative and positive samples were sealed with parafilm and a lid - and were kept in the scent detection facility to be used for sample preparation. Each bottle was kept in a resealable plastic bag to prevent spillage and cross-contamination between samples. Solutions were kept for a maximum of two weeks before being disposed of, at which point new solutions were prepared.

2.6.2 Solution preparation during experimental stage.

Preparation of all solutions in the experimental stage followed the same procedures as the abovementioned preliminary training stage. Chemical solutions were prepared by pouring 50 ml of deionized water into a 100 ml volumetric flask. 50 µL of chemical concentrate was added to the volumetric flask and shaken to ensure the solution is mixed well. Deionized

water was poured into the volumetric flask to create 100 ml of the chemical solution, and poured into the allocated 250 ml Schott glass bottle. The bottles were then sealed and placed individually into a resealable plastic bag.

2.7 Sample Preparation

2.7.1 Sample preparation during preliminary training.

All samples were prepared in the scent detection facility on the University of Waikato, Hamilton campus. Sample preparation procedures are outlined in Appendix F. The preparation bench was first cleaned with an isopropanol solution (60% isopropanol and 40% water) - and disposable paper towels. Negative samples were prepared first to prevent cross-contamination. Clean vial holders were placed on fresh paper towels. The sample vials used in the experiment were 6 cm and 1.5 cm wide. Self-adhesive labels were placed horizontally on the outside of S- vials. 2 ml of negative solution were added to the vials using a pipette. Once prepared, the vials were placed in randomly selected separate segments on the carousel of the apparatus. Schott bottles were resealed with parafilm and the lid. Once sample preparation for negative samples was completed, the preparation table and pipette were cleaned again using the isopropanol solution and disposable paper towels.

Positive samples were prepared next, and self-adhesive labels were placed on the bottom side of the vials. Two ml of amyl acetate solution was added to the vials and placed in the remaining segments on the apparatus. The lid of the apparatus was then placed on top of the segments, and the samples were left for fifteen minutes before starting an experimental session to allow for the dissipation of VOCs within the headspace of the segment. Positive samples were disposed of and replaced with fresh samples every four hours.

The segments were randomly selected and allocated to a specific sample within the same day to prevent cross-contamination between samples. In preliminary training sessions,

samples that held S- samples during the morning sessions could be used to hold S+ samples during afternoon sessions. However, segments that had been used for S+ samples during morning sessions were not used for S- samples during afternoon sessions. This ensured that the VOCs from the S+ samples that were placed in the segment did not affect later S- samples.

2.7.2 Sample preparation during experimental stage.

The preparation bench was cleaned following the same procedures in the preliminary training phase. The preparation order of chemicals was randomised every day. The VOCs that emanated from the chemical solutions dissipated over time as the chemicals that were present in the solution vaporized. The randomisation of the order of preparation ensured that the dogs did not learn any specific order effects. If the target scent was always prepared first, the concentration of VOCs within the target scent would be lower in comparison to other scent samples.

The chemicals used in the experiment were separated into two lists. The first list consisted of ten potential target scents. Once the target scent was randomly selected from the list, the remaining nine chemicals formed a list of non-current target scents, three of which were randomly selected and presented in each session. The original research design was to present all ten chemicals as potential target scents. However, the study was concluded earlier than planned due to Covid-19. The second list of scents consisted of the remaining three distractor chemicals that would never be used as a target or non-current target scents in this experiment. One distractor scent was randomly selected and presented in each session.

Self-adhesive labels were placed on each vial (Figure 6). A rectangle self-adhesive label was placed on the bottom side of a vial to indicate the vial contained a target (T) sample. The distractor samples were labelled with a triangle self-adhesive label on the side of

the vial. All non-current target samples were labelled differently. The first non-current target sample (NC_A) was labelled with a rectangle self-adhesive label placed horizontally on the side, near the top of the vial. The second non-current target sample (NC_B) was labelled with a rectangle self-adhesive label on the side of the vial, near the bottom of the vial. The third non-current target sample (NC_C) was labelled with a rectangle self-adhesive label placed vertically on the side of the vial.

Clean vial holders were placed on fresh paper towels. Vials (550 mm x 180 mm x 200 mm) were individually labelled with their corresponding self-adhesive label and placed in the vial holder. Two ml of chemical solution was added to the vials. New pipette tips, gloves, and paper towels were used for each new chemical solution - and disposed of after use. During the experimental stage, all segments were randomly selected at the beginning of each day, and were kept to use with their allocated chemical solution for both morning and afternoon sessions. All segments used for a specific chemical (e.g., propionic acid) were kept together on a fresh paper towel in an allocated corner of the experimental room. Once all samples were prepared and placed in the apparatus, the lid of the apparatus was placed over the samples and they were left for 15 minutes before starting an experimental session. All samples were disposed of and replaced with fresh samples after four hours.

Target Sample | Non-current target sample A (NC_A) | Non-current target sample B (NC_B) | Non-current target sample C (NC_C) | Distractor Sample (D)

Figure 6. Placement of self-adhesive labels on vials

2.8 Cleaning Procedures

2.8.1 Cleaning of apparatus and facility.

Sample arrangements were re-randomised after four sessions. At this point, both vials and segments were removed from the apparatus and placed on fresh paper towels. Segments used for a specific chemical (e.g., hexanol) were placed on the same paper towel in allocated corners of the experiment room. The lid and carousel of the apparatus were then cleaned with isopropanol solution and paper towels. Vials and their respective segments were placed back on the carousel following a re-randomised arrangement and covered with the lid of the apparatus. The samples were left for 15 minutes before the commencement of the next session. At the end of the day, vials and segments were removed from the apparatus, and the apparatus was cleaned using isopropanol solution to eliminate any residual odours. The tray of the feeder was also cleaned to remove residual saliva and bits of kibble.

All segments were washed individually at the end of each day. Boiling water was poured over the preparation bench and sink twice. It was then cleaned with an isopropanol solution with paper towels. A Sunlight® Power Max dishwashing tablet was dissolved in hot water, and the segments were individually cleaned using hot soapy water. The soap was

rinsed off, and the segments were dipped into an isopropanol solution bath and left to dry on the bench. Vial holders were cleaned in the same way.

2.8.2 Cleaning of glassware.

All glassware used in the preparation, storage, and presentation of the chemical solutions were brought to the chemistry lab for cleaning. The adhesive labels that were placed on the side of the vials were removed and disposed of. All glassware was cleaned by placing in an acid bath solution of concentrated nitric acid (HNO_3) for at least 24 hours. Acid washing procedures are detailed in Appendix G. The glassware was taken out of the acid bath, rinsed using distilled water, and placed in a Contherm thermotech 2000 oven to dry at 60-degree Celsius for 24 hours.

2.9 Testing Protocols

2.9.1 Testing Protocols in preliminary training stage.

The researcher employed the method of shaping, which established a behaviour by providing reinforcement for successive approximations to the target behaviour, and placed previous approximations of the behaviour under extinction until the subject performed the next closer approximation to the target behaviour (Martin & Pear, 2015).

Six dogs participated in the preliminary training, and the steps are outlined in Appendix H. In step one, the dog was brought into the experiment room and trained to approach the feeder to consume the kibble provided. The researcher used the wireless remote to deliver kibble, which was dispensed from the feeder into a tray. The dog had to be able to reliably approach the feeder within three seconds, three times before proceeding to the next stage. Occasional gestural prompts (towards the port or lever) or verbal prompts (to direct their attention) were used to facilitate the training process. The researcher had to take caution to avoid eye contact with the dogs in order to prevent providing unintentional cues. As the

performance of the subject increased, prompts were systematically faded to encourage independent learning. All prompts had to be faded before moving on to the next step of training.

In the second step, food was only delivered when the dog turned and moved away from the feeder, moving closer towards the apparatus. When the dog moved toward the apparatus and put their nose in the port, a continuous 'beep' sound indicated that the infrared beam had been broken. Reinforcement was then delivered for every instance the beam was broken and a "beep" sound was made, and then for pushing the segment flap open to gain access to the sample. The threshold for an observation response was set (501 ms) to ensure that the dog had interacted with the sample. When the dog was reliably performing the observation response, all segments were loaded with S+ samples. The dogs were then further trained to perform the indication response, where the dogs held their nose in the port to meet a response duration (starting at 501 ms) for reinforcement delivery. The duration requirement was gradually increased in increments of 250 ms to 500 ms until the response duration requirement reached 1500 ms.

At this point, the apparatus was turned off, and the researcher used the wireless remote to manually control reinforcement delivery. Reinforcement was delivered to shape lever-press behaviour. Dogs could perform the lever press using any part of their body (i.e., paw, snout, or body). Once the dogs were reliably pressing the lever independently, negative samples were introduced.

In this study, four outcomes were possible (Table 6). Hits were achieved when the dog correctly indicated on an S+ sample by holding their nose in the port of the apparatus for the predetermined response duration time (5001 ms), while misses were observed when the dogs failed to do so when the S+ sample was present. False alarms occurred when the dog

performed an indication response in the presence of an S- sample. Correct rejections occurred when the dog broke the infrared beam to perform an observation response (501ms) and proceeded to press the lever before meeting the indication threshold in the presence of an S- sample. Any response duration between the two thresholds was considered a rejection response. The lever press rotated the carousel to present the next sample. Of these four possible outcomes, reinforcement was only delivered for hits.

Table 6
2 x 2 contingency table of stimuli and responses

	"Yes" response (5,001ms indication response)	"No" response (Lever press)
Target present (S+ sample)	Hit (Reinforcement)	Miss
Target absent (S- sample)	False alarm	Correct rejection

Mastery criteria were used to evaluate skill acquisition at each step of training. Hit rates and correct rejection rates were selected as they reflected the dogs' performance with positive and negative samples, respectively. The hit rates represented the percentage of trials where the dog had correctly indicated on an S+ sample divided by the total number of S+ samples presented within a session. The correct rejection rate calculated the percentage of trials where the dog had correctly rejected an S- sample divided by the total number of S- samples presented within a session.

In the fourth step of training, the dog began discrimination training. Nine S+ and eight S- samples were arranged in an alternate pattern, where an S+ sample preceded and followed each S- sample (Table 7). The apparatus was switched on, and the software programme delivered reinforcement. The duration of indication responses was increased in increments of 500 ms from 1500 ms until the duration requirement reached 5000 ms. The dog proceeded to

the next step of training when it independently reached the criterion threshold of hit and correct rejection rates above 80% in a session, for 4 out of 5 sessions. The randomisation of all S+ and S- samples occurred in the fifth step of training, and prevented sequence learning effects from the previous positive/negative set pattern.

Table 7

Allocation of scents in a pattern arrangement within the 17 segments, where "P" denotes a positive sample, and "N" denotes a negative sample

Segment number	Scent
1	P
2	N
3	P
4	N
5	P
6	N
7	P
8	N
9	P
10	N
11	P
12	N
13	P
14	N
15	P
16	N
17	P

Once the dog did not require any prompts from the researcher, the researcher then gradually moved out of the room. The researcher took steps closer to the door, and eventually stepped out and closed the door slightly with every session. The next step had the door completely closed, and the dog working independently on the task. The number of S+ samples within a session was decreased once the dog met the mastery criteria (above 80% for both hit rate and correct rejection rate for four out of five consecutive sessions). As the number of S+ samples decreased, the number of S- samples increased proportionately. All dogs began discrimination training with nine S+ and eight S- samples. Training and testing

continued until all dogs met the criterion threshold with five S+ samples and twelve S- samples.

For each dog, two sets of four sessions (eight sessions in total) were conducted an hour apart. Each session consisted of seventeen trials, where a trial referred to the presentation of an odour sample in a segment. The arrangement of samples was randomized after each set of sessions.

2.9.2 Testing protocol in odour discrimination stage.

During the preliminary training stage, the dogs were trained to discriminate between amyl acetate and deionized water. Deionized water does not have a specific scent and was presented as a neutral negative sample. Thus, it is possible that the dogs could be indicating on the presence of any odour, rather than the scent of amyl acetate specifically.

Therefore, the researcher introduced another chemical as a negative sample to test the accuracy of the dog's odour discrimination. The testing protocol for this stage followed the previous stage, and the same ratio of five S+ samples and twelve S- samples were presented. However, half the S- samples consisted of the previously used S- sample (deionized water) and the other half consisted of the newly introduced chemical.

Hexanol was randomly selected from the list of the distractor chemicals - and was presented as an S- sample in this phase. A non-target distractor sample was selected so that the dogs would not be exposed to a sample type that might be a target in the upcoming experiment. All samples presented in this phase were placed in a randomized arrangement on the apparatus. The dogs were required to meet the mastery criteria of a minimum of 80% for both hit and correct rejection rates, for 4 out of 5 sessions, before progressing to the experimental stage. Testing at this stage would determine if the dogs were able to correctly

indicate the presence of the previously used positive sample, amyl acetate in the presence of another novel odour.

2.9.3 Testing protocol in experimental stage.

The experimental sessions began once all dogs (N = 5) were working independently at a ratio of five S+ and twelve S- samples. Experimental stage procedures are outlined in Appendix I and were carried out in four separate phases. Amyl acetate, which was used as a target scent in the training phase, was not used in the experimental stage. Seventeen vials were placed in each of the segments on the apparatus. In each session, five different scents were presented to the subjects; one target scent, three non-current target scents (NC_A, NC_B, NC_C), and one distractor scent (Figure 7). Of the 17 vials on the carousel, 5 vials contained target scents, and three vials contained distractor scents. The remaining nine vials were allocated for the three non-current target scents. Each non-current scent occupied three segments.

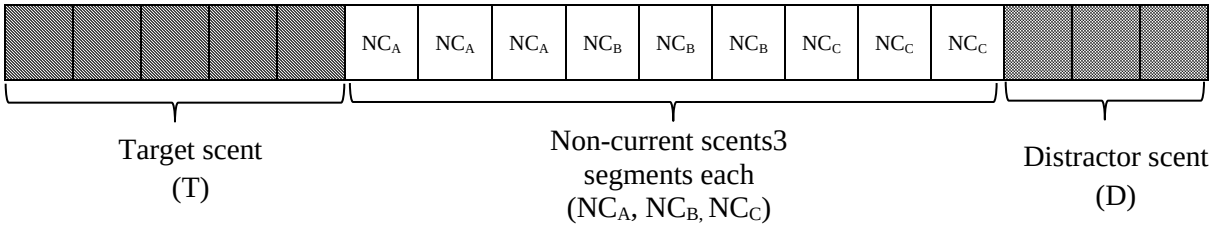

Figure 7. Visual representation of the proportion of scent samples among the 17 segments. Non-current scents refer to the list of scent samples that were not currently the target scent but would potentially be target scents in later phases. The distractor scents refer to the scent samples that would never be presented as a target scent in this experiment.

Two mastery criteria were set for this phase (Table 8). The first mastery criterion remained the same as the first phase, a minimum of 80% for correct indication responses on S+ trials, for four out of five sessions. The second mastery criterion was a minimum of 80% for correct rejection responses for the remaining S- trials (both non-current targets and distractor scents), for four out of five sessions. This criterion permitted the subject to make

two incorrect indication responses on S- trials in a session, and still meet criteria. However, the subject was only permitted to indicate once on the previously trained target scent to meet criteria. If the subject indicated more than once on the immediately previously trained target scent, the criteria would not have been considered met. The experimental procedure was repeated in every phase of the experiment. In every phase, the most immediately previous target scent was presented until criteria was met. The remaining two non-current target scents were chosen by sampling without replacement.

The target scent selected for an experimental phase was presented in every session within that experimental phase. During the next phase, the target scent from the previous phase was presented until mastery criteria were met. Once mastery criteria were met, the target scent from the previous phase would only be presented as a non-current target scent when chosen in the sampling selection process. Non-current target scents and distractor scents was chosen at random from two separate lists, following the method of "sampling without replacement". In this method, each sample scent in the full array of ten chemicals was only selected once. Once a sample was selected, it was not replaced in the sample set, and was not available for further selection until all samples had been selected. At this point, the array was repopulated and random sampling of all chemicals began. The other non-current target scents were also chosen in this manner, except for the target scent from the previous phase, which had to first meet the extinction criteria. This process was done to approximate counterbalancing of the presentation of non-current target scents, and to ensure that each dog was similarly exposed to each chemical.

The details of each phase in the experiment are outlined in Table 8. In the first phase of the experiment, 2-phenylethanol was randomly selected as the target scent from the list of ten potential target scents. The remaining non-current target scents and distractor scents for

each session was selected following the method of "sampling without replacement", from the two lists, as described above. At the start of this phase, the response duration of this indication response was reduced to 501 ms. Once the dog met the minimum of 80% hit rate in a session, the duration of the of the indication response was increased in 500 ms increments. The minimum positive indication time was increased to reach a final indication threshold of 5001 ms. The response accuracy for each session was recorded, and reported as hit rates and correct rejection rates for each session.

In the second phase of the experiment, cinnamaldehyde was randomly selected as the target scent from the remaining eight potential target scents. The selection and presentation of non-current target and distractor scent samples followed the same procedures as mentioned in the first phase of the experiment. The target scent presented in the previous phase (2-phenylethanol) was presented in every session alongside other non-current scents, until the subject did not indicate on it more than once for three sessions in a row. Once the criterion was met, the previously encountered target was only presented as a non-current target scent when chosen in the sampling selection process.

Table 8

Experiment phases

Phase 1. Target scent: 2-phenylethanol Criteria: Minimum of 80% hit rate and correct rejection rate across four out of five consecutive sessions.
Phase 2. Target scent: Cinnamaldehyde Criteria: Minimum of 80% hit rate and correct rejection rate, and no more than one indication response on previous targets, across four out of five consecutive sessions.
Phase 3. Target scent: Linalool Criteria: Minimum of 80% hit rate and correct rejection rate, and no more than one indication response on previous targets, across four out of five consecutive sessions.
Phase 4. Target scent: Ethyl butyrate Criteria: Minimum of 80% hit rate and correct rejection rate, and no more than one indication response on previous targets, across four out of five consecutive sessions

2.10 Data analysis

SPSS (IBM, Version 26.0, Armonk, NY) was used for all analyses. Data from the experimental sessions were recorded and analysed to derive the descriptive statistics of their performance. The specified alpha for all statistical tests was set at 0.05. The hit rates were calculated by dividing the number of correct indications on S+ trials by the total number of S+ trials presented within a session. Likewise, the false alarm rate was calculated by dividing the number of indications on S- trials by the total number of S- trials presented within a session.

$$False\ alarm\ rate = False\ alarm \div (Correct\ rejection + False\ Alarm)$$

Chapter 3
Results

3.1 Preliminary Training Stage

All six dogs that participated in the preliminary training stage met the mastery criteria (above 80% for both hit rate and correct rejection rate, for 4 out of 5 sessions). Correct rejection rates (CRR) were used as the criteria for phase changes during data collection. CRR and false alarm rates (FA) are inverse of the other as they represent the two available responses when presented with the S- samples. FA rates were used in the presentation of results as it provides clarity for visual analysis of the graphs. Figure 8 shows the percentage of correct indications on target S+ (amyl acetate) samples and false alarms on S- (deionized water) samples per session, across a decrease in the number of S+ samples presented in a session

The six dogs were able to successfully discriminate between S+ and S- samples across a decreasing number of S+ samples (Figure 8). Almost all dogs demonstrated high false alarms at the start of training, which then decreased rapidly across this phase. Initial odour discrimination training started with a ratio of nine S+ samples to eight S- samples. It was found that the dogs took fewer trials to meet criteria as the number of S+ samples decreased. During the initial training, the average number of trials taken to meet criteria was 17 trials, which decreased to 5.83 trials at the end of the phase.

The dogs had a two-month hiatus from December 2019 to February 2020, due to renovations occurring at the scent detection facility. A sharp increase in false alarms was observed when the dogs resumed training in February. However, the dogs also showed an abrupt decrease in false alarms and maintained a low false alarm rate for the rest of the phase. Mika displayed the highest increase in false alarms, displaying a maximum of 75% false alarm responses in a session). Jasper and Hollie1 also displayed abrupt increases of false

alarms, showing a maximum of 58.33% and 41.7% false alarm indications within a session, respectively. However, both dogs maintained zero false alarms for the rest of the stage. The other two dogs (Scout and Hollie2) that resumed training in February displayed a smaller increase with a maximum of 25% of false alarm indications within a session. However, all dogs except Jasper maintained a 100% correct indication rate during these sessions. Jasper missed an S+ sample in one session and achieved a minimum of 80% correct indications within that session.

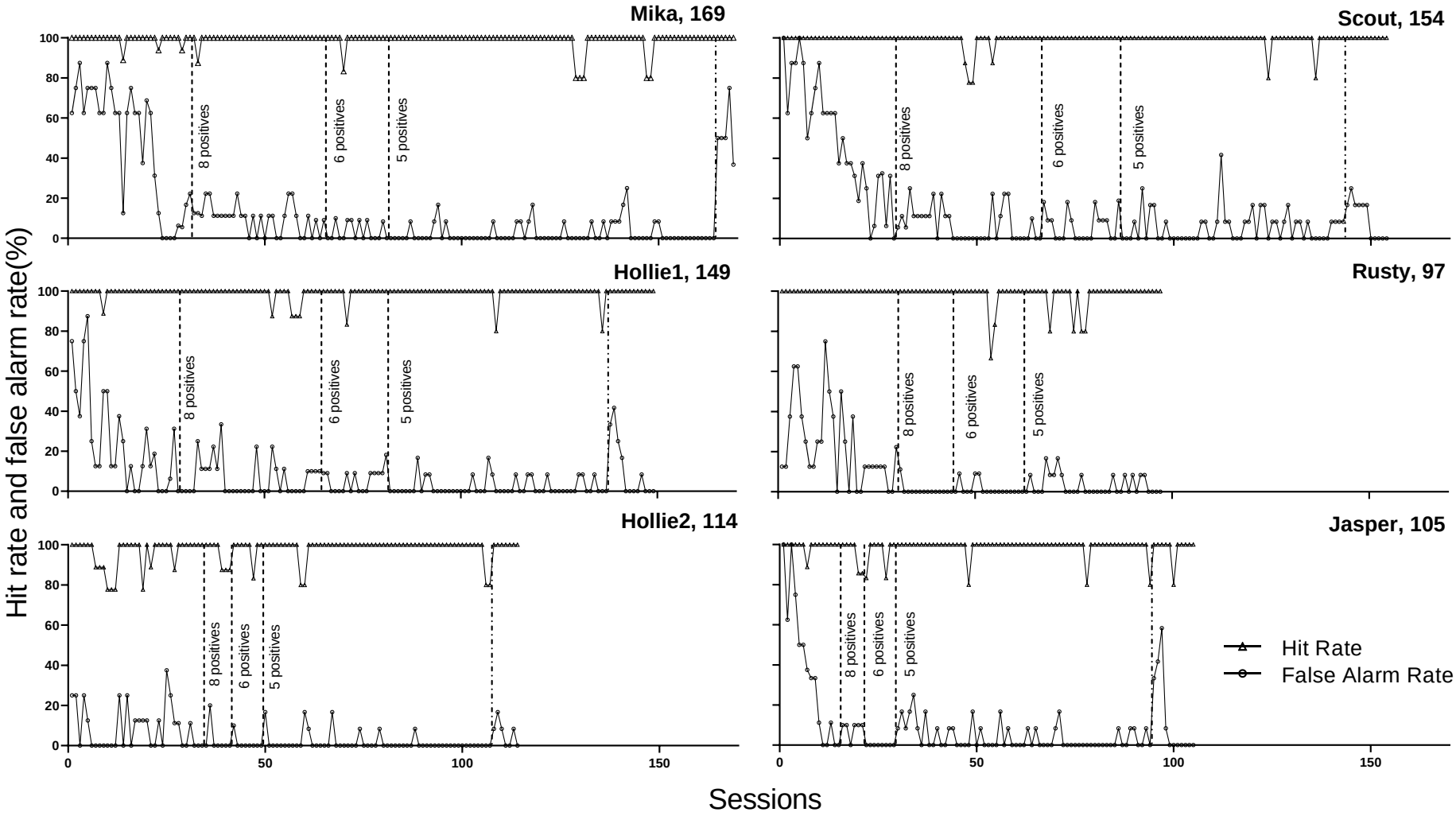

Figure 8. Performances of all dogs across S+ (i.e., amyl acetate), and S- samples (i.e., deionized water) during the preliminary training stage. Each graph is labelled with the dog's name and the number of sessions that were conducted in this stage. The first three phase lines indicate the decrease in the number of S+ samples presented within each session and the last phase line denotes the break between December 2019 and February 2020.

3.2 Odour Discrimination Stage

Rusty was withdrawn from the experiment prior to the beginning of the odour discrimination stage due to personal reasons. Therefore, his data from the preliminary training stage will not be discussed henceforth.

This stage of the experiment assessed whether the dogs were able to successfully discriminate between the target scent (amyl acetate) and the scent of a novel chemical, hexanol (Figure 9). The 12 S- trials consisted of six deionized water samples and six hexanol samples. All dogs that participated in this stage met the criteria within eight sessions (Figure 9). All dogs achieved high hit rates (M = 96.5, SD = 8.93) and low false alarm rates (M = 0.833, SD = 3.66). Mika displayed 100% correct indication response rate, whereas Jasper displayed the largest variability of correct indication responses after hexanol was introduced (min = 60, max = 100).

In the eight sessions, two dogs displayed no false alarms, whereas three dogs falsely indicated on S- trials (i.e., Mika, M = 4.18; Scout, M = 1.04; Hollie2, M = 1.04). However, the S- samples that were indicated were deionized water samples, not hexanol samples. Therefore, all dogs correctly rejected all S- trials where hexanol was presented.

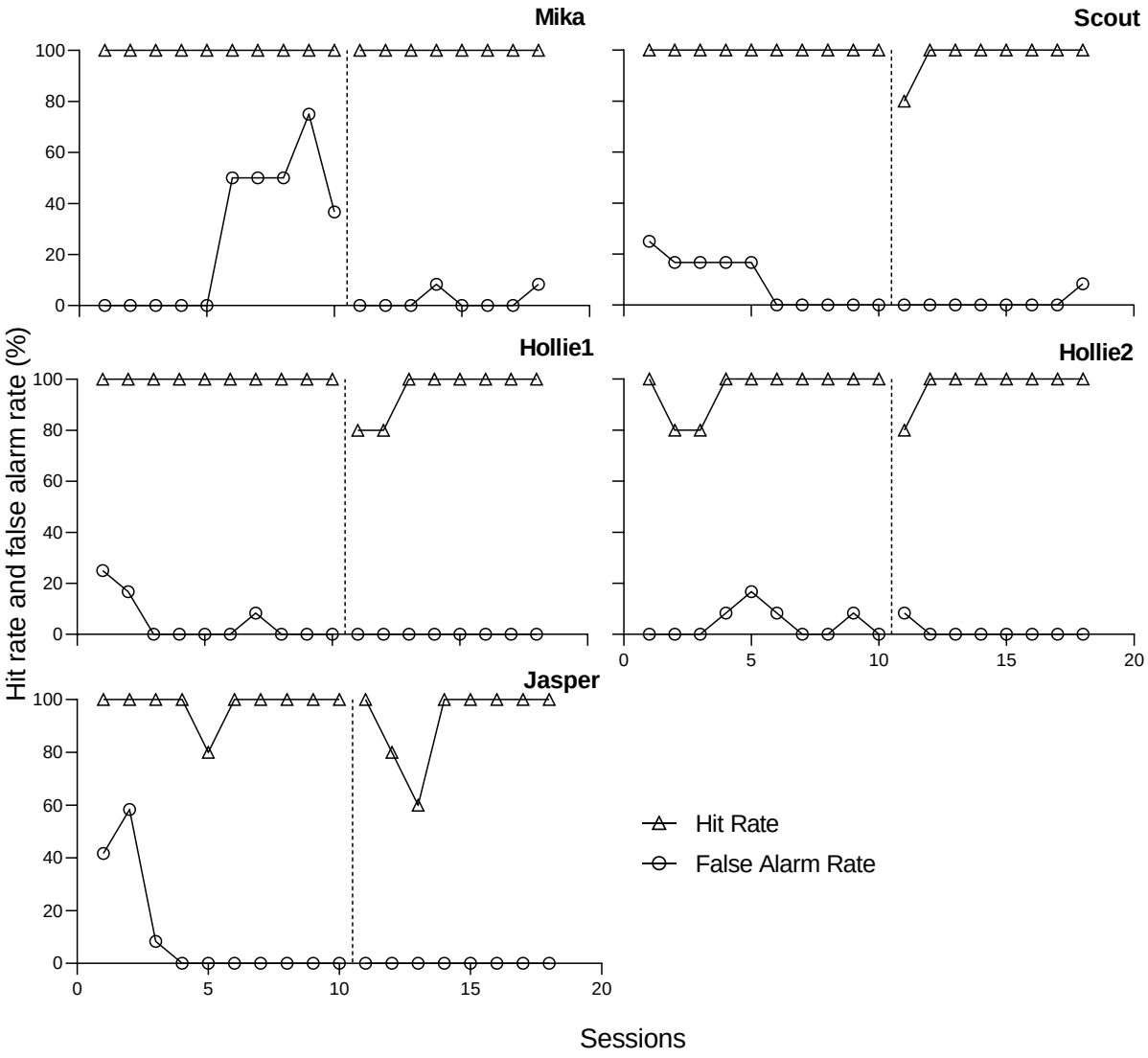

Figure 9. Performance across S+ (i.e., amyl acetate) and S- samples. Phase lines indicate the introduction of Hexanol as an S- sample.

3.3 Experiment Stage

3.3.1 Performance across phases.

Figures 10 to 14 detail the sequential performance of correct indications on the target scent (S+) and false alarms on all non-target scents (S-) for each session. The target scents that were presented in each phase were 2-phenylethanol, cinnamaldehyde, linalool, and ethyl butyrate, respectively. All dogs were required to meet criteria before progressing to the next phase.

With the introduction of each new target scent (i.e., a new phase), an immediate change in the responses was observed; some of the dogs displayed more variability in their hit rate, and there was a sharp increase in false alarm responses. The hit rate and correct rejection rates in each phase generally followed the reinforcement contingencies presented with each phase. For example, it was observed that the hit rate of the target scent within each phase was the highest compared to the indication responses on non-target scents. When reinforcement was no longer provided for indication responses on the target scent in the subsequent phases, the response rates towards the previously trained target scent would decrease.

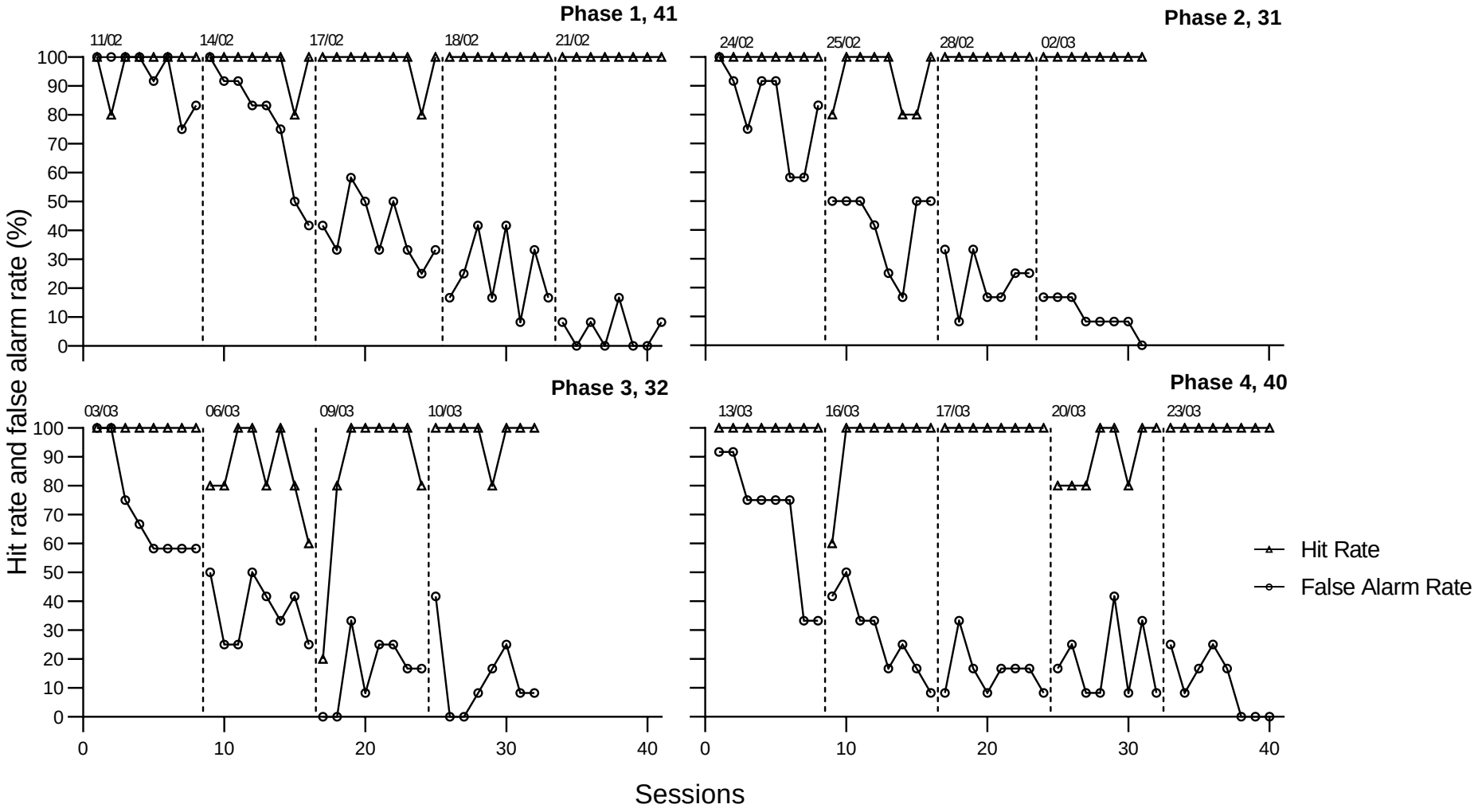

Figure 10. Mika's percentage of hit rate and false alarm responses on S+ trials, across phases. The S+ samples across the phases were 2-phenylethanol, cinnamaldehyde, linalool, and ethyl butyrate. Phase lines indicate the start and end of each experimental day, and are indicated by the dates above. Each graph is labelled with the phase number and the number of sessions required to meet the criteria for that phase.

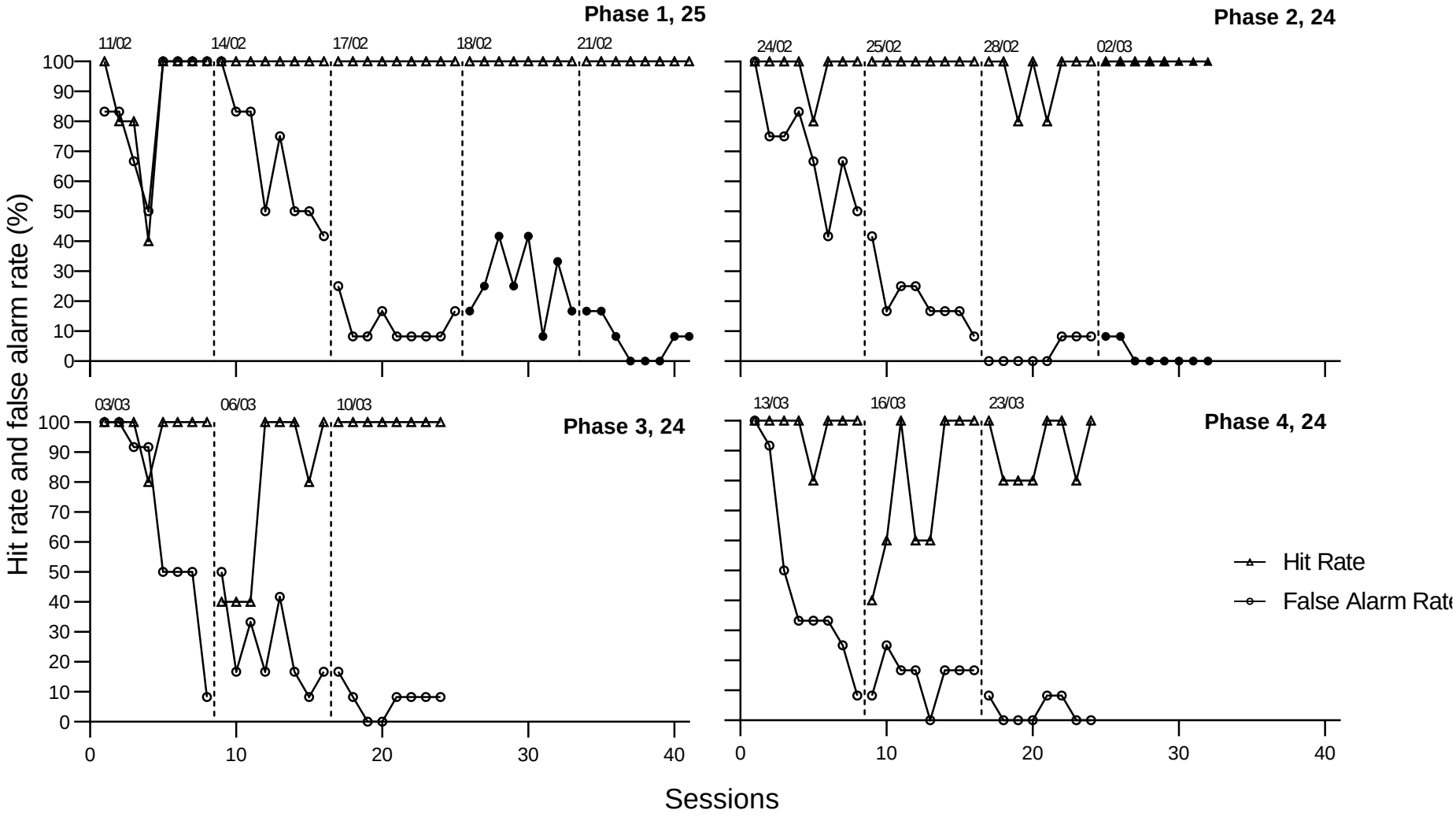

Figure 11. Scout's percentage of hit rate and false alarm responses on S+ trials, across phases. The S+ samples across the phases were 2-phenylethanol, cinnamaldehyde, linalool, and ethyl butyrate. Phase lines indicate the start and end of each experimental day, and are indicated by the dates above. Each graph is labelled with the phase number and the number of sessions required to meet the criteria for that phase. Filled data points represent sessions that were conducted after criteria was met.

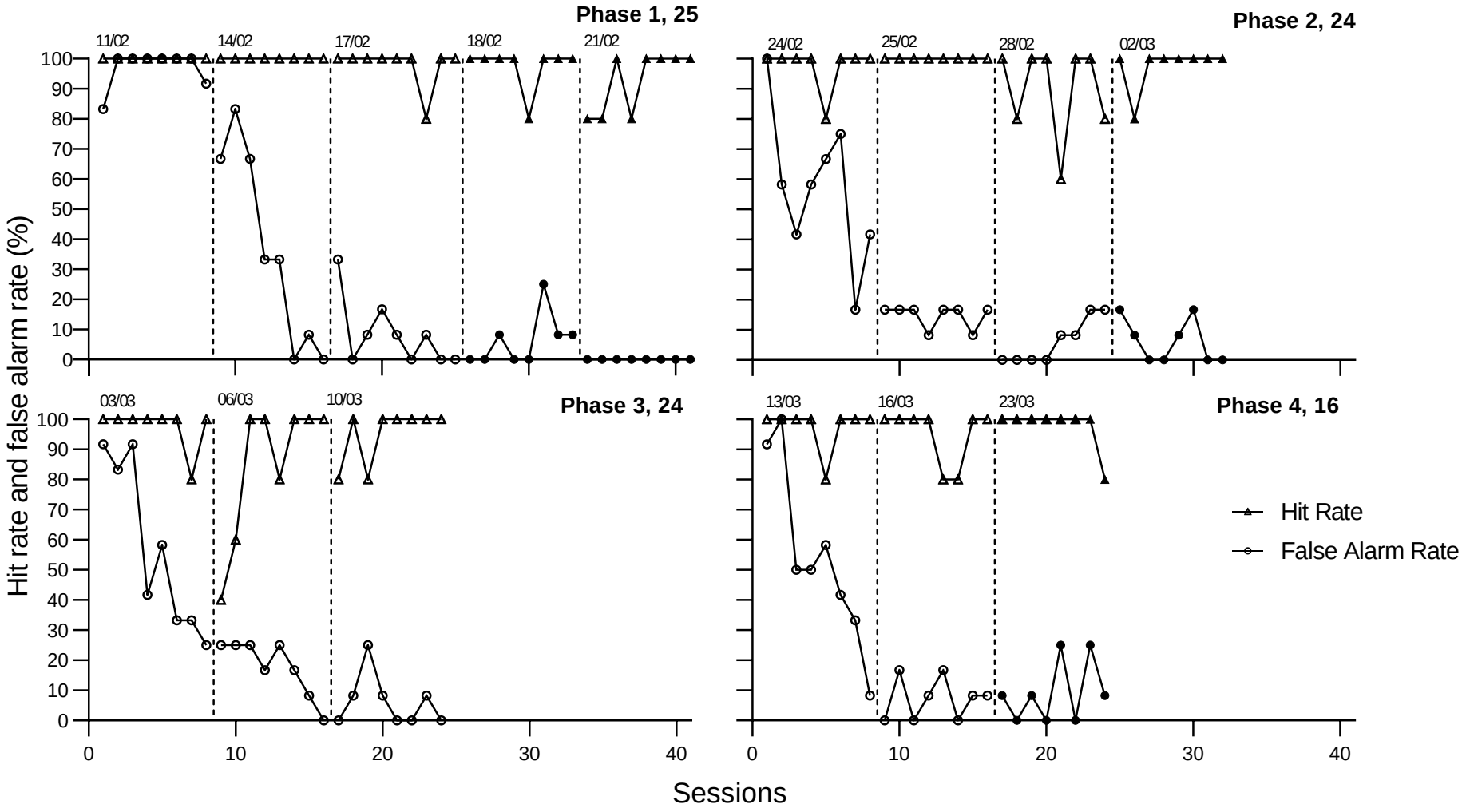

Figure 12. Hollie1's percentage of hit rate and false alarm responses on S+ trials, across phases. The S+ samples across the phases were 2-phenylethanol, cinnamaldehyde, linalool, and ethyl butyrate. Phase lines indicate the start and end of each experimental day, and are indicated by the dates above. Each graph is labelled with the phase number and the number of sessions required to meet the criteria for that phase. Filled data points represent sessions that were conducted after criteria was met.

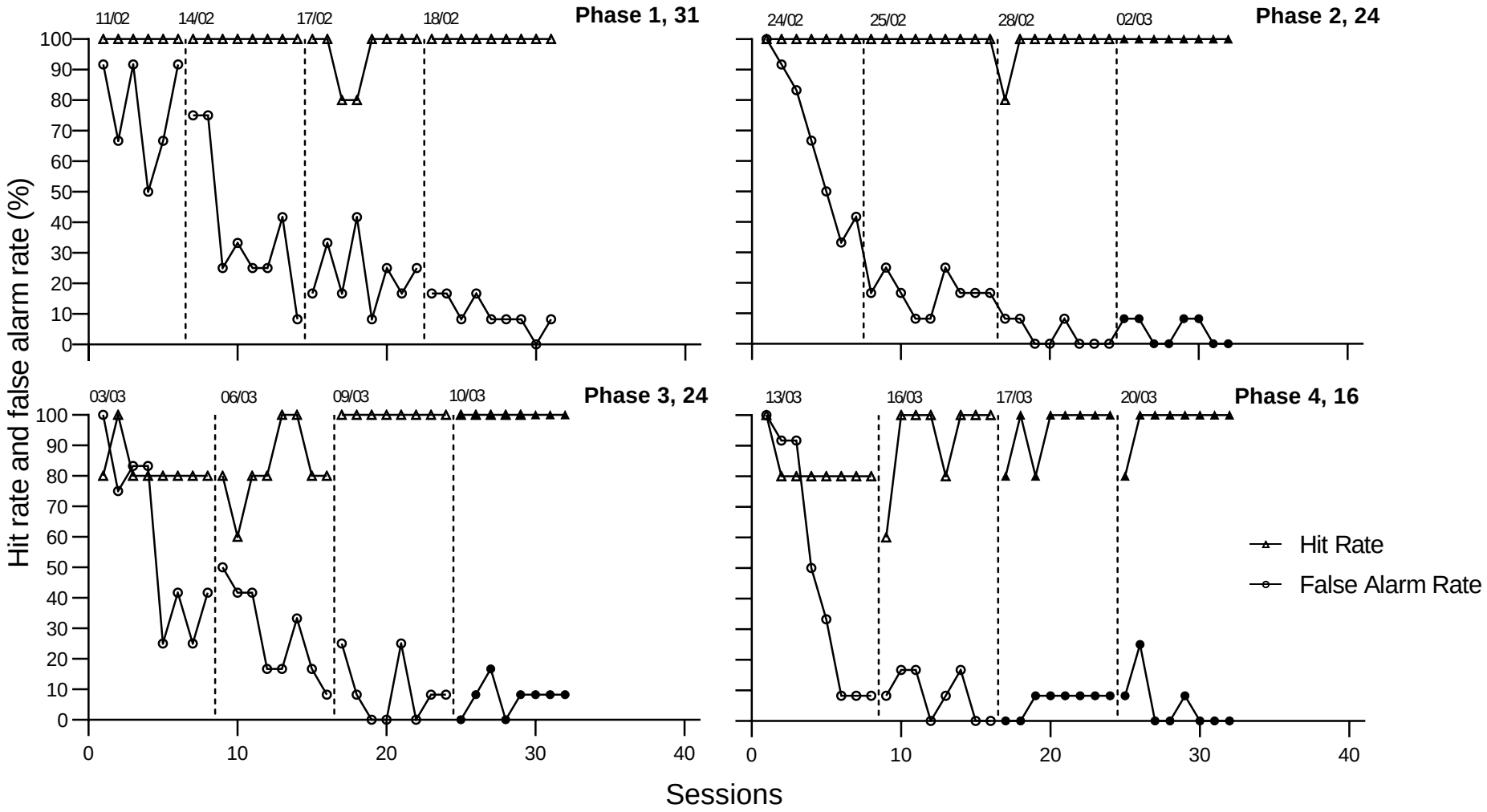

Figure 13. Hollie2's percentage of hit rate and false alarm responses on S+ trials, across phases. The S+ samples across the phases were 2-phenylethanol, cinnamaldehyde, linalool, and ethyl butyrate. Phase lines indicate the start and end of each experimental day, and are indicated by the dates above. Each graph is labelled with the phase number and the number of sessions required to meet the criteria for that phase. Filled data points represent sessions that were conducted after criteria was met.

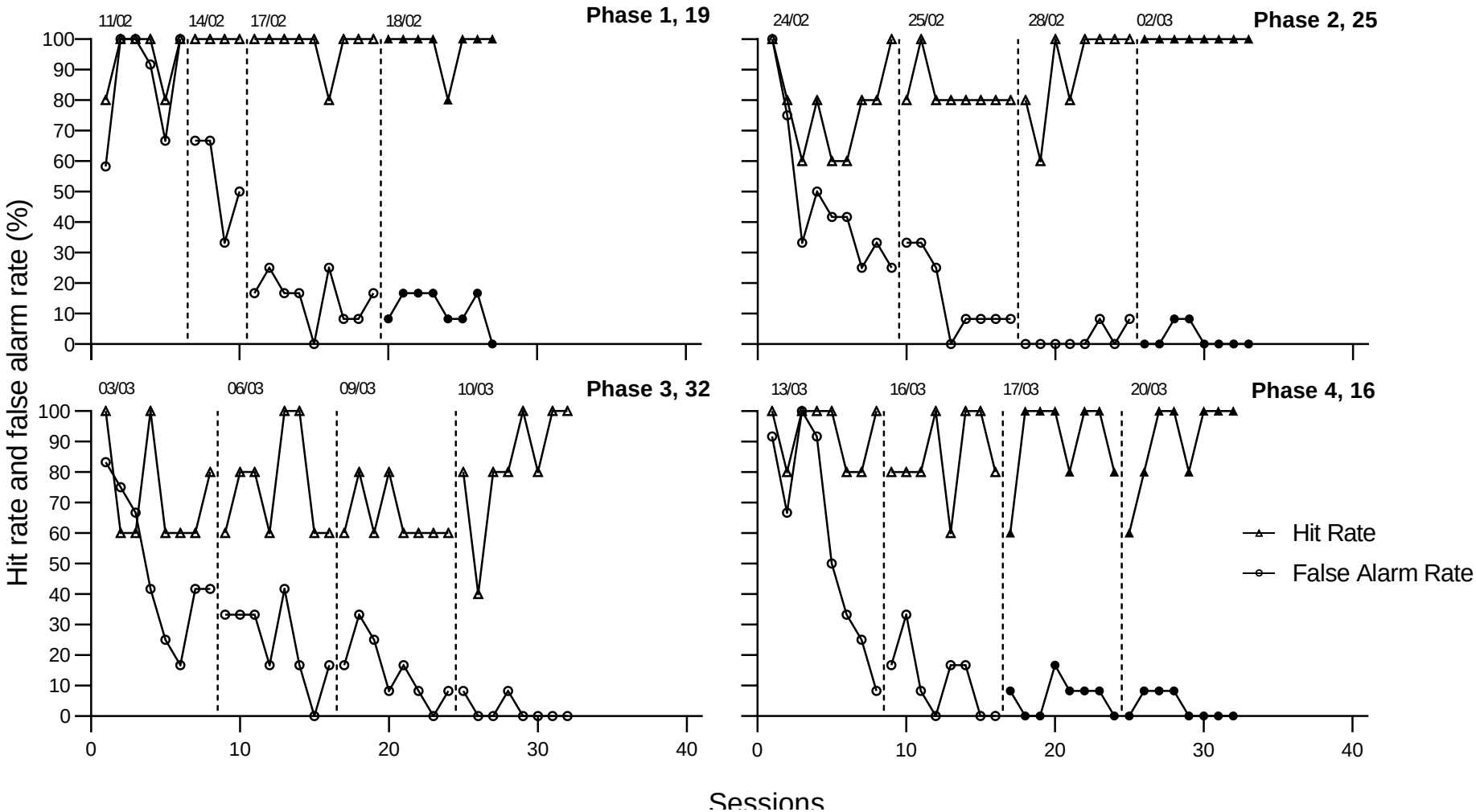

Figure 14. Jasper's percentage of hit rate and false alarm responses on S+ trials, across phases. The S+ samples across the phases were 2-phenylethanol, cinnamaldehyde, linalool, and ethyl butyrate. Phase lines indicate the start and end of each experimental day, and are indicated by the dates above. Each graph is labelled with the phase number and the number of sessions required to meet the criteria for that phase. Filled data points represent sessions that were conducted after criteria was met.

The figures show that while the hit rate across all dogs was consistently high in the first phase, the hit rate showed more variability in the later phases. A Friedman test was conducted to determine the difference between the average hit rates of each phase. The results showed that the hit rates were statistically significantly different across phases of the experiment, $\chi^2(3) = 9.72$, $p = .021$. Post hoc analyses showed that there were statistically significant differences between the hit rates in Phase 1 (*Mdn* = 97.56) and Phase 3 (*Mdn* = 91.25) of the experiment ($p = .02$). The figures also depict a rapid decrease in the percentage of false alarm responses within the first few sessions of each phase. The false alarm rates were significantly different across phases of the experiment, $\chi^2(3) = 12.12$, $p = .007$. Post hoc analyses showed that there was a statistically significant difference between the false alarm rates in Phase 1 (*Mdn* = 35.5) and Phase 4 (*Mdn* = 21.52) of the experiment ($p = .009$).

Mika displayed the highest average for both hit rate (*M* = 96.35, *SD* = 31.68) and false alarm rate (*M* = 31.68, *SD* = 26.22), showing the highest accuracy of correct indication responses when presented with S+ trials, and the lowest accuracy in specificity when presented with S- samples throughout the experiment. In contrast, Jasper displayed the lowest average for both hit rate (*M* = 86.45, *SD* = 15.83) and false alarm rate (*M* = 23.32, *SD* = 27.59), showing the lowest accuracy of correct indication responses, and the highest accuracy in specificity when presented with S- samples.

All dogs demonstrated variability in their hit rates across phases. However, all dogs demonstrated the most varied hit rate performance in Phase 3, where the target scent was Linalool. Scout displayed the largest range in the percentage of responses within that phase (*SD* = 20.41) and Hollie2 displayed the lowest (*SD* = 11.29), which were significantly different from the rest of the phases.

Mika, Hollie1, and Jasper displayed similar correct indication responding patterns, showing a consistent decrease in hit rate from Phase 1 to Phase 3. However, all three dogs achieved a higher hit rate in Phase 4, when they were introduced to ethyl butyrate. Figure 10 shows Mika's accuracy of correct indication responses were similar in Phase 1 (M = 97.56, SD = 10.19) and 2 (M = 98.13, SD = 5.92). However, the accuracy rates dropped in Phase 3, ranging from 20% to 100% (M = 91.88, SD = 34.37). Visual inspection of the graph showed that day two of the third phase accounted for most of the variability in Mika's performance. Accuracy rates dropped to a low of 20%, before returning to stable high levels in phase 4 (M = 97, SD = 8.53).

Hollie1 displayed a high and relatively stable hit rate across all phases, with the highest average hit rate in phase 1 (M = 97.56, SD = 39.14) which decreased through phase 2 (M = 96.25, SD = 9.18) and phase 3 (M = 92.5, SD = 15.39). Visual inspection of Hollie1's graph also showed that day two of the third phase accounted for most of the variability in Hollie1's performance, with a low of 40% of correct indications in one session. However, there was an increase in hit rate in phase 4 (M = 96.67, SD = 7.61).

On the other hand, Scout and Hollie2 had displayed similar correct indication responding patterns across phases. Both dogs demonstrated high hit rates in Phase 1 (Scout, M = 97.56, SD = 10.19; Hollie2, M = 98.71, SD = 4.99) followed by an increase in Phase 2 (Scout, M = 98.13, SD = 5.92; Hollie2, M = 99.38, SD = 3.54). However, a significant decrease was observed in the later phases, as there was more varied responding, ranging from 40% to 100% of correct indications in those phases.

Scout was the only subject that displayed an abrupt and varied decrease in both hit rate and false alarm rate in the first experimental day of the first phase. An abrupt decrease was observed in the second to fourth session of the day, as the hit rate had decreased to 40%,

before abruptly increasing and maintaining at 100% of correct indications for the rest of the phase.

It was observed that there was a similar trend among all dogs (except Mika). While there was an overall decrease in the percentage of false alarms from the first phase to the last phase, it was found that four of the dogs displayed a significant increase in false alarms in Phase 3. The largest increase was observed in Scout's performance when the percentage of false alarms increased by 9.37%, from 23.96% in Phase 2 to 33.33% in Phase 3.

When comparing the decreasing trend of false alarms, it was observed with all but one dog (Jasper), there was a more abrupt decrease in false alarms in the first experimental day of the Phases 3 and 4, as compared to the two earlier phases. This pattern of responding was demonstrated in Hollie1's data as it shows the largest difference of first day responding between the first and last phase, and decreased consistently between phases (M = 96.88for Phase 1, M = 57.3 for Phase 2, M = 57.29 for Phase 3, M = 54.17 for Phase 4). These results also show that the variability of false alarms increased throughout the phase.

3.3.2 Sessions to meet criteria.

All dogs were introduced to four target chemicals throughout the experimental phase, and met criteria for each phase within 41 sessions (*M* = 25.85, *SD* = 6.97) (Figure 15).

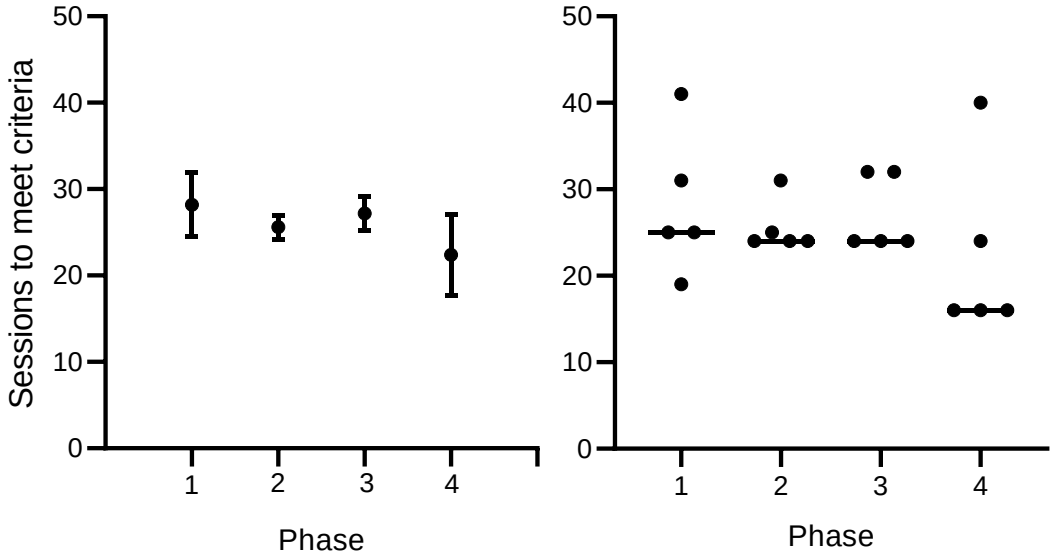

Figure 15. Number of sessions taken to meet criteria by all dogs for each new odour discrimination. Left: mean number of sessions across all dogs, with bars indicating standard error. Right: number of sessions each dog took to meet criteria for each new odour discrimination – with the horizontal line indicating the median.

A non-parametric Friedman test was conducted to determine the differences between the number of sessions the dogs took to meet criteria across the four phases of the experiment. The results showed that there were no statistically significant differences, $\chi^2(3) = 7.227$, $p = .065$ (Figure 15). Three dogs (Hollie1, Hollie2, and Jasper) met criteria in fewer sessions in Phase 4, compared to the number of sessions needed to meet criteria in Phase 1.

3.3.3 Re-emergence of indications of previously trained target scents.

The indication response rates on a target chemical in the last five sessions under extinction conditions were compared to the first five sessions when the chemical was next presented in the subsequent phase. In this study, "renewal" is defined as the relapse of an extinguished response in a context different from that in which extinction took place. In this analysis, an ABC renewal model was used to examine the changed of detection performance when acquisition, extinction and testing occurred in different contexts. Renewal was examined with the first two target chemicals presented – 2-phenylethanol and cinnamaldehyde, as these are the only two chemicals that the dogs were exposed to across at least three (ABC) conditions.

Figure 16 to 20 show the dogs' hit rates and false alarms in response to each target chemical across the four phases. Target scents across the four phases are 2-phenylethanol (T1), cinnamaldehyde (T2), linalool (T3), and ethyl butyrate (T4). Reinforcement was delivered for indication responses on T1 (target scent in the first phase, 2-phenylethanol) in Phase 1. However, responding to T1 was placed under extinction conditions in Phase 2, while a new target scent in Phase 2 (T2, cinnamaldehyde) was reinforced. As such, T1 was re-defined as a previously trained target sample and non-target scent in Phase 2. The context in which T1 was presented after Phase 2 was defined as the testing phase.

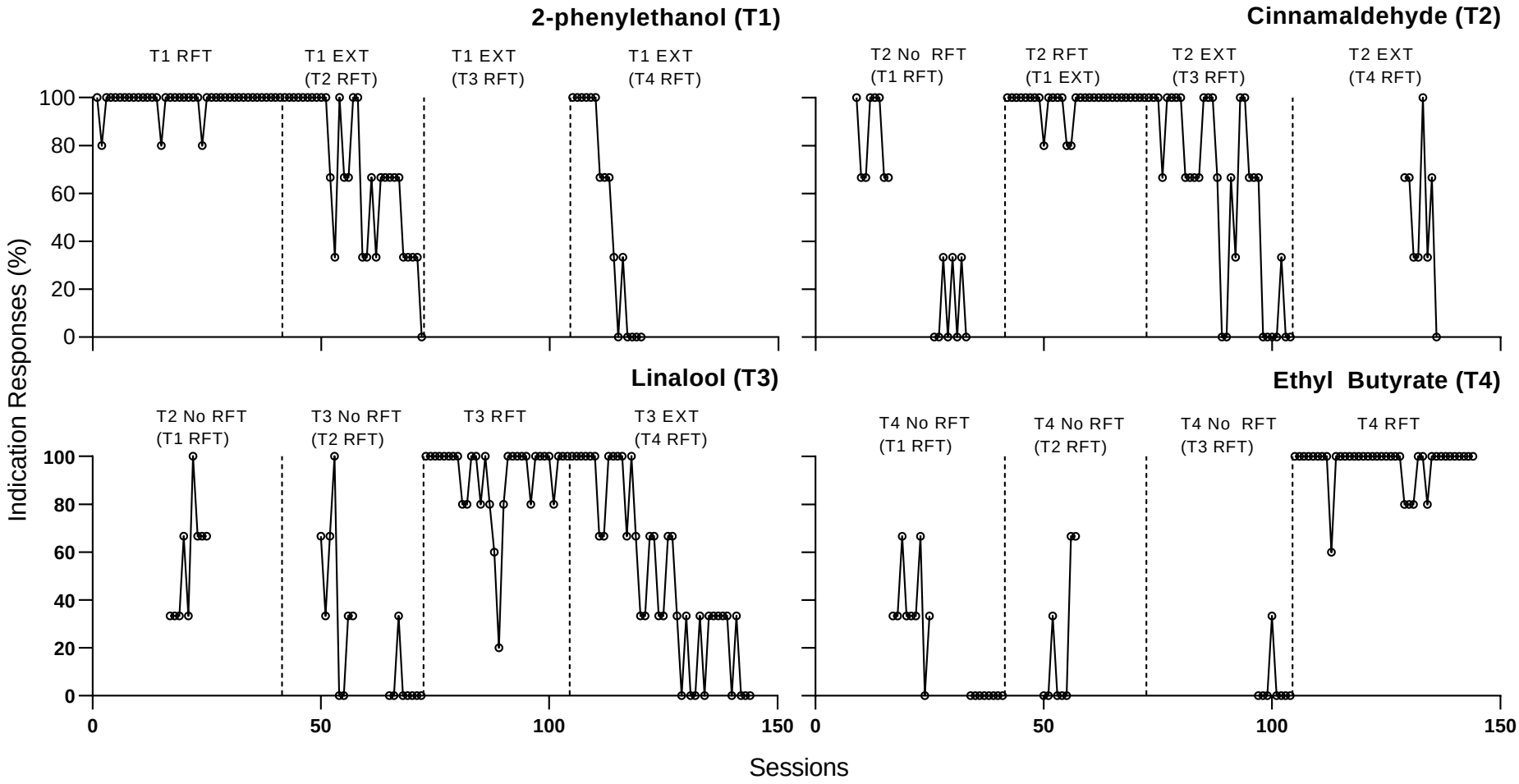

Figure 16. Mika's indication performance on the four chemicals used as target scents, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-). The graphs are labelled with the name of the chemical and the phase in which the chemical was the target scent (T1, T2, T3, and T4).

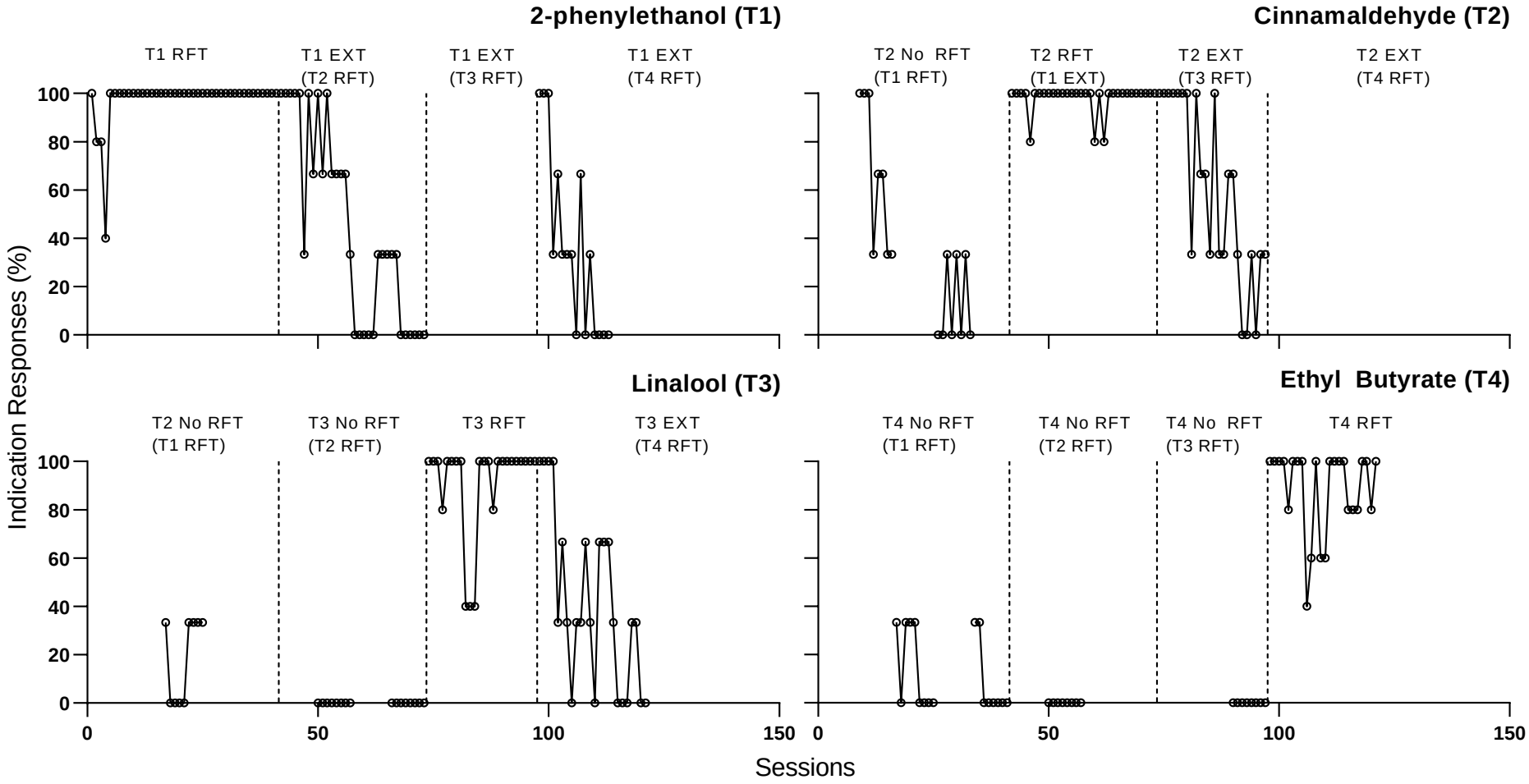

Figure 17. Scout's indication performance on the four chemicals used as target scents, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-). The graphs are labelled with the name of the chemical and the phase in which the chemical was the target scent (T1, T2, T3, and T4).

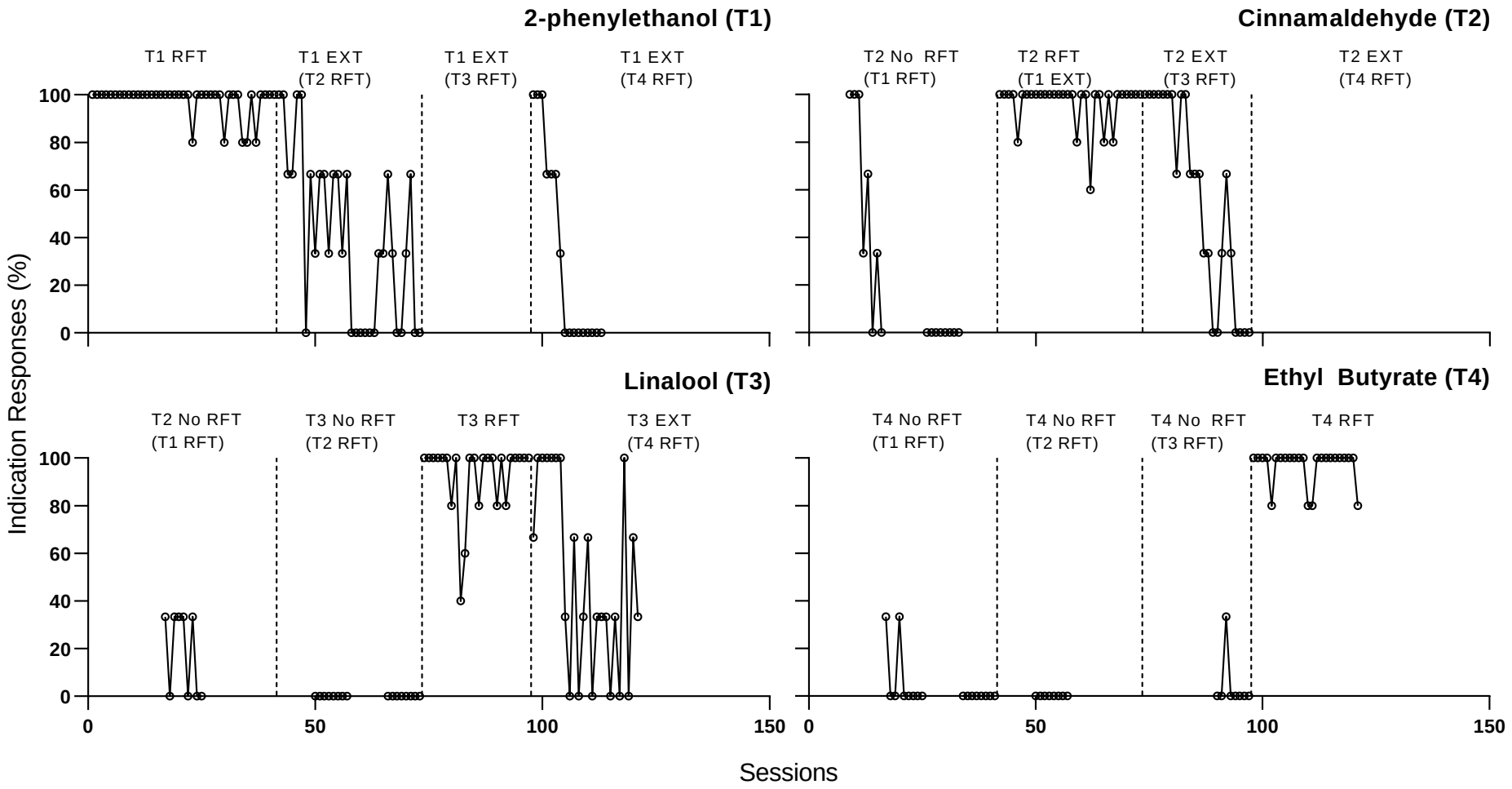

Figure 18. Hollie1's indication performance on the four chemicals used as target scents, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-). The graphs are labelled with the name of the chemical and the phase in which the chemical was the target scent (T1, T2, T3, and T4).

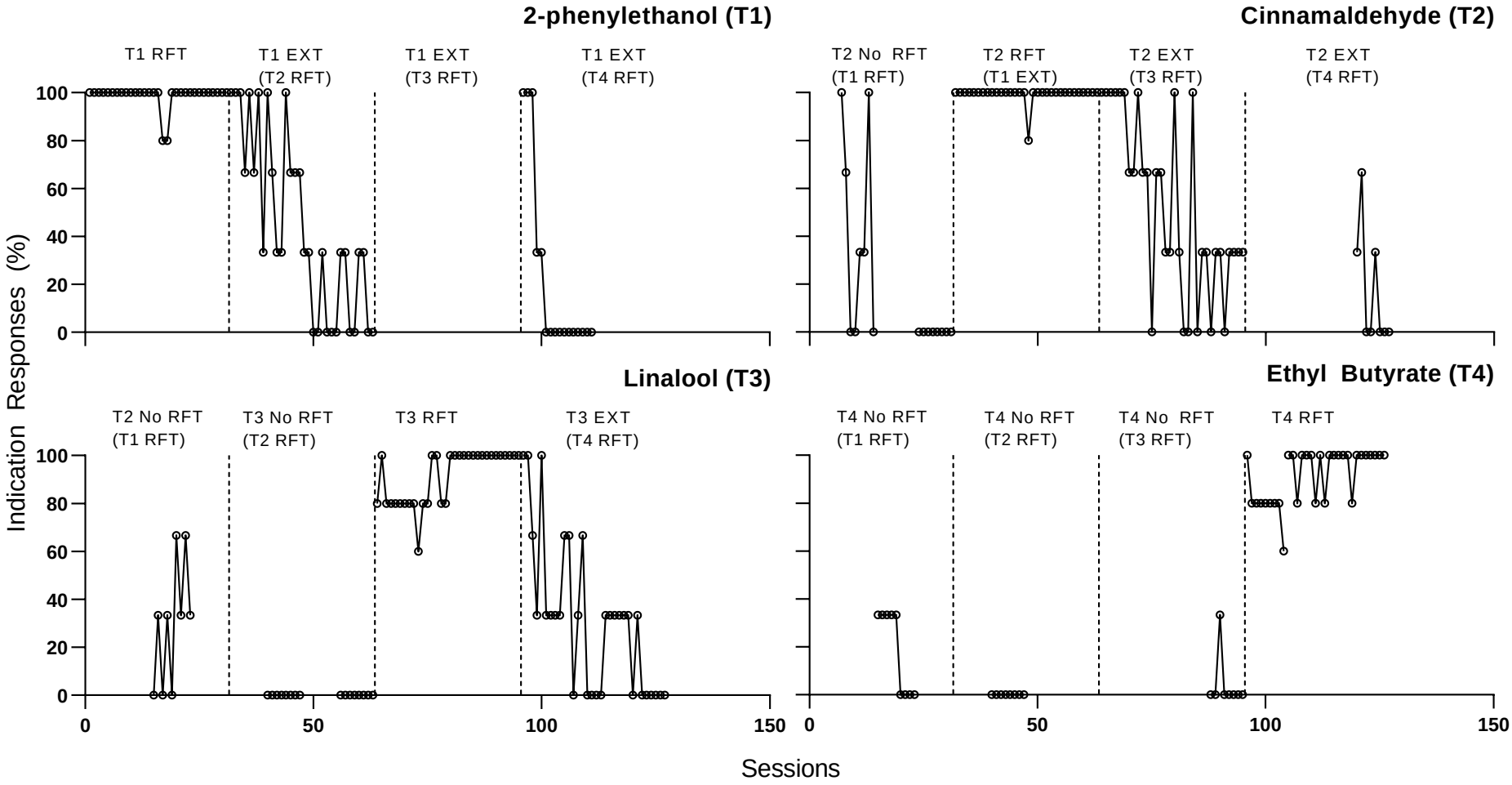

Figure 19. Hollie2's indication performance on the four chemicals used as target scents, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-). The graphs are labelled with the name of the chemical and the phase in which the chemical was the target scent (T1, T2, T3, and T4).

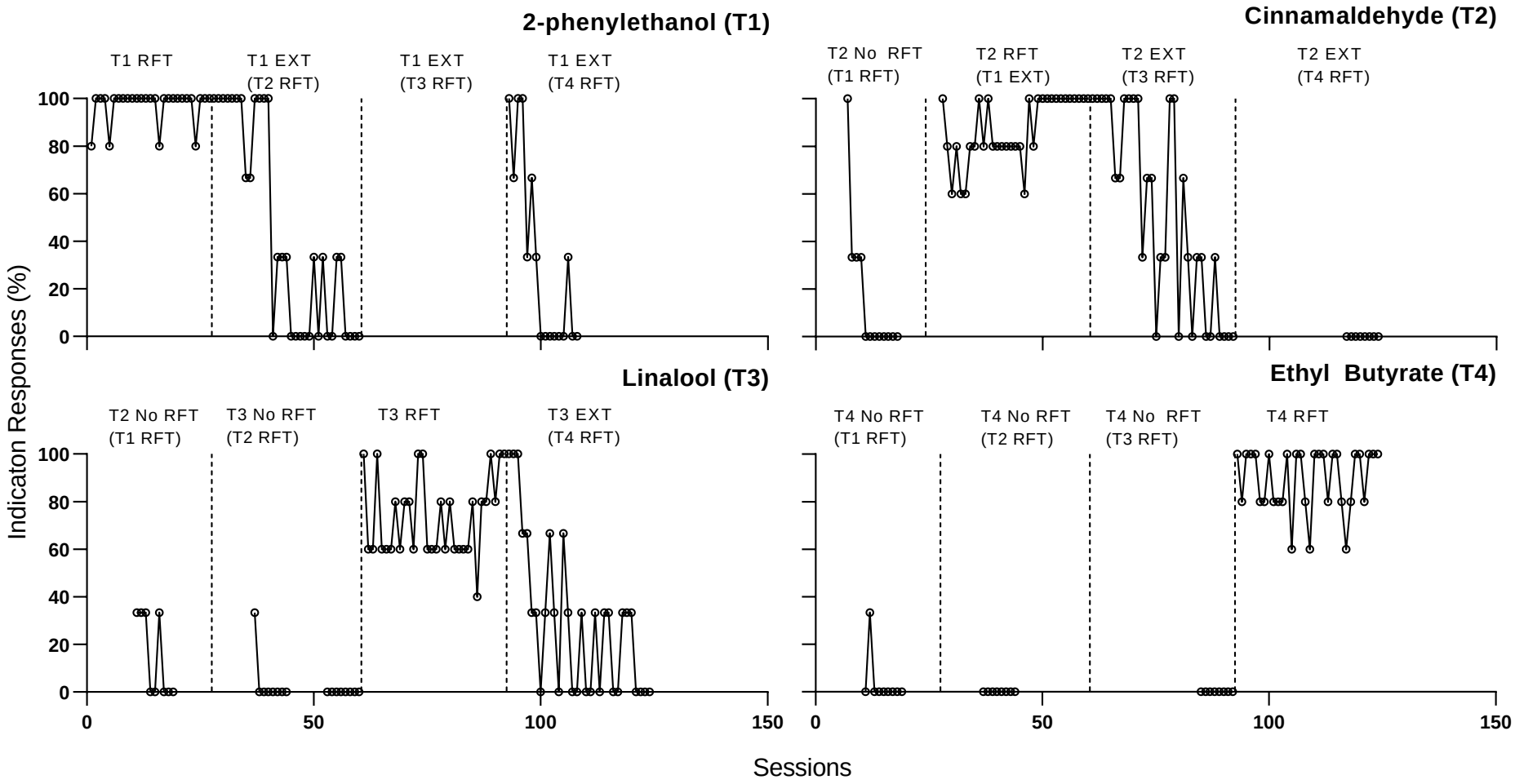

Figure 20. Jasper's indication performance on the four chemicals used as target scents, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-). The graphs are labelled with the name of the chemical and the phase in which the chemical was the target scent (T1, T2, T3, and T4).

Figures 16 to 20 depict the magnitude of renewal across all dogs when T1 and T2 were next presented in Phase 4 after responding to both target chemicals were extinguished in previous phases. Mika displayed the highest percentage of indication responses (false alarms) when T1 was next presented in Phase 4 after responses were previously extinguished in Phase 2 (M = 100, SD = 0). Indication responding increased to 100% for the first six sessions before Mika began to correctly reject trials where T1 was presented. On the other hand, Hollie2 displayed the lowest percentage of false alarms as compared to the rest of the dogs, when T1 was next presented in Phase 4 (M = 73.32, SD = 36.53). Indication responses returned to 100% for the first three sessions before Hollie2 began to correctly reject trials where T1 was presented. It was observed that Hollie1 and Hollie2's responding patterns to T1 were similar as both dogs' indication responses increased to 100% for the first few sessions of Phase 4 before rapidly decreasing and maintaining zero indication responses on T1 for the rest of the phase. This recurrence of responding to the previously extinguished target, T1, illustrates a renewal effect across all dogs.

Cinnamaldehyde (T2) was not presented to two dogs (Scout and Hollie1) after extinction due to missed testing days. As a result, their data will be excluded when testing for renewal on cinnamaldehyde. The dogs' responding patterns on each chemical differed when comparing the phase where responding for each chemical was extinguished to the phase where the chemicals were next presented after extinction. It was interesting to note that none of the dogs' indication responses returned to 100% during the first few sessions when T2 was next presented after extinction as it had when T1 was next presented after extinction. Mika showed the highest magnitude of renewal when T2 was presented in the phase after responding to T2 had been extinguished (M = 39.96, SD = 27.86). Mika was also the only dog whose indication responses to T2 after extinction were lower compared to the average indication response to T1. However, there was a larger difference in the percentage of

indication responses between extinction conditions and the next presentation of the previously trained target scents when T2 was presented (73.28% increase), as compared to when T1 was presented (33.3% increase).

Neither Hollie2's nor Jasper's indication responses towards T2 returned to 100% within a session when T2 was presented after responding was extinguished, as it did when T1 was presented after responding was extinguished. Hollie2's average indication response when T2 was presented both during (M = 26.64, SD = 14.89) and after (M = 26.64, SD = 27.86) extinction conditions remained the same. The responding pattern for Jasper was the opposite of Mika's performance. Jasper did not make any indication responses towards cinnamaldehyde when it was presented after extinction thereby displayed a decrease in the percentage of indication responses.

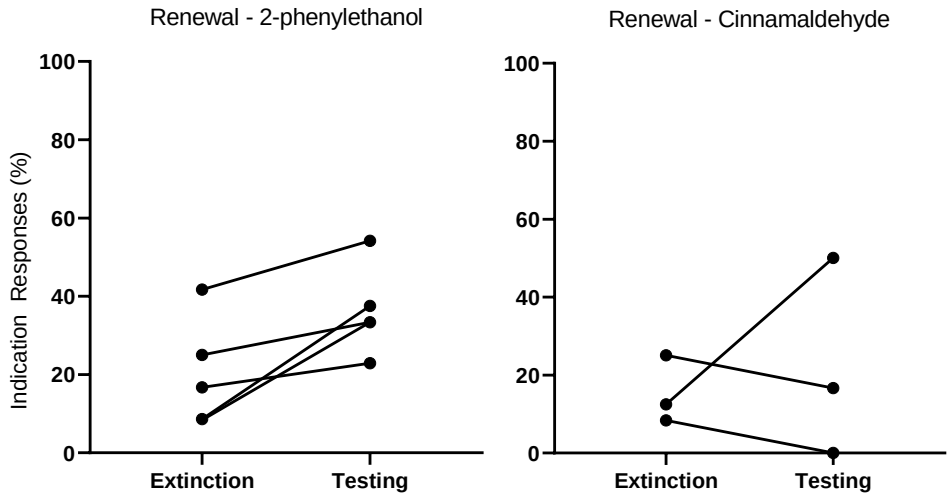

Figure 21. Renewal upon next presentation after extinction. Left: mean responding by all dogs during extinction and when 2-phenylethanol was presented next as a non-target scent. Right: mean responding by all dogs during extinction and when cinnamaldehyde was presented next as a non-target scent.

Overall, there were differences in the responding patterns on T1 and T2. In Phase 2, the levels of responding to T1 decreased when reinforcement was not delivered for indication

responses. As such, the responses to T1 decreased to eventual cessation, while the responses to T2 increased. In the last five sessions of Phase 2, indication responses to T1 were low across all dogs, meeting the criteria of less than indication response on the previously trained target scent in a session. Resurgence/renewal of responding on T1 occurred for each dog when T1 was next presented in a different phase. It was observed that the indication responses to T1 returned to 100% for the first few sessions (Figure 21). It was observed that all dogs displayed similar increasing trends across indication responses in Phase 2, as compared to when T1 was next presented in Phase 4. With extended exposure to extinction conditions, response rates to T1 decreased for each dog. The percentage of indication responses to T1 was higher in Phase 4 (M = 83.98, SD = 10.12) as compared to the percentage of indication responses during the last five sessions of Phase 2 (M = 13.34, SD = 10.56). There were statistically significant increases in indication responses on T1 between Phase 2 and when it was next presented in Phase 4 (M = 70.65, SE = 3.39), 95% CI [61.22, 80.07], $t(4) = 20.813$, $p < .001$.

The analysis of the data shows that there are mixed results regarding the presence of a renewal effect across all dogs. The recurrence of responding to the first previously extinguished target scent (2-phenylethanol, T1) illustrates a renewal effect across all dogs. In contrast, the variability in responding patterns towards the second previously extinguished target scent (cinnamaldehyde, T2) displays mixed results. The performance of both Mika and Hollie2 demonstrate a renewal effect when presented with T2. Jasper did not make any indication responses towards T2 when it was presented after responding was extinguished, and thereby demonstrates no renewal effect towards T2 for Jasper. The results further demonstrate that the experiment produced a more significant renewal effect with T1 (2-phenylethanol) as compared to T2 (cinnamaldehyde).

However, the indication responses could be affected by the part of the phase the chemical was presented. The threshold duration for an indication response to be recorded was lowered at the start of each phase to 501 ms – and would be increased to the maximum of 5001 ms. It would be more likely for the dogs to perform an indication response under a lower threshold at the beginning of the phase as compared to the higher threshold. It was observed that there were abrupt increases of false alarms and decreases in hit rates at the beginning of each phase. This could be partially attributed to the lower threshold duration at the beginning of the phase, instead of the re-emergence of indication behaviour on non-target scents.

3.3.4 Indication Responses on non-target chemicals.

Of the 13 chemicals that were presented throughout the experiment, nine chemicals were non-targets (S-) and were not presented as a target scent (S+) throughout the experiment.

A Friedman test was conducted to determine if there were any differences in the percentage of indication responses on the nine non-target chemicals throughout the experiment. The results showed that there were statistically significant differences, $\chi^2(8) = 36.85$, $p < .001$ (refer to Table 9 for the results of the paired comparisons). The chemicals that had the highest percentage of indication responses were ethyl acetate ($M = 57.08$, $SD = 11.92$), benzaldehyde, ($M = 22.6$, $SD = 21.92$), and methyl propionate ($M = 48.59$, $SD = 10.85$). There were also significant differences between ethyl acetate (highest indication responses) and butyl acetate ($M = 15.36$, $SD = 11.03$, $p = 0.44$), hexanol ($M = 7.28$, $SD = 3.41$, $p = .003$), and cyclohexanone ($M = 1.09$, $SD = 1.76$, $p < .001$).

Table 9

Pairwise comparisons of indication responses to non-target chemicals

				Comparison (p-value)								
Chemical	Mean (%)	Median (%)	SD	Ethyl acetate	Methyl propionate	Benzyl acetate	Methyl acetate	Propionic acid	Benzaldehyde	Butyl acetate	Hexanol	Cyclohexanone
Ethyl acetate	57.08	55.56	11.93	..								
Methyl propionate	48.59	44.44	10.85	1.000	..							
Benzyl acetate	41.64	47.03	10.21	1.000	1.000	..						
Methyl acetate	24.08	24.85	5.35	.753	1.000	1.000	..					
Propionic acid	21.13	21.88	3.62	.551	1.000	1.000	1.000	..				
Benzaldehyde	22.60	21.92	1.76	.399	1.000	1.000	1.000	1.000	..			
Butyl acetate	15.36	11.31	11.03	.044*	.399	1.000	1.000	1.000	1.000	..		
Hexanol	7.28	7.57	3.41	.003**	.044**	.140	1.000	1.000	1.000	1.000	..	
Cyclohexanone	1.09	.000	1.76	.000**	.003**	.012*	.753	1.000	1.000	1.000	1.000	..

SD = standard deviation

* Denotes $p < .05$

** Denotes $p < .01$

Figure 22 details the indication performance of all dogs on each non-target chemical that was presented within a phase. The mean percentage of indication responses were calculated by dividing the number of indication responses on a specific chemical by the total number of opportunities to do so throughout the experiment and multiplying the result by 100. The dogs' displayed a decreasing trend in indication responses on a majority of the chemicals across phases and maintained low levels of indication responses in later phases.

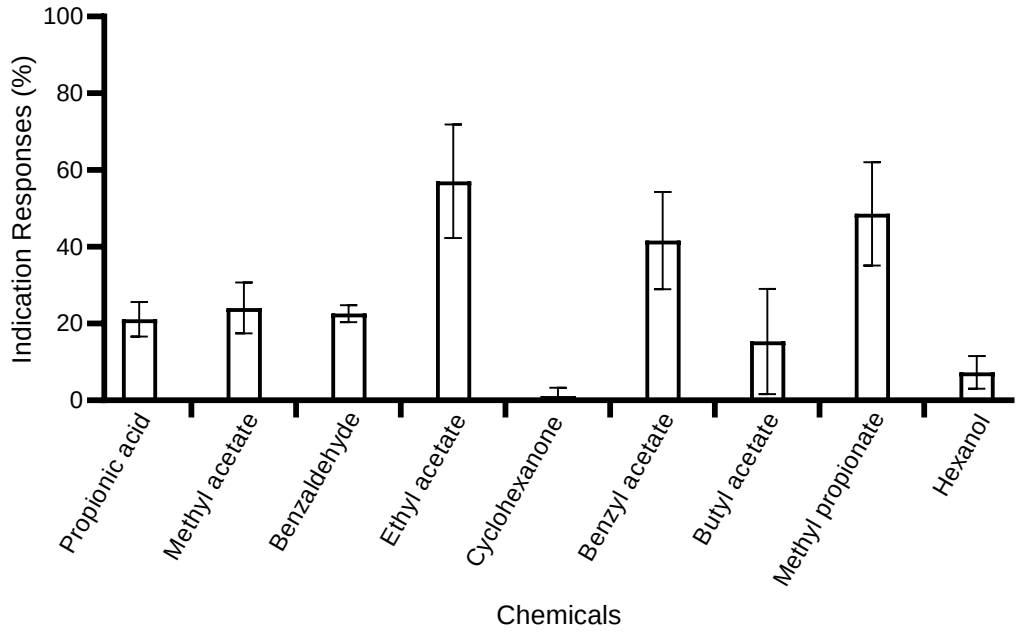

Figure 22. Mean percentage of indication responses on each non-target (S-) chemical throughout the experimental stage.

As expected, the indication responses for the specified target scent in each phase had the highest level of responses within the phase. A visual inspection of the graphs showed that there was a decreasing trend in indication responses on each chemical across phases (Figure 23). The graphs also showed that the percentage of indication responses within the last 15 trials presented were decreasing across phases.

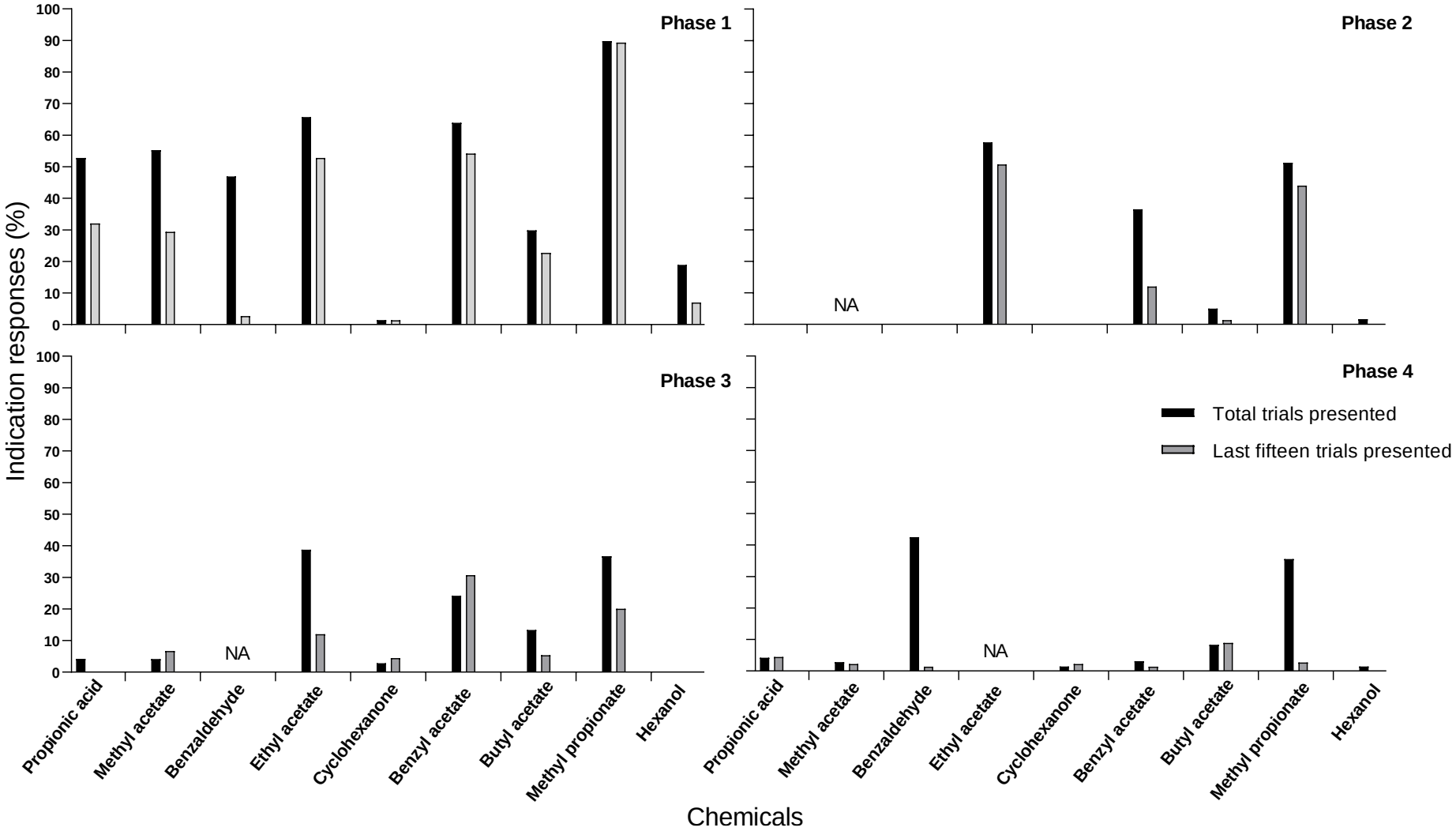

Figure 23. Indication of all dogs across all chemicals presented within each of the four phases. The black bars indicate the percentage of indication responses, while the grey bar indicate the percentage of indication responses on the last 15 trials presented in each phase. “NA” denotes the chemicals that were not presented to all dogs in that phase.

3.3.5 Indications of non-target scents across phases.

In this experiment, the re-emergence of behaviour on non-target scents was observed across phases. A few non-target chemicals were not presented in all four phases, resulting in a gap in the presentation of the chemicals. As reinforcement was not delivered for indication responses on all non-target chemicals, the phase in which the chemical was not presented allows the researcher to assess the influence of time on the indication responses on non-target chemicals. This analysis evaluated the accuracy of FA rates occurring when the chemicals were presented in the subsequent phase after not being presented in a phase.

Figures 24 to 28 show each dogs' performance on non-target scents. Methyl acetate was presented in Phase 1 and in Phase 3, leaving a gap in the presentation in Phase 2. There was high indication responding to methyl acetate across all dogs during Phase 1 (M = 55. 29, SD = 36. 32). Mika displayed an increase in responding during the first session when methyl acetate was next presented in Phase 3 (Figure 24). Responding then decreased to zero for the remaining seven sessions within that experimental day (M = 8.34, SD = 23.58). However, when it was presented on a separate experimental day, Mika displayed an overall increase in responding (M = 23.58, SD = 17.29).

There was an overall decrease in responding to methyl acetate across all dogs when it was presented again in Phase 3 (M = 4.17, SD = 10.14). Three of the dogs (Scout, Hollie1, and Hollie2) displayed low levels of responding on methyl acetate, indicating only once out of all opportunities presented within Phase 3 (M = 2.09, SD = 8.35). Jasper did not perform any indication responses on methyl acetate when it was next presented after extinction. The results of this experiment show a significant decrease in responding on methyl acetate across phases, $t(4) = 15.681$, $p < 0.001$.

Benzaldehyde was another chemical that was not presented in all four phases. It was not presented in Phase 3 and was next presented in the first few sessions of Phase 4. As the duration required to perform an indication response was decreased to 501 ms at the start of each phase, an increase in indication responses on benzaldehyde was expected. The results of this experiment display a statistically significant difference in responding when benzaldehyde was next presented in Phase 4, $t(4) = 17.395$, $p < 0.001$. Despite an increase in responding, all dogs displayed an abrupt decrease in responding within the first few sessions of presentation and maintained low levels of indication responses on benzaldehyde.

Cyclohexanone was not presented in Phase 3 for two dogs (Scout and Hollie1). An interesting finding was that while both dogs did not show an increase in responding on cyclohexanone when it was next presented after a gap in presentation, the two dogs did not perform a single indication response on cyclohexanone throughout the experiment. Therefore, this experiment demonstrated varying results regarding the re-emergence of indication behaviour on non-target scents.

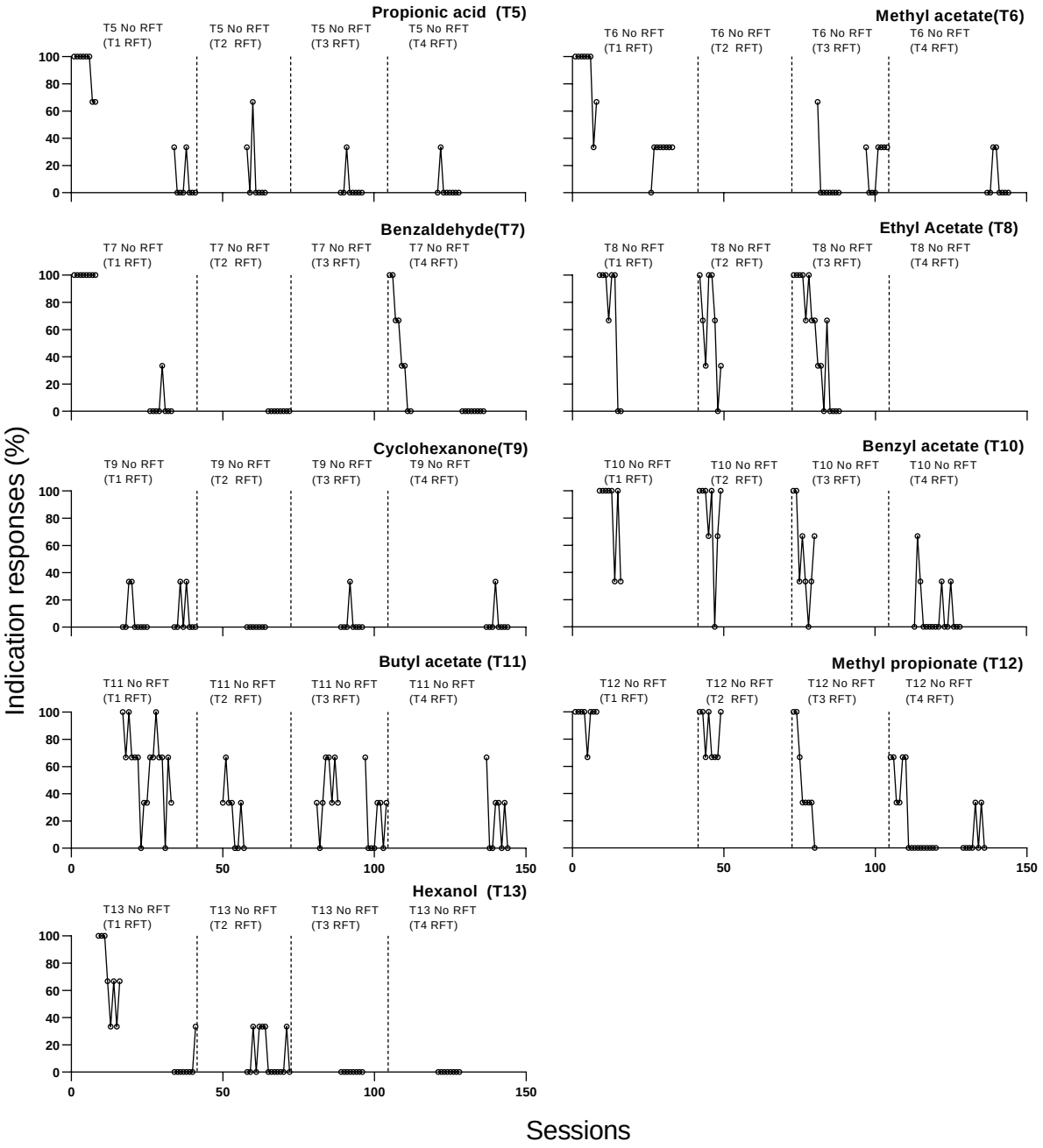

Figure 24. Mika's performance on all non-target chemicals, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-).

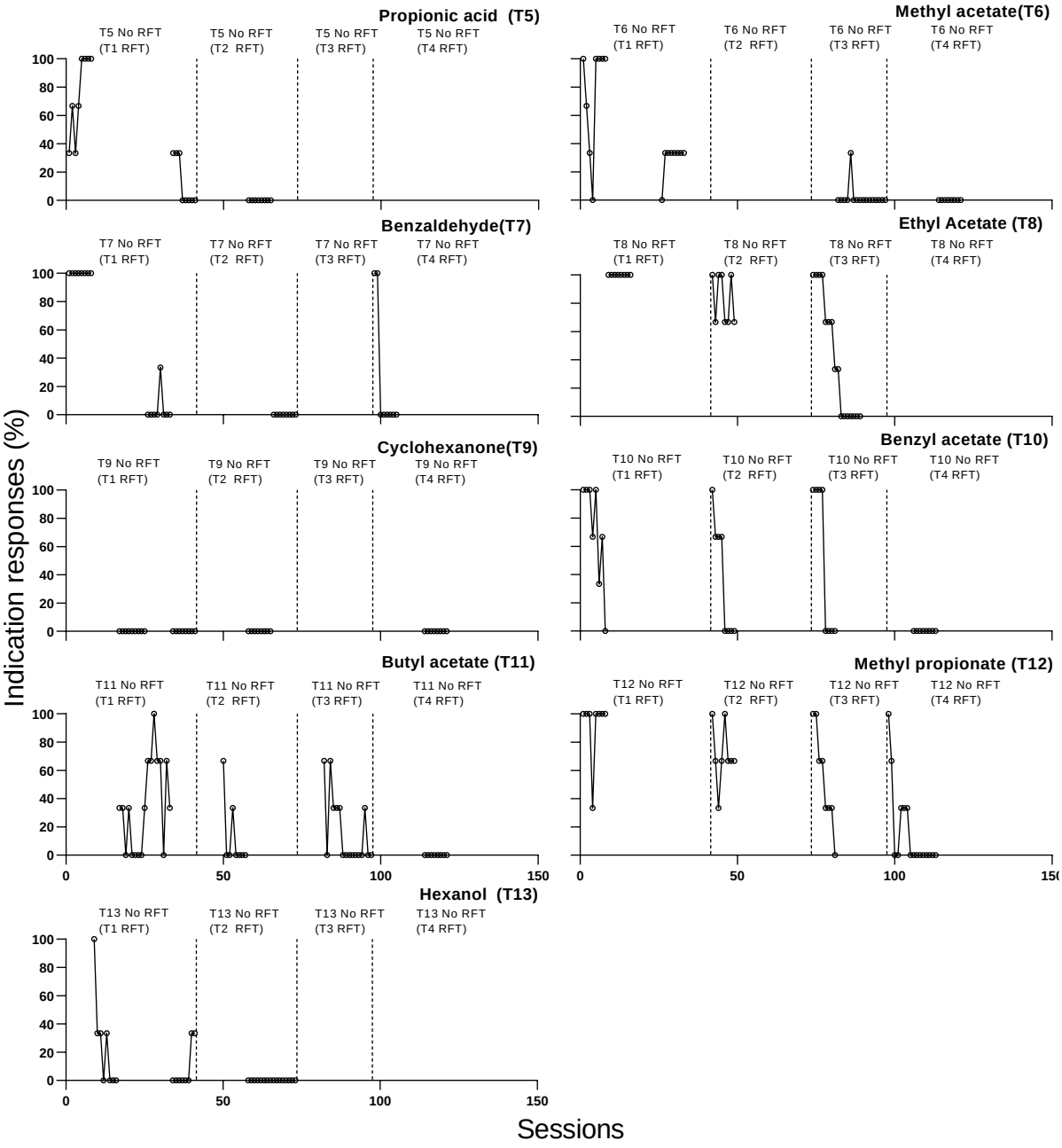

Figure 25. Scout's performance on all non-target chemicals, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-).

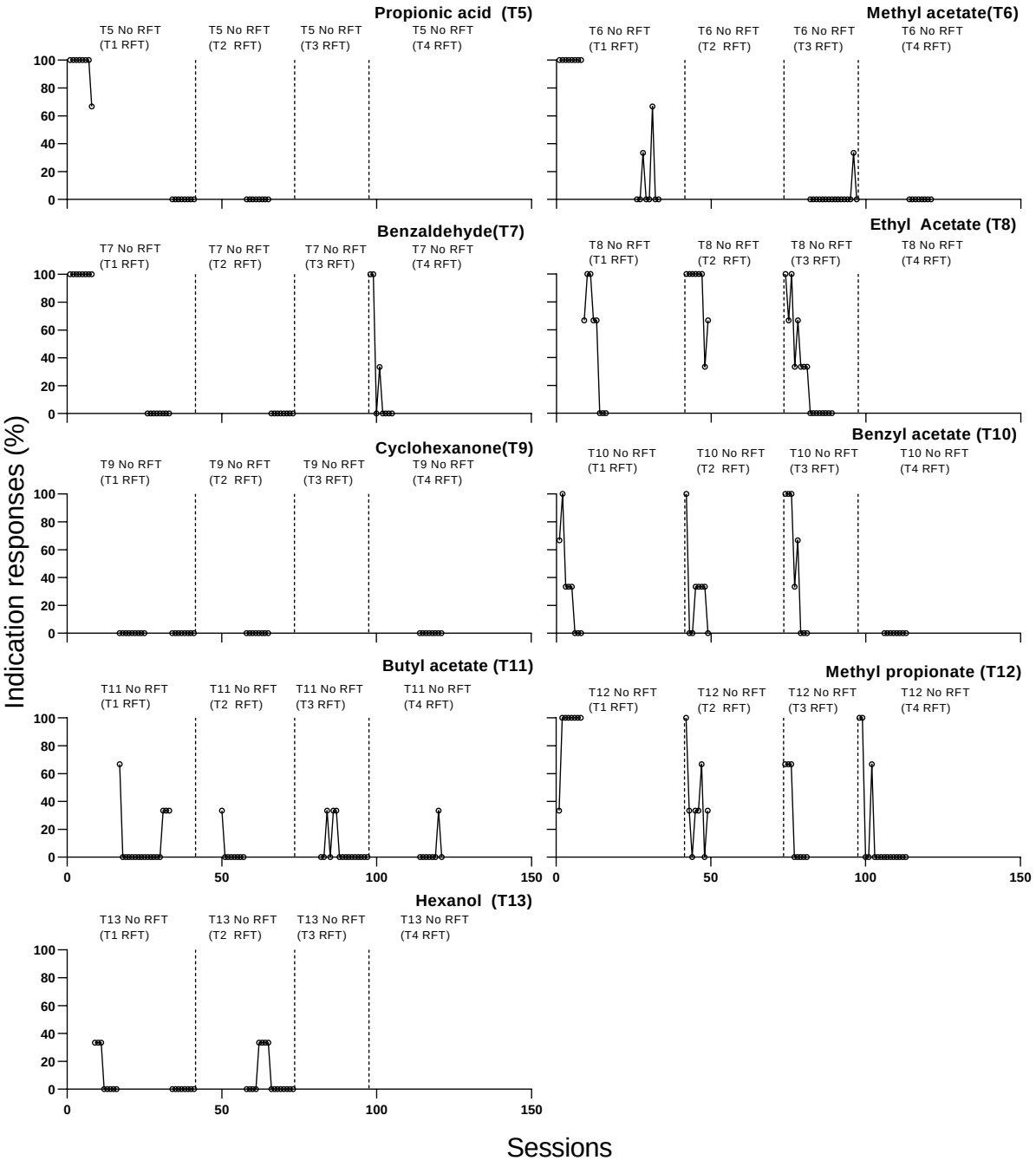

Figure 26. Hollie1's performance on all non-target chemicals, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-).

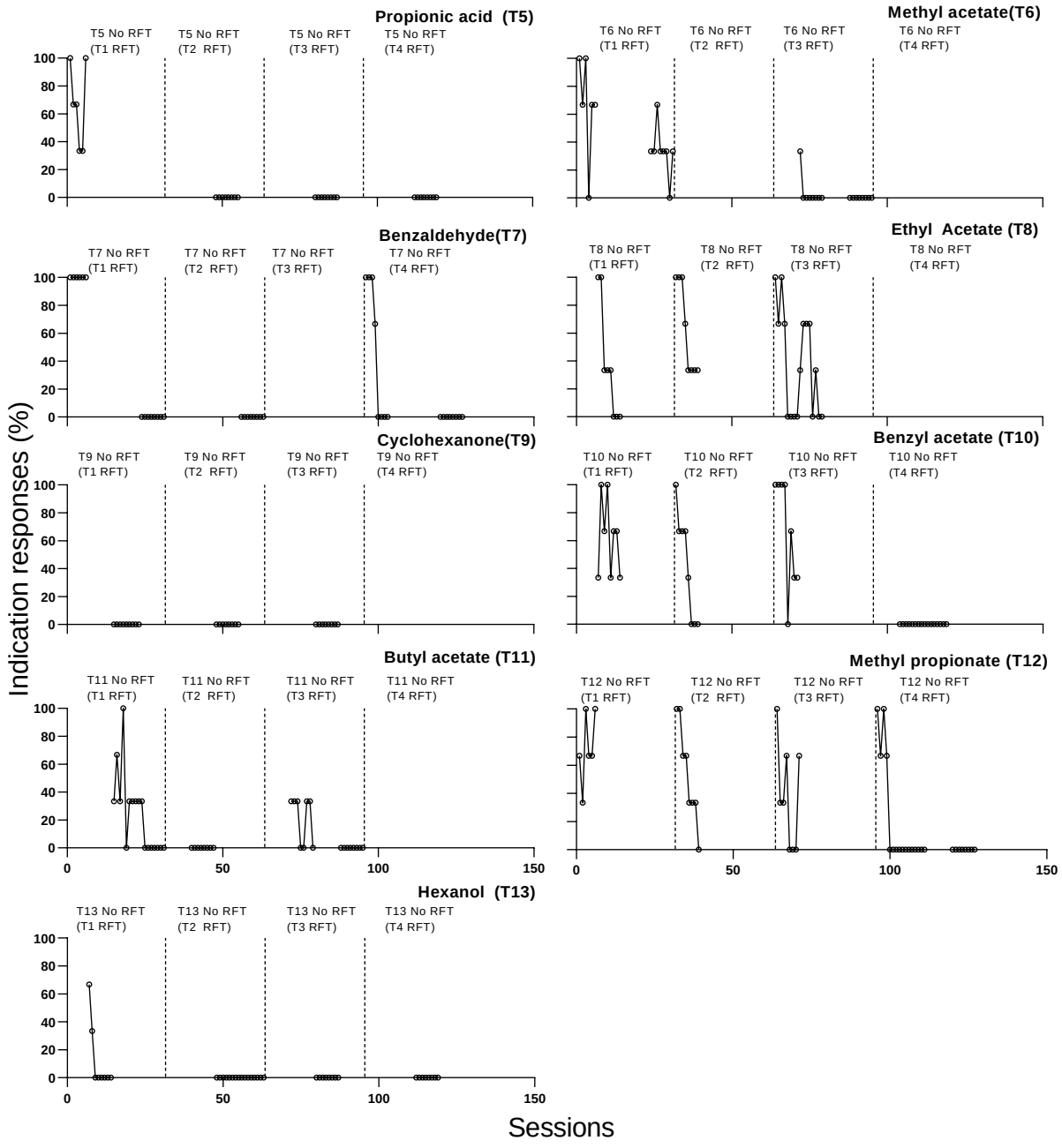

Figure 27. Hollie2's performance on all non-target chemicals, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-).

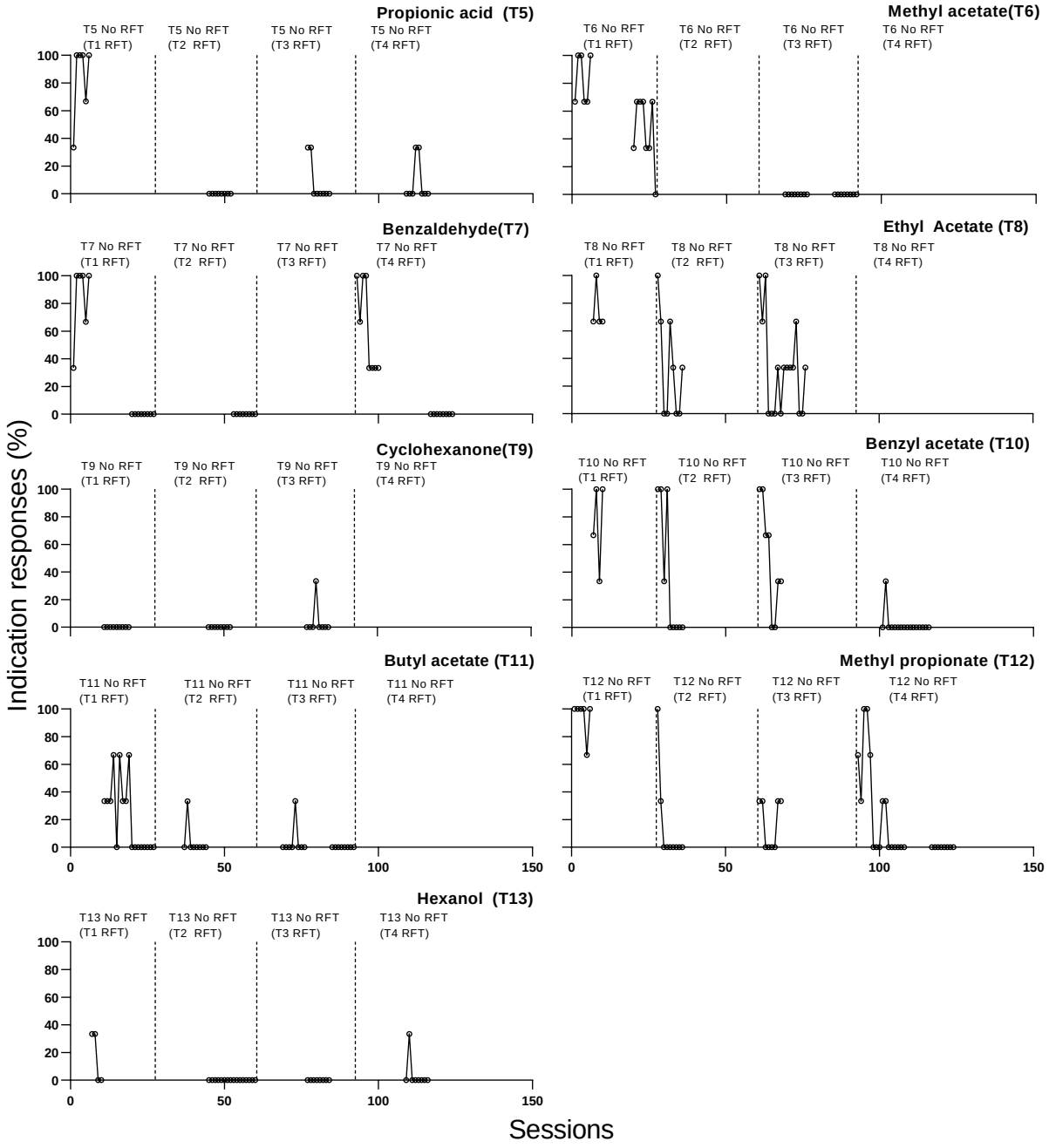

Figure 28. Jasper's performance on all non-target chemicals, per session, across phases. Phase lines indicate the start of the next phase where a new target scent (S+) was introduced and the target scent used in the previous phase would be a non-target (S-).

Chapter 4
Discussion

The overall aim of this present study was to investigate dogs' ability to learn to identify a new target odour, in the presence of previously trained target odours that were redefined as non-target odours. This appears to be the first study to systematically evaluate dogs' detection performance on rapidly changing targets. The five dogs that participated in this experiment demonstrated the ability to successfully discriminate between the specific target scent introduced in a particular phase and previously trained target scents.

4.1 Preliminary Training Stage

All six dogs that participated in this stage of the experiment were able to successfully discriminate between S+ (amyl acetate) and S- (deionized water) samples. This was not unexpected as this was simple discrimination between the presence and absence of the target scent. The dogs demonstrated high accuracy rates in this preliminary training phase, despite the decreasing proportion of S+ samples presented within a session. It was observed that the average number of trials needed to meet criteria decreased as the proportion of S+ samples also decreased. Webb et al. (2020) found that despite not receiving training sessions for two weeks, the rats in the study did not display any significant differences in overall sensitivity or specificity. However, the dogs' detection performance in this present study dropped significantly following the two-month training hiatus. When training resumed, there was an abrupt increase in false alarms, followed by an abrupt decrease. This suggests that while the dogs were still able to accurately indicate on S+ samples, the specificity with S- samples were affected by the training hiatus.

During the first part of the experiment, the ratio of S+ to S- samples was almost equal, with nine and eight samples, respectively. The dogs were able to meet the criteria in the preliminary training phase and progressed to the next stage within 169 sessions (minimum: 97, maximum: 169). The false alarm rate decreased most significantly when the proportions of S+ samples decreased to eight samples, as compared to the S+ reductions. After the initial increase in accuracy under the highest S+ to S- sample ratio, there were no significant differences in sensitivity or false alarms across stages.

4.2 Odour Discrimination Stage

The aim of this stage of the experiment was to determine the sensitivity and specificity of the dogs' detection performance in discriminating between S+ and S- samples when presented with a novel chemical. The previous stage of preliminary training presented only two samples, amyl acetate and deionized water. Deionized water was present in both sample types, so the dogs were being trained on discrimination between the presence or absence of the target scent. This raised the question of whether the dogs were indicating on amyl acetate trials because of the presence of the specific scent or simply due to the presence of any scent. If the dogs were indicating on amyl acetate because it was the only trial that presented a sample with a distinct smell, the introduction of the novel scent would result in a high false rate for hexanol. However, if the dogs were indicating on amyl acetate trials due to the specific scent of amyl acetate, the dogs would proceed to correctly reject trials where hexanol was presented resulting in high hit rates for amyl acetate trials, and low false alarm rates for hexanol trials.

Gadbois and Reeve (2016) posited that there would be a high likelihood that the dogs would spend more time investigating the novel scent when it was first presented to the dogs. The results of this stage of the experiment demonstrate that the dogs were able to successfully

discriminate between S+ and S- samples, and correctly reject all trials where the novel scent, hexanol was presented without any additional training. It was interesting to note that the only false alarms that did occur occurred on deionized water samples, rather than the novel scent, hexanol. As such, the results of this stage are in contrast to the results of Gadbois and Reeve (2016). One explanation for this could be that the samples used in the abovementioned study were snakes of the same genus which could have possibly contributed to the dogs' interest in the novel scent. Additionally, the odour of snakes is a complex bouquet of odours and may be naturally attractive to dogs, whereas hexanol may not have the same effect. Hexanol was a pure chemical (dissolved in deionized water) and had a distinct smell. The chemicals in this study were selected based on the assumption that the dogs would not have had any previous experience with the chemicals in the environment, nor would they serve any natural species-specific function. As such, it may have been less likely for the dogs to display interest in the novel scent.

4.3 Experimental Stage

4.3.1 Performance across phases.

In this phase, each dog's detection performance was compared based on their sensitivity to new target scents, and the percentage of false alarms that were emitted on S- samples. The hypothesis was that the dogs would be able to rapidly learn to indicate the presence of each new target scent, whereas the rejection of the previously trained target scents would not occur as rapidly.

All five dogs that participated in this experiment were able to discriminate between the target and non-target scents in each phase. The dogs were able to complete four phases of the experiment within 144 sessions (minimum: 121, maximum: 144). There were abrupt increases in false alarms at the beginning of every phase due to the programmed reduction of

the indication response requirement. Within each phase, false alarms decreased quickly for all dogs. This decrease could be a product of both the increased indication threshold and the increased tendency of the dogs to reject non-target samples as the phase progressed.

According to the hypothesis regarding the dogs' anticipated tendency to continue to indicate on previous targets, the number of sessions was expected to increase significantly across phases as the dogs were required to reject all previously trained target scents while learning to detect a new target scent simultaneously. However, contrary to our predictions, there were no significant differences in the dogs' detection performance, and all dogs met the accuracy criteria in each phase.

4.3.2 Sessions to meet criteria.

Each dog's detection performance was compared based on hit rates and false alarm rates, and the number of sessions required to meet mastery criteria. In this present study, the dogs were required to reject all previously trained target scents, while simultaneously learning to detect a new target scent. There was no significant difference between the number of sessions it took to meet the criteria for each target. While the rest of the dogs did show a minimal decrease in the number of sessions it took to meet criteria across successive targets, the number of sessions Mika required to meet criteria was consistent at 40 sessions in both the first and last phase of the experiment.

Both Webb et al. (2020) and Williams and Johnston (2002) conducted experiments where the new target scents were added cumulatively to the array of targets. However, the animals were not required to learn to stop responding to previous targets. Therefore, it was predicted that the tasks in the present study would be more difficult compared to the tasks in these two studies and that the dogs would require a longer training time to meet criteria for each new target scent. While both studies demonstrated a decrease in training time as

additional targets were introduced, the dogs in those studies were only required to indicate on all accumulated and new target scent. Therefore, if the dog had encountered any of the previously trained chemicals, it would perform the indication response. However, this is not always desirable in application settings.

Despite finding no statistically significant differences in the time required to meet criteria in the present experiment, it was observed in the data that new targets were being acquired more quickly. However, due to the premature termination of the project, it is unknown whether a significant decreasing trend would emerge with the addition of further targets.

4.3.3 Re-emergence of behaviour.

This present study investigated the dogs' detection performance and the response persistence under extinction conditions. In this study, response persistence refers to the continued response to a previous target, even after reinforcement is no longer delivered for the response. Response persistence was observed when a new target scent was introduced (i.e., the context was changed). The persistence of previously trained target behaviour decreased rapidly across phases. It was also observed that upon changing to a new target scent, there was an abrupt increase in the percentage of indication responses on the new target scent. This could be explained by the decrease in threshold duration at the start of each phase. As a result, the likelihood of a response being recorded as an indication was higher at the beginning of the phase. The threshold duration was lowered at the start of each phase so that the dogs were able to contact reinforcement when presented with the new target scent. These were incremental changes to denote transition between previously trained target scents and the new target. When the dogs were able to correctly perform an indication response on the new target

scent, the threshold duration would be increased gradually to meet the final threshold of 5001 ms.

The present experiment included some procedural features of both renewal and resurgence. Both renewal and resurgence involve increases in the frequency of a previously extinguished behaviour following contextual changes. With resurgence, the contextual change is the discontinuation of reinforcement for a second behaviour, whereas the term renewal applies more generally to the re-emergence of the extinguished behaviour following any contextual change. The present study confirmed that responding to the target scent re-emerged when the context was changed after extinction.

The dogs' indication response rates on the target scent were the highest during Context A as reinforcement was delivered for each indication response. The target scent was then redefined in Context B as a non-target scent – and reinforcement was not delivered for indication responses on the previously trained target scent. As such, a decrease in indication responses was observed in both Context B and C. The first context change from Context A to Context B was predicted to result in a decrease in responding as reinforcement would not be delivered for indication responses on the chemical. Responding to T1 (2-phenylethanol) was extinguished in Context B as T1 was no longer the target scent. As expected, when indications to the previously trained target chemical were not reinforced, these indication responses decreased. The second context change from Context B to Context C resulted in a re-emergence of indication responses. Responding re-emerged when T1 (2-phenylethanol) was extinguished when T1 was no longer the target (first context change) and the indications on T1 re-emerged when the target changed for a second time (second context change).

These results appear to be a demonstration of ABC renewal as the change from the acquisition (A) and extinction (B) context to the Context C resulted in an increase in the

originally trained response. This is also consistent with the theory that the new context-dependent inhibitory learning behaviour that is largely specific to the extinction context results in the re-emergence of behaviour when exposed to another context (Todd, Vurbic, & Bouton, 2014). The results of the study aligned with the hypothesis there would be a re-emergence of indication responses when previously trained target scents were presented in Context C. However, the potential issue of the threshold duration should be considered as the decrease in threshold duration at the beginning of each phase may affect data analysis of the results of the study.

4.4 Limitations and Future Recommendations

A few procedural limitations should be noted. The first limitation of the study was that the dogs were trained on each chemical for a shorter period as compared to the amount of time that working dogs might be expected to work with a target scent. The dogs in this study were exposed to each of the target scents in each phase for less than two weeks at a time. This may affect the applicability of the results to these scenarios. The longer history of reinforcement would likely affect the dogs' performance during extinction and transitions to new target scents. However, one exception may be search and rescue dogs that search areas to locate missing individuals, as these dogs are exposed to a new scent when required to search for a new individual. These dogs usually work from an article of clothing to detect and follow a matching odour trail to locate the specific person. The dogs would also be required to reject all other odours present within the environment. These search and rescue dogs are not trained on a particular scent for an extended period, instead they are trained to match the odour trails only once.

Another limitation of the study was the technical issues faced when using the equipment in the study. The researcher encountered a few technical issues while using the

apparatus as it was a working prototype. On occasion, the carousel of the apparatus would get jammed while rotating. As a result, the dogs were pulled out of the session and work had to be discontinued until the issue was fixed. Another technical issue occurred when the dogs placed their nose through the port before the segment had fully moved into place. The apparatus was programmed to stop if the dogs placed their nose through the port while the carousel was still moving to prevent any injuries. In this situation, when the dogs placed their nose through the port and encountered an S+ sample, they would hold their nose in to perform an indication response. However, any response to an out-of-place segment would not be recorded, and no reinforcement would be delivered even if the dog had performed an indication response to an S+ sample. This may have interfered with the acquisition of the target scent because the delivery of reinforcement followed an intermittent reinforcement schedule. Visual and auditory cues were provided to signal that the segment was in place. The clear front panel of the apparatus provided visual feedback for the dogs and enabled them to see when the carousel had stopped turning. As the carousel rotated, a buzzing sound provided auditory feedback for the dog. The apparatus also produced two short "beep" sounds, which indicated the opportunity for the dog to respond. However, these did not seem to be equally effective across all dogs as some dogs would continue to place their nose through the port when the carousel was rotating.

The feeder that was used in the experiment to deliver the food reinforcement (meat paste) presented a few technical issues. The feeder was programmed to deliver one piece of food each time the dogs performed a correct indication response. The feeder would then produce a "beep" sound to signal the delivery of reinforcement and dispense the programmed number of food reinforcement into the feeder tray. However, the feeder would dispense an inconsistent amount of food pieces, ranging from zero to ten pieces at once. As the number of treats dispensed was inconsistent and intermittent, the dogs would lie in front of the feeder

waiting to receive more pieces of food without returning to the apparatus. This disrupted the sessions and the dogs required prompts from the researcher to return to the apparatus.

It was also interesting to note throughout the course of the experiment that the individual differences between each dog would affect how they performed within the experiment. Although all dogs were trained to eventually work independently in the room without any additional prompts from the researcher, it was found that some dogs worked better when a human was physically present or within line of sight. One example would be Hollie1. While moving out of the room and closing the door, Hollie1 would start to stare at the door and lie down waiting for the researcher to return. While Hollie1 would not approach the apparatus when the researcher was completely out of the room, it was found that she would return to the apparatus and continue working when the door was slightly ajar and the researcher was standing outside the room within line of sight. In these situations, the researcher would not make any eye contact or provide any prompts to the dogs. This was a limitation to the study as it demonstrated that while dogs may be capable of scent detection work, not all dogs were suitable for working alone in a room without people present. Therefore, for future research using an automated approach, it would be prudent to screen dogs for such behaviour before continuing with training.

Another procedural limitation was the presentation of chemicals throughout the experiment. The dogs were able to progress to the next phase when all dogs met the mastery criteria. However, this usually occurred before all non-target chemicals were presented in a phase. This led to difficulty in data analysis. It is recommended that all chemicals should be presented at least once within each phase before all dogs progressed to the next phase. Doing so would allow the researchers to conduct a thorough analysis of the trends of indication responses on each target and non-target chemical in each phase.

One major limitation of this study was that the researcher was unable to complete the programmed research as originally planned, due to the Covid-19 pandemic. The first coronavirus case arrived in New Zealand on the 28th of February 2020. New Zealand implemented lockdown rules on the 25th of March 2020. Laboratory work at the scent detection facility was stopped for the duration of the lockdown and was unable to continue until the country entered a lower state of alert. As a result, the researcher was unable to finish the study as intended. Therefore, the study evaluated the dogs' detection performance on four chemicals (2-phenylethanol, cinnamaldehyde, linalool, and ethyl butyrate) as compared to the initially proposed ten chemicals. In order to further improve this researcher and explore the ability to retrain dogs. It would be valuable to repeat the experiments with more targets and some of the improvements recommended herein. This would allow researchers to test the dogs' detection performance when the dogs were presented with all ten target chemicals. This would also provide information on multiple targets and allow for researchers to come to more robust and convincing conclusions regarding dogs' ability to be retrained for detection of new targets.

Another recommendation for the study would be to introduce amyl acetate (or the original training target) as a non-target sample later in the study to observe the dogs' response to previously trained scent. As amyl acetate was used as the target scent for the longest period of time in the preliminary training phase (four months), introducing amyl acetate later in the study would enable researchers to evaluate the effects of a longer reinforcement and extinction history that were present with amyl acetate.

4.5 Conclusion

Training a dog to detect a new target may sometimes be necessary due to the conclusion or change in the focus of studies. However, there is a dearth of information in the literature

explaining how dogs might respond when targets change, particularly when they change multiple times. The results of this study provide evidence that dogs can be trained to successfully discriminate between a target scent in the presence of previously trained target scents. Animal scent detection programs may be able to expand the impact of their animals and thereby increasing the longevity and flexibility of each scent detection animals' career. The general methodology of the present study may be used to provide the initial framework for the investigation of retraining dogs to detect new target scents and promote lasting suppression of responding to the previously trained target scents. Future research could also be conducted to replicate these results in an operational setting with working scent-detection dogs.

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Appendix A

DOG BEHAVIOUR RESEARCH
Initial Enquiry Form

Thank you for your interest in our dog behaviour research. We are looking for dogs who enjoy going to new places and meeting new people – and who really like working for food. We have some other criteria for potential research participants, so if you are interested in your dog possibly taking part, please provide the following information.

Is your dog fully vaccinated (standard vaccines: distemper, hepatitis, parvovirus)? **Yes / No**

If **no**, please explain briefly:

_____

_____

Does your dog enjoy meeting new people? **Yes / No**
E.g., are they friendly and comfortable around strangers?

If **no**, please explain briefly:

_____

_____

Is your dog comfortable being handled by other people? **Yes / No**
E.g., Is your dog happy to be touched on their body, neck, head, tail, paws, etc.?

If **no**, please explain briefly:

_____

_____

Is your dog comfortable going to new places? **Yes / No**
E.g., is your dog relaxed and happy (showing no signs of stress) when you go somewhere new?

If **no**, please explain briefly:

_____

_____

Is your dog comfortable when you leave them, including at home alone and new places? **Yes / No**
E.g., is your dog relaxed and happy (showing no signs of stress) when you leave them?
(Dogs will not be left alone at our training facility, but we would like to know if they might have any separation-type anxieties.)

If **no**, please explain briefly:

_____

_____

Does your dog like working for food? **Yes / No**

If **no**, please explain briefly:

_____

_____

Can your dog eat any food, including kibble (biscuits) and different kinds of meat products? **Yes / No**

If **no**, please explain briefly:

_____

_____

If your dog comfortable with people getting near their food? **Yes / No**

E.g., if your dog has shown any aggression (freezing, growling, snarling, biting) around food, please select 'no'.

If **no**, please explain briefly:

_____

_____

Is your dog friendly towards other dogs? **Yes / No**

E.g., if your dog has shown any aggression or fear towards other dogs, please select 'no'
(We will not necessarily have more than one dog at the training facility at once. If we do, it will be with permission of all owners and the dogs will be contained separately.)

If **no**, please explain briefly:

_____

_____

Is your dog comfortable with unexpected/loud noises, such as beeping sounds? **Yes / No**

If **no**, please explain briefly:

_____

_____

Is your dog free of medical conditions that could be aggravated by repetitive walking? **Yes / No**

E.g., if your dog has any joint or other problems that might be affected, please select 'no'.

If **no**, please explain briefly:

_____

_____

Would you be able to drop off and pick up your dog in the morning/afternoon so that your dog spent just half a day with us (our facility is at the University of Waikato main campus)? **Yes / No**

Please indicate which times are more convenient:

_____

_____

We want to make sure that all dogs enjoy participating in our research. If you answered "no" to any of these questions, this may indicate that your dog is not suitable for some of this research. However, it does not necessarily exclude them from taking part. A researcher will be in touch with you to discuss the information you have provided here. Thank you for taking the time to complete this form.

Please email this form to: mcsy1@students.waikato.ac.nz

Appendix B

DOG BEHAVIOUR RESEARCH

Initial Enquiry Form

Thank you for your interest in our dog behaviour research.

Please provide us with the following contact information:

Owner's name	
Mobile phone	
Home phone	
Email address	
Home address	
Most convenient days & times to drop off/collect your dog?	
How did you hear about this research?	

Please provide us with the following information about a nominated emergency contact person (in case we cannot contact you):

Nominated contact person	
Mobile phone	
Home phone	
Email address	
Home address	

Please provide us with the following information about your dog's normal veterinarian:

Normal vet clinic	
Normal veterinarian	
Clinic phone	
Clinic address	

Please provide us with the following information about your dog:

Dog's name	
Breed	
Date of birth	
Age	
Sex	
Are they de-sexed?	
Weight	
Colour & distinguishing features	
Fully vaccinated?	
When are their next vaccinations due?*	
Normal food type	
Normal meal times/amounts	
Favourite food type	
Any aggression around food?	
Allergies/illnesses	
Behaviour issues	
Other likes/dislikes (e.g., other dogs, being touched, being alone, noises, etc.)	

* Please bring your dog's vaccination records with you for your first visit to the training facility.

Thank you for taking the time to complete this form.

Please email this form to: mcsy1@students.waikato.ac.nz

Appendix C

DOG BEHAVIOUR RESEARCH

Researcher's Copy

These protocols have been approved by the Animal Ethics Committee of the University of Waikato.

As the owner or duly authorized agent for the owner of _____, you are being asked to have your pet participate in the project evaluating the ability to re-train dogs to detect new target odours. Before giving your consent to your pet's participation, please read the following, ask as many questions as needed to understand what your participation involves, and sign and date the statement at the end of this document.

PRINCIPAL INVESTIGATORS

Maria Chia, 022 625 4505, mcsy1@students.waikato.ac.nz
Dr Tim Edwards, 07 837 9409, tim.edwards@waikato.ac.nz
Dr Clare Browne, 07 837 9394, clare.browne@waikato.ac.nz

PURPOSE OF THE PROJECT

1. I certify that I am over the age of 18 and hereby grant permission for my pet to participate in a research project designed to evaluate the re-training of dogs to detect new target odours.
2. I have been informed about the purpose of the project and what my dog is going to do.

DESCRIPTION OF PROCEDURE

Samples will be presented to dogs via an automated carousel apparatus that turns, presenting multiple samples, one by one. The dogs will be trained to sniff each sample, and to indicate if the samples do/not contain certain chemicals commonly used in scent detection research. Training will be achieved using food treats as positive reinforcement.

I understand that my dog will only participate in the project if willing to do so and will be humanely treated at all times as described in the Standard Operating Procedures for Handling and Care of Pet Dogs for Research, which has been approved by the University of Waikato Animal Ethics Committee.

COSTS TO OWNER

I shall be responsible for all costs related to illness or treatment of problems unrelated to the experiment.

WITHDRAWING MY PET FROM THE PROJECT

I understand that participation in this project is entirely voluntary and that I may withdraw my pet at any time without any negative consequences. I understand that my dog might be withdrawn from the project if a vet finds it is necessary and in my dog's best interest.
If I have additional questions regarding this project, I may phone or email the principal investigators.

ADDITIONALLY

I understand that participation in this project involves a commitment to bring my pet to the dog facility according to a schedule realised in cooperation with the researchers. Upon completion of the research, I will have access to my dog's data and the general findings from the research project.

AUTHORISATION

I have read and understand the foregoing statements and agree to allow my pet to participate in this project. Upon signing below, I will receive a copy of this consent form.

I give consent for my dog to be at the research facility in the presence of other dogs:	**Yes / No**
My dog is friendly towards other dogs:	**Yes / No**
I give consent for videos of my dog to be shown for other purposes (presentations, lectures, etc.):	**Yes / No**

Pet's name: _____

Owner's name: _____

Owner's signature: _____ Date: _____

Appendix D

Standard Operating Procedure: Dog Recruitment

1. **Purpose**

   This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted when recruiting dogs for scent detection projects at the University of Waikato Hamilton campus.

2. **Recruitment**

   2.1. Dogs may be recruited by talking to other dog owners by handing out flyers or posting to social media platforms approved by the project supervisors/university.

   2.2. All initial contact with the project will be established via the official scent detection group email address: dogs@waikato.ac.nz, which is managed by Dr Clare Browne.

   2.3. Dr Clare Browne will forward the message to the researcher in charge of recruitment at that time.

   2.4. A preliminary email from the researcher will be sent to the owner of the prospective participant. Owners will be required to completed two initial enquiry forms to provide biographical, behavioural details and preferences of their dogs.

3. **Inclusion/Exclusion Criteria**

   3.1. When the forms have been filled out and returned, the researcher in charge of the project will ascertain if the dog is a suitable fit for the programme.

   3.2. On the initial enquiry form, the researcher will have to ensure the following:

      3.2.1. The dog is fully vaccinated. Standard vaccinations include distemper, hepatitis, and parvovirus.

      3.2.2. The dog is comfortable being in new/unfamiliar places, and handled by other individuals.

      3.2.3. The dog is comfortable being away from their owner and displays no signs of distress (e.g., separation-type anxieties).

      3.2.4. The dog likes to work for food and does not have any special dietary restrictions. The researcher will have to clarify that the dog is able to be fed kibble available at the dog lab (specific brand of kibble).

      3.2.5. The dog is comfortable around other dogs and does not display any aggressive tendencies around them.

      3.2.6. The dog is healthy, and free of any medical conditions that could be aggravated by repetitive walking.

   3.3. The researcher will then have to coordinate the owner's available dates and times with their project days.

   3.4. The researcher will then send a reply with an offer of an initial meeting at the scent detection facility.

4. **Initial Interview**

   4.1. Children are not at the lab. However, owners are welcome to bring any other interested adult who may be involved in picking up or dropping off the dog for sessions.

   4.2. The researcher will introduce themselves and explain their role.

4.3. They will provide the owner will details about their project, individuals involved and the requirements to participate in the project (e.g., the level of commitment required).
4.4. During the meeting, the dog is let off-lead in the workroom.
4.5. The owner and researcher will have the opportunity to observe the dog's behaviour in an unfamiliar environment.
    4.5.1. The dog will be placed in a crate.
    4.5.2. The dog will be separated from their owner for a brief period of time (the owner will leave the workroom).
    4.5.3. The dog's level of arousal and their responsiveness to kibble will be observed.
4.6. The dog should not display signs of distress while being in the workroom.
4.7. A weekly timesheet will be filled out by the owner to indicate their availability to participate in the project.
4.8. After the meeting, the owner and researcher will agree on the dog's participation in the project. The owner will then be asked to sign a form that consents to their dog's participation on the project.

5. Confirmed participation on a project

5.1. The researcher will take note of each dog's gear. Photograph any gear that arrives with a dog so as to avoid confusion.
    5.1.1. If the dog wears a harness, ensure the owner demonstrates the proper way to put it on.
5.2. Take a picture of the dog in their working dog jacket for the owner.
5.3. Create a profile card for the dog and pin it on the board at the scent detection lab. The owner's contact details should be printed on the back of the card for access during an emergency.
5.4. Share specific details about activities, performance criteria and the dog's performance on the project so as to cue the owner about the suitability of the dog. **No owner should be surprised if their dog is being dropped from the programme.** Ample warning and a 'countdown' timeframe should be provided to the owner, as soon as the researcher is aware of any situation that may preclude the dog from participating in the project.
5.5. Incidents need to be reported immediately to owners upon pickup or via phone calls during the day if serious.
5.6. Supervisors need to be alerted to serious incidents, and be given an opportunity to support you and the owner at handover, if required.
5.7. In extreme cases, an incident report may need to be written, and submitted to the supervisors.

Appendix E

Standard Operating Procedure: Solution Preparation

Guidelines for Solution Preparation

1. **Purpose**

   This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during preparation of solutions during training and experimental phases of the experiment.

2. **Solution Preparation for Initial Training Sessions**

   Solutions are prepared at the chemistry lab located in E block on the University of Waikato campus under a fume hood. Covered shoes and personal protective equipment (goggles and a lab coat) are to be worn on the premises at all time. Disposable gloves are worn when handling chemicals, and changed between handling new chemicals.

   Preparation of Negative Solution

   2.1 Wear a new pair of disposable gloves
   2.2 Cut a piece of parafilm
   2.3 Pour deionized water into a 100mL volumetric flask. Use a plastic 10mL syringe to obtain a precise amount.
   2.4 Pour deionized water into Schott bottle
   2.5 Repeat process to obtain 200mL of solution
   2.6 Seal mouth of Schott bottle with parafilm. Cover with lid
   2.7 Dispose of gloves

   Preparation of Positive Solution

   2.8 Wear a new pair of disposable gloves
   2.9 Retrieve bottle of amyl acetate concentration from storage cabinet, and pour a small amount into the 5mL beaker
   2.10 Fill half of volumetric flask with deionized water (approximately 50mL)
   2.11 Use the pipette to extract 25 microlitres (µL) of amyl acetate concentrate from the 5mL beaker, and add it to the volumetric flask. Shake flask well to ensure mixture of solution. Using a plastic 10mL syringe, fill the volumetric flask with deionized water to accurately obtain 100mL of chemical solution
   2.12 Pour shaken mixture into a Schott bottle. Repeat process obtain 200mL of amyl acetate solution
   2.13 Seal the mouth of Schott bottle with parafilm. Cover with lid. Dispose of gloves and pipette tip

3. **Solution preparation for Experimental Sessions**

   Preparation of Samples

   Chemicals are prepared individually. New volumetric flasks, 5mL beakers, 250mL beakers, and pipette tips are used in the preparation of each chemical solution. Paper towels are laid down for preparation in the fume hood. Gloves are disposed of, and changed between the handling of each chemical. Cut the required number of parafilm

prior to preparation of samples.

3.1 Wear a new pair of disposable gloves
3.2 Lay down paper towels prior to preparation of samples. Retrieve a clean set of glassware: a volumetric flask , a 5mL beaker and a 250mL beaker . Place glassware on paper towel. Fit the pipette with a clean tip.
3.3 Retrieve chemical concentrate from storage cabinet and pour a small amount into the 5mL beaker.
3.4 Fill half the volumetric flask with deionized water (approximately 50mL)
3.5 Use the pipette to extract 25 microliters (μL) of chemical concentrate from the 5mL beaker, and add it to the volumetric flask. Shake flask well to ensure mixture of solution. Using a 10mL plastic syringe, fill the volumetric flask to obtain 100mL of chemical solution
3.6 Pour shaken mixture into Schott bottle. Repeat process to obtain 100mL of chemical solution
3.7 Seal Schott bottle with parafilm and cover with lid. Place Schott bottle into separate plastic bags to prevent any cross-contamination between chemical solution.
3.8 Dispose of pipette tip and change gloves before preparation of the next chemical solution
3.9 Place all used volumetric flasks and beakers into a separate holding container. All used glassware will be placed into acid bath for cleaning.

4. Disposal of Chemicals

All remaining concentrates that were not used in the preparation of the chemical solutions were disposed of into chemical waste bottles in the chemistry lab. Vanillin, propionic acid, benzaldehyde and cinnamaldehyde would be disposed of into separate waste bottles.

5. Cleaning Procedures

The preparation table is cleaned before any samples are prepared with an isopropanol solution (50% isopropanol, 50% water) and disposable paper towels.
Place all glassware used in the preparation of samples in nitric acid bath for at least 24 hours.
The body of the pipette is cleaned with isopropanol solution, and disposable paper towels after preparation of each chemical.
If any spillage of solution occurs on the preparation table, this is cleaned with isopropanol solution and disposable paper towels. Gloves must be changed between cleaning the spillage and handling the next chemical solution.

Appendix F

Standard Operating Procedure: Sample Preparation

Guidelines for Sample Preparation

1. **Purpose**
   This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during preparation of samples during both training and experimental phases of the experiment

2. **Sample Preparation for Initial Training Sessions**
   Samples are prepared at the canine scent facility located at TTH4 on the University of Waikato campus. Only those with prior training are authorised to prepare samples. Preparation table is cleaned with an isopropanol (IPA) solution and disposable paper towels before preparation begins. Cut required amount of parafilm before sample preparation

   Preparation of negative samples

   2.1 Wear a new pair of disposable gloves
   2.2 Place paper towel on the preparation table
   2.3 Place vial holders on the paper towel, and place vials in the holder
   2.4 Place a rectangle self-adhesive horizontally on the side of the vial to indicate it contains a negative sample
   2.5 Uncap Schott bottle containing negative sample, dispose of used parafilm and gloves
   2.6 Using a new pair of gloves, retrieve a new pipette tip from the storage container, and fit it onto the body of the pipette. Fill the vials with 2mL of solution. Place vials in the pre-determined arrangement in separate segments of the apparatus.
   2.7 Seal Schott bottle using a new piece of parafilm and the lid
   2.8 Place used vial holders in a plastic bag for cleaning, and dispose of paper towel
   2.9 Clean body of pipette before preparing the next batch of samples
   2.10 Dispose of gloves

   Preparation of positive samples

   2.11 Wear a new pair of disposable gloves
   2.12 Place paper towel on the preparation table
   2.13 Place vial holders on the paper towel, and place vials in the holder
   2.14 Place a rectangle self-adhesive on the underside of the vial to indicate it contains a positive sample
   2.15 Uncap Schott bottle containing positive sample, dispose of used parafilm and gloves
   2.16 Using a new pair of gloves, retrieve a new pipette tip from the storage container, and fit it onto the body of the pipette. Fill the vials with 2mL of solution. Place vials in the pre-determined arrangement in separate segments of the apparatus.
   2.17 Seal Schott bottle using a new piece of parafilm and the lid
   2.18 Place used vial holders in a plastic bag for cleaning, and dispose of paper towel and gloves

3. **Sample Preparation for Experimental Sessions**

Preparation table is cleaned with an isopropanol (IPA) solution and disposable paper towels before preparation begins.

Ensure that the order in which the chemical samples are prepared is randomised every session.

Cut required amount of parafilm before sample preparation

3.1 Wear a new pair of disposable gloves

3.2 Place paper towel on the preparation table

3.3 Place vial holders on the paper towel, and place vials in the holder

3.4 Label vials using adhesive label

- Place a rectangle adhesive label along the bottom of the vial to indicate that the vial contains a target sample (T)
- Place a rectangle adhesive label horizontally across the top of the vials to indicate that the vials contains the first non-current target sample (N_A)
- Place a rectangle adhesive label horizontally across the bottom of the vials to indicate that the vials contains the second non-current target sample (N_B)
- Place a rectangle adhesive label vertically at the top of the vials to indicate that the vial contains the third non-current target sample (N_C)
- Place a rectangle adhesive label vertically at the bottom of the vial to indicate that the vial contains a distractor sample (D)

3.5 Uncap Schott bottle containing the chemical solution, dispose of parafilm and gloves.

3.6 Using a new pair of disposable gloves, retrieve a new pipette tip from the storage container, and fit it onto the body of the pipette. Fill the vials with 2ml of solution. Place vials in the pre-determined arrangement in separate segments of the apparatus.

3.7 Seal Schott bottle using a new piece of parafilm and the lid

3.8 Place used vial holders in a plastic bag for cleaning, and dispose of paper towel and gloves

3.9 Using a new pair of disposable gloves, clean body of pipette before preparing the next batch of samples. Dispose of gloves when complete.

3.10 Repeat the procedure for all 5 chemical solutions

4. **Disposal of Chemicals**

The chemicals that were used in experimental sessions are poured into separate Schott bottles, and disposed of in the chemistry lab. Vanillin, propionic acid, benzaldehyde and cinnamaldehyde would be disposed of into 4 separate waste bottles. The rest of the chemicals are disposed into the same waste bottle labelled "non-chlorinated and non-halogenated solvents". Deionized water is disposed of in the sink.

5. **Cleaning procedures**

The preparation table is cleaned before any samples are prepared with an isopropanol solution (50% isopropanol, 50% water) and disposable paper towels.

If any spillage of solution occurs on the preparation table, this is cleaned with isopropanol solution and disposable paper towels. Gloves must be changed between cleaning the spillage and handling the next chemical solution.

The body of the pipette is cleaned with the isopropanol solution, and disposable paper towels after preparation. New gloves are to be worn before cleaning the body of the pipette, and are disposed when complete.

Appendix G

Standard Operating Procedure: Acid Washing

Guidelines for Acid Washing Glassware

1. **Purpose**
   This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during acid washing of glassware during scent detection projects in the chemistry lab on the University of Waikato Hamilton campus. Only those with prior induction training are authorised to do this.

2. **General Rules**
   Acid baths are located in the chemistry lab under a fume hood. Covered shoes and personal protective equipment (goggles and a lab coat) are to be worn on the premises at all time. Disposable gloves are worn when handling chemicals, and changed between handling new chemicals. Glassware are to be left in acid overnight.

3. **Placing Glassware into Acid**
   All glassware used in the preparation and presentation of samples must be washed in nitric acid. Schott bottles used for storage of diluted chemical solutions have to be placed into nitric acid prior to use.

   All used glassware must be rinsed thoroughly using distilled using distilled water to remove residual chemical solutions, before being placed in nitric acid baths.

   3.1. A lab coat, safety glasses, disposable gloves, long green gloves, and a protective apron must be worn when interacting with the acid.
   3.2. Check the pair of long green gloves thoroughly for any holes. If gloves are deteriorated, replace with new ones.
   3.3. Remove glass lid from the acid bath
   3.4. Using tongs, carefully place the glassware into the acid bath. Ensure that there are no trapped air bubbles, and the glassware is fully submerged.
   3.5. Replace the lid on the acid bath
   3.6. Rinse tongs and long green gloves thoroughly in the sink. Take caution to ensure fume hood is properly closed
   3.7. Remove protective apron and hang it on the hook
   3.8. Dispose of gloves

4. **Taking Glassware out of Acid**
   4.1. A lab coat, safety glasses, disposable gloves, long green gloves, and a protective apron must be worn when interacting with the acid.
   4.2. Fill rinsing buckets with tap water prior to interacting with acid.
   4.3. Check the pair of long green gloves thoroughly for any holes. If gloves are deteriorated, replace with new ones.
   4.4. Remove glass lid from the acid bath.
   4.5. Using tongs, carefully remove glassware from acid bath. Empty glassware of acid as much as possible, and then submerge in the rinsing bucket

4.6. Replace the acid bath lid
4.7. Rinse the tongs and long green gloves thoroughly in the sink. Take caution to ensure fume hood is properly closed
4.8. Remove protective apron.
4.9. Dispose of gloves and replace with new pair.
4.10. Retrieve metal mesh tray from the cupboard, and line it with aluminium foil.
4.11. Ensure bottle of distilled water is filled prior to rinsing procedure.
4.12. Glassware must be submerged in the rinsing bucket for 10 minutes prior to being rinsed with distilled water.
4.13. Place rinsed glassware in metal mesh tray.
4.14. Play glassware (in tray) in the incubator.
4.15. Dispose of water in rinsing bucket, and place in storage area.
4.16. Dispose of gloves.
4.17. Leave glassware in incubator overnight to dry

5. Taking Glassware out of incubator

5.1. A lab coat, safety glasses, and disposable gloves must be worn.
5.2. Retrieve storage tub for clean vials, and line it with a paper towel
5.3. Retrieve glassware in metal mesh tray from incubator, and place on bench.
5.4. Place clean glassware into storage tub
5.5. Dispose of aluminium foil, and place metal mesh tray in storage area.
5.6. Dispose of gloves.

Appendix H

Standard Operating Procedure: Preliminary Training Phase

Guidelines for Training Dogs for Scent Detection Work Using Automated Apparatus

1. **Purpose**
   This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during the initial training phase of the experiment.

2. **Apparatus Setup**
   Position the apparatus in a room without other objects that might distract the dog. Only the front panel should be accessible to the dog, a ramp may be required so the dog can access the sample port hole. Movable partitions may be used to block access to the other sides of the apparatus. The room must have a door that closes/latches, and should be equipped with one or two cameras to monitor the dog. The computer(s) used to control the apparatus and monitor the dogs should be positioned in an adjacent room.

3. **Initial Training Phase**
   This section outlines the basic training hierarchy for shaping by successive approximations. As a general rule, each step must be completed independently 3 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 (under 5 minutes), and finish on a positive note when possible to ensure that the process is enjoyable for the dog.

   3.1. Introduction
   Once the dog has been habituated to the environment and the researcher(s), the research can proceed with training sessions. During the shaping and early training process, at the first sign of fatigue to disinterest, the session should be terminated, ideally immediately following a correct response and reinforcement. Early shaping/training sessions should not exceed 10 minutes. Dogs should be given a short break between sessions.

   3.2. Conditioned Reinforcer Establishment
   The researcher should enter the experimental room with the dog, and stand to the side of the apparatus (the side closest to the door is preferred if possible). The researcher should stand with their hands crossed either in front of their body or behind their back (whichever is more comfortable), holding the wireless remote of the feeder out of view of the dog. The dog should be allowed to explore the experimental room freely.
   Researcher to 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 maybe used to facilitate training, but these should be used only as needed as they must be faded out before training is complete.
   Dispense food from the automatic feeder using the wireless remote until the dog immediately approaches the feeder upon hearing the sound made when the feeder is

activated. Take care not to trigger the feeder if the dog is only sitting and staring at the feeder. The dog should approach the automatic feeder, and consume the food within 3 seconds of activation 3 times in a row to continue to the next stage of training.

3.3. Shaping – Nose to Port

Once the sound of the feeder is established as a conditioned reinforcer, the wireless remote is used to train the dog to put its nose into the sample port of the apparatus. Use the method of differential reinforcement of successive approximations to target this behaviour (see appendix). For initial sessions, the apparatus should be turned off. Empty segments may be placed on the apparatus so that the dog can push the flap open. The sound of the flap closing makes a sharp tap noise, which may initially startle the dog. Prompting (e.g. pointing) may be used, but the prompt must be faded and removed before proceeding to the next step (lever activation). As soon as the dog is comfortably placing its nose into the port far enough to open the flap of the segment fully, the apparatus should then be loaded with positive samples and turned on. The subject's configuration file on the computer should then be edited to set the arrangement of samples on the carousel, and the indication response time of 1000ms and an observation response time of 500ms. The apparatus will make a continuous "beep" sound when the dog places its nose in the port. Continue shaping as required until the dog begins to trigger the feeder automatically. Once a run (17 samples) at the 1000ms threshold is complete, increase the threshold in 100 to 500ms intervals to 1500ms. Once a run is completed at 1500ms, continue to the next step.

3.4. Shaping – Lever Activation

With the apparatus unloaded and turned off, use the method of differential reinforcement of successive approximations to shape lever pressing (see Appendix). Depending on the size and behavioural tendencies of the specific dog, an appropriate topography should be selected for shaping (e.g. use of a paw or nose to activate the lever). Prompting (e.g. pointing) may be used, but the prompt must be faded and removed before proceeding to the next step. Once the lever has been pressed 10 times without prompts (and reinforced via manual activation of the feeder), proceed to the next step.

3.5. Discrimination Training

Load the apparatus with approximately half positive and half negative samples (e.g. 9 positive samples, and 8 negatives samples), alternating positive and negative sample placement on the carousel, with a positive sample starting in the first position. The arrangement of the samples on the carousel should then be updated in the subject's configuration file.

Bring the dog into the experiment room and stand in place beside the apparatus. If the dog does not respond to the apparatus within 20 seconds, prompt as required. When the dog encounters the first negative sample, allow 20 seconds before prompting to see if lever press occurs without prompt. Continue prompting when necessary, but fade out prompts as soon as possible (e.g. wait for increasing amounts

of time before prompting). Be sure to prompt with a consistent cue.

Once one run has been completed without prompting, randomise the arrangement of samples in subsequent sessions, and update the subject's configuration file. The same randomisation pattern may be used for up to a maximum of 5 sessions in a row before it is needed to be randomised again. Continue until hit rate (correct positive indication) and correct rejection rate (correct lever pressing) is above 80% without prompt.

At this point, the researcher should gradually remove themselves from the room, and systematically increase the indication threshold in 100 to 500ms increments once the dog is working successfully independently. The indication response threshold is increased until the target threshold is reached (5000ms is generally optimal based on preliminary research, but this may vary depending on dog/application). With a standard sample (e.g. amyl acetate), hit rate and correct rejection rate should be reach and stay at approximately 100%.

3.6. Advanced Training

3.6.1. Decreasing Number of Positive Samples

If the dogs are encountering problems with the decrease in the number of positive samples, it may be necessary to re-enter the room and provide prompts. All prompts have to be faded as soon as possible, and the researcher has to gradually remove themselves from the room again.

Appendix: Troubleshooting tips

If the dog is performing poorly in training:

- Ensure that the dog is healthy. Deal with any health related issues first.
- Confirm that there have been no significant changes in the dogs' home routine (e.g. owner away for an extended period of time, changes in routine, diet, or household).
- Confirm that food is an effective reinforcer by evaluating approach and consumption. If confirmed, try selecting different food.
- Return to earlier stages of training as required (e.g. if the lever press is not occurring reliably in discrimination training, conduct another lever press shaping session).
- If the dog continues to perform poorly, consult with supervisor. The dog may need to cease participation in the study.

If the dog is putting its nose in the port too early (while the apparatus is still moving):

- Use a sandwich board to create an obstacle the dog must navigate around in order to reach the lever, and return to the port.

Appendix: Guide for shaping

Procedure

1.1. Shaping of sample port entry

1.1.1. For initial sessions, the apparatus should be turned off. Empty segments may be placed on the apparatus so that the dog can push the flap open. The sound of the flap closing makes a sharp tap noise, which may initially startle the dog.
1.1.2. Reinforce moving further and further away from the feeder, until the dog is reliably approaching the side of the room near the apparatus
1.1.3. Reinforce attending to the apparatus (putting nose near or on any part of the front panel).
1.1.4. Reinforce nose near port
1.1.5. Reinforce nose in port
1.1.6. Reinforce nose touching and opening the flap (indicated by a tap noise as it closes)
1.1.7. Reinforce pushing flap inwards
1.1.8. Turn the apparatus on – when the sample port beam is broken, it will now produce a continuous "beep" sound
1.1.9. Continue to reinforce beam breaks and pushing the flap inwards, until the dog is fully opening the flap (nose is fully inside the port).

1.2. Shaping of lever press

1.2.1. Turn apparatus off. Do not have apparatus loaded with samples
1.2.2. Reinforce any movement towards the lever
1.2.3. Reinforce movement of nose or paw towards the lever (as appropriate)
1.2.4. Reinforce any contact with the lever (nose or paw, as appropriate)
1.2.5. Reinforce any movement of the lever
1.2.6. Reinforce movement of the lever that produces a "click" (circuit switch close)

Appendix I

Standard Operating Procedure: Experimental Stage

Guidelines for Experimental Phase

1. Purpose

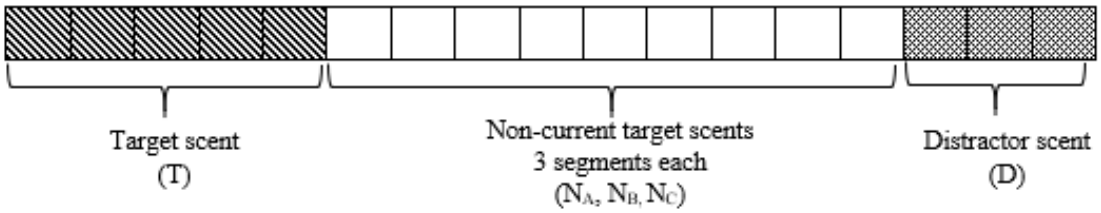
This standard operating procedure (SOP) provides guidelines and standardised procedures to be adopted during experimental phases of the experiment.

2. Testing Protocols of Experiment

This section outlines the procedures to be adopted during the experimental phase. After the initial training phase is completed, the experimental sessions may begin. All dogs should be working independently in the experimental room alone. Amyl acetate, which is used as a target scent in the training phase, will not be used in the experimental phase. All dogs should be working at the same ratio of positive and negative samples (5 positives and 12 negatives) before proceeding with the experiment.

The researcher conducts a maximum of 10 sessions for each dog per experimental day. Each session consists of 17 trials, where a trial refers to the presentation of an odour sample in a segment. In the first experimental phase, 1 target scent is chosen at random from the list of 10 potential target scents. The remaining scents form a list of non-current target scents, 3 of which will be randomly selected and presented in each session. While they are not currently target scents in this phase, they will be selected as target scents for later experimental phases. 3 scents form a list of distractor scents, which will never be presented as a target scent or non-current target scent during the experiment.

In each session, 5 odour samples will be presented to the subjects. 1 target scent, 3 non-current target scents and 1 distractor scent. Henceforth, the term "non-targets" will refer to both non-current target scents and distractor scents.



Of the 17 vials on the carousel, 5 vials will contain the target scent, 3 vials will contain the distractor scent, and the remaining 9 vials contained 3 non-current target scents. Each non-current target scent occupies 3 vials.

The target scent selected for an experimental phase will be presented in every session within that experimental phase. During the next phase, the target scent from the previous phase will be presented in the current phase until mastery criteria are met. Following which, that scent will only be presented as a non-current target scent when selected in the sampling selection process. Selection of non-current target scents and distractor scents will be selected at random from two separate lists, following the method of "sampling without

replacement". The other "non-current target scents" will be selected in this manner, with the exception of the target from the previous phase, which must first meet extinction criterion.

Each experimental phase starts by presenting the samples in a pattern for the first 5 sessions. This configuration pattern starts with presenting the target scent in the first trial, and every subsequent fourth trial. The rest of the scents are presented randomly between target scent trials. After these 5 sessions, all samples will be randomised. The same randomised configuration pattern may be used for a maximum of 4 sessions in a row before it is needed to be randomised again. The subject's configuration file should be edited before the start of each session to change the arrangement of samples on the carousel.

2.1. Phase 1 of experiment

The target scent for this experimental phase is chosen at random from the list of 10 potential target scents. The remaining non-current target scents and distractor scents for each session will be selected following the method of "sampling without replacement", from the two lists. Each time a sample is randomly selected from either list, that chemical cannot be selected again until all chemicals remaining on that list have been selected, and the list has been repopulated.

2.1.1. The first experimental phase randomly selected 2-phenylethanol as a target scent. 2-phenylethanol was selected at random from the list of 10 potential target scents. Minimum positive indication time is reduced to 501ms, and is increased in 500 to 1000ms increments. The minimum positive indication time is increased when the subject meets a minimum of 80% hit rate (correct indication response for target scents), in a session. The minimum positive indication time is increased to reach a final indication threshold of 5001ms.

2.1.2. The same randomisation pattern may be used for a maximum of 4 sessions in a row before it is needed to be randomised again. Continue until a minimum of 80% hit rate (correct positive indication) and correct rejection rate (correct lever pressing)

2.1.3. 2 mastery criteria are set for this phase of the experiment. The first criterion is a minimum of 80% for the hit rate, for 4 out of 5 sessions. The second criterion is a minimum of 80% for correct rejection responses on the remaining non-target scent samples (both non-current target and distractor) for 4 out of 5 sessions.

2.2. Phase 2 of experiment

The target scent for this experimental phase is chosen at random from the remaining chemicals available for selection. The remaining distractor and non-target scents for each session will be selected following the method of "sampling without replacement"

2.2.1. The second experimental phase randomly selected Cinnamaldehyde as a target scent, from the list of remaining potential target scents. Minimum positive indication time is reduced to 501ms, and is increased in 500 to 1000ms

increments. The minimum positive indication threshold is increased when the subject meets a minimum of 80% hit rate (correct indication response for target scents) in a session. The minimum positive indication time is increased to reach a final indication threshold of 5001ms.

2.2.2. The same randomisation pattern may be used for up to a maximum of 4 sessions in a row before it is needed to be randomised again. Continue until a minimum of 80% hit rate (correct positive indication) and correct rejection rate (correct lever pressing)

2.2.3. The target scent presented in the previous phase (2-phenylethanol) will be presented in every session until the subject does not indicate it more than once, three sessions in a row. Once the criteria is met, the previously encountered target scent (2-phenylethanol) will only be presented as a non-current target scent when selected in the sampling selection process.

2.2.4. 2 mastery criteria are set for this phase of the experiment. The first criterion is a minimum of 80% for correct indication response for target scents, for 4 out of 5 sessions. The second criterion is a minimum of 80% for correct rejection responses on the remaining non-target scent samples (both non-current target and distractor), without performing an indication response more than once on the previous target scent, for 4 out of 5 sessions.

2.3. Phase 3 of experiment

The target scent for this experimental phase is chosen at random from the remaining chemicals available for selection. The remaining distractor and non-target scents for each session will be selected from two separate lists, following the method of "sampling without replacement".

2.3.1. The third experimental phase randomly selected Linalool as a target scent, from the list of remaining potential target scents. Minimum positive indication time is reduced to 501ms, and is increased in 500 to 1000ms increments. The minimum positive indication threshold is increased when the subject meets a minimum of 80% hit rate (correct indication response for target scents), in a session. The minimum positive indication time is increased to reach a final indication threshold of 5001ms.

2.3.2. The same randomisation pattern may be used for up to a maximum of 4 sessions in a row before it is needed to be randomised again. Continue until a minimum of 80% hit rate (correct positive indication) and correct rejection rate (correct lever pressing)

2.3.3. The target scent presented in the previous phase (Cinnamaldehyde) will be presented in every session, until the subject does not indicate it more than once three times in a row. Once the criteria are met, the previously encountered target scent (Cinnamaldehyde) will only be presented as a non-current target scent when selected in the sampling selection process.

2.3.4. 2 mastery criteria are set for this phase of the experiment. The first criterion is a minimum of 80% for correct indication response for target scents, for 4 out of 5 sessions. The second criterion is a minimum of 80% for correct rejection responses on the remaining non-target scent samples (both non-current target

and distractor), without performing an indication response more than once on the previous target scent, for 4 out of 5 sessions.

2.4. All remaining phases of experiment

2.4.1. Selection and presentation of all chemicals will be selected using the procedures outlined above. The mastery criteria will remain the same for all remaining phases.

2.4.2. Chemicals selected for each phase are outlined in the table below

Phase	**Chemical**
Initial Training Phase	Amyl acetate
Target or distractor scents	
Phase 1	2-phenylethanol
Phase 2	Cinnamaldehyde
Phase 3	Linalool
Phase 4	Ethyl butyrate
Non-target scents	
Butyl acetate Methyl propionate Hexanol	

Appendix: Troubleshooting tips

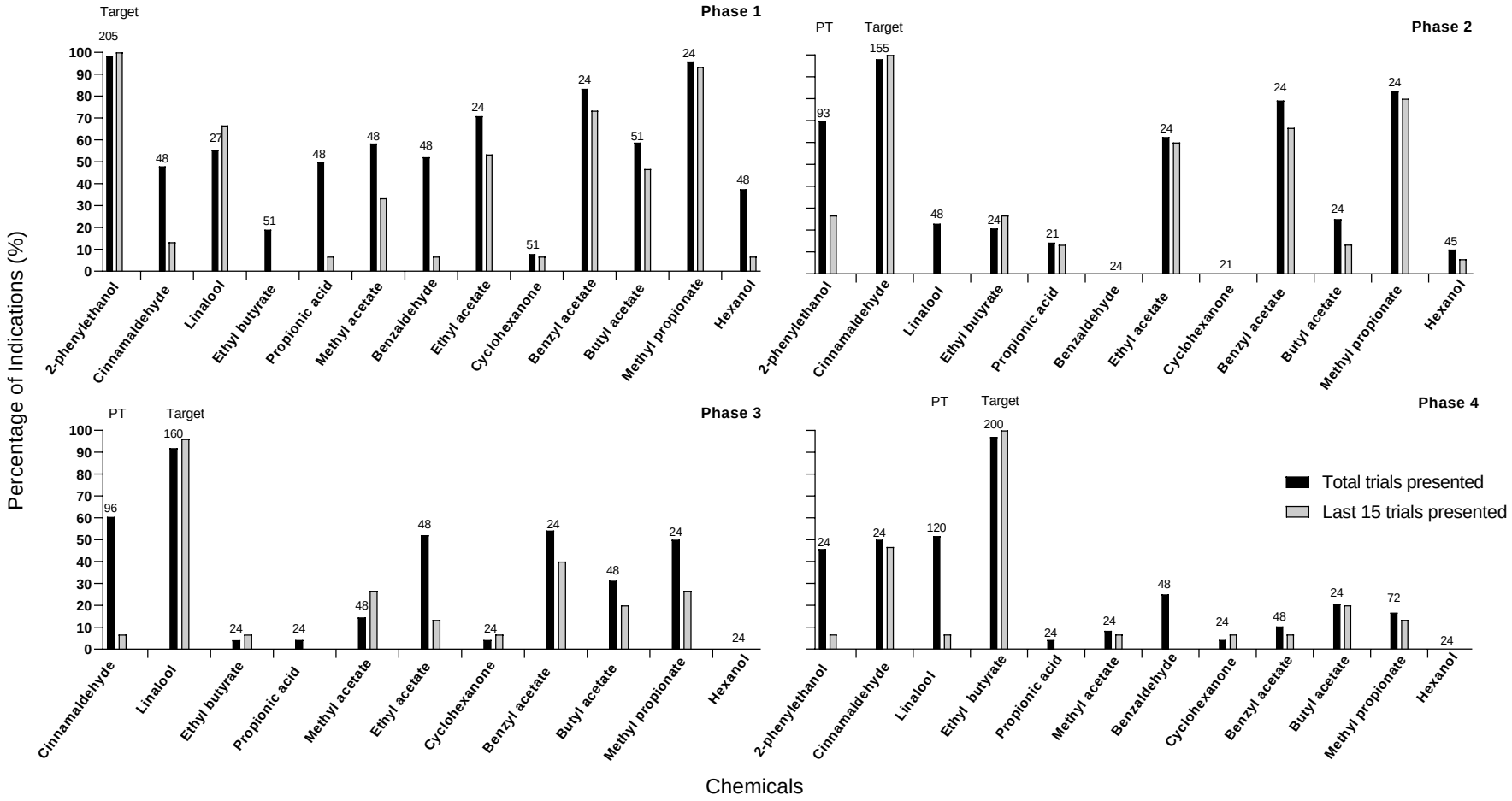
If the dog is performing poorly:

- Ensure that the dog is healthy. Deal with any health related issues first.
- Confirm that the dog was not fed by the owner at least 2 hours prior to training.
- Confirm that there has been no significant changes in the dogs' home routine (e.g. owner away for an extended period of time, changes in routine, diet, or household).
- Check factors related to sample quality. Ensure that samples have been prepared and arranged as specified in the specific sample preparation SOP.
- If the dog continues to perform poorly, consult with supervisor. The dog may need to cease participation in the study.

The researcher may decide to return to the experimental room for prompting if:

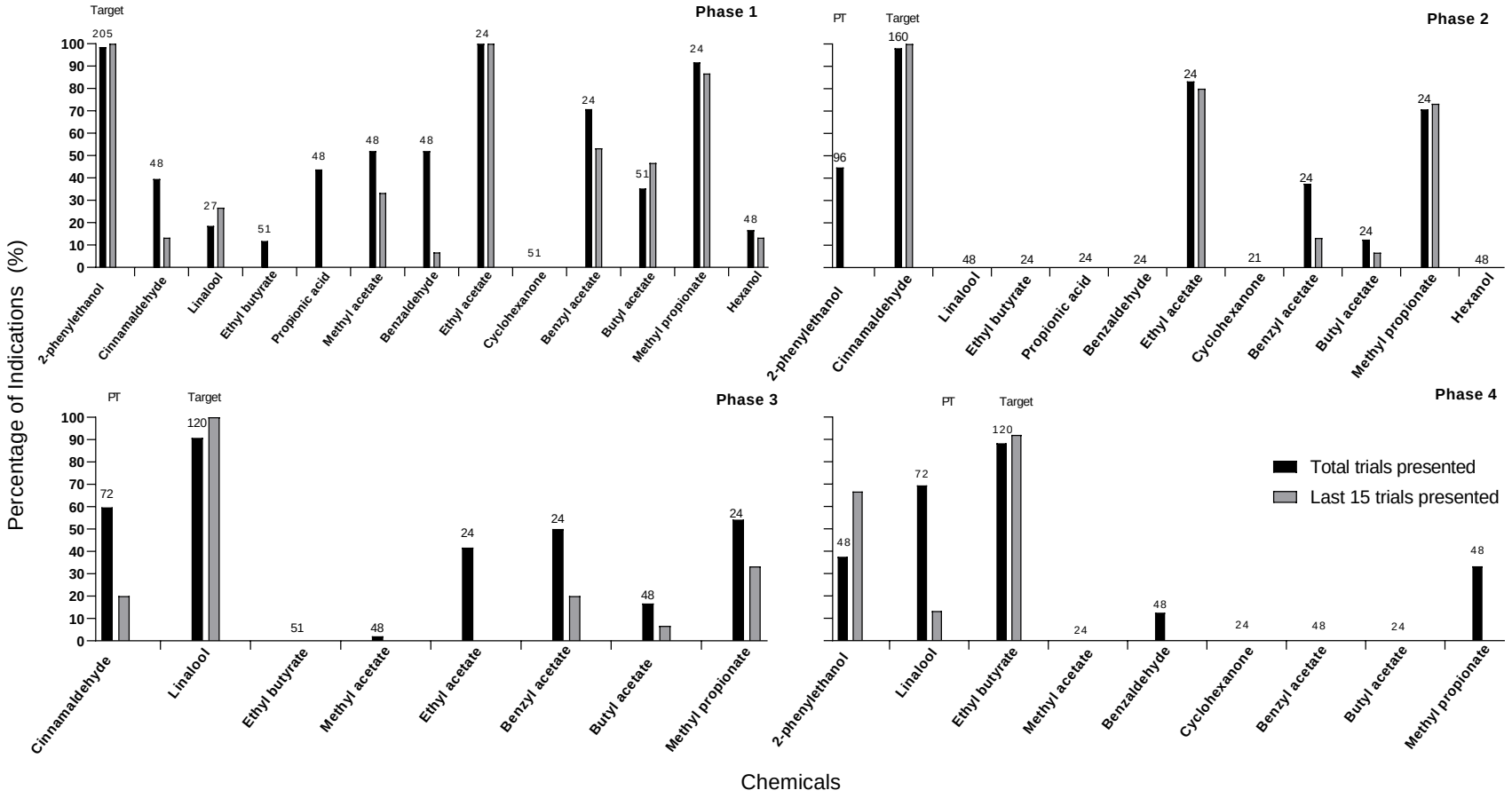
- The dog does not return to the apparatus after 1 minute despite prompting using "beep" function on the apparatus, controlled on the computer program.
- The dog places nose in the port when presented with a scent that was a previous target (and is currently a distractor scent), and does not proceed to press the lever for more than 1 minute.
- The dog displays signs of distress during the session.

Appendix J



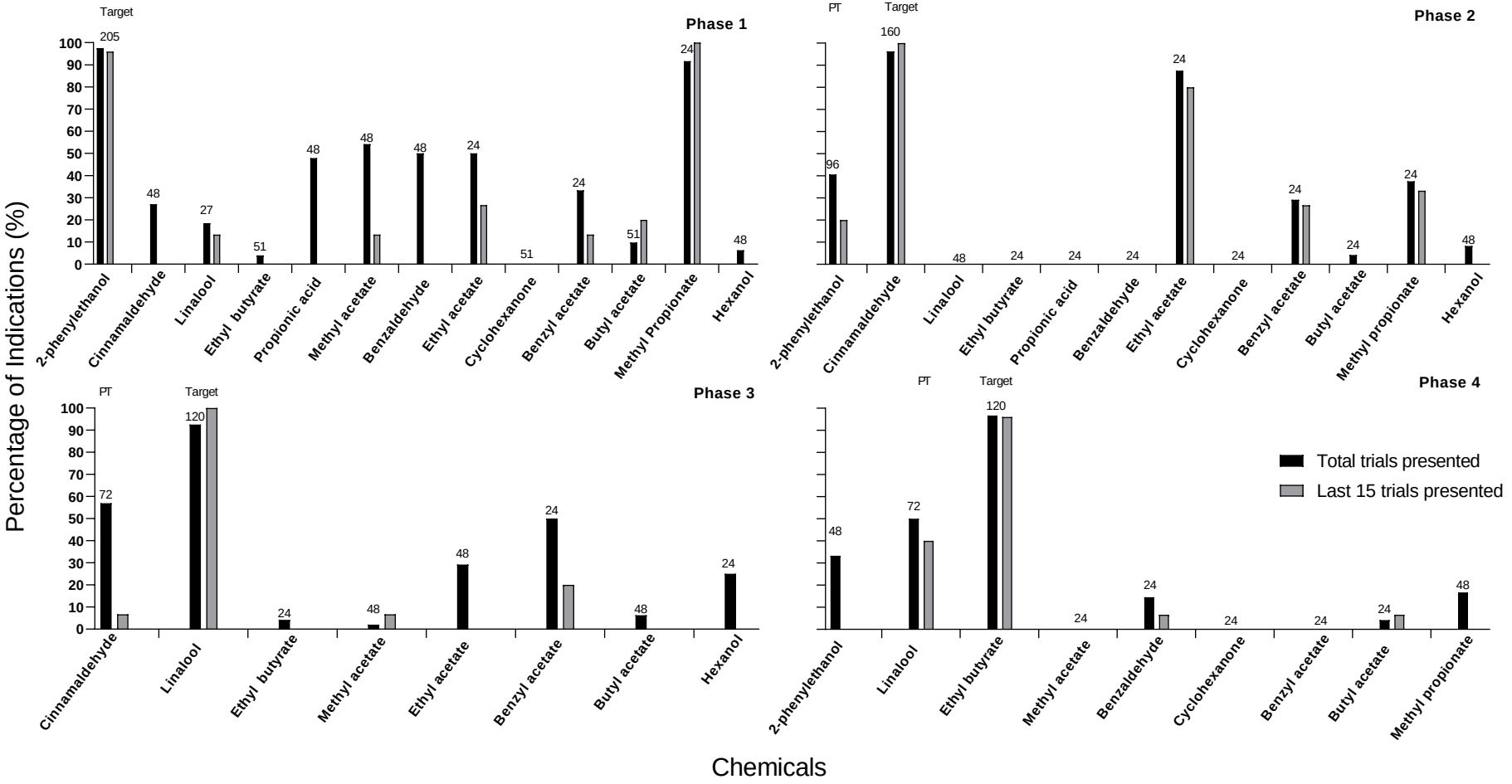
Mika's Indication performance across all chemicals presented within each of the four phases. The black bars indicate the percentage of indication responses, while the grey bars indicate the percentage of indication responses on the last 15 trials presented in each phase. The label "PT" refers to a previously trained target scent that was a target scent in the previous phase, and "target" refers to the target scent in that phase.

Appendix K



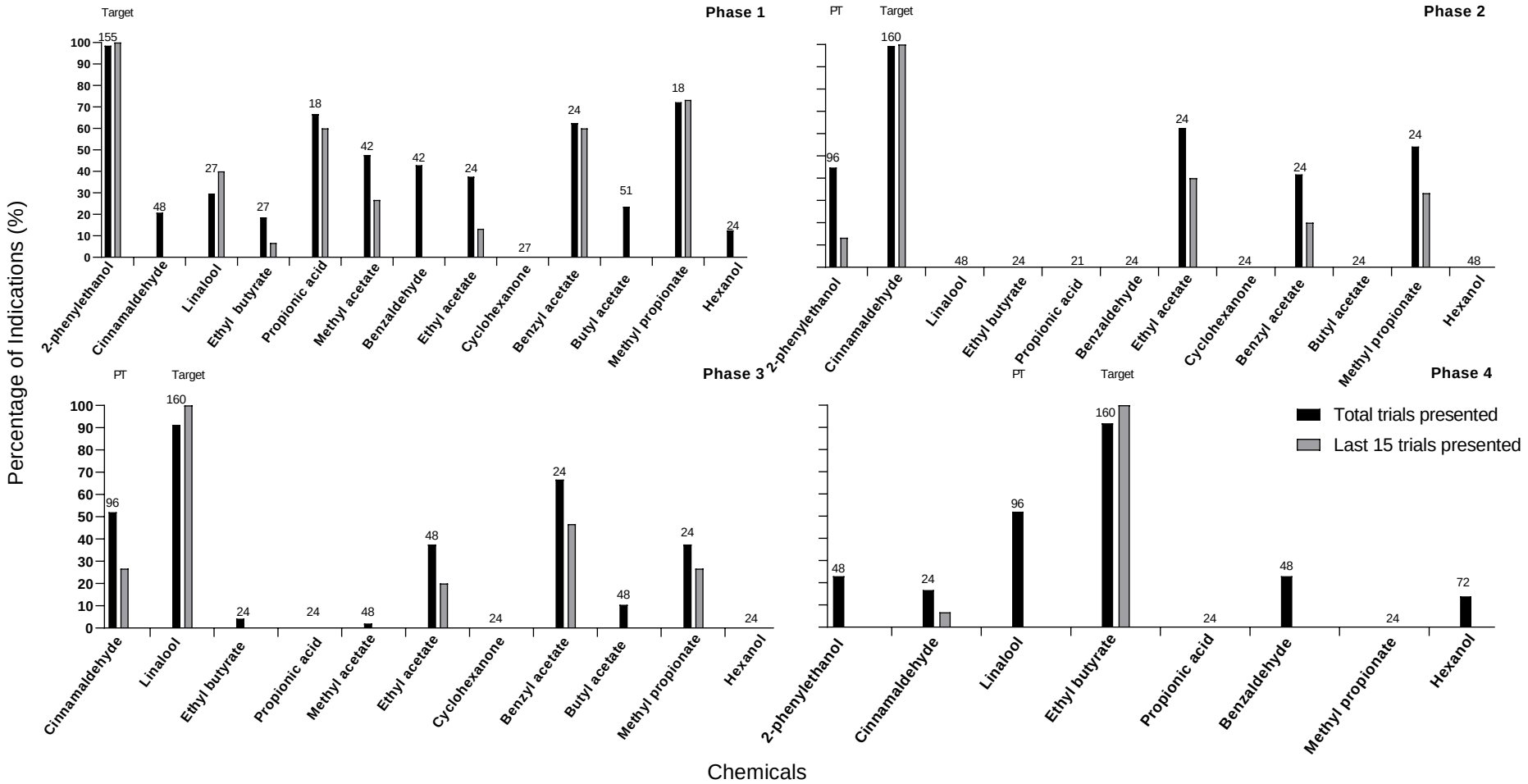
Scout's indication performance across all chemicals presented within each of the four phases. The black bars indicate the percentage of indication responses, while the grey bars indicate the percentage of indication responses on the last 15 trials presented in each phase. The label "PT" refers to a previously trained target scent that was a target scent in the previous phase, and "target" refers to the target scent in that phase.

Appendix L



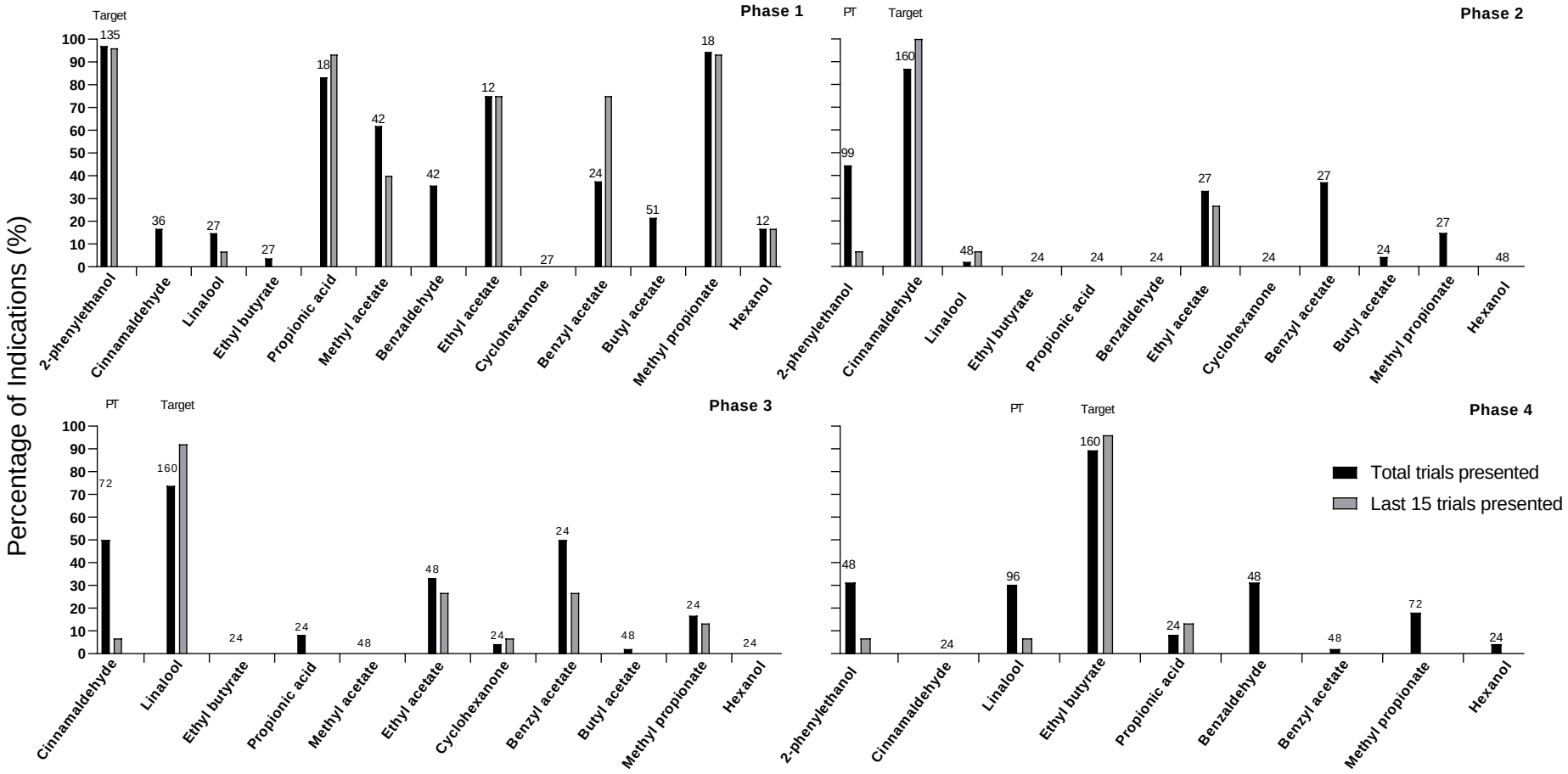
Hollie1's indication performance across all chemicals presented within each of the four phases. The black bars indicate the percentage of indication responses, while the grey bars indicate the percentage of indication responses on the last 15 trials presented in each phase. The label "PT" refers to a previously trained target scent that was a target scent in the previous phase, and "target" refers to the target scent in that phase.

Appendix M



Hollie2's indication performance across all chemicals presented within each of the four phases. The black bars indicate the percentage of indication responses, while the grey bars indicate the percentage of indication responses on the last 15 trials presented in each phase. The label "PT" refers to a previously trained target scent that was a target scent in the previous phase, and "target" refers to the target scent in that phase.

Appendix N



Jasper's indication performance across all chemicals presented within each of the four phases. The black bars indicate the percentage of indication responses, while the grey bars indicate the percentage of indication responses on the last 15 trials presented in each phase. The label "PT" refers to a previously trained target scent that was a target scent in the previous phase, and "target" refers to the target scent in that phase.