Assessing Flexible Information Use in the European Honey Bee (*Apis mellifera*)

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DECLARATION

This thesis is formatted to the style of peer reviewed journal Animal Cognition, with exceptions to figures embedded into the body, font sizing as 12 pt, 2 cm margins, and spacing as 1.5x, as requested by the Biological Sciences department of Macquarie University.

All work contained in this thesis, with the expectation of where due acknowledgement has been made, is that of the author. This thesis has not been submitted in full or partially to any other institution.

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ABSTRACT

The European honeybee (*Apis mellifera*) has been shown to be capable of solving a wide range of cognitive tasks. It is currently unknown what the limitations of their cognitive abilities are. Given the bee has a relatively tiny brain with less than one million neurons, it is surprising that these limitations have not been well characterised. To explore the nature of limits to bee cognition here we explored the capacity of bees to solve conflicting information in two different delayed conditional discriminations and a sequential conditional discrimination. In addition we explored whether there was any evidence of bees learning from their errors by assessing for a post-error slowing effect, a phenomenon found in vertebrates. These general learning abilities underpin a range of cognitive tasks, and have been considered hallmarks of intelligent behaviour.

It was found that bees performed above chance levels in all three discrimination tasks, with their accuracy improving over learning trials, demonstrating that bees are capable of resolving conflicting information using conditional information. The reaction time in post-error trials were found to not display a significant slowing phenomenon. This suggests that animals with small brains can adapt to conflicting information but with some limitations in that they do not adapt their behaviour to their errors.

1. Introduction

The ability to adaptively learn from a past experience and apply it in present situations is found across many animal taxa with all animals able, to some extent, respond to stimuli in an environment that is changing (Nairne et al., 2012; Thornton et al., 2012). The capability for learning is found throughout most species evidently suggesting its importance (Johnston, 1982). The basic mechanisms of adaptive learning appear across taxa even in those that are phylogenetically distant (Perry et al., 2013; Poli, 1988). Despite similarities in these mechanisms, the degree and complexity of learning between taxa is quite diverse (Enquist et al., 2016; Heyes, 1994). One type of learning found in disparate taxa is the ability to respond and learn from the relationships between two stimuli or responses is characteristic of elemental learning (Couvillon and Bitterman, 1988). Elemental forms of learning are found throughout most animals, from those considered more cognitively advance such as mammals, to those considered neurologically simple such as decapitated insects and flatworms (Furedy and Riley, 1987; Horridge and Wigglesworth, 1962; Thompson and McConnell, 1955). The presence of elemental forms of learning throughout the animal kingdom therefore suggests that the neuronal mechanisms underlying this type of cognition serves a generalised function rather than a more specific complex one, having either appeared early on in the evolution of multicellular organisms or independently evolved across taxa due to evolutionary selection pressures. While elemental learning is common in that it is found in the majority of animals, non-elemental learning is less so. Non-elemental learning allows animals to learn the complex relationships in their environment and respond to them appropriately (Kleyko et al., 2015). Non-elemental learning is found throughout many taxa including primates, rodents, birds and invertebrates such as crickets (*Gryllus bimaculatu)*, cockroaches (*Periplaneta americana*), and bumblebees (*Bombus terrestris)* (Archer and Sjödén, 1980; Bateson et al., 2003; Matsumoto and Mizunami, 2004; Sato et al., 2006; Sommerlandt et al., 2014).

Intelligence as it is often defined, encompasses a wide variety of these non-elemental learning and adaptive behaviours, all of which range in complexity making intelligence increasingly difficult to measure (Pearce, 2008). Cognitive experiments tend to measure intelligence by assessing the multitude of behavioural and cognitive functions of an individual and its ability to apply them appropriately depending on the situation (Roth and Dicke, 2005). While there has yet to be a universally accepted definition of intelligence within neurobiology, the generally accepted description of intelligence is an individual's ability to selectively apply new information in a way that is fluid and appropriate as dependent on the surrounding environment. This definition has been

used in past research, and is able to be applied to a variety of species across taxa and expanded as needed (Pearce, 1997; Roth and Dicke, 2005).

The European honeybee (*Apis mellifera)* is an invertebrate species commonly used to study animal cognition and learning due to its impressive cognitive abilities. In terms of animal cognition honey bees are considered an outlier in that they have continued to defy assumptions of the cognitive abilities of invertebrates. They have demonstrated not just the capacity for basic forms of elemental learning as in most insect species, but the capacity for more complex non-elemental learning (Collett and Baron, 1995)*.* Honey bees display forms of non-elemental learning such as: contextual discrimination learning, conceptual learning and spatial navigation suggesting that they are capable of complex cognition (Dukas, 2008; Hammer, 1993; Theobald, 2014; Whitlow and Wagner, 1972; Zhang et al., 1999). These cognitive abilities in bees are unexpected with the assumption being that the $\sim$1 mm^3 brain of bees is much too small to contain the adequate number of neurons needed for complex learning (Adamo, 2016; Roper et al., 2017). The honey bee brain has just under a million neurons, compared to humans with 84 billion neurons, who are capable of complex learning (Herculano-Houzel, 2016; Lent et al., 2012). The fact that bees demonstrate complex behaviours using relatively simple neural circuit's means they are often used models for the development of computational neural network models (Ardin et al., 2016; Fernando and Kumarasinghe, 2018; Roper et al., 2017). This demonstrates that even if an animal exhibits a behaviour that appears to be complex it does not necessarily mean it requires a complex neural system to achieve it (Cope et al., 2018; Giurfa, 2003). Bees have therefore become an important model for exploring these mechanisms of cognition in non-human animals (Menzel, 2012).

While the honey bee is an important model for cognitive research little is still known about what limitations there are to their cognitive abilities, with a lack of a structured assessment into such possible limitations. This project proposes looking into a new aspect of intelligence in bees by assessing if they have the cognitive capacity to solve a task in which the information given is conflicting, in that sometimes a stimulus is rewarding but other times it is punishing. Three different conditional discrimination tasks were used, which required the bee to seek out additional information in the form of a contextual cue. Conditional discrimination tasks require a subject to learn that in one context (C1) stimulus A is rewarded while stimulus B is not and the opposite is true in a second context (C2) wherein stimulus B is rewarded and A is not (Giurfa, 2003). This problem requires the use of non-elemental learning processes, as the elements alone do not allow for the subject to solve the task, due to each stimulus (A and B) being equally presented as rewarded or

unrewarded during training, as is either context (C1 or C2). These tasks mimic possible ecological tasks that bees are likely to encounter within their niche (Cauchoix and Chaine, 2016). Foraging bees, for example, will often encounter flowering plants that offer a high incentive food resource and are rewarding but vary in the likelihood and availability of the reward (Goulson et al., 2001; Tedjakumala and Giurfa, 2013).

Two such conditional discrimination tasks were presented to bees within this project; a delayed conditional discrimination (DCD) and a sequential conditional discrimination (SCD) task. Like that of a conditional discrimination task a subject must learn that in certain contexts either stimulus A or stimulus B is rewarded, however a DCD introduces a temporal element to the context in order to solve the task. A contextual cue (C1) is presented for only a short period of time and then is removed before the stimuli are simultaneously presented which of stimuli A or B is rewarded requiring the subject to form an association between the initial contextual cue (C1) and the rewarded stimulus (A). The delay is therefore the absence of the contextual cue when the subject is presented with the stimuli, requiring a recall of the cue in order to select a choice. Similarly, for a contextual cue (C2) bees must use working memory in order to remember the cue and associate it to stimulus B which is now the rewarded stimulus (Herremans et al., 1994). DCD tasks have been conducted on vertebrate species such as primates (*Macaca mulatta*), brown rats (*Rattus norvegicus*) and pigeons (*Columba livia domestica*) using contextual cues in the form of auditory and visual cues. These animals were found to be capable of solving this conflicting information task (Herremans and Hijzen, 1997a; Honig and Dodd, 1983; Watanabe, 1981). No such experiments have previously been done on any invertebrate species. Bees have likewise not been shown to solve a SCD task before. A SCD does not use a visual contextual cue but rather a conceptual cue in the form of a sequence that the subject must learn in order to determine if stimulus A or B is rewarded at a time. This task requires an animal to have the cognitive ability to either count the sequence to anticipate a switch in contingency, or to employ another strategy such as a win-stay/ lose-shift to rapidly adapt to a switch in contingency. Due to their natural foraging behaviours bees have been suggested to be capable of basic numerical counting of upwards to four and employing a win-stay/ lose-shift strategy in deciding between flowers (Bar-Shai et al., 2010; Chittka et al., 1999; Chittka and Geiger, 1995; Komischke et al., 2002a).

Animals that have been shown to solve conditional discrimination tasks have also displayed a post-error adjustment phenomenon called post-error slowing, in which the reaction time they take to make a choice slows following an error (Notebaert et al., 2009; Wickelgren, 1977). Errors elicit shifts

in behaviour that can cause differences in reaction time and accuracy, which can result in behavioural post-error adjustments (Danielmeier and Ullsperger, 2011). This slowing behaviour therefore suggests the ability to learn from one's mistakes by taking the additional time to avoid the same potential errors having just been made. Post-error slowing is one phenomenon of the Rabbitt Effect, a post-error adjustment that is considered a hallmark of human intelligence (Laming, 1979a; Notebaert et al., 2009; Rabbitt, 1966). The Rabbitt effect states that the subject's reaction time following an error, will slow compared to that of a correct response and that this slowing will increase the accuracy of correct choices (Rabbitt and Rodgers, 1977). While rats have been suggested to potentially show post-error slowing of the Rabbitt Effect, it is uncertain if an invertebrate species has the neural capabilities for this type of learning (Narayanan and Laubach, 2008; Summerfield and Yeung, 2013). If bees are found to be capable of post-error slowing this would suggest that they are capable of learning from their past errors. Furthermore showing that they meet at least one aspect of the Rabbitt Effect would lay the foundation for further research into whether bees are cognitively capable of this hallmark of human intelligence.

1.1 Thesis Outline

This research project's aim is to quantify an aspect of intelligence in *Apis mellifera* by assessing how they are able to manage conflicting information. In some training instances the stimulus that is offered is a reward but other times a deterrent. Three experimental tasks were therefore presented: two DCD tasks that offered a contextual cue in the form of a visual cue and a SCD task offering a cue in the form of a sequence. The DCD tasks were presented as a three-choice task which offered three stimuli: a reward, a punishment and an unrewarded stimulus, and a binary-choice task offering just a rewarded and a punished stimulus. This project will therefore address the following questions:

1. **Are bees capable of solving conflicting information?**

Invertebrates have not yet been shown capable of solving conflicting information in the form of DCD and SCD tasks. Two DCD tasks were presented that both offered a rewarded and a punished stimulus, with the three-choice task introducing a third unrewarded stimulus. In order to solve this task bees were given one of two possible visual contextual cues. The third task was presented as a SCD requiring the bees to solve a sequence to determine which stimulus was a reward or punishment. The first thesis aim of this project was to assess if bees were capable of learning to solve the conflicting information of these tasks.

2. Do bees display a post-error slowing effect?

Post-error slowing demonstrates the ability for an animal to slow down following an error in order to become more accurate, therefore learning from their past mistakes. While this phenomenon has been shown in vertebrates it has yet to be in an invertebrate species such as honey bees. Assessing the reaction time of bees in trials following errors compared to trials following correct choices will determine if bees display this slowing effect, and therefore are learning over time from these errors.

2. General Methods and Pilot Studies

2.1 Bees and Experimental Set-up

A single bee hive (standard 8 frame Langstroth) from Macquarie University, Sydney NSW was relocated from the bee yard into a flight cage (25m x 10m). It was placed at one end of the flight cage (fig. 1), positioned with the entrance of the hive facing the opposite end. A meter from the entrance the colony was provided with food in the form of a sugar feeder containing 20% sucrose solution and a plate of ground pollen, that were replaced every two days (fig. 1). The hive was regularly checked for health and motivation by monitoring the number of workers, flight activity, and inspecting the hive for production of honey and brood. If needed the hive was replaced with a new one from the yard every 3-6 weeks during the period of the experiments (January through to May 2019).

Figure 1: experimental set-up of the flight cage with the hive at one end facing the feeding table, offering sucrose solution of 20% and a plate of pollen. At the opposite end was the experimental table and testing chamber.

Three experimental chambers were built using polypropylene sheets for the sides and walls (Project Panel, Australia) and fibreglass insect mesh for the top of the lid (Cyclone, Australia). The chambers were constructed from three main parts; the antechamber, main chamber and lid (fig. 2).

Figure 2: Structural diagram of experimental testing chamber with the measurements used in construction

The antechamber had an entrance of approximately 5cm allowing for individual bees to fly inside once trained. The chamber contained a two-door system, one at the entrance and a second door at the entrance into the main chamber. With this door system an individual bee could be isolated and contained in the antechamber allowing for the recording device to be turned on, before the bee could then enter the main chamber. The top of the antechamber was covered in insect mesh to keep the chamber well ventilated and was detachable to allow for the quick release of any untagged bees that would accidently enter the antechamber. The top of the lid of the chamber was fixed with insect mesh and a platform so that a recording device (iphone 6, Apple Inc. US) could sit on top and record the experimental task. Once finished the bee would naturally fly up towards the sunlight coming through the top of the chamber where the lid could be removed to allow the bee to return to the hive.

The inside of the main testing chamber was covered in a random textured background giving the bees better optic flow for flight stabilisation within the arena (fig. 3). This specific background was selected due to bees seeing red as grey, as they lack the photoreceptor to see the red end of the visual spectrum. Instead red appears to bees as shades of grey. The visual complexity and contrasting colour of the background further allowed for the bee to be better differentiated against it, making it easier to measure the movement of the bee precisely once run through a MatLab code (Mathworks, Australia) which was used to extract data from the recorded trials.

A pink stimulus (CMYK: 0, 35, 0, 0) was used for the pre-training of the bees due to appearing grey to them and as not to bias the conditional discrimination experimental tasks (fig. 3a). Three types of coloured stimuli were u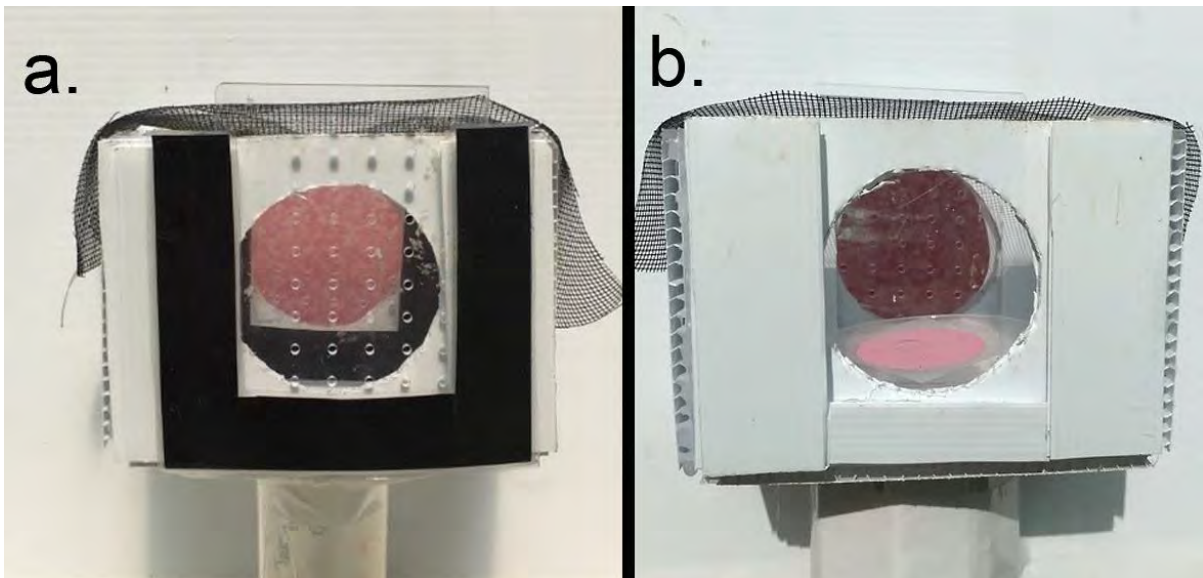sed for training and testing: a blue stimulus (CMYK: 100, 0, 0, 0), a yellow stimulus (CMYK: 0, 0, 100, 0) and a green stimulus (CYMK: 100, 0, 100, 0). Each of these three stimuli options offered a different solution to the bees, yellow and blue offered either a rewarded stimulus in the form of 50µl of 60% sucrose solution or a punishment in the form of 50µl of 50mM quinine solution, depending on the context it was given in. The green stimulus was an unrewarded option offering 50µl of water in both stimuli. Quinine was selected as a punishment due to being highly aversive to bees and 60% sucrose as a reward as it is a greater incentive compared to the 20% sugar feeder (De Brito Sanchez et al., 2014). It is additionally well documented that bees are able to be rapidly trained in an experiment with the use of sucrose and quinine (Sanchez et al., 2015). Each colour stimuli were printed on 160gsm printer paper printed using an ink-jet printer and cut into circles 4.5cm in diameter before being laminated. For the tasks they were placed on the top of 70ml specimen containers: 5.5cm in height from the floor of the arena, 5cm from the wall of the experimental arena and 10cm from the other stimuli (fig. 3b). They were positioned within the main testing chamber pseudo-randomly to ensure the bees would be unable to use the position of the stimuli as spatial learning cues. Positions of the stimuli changed randomly between each trial while maintaining the described placement rules.

Figure 3: Example of the inside of the testing chamber covered in the red-patterned background with the stimuli inside. a) The pink stimulus used throughout the pre-training phase. b) The three stimuli (blue, green and yellow) used through the experimental tasks. The stimuli were spatially arranged pseudo-randomly 5cm from the walls of the main testing arena and at least 10cm from each other.

Two contextual cues were used for both the three-choice and binary-choice DCD tasks; a black cue or a white cue. The cue was fashioned to the front entrance of the antechamber and a second cue was placed inside the antechamber, on the front entrance of the main chamber (fig. 4). The black cue used was created using two pieces of black paper cut to the 12cm x 12cm dimensions of the antechamber (fig. 4a). They were laminated and attached to the chamber entrances using hooks made from 1.57mm galvanised tie wire (Whites, Australia) secured to the back. The white cue was a contextual cue in the form of a distinct white visual signal, in contrast to the black cue (fig. 4b).

Figure 4: The two visual contextual cues for the three-choice and binary-choice tasks. a) The black contextual cue. b) The white contextual cue

Each of the coloured stimuli used throughout the main experimental trials, the pink stimuli used in the training trials, the red-patterned arena background, the white of the testing chamber and the contextual cues were all subject to spectrophotometry readings (table 1). Using the PAVO 2 package in RStudio these readings were processed to display the reflectance within the hexagon colour space of the honeybee visual system (Maia et al., 2019; RStudio Team, 2015) (fig. 5). The hexagon colour space is a chromaticity model of the trichromatic vision of hymenopterans. (Backhaus and Menzel, 1987; Chittka, 1992). The colour hexagon is based on how colour is processed as a generalised colour opponent space and has been applied extensively across hymenopterans, including *Apis mellifera* (Chittka, 1999). The use of this model is further validated as the colour distances within the hexagon for the honey bee is a reliable measure of perceptual distance due to having been comprehensively upheld against behavioural studies (Avarguès-Weber et al., 2010; Chittka, 1992; Dyer et al., 2008).

Table 1: Human visual depiction of all the colours used throughout the experimental tasks, with the CYMK values and equivalent RGB values and the resulting bee vision loci.

Human Colour							
CYMK	100, 0, 0, 0	100,0,100,0	0, 0, 100, 0	0, 35, 0, 0	0, 85, 85, 0	0, 0, 0, 89	0, 0, 0, 3
RGB	0, 174, 239	0, 166, 81	255, 242, 0	247, 183, 211	225, 37, 37	28, 28, 28	248, 248, 248
Hexagon loci (x,y)	0.041, 0.132	0.160, -0.026	0.196, -0.157	-0.095, 0.040	-0.016, 0.050	-0.190, 0.041	0.081, 0.083

Figure 5: Colours of each of the stimuli, contextual cues and background used in the experimental tasks. a) Bee vision hexagon plot of the all the colours bees encountered during the tasks. b) Spectral reflectance plot of each of the colours used.

These measurements were taken to ensure that the colours used were visually distinct from each other, specifically between the stimuli colours; yellow, green and blue, with the unrewarded green stimulus intended to be an intermediate between the yellow and blue stimuli. For a 75% discrimination honey bees require a colour distance of at least 0.008 hexagon units (Dyer and Neumeyer, 2005). The Euclidean distance between the blue and green stimuli was found to be 0.025, while blue and yellow had a distance of 0.023, and yellow and green a distance of 0.011. As the distance between these stimuli is greater than 0.008 hexagon units this therefore shows bees should be capable of easily distinguishing between colours used in this experiment.

2.2 Pre-training

In order to subject bees to the contextual discrimination tasks, they first had to be trained to travel from the hive to the experimental table and into a testing chamber. On the opposite end of the flight cage from were the hive was placed, three experimental tables were set up for each testing chamber. A pink stimulus was placed on top of a 120ml specimen jar, 11cm in height, with 100µl of 60% (w/v) sucrose solution pipetted on the surface. The concentration and quantity were specifically chosen for training due to the fact bees can store up to 70mg of nectar inside their honey crop and that 60% sucrose is a high-incentive reward for honeybees (Combs, 1972; Simcock et al., 2018). Offering a high reward in both concentration and volume therefore allowed for effective and rapid training of individual bees.

Foraging bees were lured from the 20% sucrose solution on a sugar feeder to the experimental table, using a cotton tip (Help@Hand, Australia) soaked in 60% sucrose solution. The end was pressed lightly to the antennae until they exhibited a proboscis extension response. The bee could then be coaxed onto the cotton tip and transferred (~20m) to the experimental table and then maneuvered onto the pink stimulus. The bee was allowed to drink the 60% sucrose reward before returning to the hive. Once a bee had returned to the pink stimulus by itself it was marked with a coloured paint pen on the thorax and/or abdomen using acrylic high-pigmented paint markers (Posca, Japan). The pink stimulus was then moved to sit right in front of the entrance to the antechamber of the testing chamber with another 100µl of 60% sucrose solution pipetted on the surface. Once the bee foraged from this location twice, the stimulus was then moved into the centre of the antechamber allowing the bee to forage for another two separate visits. The stimulus was then moved into the large testing chamber on top of a platform 11cm in height, positioned in front of the inner antechamber door so as to be visible to the foraging tagged bee from the antechamber. Once it had foraged two separate times the stimulus was moved into the centre of the chamber and the bee allowed to forage for another two trips. The 11cm platform was swapped for a 70ml specimen jar, 5.5cm in height with the bee foraging from it two times before two additional 5.5cm jars each were spatially arranged in a pseudo-random manner, to ensure that the bees would not just learn the position of the platforms. Each of these three stimuli offered 20µl of 60% sucrose solution, in order to encourage the tagged bee to forage from multiple platforms during a visit.

2.3 Colour Bias Test

To determine if there was an innate preference in learning, either the yellow or blue stimulus bees (n = 20) were presented with a task in which half (n = 10) were offered blue as a rewarded stimulus

and yellow as punishment for a total of 10 trials, and the other half (n = 10) were offered the reverse contingency. Individual bees were offered three stimuli: a punished stimulus of quinine, a rewarded stimulus of sucrose solution and an unrewarded stimulus of water. A single trial was defined as a bee entering the main testing arena and having the sequence of stimuli it landed on recorded until it landed on the rewarded stimulus and drank its sucrose reward. Once the bee finished, it was released through the top of the chamber and allowed to return to the hive. The bees were put through a total of 10 trials with each trial being recorded. Between each trial the stimuli were each cleaned thoroughly using a 70% ethanol solution to ensure there was no cross contamination of residual solution left from the sucrose or quinine, and to ensure bees were not leaving chemical signals left by footprints in previous trials. The platforms were spatially rearranged pseudo-randomly between each trial, with a 10cm space between each stimuli and that all stimuli where at least a 5cm from the walls of the chamber.

In this experiment bees, regardless of if they received a blue or yellow stimulus as the reward, learnt within only three trials to repeatedly return to the rewarded stimulus over the punished (fig. 6). By trial 5 bees in both contingencies had a mean frequency of correct choices of above 70%, compared to their first experience in trial 1 with both below 35%. By trial 10 both contingencies returned frequencies of above 75%, with bees given a blue stimulus was the reward, scoring as high as 100% in trial 9.

Figure 6: Frequency of correct first choices made between two sets of bees (n=20). Blue line indicating those (n=10) given blue as a reward and yellow line indicating those (n=10) given yellow as a reward.

Binomial linear models were run to further determine if bees were able to solve this task and if there was any notable difference in learning between trials with blue as the reward against trials with yellow as the reward. In both cases trial explained a significant amount of variation in likelihood of making a correct choice. Bees that were given a blue rewarded stimulus returned a significant P-value of 0.00275 (Z = -2.995) and those given a yellow rewarded stimulus returned a significant P-value of 0.0005 (Z = -3.481), therefore indicating a significant change in likelihood of correct choices with successive trials. A second linear model run on the frequency of correct choices against trial and whether the reward was either blue or yellow returned an insignificant value (P = 0.1055, Z = 1.619) therefore there was no difference in learning between the two contingencies.

2.4 Data Analysis

All experimental trials were recorded from a single overhead angle using a recording device (iPhone 6). These recordings were intended to be used to collect timing and choice data through an automated code written in Matlab R2019a (9.6.0.1072779). Due to time restraints and technically difficulties resulting in inaccurate data, the output extracted using this automated code was ultimately not used. Data were instead extracted manually using the datasheets taken during the

experiments and comparing them against the video recordings of each trial for each bee over all three of the tasks to ensure the data was accurate. A total of 660 videos per task for a total of approximately 1900 videos were analysed for data such as contextual cue colour, rewarded and punished stimuli colour and all choices made across 30 trials.

To extract timing data for the post-error slowing analysis the reaction time was documented using Solomon coder beta v. 19.08.02 (Péter, 2012). Due to time constraints 2 trials were analysed per bee over both the three-choice and binary-choice tasks, for a total of 88 videos examined. A trial in which the bee was incorrect between trials 10 – 20, followed by correct in the following trial were selected via a random number generator in Matlab. Using these videos the behavioural parameters of; entry, hovering, landing and stimuli choice were recorded (table 1). A "hover" was counted when the bee flew within ~4cm of a stimulus with its body facing towards the stimulus, slowing to a stop or adopting a side-ways motion around the stimulus staying within ~4cm of it, occurring for at least ~0.1 sec. A "landing" was recorded when the four forelegs of the bee touched the surface of the stimulus for > 0.1 sec.

Table 1: The definition of the behavioural parameters used when scoring the recordings from the experiments.

Behaviour	Description
Entry	The full length of the bee's body crosses over the entrance, from the ante-chamber into the main testing arena.
Hovering	The bee approaches a stimulus, flying within the diameter length of ~4cm with its body facing towards the stimulus. Moving in a side-ways motion or slowing to a stationary stop that occurs for ~0.1 sec.
Landing	The bee places at least four of its forelegs onto the surface of the stimulus, lasting for >0.1 sec
Stimuli choice	The colour of stimulus a bee selected by landing on the stimulus.

3. Conditional Discrimination

3.1 Introduction

Conditional discrimination tasks depend on the conditional relationship between stimuli, in that a response is reinforced in the presence of a given context only (Catania, 1973). This is distinguished from simple discrimination in which there is no context influence and given a stimulus is always rewarded or punished, whilst in conditional discrimination a stimulus is rewarded or punished depending on context. In conditional discrimination tasks there are at least two contexts (McIlvane, 2013; Pérez and Polín, 2016). Forms of conditional discrimination tasks include matching-to-sample tasks, which have been used to assess cognitive ability in a wide range of animals, including honey bees (Couvillon and Bitterman, 1988; Paine and Olmstead, 2004; Sidman and Tailby, 1982). Conditional discrimination tasks such as sequential conditional discrimination (SCD) and delayed conditional discrimination (DCD) tasks are less common, and have only been presented to a handful of vertebrate species such as primates, rodents and birds (Cohen et al., 1988; Honig and Dodd, 1983; Watanabe, 1981).

DCD tasks are much more complex and therefore difficult to solve in comparison to established condition discrimination tasks. This is due to the temporal aspect which requires the subject to access their working memory in order to use a contextual cue that is no longer present when the decision is being made (Herremans and Hijzen, 1997b). Bees have been shown to solve some conditional discrimination tasks which require working memory to solve, for instance delayed matching-to-sample tasks (DMTS) using such concepts like "sameness" to do so (Brown et al., 1998; Giurfa et al., 2001). DMTS tasks require the subject to retain memory of a sample stimulus after a delay and go on to match the sample to the stimulus. Similar to a DMTS task, DCD tasks have a delayed component that requires bees to use working memory to solve. Working memory is thought to underline more complex cognitive abilities, therefore giving a better indication into the complex cognitive abilities of bees. Delayed conditional discriminations have currently only been shown in vertebrates, namely in rats (*Rattus norvegicus*) and pigeons (*Columba livia domestica*). In one such DCD task in rats, the subjects were given a contextual cue in the form of either an auditory tone or a light, after which there was a delay interval (Herremans and Hijzen, 1997a). Following this delay the subjects were presented two stimuli, given as either a left or a right lever. To determine which lever to press, rats had to learn which contextual cue indicated which lever. The set-up of this task as a result did not allow for the rats to use positional strategies for mediate choice responding. Another such DCD task presented rats with two contextual cues rather than just one. The cues were

given as four possible sequence combinations made up of an auditory tone and / or a light (light-light, tone-tone, light-tone and tone-light) (Cohen et al., 1988). Between each of the two contextual cues, a delay retention interval was given, for a total of two delays. Increasing the interval time of the first delay resulted in more errors made and therefore greater decreases in accuracy, however an increase in second delay did not have any effect on accuracy. The results of this suggest that recall of the first stimuli occurs during the first delay and is absent during the second delay. There is yet to be any indication if invertebrates are capable of solving the conflicting information of a DCD task.

Solving the conflicting information of a SCD task, likewise, has yet to be shown possible in an invertebrate (Snodgrass and McMillan, 1989). A SCD task offers a conceptual cue in the form of a sequence rather than in the form of a perceptual cue that is perhaps visual, auditory or olfactory like that of a DCD task. So that a certain stimulus may be rewarded for a sequence of three times before it is switched to be unrewarding and so on, requiring a subject to learn this pattern in order to solve the task. This makes this task increasing difficult compared to offering a visual contextual cue as it requires either the numerical ability the count or the use of an alternative strategy such as a win-stay/ lose-shift strategy. In discrimination learning a win-stay/ lose-shift strategy is a heuristic learning phenomenon in which the subject will continue to give the same response providing that it continues to be rewarded for doing so (win-stay) (Imhof et al., 2007; Rawlins et al., 1988). Once they are no longer rewarded the subject will change responses (lose-shift). It is therefore possible to employ this strategy of continuing to select a rewarded stimulus until it becomes unrewarded and simply shift to selecting an alternate stimulus. The use of counting to solve this task would be a perfect solution, with the results reflecting this as a linear increase in performance over time and a low frequency of errors (Dacke and Srinivasan, 2008). A win-stay/ lose-shift strategy however would at best only allow for systematic errors as the subject must eventually make an error in order to shift to the correct choice (Simon et al., 2008).

Performance in such conditional discrimination tasks have been shown to be affected by factors such as the length of the retention interval or introduction of an interfering stimulus, suggesting that the performance is sensitive to interference. Interference of working memory is a phenomenon in which information that is either similar or conflicting in some way, interferes with information that is trying to be recalled (Wright et al., 2018). Conditional discrimination tasks have been shown to produce both retroactive and proactive interference in animals (Herman, 1975; Jans and Catania, 1980; White et al., 2004). Retroactive interference is when newly encountered information

interferes with older information that is being recalled. Proactive interference is the opposite, wherein older information prevents the recall of more recent information (Crossley et al., 2019; Köster et al., 2002; Wright, 2007). These behavioural phenomena that affect performance are similar to those that are known to affect working memory performance in humans, suggesting that working memory processes are involved in the ability to retain memory of the sample stimulus (Herremans and Hijzen, 1997a). The world around us presents an endless and near constant stream of information in the form to stimuli. Animals have consequently developed efficient neural mechanisms in order to determine between information that is relevant or irrelevant, such as selective attention; in which a stimulus can be identified as irrelevant and therefore inhibiting access of the irrelevant stimuli to the neural response system (Bivort and Swinderen, 2016; Paulk et al., 2014; Swinderen, 2007). Sometimes however, a stimulus is distracting in that it can cause retroactive or proactive interference. To account for this a distracting stimulus can be purposely introduced to determine if the behaviour in which the subject selects the target stimulus is altered. Previous literature has shown bees are able to easily determine a distractor stimulus from that of the target stimulus, learning to effectively avoid it rather rapidly (Chittka and Raine, 2006; Dyer et al., 2008). In some cases, however, a novel or distracting stimulus can be subject to generalisation, in which the behaviour of the reinforced stimuli shifts to the novel stimulus if the stimuli share similar properties established by conditioning (Andrew et al., 2014; Perry et al., 2013; Spence, 1937). In this case, as the blue and yellow stimuli are equally rewarded and punished, the subject may then begin to generalise broadly the way in which they respond to this information onto the green stimulus, which is the perceptual intermediate between yellow and blue colours. This use of past learning in present learning conditions therefore would result in bees choosing the green stimulus at a low frequency close to chance. The unrewarded green stimulus was therefore introduced as a purposely distracting stimulus in the three-choice DCD task. This task was then able to be compared to the binary-choice DCD task that did not have this third unrewarded stimulus option.

This project presented European honey bees with two novel conditional discrimination tasks; a three-choice and binary-choice DCD tasks and a SCD task in order to assess if bees can learn to solve conflicting information despite an added temporal delay, or with the use of a sequence rather than a visual contextual cue. If bees can be found to successfully complete the above tasks, this will provide a greater insight into the cognitive abilities of bees and the strategies they may employ in order to deal with these conflicting information tasks.
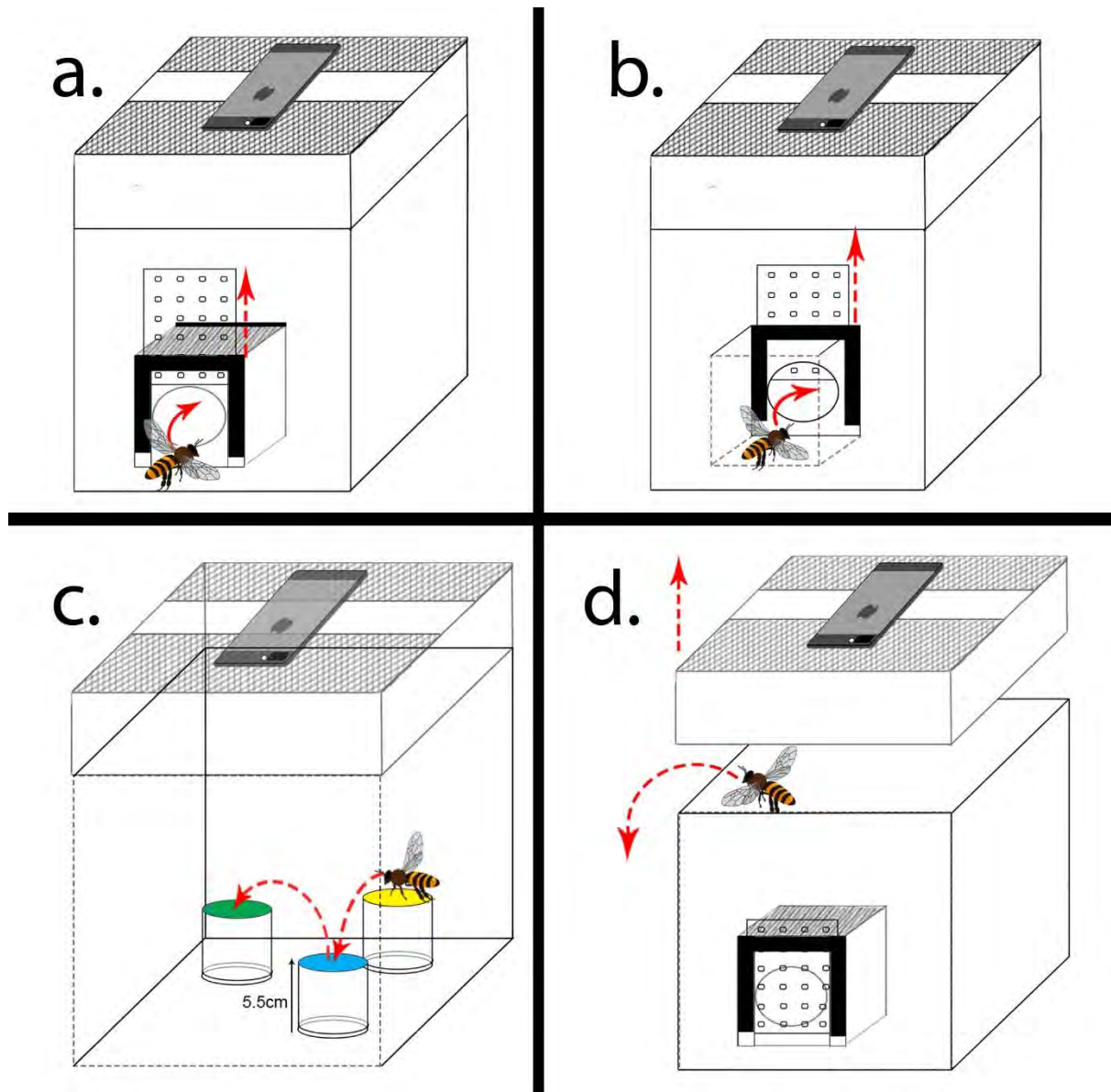
3.2 Methods

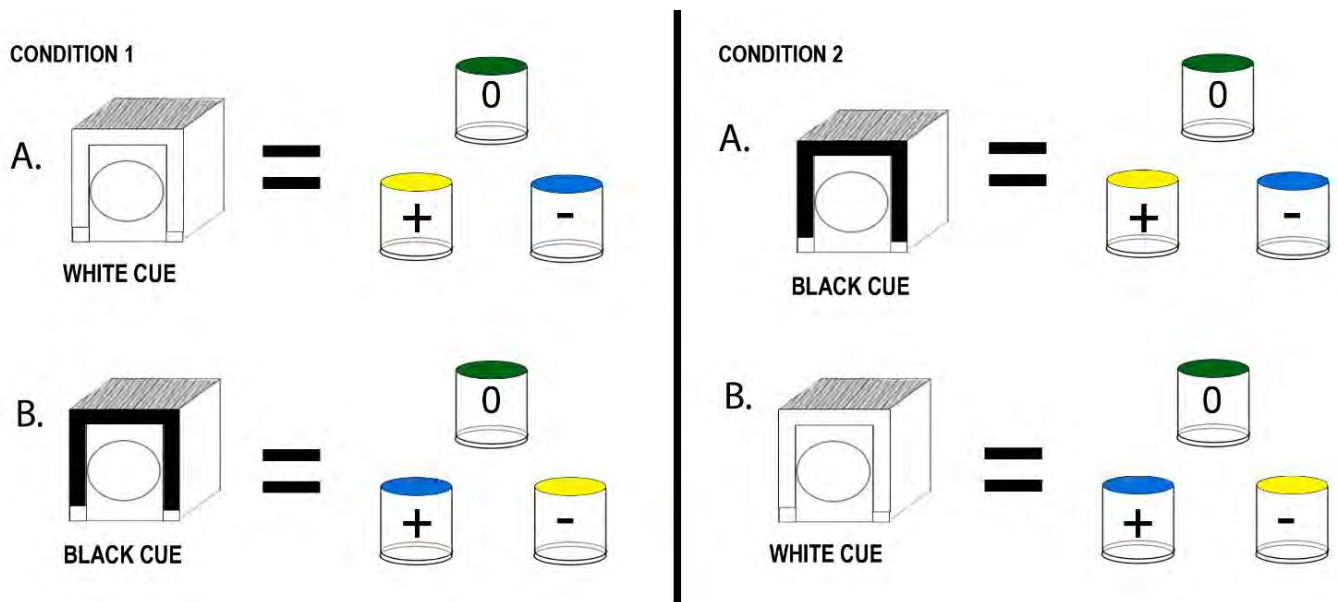### 3.2a Three-Choice Delayed Conditional Discrimination Task

Individual painted bees (n=22) were presented with a delayed conditional discrimination task that presented conflicting stimuli offering either a sucrose reward or a quinine punishment. This task was presented with three stimuli; a reward of sucrose and a punishment of quinine on either a blue or a yellow stimuli and a third unrewarded option of a green stimulus that always offered water. Green is perceptually intermediate between blue and yellow, therefore as the blue stimulus and yellow stimulus are both rewarded and punished the bee may come to prefer this unrewarded green option because it is never punished (fig. 5)(Dyer and Chittka, 2004; Perry et al., 2016). As such, the rationale for adding this third green option was for it to act as a distractor and to determine if bees would begin to generalise the green stimulus (Spence, 1937).

In order to solve the conflicting information task bees were given a contextual cue, in the form of either a black or a white entrance on the antechamber that would indicate if a stimulus was rewarded or punishment. A painted bee would approach the entrance of the antechamber in which they were given one out of the two contextual cues. The entrance door was opened allowing the bee to enter the antechamber, and closed behind them to isolate the individual bee (fig. 7a). The bee remained inside the antechamber until the recording device was turned on. While inside the antechamber bees encountered the contextual cue again, as the cues were placed both on the outside entrance of the antechamber and additionally inside the antechamber to account for the delay period between entering the antechamber and entering the main testing arena. Once the recording device was ready the second door was opened to allow the bee into the main testing arena (fig. 7b). Once inside the bee would be allowed to make its choice by landing on the surface of one of three possible stimuli, counting as a single choice (fig. 7c). The bee's sequence of choices was recorded until they had landed on the rewarded stimulus or began to bump against the mesh lid of the chamber continuously for over 5 minutes. At this point they were either allowed to leave the chamber or were forcefully placed on the rewarded stimulus by trapping them in a jar and placing this atop the surface of the rewarded stimulus until the bee landed and began to drink. Once the bee had finished the sucrose reward it would fly upwards where the lid of the chamber could be removed to allow it to return to the hive (fig. 7d). This would be counted as a single trial. In total each individual bee was subjected to 30 trials.

Figure 7: a) the first door opened to allow the individual painted bee into the antechamber. b) Once contained inside the antechamber the second door opened to allow the bee into the main arena. c) The bee was recorded until it landed on the rewarded stimulus. d) Once it had filled its crop the bee flew upwards where it was released by removing the lid of the chamber.

Half of the 22 sample size were tested under condition one (n=11) and half under condition two (n=11). If given condition one the bees would be given a white cue presenting contingency A and a black cue presenting contingency B (fig.8). For the bees presented with condition two the cues for the contingencies would be reversed, with a white cue now presenting contingency B and a black cue presenting contingency B.

Figure 8: Three-choice cue trial which offered two different conditions and two different contingencies. Condition one: A) In condition one bees viewed a white cue prior to entering the main chamber and finding contingency A. B) In condition one bees viewed a black cue prior to entering the main chamber and finding contingency B. Condition two: A) In condition two bees viewed a black cue prior to entering the main chamber and finding contingency A. B) In condition two bees viewed a white cue prior to entering the main chamber and finding contingency B.

The sequence for which contextual entrance cue (black or white) was given to the bees over the 30 trials was decided using a pseudo-random sequence in MatLab random number generator, so no more than two of the same cue occurred in a row.

3.2b Binary-Choice Delayed Conditional Discrimination Task

Individual bees (n=22) followed the same protocol as per section 3.2a but instead of being presented with three stimuli options, bees were given just two stimuli to choose from; a reward or a punishment. The reward and punishment was presented on either a blue or yellow stimulus depending on the cue given to the bee for two conditions (fig. 9).
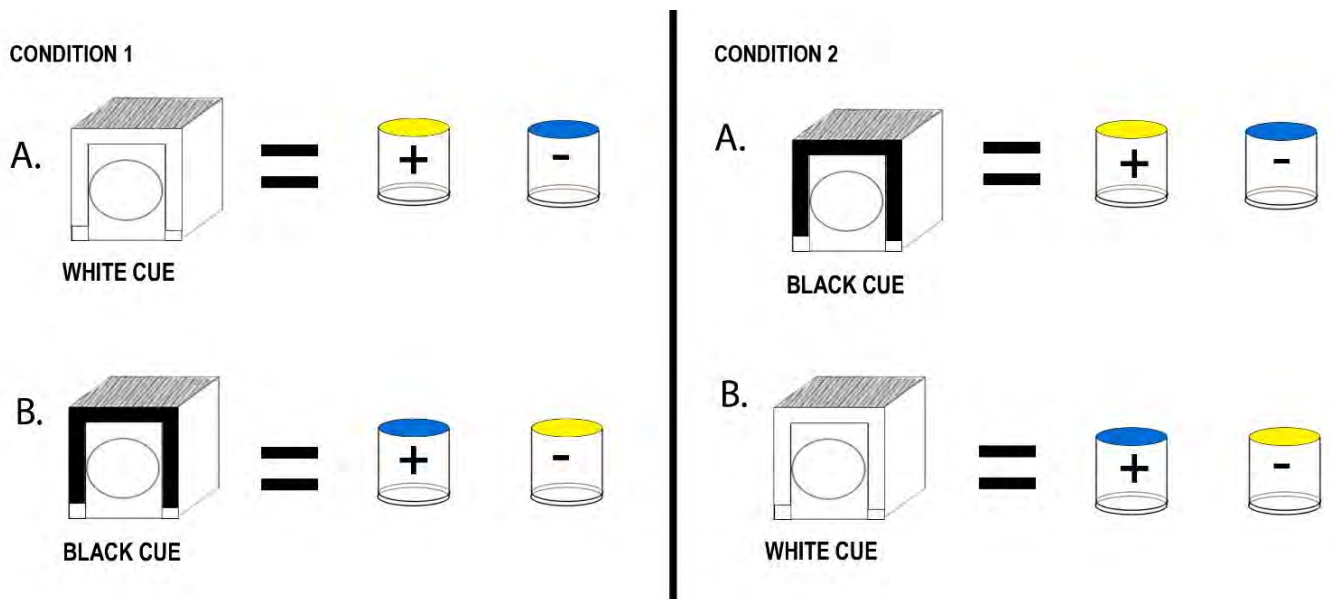
Figure 9: Binary-choice cue trial which offered two different conditions and two different contingencies. Condition one: A) In condition one bees viewed a white cue prior to entering the main chamber and finding contingency A. B) In condition one bees viewed a black cue prior to entering the main chamber and finding contingency B. Condition two: A) In condition two bees viewed a black cue prior to entering the main chamber and finding contingency A. B) In condition two bees viewed a white cue prior to entering the main chamber and finding contingency B.

Each bee was subjected to a total of 30 trials with each being recorded for later data analysis. The order of the entrance cue and treatment were randomised, with no more than two of the same cues presented in a row.

3.2c Sequential Conditional Discrimination Task

Bees (n=22) were offered three choices as per section 3.2a: a rewarded stimulus, a punished stimulus and an unrewarded water stimulus. To solve the task the bees were given a contextual cue in the form of trials in a fixed sequence: three trials of contingency A before switching to three trials of contingency B and so on until 30 trials had been completed (fig. 10).

Figure 10: The SCD task which offered two different contingencies. A) Bees entered the main chamber and found contingency A. B) Bees entered the main chamber and found contingency B. Bees were presented with one of the contingencies for three trials after which the contingency was switched for another three trials and so on.

3.3 Results

3.3a Three-Choice Delayed Conditional Discrimination Task

When presented with a three-choice task bees (n = 22) were able to learn to solve this task by choosing a rewarded stimulus over a punished or unrewarded stimulus. Over 30 trials the mean frequency increased from near chance (33.33%) at 28% to consistently above chance by trial 6 at approximately 40% (fig. 11). With the highest returned frequency of correct choice at 76% at trial 17 and staying above 50% for the remainder of the trials. A binomial linear model was run to determine the linear relationship between the frequency of correct choice and trials, returning a P-value = 0.0005 and a Z-value = 3.479. This suggests that the correct choice increases over the trial, further indicating that the bees learnt to solve the task over time. The choice of an unrewarded stimulus given as a green option was found to remain below chance at a mean frequency of 22.72% when first encountered in trial 1 (fig.11). Frequency of choice of the unrewarded stimulus was recorded at its highest at 31.82% during trial 2, before steadily decreased over the 30 trials, with a lowest frequency at 0% in both trials 16 and 24. Towards the end of the trials there was a general decline in the frequency correct, decreasing from 72.73% in trial 27 to 45.46% in the following trial.

Figure 11: Acquisition curve for the three-choice DCD task over 30 trials. The black curve indicates the mean percentage of correct first choices made (std error ± 0.00916), the green curve indicates the mean percentage of green first choices made and the red line indicates the performance level expected by chance at 33.33%.

Binomial linear models were run on other possible variables to determine if there was a statistically significant relationship between the variables and the correct choice rate. There was found to be no significant difference (P = 0.912, Z = -0.111) between a performance of bees given contingency A or contingency B (fig. 8.). The condition a bee received likewise, was shown to not be significant (P = 0.585, Z = -0.546) between condition one and condition two. This suggests that these variables did not affect the frequency of correct choices with no one variable returning a better rate of correct choices compared to the other. Entrance colour, regardless of reward colour, however did return a marginally significant relationship (P = 0.0302, Z = 2.168) between the contextual cue given (black or white) and the frequency of correct choices in this task. To further assess this the sum of correct choices for each cue were calculated. The black contextual cue had a total of 158 correct first choices (45.53%) in comparison to the white cue with 189 correct choices (54.47%). This shows that there was only marginally more correct choices when bees were given a white cue instead of a black cue.

3.3b Binary-Choice Delayed Conditional Discrimination Task

When the unrewarded green choice was removed in the binary-choice task bees (n = 22) were nevertheless still able to solve the given DCD task over time. The mean frequency of correct first choices across 30 trials increased from near chance (50%) to consistently above chance by trial 14 at approximately 73.33% (fig.12). To further determine whether bees were learning over training trials a binomial linear model was run on the relationship between correct choice and trials, which returned a significant value (P = 0.0001, Z = 3.789).
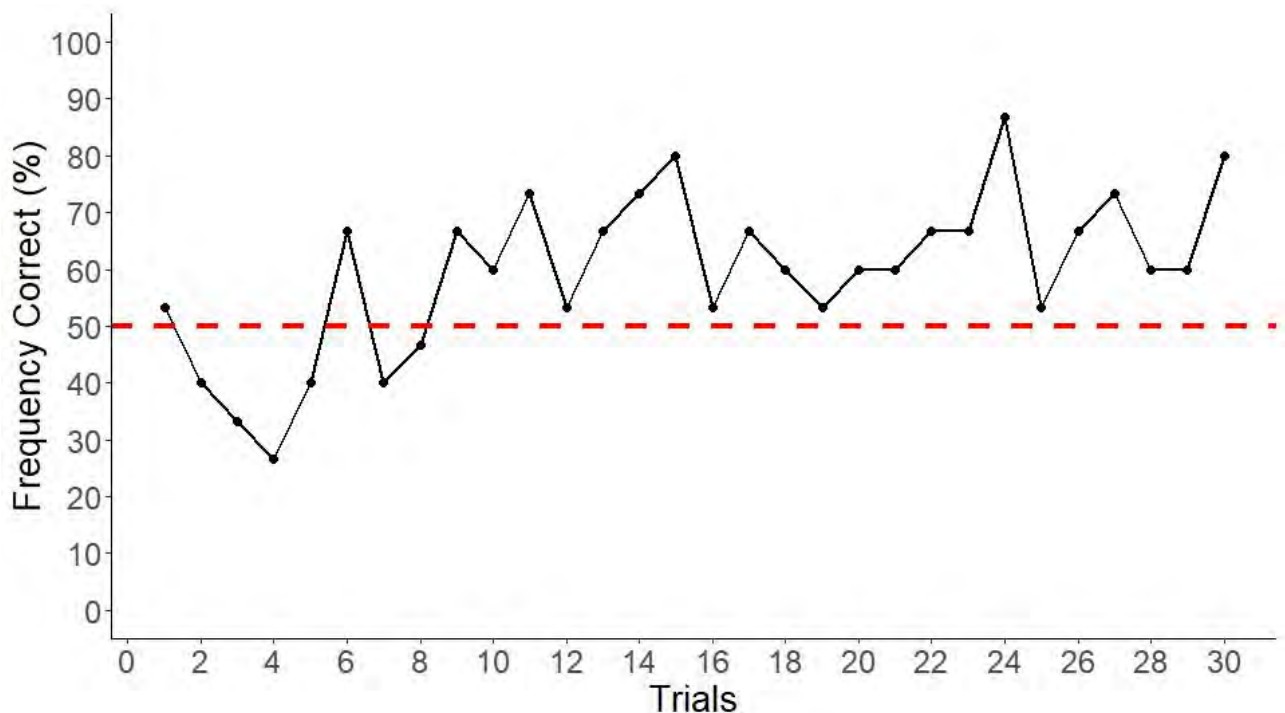
Figure 12: Acquisition curve for the binary-choice DCD task over 30 trials. The black curve indicates the mean percentage of correct first choices made (std error ± 0.00947), and the red line indicates the performance level expected by chance at 50%.

To further determine the significance of performance over trial additional binomial linear models were run on other numerous variables within the dataset. There was found to be no significant difference (P = 0.321, Z = -0.993) in the frequency of correct choices between contingencies (fig. 9). Between conditions, likewise there was found to be no significant difference (P = 0.6564, Z = 0.440). Like in the previous section 3.3a, the lack of significance between these variables suggests that they did not influence the frequency of correct choices. Unlike that in the aforementioned three-choice DCD task, the colour of the contextual cue given (black or white) did not return a significant

difference (P = 0.2194, Z = 1.228), suggesting that in this case the colour of the contextual cue did not have a significant effect on the frequency of correct choices made between trials.

Comparing the latter two three-choice and binary-choice DCD tasks found that they both started at chance, with the three-choice starting at its chance 33.33% and binary-choice at chance at 50% (fig. 13). By trial 10 the frequency of correct choices remained over chance for both the tasks therefore suggesting that bees had by then learnt the task. The three-choice task saw a performance height of 76% at trial 17 compared to the binary-choice task at 86.67% in trial 24. This comparison between the two DCD tasks suggests that the addition of the green unrewarded stimulus did not work as a distractor.

Figure 13: Acquisition curves for both the three-choice and binary-choice DCD tasks over 30 trials. The purple curve indicates the percentage of correct first choices made in the three-choice task (std error ± 0.00916) with the dark purple line of performance level expected by chance at 33.33%. The orange curve indicates the mean percentage of correct first choices made in the binary-choice task (std error ± 0.00947) with the and the dark orange line the performance level expected by chance at 50%.

No further stats were done on this comparison between the three-choice and binary-choice DCD tasks due to the many confounds that would potentially bias these results, such as the two tasks being done at different times of the season (fig. 13).

3.3c Sequential Conditional Discrimination Task

When given a conceptual cue in place of a visual contextual cue bees (n = 22) were found to be able to solve this SCD task using a sequence as a cue. The frequency of correct first choices across 30 trials increased from a start of 45.46% before decreasing to the lowest correct frequency at 13.64% when bees were given the first trial that switched the contingencies; reversing the colours for the rewarded and punished stimuli (fig. 14). The jagged appearance of the acquisition curve reflects these regular switches in contingencies every three trials. The frequency of correct choices remained constantly above chance (33.33%) after trial 5, with the highest frequency at 90.91% of correct choices at trial 18.

Figure 14: Acquisition curve for the SCD task over 30 trials, with the grey lines indicating the switch in contingencies. The black curve indicates the mean percentage of correct first choices made (std error ± 0.00935), and the red line indicates the performance level expected by chance at 33.33%.

To assess if bees were in fact learning to solve the task over trials a binomial linear model was run on the relationship between correct choice and trials, and returned a significant value (P = 3e-5, Z =4.121). It was found however, that the sequence of a trial, whether it was a switch trial, one after the switch or two after the switch, had a significant effect (P = 0.0006, Z = 3.403) on the frequency of correct choices, prompting a more detailed look into this variable.

The performance of the bees in trials in which the contingencies where switched, reveals that the first experience of this switch at trial 4, saw a decline in the frequency of correct choice well below chance (33.33%) at 13.64% (fig.15). Compared to these switch trials (trials 4, 7, 10, 13, 16, 19, 22, 25, 28), the performance on trials one after the switch trials (trials 5, 8, 11, 14, 17, 20, 23, 26, 29) were found to be consistently higher. The second switch trial at trial 7 saw 36.36% of bees select the correct choices, placing bee's performance now marginally above chance. Performance of the trials two after the switch (trials 6, 9, 12, 15, 18 21, 24, 30) showed the highest frequency of correct choice, scoring a performance of 90% correct in trial 18 and remaining well above chance over the experimental task.

Figure 15: Acquisition curve of the trials on the switch, one after the switch and two after the switch with the grey lines indicating the switch in contingencies. The purple curve indicates the percentage of correct first choices made on the switch trials, the green curve indicates the percentage of correct first choices made on trials one after the switch and the pink line indicates the percentage of correct first choices made on trials two after the switch. The red line indicates the performance level expected by chance at 33.33%.

Considering just the trials in which a switch in contingency was made (fig. 15, purple curve), the frequency of correct choices increased with trial number, with a binomial linear model returning a significant value (P = 0.0286, Z = 2.189). The trials one after the switch in contingency (fig. 15, green

curve), also were found to significantly (P = 0.0213, Z = 2.302) increase in frequency of correct choices. In contrast to the previous mentioned trials, the trials two after the switch (fig. 15, pink curve) were found to not significantly (P = 0.481, Z = 0.704) increase in frequency over the trials. Figure 14 however shows a non-linear correlation therefore the data could be skewed due to the variation in frequency, specifically, the decline in performance after trial 21. A second binomial linear model was therefore run on the two trials after switch data using just the first 21 trials, right before the decline in performance. This model now found a significant (P = 0.00614, Z = 2.740) increase in frequency of correct choices over trial. All trials in the sequential task returned significant values providing evidence of change with trial and therefore change of experience suggesting bees were learning to solve this SDC task.

3.4 Discussion

Bees were shown to not be making choices at random but rather consistently selecting the rewarded stimulus when presented with either a binary or three-choice option (fig. 11 & 12). The frequency in which the rewarded choice was selected increased over individual trials, therefore suggesting that bees were able to learn (fig.13). This was further assessed by excluding the contributions of other variables within the statistical analyses. Namely, excluding the colour of stimuli, individual bees and contingency. The colour bias test further supported that stimuli colour was likely to not have an effect on correct choice in later conditional discrimination tasks (fig. 10). One variable however, the entrance colour of the contextual cue, did returned a significant value in the three-choice DCD task but not in either the binary-choice DCD or SCD tasks. A further assessment of this significance however, found it to be only marginally significant. The white contextual cue had only a borderline higher frequency of correct choices in comparison to the black contextual cue. Bees appear to be able to conceptualise the white contextual cue easier than the black cue. Considering the insignificance of contextual cue colour in the other tasks and this marginal significance, however it would appear that this was a false positive.

The frequency in which bees selected the rewarded stimulus over all others in the sequence task, suggests that they are in fact able to use a contextual cue in the form of a sequence to solve a conflicting information task (fig. 14). Excluding other variables such as contingency and colour assigned to stimuli further suggests that bees are in fact using the sequence to solve the task. Accuracy, however, decreased on each trial in which the contingencies were switched before increasing rapidly again in the following two trials (fig. 15). The distribution of the data suggests that the bees are not however, using a pure sequence strategy due to the lack of a gradual steady

increase in performance over trials (fig. 14 and 15). This suggest bees appear to be implementing a multiple of possible strategies in order to solve the conflicting information of a SCD task.

3.4a Presence of Green Stimulus

The rationale for including an unrewarded green stimulus was for it to act as a distractor stimulus, in order to determine if bees would begin to choose this unrewarded stimulus more as the trials went on, with green being selected colour wise as it shares properties of both the blue and yellow stimulus. If chosen in trials this would suggest that the information between the yellow and blue stimulus were being generalised and their properties should extend onto the green stimulus. As the blue and yellow stimuli are equally rewarding and punishing, bees would begin to generalise both this reward and punishment to the green stimulus resulting in it being selected at a low frequency. The presence of this unrewarded option offered in the three-choice task, however, did not influence the frequency of correct choices in comparison to the binary task. As the binary task offered no unrewarded green option yet the accuracy rate did not differ to the three-choice task that did offer this third green option (fig. 11 & 12). Bees appeared to learn swiftly that this stimulus did not offer a reward and the frequency in which they chose the green stimulus decreased within the first 6 trials and remained well below chance (fig. 11).

A comparison in learning between the three-choice DCD task that offered the unrewarded green stimulus against the binary-choice DCD task that did not, found there was no difference in learning (fig. 11 & 12). The presence of an unrewarded stimulus therefore did not result in bees learning at an increased rate, further supporting the evidence that the green stimulus did not cause any interference. The bees therefore did not begin to generalise the green stimulus but instead learnt to avoid it over successive training trials in favour of the target rewarded stimulus.

3.4b Conflicting Information as a Sequential Task

The results suggest that bees are capable of solving a SCD task when given a sequence. This could furthermore suggest several strategies bees are implementing in order to solve this task (fig. 13). One such interpretation is bees are implementing a win-stay/ lose-shift strategy (Rawlins et al., 1988). This is supported by a decline in performance in the trials in which the contingencies were switched followed by an increase in the subsequent trials following this switch (fig. 14). The decrease in performance during the switch trials, followed by a steady increase in the subsequent trials could be a possible indication that bees were utilising this win-stay/ lose-shift strategy in order to solve the task. The use of this strategy has been widely used to assess the ability of an animal in the use

of spatial memory, particularly when it comes to foraging (Laughlin and Mendl, 2000). Ungulate species such as pigs (*Sus scrofa*), cattle (*Bos Taurus*), and sheep (*Ovis aries*) all adopt a win-stay strategy, as do some primates, (Hosoi et al., 1995a, 1995b; MacDonald et al., 1994; Mendl et al., 1997). Rats (*Rattus norvegicus)* and pigeons (*Columba livia*) tend to implement a win-shift strategy, instead avoiding stimuli in which they had previously been rewarded (Gittis et al., 1988; Komischke et al., 2002b; Randall and Zentall, 1997). A species propensity to a win-shift strategy could likely provide an advantage in foraging, wherein a food source needs to be replenished (Bar-Shai et al., 2010; Olton and Samuelson, 1976).

A win-stay/ lose-shift strategy in contrast, dictates that an animal will repeatedly select a reinforced stimulus until said stimulus is no longer rewarded, in which case their response shifts to the alternative stimulus. Repeated selection of this alternative stimulus will continue as long as it is rewarded (Evenden and Robbins, 1984). Animals will often employ this strategy when confronted with multiple discrimination information (Rayburn-Reeves et al., 2013). As they continue to receive an increasing amount of conflicting information they switch from using a stimulus-response associations to a less specific and more general strategy, which can be more effective (Purdy, 2012). Primates such as chimpanzees have shown consistent evidence of adopting this strategy, when presented with a successive discrimination-reversal task that presented a series of 180 discrimination tasks. The chimpanzees experience within these tasks, several reversals of contingencies and implemented a win-Stay/ Lose-Shift in order to deal with this large amount of conflicting information given in rapid succession (Schusterman, 1962).

While bees have been shown to adopt both a win-shift and win-stay strategy when foraging they have been found to begin to shift their behaviour to a win-stay/ lose-shift the greater the magnitude of the reward of the information they are given (Townsend-Mehler et al., 2011). As bees were given a high incentive reward of 60% sucrose compared to the 20% sucrose solution in the sugar feeder it is possible that bees are shifting to a win-stay/ lose-shift strategy. As shown in the decline of accuracy in the switch of contingency trials followed by the increase in trials after the switch further (fig. 14). Showing that bees made more errors on the switch trials which could reflect this Lose-Shift effect, in which bees continued to select the same rewarded stimulus until it was switched. Bees could therefore be shifting from a win-stay strategy seen in DCD tasks to a win-stay/ lose-shift strategy in the SCD task, as it does not give a clear visual contextual cue but a sequential pattern which is more difficult to solve. These declines in performance however do not decrease below chance as would be expected in a win-stay/ lose-shift strategy, therefore it appears bees were not

exclusively implementing this strategy (fig. 14 and 15). It is possible that bees are instead implementing multiple strategies in order to solve the conflicting information in the sequential task, a combination of a win-stay/ lose-shift and numerical counting strategy.

3.4c Numerical Cognition

Another such interpretation for the bees' ability to solve the sequence task, could be that they are counting the trials and therefore can anticipate when the rewarded and punished stimuli will be switched. Individual bees in this project were observed to have a long running score of consecutive correct trials in a row. Some up to as many as 8 trials, anticipating the switch multiple times. A majority of studies on numerical cognition ability have focused solely on vertebrate species, with limited quantity discrimination and number comprehension found in dogs and wolves, primates and birds (Brannon and Terrace, 1998; Pepperberg and Gordon, 2005; Range et al., 2014; West and Young, 2002; Woodruff and Premack, 1981). There is now, however, evidence that these numerical abilities are not just restricted to vertebrates. Studies using jumping spiders (*Portia Africana*) demonstrated their use of quantitative concepts for araneophagic predatory behaviour based on the number of conspecifics present near their prey species' nests (Jackson and Nelson, 2012; Nelson and Jackson, 2012). Male mealworm beetles (*Tenebrio molitor*) have been shown to use numerical cues in order to assess quantities. Keeping a running score of competitor males they encounter in order to adjust the investment into reproductive behaviours such as mate-guarding (Carazo et al., 2012).

While reports have begun to show evidence of invertebrates' numerical ability, the research tends to focus primarily on eusocial insects due to the greater demand in navigational problems and therefore benefiting from a numerical sense. One such social insect species, the buff-tailed bumblebee (*Bombus terrestris L.*), have shown the ability to count the number of nectar rewards they had received in order to avoid revisits to nectaries they had already previously depleted (Bar-Shai et al., 2010). Bumblebees learn to then leave a foraging patch after they have foraged a fixed number of a food source, something that has been previously theoretically proposed (Green, 1980; Pyke, 1984). This patch departure pattern and fixed number behaviour displayed by bumblebees furthermore contradicts the use of a win-stay/ lose-shift rule.

There is evidence that bees are capable of object-independent counting, with their behaviour meeting some, but not all the criteria of true counting (Chittka and Geiger, 1995). True counting is defined as the ability for order irrelevance, cardinality, abstraction, correspondence and a stable order. In this sense bees do not display the level of cognitive sophistication of strict true counting

but rather a number competence described as proto-counting (Gelman and Gallistel, 1978; Pahl et al., 2013). Provided that the number does not exceed four, bees display the numerical competence of being able to count objects when encountered one after the other, called sequential counting (Dacke and Srinivasan, 2008). This requires the ability to recall past encounters and keep a running score, incrementing by one after each new encounter (Dehaene et al., 1990). This ability to sequentially count when navigating is likely beneficial in tallying prominent landmarks en route to a food source allowing the foraging bee to track its own progress along the route and its vicinity to the destination (Chittka et al., 1995; Vladusich et al., 2005). This experiment requires bees to be able to count three separate trials in order to predict the switch in contingencies, therefore suggesting that bees may be using both proto-counting in combination with another strategy to solve the conflicting information of the SCD task.

3.4d Proactive Interference

Throughout all three trials there is a noteworthy decline in performance towards the end of the experimental tasks, specifically around trials 25 to 27 (fig. 11, 12 and 14). A speculative explanation for this decrease in performance could be an interference of the working memory of bees, in which information that is similar in format is interfering with information that is attempting to be recalled (Baddeley and Hitch, 1974; Wright et al., 2012). In this case the interfering effect is called proactive interference, wherein previous information prevents the recall of new information (Crossley et al., 2019; Wright, 2007). Proactive interference in humans has been shown to rapidly increase in long-term retention tasks, producing a dramatic decline in accuracy (Keppel and Underwood, 1962). Furthermore, delayed matching-to-sample tasks have been found to proactively interfere within experimental trials using animals across taxa (Edhouse and White, 1988). One such animal study using pigeons found they displayed a time-based proactive interference in delayed matching-to-sample tasks. With even a 1 sec delay producing a considerable interference, especially in the trial following the delay. It was found the longer the delay the greater proactive interference and effect on overall accuracy (Wright et al., 2018, 2012). Rhesus and capuchin monkeys, in contrast, were shown to display an event-based proactive interference, rather than displaying no time-based interference. When given a delay there was shown to be evidence of a substantial proactive interference (Devkar and Wright, 2016). The interference, however, did not fluctuate based on different delay periods thus concluding that the interference was event-based rather than time-based (Wright et al., 2018). Short-term memory in primates, therefore, appears to be highly susceptible to proactive interference (Bigelow and Poremba, 2013). Delayed conditional

discrimination tasks in rats and pigeons likewise have reported evidence of proactive interference. As the delay interval reduced the accuracy of correct choice was found to decrease, therefore increasing the interference effect (Herremans et al., 1994). With an increased delay interval it was found that the preceding stimuli no longer actively exerted discriminative control therefore no longer interfering with the following trial. The bees when subject to a delayed conditional discrimination task similarly showed evidence of this proactive interference (fig. 11, 12, 14).

It is not just vertebrates that have displayed proactive interference in cognitive tasks. A study of serial visual reversal learning in octopus *(Octopus vulgaris)* found that variation in the learning between individuals could be explained by proactive interference. Which saw a significant decline in performance of some individuals against others in which there was no decline (Bublitz et al., 2017). A similar experiment that assessed habit reversal in goldfish (*Carassius auratus*) found that the fish showed no decrements in retention and lacked any progressive improvement, a result of proactive interference impairing the retention of a subsequently learnt stimulus when taught a new novel stimulus (Gonzalez et al., 1967). Pond snails (*Lymnaea stagnalis)* were also shown to display proactive interference, retaining the memory of the older information at the expense of the newer information (Crossley et al., 2019).

Previous experiments using the common eastern bumblebee (*Bombus impatiens)* found that the bumblebees displayed evidence of retroactive interference that correlated with the size of the bee (Worden et al., 2005a). It was found larger bumblebees showed a greater interference effect compared to that of smaller bees. As larger bumblebees are typically foragers compared to those smaller, it is possible that these bees are capable of faster learning in order to learn colour associations needed for effective foraging (Goulson et al., 2002; Muth et al., 2015). As a result they undergo a retroactive interference effect at a sooner and greater rate as a trade-off for more rapid learning abilities. It has been suggested that bumblebees use retroactive interference, even within the presence of a contextual cue, in order to forget old information in the presence of new novel information (Worden et al., 2005b). This could instead suggest the bees experienced performance fatigue or decline in foraging motivation rather than a form of interference. A proactive interference effect, however, appears to be a more likely explanation, as the decline in correct choice remained consistent across all three of the tasks. Similar interference effects have furthermore been found in previous literature on conditional discrimination tasks in animals (Crossley et al., 2019; Edhouse and White, 1988).

Further examination into what type of memory bees employ in order to solve these delayed contextual tasks would be a logical next step in further studies. Humans and non-human primates have been shown to use long-term memory for these tasks, being able to retain the information and associations of the contextual cues so that when presented with the same task some time later, they are still able to solve it (Carruthers, 2013; Goldman-Rakic, 1995). When it comes to presenting a delayed task to an insect species, it is unknown if they are also capable of this type of long-term memory retention and are only able to working memory. Extending the delay given to different time intervals and comparing these could provide greater insight into if bees can use long-term working memory to solve conflicting information tasks days later, similar to that in primates. The use of a computational model of the neural circuitry and mushroom body (corpora pedunculata) of the honey bee brain could provide further evidence that bees are capable of solving a DCD and SCD task. Past literature has used similar models of the mushroom body to explain how bees can solve seemingly complex cognitive tasks using their relatively simple brains (Cope et al., 2018; Peng and Chittka, 2017).

Determining which strategy bees used in order to solve the sequence task would be a topic for future study. Both the use of a win-stay/ lose-shift and sequential counting strategy have been shown before in bees and it appears that they may be implementing a combination of both strategies in order to solve the SCD task, or another unconsidered strategy (Bar-Shai et al., 2010; Townsend-Mehler et al., 2011). To either confirm or eliminate the possibility of counting within the delayed tasks, future tasks could introduce trials in which the switch of the reward and punished stimuli is extended from three. Introducing trials with a switch on every forth trial, and a switch on every fifth trial. As bees have only displayed the ability for numerical competence up to four, if bees are counting there should be a decline in performance when given a task requiring them to count beyond this (Chittka and Geiger, 1995; Dacke and Srinivasan, 2008). If bees are using a win-stay/ lose-shift strategy this change in contingency switch will not show a significant difference in behaviour between a switch at every third trial to a switch at every fifth trial. The rapid decline in the trials towards the end of the sequence task would additionally be something to further determine in future research, whether this phenomenon is a result of proactive interference or perhaps performance fatigue. Further analysis of the reaction time data already collected would be able to provide further insight into this. If bees were in fact experiencing proactive interference the data will return an increase in reaction time with this sudden decrease in accuracy compared to the reaction time of trials before this decrease. With an increase in speed caused by the interference of

the stimuli causing bees to become confused and therefore take longer to select a choice. If this decline is instead caused by performance fatigue or a similar effect, reaction time should instead decrease with the decline of accuracy, as bees are making choices hastily without actively considering the stimuli. The effect of interference has been shown to be significantly reduced when the interfering information is learnt in a different context compared to new novel information (Dallett and Wilcox, 1968; Hockley and Bancroft, 2015). Studies using bumblebees found that providing the bee with different multiple contextual cues can reduce the effect of interference. In a natural situation bumblebees may actually require the use of multiple contextual cues for any one given task in order to reduce the effect of interference in their performance, compared to laboratory experiments which often only offer a single contextual cue (Worden et al., 2005b). Presenting honeybees with multiple delayed contextual cues rather than a single one may return similar results and would be an additional factor to consider for future studies.

4. The Post-Error Slowing Effect

4.1 Introduction

All features of the Rabbitt Effect has only been shown in humans and is considered a hallmark of human intelligence that has yet to be found in other taxa (Rabbitt and Rodgers, 1977). Post-error slowing is a feature of this phenomenon and is suggested to be a temporary measure to support cognitive processing to improve accuracy in post-error trials (Cho et al., 2009). In humans, reaction times have been found to gradually decrease through trials until the subjects make an error. Once an error has occurred subjects will show an increase in reaction time (Laming, 1979b; Narayanan et al., 2013). As of yet non-human animals have not been shown to display all features of the Rabbitt effect with certainty, however research with rats (*Rattus norvegicus*) have suggested that they do display a post-error slowing effect (Beard et al., 2015, 2015; Narayanan and Laubach, 2008). In a simple reaction time task, rats were trained to hold down a lever for a 1.0 sec delay, with an error counted as when the lever was released too early or too late (Narayanan and Laubach, 2008). In trials that followed an error having occurred, rats were found to have an increase in reaction time compared to trials following a correct response. Non-functional explanations have proposed this slowing is due to reduced cognitive processing that results in a decrease in post-error accuracy (Dudschig and Jentzsch, 2009; Jentzsch and Dudschig, 2009). There are a number of reduced cognitive processing explanations that have been put forward and tested in human trials (Dutilh et al., 2012). One such explanation is priori bias, which states that following an error the subject becomes negatively biased against the stimuli they chose (Rabbitt and Rodgers, 1977). This potential negative bias hinders repetitions to stimuli and encourages a decrease in reaction speed. Another possible explanation is that this post-error slowing is caused by perceptual distraction, in that a subject is momentarily distracted. Due to errors being infrequent and unanticipated, following an error in the subsequent trial the subject becomes distracted due to the shock of the error (Notebaert et al., 2009). Recent studies found however, no evidence that post-error slowing was a result of these non-functional explanations, suggesting it's cause could be functional (Dutilh et al., 2012).

In conditional discrimination tasks improved accuracy often comes at the cost of reaction time as animals are unable to simultaneously maximise speed as well as the accuracy of their decisions (Chittka and Spaethe, 2007). This speed-accuracy trade-off has been documented in a range of animals across taxa, with it being shown first in primates such as humans (Wickelgren, 1977). Rodents such as rats (*Rattus norvegicus*) and mice (*Mus musculus*) have also been shown to experience this speed-accuracy trade-off (Berberi and Careau, 2019; Uchida et al., 2006). As well as

other insects such as bumblebees (*Bombus terrestris*), ants (*Temnothorax albipennis)* and honeybees (Chittka et al., 2003; Franks et al., 2009; Seeley et al., 2006). This ubiquitous behavioural phenomenon suggests that the trade-off between reaction speed and decision accuracy is important in decision-making (Heitz, 2014). Accuracy depends on gathering time-consuming information in order to make a decision with fewer errors. Rock-ants for example, were found to prefer slow tandem running when recruiting a sufficient number of nest-mates (quorum threshold) to a potential new nest-site. While ants could recruit by carrying nest-mates which was three-times faster, the slower tandem running allowed for one ant to effectively teach the route between nest sites to new ants. So while overall tandem running has a slower reaction time, it allows for greater gathering of information and therefore greater accuracy. Honeybees have been shown to also display this trade-off of speed for an increase in accuracy (Seeley et al., 2006).

This chapter will determine if honey bees (*Apis mellifera*) display evidence of a post-error slowing phenomenon by determining if their reaction time slows in trials following an error. By assessing reaction times of trials following errors against the reaction time of trials following correct choices it can be determined if their speed increases or decreases. If bees are found to increase their reaction time this will support evidence of one aspect of the Rabbit Effect in an invertebrate species, showing that this is not a hallmark of human intelligence.

4.2 Methods

To determine if bees displayed a post-error slowing effect, the reaction time of two trials from the DCD tasks were recorded as per the data analysis methods outlined in section 2.4. Reaction time was determined as when the bee entered the main testing arena to until it had landed with at least four legs down on the correct stimulus choice and began to drink. The trials used were those in which the first-choice bees selected were either the punished or unrewarded stimulus (incorrect choice) followed by a subsequent trial in which bees' first choice was the rewarded stimulus (correct choice) (fig.16). For the incorrect trial, the reaction time of the first choice made was recorded, followed by reaction time of the bees' second choice and so on until the last choice made. In the subsequent trial bees' first choice was correct therefore the reaction time of only this first choice was recorded.
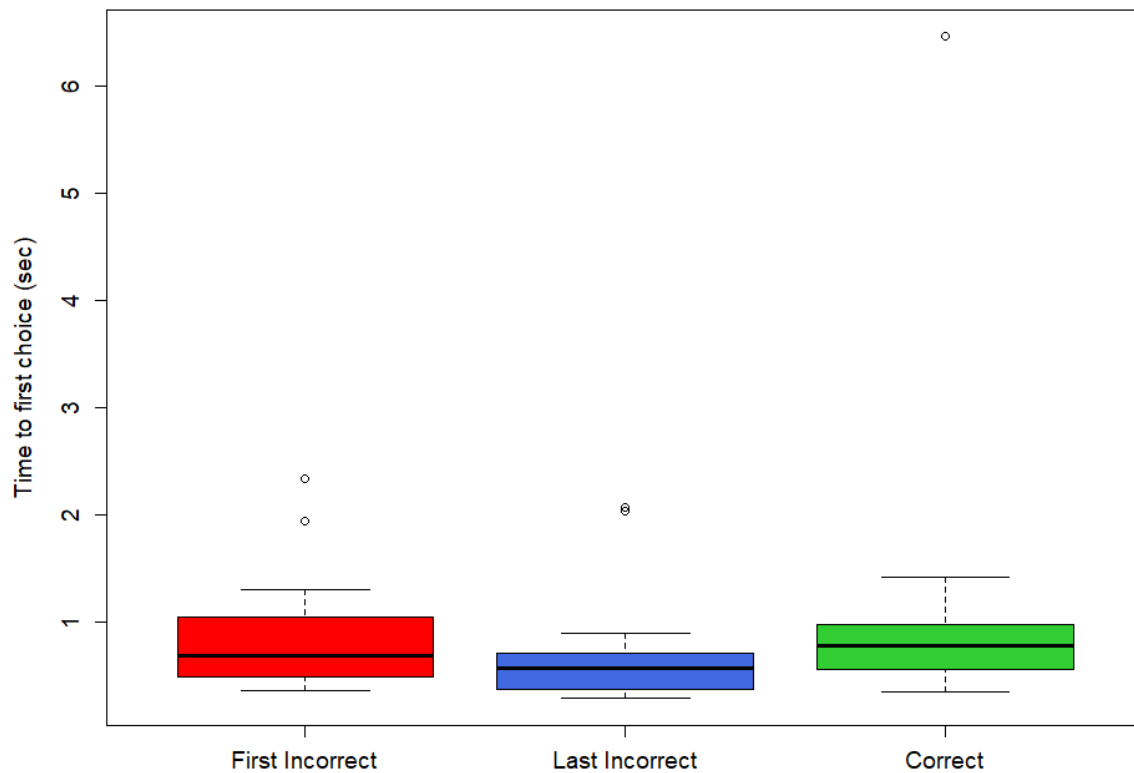
Figure 16: The two trials selected between trials 10-20 per bee per DCD task. The first in which the bees' first choice was either the punished or unrewarded stimulus and therefore incorrect followed by the next trial in which the bees' first choice was the rewarded stimulus and therefore correct.

These data were initially planned to be collected using an automated MatLab code which would track the bees in the video recordings and collect a multitude of data such as hovering time, landings, flight path and direction of the body. The automated code however, did not work as the threshold to identify landings and hovering was incorrect and could not be fixed in the allotted time therefore data had to be manually extracted. Future research on this project would aim to correct the programming bugs and use the automated code to collect additional and more precise data.

4.3 Results

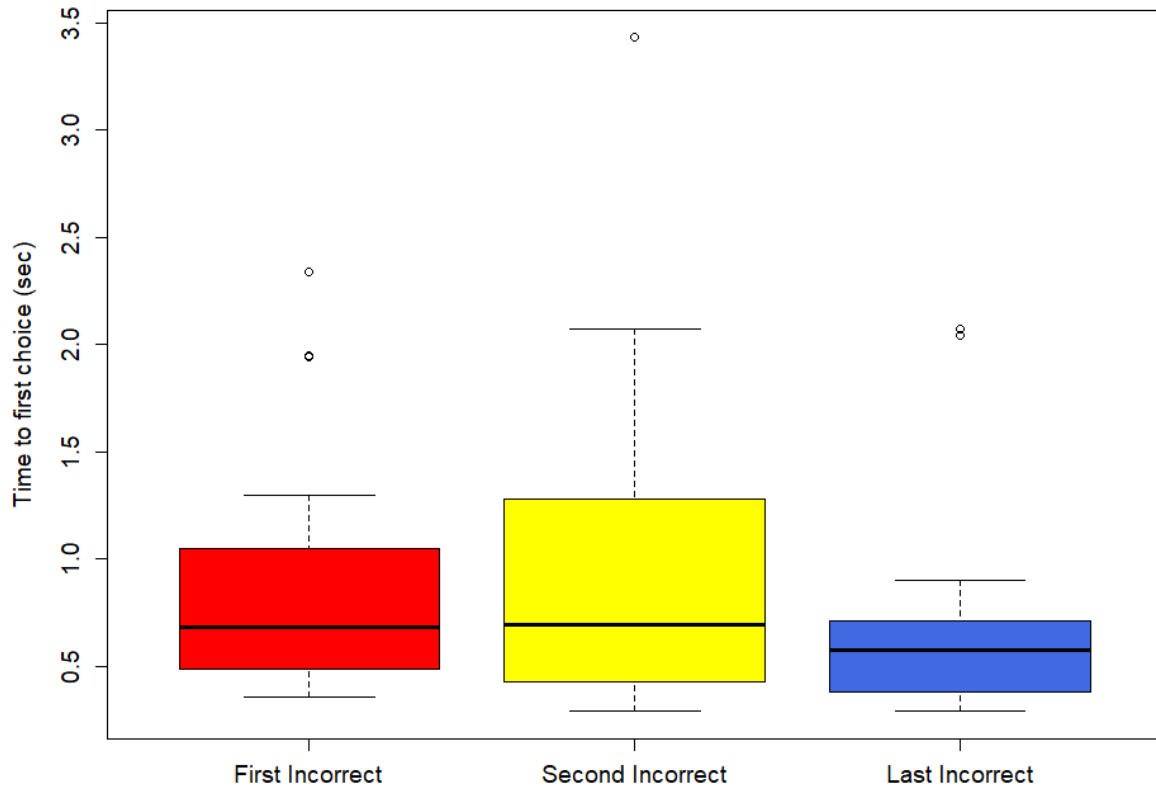### 4.3a Three-Choice Delayed Conditional Discrimination Task

An across-trial comparison assessed the reaction time of bees (n=22) within two trials, wherein either the punished or unrewarded stimulus were selected first (incorrect) followed by a subsequent trial in which the rewarded stimulus was selected (correct) (fig. 17).

Figure 17: The reaction time of stimulus choices made within an incorrect trial and a subsequent correct trial. First Incorrect) Time to first choice in an incorrect trial with a mean of 0.65 sec. Last Incorrect) Time to last choice made in the same incorrect trial with a mean of 0.56 sec. Correct) Time to first choice made in the subsequent correct trial with a mean of 0.8 sec.

A Welch Two Sample t-test between the reaction time for the first incorrect trial compared to the reaction time in the subsequent correct trial (n = 22) found that there was no significant difference (P = 0.4846, T = -0.7080) between these times (fig. 17). Bees therefore did not appear to show post-error slowing in the subsequent correct trial. Furthermore, the reaction time of the last incorrect against the reaction time of the subsequent correct trial was found to not be significant (P = 0.1872, T = -1.3532), meaning there was no difference in timings between these trials and bees' therefore did not show a post-error slowing effect. Due to the skew of the outliers' a non-parametric Wilcoxon signed rank test was conducted The comparison between reaction time in the first incorrect trial against the subsequent correct trial nonetheless returned an insignificant value (P = 0.3299, W = 200). Likewise, the reaction time in the last choice of the incorrect trial against the following correct trial returned an insignificant value (P = 0.2349, W = 145).

An inter-trial comparison assessed the choices made within the trial where a bee (n = 22) selected the punished stimulus, making an incorrect first choice: comparing the reaction time of the first choice made, the second choice made and the last choice for which the bee selected the punished stimulus (fig. 18).

Figure 18: The inter-trial reaction time of stimulus choices made within the same incorrect trial. First Incorrect) Time to first choice in an incorrect trial with a mean of 0.86 sec. Second Incorrect) Time to second choice in the incorrect trial with a mean of 0.96 sec. Last Incorrect) Time to last choice made in the same incorrect trial with a mean of 0.68 sec.

A Welch Two Sample t-test between the first choice and last choice made returned an insignificant value (P = 0.2853, T = 1.0825) and the first choice against the second incorrect choice also returned an insignificant value (P = 0.5886, T = -0.5436).

4.3b Binary-Choice Delayed Conditional Discrimination Task

As in section 4.3a an across-trial comparison assessed the reaction time of bees (n=22) within two trials; an incorrect trial wherein bees select the punished stimulus first, followed by the subsequent correct trial where the rewarded stimulus was selected (fig. 19).

Figure 19: The reaction time of stimulus choices made within an incorrect trial and a subsequent correct trial. First Incorrect) Time to first choice in an incorrect trial with a mean of 0.93 sec. Last Incorrect) Time to last choice made in the same incorrect trial with a mean of 0.7 sec. Correct) Time to first choice made in the subsequent correct trial with a mean of 0.76 sec.

Reaction times for the first choice in an incorrect trial were compared with the reaction time for the correct choice in the subsequent trial with Welch Two Sample t-test (fig. 19). There was found to be no significant difference (P = 0.1513, T = 1.4803) in between these reaction times. Bees' reaction speed did not slow following an error but remained rather consistent. The reaction time for the last choice in the incorrect trial against the time in the subsequent correct trial likewise was found to be insignificant (P = 0.5254, T = 0.6246). Bees in the DCD task therefore did not differ in reaction time between trials in which they were incorrect compared to trials in which they were correct.

As in section 4.3a, the inter-trial reaction times of the incorrect trial were compared together. The first choice bees (n=22) made within the incorrect trial was compared against the second choice they made immediately after and to the last choice made during the same trial (fig. 20).
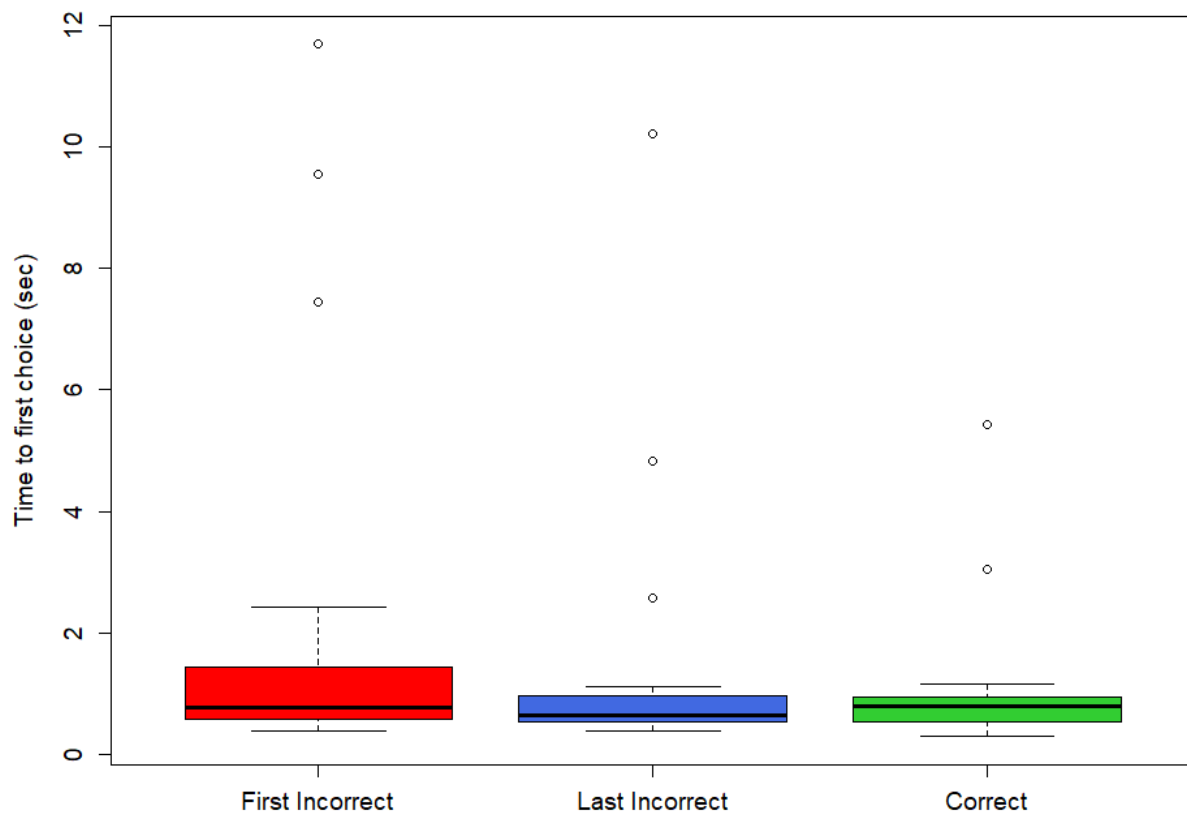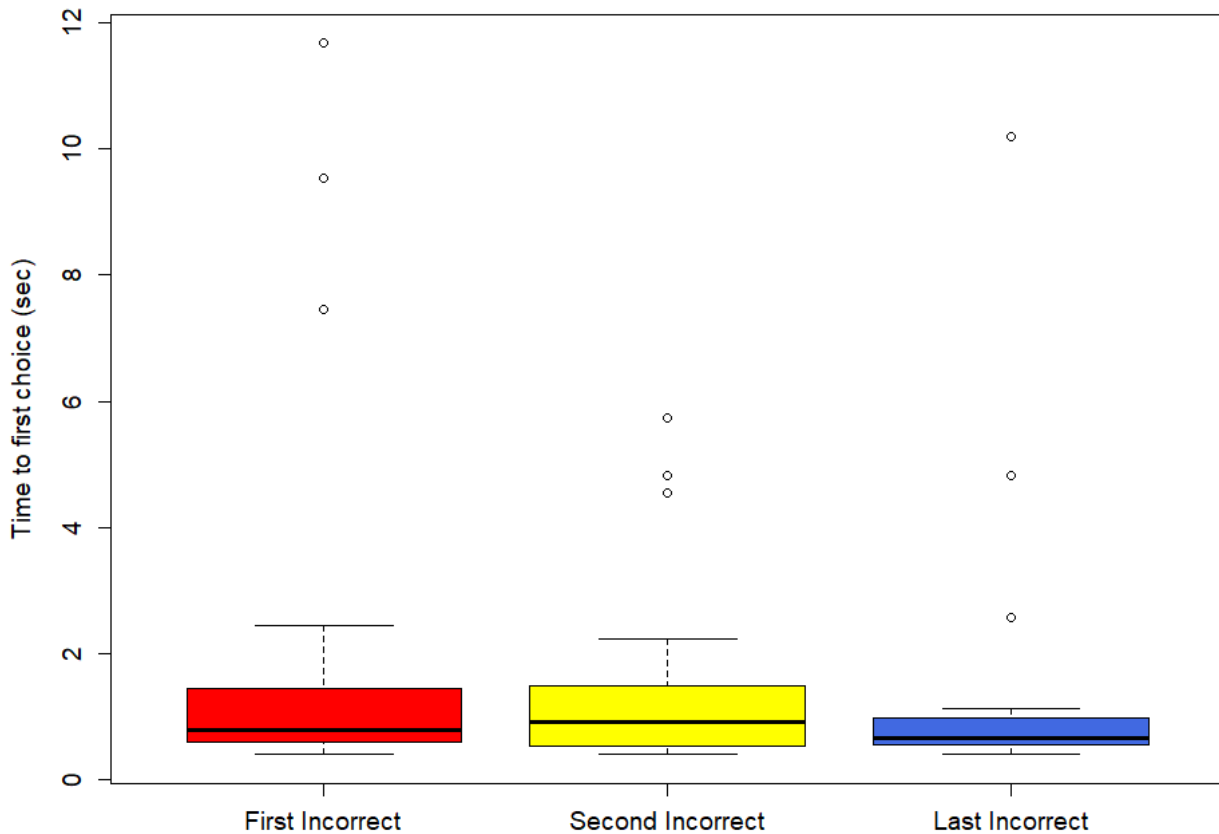
Figure 20: The inter-trial reaction time of stimulus choices made within the same incorrect trial. First Incorrect) Time to first choice in an incorrect trial with a mean of 0.93 sec. Second Incorrect) Time to second choice in the incorrect trial with a mean of 0.9 sec. Last Incorrect) Time to last choice made in the same incorrect trial with a mean of 0.7 sec.

The reaction time for a first incorrect choice was compared against the time of the second choice made in the same trial with a Welch Two Sample t-test (fig. 20). Between the two choices no significant difference (P = 0.3956, T = 0.8623) in reaction time was found. Reaction time between the first incorrect choice and the last incorrect choice was likewise found to not show any significant difference (P = 0.3883, T = 0.8741). The time between choices within the same trial therefore remained consistent and did not appear to display a slowing phenomenon.

4.4 Discussion.

The results indicate that the difference between trials following correct choices compared to trials following those in which bees made an error were not significant, however there does appear to be a trend in which the bees' reaction time does slow following an error (fig. 17 & 19). In both the three and binary-choice trials bees took approximately less than 0.2 of second longer in the post-error trial as they did in the previous trials. Furthermore inter-trial reaction times found that in

comparison to the first choice made, bees' reaction time increased in the following choice, within the same trial (fig. 18 & 20). While these variances in reaction times may at first seem too minute to consider, it is worth noting the time bees require to make decisions. A foraging bee is able to detect and inspect several potential food resources per second using a number of sensory cues (Benard et al., 2006; Chittka et al., 1999). To maximise the optimal fitness of the colony, bees need to forage from food sources that offer high nectar and pollen rewards in as little time as possible all while avoiding potential errors (Pyke, 1984). Since bees are capable of rapid decision-making within seconds the differences in reaction times found within this study should not be discounted. Furthermore research on post-error slowing in humans found the difference in reaction time between trials following errors and those following correct choices, were between 0.1 – 0.2 sec, similar to the reaction times found in bees in this project (Rabbitt, 1966). Reaction times furthermore decreased when the final choice was made within the same trial in comparison to the first choice made. The addition of the green stimulus in the three-choice trial appears to have resulted in bees overall taking less time to select a choice. Outliers in the three-choice trials remain under 6 sec while those in the binary task returned values of as high as 12 sec. Overall, these results show little significant difference in reaction times between the two tasks. The reaction time between an error trial and a post-error trial returned even less of difference.

4.4a Caution Response

One such functional explanation for post-error slowing states that it is due to an increased response caution, in which the subject becomes more cautious and therefore takes more time in response in order to avoid errors therefore resulting in a post-error accuracy increase (Botvinick et al., 2001; Laming, 1968). An error therefore, prompts the need to accumulate more information to make a more informed decision, increasing reaction time. This explanation suggests that response thresholds can be adaptively changed to an optimal state based on previous choices (Smith and Brewer, 1995). The threshold slowing post-error that was found in this project although not significant, could be a result of this caution response in which bees slowed to avoid making further potential errors in later trials (fig.17 and 19).

Further examination on the reaction times of bees rejecting stimuli could be assessed in future studies using the recordings already collected. This could provide further insight into how bees respond and shift their behaviour following an error, providing evidence that could suggest bees are displaying this caution response behaviour by assessing if they are rejecting stimuli at a higher rate following a post-error trial.

4.4b Speed-Accuracy Trade-off

Although bees did not show a significant slowing effect following an error, they did display a threshold of increased speed in post-error trials (fig. 17 and 19). This may not be a post-error slowing effect but rather the result of another phenomenon; like that of a speed-accuracy trade-off. Additionally, if an aversive cost is introduced, such as a quinine solution like that used in this project, bees show a significant improvement in accuracy at the cost of speed (Chittka et al., 2003). Due to time restrictions, reaction time over all trials was unable to be analysed in order to determine if speed was reducing as accuracy increased which would further imply a trade-off between speed and accuracy.

The reaction times in both the post-error trials and correct trials returned a large variation in times, with some individual bee's reaction times falling well outside the average as outliers (fig. 17, 18, 19 and 20). Colour discrimination tasks with bumblebees (*Bombus terrestris)* have shown that there is variation in the speed-accuracy trade-off between individual foraging bees from the same colony (Burns and Dyer, 2008). Due to Individual bees having pronounced differences in the problem solving strategies they implement, with some bees investing more time into solving the task while other's make rapid decisions (Skorupski et al., 2006; Wang et al., 2018). Those that invested time had a higher accuracy compared to those that rapidly made choices and were, as a result more error-prone (Burns and Dyer, 2008; Chittka et al., 2003). Previous literature found that bees that were faster but more inaccurate collected more nectar compared to bees that had slower yet more accurate foraging behaviour (Burns, 2005). Despite the difference in time taken for a task, there are still individual bees that forage using both behaviours rather than just the more efficient fast-inaccurate behaviour. This suggests that this variation in behaviour acts to reduce variability in nectar acquisition. The outliers within the tasks could, possibility, be the result of this variation in foraging techniques between individual bees.

Due to unforeseen issues with the threshold of landing for the automated code and time restraints, the accuracy following errors and those following correct choices, were unable to be fully analysed. Future study will work on correcting these programming bugs in the code in order to fully analyse the reaction time of all the trials. It is therefore uncertain if the bees meet the all criterion of the Rabbitt effect which states that accuracy should increase following an error (Laming, 1979b; Rabbitt, 1966). Future research could determine if there is any significant difference in the accuracy of the bees following errors and if so, whether this increases or decreases with reaction time to further reject the Rabbitt effect in bees and therefore suggest a limitation in their cognitive ability.

Further study into the metacognition processes that result in a lack of a post-error slowing phenomenon in bees would be an interesting next step, including further assessment into why bees do not show significant difference in reaction time compared to humans, providing better insight into these mechanisms (Perry and Barron, 2013). Comparing this post-error reaction time against other species of invertebrates and perhaps vertebrates could additionally conclude this. As it is unclear if bees are sensitive to errors, not displaying substantial evidence of a post-error slowing, it is possible that they are not able to learn and respond to errors in the same way as mammals, revealing a possible limitation in their cognition.

5. Conclusion

The honey bee continues to defy expectations as to what is cognitively capable of an invertebrate. This project was no exception, with bees confirmed as capable of solving a task presenting conflicting information in the form of a delayed conditional discrimination (DCD). Bees' performance in the visual three and binary- choice DCD tasks was well above chance suggesting learning of the task within 10 trials. The ability to solve this DCD task suggests that bees use working memory to do so, which is often regarded as a factor that underlines complex cognitive abilities in animals (Cohen et al., 1988; Watanabe, 1981). The presence of an unrewarded stimulus given as a green intermediate choice between the blue and yellow stimuli, was shown to have no effect on the three-choice and sequence DCD tasks compared to the binary-choice trial that offered no such choice. Bees learnt rapidly that this stimulus only offered an unrewarded solution and were never punished nor rewarded and therefore tended to avoid landing on the green stimulus after as little as three trials. Bees were found to not only be able to solve tasks with a visual contextual cue but also by a conceptual cue in the form of a sequence. To solve this task, bees would have had to implement one of two different strategies using either win-stay/ lose-shift or numerical counting abilities. With win-stay/ lose-shift strategy bees would continue to choose the same colour stimulus as long as it is rewarded (Rayburn-Reeves et al., 2013). Once the stimulus they choose is punished they will shift to the other stimulus and continue to choose this until it is punished and so on (Imhof et al., 2007). Alternatively bees may instead be able to count the trials and learn to anticipate when the contingencies will be switched, such as found in previous studies suggesting that bees have the numerical capability to count to at least four (Chittka and Geiger, 1995; Dacke and Srinivasan, 2008). Towards the end of the trials in the two DCD tasks bees displayed a general decline in performance contributed to proactive interference in which information in the previous trial prevents the recall of new information in the following trials. Proactive interference is often a consequence of discrimination tasks that require the use of working memory and have been found in past DCD tasks using rats and pigeons (Cohen et al., 1988; Sherburne and Zentall, 1993; Smith and Brewer, 1995). This decline in accuracy however could be attributed to performance fatigue or a loss of foraging motivation.

Bees were found to show no significant evidence of the post-error adjustment known as the post-error slowing effect as demonstrated in humans, with bees shown to only slow in only a few seconds. This lack of post-error slowing in reaction time in post-error trials is one phenomenon of the Rabbitt effect found in full effect currently only in humans (Rabbitt, 1966). A functional

explanation for this slight insignificant slowing is an increased caution response. In order to avoid making errors in impending trials bees reduce their speed, increasing their reaction time in order to collect more information and make an informed decision. So while a post-error slowing was not shown to be significant for bees within these tasks, it is possible that the slight slowing was a speed-accuracy trade-off effect. Accuracy requires gathering information in order to avoid errors which is often time-consuming. As such a trade-off between the decision speed and performance is often found in similar discrimination tasks, as it is not possible to maximise both speed and performance. This project further supports this trade-off phenomenon in bees that has been found in previous studies before. This evidence paired with a lack of evidence of a post-error slowing effect provides further insight into decision-making in foraging bees, in that they appear to show an increase in performance at the expense of a more rapid reaction time.

This project shows evidence that bees are capable of solving a SCD task that presented a sequence in the form of a contextual cue. It appears that bees were not implementing a pure win-stay/ lose-shift strategy or counting the sequence but a combination of multiple strategies, suggesting that bees do have the numerical competence of sequential counting. This demonstrates that bees have the non-elemental learning abilities to solve conflicting information when given complex contextual cues that are temporal and cues that are sequential. This type of learning is therefore not just limited to vertebrates as once thought, showing that bee brains are just as capable of handling information that is conflicting (Herremans and Hijzen, 1997b; Honig and Dodd, 1983). It is, however, no clearer if they are capable of learning from the errors they make within these conflicting information tasks. Vertebrates show a post-error slowing effect that bees do not, suggesting a possible limitation of cognitive ability in bees (Jentzsch and Dudschig, 2009; Narayanan and Laubach, 2008). This may suggest bees have evolved a different sort of intelligence that makes them less sensitive to errors and limits their ability to learn from them, unlike in mammals. Future studies could delve deeper into possible taxonomic differences in cognitive ability and the neural processes that explain why this post-error slowing effect is shown in humans but not in full effect in bees. Understanding the cognitive capabilities and the limitations of an invertebrate species provides further comprehension into the evolution of complex intelligence across taxa and how it differs in an animal with a brain that is 0.0002 per cent smaller in volume than our own.

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