A visual discrimination task to identify judgement bias in hens (*Gallus gallus domesticus*).

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ABSTRACT

This study aimed to investigate whether the use of a judgement bias task with differential reinforcement was suitable to assess emotional states (positive or negative affect) in domestic hens. Six hens were trained to discriminate between a vertical and horizontal rectangle using an operant procedure with differential reinforcement to each stimulus in order to induce a positive or negative affect. Three ambiguous rectangles were introduced at intermediate angles and responses to these and the two learned rectangles were used to measure for judgement bias. Initial judgement bias probes measured response bias using three different foods as reinforcement; poultry pellets, wheat and puffed wheat in a one food magazine operant chamber. The chamber was then modified from a single food magazine to a double food magazine allowing six different food combinations with the three foods and differing reinforcement periods. Judgement bias probes measured response bias to the learned and ambiguous rectangles using combinations of each food type. Overall response latencies revealed a consistent pattern across all six hens with the fastest response times to the vertical (positive) stimulus and slowest response times to the horizontal (negative) stimulus. Results indicate that judgement bias was best measured and identified in the one food magazine chamber with four of the six hens consistently demonstrating positive affect and two hens demonstrating slight negative affect. It was found that results varied in the double magazine chamber revealing both response bias and food preference.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Judgement Bias

Judgement bias was initially found within an area of study relating to human cognition, however it has more recently been introduced into animal welfare. This work has its roots in many aspects of modern psychology and ethology, and a background that draws on animal welfare, psychophysics, cognitive bias and discrimination learning based on operant procedures. The following seeks to provide a brief insight into the history of how judgement bias procedures have been established within the science of animal welfare as an enquiry into animal emotions, before progressing onto current research in this area. Information pertinent to the animals that are the focus of this current study – domestic hens (*Gallus gallus domesticus)* is also provided.

Animal Welfare

Animal welfare is an age-old concept, with the first ever recorded debate over the treatment of animals understood to have taken place in the sixth century BC in Greece (Fraser, 2008). Historically, the concerns regarding animal welfare were moral, in recognition of the dependency upon, and kinship with animals. Animals featured in all aspects of human living; as a means of transport, pulling ploughs through fields for farmers, competition racing, providing sustenance through meat, milk and cheese, anatomical research (Fraser, Friendship & Martineau, 1994) and also guarding, warfare and companionship (Bodson, 2008). Although social debate for the ethical treatment of animals had a revival in the late 1800's (Turner, 1964), this was put on hold due to the period that included two World Wars and the Great Depression, where human survival became of foremost concern.

During the 1960's, following a period of affluence, attention returned to animal suffering. This came about for several reasons but one particularly important contribution was Ruth Harrison's book Animal Machines published in 1964. This led to a review of animal welfare in the farming and production sector undertaken in the United Kingdom in 1965 by the Brambell Committee. The review resulted in recommendations for animal husbandry methodology, the basic freedoms for animals - to stand, lie, turn around, groom and stretch (McCullough, 2012), but also an appeal for research. With the support of government resulting in the provision of funding (Fraser, 2008), the science of animal welfare was established, based on the premise that animals should be able to live freely, naturally and safe from the predations of mankind, or at least as much as possible if used for farming, breeding, experimental research or kept in captivity.

Judgement bias research has been adopted within animal welfare science, as a procedure and methodology to enable the study of animal emotion. Measurement of negative and positive affect in animals enables the improvement of animal husbandry procedures, environmental enrichment and expanding knowledge of what stimuli may be rewarding or aversive, thereby reducing or eliminating negative aspects and increasing opportunities for positive affect.

Choice and Preference

Providing options of choice is a useful determinant of preference and is a behavioural method employed to assess stimuli that may be effective reinforcers for both humans and animals. Preference Assessments were initially created to provide a non-verbal method of asking people with developmental disabilities which of the stimuli provided (edible/tangible/pictures/music) they preferred that may be used as a reinforcer in learning opportunities, and has been a useful adaptation within animal research. Preference indicates what is preferred or offers the most value at that time, and as such, preference can vary with day, time, environment, demands, deprivation or satiation.

Whilst there are various types of preference assessments for humans the two methods relevant here are: Single Stimulus (SS) presentation (Successive Choice method), a presentation of a stimulus and the consequent reaction noted – approach, touch, eat, or ignore (Pace, Ivancic, Edwards, Iwata & Page, 1985); Paired Stimuli (PS) presentation (Forced Choice method) is the simultaneous presentation of two stimuli, each matched with all other possible combinations in a set (Fisher, Piazza, Bowman, Hagopian & Owens, 1992), with stimuli rank-ordered as high, medium or low preference, dependent upon the number of choices made for each stimulus. The Successive Choice and Forced Choice procedures are also well used methods within animal research although in a slightly different format. The Successive Choice is similar to the Go/No-Go task that utilises an approach and avoidance procedure, likewise the Forced Choice procedure is similar to that of the Go/Go (Active Choice) with high and low reinforcement opportunities (further details on page 11).

Much animal welfare research has been undertaken in the animal domain on preference of food options and housing (environment enrichment). Animal food preferences can be assessed by offering choice opportunities or implementing concurrent schedules of reinforcement with a changing response requirement and the subsequent measures of response bias. Response requirements have been shown to determine food preference with the preferred food becoming evident as schedules of reinforcement increase.

Food preference research with hens, indicates that overall there is an appetitive preference for hens to wheat and pellets over puffed wheat when working with an increasing schedule of reinforcement (Flevill, 2002; Bruce, 2007;) and with changes in maintenance diet (Schroeder, 2013).

Behavioural Observation

The genesis of a multidisciplinary branch of animal welfare science has led to a variety of methods and approaches being implemented to undertake measurements of animal welfare, and includes aspects of physiology, pathology, epidemiology and observation, and where possible, a combination of these. Observation of animals can be classified as clinical, physiological and behavioural and signs of both illness and good health can be identified with these methods. Behavioural observations are useful in determining internal states (fear, hunger, pain) and are also the least invasive method. Behaviours indicating fear may include immobility, hiding and also excessive vocalisations in piglets (Weary & Fraser, 1995b), chickens (Koene & Wiepkema, 1991), and macaques (McCowan & Rommeck, 2007), changes of gait in cattle may indicate pain (Weary, Niel, Flower & Fraser, 2006; Flower, Sedlbauer, Carter, von Keyserlingk, Sanderson & Weary, 2008; Bernardi, Fregonesi, Winckler, Veira, von Keyserlingk & Weary, 2009), and ear and tail postures of sheep are useful in determining both negative, intermediate and positive emotional states (Reefmann, Kaszàsa, Wechsler & Gygax ,2009).

Psychophysics

Psychophysics is another non-invasive method, originally developed by Gustav Fechner in 1860 to study human perception, and by applying two of the methods used for humans (method of limits and method of constant stimuli), animal behaviour can be measured and quantified, specifically the sensory functions that include vision, hearing, touch, taste, smell and timing (Kingdom, 2012; Lazareva, Shimizu & Wasserman, 2012). Measurements of animal sensory abilities inform the understanding of animal capabilities and also assist with an understanding of welfare by indicating which stimuli animals show a preference for or find aversive, and is currently the most preferred methodology in judgement bias research due to the ability to measure positive and negative responses (judgements) objectively.

Discrimination

The ability to discriminate requires involvement of a group of cognitive processes, including but not limited to, perception, attention and learning, all of which can be measured by behavioural responses (psychophysical scaling). Discrimination training procedures with animals usually include operant tasks with differential reinforcement for making different responses to two or more stimuli, presented either simultaneously or successively (the judgement bias task).

Visual discrimination abilities – Vertical and Horizontal rectangles

It was in the late 1950's that research was initially undertaken on animals' ability to discriminate shapes that included horizontally, vertically and obliquely presented rectangles (lines and parallelograms) with subjects that included cats, octopi, goldfish, monkeys and rats. Sutherland (1963) demonstrated that cats were able to successfully discriminate between a horizontal and vertical rectangle as well as two oblique rectangles at opposite angles, taking just 11 days on average to learn to discriminate between vertical/horizontal, and 10.5 days on average to discriminate between the two oblique rectangles. Attempts to demonstrate monocular discriminations by octopi revealed success in horizontal/vertical rectangle discriminations but not with the oblique rectangle discriminations (Sutherland, 1957; Sutherland, 1969; Messenger & Sanders, 1971). Goldfish were able to discriminate between vertical/horizontal and two mirror image oblique rectangles, although acquisition rate was twice as fast for the vertical and horizontal, than the oblique discrimination (Mackintosh & Sutherland, 1963). Sutherland's (1969) comparative study found that whilst goldfish were able to discriminate oblique shapes, they were unable to discriminate between oblique rectangles and parallelograms. Monkeys were able to discriminate between a vertical/horizontal line (Ganz & Wilson, 1967) and rats discriminated oblique and vertical/horizontal rectangles and parallelograms (Sutherland, 1969).

The varying visual discrimination abilities of species is an important aspect to consider with regard to creating visual tasks that may be implemented as judgement bias tasks. Judgement bias tasks are intended to be simple and time efficient with regard to the discrimination learning and the resulting judgement bias, and therefore test designs must be appropriate for the species under study.

Visual perception in hens (gallus gallus domesticus)

Hens have two visual systems, one monocular and lateral, and the other frontal (Shimizu & Karten, 1993). Hodos (1993) describes the monocular lateral field as being utilised in detecting distant and moving objects, and the frontal for recognition of static and near objects and conspecifics (Dawkins, 1995). Dawkins and Woodington (1997) found that distance is an important consideration for discrimination tasks for hens as visual task and also colour (red vs. blue) discriminations improved for hens when viewing stimuli from 5–25cm from those at 120cm, and accuracy was enhanced when the hens were allowed to approach the stimulus objects.

Cognition

Cognition in both humans and animals is the mental process of perception and learning that mediates the relationship between environment and behaviour (Timberlake, 2002; Shettleworth, 1998). Cognitive human studies require the recording and measurement of responses to stimuli but can also include verbal reports. Comparative cognition studies are concerned with the cognitive processes of animals but rely solely on behaviour to generate probable representational processes from those based in human studies (Wasserman, 1984). Theorisation is a co-requisite of these studies as the behavioural responses of an animal that are the equivalent or similar to that of a human response, are regarded as representing or suggesting a similar subjective state. It is unknown whether the systems of consciously processing information are the same for humans and non-human animals, but well-defined behavioural responses are useful indicators of same/different responses to environmental conditions.

Cognitive Bias

In the early 1970's, Amos Tversky and Daniel Kahneman introduced the term of cognitive bias as a result of their heuristics and biases program that researched decision making by humans with limited resources and the resulting defective responses to judgements and decisions (Wilke & Mata, 2012). The systematic deviations (biases) that were found between the responses made and expected solutions, were attributed to cognitive limits, motivational factors and/or environmental adaptation. An extensive catalogue of norm violations was created (eg. fundamental attribution error, hindsight bias, in-group bias) under classifications of decision-making, belief, behaviour, social and memory biases. Cognitive bias occurs when humans experience a change to their emotional state, changing the way they think about or experience their environment. Cheerful and content people tend to make positive judgements, over-estimating pleasing outcomes of future events and interpreting ambiguous stimuli in a favourable light, also known as the Optimism Bias. Depressed and/or unhappy people often make negative judgements, over-estimating the likelihood of negative outcomes and interpret ambiguous stimuli in a negative frame (Wright & Bower, 1992), also known as the Pessimism Bias.

Affective states in humans

Affect, also known as emotion and feelings (including optimism and pessimism), is considered to be another influencer of cognitive processes and resulting human behaviour. Forgas (2012) suggests that it's necessary to distinguish between two kinds of affect – emotions and moods. Moods are understood to be low-intensity, enduring and with no obvious cause whereas emotions are considered intense, less durable, and are cognitively conscious. Behaviourally, emotions are demonstrated less consistently and are situational and context specific. A further divergence is seen with regard to research into moods and emotions whereby studies of emotion investigate cognitive antecedents that trigger the emotional response, and studies of mood look at the cognitive consequences of affective states upon thinking, attention, memory and judgements. These states are known to interact with and influence each other, as emotions can leave a prolonged mood state, and conversely moods can impact on the emotional responses generated (Forgas, 2001).

Emotions are valenced, they are either experienced as positive (rewarding) or negative (punishing). While emotions can range from highly arousing (fearful - excited) to low in arousal (sad - relaxed), they also range from low valence (unpleasant) fearful – sad, to high valence (pleasant) excited - relaxed. It's the combination of the valence and arousal dimensions that determines core affect. Human studies have revealed that stimuli are best remembered if they're emotionally arousing, and findings indicate it's the emotional arousal and not the valence that is the main factor in consolidating information into long term memory (Forgas, 2001).

Affect congruence is the relationship demonstrated in humans when feeling good results in making positive judgements, and the opposite is witnessed when feeling bad, with a tendency to make negative judgements. In earlier years, affect was considered to be disruptive to cognitive processes, however more recent research suggests it may also be advantageous in demonstrating rational behaviour (Adolphs & Damasio, 2001; Ito & Cacioppo, 2001). Affective state effects are considered to be context specific, as oftentimes affective state may facilitate quality decision making (Isen & Means, 1983), yet at other times may result in judgment and decision errors (Forgas, 1998a). In order to demonstrate the effect of affect upon cognition, an early study undertaken by Razran in 1940, induced states upon participants by either exposing them to an offensive smell or providing a free lunch. Results revealed a significant influence of affect congruence upon subsequent social judgments.

Measuring affective states in animals

Emotional states are composed of physiological, behavioural, cognitive and subjective facets. While a conscious experience of emotion can be conveyed in the form of verbal behaviour by humans, this method of reporting is not directly available with animals, however the detection of aspects of emotion is possible through the measurement of behavioural and physiological changes.

There are limitations with these methods within the interpretation of the demonstrated physiological and behavioural changes as while emotional arousal is evident, it's not definitive as to whether that arousal is pleasant or unpleasant (Paul, Harding & Mendl, 2005).

Emotions are considered to be an important aspect to influencing an organism's survival and reproduction. Rolls (2014) theorises that animals behavioural decisions are guided by emotion in significant situations as a means of enhancing survival when making choices of attaining resources or avoiding harm (Mendl, Burman, Parker & Paul, 2009). The differing affective responses by species of animals to a range of rewards (food, water) and punishers (predators) are believed to have evolved over time, supporting and guiding animals in their survival. Empirical studies indicate that the high arousal/high valence affective states are associated with appetitive motivation and seeking and obtaining rewards, and core affect has a functional capacity in that it's related to the success or failure of procuring resources (Forgas, 2001).

There are two trains of thought with regard to cognition and emotion in humans and animals. Whilst there is the view that cognitive and emotional processes are linked or connected and have some aspect of co-dependence (Forgas, 2000; Lazarus, 1999; Mendl et al, 2009), the other view is that cognitive and emotional processes are separate and independent of the other (Panksepp, 1998; Zajonc, 2000).

Research on animal emotion has had a major focus on the discrete emotion approach, and how animals respond to situations where a specific emotion has been induced, such as fear or anxiety, using behavioural and physiological methods. This approach is limiting with regard to the specificity of the emotion being induced but its focus has also been on negative states, rather than positive ones. The judgement bias task seeks to cover both ends of the spectrum with a focus on both positive and negative states. Other limitations of the discrete emotion approach include the use of physiological measurements, as the sampling/collection methods can increase the emotional response or conversely provoke conflicting emotions (Mendl et al, 2009; Mellor, 2015); different affective states reveal the same physiological responses - rising cortisol levels can indicate fear, sexual activity or increased activity within a neutral situation, likewise an increasing heart rate not only indicates activity but also expectancy of an aversive or reinforcing stimulus (Paul et al, 2005). Broom and Johnson (1993) suggest that observations of behaviour should be included with physiological measures to help ascertain an animal's affective state.

The significance of behaviour is also determined by evaluation of the environment in which it occurs. Behaviours that occur regularly in a number of settings and appear to have the same valence and don't occur in the opposite valenced setting are probably a reliable indication of affect. Freezing or attacking provides an indication of function and affect, as well as exploratory, play and feeding behaviour. Approach and avoidance behaviours to a specific stimulus are useful in determining whether animals consider it positively or negatively, conversely, approach behaviour in animals can also be seen in threatening situations with predators and contesting conspecifics (Paul et al, 2005). Further challenges to interpretation include whether activity in an open space is escape or exploratory behaviour.

Both unconditioned and learned response tasks have been developed to determine animals' affective or emotional states. The learned response tasks are those that are used to determine whether an animal considers a stimulus to be a positive or negative reinforcer (preference/avoidance), but have been further developed to ascertain how hard animals will work to get at or avoid reinforcing stimuli.

The previous brief overview provides the background to the numerous aspects that have been brought together, enabling development of a procedure that is promising to be particularly valuable in the area of animal welfare research with regard to the ability to measure or detect features of animal emotion.

Cognitive/Judgement Bias

Judgement bias is the influence of affect on the interpretation and response to ambiguous stimuli. Positive emotion is reflected by more positive judgements (glass half full) about ambiguous stimuli and negative emotion is reflected in more negative judgments about ambiguous stimuli (glass half empty).

Paul et al (2005) propose that the ways that animals appraise, evaluate and weight the importance of stimuli will likely have an affect upon their emotional response. Any bias occurring in these cognitive processes will be indicated by the direction of any effects shown.   Human research studies into effects of emotion upon judgement have been undertaken in the areas of risk-taking, expectations about the future and interpretations of ambiguous stimuli. Interpretations of ambiguous stimuli was implemented into animal studies by Harding, Paul & Mendl (2004) as a new approach to measuring affect, using a method based on an operant discrimination task.

Harding et al (2004) utilised the knowledge of the interaction between affect and cognitive bias from human studies to undertake the first cognitive (judgement) bias experiment with rats to endeavour to assess affective states in animals. Rats were trained to press a lever in response to a tone indicating a positive event (food) and not to press a lever that indicated a negative event (white noise). One group of rats was allocated to unpredictable housing which included zero to two negative interferences each day within their environment at random times but within two hours before or after trials – tilting the cage, introducing an unknown conspecific, interrupting the light/dark cycle or leaving damp bedding in the cage. The other group was allocated to predictable housing with no interference. The latencies and responses made to ambiguous stimuli by the rats in unpredictable housing indicated reduced anticipation of positive events – these rats were slower to respond to the ambiguous tones than the rats in predictable housing, and also made less responses to the ambiguous tones close to the positive event as well as to the actual food tone itself.

Judgment bias animal research, discrimination tasks & ambiguous cues

Animal research on cognitive bias was initiated by Harding et al’s (2004) judgement bias in rats experiment as mentioned in the previous paragraph. The term judgement bias as opposed to cognitive bias was also adopted due to the judgement of ambiguous stimuli being the method in which these biases are being studied. The judgement bias task has become a popular choice with regard to studying animal welfare due to the simplicity of design and response measures being suitable to numerous species and contexts (Bethell, 2015). The terms of optimism and pessimism biases have also been included due to the nature of the choices made that may indicate a positive or negative outlook/expectation of future events.

There are three types of judgement bias tasks – The Go/No-Go, Go-Go (Active Choice with high and low reward) and Active Choice with positive and negative reinforcement. The Go/No-Go task has utilised spatial, location, visual, and tone cues with animals that have included goats (Briefer & McElligott, 2013), sheep (Sanger, Doyle, Hinch & Lee, 2011), pigs (Douglas, Bateson, Walsh, Bédué, & Edwards, 2012), horses (Briefer Freymond, Briefer, Zollinger, Gindrat-von Allmen, Wyss & Bachmann, 2014), dogs (Starling, Branson, Cody, Satling & McGreevy, 2014; Müller, Riemer, Rosam, Schöβwender, Range & Huber, 2012), cats (Tami, Torre, Compagnucci & Manteca, 2011), rats (Burman, Parker, Paul & Mendl, 2008; Harding et al, 2004), starlings (Bateson & Matheson, 2007) chicks (Salmeto, Hymel, Carpenter, Brilot, Bateson & Sufka, 2011) calves (Daros, Costa, von Keyserlingk, Hötzel & Weary, 2014; Neave, Daros, Costa, von Keyserlingk, & Weary, 2013) and mice, hamsters and marmosets. Animals were trained to approach one location/container for reinforcement (food, conspecific) and to avoid approaching the other known location/container with an aversive or negative reinforcer (unpalatable or no food, airblower, predator). The ambiguous/intermediate locations are spaced (usually equi-distantly) between both learned discriminations and the tendency and latency to approach these locations/containers are measured. Mendl et al (2009) propose that the limitations that have been detected with this type of task in the occurrence of no-go responses, is possibly due to a number of factors including reduced motivation to eat or drink, distraction, confusion and arousal.

The Go-Go task, also known as Active Choice with positive reinforcement (high and low) emerged from the necessity to address the interpretation difficulties found with the previous task (Go/No-Go). Animals are trained to discriminate between cues that provide a high and low reward and are then tested on their responses to the intermediate cues/stimuli. Operant tasks have included visual, audio, spatial and tactile discriminations with capuchin monkeys (Pomerantz, Terkel, Suomi & Paukner, 2012) grizzly bears (Keen, Nelson, Robbins, Evans, Shepherdson & Newberry, 2014), hens (Hernandez, Hinch, Ferguson & Lee, 2015), pigs (Murphy, Nordquist & van der Staay, 2013), rats (Parker, Paul, Burman, Browne & Mendl, 2014) starlings (Matheson, Asher & Bateson, 2008) and honeybees (Bateson, Desire, Gartside & Wright, 2011). The limitations found with this type of task is the difficulty in training the difference between both reinforcement schedules, as both stimuli effectively provide positive reinforcement, and with this it may be that detection of a negative affect is limited (Mendl et al, 2009).

The Active Choice task with negative reinforcement, includes a discrimination task that provides positive reinforcement (food) for a correct response to the CS+ stimulus but also a negative reinforcement, whereby the correct response to the CS- inhibits the aversive reinforcement (electric shock). This option has been used in a number of rat experiments (Papciak, Popik, Fuchs & Rygula, 2013; Rygula, Pluta & Popik, 2012) but is not generally used within animal welfare research.

Judgement bias studies require a discrimination task which may include visual stimuli, tone frequency or spatial location, however what is essential is that there are intermediate stimuli to the learned discriminations, as it's the response to these initially novel and ambiguous stimuli that provide the measures of effect; and whether the response is as would be expected to the positive or negative stimulus. Response latencies are also utilised as a measurement as the expectations are that positive stimuli will have a reduced latency to that of the negative stimuli and the ambiguous stimuli will be somewhere in the middle, and data will result in an S shaped curve or Sigmoid function.

A further variable that is included in many judgement bias studies is the addition of environmental manipulation to induce affect. Generally, this is done by housing experimental animals in small unenriched or unpredictable housing (Parker et al, 2014) and the control group in large enriched housing (Matheson et al, 2008; Burman et al, 2008). Bright lighting versus normal lighting (Boleij, Van't Klooster, Lavrijsen, Kirchhoff, Arndt & Ohl, 2012) as well as social isolation versus socialisation (Salmeto et al, 2011) and psychosocial stress (Papciak et al, 2013) have also been used to influence affect.

Happy Hen pilot study

The current experiment was based on a partial replication of the Happy Hen pilot study (Edwards & Bizo, 2012 unpublished), which was undertaken to develop a methodology to assess judgment bias in laying hens. Six hens were trained to discriminate between horizontal and vertical rectangles in the experimental procedure, where correct discrimination of the vertical rectangle resulted in 3 seconds (positive) access to food and correct discrimination of the horizontal rectangle resulted in 1 second (negative) access to food. Judgement bias of the vertical-positive and horizontal-negative cues were tested, using five rectangles at intermediate (ambiguous) angles to the learned cues at 0 and 90 degrees (seven angles overall). Hens decision-making behaviour was tested under two housing conditions, alternating between three days in the aviary and three days in the individual cage, (as used in commercial egg production), expected to alter the emotional state of the laying hens. It was predicted that hens housed in a barren environment (caged) would show negative judgement bias in comparison to hens housed in an enriched environment (aviary). No significant difference was found however in judgments made between caged and aviary housed hens, and it was considered possibly due to the task being too complex, the housing conditions too brief, and the negative cue not being negative enough.

On review of the procedure there were a number of other potential confounding factors that may have contributed to the lack of any effect which we addressed in order to improve methodology of the current experiment:

- There was an excessive number of presentations of ambiguous stimuli. For every ten trials, all seven non-reinforced probe stimuli were presented and three presentations of the two learned vertical and horizontal discriminations, making this a lean reinforcement schedule of 30%. In the current experiment the number of intermediate stimuli was reduced from seven to five, this included three intermediate stimuli and the two learned vertical and horizontal discriminations with only two non-reinforced probe stimuli presented in every ten trials with 80% reinforcer availability.
- The 40 minute probe trials (2400secs) were almost three times longer than that of the training sessions. In the current experiment the probe trials took approximately the same time as the discrimination trials.
- Three of the hens had high accuracy rates of 90%+ of the discrimination, and the remaining three achieved less than 70% accuracy. All hens in the current experiment were required to achieve 80-100% accuracy rates in training/habituation in order to increase the validity of probe trial responses.

The current experiment is a visually based experiment and includes horizontal, vertical and oblique rectangle stimuli; the operant task required is a key peck response to the left or right under appetitive control indicating discrimination between the horizontal and vertical rectangles. The method of constants is applied as the stimuli are pre-selected and presented in random order. The analysis for measurement is the One Alternative Forced Choice (1AFC) with measures of proportion correct responses in both training and probe trials, as well as latencies and bias responses that are neither correct nor incorrect in the probe trials. Although the Happy Hen pilot study included differing housing conditions to manipulate mood and therefore judgement bias responses, the current study did not. It was anticipated that the discrimination task stimuli (antecedents) and the resulting differential reinforcement opportunities (consequences) would result in an association of high or low reward. The presentation of the vertical rectangle was anticipated to promote positive affect, with 3 seconds of feed time (approx. 3-5 pecks), and a negative affect on presentation of the horizontal rectangle with a limited feed time of only 0.75 seconds (1 peck).

In the following experiment it was hypothesized that the initial probe trials with the intermediate novel stimuli using a one food magazine chamber, would reveal a judgement bias in hens through an increased tendency to select the vertical-positive key as a response to ambiguous stimuli, due to the longer interval of food access, and therefore inducing a positive affect. The horizontal-negative key was associated with a short interval of food access and it was therefore expected that a negative affect would be a result due to the reduced/minimal eating time. Response latencies were expected to be shorter for the vertical-positive, and longer for the horizontal-negative, with intermediate latencies for the unlearned stimuli with data potentially revealing a Sigmoid function.

Following a change from a single to a two food magazine chamber, both bias and preference were expected to reveal a degree of interaction, as all combinations of the three foods (pellets/wheat/puffed wheat) were provided (six presentations of alternate foods and food access time) with biased responses anticipated to preferred foods in different presentations to both vertical-positive and horizontal-negative. It was also considered that during the 5 day probe trials, there would be learning that the three intermediate stimuli were not reinforced, resulting in an effect on approach latencies to these.

EXPERIMENT

Method

Subjects

Six Brown Shaver hens (*Gallus gallus domesticus)* aged 45 months at commencement of experiment were numbered 12.1 to 12.6. All hens had prior handling and experience on operant tasks that included key pecking and eating wheat from a food magazine. Hens were housed individually in cages measuring 44cm (height), 51cm (width) and 46.5cm (depth), for the duration of the study. Lighting in the laboratory was on a fixed setting of 12 hours light and 12 hours darkness, and ventilation was provided 24 hours per day. After establishing a target body weight at 85% (+/-) of their outdoor enclosure weight, through gradual weight reduction that was below laying weight, they were weighed daily and fed a ration of poultry pellets to keep their weight consistent, grit and vitamins were provided weekly, and water was always available. Hens were fed additional pellets following the experimental task, if their weights prior to commencing the experimental task, were below their 85% (+/-) target body weight. Throughout the discrimination training period, hens obtained at least 50cc of pellets per day.

Ethics

Ethics approval from the University of Waikato Animal Ethics Committee, protocol number 927.

Apparatus

A chamber made from particle board, measuring 53cm (h), 56.5cm (w) and 40cm (d) was used for the experimental sessions. The door was one of the two widest sides with the opposing widest side being fixed to the wall (see figure 1.1). Internal chamber equipment included an upper 5 x 4cm central screen on which visual stimuli were presented, situated within a 13.2 x 11.2cm white frame. Manipulanda included two circular 3cm diameter response keys made from translucent perspex, each located at the lower end of a vertical 7 x 3.5cm rectangular aluminium strip, these were 22cm apart and situated either side of the central screen (see figure 1.2). The response keys had backlights that illuminated each key requiring a peck force of at least 0.1N to close a micro-switch behind the key, followed by a brief audible beep. Directly below the central upper screen was a rectangular 11.5 x 8cm opening, the distance from the base of the opening measuring 11cm to the chamber floor (see figure 1.3), providing access to food when the magazine was activated by a correct response. A MedPC program produced the experimental events and recorded responses for each hen in every session. Data was discarded if an egg was laid in the chamber during the session, or in the case of equipment failure.

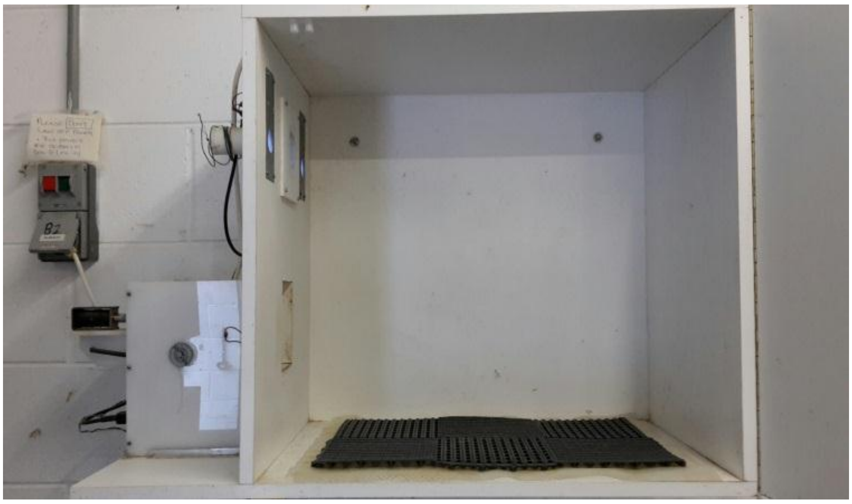


Figure 1.1. Side view of operant task chamber and external food magazine on bottom left.

Discrimination Task Visual Stimuli

A black rectangle (8 x 28mm) was presented on the upper midcentral screen on a white circle background with black surround. Response keys were equidistant either side of the screen. The two stimuli presented successively were equivalent in size, shape and colour, but differed in the dimension of orientation only - horizontal or vertical presentation (see figures 1.2 & 1.3 for vertical presentation).

Figure 1.2. Side view of open operant task chamber.

Figure 1.3. Operant chamber screen (centre), showing vertical rectangle (positive cue) presentation, response manipulanda to left and right of screen and food access opening below the screen, on short wall.

Experimental Design

PART I

<u>Discrimination Training (condition 1, pellets):</u>

Discrimination task training of two visual stimuli using a one alternative forced choice (1AFC) psychophysical procedure on a continuous reinforcement schedule (CRF) with correction method as per Edwards & Bizo, 2012.

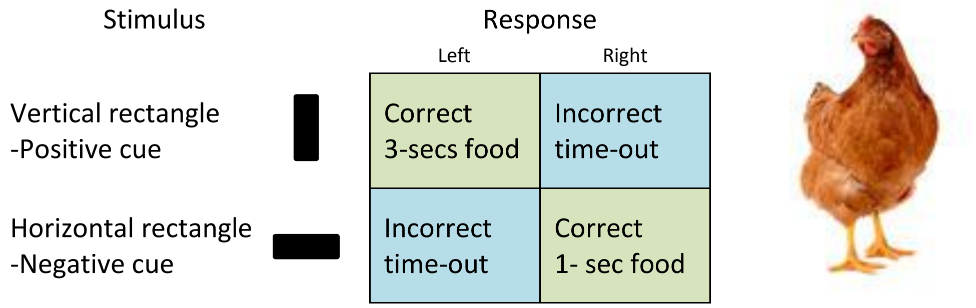


Figure 1.4. The stimulus-response matrix of the alternative forced-choice training procedure for hens 12.1 to 12.3. The cell entries represent the four possible outcomes of each trial.

On presentation of the visual stimulus (vertical or horizontal rectangle) which remained on the central screen until a response was made, following a 2 second delay, both the left and right response keys lit up simultaneously, remaining lit until one of the response keys was pecked (see figure 1.3). Following presentation of the vertical rectangle, hens 12.1 to 12.3 received 3 seconds access to poultry pellets (home diet) if they pecked the left lit key and following presentation of the horizontal rectangle received 1 second access to pellets if they pecked the right lit key. Trials were H (horizontal) or V (vertical) and presented pseudo-randomly with the same number (+/-1) of each orientation of rectangle presented. The timing of magazine/food access (1 or 3 secs) started once the hen's head entered the food access opening. A 5 second (inter-trial interval) ITI followed at cessation of each food reinforcement of 1-3 seconds or 3 seconds time-out for an incorrect response. In order to identify any potential for side bias, hens 12.4 to 12.6 received food reinforcement on the opposing sides (right key, vertical and left key, horizontal). Each session ended after 40 minutes or 50 correct responses, whichever occurred first. Mastery criteria was set at a minimum of 80% accuracy of correct key peck responses to the horizontal or vertical stimuli presentations over five consecutive days.

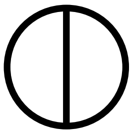
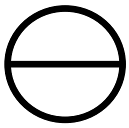
Correction method: the consequence immediately following an incorrect response was a time-out period of 3 seconds, 5 second ITI, followed by a repeated presentation of the same stimulus in the next trial (schedule 1).

A continuous reinforcement schedule with an extra correction procedure was implemented for one hen (12.5) on days 38-47 as response accuracy was below 60% (all other hens had achieved at least 70% at this stage). The change was made to the programme for hen 12.5 to assist with discrimination learning in order to improve accuracy. Following presentation of either the horizontal or vertical rectangle, only the correct key became available (lit) for a response. A daily session continued until 50 reinforcement opportunities had been provided, 25 for both stimuli, for 10 days. Hen 12.5 resumed Condition1 on day 48.

By day 90 of Condition 1, three of the six hens had achieved mastery of this task - Hens 12.3, 12.4 and 12.6 respectively on days 32, 88 and 41. Hens 12.1, 12.2 and 12.5 had not achieved mastery and managed a consistent response accuracy of only 60-70%. A modification was made to the procedure for all hens in order to improve discrimination accuracy for hens 12.1, 12.2 and 12.5. The continuous reinforcement procedure continued but with removal of the original correction procedure as it was suspected this had contributed to the failure of the three hens in learning the discrimination. Implementation of the non-correction method meant that an incorrect response would now result in a time-out period of 3 seconds, then 5 second ITI, followed by a random presentation of either stimulus (schedule 2).

At day 15 of the procedure with the non-correction method, hens 12.3, 12.4 and 12.6 continued to demonstrate an 80%+ response accuracy. Hens 12.1, 12.2 and 12.5 however demonstrated a decrease in accuracy to that of a 50% average accuracy rate. In further consideration that these hens would benefit from a change of schedule that was known to be effective in the hen lab with other discrimination training hen experiments, a new schedule was implemented that provided side allocation intermittent reinforcement (schedule 3). This provided pseudo-random intermittent allocation of reinforcement opportunities for correct responses to vertical and horizontal stimuli.

Table 1.1. Representation of decision making process occurring in response to presentation of each stimulus within the test box for hens 12.1 – 12.3.

Stimulus (SD) & Event Predicted	**Choice**	**Event** ***Schedules 1 & 2*** ***1 Mag***	**Event** ***Schedule 3*** ***1 Mag***	**Event** ***Schedule 3*** ***2 Mag***	**Behavioural consequence**
 Positive (large reward)	Peck left key	No food, time out	No food, time-out	No food, time-out	Extinguish
	Peck right key	3 secs access to food	3 secs access to food	3 secs access to food in left magazine	Reinforce
 Negative (small reward)	Peck left key	1 sec access to food	0.75 secs access to food	0.75-secs access to food in right magazine	Reinforce
	Peck right key	No food, time out	No food, time-out	No food, time-out	Extinguish

*Left/right keys reversed for Hens 12.4 - 12.6.

Hens 12.3, 12.4 and 12.6 achieved mastery with the new schedule at 19, 10 and 5 days respectively. Hens 12.1, 12.2 and 12.5 continued to remain at a stable accuracy rate of 50-60% over the initial 30 days, with no indication of successfully learning this task, therefore a supplementary ethics request was made to the Ethics Committee requesting replacement of these hens.

Hens 12.1, 12.2 and 12.5 ceased discrimination training and returned to the aviary. With Ethics Committee consent, three Brown Shaver hens, all aged 50 months commenced the experiment as 12.1, 12.2 and 12.5 (same hatch dates as 12.3, 12.4 and 12.6). All three hens had previous experience of making key peck responses in operant chambers. New hen 12.2 had participated in the Happy Hen pilot study achieving mastery of the discrimination task at that time (response accuracy ranging from 90-98% for the final 10 days), responding right vertical, whereas in this study she was required to respond left vertical.

A further modification was made to the method with the food access time for the "negative" (horizontal) key peck response being reduced from 1 second to 0.75 seconds when the three new hens commenced discrimination task training. This was to assist in discrimination learning between the positive and negative response, due to a finding from the pilot study that considered the negative key wasn't "negative enough". Observation by the experimenter confirmed that the short reinforcement duration of 0.75 secs was sufficient for all hens to obtain at least one poultry pellet (later wheat and puffed wheat).This was at day 34 of this condition for hens 12.3, 12.4, and 12.6.

Fig 1.5a. Hen 12.6 waiting for stimulus to appear

Fig 1.5b. Pecking right key (correct response to positive stimulus for hens 12.4 – 12.6)

Fig 1.5c. Consequence: reinforcement

Fig 1.5d. 3 seconds food access

Hen 12.4 died ninety-nine days after commencing the new schedule (and 3 days of probe baseline), and a new hen 12.4 was brought in to commence the experiment eighty-four days after the three new hen replacements. Hen 12.4 (new) was a Barnevelder, aged 41 months with previous chamber and key peck response experience. New hen 12.4 commenced the discrimination training which continued for all hens until this hen had met the criteria for mastery. New hen 12.1 mastered this condition at day 14, and new hens 12.2 at day 74 (previous participant in Happy Hen pilot), 12.5 at day 118 and 12.4 at day 106.

Probe (Judgement Bias Baseline):

Introduction:

The hens commenced probe trials (reinforcement: poultry pellets) after meeting the mastery criterion of five days at 80%+ accuracy for the discrimination task. Three hens commenced probe trials on the same day: Hens 12.3 and 12.6 on day 81, and 12.1 which was day 46 (see table 1.3) for this hen. New hens 12.2 and 12.5 commenced probes on day 189 and 12.4 respectively on day 117. The probe trials were implemented to test for judgement bias responses to the two learned rectangles and the three ambiguous rectangles (horizontal close 22.5^{o}, neutral 45^{o}, vertical close 67.5^{o}) at intermediate angles to the vertical-positive (0º) and horizontal-negative cues (90º).

Method:

Discrimination task with reinforcement of 0.75 or 3 seconds access to poultry pellets for correct responses to the two learned rectangles, and five probe stimuli presentations (with no reinforcement) that included the two learned horizontal and vertically presented rectangles and three intermediate ambiguous oblique rectangles; vertical close, neutral and horizontal close.

The learned discriminations of the 0 (horizontal) and 90 (vertical) degree stimuli were intermittently reinforced due to presentation either as a probe stimulus (unreinforced) or as a learned stimulus (reinforced). A total of 75 trials were given each session that included 60 presentations of horizontal or vertical rectangles on a CRF schedule, and 15 probe angles (unreinforced presentation of the five orientations of rectangles three times each during the session). Pseudo-random presentation of one probe in five trials that was comprised of four presentations of either the horizontal/vertical rectangle with access to reinforcement and one of the five probe presentations with no reinforcement. Percent correct accuracy of the learned discriminations, latency to respond to the five probe stimuli presentations and the responses made (horizontal-negative or vertical-positive) to the probe stimuli (3 ambiguous, 2 learned) were recorded.

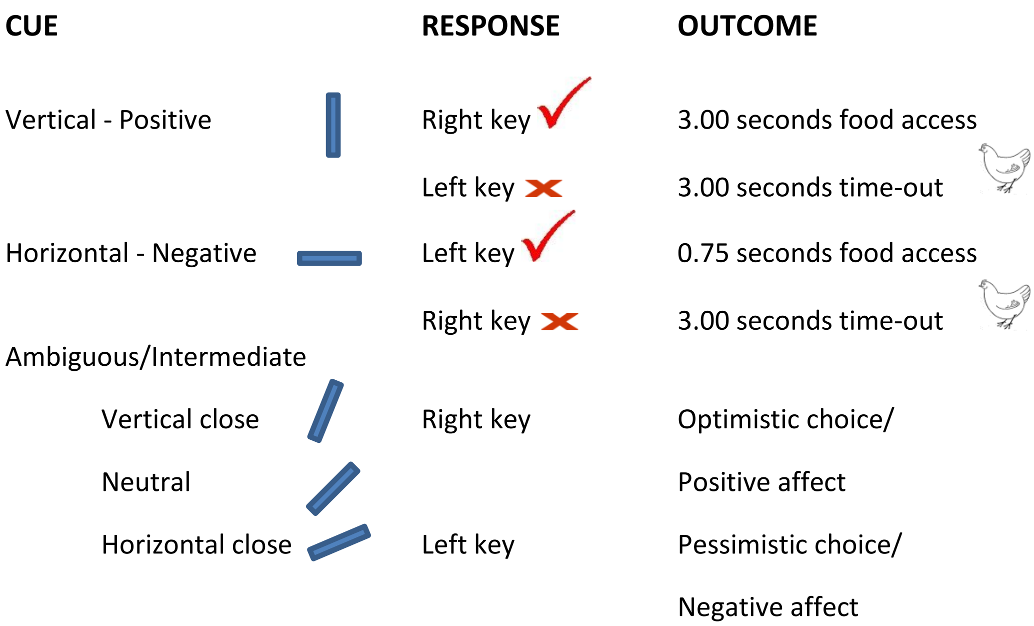


Figure 1.6. The cue-response-outcome of the alternative forced-choice training and probe conditions for hens 12.4 – 12.6.

Habituation (Condition 2) + Probe (wheat):

All hens continued with the discrimination task as per previous method with implementation of a novel food (wheat). Hens commenced with 7 days of habituation with the new food, followed by 5 days of probe trials, and 2 days of maintenance.

Habituation (Condition 3) + Probe (puffed wheat):

All hens continued with the discrimination task as per previous method with implementation of another novel food (puffed wheat). Hens commenced with 3 days of habituation, followed by 5 days of probe trials, and 2 days of maintenance.

PART II

Training (Conditions 4–9) + Probes (pellets/wheat/puffed wheat):

Subjects

Six hens: Five Brown Shaver hens, now 58 months old and one Barnevelder (12-4) aged 47 months. All hens had completed the three food conditions in the one food magazine chamber with pellets/wheat/puffed wheat and five days of probe trials for each condition. Hens remained in the same laboratory cages for the duration of the study, and lighting, freely available water and postfeed continued as noted previously. Hens obtained 50-60cc of pellets/wheat/puffed wheat per day during the daily session and received post-feed also as necessary.

Apparatus

The operant task chamber utilised for conditions 1-3 underwent modification for conditions 4-9. The change implemented was to close over the lower central opening that provided food access, and the creation of two lower central openings on either side of the original opening (see figure 1.7), providing access to two food magazines when activated by a correct response to the response keys. Magazines contained different foods for reinforcement (see figures 1.8 & 1.9). For hens 12.1 - 12.3 the left magazine contained the positive reinforcement (3 secs food access) and the right magazine contained the negative (less positive) reinforcement (0.75 secs food access), and for hens 12.4 - 12.6, the right magazine contained the positive reinforcement, and the left magazine contained the negative (less positive) reinforcement. A MedPC program provided the schedule of tasks and recorded event data for each hen in every session.

Experimental Design

<u>Training:</u>

(20 days – pellets 3 secs, pellets 0.75 secs reinforcement) Discrimination task training on side allocation intermittent reinforcement schedule (partial reinforcement schedule) with newly implemented double magazine food access (pellets long/positive, pellets short/negative).

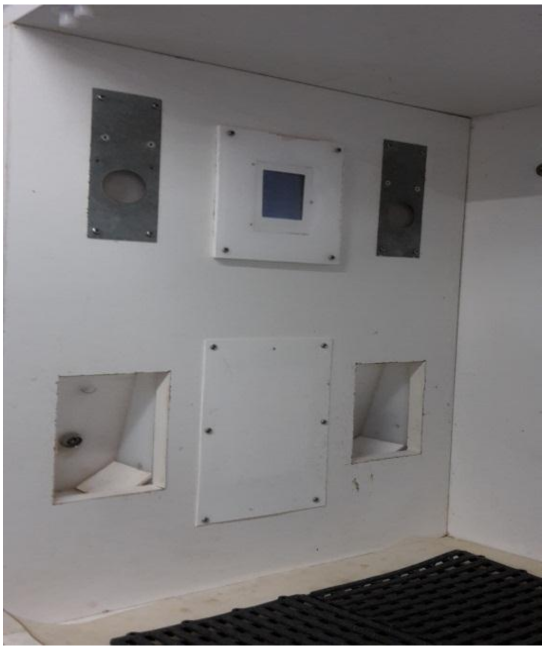


Figure 1.7. Modified chamber with access to two food magazines

Figure 1.8. External view of the two food magazines

Figure 1.9. Birds-eye view of the two external food magazines (left, wheat and right, puffed wheat).

Conditions 4-9:

A requirement of the double magazine with differing foods necessitated that all hens commenced and completed each condition with the new food combination on the same day. Habituation sessions for the new combination of foods had a maximum time-out of 40 minutes (2400 seconds) or 50 reinforced correct responses (0.75 or 3 seconds access to poultry pellets), whichever was reached first.

Following three days of habituation to each food combination, all hens completed five days of probe trials with the same food combination, and when completed, they then commenced the next food combination habituation period over the following three days, followed by probe trials of the same, until all six food combinations had been completed (pellets long with wheat short and puffed wheat short, wheat long with pellets short and puffed wheat short, and puffed wheat long with pellets short and wheat short) (see table 1.2).

Table 1.2. Food combination schedule for hens for Conditions 4-9 with double food magazine.

Condition	Habituation Days	Hens	Food (3.00 secs)	Food (0.75 secs	Probe days	Accum. total days
Training		1-6	Pellets	Pellets		20
4 6	3	1-3 4-6	Pellets Wheat	Wheat Pellets	5	28
5 8	3	1-3 4-6	Pellets P.Wheat	P.Wheat Pellets	5	36
6 4	3	1-3 4-6	Wheat Pellets	Pellets Wheat	5	44
7 9	3	1-3 4-6	Wheat P.Wheat	P.Wheat Wheat	5	52
8 5	3	1-3 4-6	P.Wheat Pellets	Pellets P.Wheat	5	60
9 7	3	1-3 4-6	P.Wheat Wheat	Wheat P.Wheat	5	68

Probes:

Judgement bias responses (and latencies) probed for each combination of foods in conditions 4-9. Discrimination task with reinforcement (0.75 or 3 seconds access to pellets, wheat or puffed wheat) + five probe stimuli (no reinforcement) that included the two learned horizontal (0^o) and vertically (90^o) presented rectangles and rectangles presented at three intermediate ambiguous (oblique) angles (horizontal close 22.5^o, neutral 45^o, vertical close 67.5^o).

The learned discriminations of the 0 (horizontal) and 90 (vertical) degree stimuli were intermittently reinforced (PRF) due to presentation either as a probe stimulus (unreinforced) or as a learned stimulus (reinforced). As previously, a total of 75 trials were given each session that included 60 presentations of horizontal or vertical rectangles on a CRF schedule, and 15 probe angles (unreinforced presentation of the five angle orientations three times each during the session). Pseudo-random presentation of one probe in five trials that was comprised of four presentations of either the horizontal/vertical rectangle with access to reinforcement and one of the five probe presentations with no reinforcement. Percent correct accuracy of the learned discriminations, latency to respond to the five probe stimuli presentations and the responses made to the intermediate stimuli (horizontal-negative or vertical-positive key peck) were recorded.

Hens 12.1 – 12.3 completed conditions 4-9 in that order and hens 12.4 – 12.6 completed these same schedules but in the order of 6, 8, 4, 9, 5, 7 (see Table 1.2). This was due to the counterbalancing side requirements for the negative and positive response keys, i.e. the left magazine provided the positive (pellets) food access time of 3 seconds and right magazine the negative (wheat) access time of 0.75 seconds for hens 12.1 - 12.3, and for hens 12.4 - 12.6 the right magazine was positive (wheat) and the left magazine was negative (pellets).

All hens completed the same number of probe trials for both the one and two magazine chambers. Training days ranged from 125-346 for the hens as mentioned previously with the overall number of days that hens were involved in the study ranging from 208-429 (see table 1.3).

Table 1.3. Overall number of days hens in training and probe trials.

Hen	**Training 1 mag**	**Probes 1 mag**	**Training 2 mag**	**Probes 2 mag**	**Total Days**
12.1n	208	15	38	30	291
12.2n	208	15	38	30	291
12.3	346	15	38	30	429
12.4n	125	15	38	30	208
12.5n	208	15	38	30	291
12.6	346	15	38	30	429

RESULTS

Discrimination Task Training

Six Brown Shaver hens commenced the visual discrimination task training of the vertically and horizontally presented rectangles with the correction method and initially completed 90 days training. Hens 12.3, 12.4 and 12.6 were the only three hens to achieve mastery, attaining at least 80% accuracy. Hens 12.3 and 12.6 were the first to master the discrimination task respectively on days 32 and 41, and 12.4 on day 88 (and scored 100% on day 89). Hens 12.1, 12.2 and 12.5 did not master the discrimination task (see figure 2.1).

An extra procedure was added for Hen 12.5 due to performance remaining under 60% accuracy in a stable pattern. The extra correction method that was added for 10 days (from day 38 – 47), ensured that the hen achieved 100% accuracy by providing only the correct response key (lit up) as the available option. The 10 days prior to this Hen 12.5 achieved a mean accuracy rate of 52%. Following a return to the procedure that the other hens had remained on during this time, a small increase in accuracy was noted for Hen 12.5 over the following 10 days with an average accuracy rate of 57.5% (see figure 2.1).

Over days 80 – 90 of the correction method, hens 12.1, 12.2 and 12.5 respectively achieved an average accuracy rate of 68%, 68% and 63%. Due to concerns that these three hens were not going to achieve mastery with the correction method procedure, as the data indicated that it was likely that they had learned to switch keys following an incorrect response and not the discrimination, the correction method was removed for all hens on day 90. With removal of the correction method, (now the non-correction method) data remained stable for the three hens that had learned the discrimination task (12.3, 12.4, 12.6), however hens 12.1, 12.2 and 12.5 showed a decrease in accuracy rates respectively to 51%, 50% and 50% over the 15 days of this procedure. The decrease in accuracy back to that of chance confirmed their practice of switching with the correction method, however due to the unlikelihood of learning the discrimination under this schedule, a new one was implemented (see figure 2.1).

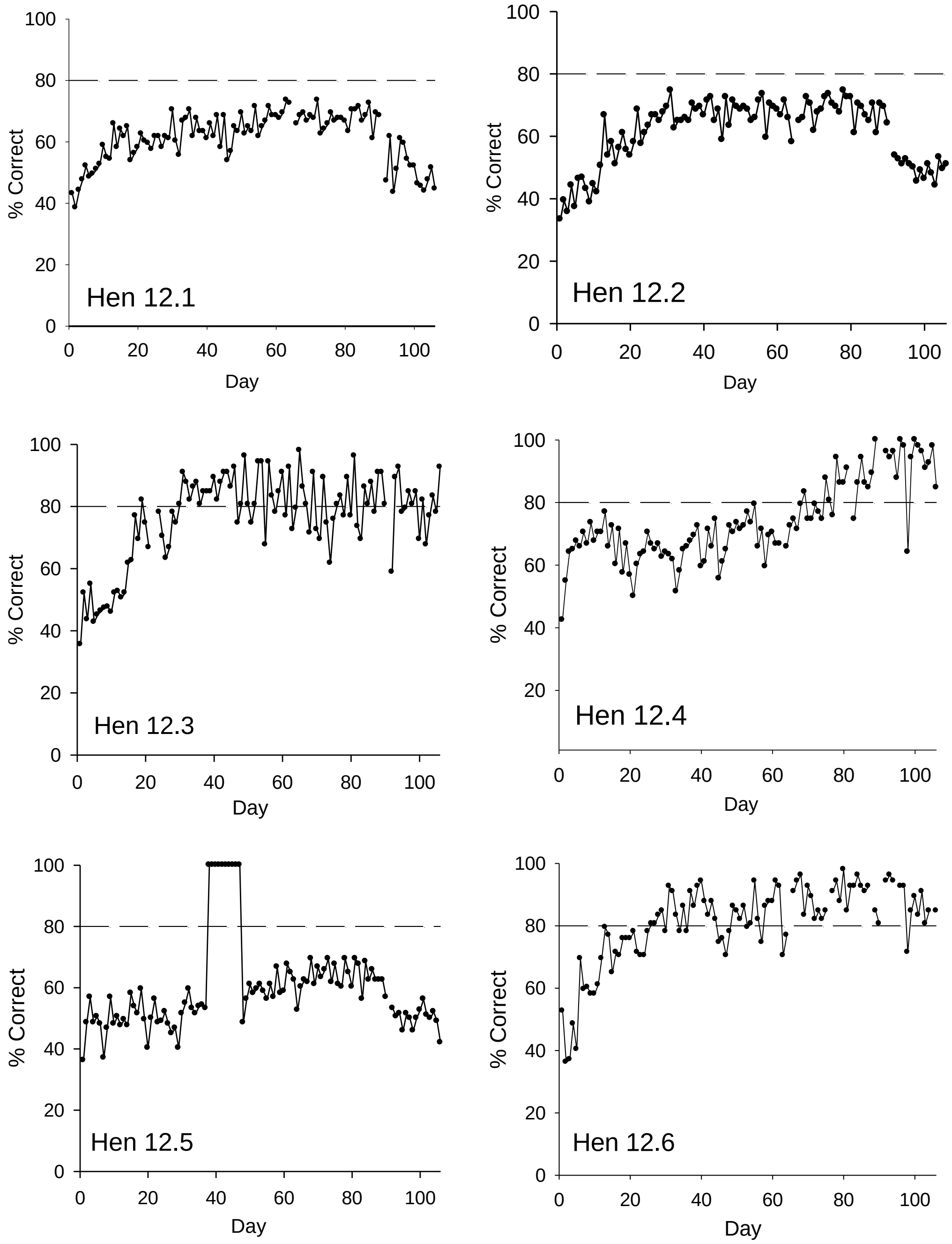


Figure 2.1. Discrimination task training percentage of correct responses days 1-90 on a continuous reinforcement schedule (CRF) with correction method (*12.5 only, days 38-47, CRF schedule with extra correction method), and days 91-105 on CRF with non-correction method.

A reinforcement schedule procedure was introduced that had previously been used in the animal lab with success in training discriminations - side allocation intermittent reinforcement (partial reinforcement schedule). This schedule uses a pseudo-random pre-selection of allocation of reinforcement. All six hens commenced this procedure. Hens 12.3, 12.4 and 12.6 continued to demonstrate mastery of the discrimination task and as data remained stable they were considered to be in a maintenance phase. Hens 12.1, 12.2 and 12.5 continued to show a poor performance in discrimination, achieving average accuracy rates respectively at 52%, 50% and 51% over 10 days (24 – 33) (see figure 2.2). Due to the increasing unlikelihood of the three hens learning the discrimination, having received 138 days of training, the decision was made to replace them and three new hens commenced this procedure on what was day 34 of the new side allocation intermittent reinforcement schedule for hens 12.3, 12.4 and 12.6 (see figure 2.3).

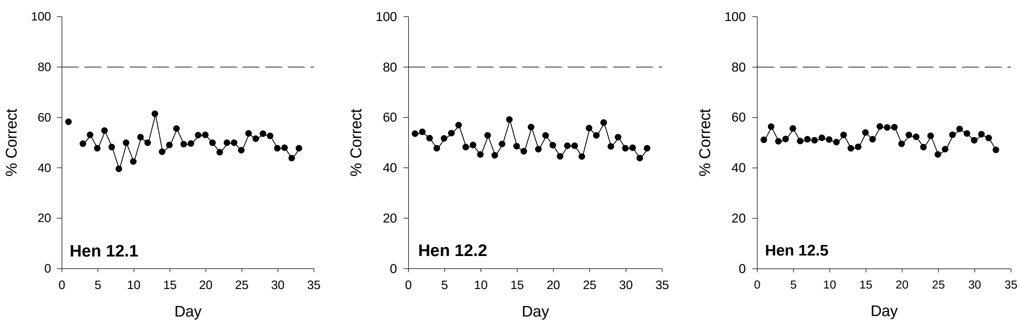


Figure 2.2. Discrimination Task Training percentage of correct responses for 33 days for hens 12.1, 12.2 and 12.5 on the side allocation intermittent reinforcement schedule.

Hen 12.4 died suddenly on day 99 of this new procedure and another new hen was brought into the experiment. New hen 12.4 commenced on what was day 84 of the training for new hens 12.1, 12.2 and 12.5 and day 117 of maintenance for hens 12.3 and 12.6.

Table 2.1. Number of Days taken for Hens to Achieve Mastery of Discrimination Task, Probe Commencement and Total Training Days for Conditions 1-3

Hen	Day Mastery Achieved	Day Probe Commenced	Days Training Total
12.1 (n)	14	46	208
12.2 (n)	74	189	208
12.3	32	81	241
12.4 (n)	106	117	125
12.5 (n)	118	189	208
12.6	41	81	241
	$\bar{x} = 64, s^2 = 38$		

The number of days it took all hens to learn the discrimination ranged from 14 - 118. New hen 12.1 was the fastest of all hens in learning the discrimination task at just 14 days, then 12.3 at 32, 12.6 at 41, 12.2 at 74, and the two slowest hens 12.4 at 106 days and 12.5 at 118. Hen 12.5 demonstrated response accuracy of over 80% at day 33 and over twelve random days after this, however consistency was not achieved until 118 days (see table 2.1).

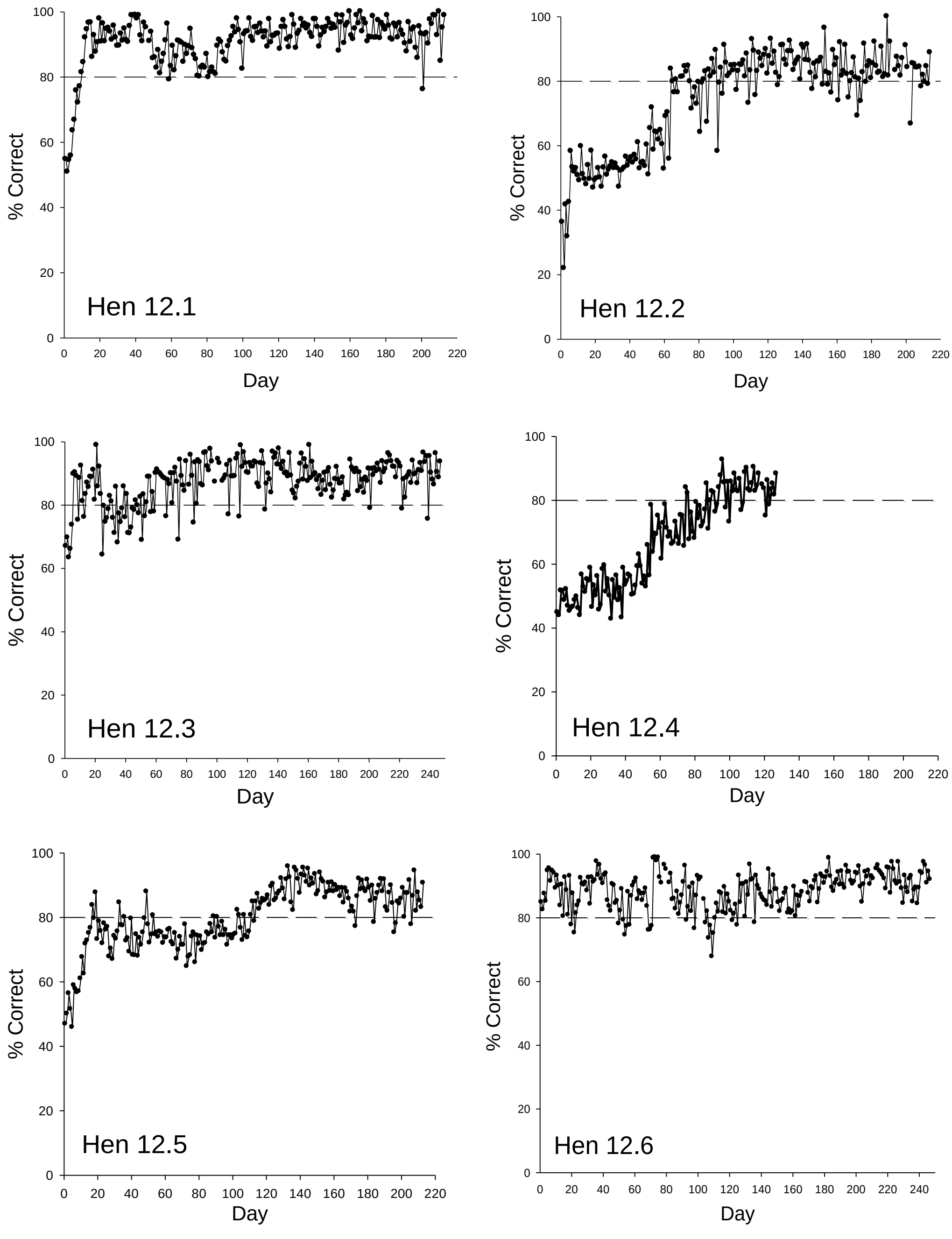


Figure 2.3. Discrimination task training percentage of correct responses for new hens 12.1, 12.2, 12.4, 12.5 and percentage of correct responses on discrimination task maintenance for hens 12.3 and 12.6 on the side allocation intermittent reinforcement schedule (pellets).

Probe Trials for Judgement Bias

The three hens 12.1, 12.3 and 12.6 undertook 3 days of probe trials of judgement bias prior to the other hens, due to having learned the discrimination at an earlier stage than the other three hens. Initial probe sessions introduced the novel/ambiguous stimuli of rectangles at the three intermediate angles (22.5, 45, 67.5 degrees) between the horizontal (0 degrees) and vertical (90 degrees) angles, reinforcement included pellets vertical/3 secs, pellets horizontal/0.75 secs. The remaining three hens undertook 3 days of probe trials 143 days following these hens, after having learned the discrimination at a later time. Twenty days later all hens then completed a further two days of probe trials for the pellets vertical and pellets horizontal condition, all completing five days each of probe sessions.

The reinforcement food was then changed from the home diet of poultry pellets to a novel one of wheat. Discrimination task maintenance continued for 7 days with wheat to provide a habituation period for the hens with the new food, prior to completing 5 days of probe sessions with wheat, and then followed with a further 2 days of discrimination task maintenance. The reinforcement food was then changed again from wheat to a further novel one of puffed wheat. Discrimination task maintenance was undertaken for 3 days as a habituation period prior to the 5 days of probe sessions with puffed wheat. This completed the three probe conditions of pellets, wheat and puffed wheat with the one food magazine.

It was an expectation that results would show responses to the vertical rectangle as mostly 1 or close to, and responses to the horizontal rectangle as mostly 0, or close to, due to these two angles of rectangle being the learned discriminations. The neutral rectangle at the absolute intermediate angle is expected to demonstrate in which direction the judgement bias lies, above 0.5 suggesting a positive/optimistic outlook and lower than 0.5, a negative/pessimistic outlook.

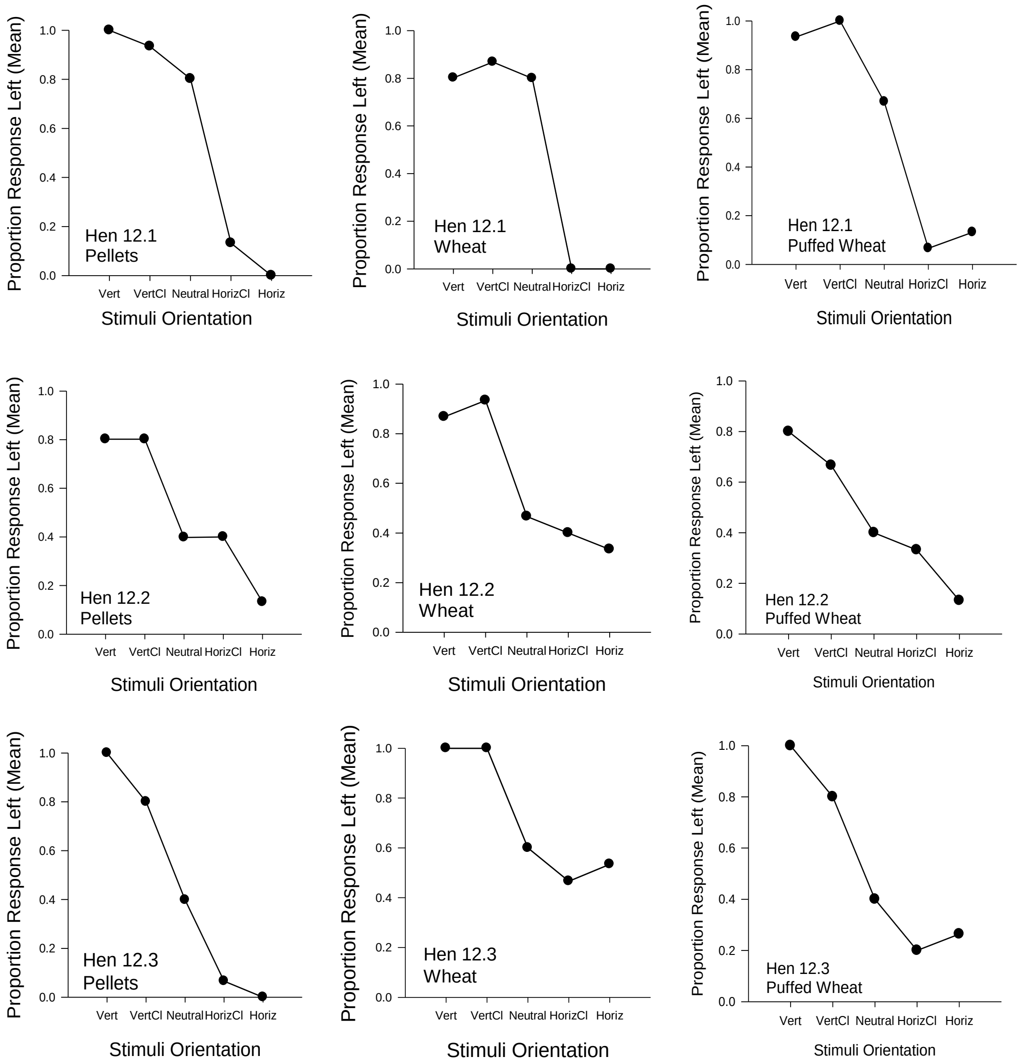


Figure 2.4. Hens 12.1-12.3 Judgement bias probe trials, proportion of responses to left (vertical) key for pellets, wheat and puffed wheat (single magazine).

Hen 12.1 demonstrated a consistent positive affect with responses to the vertical, vertical close and neutral stimuli from 0.6 – 1.0, and the horizontal close and horizontal angles 0 to 0.2 across all food options (see figure 2.4).

Hen 12.2 responded from just over 0.6 to just under 1.0 for the vertical and vertical close angles only. The neutral, horizontal close and horizontal responses range from just under 0.2 to just under 0.5. The neutral angle has negative responses under 0.5 across all food types, indicating negative affect, however the horizontal close and horizontal do not score 0 at all, suggesting a propensity to respond to them positively or a preference for the longer food access. Responses to wheat indicates a slight negative affect as the neutral, horizontal close and horizontal stimuli have been responded to as vertical for just over a third of the time and are close in range with responses varying from 0.3 to just under 0.5 (see figure 2.4).

Hen 12.3 responded to the vertical and vertical close from 0.8 – 1.0 for all foods, and from 0 – 0.4 for neutral, horizontal close and horizontal for pellets and puffed wheat. Responses to wheat for neutral, horizontal close and horizontal are similar to that of Hen 12.2, ranging from 0.4 – 0.6, indicating slight negative affect (or a preference for wheat) (see figure 2.4). The scores of 1 for both vertical and vertical close for wheat is another strong indicator of preference/positive bias with 100% of responses vertical for both angles. The neutral, horizontal close and horizontal responses for pellets reveal negative responses. The neutral response for puffed wheat also indicates a negative judgement, however horizontal close and horizontal responses indicate preference for the longer food access.

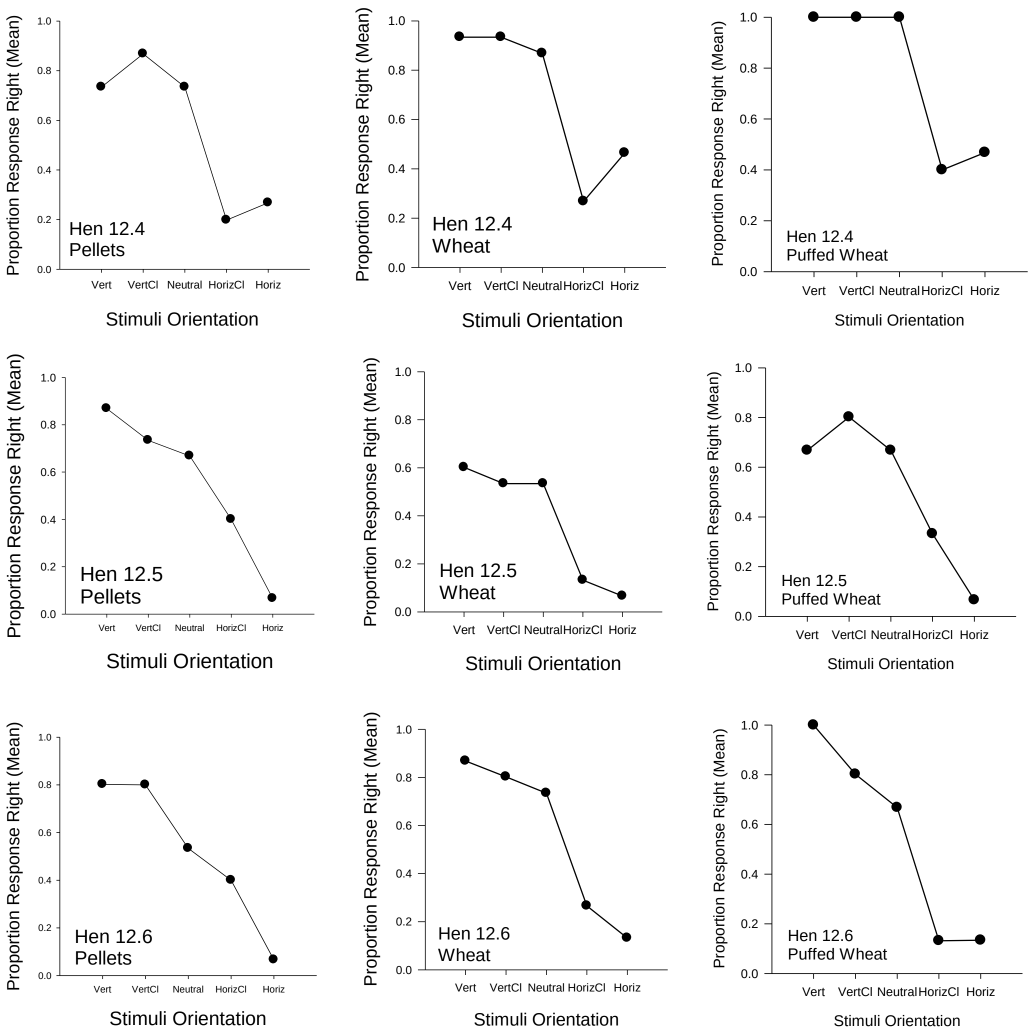


Figure 2.5. Hens 12.4-12.6 Judgement bias probe trials, proportion of responses to right (vertical) key for pellets, wheat and puffed wheat (single magazine).

Hen 12.4 demonstrated a consistent positive affect with responses to the rectangles at vertical, vertical close and neutral angles from 0.7 – 1.0, and the horizontal close and horizontal angles from 0.2 – 0.5 across all food options. As there are no responses between 0 and 0.2 for the horizontal/close stimuli, this suggests an inclination to respond vertical/positive (see figure 2.5).

Hen 12.5 has responded from 0.5 – 0.9 for vertical, vertical close and neutral angles for all food types, suggesting positive affect, however with the wheat option, all responses are lower than the other food types, except for the horizontal which is the same as all other food types and reveals the least range of responses from 0.06 - 0.6 indicating slight negative affect (see figure 2.5).

Hen 12.6 demonstrated consistent positive affect with responses to the stimuli at vertical, vertical close and neutral angles from 0.53 – 1.0, and the horizontal close and horizontal angles from 0.06 – 0.4 across all food options. Although no 0 responses (but close to) for the horizontal stimuli, responses to horizontal close have trended downwards to that of the horizontal across the food conditions (see figure 2.5).

Single Magazine Probe Trials Summary

In Condition 1, four of the six hens (12.1, 12.4, 12.5, 12.6) demonstrated positive affect and two hens (12.2, 12.3) demonstrated a slight negative affect. In Condition 2, five hens (12.1, 12.3, 12.4, 12.5, 12.6) demonstrated positive affect, this was moderate however for Hen 12.5 and Hen 12.2's scores indicated a preference to respond vertical for this new food type. In Condition 3, the same four hens (12.1, 12.4, 12.5, 12.6) that demonstrated positive affect with pellets, also demonstrated positive affect with puffed wheat, and with a higher range of scores than that of pellets. Hens 12.2 and 12.3 also again demonstrated negative affect.

Overall, hens 12.1, 12.4, 12.5 and 12.6 demonstrated consistent responses indicating positive affect across all three food types. One exception was for hen 12.5 with all responses to stimuli for wheat being lower in proportion than pellets and puffed wheat and one explanation may be that this hen's responses changed with the introduction of a novel food, as up to this point the hens received pellets as their reinforcement as well as this being their home diet. Hen 12.2

demonstrated consistent responses indicating negative affect across all three food types and hen 12.3 demonstrated negative affect for two of the three conditions.

With regard to the anticipated S shape curve (responses ranging from 0 to 1), hens 12.1 and 12.3 demonstrate this, but only for pellets, therefore there is no expectation of these being seen in the next judgement bias probes with the double food magazines. It is an expectation however that responses to the horizontal and horizontal close rectangles are similar to each other and the same for the vertical and vertical close rectangles as this is a pattern that has been revealed in these probe trials.

Conditions 4-6 - Judgement Bias probes

Hens 12.1-12.3 completed all six probe trials for judgement bias in the same order with the double food magazine (Conditions 1-6). Pellets (P) long with wheat (W) short (Condition 1) and pellets long with puffed wheat (PW) short (Condition 2). Wheat long with pellets short (Condition 3) and wheat long with puffed wheat short (Condition 4), puffed wheat long with pellets short (Condition 5) and puffed wheat long with wheat short (Condition 6). Hens 12.4 – 12.6 completed the probe trials in the order of condition 3, 5, 1, 6, 2 and 4.

Please note: the first letter in the following graph legends indicates the long food access and second letter the short food access, i.e., P vs.W designates pellets 3 seconds access, and wheat 0.75 seconds access.

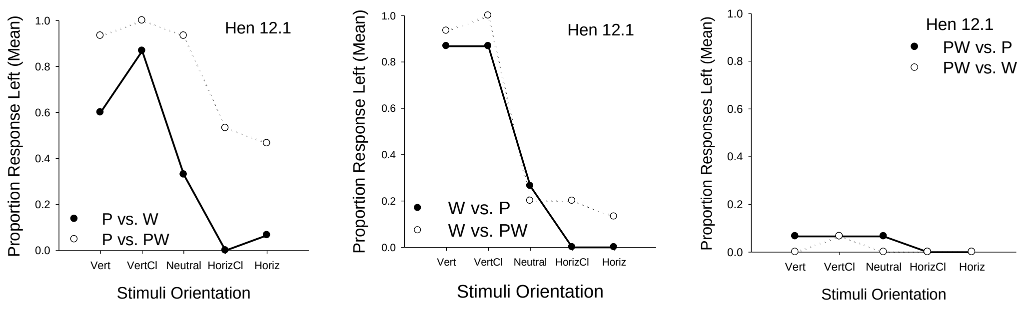


Figure 2.6. Hen 12.1 Judgement bias probe trials, proportion responses left (vertical) for combinations of pellets (P), wheat (W) and puffed wheat (PW).

Hen 12.1 demonstrates a similar pattern of behaviour within each food type. A strong preference for pellets over puffed wheat (P vs. PW) is demonstrated as both the horizontal close and horizontal have been responded to more than half the time as the vertical/positive. Pellets vs. wheat (P vs. W) reveals expected responses to the horizontal, horizontal close and vertical close stimuli, however lower than expected responses for vertical and neutral indicates a slight negative affect. Wheat (long) responses are similar with each (short) food type with negative affect revealed to both pellets and puffed wheat (W vs. P, W vs. PW) with responses to neutral, horizontal close and horizontal ranging from only 0 – 0.2. Puffed wheat (long) reveals that hen 12.1 has a clear preference for pellets and wheat over puffed wheat (PW vs. P, PW vs. W) with all responses ranging from only 0 – 0.06 (see figure 2.6).

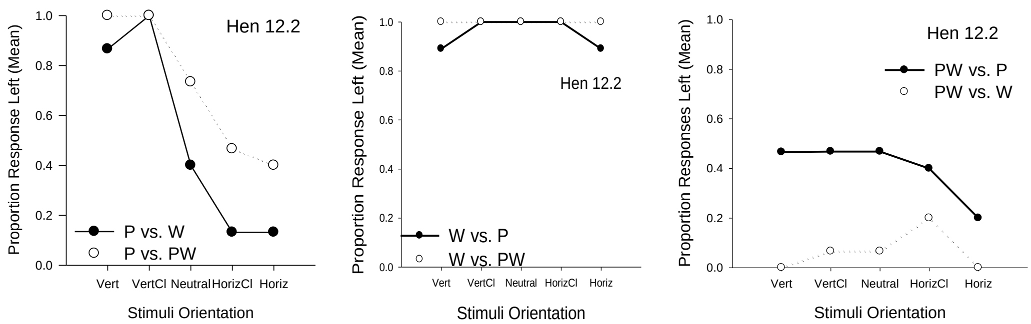


Figure 2.7. Hen 12.2 Judgement bias probe trials, proportion responses left (vertical) for combinations of pellets (P), wheat (W) and puffed wheat (PW).

Hen 12.2 demonstrates consistent patterns within each food type. Although hen 12.2 has responded ‘vertically’ to the vertical and vertical close for pellets (long), the neutral, horizontal close and horizontal have all been responded to negatively with wheat (P vs. W), however these three angles for the puffed wheat (P vs. PW) option show increased vertical/positive responses, indicating a preference for pellets. The wheat with both pellets (W vs. P) and puffed wheat (W vs. PW) options demonstrates a definitive preference with all responses ranging from 0.9 – 1. The puffed wheat (long) with both pellets (PW vs. P) and wheat (PW vs. W) indicates preference for low reward food with all responses ranging from 0 – 0.47.

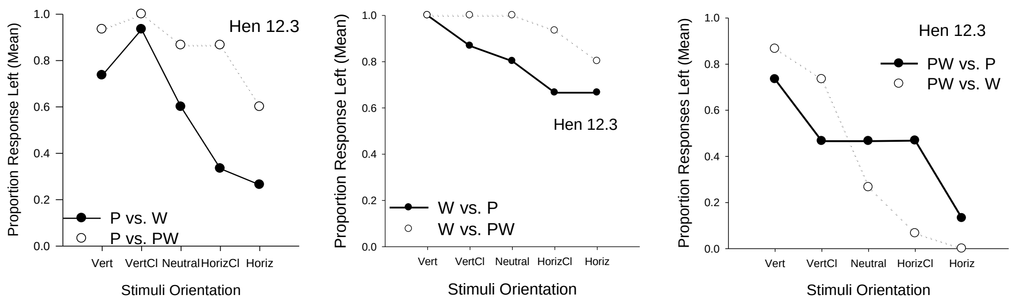


Figure 2.8. Hen 12.3 Judgement bias probe trials, proportion responses left (vertical) for combinations of pellets (P), wheat (W) and puffed wheat (PW).

Hen 12.3 also demonstrates patterns of consistent behaviour across the three food types. The pellets option indicates preference with all responses to puffed wheat (P vs. PW) ranging from 0.6 – 1. Responses to wheat (P vs. W) for vertical, vertical close and neutral are all above 0.5 and the horizontal close and horizontal have also been responded to as vertical for at least a third of the time, indicating a food preference. The wheat options (W vs. P, W vs. PW) demonstrate a definitive food preference with all responses from over 0.6 – 1, with the puffed wheat (W vs. PW) option even more so with responses from 0.8 – 1. The puffed wheat (long) condition reveals a change in affect with the pellets option (PW vs. P) indicating negative affect for all angles except vertical, and although the wheat option (PW vs. W) reveals positive responses for both the vertical and vertical close, the neutral, horizontal close and horizontal angles responses (0 – 0.3) suggest negative affect (see figure 2.8).

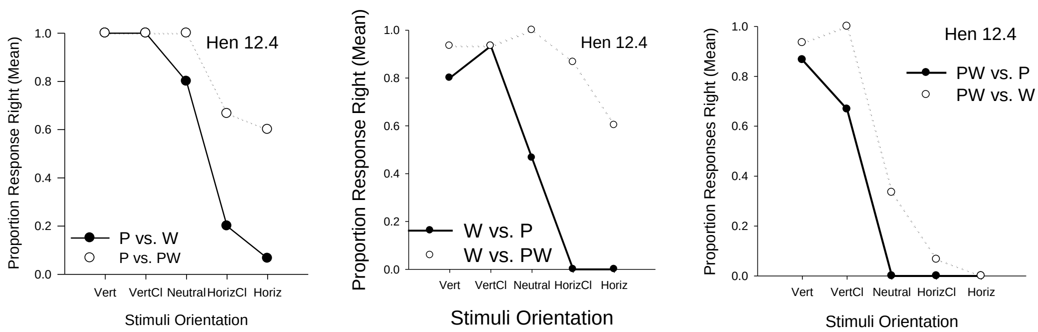


Figure 2.9. Hen 12.4 Judgement bias probe trials, proportion responses right (vertical) for combinations of pellets (P), wheat (W) and puffed wheat (PW).

Hen 12.4 demonstrates a consistent pattern of behaviour with pellet responses across all food types ranging from 0 – 1, although a decrease from a positive to negative affect is seen across the food types with responses to the neutral angle (P vs. W, W vs. P, PW vs. P). Both the pellets vs. puffed wheat (P vs. PW) and wheat vs. puffed wheat (W vs. PW) options demonstrate a high reward food preference as all responses range upward from 0.6 – 1. A negative affect is seen in the puffed wheat option (PW vs. P and PW vs. W) with neutral, horizontal close and horizontal responses 0 – 0.3 (see figure 2.9).

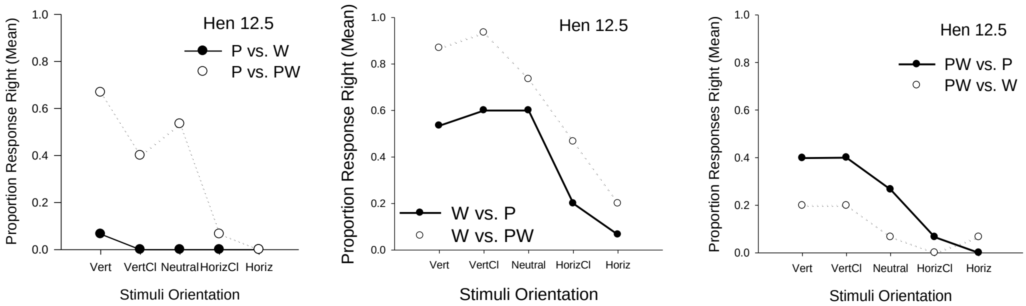


Figure 2.10. Hen 12.5 Judgement bias probe trials, proportion responses right (vertical) for combinations of pellets (P), wheat (W) and puffed wheat (PW).

Hen 12.5 reveals a strong low reward food preference with the pellets vs. wheat (P vs. W) option, however these results should be interpreted with caution due to a side bias for the left/horizontal key that commenced just days after meeting the mastery criterion in Condition 1. Prior to this, incorrect responses were low for the vertical key and high for the horizontal key. There was an expectation that if any side bias would be demonstrated it would be for the vertical/long food access option, therefore the bias for the horizontal/short food access was unanticipated. Following a failed normality (Shapiro-Wilk) test ($P < 0.050$), a t-test for dependent means revealed a significant difference ($t=8.012$) between the incorrect responses to the left/horizontal and right/vertical keys ($P<0.001$) for the final 100 days of discrimination maintenance trials. A slight negative affect is also seen with the pellets vs. puffed wheat (P vs. PW) with a lower than expected vertical (0.7) and vertical close responses (0.4), although neutral is just over 0.5, the horizontal close and horizontal are as expected at 0 – 0.1. Positive affect is shown with the wheat vs. puffed wheat (W vs. PW) option with the vertical, vertical close and neutral responses, and the horizontal close and horizontal responses at 0.4 and 0.2. Very slight positive affect is seen with the wheat vs. puffed wheat (W vs. P) option as the vertical, vertical close and neutral angle responses are all just above 0.5 – 0.6. The puffed wheat option reveals a low reward food preference with both pellets (PW vs. P) and wheat (PW vs. W) as all responses range from 0 – 0.4, and particularly so with the wheat option only ranging from 0 – 0.2 (see figure 2.10).

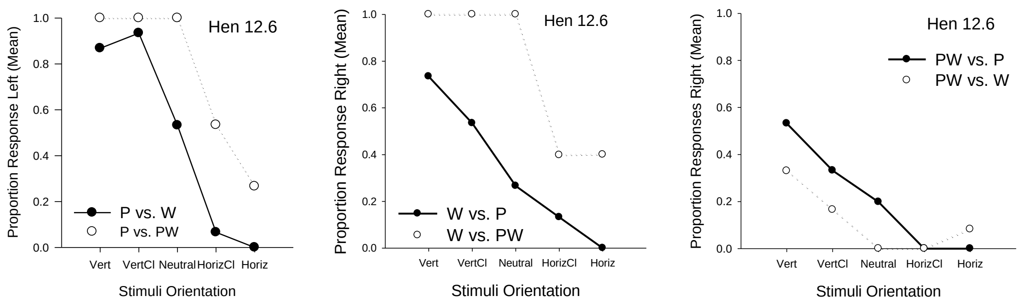


Figure 2.11. Hen 12.6 Judgement probe trials, proportion responses right (vertical) for combinations of pellets (P), wheat (W) and puffed wheat (PW).

Hen 12.6 demonstrates a shift in positive affect from moderate to strong with the pellets vs. wheat (P vs. W) option and the neutral response at 0.5 to the pellets vs. puffed wheat (P vs. PW) option with the neutral (and vertical and vertical close) responses all increasing to 1. The horizontal close has been responded to vertically for just over half the time and horizontal responses for at least a third of the time indicating a high reward food preference. Both a positive and negative affect is seen in the wheat options. The responses to pellets (W vs. P) reveal negative affect as the horizontal, horizontal close and neutral angles have all been responded to from between 0 – 0.3. The responses to puffed wheat (W vs. PW) indicate positive affect/high reward food preference, with the vertical, vertical close and neutral all at 1 and the horizontal close and horizontal being responded to over a third of the time as 'vertical'. A low reward food preference is then demonstrated in the puffed wheat options with both pellets (PW vs. P) and wheat (PW vs. W) with all responses ranging from 0 to just 0.5 (see figure 2.11).

Double Magazine Probe Trials Summary

Pellets was paired with both wheat (Condition 4) and puffed wheat (Condition 5). Data was consistent across all six hens in that pellets vs. wheat revealed less proportion vertical responses than pellets vs. puffed wheat. It's known that puffed wheat is often the least preferred food when paired with pellets and wheat, and the results indicate that hens responded vertically on the whole with the pellets vs. puffed wheat across all stimuli revealing short and shallow S curves. All hens demonstrated what appears to be positive affect with the pellets vs. puffed wheat, except for hen 12.5. Three hens (12.1, 12.2, 12.5), demonstrated negative affect with pellets vs. wheat. Hen 12.5's data differed again in that there were almost no vertical responses to pellets vs. wheat, indicating that this hen preferred wheat on low reinforcement over pellets at high reinforcement.

Wheat was paired with both pellets (Condition 6) and puffed wheat (Condition 7). Data was consistent again across all six hens in that wheat vs. pellets revealed less proportion vertical responses than wheat vs. puffed wheat, indicating that puffed wheat was the least preferred food option. Wheat vs. pellets revealed that hens were evenly divided in demonstrating negative and positive affect. Hens 12.1, 12.4 and 12.6 showed negative affect with the wheat vs. pellets appearing as normal curved data. Hens 12.2 and 12.3 however reveal a strong preference to respond vertically to wheat when combined with both pellets and puffed wheat. Hen 12.1 only demonstrated negative affect when wheat was paired with puffed wheat.

Puffed wheat was paired with both pellets (Condition 8) and wheat (Condition 9). Data was consistent here also for all six hens with a negative affect shown with both food combinations, indicating that when puffed wheat was the high (vertical) reinforcement option, all hens selected the horizontal/low reinforcement "other' food option. Hens 12.1, 12.2, 12.5 and 12.6's responses indicate preference for the low reward food. Hens 12.3 and 12.4's data reveals normal curves, indicating a negative judgement bias.

The food combination graphs reveal two differing representations of data. Where data shows an S shaped curve, it can be interpreted as judgement bias, however where data is flat or shallow, then this data should be interpreted as revealing a food preference due to only one (or neither) of the learned stimuli being responded to as correct. Generally the food combination results vary considerably between and within hens. Hen 12.4 reveals some consistency across the food conditions when pellets and wheat are paired and when both are paired with puffed wheat.

Response latencies

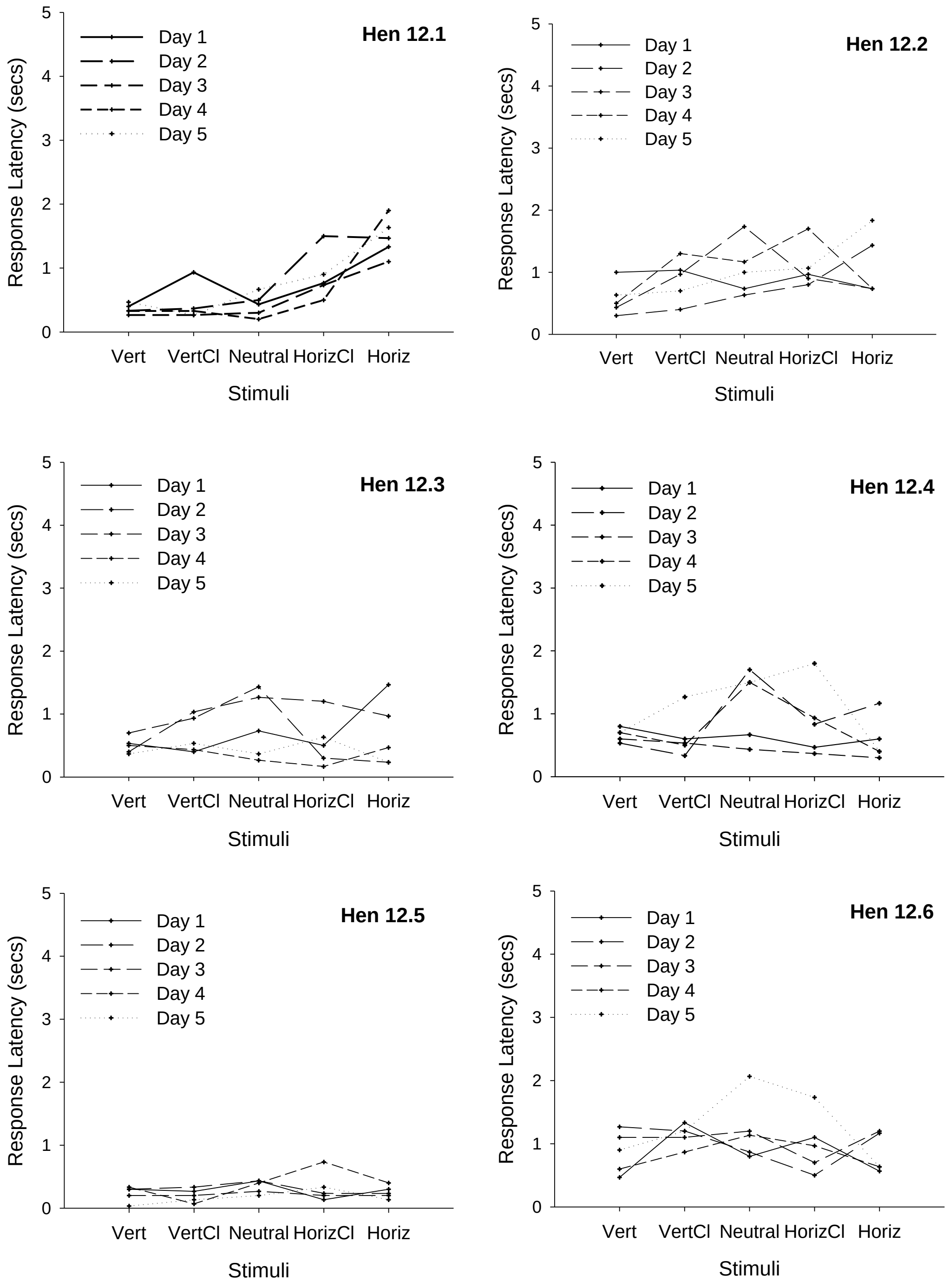


Figure 2.12. Response latencies (seconds) to probe stimuli for all hens for initial probe trials in one magazine chamber (Condition 1, pellets).

Response latencies in initial probe trials (Condition 1 - see figure 2.12): Hen 12.1 shows a clear trend in responses to the five probe stimuli, with shorter latencies in responding to the vertical stimulus, latencies increasing over the three intermediate stimuli, and the longer latencies in responding to the horizontal stimulus. Hen 12.2 also shows the same trend in the shorter latencies in responding to the vertical stimulus, and longer latencies in responding to the horizontal stimulus, however with more variance to the intermediate stimuli. Hens 12.3 and 12.4 show similar response trends with a very small range of response latencies to the vertical stimuli and this range increasing for response latencies to the horizontal stimuli, however the response latencies are mixed between these two extreme stimuli. Hen 12.5 demonstrates the fastest response latencies across all stimuli, all under 1 second, but also very stable tracking of behaviour. Hen 12.6's response latencies are not dissimilar to that of 12.3 and 12.4 although range slightly more in response latencies to the vertical stimulus.

Response latencies in final probe trials for hens 12.1 – 12.3 (Condition 9 - see figure 2.13): Hen 12.1's response latencies are all under 1.5 seconds with response times to both vertical and vertical close remaining under 1 second as seen in the initial probe trials. The most variability is seen with response time to the neutral stimulus (0.2-1.3secs). In all but one trial the vertical stimulus has been responded to faster than the horizontal stimulus. Hen 12.2 shows delayed response times of over 3 seconds on day one of these trials to the vertical close and horizontal close, all other responses remain under 2 seconds, with variability across all stimuli, and response times to the horizontal stimulus being slightly less than, if not the same, as to the vertical stimulus. Hen 12.3 shows a fast response time across all stimuli of under 1 second, except for two responses on day four between 1 and 2 seconds. All responses to the vertical stimulus are all slightly faster (with one the same) than that of the horizontal stimulus.

Response latencies in final probe trials for hens 12.4 – 12.6 (Condition 7 - see figure 2.13): Hen 12.4 shows variability in response times across all stimuli from 0.4 to 4.5 seconds, although response times overall to the neutral stimulus show the most delay. Hen 12.5's responses show an increase in time and variability across all stimuli in comparison with the initial probe trials, however all remain under 1.5 seconds. Hen 12.6 shows consistent responses to the neutral and horizontal stimuli, and variability of response times to the remaining stimuli with two responses on day four taking over 5 seconds. While hens 12.1, 12.3 and 12.5 show relatively contained response times and variation, and hen 12.2 with the exception of the two outlier response times, there is no systematic commonality seen for hens 12.4 and 12.6 over these five days.

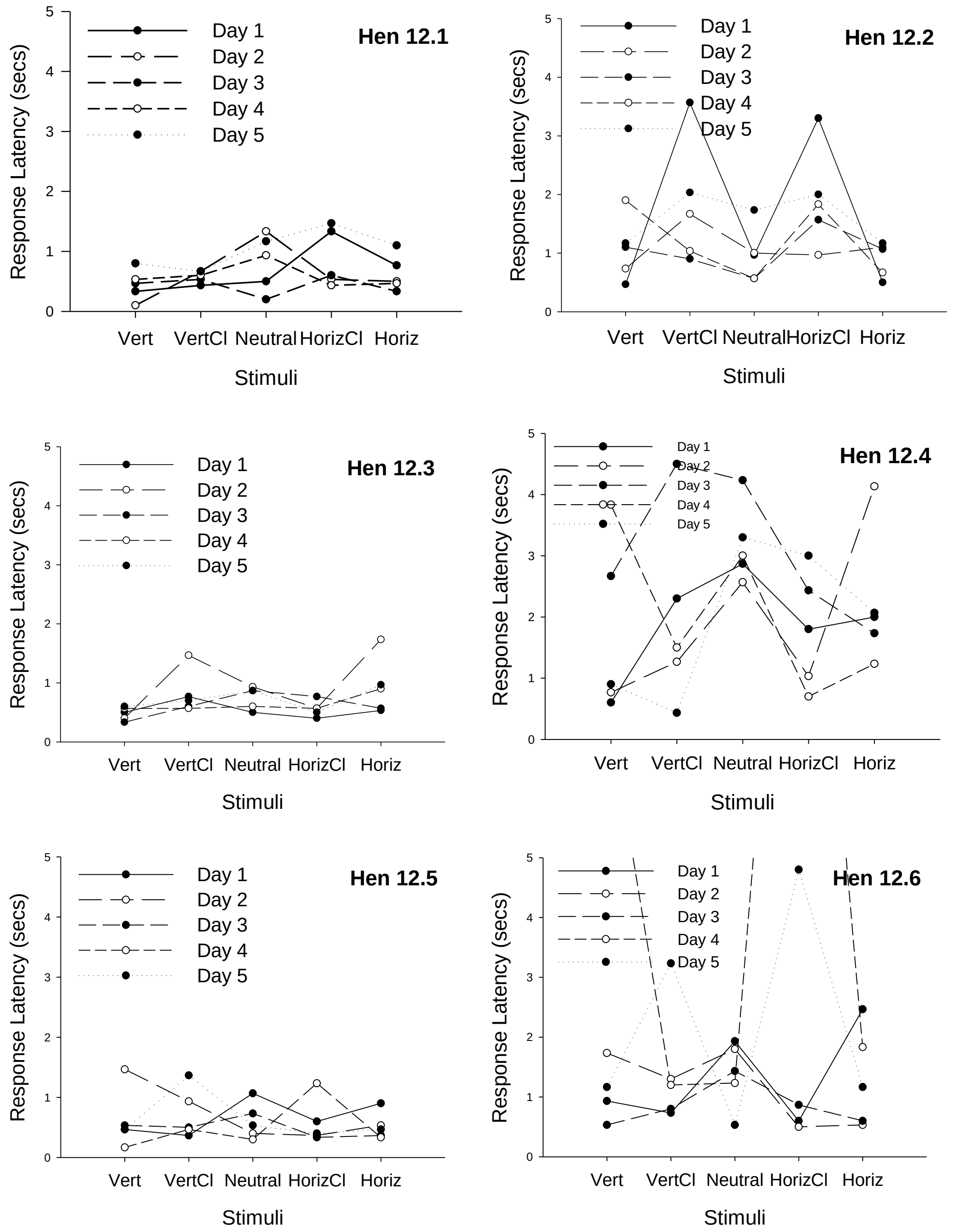


Figure 2.13. Response latencies (seconds) to probe stimuli for all hens for final probe trials in double magazine chamber (hens 12.1-12.3 condition 9, puffed wheat vs. wheat and hens 12.4-12.6, condition 7, wheat vs. puffed wheat).

A comparison of the initial and final probe trial latencies reveal changes in response behaviour. Hen 12.1's response times to the learned discriminations have decreased, Hen 12.2's responses to vertical have increased however horizontal stimulus latencies have reduced. Hen 12.3 has consistent response latencies for both initial and final probe trials. With the knowledge that wheat is preferred over puffed wheat, it's expected that response latencies to the horizontal stimulus may be less than that of the vertical, however this is only demonstrated by hen 12.2. Both hens 12.1 and 12.3 show reduced response latency to puffed wheat (long) than to wheat (short). This may be due to the learned association that the vertical key provides the longer food access time and is therefore considered the high reward option. However, on review of the probe trial proportion response vertical for puffed wheat vs. wheat, it's noted that hens 12.1 (see figure 2.6) and 12.2 (see figure 2.7) have responded horizontal to almost all stimuli, therefore latency response time is not based on a correct response, but a response time only. Ergo the unexpected reduced latencies to respond vertical, have likely been an incorrect 'horizontal' response to the vertical stimulus, indicating preference for the wheat option. Hen 12.3's normal curved data shown in the probe trial proportion response vertical for both pellets and puffed wheat vs. wheat reveals negative affect in both conditions 1 and 9 as well as consistent response latencies in the initial and final probe trials and supports validity of these findings for this hen.

Hen 12.4 shows changes in response latencies from first probe trials to final probe trials where latencies have increased to the learned discriminations. Hen 12.5 revealed the fastest response latencies to both vertical and horizontal stimuli at less than 0.5 seconds in the initial probe trials, however these increased slightly, but otherwise consistent on the final probe trials. A large increase in latency to vertical was demonstrated by hen 12.6 in final probe trials and a small increase with horizontal. Knowing that wheat is preferred over puffed wheat, response latencies were expected to be less to the vertical (wheat) stimulus than that to the horizontal (puffed wheat) stimulus. The opposite is seen with all three hens, with reduced latencies to the horizontal (puffed wheat) stimulus. For all three hens, most stimuli were responded to as vertical indicating a strong preference for the wheat option and yet on presentation of the vertical stimulus, response latency is longer to the vertical stimulus than the horizontal.

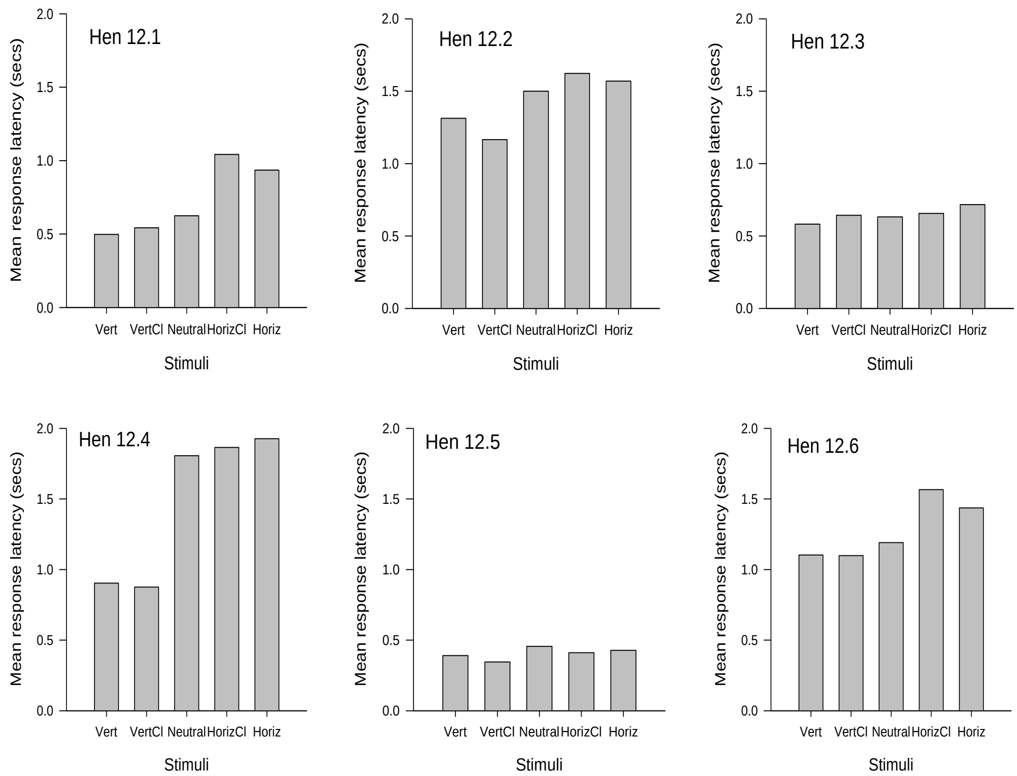


Figure 2.14. Average response latency (seconds) to stimuli for all probe trials.

Overall, all hens demonstrate faster response latencies to the vertical and vertical close stimuli to that of the horizontal and horizontal close stimuli. The differences for each hen between the vertical and horizontal stimuli range from 0.04 secs (hen 12.5) to 1.02 secs (hen 12.4). Four (12.1, 12.2, 12.4, 12.6) of the six hens reveal the neutral stimulus with the median response latency. Both hens 12.1 and 12.6 show a shorter latency to the neutral stimulus, similar to vertical and vertical close responses. Both hens 12.3 and 12.5 show a very level and stable pattern of latencies with minimal variance, however hen 12.3 shows similar response latencies across all stimuli. Hen 12.4 shows a longer latency to the neutral stimulus, similar to horizontal and horizontal close responses, and also the most variance in response latency with a significant effect ($p<0.05$). Following a failed normality (Shapiro-Wilk) test ($P < 0.050$), a one sample t-test revealed a significant difference ($t=6.152$) between response latencies.

DISCUSSION

The discrimination task took 14 – 118 days (2-16 weeks) to learn, revealing large individual differences in learning the skill. This considerable variation and long period of time indicates this was a difficult discrimination. Several changes of schedule and hens were required in order that the discrimination accuracy criterion was met, which ensured stimulus control and probe trial response validity. Several factors may have contributed to this longer than anticipated learning period, including the reinforcement schedule and discriminative stimuli. With only 50% of the subjects initially successfully learning the discrimination using the correction method, reservations regarding its use resulted in removal and a non-correction method implemented. Due to accuracy decreasing for three hens on the non-correction method, the side allocation intermittent reinforcement schedule was then introduced.

It's possible that the three hens that didn't learn the discrimination, had learned inappropriate response chains from the correction method where they were immediately reinforced when a chance correct response was made, and when not reinforced, they followed the habit of swapping their response to the other key and receiving delayed reinforcement (i.e. peck A, and if no reinforcement, peck B and vice versa, peck B, and if no reinforcement, peck A). It may be that some feature of the discrimination was problematic for these three hens, but it's also possible that no attention was given to the visual stimuli, but ultimately the cause is unknown.

The stimuli used in the discrimination task differed by only one feature, the angle of presentation/orientation. Similarity in stimuli negatively effects the speed of discrimination learning. If stimuli differ in several features, providing redundant cues, acquisition is faster than if they differ in only one feature (Mackintosh, 1974). In most discrimination tasks the animals will learn to focus on relevant dimensions (Pearce, 1987), this becomes problematic when both stimuli have the same dimensions (two short sides, two long sides) and the same colour (black), and the same colour surround (white) and both indicate reinforcement. It could be argued that the white background to the black rectangles was an extra cue for discrimination, increased volume of white showing to the left and right for the vertical rectangle, and increased volume of white showing top and bottom for the horizontal rectangle, however it's uncertain if any of the hens applied this extra cue.

Aspects known to expedite discrimination learning were included in the method; a continuous reinforcement schedule, differential reinforcement and correction procedure. A continuous reinforcement schedule is useful when shaping a new behaviour or a chain of responses and typically leads to rapid learning (Chance, 1999), however only one hen could be considered to have learned rapidly in this experiment.

Differential reinforcement was implemented through the contingency of 3 seconds access to food vs. 0.75 seconds. The differential outcomes effect is found where the use of different stimuli requiring different responses with differing consequences, results in faster discrimination learning and a higher level of accuracy (Chance, 1999). Again, only one hen met the criteria for faster learning.

Shettleworth (1998) suggests the correction method may be useful in go/no-go discrimination training with successive presentations, where reinforcement of chance correct responses outweighs the effects of unreinforced trials, and correction procedures are helpful in exposing the animal to the associations that are to be learned. It's considered that the correction method contributes to slow learning as essentially the animals are always reinforced, albeit delayed, in the event where an incorrect response is always followed with a correct response. A study by Leising, Wolf & Ruprecht (2013) found that a brief implementation of the correction method was beneficial in discrimination training for rats that had not learned the discrimination with the non-correction method.

Kalish (1946) investigated the differences between the correction method and non-correction method and found that the non-correction method was the best option for discrimination learning as the correction method allowed animals (rats) to correct their errors by not learning the two responses, but learning alternatives in which to reach their goal (reinforcement) due to both possible behaviour sequences ending with the same goal, effectively learning a response chain (Sutherland & Mackintosh, 1971). Besch, Morris & Levine (1963) also undertook a comparison study between the correction and non-correction methods and found the non-correction method to be the most effective.

Several conclusions were provided by the Happy Hen pilot suggesting modifications to methodology and outcome of the judgement bias procedure due to not being able to determine any judgement bias effect using the alternative enriched and barren housing. The recommended changes included addressing the negative cue as not being ‘negative’ enough, the task being too complex, and recommending a simpler and shorter method. These findings were addressed in the current study as follows.

The negative food access time was reduced from 1 to 0.75 seconds. Magnitude discrimination research has shown that animals have the ability to detect differences in quantities, size, length, duration, and concentration of comparative stimuli. With the reduction from 1 to 0.75 seconds this increased the ratio of difference in the food reinforcement period from 1:3 to 1:4 (0.75 secs vs. 3.00 secs). This change increased reinforcer discriminability as hens were able to peck foods five or six times with a correct response to the vertical stimulus, however the decreased food access duration limited hens to one peck at the food with a correct response to the horizontal stimulus.

Task complexity was addressed in the first instance by reducing the number of intermediate stimuli to be presented during probe trials. The Happy Hen pilot presented seven probe stimuli and the current study presented five. The presentation schedule was also modified, reducing the number of ambiguous and unreinforced stimuli presented, and increasing the learned stimuli presentations to a richer 80% reinforcement schedule. A further change made to the methodology included removal of the housing condition as the current study relied on evocation of affect from presentation of the positive or negative stimuli.

Any future studies that may include the same discriminative stimuli requires consideration of stimuli modification, as an improvement in the discrimination learning would be beneficial in reducing in what was an extensive learning period for three of the hens. One possible option includes adding a colour dimension to the discriminative stimuli (green vertical rectangle, red horizontal rectangle) and fading out the colours to facilitate more rapid discrimination learning. Colour discriminations are easier for hens to master than shapes (Patterson-Kane, Nicol, Foster & Temple, 1997). Railton, Foster & Temple’s (2014) study demonstrated five out of six hens took just 24 days to master discrimination between two pieces of lego, one red rectangular and the other green triangular in shape. A further discrimination between two pieces of lego that differed in shape but similarly coloured took 20 – 95 days to learn, although two of the six hens did not learn this discrimination. Another option would be to present one dark/black rectangle and the other the same colour but in a much lighter shade and the ambiguous stimuli be shaded incrementally in the intermediate hues. Learning a difficult discrimination may be commenced with the training of an easy but related one (Shettleworth, 1998), therefore a further option may be to train only one stimulus vs no stimulus at a time, prior to adding the second stimulus.

But yet another aspect requiring consideration is the reinforcement contingency schedule that is applied during the discrimination learning process. The non-correction method may be a preferred procedure to commence learning, as only correct responses will be reinforced on a continuous reinforcement schedule, enabling the most rapid learning of behaviour (Chance, 1999). Incorrect responses that result in no reinforcement aid discrimination learning as well as extinguishing these responses. Once discrimination response accuracy is well above chance and stable, introducing the side allocation intermittent reinforcement procedure as the partial reinforcement schedule to ensure ongoing high accuracy rates and reliable responding would be another viable option.

Overall in the single magazine chamber, four hens demonstrated positive affect with positive judgements made to ambiguous intermediate stimuli representing the expectation of a positive event, and two hens demonstrated negative affect due to the negative judgements to ambiguous intermediate stimuli with an expectation of a negative outcome. The consistency of responses across all three food conditions supports the judgement bias testing as being a robust and reliable method. As found in human studies, optimism (positive affect) is revealed when the likelihood of a positive outcome is overestimated and the likelihood of a negative outcome is underestimated. Conversely pessimism (negative affect) is revealed where there is a tendency to predict a realistic or poor outcome as found respectively with those who have mild and severe depression.

Mendl et al (2009) suggest that ambiguous cues perceptually close to that of the trained cue can elicit the same response due to the learned cue association and expected outcome. The findings in this study indicate this also as all hens revealed consistently similar responses to both the vertical and vertical close stimuli, and also to both the horizontal and horizontal close stimuli. In the situation where both vertical stimuli and both horizontal stimuli reveal a similar response tendency, it may be that the absolute intermediate cue (the neutral stimulus in this study) could be utilised as the indicator of positive/negative affect.

Following the chamber modification from one to two magazines, there was an expectation that data would change with the combinations of foods that may include a preferred food on low reinforcement (0.75 secs access) and a less preferred food on high reinforcement (3 secs access) and vice versa. However it was found that the measure of preference dominated the findings within the two magazine chamber, limiting the opportunity to reveal judgement bias.

A possible limitation found with the ambiguous intermediate stimuli is that they are only ambiguous during initial probe sessions, following which they lose their ambiguity and are intermediate stimuli only. As responses to these stimuli will never be reinforced, there is potential for animals to learn this association which may result in slower response times or a no approach to the ambiguous cues. Implementation however, of a partial reinforcement schedule in the training and habituation periods negates any potential response difficulties.

Response times to the learned and ambiguous stimuli are useful in supporting whether stimuli is being considered as positive or negative. Although responses to ambiguous stimuli tend to reveal an increasing response latency incrementally from the vertical to the horizontal stimuli, this pattern was not found in this study with results varying between and within hens. Overall however, the average response latencies for probe trials revealed a consistent pattern of reduced (fastest) response latency to both vertical stimuli (vertical and vertical close) and increased (slowest) response latency to both horizontal stimuli (horizontal and horizontal close) between all six hens. These findings add weight to the evocation of affect through the learned association of high (positive) and low (negative) reinforcement.

Over the course of this research, two features were identified as necessitating further assessment; the reinforcement schedule and modification of discriminative stimuli to support accelerated discrimination learning. The mastery criterion applied, the number of stimuli (two learned and three intermediate) and trials per session were appropriate in eliciting meaningful responses in the one magazine chamber. Evocation of affect from the differentially reinforced cues was established as confirmed by judgement bias indicators and the correlating response latencies. Judgement bias was identified within all six subjects with four hens demonstrating positive judgements and two hens demonstrating negative judgements, supporting the one magazine chamber and methodology as a suitable measure for judgement bias. In future research it may be useful to consider repeating the single magazine chamber probe trials, after a period of time, to detect changes or consistency in judgement bias. The conflicting responses of either judgement bias or preference in the two magazine chamber reduced the utility of the added magazine and differing foods, however could be better utilised in a judgement bias experiment with magazines containing the same foods.

REFERENCES

Adolphs, R. & Damasio, A.R. (2001). In Forgas, J.P. (ed) *Handbook of Affect and Social Cognition.* Lawrence Erlbaum Associates, London UK.

Bateson, M., Desire, S., Gartside, S.E., & Wright, G.A. (2011). Agitated honeybees exhibit pessimistic cognitive biases. *Current Biology, 21,* pp 1070-1073.

Bateson, M. & Matheson, S.M. (2007). Performance on a categorisation task suggests that removal of environmental enrichment induces ‘pessimism’ in captive European starlings *(Sturnus vulgaris). Animal Welfare, 16.*

Bernardi, F., Fregonesi, J., Winckler, C., Veira, D.M., von Keyserlingk, M.A.G. & Weary, D.M. (2009). The stall design paradox; neck rails increase lameness but improve udder and stall hygiene. *Journal of Dairy Science* 92, pp 3074-3080.

Besch, N.F., Morris, H. & Levine, S. (1963). A comparison between correction and noncorrection methods in drive discrimination. *Journal of Experimental Psychology, 64,* 4, pp 414-419.

Bethell, E.J. (2015). A “How-To” Guide for Designing Judgment Bias Studies to Assess Captive Animal Welfare. *Journal of Applied Animal Welfare Science, 18,* 18-42.

Bodson, L. (2008). Motivations for pet-keeping in Ancient Greece and Rome: A preliminary survey, pp27-41, in *Companion Animals and Us* (Podberscek, A.L., Paul, E.S. & Serpell, J.A. eds, 2005). Cambridge University Press, Cambridge, UK.

Boleij, H., van’t Klooster, J., Lavrijsen, M., Kirchhoff, S., Arndt., S.S. & Ohl, F. (2012). A test to identify judgement bias in mice. *Behavioural Brain Research, 233,* pp 45-54.

Briefer, E.F. & McGellicott, A.G. (2013). Rescued goats at a sanctuary display positive mood after former neglect. *Applied Animal Behaviour Science, 147,* pp 45-55.

Briefer Freymond, S., Briefer, E.F., Zollinger, A., Gindrat-von Allmen, Y., Wyss, C. & Bachmann, I. (2014). Behaviour of horses in a judgement bias test associated with positive or negative reinforcement. *Applied Animal Behaviour Science, 158,* pp 34-45.

Broom, D.M. & Johnson, K.G. (1993). *Stress and Animal Welfare.* Chapman and Hall, London.

Bruce, J-A. M. (2007). *The Relation Between Preference and Demand in the Domestic Hen: Does Preference Vary With Price?* Masters Thesis, University of Waikato, New Zealand.

Burman, O.H.P., Parker, R.M.A., Paul, E.S. & Mendl, M. (2008). A spatial judgement task to determine background emotional state in laboratory rats, Rattus norvegicus. *Animal Behaviour, 76,* 3, pp 801–809.

Chance, P. (1999). *Learning and Behavior (4th ed).* Brooks/Cole Publishing Company, California, USA.

Daros, R.R., Costa, J.H.C., von Keyserlingk, M.A.G., Hötzel & Weary, D.M. (2014). Separation from the Dam causes Negative Judgement Bias in Dairy Calves. *PLOS One, 9,* 5.

Dawkins, M. S. (1995). How do hens view other hens? The use of lateral and binocular visual fields in social recognition. *Behaviour, 132*, 591–606.

Dawkins, M.S. & Woodington, A. (1997). Distance and the presentation of visual stimuli to birds. *Animal Behaviour, 54,* pp 1019–1025.

Douglas, C., Bateson, M., Walsh, C., Bédué, A. & Edwards. S.A. (2012). Environmental enrichment induces optimistic cognitive biases in pigs. *Applied Animal Behaviour Science, 139,* pp 65-73.

Edwards, L. & Bizo, L. (2012). Presentation at the Unitec Auckland NZ, Research Symposium 2012. *Developing a methodology for assessing judgement bias in laying hens*.

Fisher, W.W., Piazza, C.C., Bowman, L.G., Hagopian, l.P., Owens, J.C. & Slevin, I. (1992). A comparison of two approaches for identifying reinforcers for persons with severe and profound disabilities. *Journal of Applied Behavior Analysis, 25* , pp 491-498.

Flevill, A. J. (2002). *The Relation Between Preference and Demand in the Domestic Hen.* Unpublished Masters Thesis, University of Waikato, Hamilton, New Zealand.

Flower, F.C., Sedlbauer, M., Carter, E., von Keyserlingk, M.A.G., Sanderson, G.A., & Weary, D.M. (2008). Analgesics improve the gait of lame dairy cattle. *Journal of Dairy Science* 91, pp 3010-3014.

Forgas, J.P. (1998a). On being happy and mistaken: Mood effects on the fundamental attribution error. *Journal of Personality and Social Psychology, 75*, pp 318-331.

Forgas, J.P. (2000). *Feeling and Thinking: (Introduction) the Role of Affect in Social Cognition*, CUP, Cambridge, pp 1-28.

Forgas, J.P. (2001). *Handbook of Affect and Social Cognition.* Lawrence Erlbaum Associates, London, UK.

Forgas, J.P. (2012). *Frontiers of Social Psychology: Affect in Social Thinking and Behavior.* Taylor & Francis, Oxford UK.

Fraser, D. (2008). *Understanding Animal Welfare; The Science in its Cultural Context*. Wiley-Blackwell, Herts UK.

Fraser, D., Friendship, R.M. & Martineau, G.P. (1994). Aristotle on pigs: Husbandry, health and natural history of pigs in ancient Greece. Pig News and Information 15: 77N-80N.

Ganz, L. & Wilson, P.D. (1967). Inate generalization of a form discrimination without contouring eye movements. *Journal of Comparative and Physiological Psychology, 63*, 2, pp 258-269.

Harding, E.J., Paul, E.S. & Mendl, M. (2004). Cognitive Bias and Affective State. *Nature, 427*, 6972, p 312.

Hernandez, C.E., Hinch, G., Lea, J., Ferguson, D. & Lee, C. (2015). Acute stress enhances sensitivity to a highly attractive food reward without affecting judgement bias in laying hens. *Applied Animal Behaviour Science, 163*, pp 135-143.

Hodos, W. (1993). *The visual capabilities of birds*. In: Vision, Brain and Behavior in Birds (Ed. by Zeigler, H.P. & Bischof, H.-J.), pp. 63–76. Cambridge, Massachusetts: MIT Press.

Isen, A.M. & Means, B. (1983). The influence of positive affect on decision making strategy. *Social Cognition, 2*, 18-31.

Ito, T.A. & Cacioppo, J.T. (2001). In Forgas, J.P. (ed) *Handbook of Affect and Social Cognition.* Lawrence Erlbaum Associates, London UK.

Kalish, D. (1946). The non-correction method and the delayed response problem of Blodgett and McCutchan. *Journal of Comparative Psychology, 39*, 2, pp 91-107.

Keen, H.A., Nelson, O.L., Robbins, C.T., Evans, M., Shepherdson, D.J. & Newberry, R.C. (2014). Validation of a novel cognitive bias task based on difference in quantity of reinforcement for assessing environmental enrichment. *Animal Cognition, 17,* pp 529-541.

Kingdom, F. A. A. (2012) *Psychophysics.* In Encyclopedia of Human Behavior, 2nd Edition, by V. S. Ramachandran (ed.). Academic Press, an imprint of Elsevier.

Koene, P. & Wiepkema, P.R. (1991). Pre-dustbathing vocalizations as an indicator of a need in domestic hens. In: Boehnke, E., Molkenthin, V. (Eds.), *Proceedings of the International Conference on Alternatives in Animal Husbandry*. Agrarkultur Verlag Witzenhausen, Witzenhausen, pp. 95–103.

Lazareva, O. F., Shimizu, T., & Wasserman, E. A. (2012). *How animals see the world: Comparative behavior, biology, and evolution of vision*. Oxford University Press.

Lazarus, R.S. (1999). The cognition-emotion debate; a bit of history. In: Dalgleish, T., & Power, M.J. (Eds), *Handbook of Cognition and Emotion.* Wiley, Chichester, pp 3-19.

Leising, K.J., Wolf, J.E. & Ruprecht, C.M. (2013). Visual discrimination learning with an iPad equipped apparatus. *Behavioural Processes, 93*, pp 140-147.

McCowan, B. & Rommeck, I.(2007). Bioacoustic monitoring of aggression in group-housed rhesus macaques (*Macaca mulatta*). *American Journal of Primatology* 69 (Supplement 1), 47 (abstract).

McCulloch, S.P. (2012). A critique of FAWC's five freedoms as a framework for the analysis of animal welfare. *Journal of Agricultural and Environmental Ethics, December 2012.*

Mackintosh, N.J. (1974). *The psychology of animal learning.* Academic Press, New York, USA.

Mackintosh, J. & Sutherland, N.S. (1963) Visual discrimination by the goldfish: The orientation of rectangles. *Animal Behaviour, 11,* 1, pp 137-141.

Matheson, S.M., Asher, L. & Bateson, M. (2008). Larger, enriched cages are associated with 'optimistic' response biases in captive European starling *(Sturnus vulgaris). Applied Animal Behaviour Science, 109*, pp 374-383).

Mellor, D.J. (2015). Enhancing animal welfare by creating opportunities for positive affective engagement. *New Zealand Veterinary Journal, 63,* 1, pp 3-8.

Mendl, M., Burman, O.H.P., Parker, R.M.A. & Paul, E.S. (2009). Cognitive bias as an indicator of animal emotion and welfare: Emerging evidence and underlying mechanisms. *Applied Animal Behaviour Science, 118,* pp 161-181.

Messenger, J. B. & Sanders, G. D. (1971) The Inability of Octopus Vulgaris to Discriminate Monocularly between Oblique Rectangles. *International Journal of Neuroscience, 1,* pp. 171-173.

Müller, C.A., Riemer, S., Rosam, C.M., Schößwender, J., Range, F. & Huber, L. (2012). Brief owner absence does not induce negative judgement bias in pet dogs. *Animal Cognition,* pp 1031-1035.

Murphy, E., Nordquist, R.E., & van der Staay, F.J. (2013). Responses of conventional pigs and Göttingen miniature pigs in an active choice judgement bias task. *Applied Animal Behaviour Science, 148,* pp 64-76.

Neave, H.W., Daros, R.R., Costa, J.H.C., von Keyserlingk, M.A.G. & Weary, D.M. (2013). Pain and Pessimism: Dairy Calves Exhibit Negative Judgement Bias following Hot-Iron Disbudding. *PLOS One, 8,* 12.

Pace, G. M., Ivancic, M. T., Edwards, G. L., Iwata, B. A. & Page, T. J. (1985). Assessment of stimulus preference and reinforcer value with profoundly retarded individuals. *Journal of Applied Behavior Analysis, 18,* 249-255.

Panksepp, J. (1998). *Affective Neuroscience. The Foundations of Human and Animal Emotion.* OUP, New York.

Parker, R.M.A., Paul, E.S., Burman, O.H.P., Browne, W.J. & Mendl, M. (2014). Housing conditions affect rat responses to two types of ambiguity in a reward-reward discrimination cognitive bias task. *Behavioural Brain Research, 274,* pp 73-83.

Papciak, J., Popik, P., Fuchs, E., & Rygula, R. (2013). Chronic psychosocial stress makes rats more ‘pessimistic’ in the ambiguous-cue interpretation paradigm. *Behavioural Brain Research, 256,* pp 305-310.

Patterson-Kane, E., Nicol, D.J., Foster, T.M. & Temple, W. (1997). Limited perception of video images by domestic hens. *Animal Behaviour, 53,* pp. 951–963.

Paul, E.S., Harding, E.J. & Mendl, M. (2005). Measuring emotional processes in animals: the utility of a cognitive approach. *Neuroscience and Biobehavioral Reviews, 29*, 469-491.

Pearce, J.M. (1987). *An introduction to animal cognition*. Lawrence Erlbaum Associates, Hove, UK.

Pomerantz, O., Terkel, J., Suomi, S.J. & Paukner, A. (2012). Stereotypic head twirls, but not pacing, are related to a 'pessimistic'-like judgment bias among captive tufted capuchins (*Cebus paella). Animal Cognition, 15,* pp 689-698.

Railton, R., Foster, T.M. & Temple, W. (2014) Object/picture recognition in hens, *Behavioural Processes, 104,* pp. 53-64.

Reefmann, N., Kaszàsa, F.B., Wechsler, B. & Gygax, L. (2009). Ear and tail postures as indicators of emotional valence in sheep. *Applied Animal Behaviour Science, Special Issue: Animal Suffering and Welfare 118,* Issues 3–4, pp 199–207.

Rolls, E.T. (2014). Emotion and decision-making explained: A précis, *Cortex,59*, pp 185-193.

Rygula, R., Pluta, H. & Popik, P. (2012). Laughing rats are optimistic. *PLOS One*, *7*, 12.

Salmeto, A.L., Hymel, K.A., Carpenter, E.C., Brilot, B.O., Bateson, M. & Sufka, K.J. (2011). Cognitive bias in the chick anxiety-depression model. *Brain Research*, pp 124-130.

Sanger, M.E., Doyle, R.E., Hinch, G.N. & Lee, C. (2011). Sheep exhibit a positive judgement bias and stress-induced hyperthermia following shearing. *Applied Animal Behaviour Science, 131,* pp 94-103.

Schroeder, L. C. (2013). *The Effects of Altering Maintenance Diet on Food Preferences of Hens*. Masters Thesis, University of Waikato, New Zealand.

Shettleworth, S.J. (1998). *Cognition, Evolution and Behavior*. Oxford University Press, New York, USA.

Shimizu, T. & Karten, H. J. (1993). The avian visual system and the evolution of the neocortex. In: *Vision, Brain and Behavior in Birds* (Ed. by Zeigler, H.P. & Bischof, H.-J), pp. 103–114. Cambridge, Massachusetts: MIT Press.

Starling, M.J., Branson, N., Cody, D., Starling, T.R. & McGreevy, P.D. (2014). Canine Sense and Sensibility: Tipping Points and Response Latency Variability as an Optimism Index in a Canine Judgement Bias Assessment. *PLOS One, 9,* 9.

Sutherland, N.S. (1957). Visual Discrimination of Orientation by Octopus. *British Journal of Psychology, 48,* 1, 55-71.

Sutherland, N.S. (1963). Cat's Ability to Discriminate Oblique Rectangles. *Science, New Series*, *139,* 3551, pp. 209-210.

Sutherland, N.S. (1969). Discrimination in Rat, Octopus and Goldfish: A Comparative Study. *Journal of Comparative and Physiological Psychology, 67*, 2, pp 160-176.

Sutherland, N.S. & Mackintosh, N.J. (1971). *Mechanisms of animal discrimination learning.* Academic Press, New York, USA.

Tami, G., Torre, C., Compagnucci, M. & Manteca, X. (2011). Interpretation of ambiguous spatial stimuli in cats. *Animal Welfare*, *20*, pp 185-189.

Timberlake, W. (2002). Constructing animal cognition, pp105-121, in *The cognitive animal* (Bekoff, M., Allen, C., & Burghardt, G.M. editors).

Turner, E.S. (1964). *All Heaven in a Rage*. Michael Joseph, London, UK.

Wasserman, E.A. (1984) In Shettleworth, S.J. (1998). *Cognition, Evolution and Behavior*. Oxford, University Press, New York USA.

Weary, D.M. & Fraser, D (1995b). Signaling need – costly signals and animal-welfare assessment. *Applied Animal Behaviour Science* 44, pp 159-169.

Weary, D.M., Niel, L., Flower, F.C. & Fraser, D. (2006). Identifying and preventing pain in animals. *Applied Animal Behaviour Science*, 100, pp 64-76.

Wilke A. and Mata R. (2012). Cognitive Bias. In: V.S. Ramachandran (ed.) *The Encyclopedia of Human Behavior*, 1, pp. 531-535. Academic Press.

Wright, W.F. & Bower, G.H. (1992). Mood effects on subjective probability assessment. *Organizational Behavior and Human Decision Processes, 52*, 2, pp 276-291.

Zajonc, R.B. (2000). Feeling and thinking: closing the debate over the independence of affect. In: Forgas, J.P. (Ed), Feeling and thinking: the Role of Affect in Social Cognition. CUP, Cambridge, pp. 31-58.

APPENDICES

(On accompanying CD)

Appendix 1	Ethics proposal, Ethics extension
Appendix 2	Excel file: Hen weights and post feed
Appendix 3	Excel file: Discrimination task training, Probe trials and Latency data