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**METHODOLOGIES FOR ASSESSING DOMESTIC  
HENS' PREFERENCES FOR SOUNDS**

A thesis submitted in fulfilment  
of the requirements for the degree of

**Doctor of Philosophy**

in

**Psychology**

at

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by

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**WAIKATO**  
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## ABSTRACT

A series of five experiments assessed the effects of different sounds on the choice behaviour of domestic hens and chicks using a range of procedures. The first experiment compared the effects of white noise on hens' performance under multiple-concurrent schedules with those of other sounds (an alarm call, the sounds of hens feeding and a food call) with the same subjects. The sound was shown to bias responding of hens away from the keys associated with all sounds and the magnitude of this bias was largest for white noise and the food call. Different to the other sounds, white noise suppressed the responding of hens when it was present, thus confounding response allocation. In Experiment 2, a concurrent-chains procedure was used to assess hens' preferences for the same sounds used in Experiment 1. Sound was associated with one terminal-link and hens made a choice between sound and no sound alternatives in the absence of sound. The direction of noise biases from responding in the initial-links was the same across hens for one sound only (food call) but the magnitude of these biases varied. This was different from Experiment 1 but terminal-link responding was suppressed in the presence of sound, as in Experiment 1. Terminal-link entry pauses also tended to be longer in terminal-links that contained most sounds but the effect was greater with white noise and the food call. A conditioned place preference procedure was used in Experiment 3 to assess chicks' preferences for places associated with sounds, in the absence of reinforcement or sound. Baseline preferences were assessed by measuring time spent in each compartment of a 3-compartment chamber. Groups of chicks were then conditioned to either food or a sound by being confined in one compartment. The chicks' post-conditioning test sessions showed a significant conditioned place preference towards the area associated with food and away from the area associated with white noise. This experiment showed that the conditioned place preference procedure could be used to assess the conditioning effects of sound and food on domestic hen chicks and confirms the results found with white noise. As all previous experiments identified sounds that were only not preferred, Experiment 4 attempted to make a preferred sound for hens by associating a pure tone with food. Preference for this tone was assessed in the same manner as Experiment 1. The direction of preferences across subjects was inconsistent, thus this experiment was not successful. One possible reason for the inconsistent results could have been the use of a pure tone. Experiments 1 and 2 showed that white noise and the food call affected responding of hens and so it was thought possible that associating these sounds with food would increase preferences towards them. Thus, Experiment 5 used the

same procedure as Experiment 4 to associate food with white noise and a food call. Two training phases, one with food and one without food, increased noise biases away from the keys associated with sound during the preference assessment. It was theorised that the training phase made sound unpredictable and the hens no longer had control over its presence or absence, therefore making the sound less preferred and more aversive. Overall, the results of this thesis suggest that white noise is an aversive stimulus for domestic fowl and that concurrent schedules and the conditioned place preference procedure could be used with other species to assess their preferences for environmental stimuli, which would provide information that may aid in improving their welfare.

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Intensively farmed animals, including domestic fowl, are exposed to a variety of different sounds that may affect them and research has shown that some sounds may be aversive. In New Zealand, there is a large poultry meat industry as well as a large number of commercial egg producers, with an estimated 3.2 million laying hens in 2009 (EPF, 2011). When housing layer hens it is recommended, in the *Animal Welfare (Layer Hens) Code of Welfare 2005*, that exposure to loud, constant, or sudden sounds be kept to a minimum (National Animal Welfare Advisory Committee, 2005). However, there are no recommendations about the maximum decibel level of sounds or the types of sounds that layer hens should or should not be exposed to. It is likely that hens would prefer to be in the presence of some sounds and would avoid others.

There is a long standing myth that many birds can hear higher frequencies and have more acute hearing than humans that a considerable amount of research has shown to be false (Dooling, 2002). In Panel A of Figure 1, Saunders and Salvi (1993) showed that the lowest threshold for hens was between 10 and 15 dB (SPL) and between 1000 and 2000 Hz. Temple, Foster and O'Donnell (1984) found that the frequency of sounds in the most sensitive range of hearing for domestic hens was between 3000 and 5000 Hz. In comparison to humans, chickens have a higher threshold at both higher and lower frequencies. Humans have a threshold as low as -10 dB (SPL) between 2000 and 4000 Hz (Panel B in Figure 1). Domestic fowl have been shown to have a range of hearing similar to other galliform species (turkey, quail & bobwhite quail), however, domestic fowl have a lower threshold for sounds that are very high or very low in frequency (Hz) compared to other birds (Gleich & Langemann, 2011).

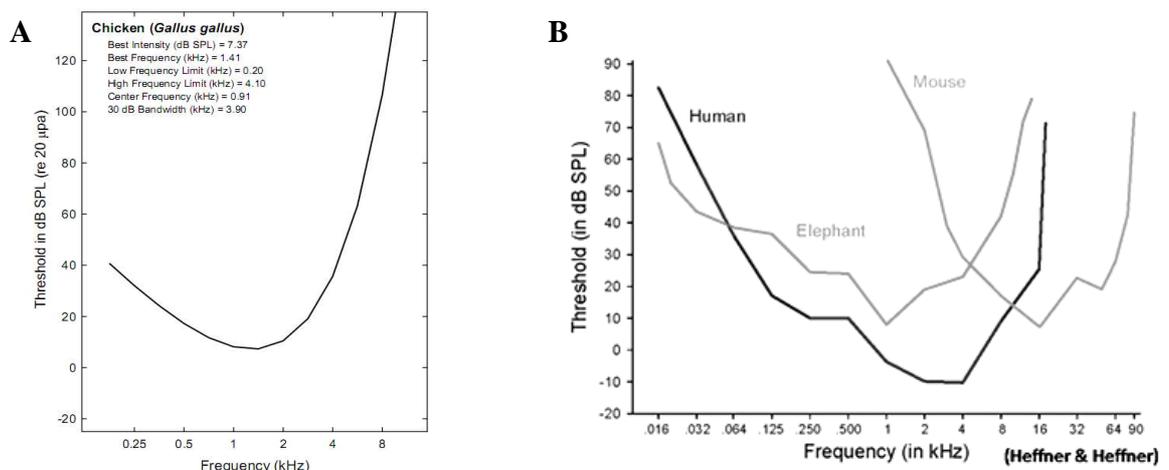


Figure 1. Panel A shows an audiogram for Chicken data derived from Saunders and Salvi (1993), cited in Dooling (2002). Panel B shows an audiogram comparing humans, elephants and mice, cited in O'Connell-Rodwell (2011).

Animals may respond to a sound as if they find them attractive or aversive, or they may be indifferent to the sound. An aversive sound is one that is avoided. Rushen (1996) has argued that aversion “can be operationally defined in terms of the ‘cost’ that an animal is prepared to pay” (p. 1993) in order to avoid a repeat of the aversive experience. Thus, the higher the cost, according to this idea, the greater the aversiveness. Nicol, Blakeborough and Scott (1991) point out that if an animal delayed its own access to reinforcement then it was paying a cost. For example, Nicol et al. found that hens reduced their response rates (i.e., delayed or gave up access to food) in order to avoid the combination of motion and noise caused by a motor. However, response rates with the noise alone did not change. The authors concluded that the motion and noise combined were aversive to these hens. In this thesis, a negative sound will be defined as a sound that is not aversive but instead is a sound that is not preferred when an animal is given an alternative (i.e., no sound). This means that an animal may prefer not to be in the presence of a negative sound but may not give up access to resources in order to avoid this sound. There are also sounds that, in certain contexts, attract animals and function as signals, for example, chicks will approach a food calling maternal hen and then peck the ground in front of the hen to search for the food (Wauters & Richard-Yris, 2002). It is likely that these sound signals are preferred to no sound. Sounds can also have a positive effect on animals and their welfare. For example, music has been shown to facilitate play behaviour in piglets (de Jong, Boleij, Baars, Dudink & Spruijt, 2008) and increase social interactions in captive chimpanzees (Videan, Fritz, Howell & Murphy, 2007). In domestic fowl, positive effects of sounds have been found from a food call, which has been shown to facilitate memory in domestic hen chicks (Field, Rickard, Toukhsati, & Gibbs, 2007; Toukhsati & Rickard, 2001), and from music, which has been reported to decrease aggression and increase productivity in chickens (Jones & Rayner, 1999).

There are a range of methods that have been used to assess animals’ responses to sounds with a variety of species and some of these have been used with domestic fowl. There can be difficulties, however, in assessing animals preferences for sounds and the appropriate procedure must be used for the type of sound that is being examined. For example, certain procedures can be used only for sounds that attract an animal while others can be used to assess sounds that animals may avoid. Other procedures assess only the damaging effects of noise. These procedures will be discussed below and, where possible, reference will be made to studies with domestic fowl.

Some research has used procedures that rely on physiological measures to assess the effects of sounds on animals. These procedures typically involve exposing an animal to sounds, usually at high intensities, and then recording blood hormone levels that are used to indicate signs of stress. For example, the ratios of heterophil to lymphocyte within the blood of chickens was found to be a reliable measure of stress (Gross & Siegel, 1983) and studies have used this measure to assess sound as a stressor for domestic fowl. Campo, Gil and Davila (2005) used measures of stress (the ratios of heterophil to lymphocyte within blood) as well as behavioural measures of fear (duration of tonic immobility) to assess the effect of noises on hens. They found that sounds of trucks, trains, and aircraft noises (at 90 dB (A)) induced more fear (longer immobility) and stress (greater heterophil to lymphocyte ratios) in birds exposed to these sounds than in birds not exposed to these sounds. Another study exposed domestic hens to slaughterhouse sounds (sounds of ventilation fans, the slaughter line, and operating carts) for 10 min (at 80 and 100 dB (A)) and found that exposure to this sound increased their plasma corticosterone levels (Chloupek et al., 2009). An increase in the ratios of heterophil to lymphocyte within blood was also found by Gross (1990) when chickens were exposed to a short duration sound (a banging metal pipe) at 104 dB (A). However, procedures that rely on physiological measures do not capture any effects of sounds on the behaviour of animals and have typically been used to assess the negative effects of sounds only.

Procedures that include escape or avoidance behaviours can also be used to assess sounds that are potentially negative or aversive. In these procedures, an animal is allowed to terminate or avoid a sound in some way and the latency to escape or move away is assumed to indicate the aversiveness of the stimulus. Such a procedure has been used to assess the effects of white noise on rats where the rats learned to escape noise in a shuttle box (Hughes & Bardo, 1981). In this study, the latency to escape decreased as the intensity of white noise increased. The avoidance of sounds has also been used with intensively farmed animals such as pigs (Hutson, Ambrose, Barnett & Tilbrook, 2000; Talling, Waran, Wathes & Lines, 1998). These studies found that pigs showed various avoidance behaviours (approach latency, turning away and longer approach distances) in the presence of a dog bark, white noise, sirens and piglet squeals (Hutson et al., 2000). Pigs were also shown to avoid a sound recording of an animal transporter when it was played intermittently (Talling et al., 1998).

Although escape or avoidance procedures can give a useful measure of animals' responses to sounds, they can be used only when the animal will escape or avoid the sound

and so, such sounds are considered negative or, perhaps, aversive. It is not clear whether faster latencies to escape or greater distances when avoiding can be interpreted as indicating greater aversiveness. Rushen (1996) points out that one limitation of procedures assessing the aversiveness of stimuli is that the animal must learn to expect the presentation of the aversive stimulus and therefore results can be affected by an animal's learning abilities. Additionally, Nicol et al. (1991) point out that an animal's avoidance of an aversive stimulus may evoke an incompatible species-specific fear reaction, such as freezing (Bolles, 1970), and this must be considered when deciding the type of technique used to assess aversiveness.

As pointed out, procedures that rely on physiological measures or on escape or avoidance have been used to assess the effects of stimuli that were generally negative or aversive. There are procedures that have been used to examine animals' preferences between a set of items or events, known as preference assessments. The most simple preference assessment involves presenting an animal with two or more alternatives and the one most often chosen, or the one the animal spends most time with, is taken as the more preferred option. Preference assessments are useful because they allow for the assessment of many different types of stimuli such as: food, social conditions, environmental events, or sounds (Sumpter, Foster & Temple, 2002). It is important to note that, regardless of the procedure used to assess preference, the resulting measures apply to only the set of alternatives offered and so are relative rather than absolute measures of preference (Duncan, 1978; Sumpter et al., 2002). Preference measures may allow one to infer that an animal prefers one environment to another, but cannot allow us to conclude that the animal likes or dislikes both environments or that the animal is suffering in the environment that is least preferred (Dawkins, 1977). Preference measures can also be influenced by an animals' previous experience with the choice alternatives, in that animals may choose to spend more time in an environment that is familiar rather than more preferred (Dawkins, 1983). A problem associated with interpreting the outcomes of preference procedures is that animals do not always choose what is in their best interests and may therefore choose an alternative that will detrimentally affect their long-term welfare (Duncan, 1992). For example, an animal may choose to eat too much and become obese as a consequence.

In spite of some of the problems associated with using preference procedures, they have proved to be useful for examining animals' responses to a wide range of stimuli, such as access to conspecifics (Tannahill, 2004) or temperature and flooring types (Vasdal, Mogedal, Boe, Kirkden & Andersen, 2010). This means that it is possible to identify the stimuli that are

most preferred by animals and this, as Dawkins (1977) points out, is important when assessing an animal's welfare. Another advantage of preference procedures is that they can allow for a graded measure of preference for the stimuli that are presented.

Observing an animal's response to sound when it is presented alone, rather than by giving the animal a choice between simultaneously presented sounds, is another way that preference for different sounds has been assessed. For example, an animal may perform behaviours when the sound is on indicating the sound has a negative or positive effect, or the animal may approach the source of the sound. One study that observed behaviour in the presence of sounds was by Pasteau, Nagle and Kreutzer (2004) who examined the song preferences of canaries. They found that canaries preferred intra-syllabic diversity (more syllables in a song) compared to intra-syllabic simplicity measured by the canaries performing more copulation solicitation displays in the presence of these songs (their indication of preference).

Other studies have used approach and time spent near a sound source as measures of preference. Preferences of quail and domestic hen chicks for maternal calls were assessed by Park and Balaban (1991). In their study, individual chicks were placed in a test cage with pressure panels located in front of two speakers. One of these speakers played the maternal calls of adult domestic hens and at the same time the other played maternal calls of adult quails. Both chicks' approach responses, as measured by presses on the pressure panel, showed that they preferred their own species' maternal call over the call of the other species. While these methods have the advantages of being simple and of being easily conducted in a laboratory setting, the proportion of time spent near or in an environment does not necessarily reflect the value of an alternative (Duncan, 1978). For example, although hens may spend a small proportion of time in a nest box laying eggs, it does not mean that access to a nest box is not important for them. Furthermore, it may not be practically possible to present some stimuli concurrently in a preference test (e.g., temperature, lighting or sounds) as the presence of one stimulus may influence the 'value' of the alternative (Kirkden & Pajor, 2006).

Preference has also been assessed by offering the animal a clear choice between two or more alternatives. In these procedures the choice may be presented using T- or V-mazes or the animal may be allowed to control whether the stimulus is present or absent. In a V-maze procedure, where a different sound was associated with each arm of the maze, cotton-top tamarins chose between species-specific feeding chirps or distress calls (McDermott & Hauser, 2004). As the animal entered one arm of the maze a sound was turned on and moving

to the other arm turned this sound off and the alternate sound on. The tamarins showed a clear preference for the arm associated with species-specific feeding chirps over the distress calls, as indicated by significantly more time spent in the arm associated with the feeding chirps. A Y-maze procedure, where only one arm was associated with a sound, has been used with cows to show that they selected the arm without sound more often than the one with milking facility noises (Arnold, Ng, Jongman & Hemsworth, 2007). Kent (1993) used a T-maze procedure to give chicks a simultaneous choice between two different sounds, each played at an end of the T-maze arms. In this study, preference was indicated by the side chosen and a chick made a choice by crossing a chalk line 35 cm from the speaker. Kent found that chicks preferred maternal clucks played at frequencies close to a normal cluck over one where the frequency had been increased by 33 %. However, presenting sounds concurrently can be problematic and it was likely in Kent's study that playing two sounds at once influenced the preference assessment.

Other 2-alternative choice preference procedures have allowed the animal control over the presence or absence of the stimulus of interest. When this is done then sound can be used to see if turning it off functions as a reinforcer (i.e., to see if the sound would serve as a negative reinforcer). This type of procedure was used in a study where mice were exposed to white noise (98 dB (A)) that they could turn off by moving off a platform-depression apparatus (Barnes & Kish, 1957). When the white noise started playing, the duration of time spent on the platform significantly decreased demonstrating that the termination of white noise at 98 dB (A) could function as a negative reinforcer.

In a similar study with hens, MacKenzie, Foster and Temple (1993) used a tilt floor to allow hens to turn a centrally located sound on or off. The hens could move freely from one end of a chamber to another where one end was associated with the sound off and the other with the sound on. MacKenzie et al. assessed the hens' responses to a variety of different sounds that ranged from pure tones and music to the sounds of other animals or machine generated sounds. When the sound played was a dog bark or the sounds of hens in a commercial poultry shed (both played at 90 dB (A)) the hens spent the majority of their time with the sound turned off. The louder the sounds of hens in a commercial poultry shed was played the more time the hens kept it off. Other sounds, such as the tones and the music, had no consistent effects on the location of the hens in the chamber, even when the sounds were increased in intensity. The authors concluded that the bark and sounds of the commercial poultry shed were aversive to these hens. In such 2-alternative choice procedures, when the

value of one alternative is greater than the other exclusive measures of preference may result. MacKenzie et al. pointed out that the fact that the proportion of time the hens spent with some sounds turned off increased as intensity of the sound was increased was surprising and unexpected as the hens had the option of simply keeping the sound off by staying at one end of the chamber.

A further method for assessing animals' preferences is based on operant conditioning. In operant conditioning an arbitrary behaviour, such as lever presses or key pecks, is controlled by consequences (i.e., reinforcers such as food) that are normally delivered according to a specified rule known as a schedule of reinforcement (see Ferster & Skinner, 1957, for a review). A schedule of reinforcement determines how frequently a reinforcer will be available. One way schedules can be used is to assess the effects of a single stimulus (such as sound) on animal's arbitrary responses (see Kilgour, Foster, Temple, Matthews & Bremner, 1991, for a review). For example, Stephens, Bailey, Sharman and Ingram (1985) trained pigs to press on a response panel to turn off either vibrations and noises presented together or the noises alone. The panel press served to escape the stimulus. This study showed that pigs would readily press the panel to turn off the compound stimulus of vibration and noise and, to a lesser degree, to turn off noise alone.

An operant procedure called passive avoidance involves baseline of responding being established and the effect of the introduction of the stimulus on this baseline is then examined. Rutter, Scott and Moran (1993) used this procedure to assess the effects of sound on responding of hens under a Variable Ratio (VR) 20 schedule of reinforcement. Under a VR 20 the number of responses required by the hen to obtain a food reinforcer varied around an average of 20 responses. Rutter et al. found that sound and the motion of a conveyor belt, and the sound alone, suppressed hens' responding. They reported that one problem with the procedure was that the measure of response suppression resulted in a floor effect where responding ceased in the presence of both sound and motion and sound alone. This meant that the authors were unable to differentiate the two levels of aversiveness between the different stimuli.

In the studies outlined above, Rutter et al. (1993) showed that sounds played alongside operant responding will suppress responding and Stephens et al. (1985) showed that an animal will turn off sounds they find unpleasant. There are studies assessing the preferences of song birds using operant procedures that allowed the animal to turn sounds on (Dobson & Petrinovich, 1973; Leadbeater, Goller & Riebel, 2005; Watanabe & Nemoto, 1998).

Watanabe and Nemoto (1998) recorded the time spent by Java sparrows on each of three available perches; one perch played no sound and when birds hopped on each of the other two perches music by different composers or white noise was played. Two Java sparrows spent more time on the perch associated with Bach than on that associated with no sound or with other composers and one bird spent more time on the perch with Bach's music than on one that turned on white noise. The authors concluded that while the sparrows showed a preference for different types of music, in that they preferred the music of one composer over the other, these preferences were specific to an individual. In a similar study, Dobson and Petrinovich (1973) found that White-crowned sparrows hopped on a perch associated with a conspecific song more than on each perch associated with white noise and that across days responding to either song "habituated", as shown by a decrease in hops to the perches associated with sound. Leadbeater et al. (2005) recorded pecks by female zebra finches on two keys, each associated with the playback of a different song motif. The birds' proportion of responding was used as the measure of preference and revealed that females preferred song motifs that did not contain inspiratory phonation (production of sound as air is taken into the lungs) over ones that did. These studies show that sound can be used in operant procedures where the animal will turn the sound on, thus demonstrating that access to sound itself can function as a reinforcer.

An operant procedure, termed concurrent schedules of reinforcement, can be used to assess preferences for different stimuli, including sounds. Under concurrent schedules of reinforcement the subject responds on two or more simultaneously available but spatially separated manipulanda, for example, keys that can be pecked. Responses made on each of these manipulanda will occasionally result in reinforcement (e.g., access to food). Concurrent schedules of reinforcement have been used to show that a loud tone had a punishing effect on rat's behaviour by suppressing their responding, especially when associated with a lean reinforcement schedule (Reed & Yoshino, 2001, 2008).

A multiple-concurrent schedule of reinforcement procedure was used by McAdie, Foster, Temple and Matthews (1993), McAdie, Foster and Temple (1996), and McAdie (1998) to assess the responses of hens to different sounds. In this procedure, two keys were simultaneously available under a Variable Interval (VI) schedule of reinforcement but the session was broken up into four 10 minute components, which constituted a multiple schedule. In the first and third components, both key lights were red and in the second and fourth components both key lights were green (the colours alternated across components). In

the red components, a sound was associated with one key (i.e., the left key), and in the green component the sound was associated with the alternate key (i.e., the right key). A response to the sound key in either component turned the sound on and it remained on until either a response to the alternative key was made or the component changed. Details of the analysis of such data will be presented later, but the procedure rests on the analysis of the degree to which behaviour is allocated to the two different schedules. A constant proportional preference for one schedule than the other, over and above any reinforcement rate differences, is termed a bias. Using the procedure outlined here allowed for the assessment of the degree of bias resulting from the sound being associated with responding on one schedule independently of any tendency to respond more on another schedule resulting from other factors (i.e., side bias).

Using this multiple-concurrent procedure McAdie and others varied the type of sound and the sound intensity. McAdie et al. (1993, 1996), using the same hens in both studies, found large biases away from a key associated with the sound of hens in a commercial poultry shed, and these biases increased as intensity of the sound was increased from 90 to 100 dB (A). Small and inconsistent biases were found with the music sample they used. McAdie (1998) used a different group of hens and found that white noise (at 105 dB (A)) also resulted in large biases in the hens' behaviour away from the key associated with the noise. Although a clear bias was shown away from responding to keys associated with certain sounds (i.e., a preference for silence), there were no consistent biases in responding towards keys associated with any of the sounds used. Thus, the hens showed either a preference away from the sound, compared to no sound, or no preference was shown.

The effects of white noise on the responding of hens found by McAdie (1998) was different from the effects of the other sounds used in the earlier studies by her. She found that white noise suppressed responding (i.e., slowed response rates in its presence) and this had not been seen for the sounds used in the earlier studies, even when large biases were generated. She also found that responding on the key associated with white noise during the seconds after the onset of white noise was suppressed more than responding on that same key after the white noise had been on for a period. This resulted in greater biases in the behaviour close to a change-over from one schedule to the other than seen in the total responding, which had not been found with earlier sounds used in McAdie et al. (1993). McAdie concluded that these two findings suggested that white noise was an aversive stimulus for hens. She suggested that this conclusion would mean interpreting the earlier data with the other sounds

as not reflective of aversiveness but rather means that other sounds were just not as preferred as no sound. However, one problem with this conclusion is that although McAdie obtained the same findings with two different groups of hens using white noise, both of these groups differed from the group used in the earlier studies and so no within hen comparison was possible. Therefore, conclusions about the effect of white noise compared to other types of sound could only be inferred. Thus, it is not known if the difference in responding between white noise and other sounds was caused by the type of sound used or simply the result of using different hens across the studies.

Another study using concurrent schedules to assess sound preferences was by Otsuka, Yanagi and Watanabe (2009). They used a concurrent-chains procedure, a procedure arranged differently from a typical concurrent schedule procedure, which will be outlined later. In their study, Otsuka et al. assessed rats' preferences for levers associated with music or other sounds. They found that preferences, as indicated by the proportion of responding on both levers, were individual across subjects but that the procedure provided a reliable way to measure preferences for sounds by rats. Although concurrent-chains procedures have not typically been used to assess animals' preferences for different stimuli, this study by Otsuka et al. showed that the procedure could be applied to assess sound preferences.

To sum, intensively farmed animals cannot escape from sounds that they are exposed to and it is important for their welfare to establish which sounds they might find aversive. A possible way to improve the welfare of these animals may be to expose them to sounds that are positive or that they prefer and to limit exposure to aversive or non-preferred sounds. Although there are a range of useful procedures for assessing the effects of sounds on domestic fowl and for assessing their preferences between sounds, not all of these procedures are appropriate for all types of sound. Some procedures are more appropriate for sounds that animals avoid or find aversive (escape or avoidance procedures) and others work better with sounds that attract an animal (measuring time spent near the sound source). Additionally, some of these procedures do not allow for the differentiation between the degrees of preference towards one sound compared to other sounds. Concurrent schedules of reinforcement have been successfully used to assess the effects of sounds on the choice behaviour of domestic fowl. They also allow for the differentiation between the degrees of preference for different stimuli because they provide information about the size of an animal's preference for one alternative over another. This means that it is possible to rank animals'

preferences (Sumpter et al., 2002). Thus, concurrent schedules are a promising procedure for identifying sounds that domestic fowl prefer or find aversive.

When assessing choice and preference in animals the most common operant procedure is concurrent schedules. Although concurrent-chains procedures have been extensively used in research, they have rarely been used to assess preferences for different stimuli (e.g., sounds). Both of these procedures can be applied to assess the effects of sounds on choice behaviour. Thus, the first aim of this thesis was to explore the use of concurrent schedules when assessing the effects of sounds on the choice and responding of domestic hens. To address this aim, the first experiment attempted to replicate and extend research by McAdie (1998) and McAdie et al. (1993), which showed that sounds biased hens' responding under multiple-concurrent schedules. Multiple-concurrent schedules were used and subjects were exposed to both white noise and other sounds in different conditions. Thus, it was possible to directly compare within subjects the effects of white noise on hens' performance under concurrent schedules with other sounds. In the second experiment of this thesis a concurrent-chains procedure was used to compare the effects of sounds on choice behaviour with those previously obtained using the same sounds. The sounds used in these experiments were selected so that they might be preferred, neutral or aversive to hens.



## EXPERIMENT 1

As previously mentioned, one way to assess hens' response to sounds is to use multiple-concurrent schedules of reinforcement (McAdie, 1998; McAdie et al., 1993, 1996). This procedure was used with hens and they showed either no biases or biases away from the key associated with the sound being on (McAdie, 1998; McAdie et al., 1993, 1996). McAdie and others suggested that these biases provided quantitative estimates of the hen's relative preferences towards or away from different sounds when compared to no sound, and gave a graded measure of hens' preferences for different sounds. That is, the larger the bias the greater the preference.

When assessing preferences using concurrent schedules, generally equal VI schedules of reinforcement are associated with the two alternatives. Under a VI schedule a reinforcer is delivered for the first response that occurs after a variable or average amount of time has elapsed since the last reinforcer was delivered (Ferster & Skinner, 1957). For example, under a VI 60-s schedule a reinforcer will be delivered for the first response that occurs after an average of 60 s has elapsed since the previous reinforcer. VI schedules are typically used because they increase the likelihood that an animal will sample both alternatives, as this maximises the rate of reinforcement delivery (Sumpter et al., 2002). The proportion of responses made and time allocated to each alternative are taken as the measures of preference.

Concurrent VI schedules can be arranged either independently or dependently. When arranged independently both schedules operate continuously throughout a session, regardless of whether a reinforcer is due on any alternative (Herrnstein, 1961). One problem with independent schedules is that an animal can receive all of its reinforcers on one alternative without ever sampling the other and therefore exclusive preference or choice may be shown by the animal (Sumpter et al., 2002). To avoid exclusive preference, experimenters commonly use dependent schedules. Here, the timing of one VI schedule stops if a reinforcer is due on the other VI schedule and is only resumed when that reinforcer has been collected (Stubbs & Pliskoff, 1969). Dependent schedules ensure that the ratios of reinforcers obtained by an animal are programmed by the experimenter and thus prevent exclusive choice to one alternative. This is because the subject must sample both alternatives in order to maximise reinforcement rates (Baum, 1979). One problem with dependent schedules, however, is that since they maintain responding on both schedules, any observed preferences seen may be smaller than the 'true' preferences (Matthews & Temple, 1979).

When using time-based schedules of reinforcement, such as VI schedules, subjects tend to switch rapidly back and forth between the alternatives (Catania, 1966). Thus, undesired adventitious reinforcement for switching behaviour may occur. To prevent this, a changeover delay (COD) is usually incorporated into the procedure (Herrnstein, 1961). A COD specifies the minimum amount of time (usually 1 to 3 s, depending on the species) that must elapse between the first response on an alternative and a reinforcer for a subsequent response on that alternative (Herrnstein, 1961).

Baum (1974) found that behaviour under concurrent VI VI schedules of reinforcement was well described by the *Generalised Matching Law* (GML). This expressed mathematically is:

$$\log \left( \frac{B_1}{B_2} \right) = a \log \left( \frac{R_1}{R_2} \right) + \log c, \quad (1)$$

where B refers to the number of responses made or the time spent responding, R denotes the number of reinforcers obtained and the subscripts 1 and 2 indicate the different response alternatives. In this equation  $a$  is a measure of sensitivity of behaviour to the reinforcement-rate differences, and  $\log c$ , termed bias, is a measure of any constant preference for one alternative that occurs over and above any reinforcement-rate differences. When the ratios of responses made, or time allocation on the alternatives, were plotted against the reinforcement ratios on logarithmic co-ordinates, the data points are well described by a straight line with a slope of  $a$  and an intercept of  $\log c$  (Baum, 1979).

When  $\log c$  is greater or less than zero, behaviour is said to be biased towards one alternative over and above any reinforcement-rate differences. This is termed inherent bias when the quality of reinforcers available on both alternatives are equal. Inherent bias is said to be due to some unmeasured factor such as differences in the response manipulanda. For example, one alternative may be easier to respond to than the other due to the degree of movement required (Baum, 1974). The researcher can also experimentally manipulate bias in order to assess an animals' preference for factors such as different food types or amounts (Baum, 1974). In such cases,  $\log c$  (Equation 1) includes a measure of both inherent bias and any bias due to variables manipulated other than reinforcement-rate. Matthews and Temple (1979) comment that it is assumed that inherent bias remains constant and, once removed from the overall bias measure, will leave a measure of bias attributable to experimental manipulation, such as different food types.

Matthews and Temple (1979) were the first to separate inherent bias successfully from bias caused by arranging qualitatively different reinforcers on the two alternatives of a concurrent VI VI schedule of reinforcement. They assessed the food preferences of dairy cows for dairy meal and chopped hay. In order to measure any inherent bias, their first experimental condition involved equal dependent concurrent VI VI schedules of reinforcement with the same food available on both alternatives. In the following four conditions, dairy meal was associated with one response alternative and chopped hay was associated with the other alternative. The reinforcement rates on both alternatives were also varied. By subtracting the initial inherent biases obtained in the first condition from the overall biases measured during the following conditions the authors were able to determine the cows' preferences for the different foods alone. The modified version of the GML proposed by Matthews and Temple (1979) that allowed separation of any inherent biases from biases resulting from experimental manipulation is expressed as:

$$\log \left( \frac{B_1}{B_2} \right) = a \log \left( \frac{R_1}{R_2} \right) + \log b + \log q, \quad (2)$$

where  $\log b$  gives a measure of inherent bias,  $\log q$  is any bias due to the qualitatively different foods and the other variables are as previously defined.

As previously mentioned,  $\log c$  can be separated into  $\log b$  (inherent bias) and  $\log q$  (bias over and above any reinforcement-rate differences) (Equation 2). A second way of calculating the separate values of  $\log b$ , and to obtain values of  $\log q$ , is to combine the data obtained in two reversed components or conditions. For example, when a preferred food is associated with the left alternative estimates of  $\log b$  and  $\log q$  can be obtained by first applying Equation 2, thus giving:

$$\log \left( \frac{P_{L2}}{P_{R2}} \right) = a \log \left( \frac{R_1}{R_2} \right) + \log b + \log q, \quad (3)$$

and also by applying Equation 2 to the preferred food being associated with the reversed alternative (e.g., the right, and assuming any bias is reversed across components) gives:

$$\log \left( \frac{P_{L3}}{P_{R3}} \right) = a \log \left( \frac{R_1}{R_2} \right) + \log b - \log q. \quad (4)$$

In the above equations,  $P_L$  and  $P_R$  refer to responses/time allocated to the left and right alternatives, respectively. The subscripts that follow these refer to the conditions/components (2 being preferred food on the left and 3 being preferred food on the right), and the other variables are as previously defined. Assuming that the reinforcement rates are equal for both

conditions, or both components, means that the log ratio of reinforcement rates will be zero for both. Thus, adding Equations 3 and 4 gives a way of calculating  $\log b$ :

$$\log b = \frac{1}{2} \left( \log \left( \frac{P_{L2}}{P_{R2}} \right) + \log \left( \frac{P_{L3}}{P_{R3}} \right) \right). \quad (5)$$

From these equations a measure of  $\log q$  can also be obtained. The degree of separation between responding to the left or right keys in reversal conditions indicates bias in responding towards the preferred food.  $\log q$  is a bias due to the preferred food over and above any reinforcement-rate differences. By using Equation 6 it is possible to get a measure of bias due to food preference only, by removing any inherent biases

$$\log q = \frac{1}{2} \left( \log \left( \frac{P_{L2}}{P_{R2}} \right) - \log \left( \frac{P_{L3}}{P_{R3}} \right) \right). \quad (6)$$

This method of analyses of bias due to a stimulus was used by McAdie (1998) and McAdie et al. (1993, 1996) to assess the bias resulting from the various sounds and white noise. McAdie and others were able to quantify the aversiveness of different sounds for hens using Equation 6, by removing the inherent and component bias (as outlined above).

In the present experiment, the sounds selected were chosen to represent sounds that may be aversive, neutral or preferred by the hens. A sound that has previously been shown to be aversive to hens is white noise. McAdie (1998) exposed hens to white noise using both concurrent and single key schedules where the noise was delivered in different ways (e.g., continuously or in bursts) that were either contingent or not contingent on the hens' responding. Their overall results showed that the behaviour of hens was biased away from the white noise, regardless of the way it was delivered. McAdie concluded that white noise was aversive to hens because its onset caused greater bias in their behaviour and because it also decreased their rates of responding. Neither effect was seen with the other sounds used in McAdie's previous experiments. Thus, white noise was selected as an aversive sound to use here. Another sound used by McAdie et al. (1993, 1996) was that of hens in a commercial poultry shed. These studies found that although this sound was less preferable to hens than no sound, it did not result in decreased response rates. They also found that bias resulting from responding that occurred during the COD timing (within-COD) was not as large as bias resulting from responding after the COD (post-COD), which was the opposite result to that found by McAdie (1998) with white noise. MacKenzie et al. (1993) had also found that all of their hens spent more than 70 % of the session time with this same sound of hens in a commercial poultry shed off, when it was played at 90 (dB (A)). Thus, the sounds of hens in a commercial poultry shed

was selected as a sound that hens find less preferable to no sound but that might not be as aversive as white noise.

It has been shown that the recordings of chick distress calls provoked more food calling in domestic hens (Hughes, Hughes & Covalt-Dunning, 1982) and elicited more clucking by mother hens (Collias, 1952). Contrary to these findings, MacKenzie et al. (1993) found that half of their hens were indifferent to the recordings of an alarm call, when it was played at 90 dB (A), while the other half spent more than 70 % of their time with this sound off, thus generally preferring not to be in the presence of an alarm call. From these contrasting results it is unclear if an alarm call would be preferred by hens to no sound or not, when it is played during multiple-concurrent schedules. Thus, this sound was selected as a sound that is neither preferred nor aversive to hens (i.e., neutral) and may be a sound that gives mixed results among subjects.

A food call has been found to attract the attention of hens. Marler, Dufty and Pickert (1986) found that hens readily approached (within at least 10 s) a rooster that made a food call upon receiving a food item. Evans and Evans (1999) played recorded food calls to domestic hens and found that the hens pointed their heads downwards and that the food calls elicited anticipatory feeding behaviour when the sound was on. Researchers have also observed hens' responses to males making food calls in a naturalistic setting (Gyger & Marler, 1988). Gyger and Marler showed that a hen's approach was highly correlated with the male food call, with hens approaching the male for on average 77 % of the calls made for an object. Additionally, van Kampen (1994) also found that hens approached a food calling male and whether the hen approached the male, and their approach speed, were influenced by the hen's peck order rank and willingness to conduct sexual behaviour, such as crouching. Based on the above studies, it seems reasonable to predict that a male's food call is a positive stimulus for hens and was selected as a sound that may be preferred by them.

The aim of this experiment was to further examine the use of a multiple-concurrent schedule of reinforcement procedure for assessing the aversiveness of, and preferences for or against, different sounds. It was also the intention to compare the effects of white noise with those of other sounds, using the same subjects. This was done by replicating McAdie et al. (1993) and McAdie's (1998) procedure and analysing the results in the same manner, using the modified version of the GML (Equation 6) to assess the bias resulting from the sounds. Each sound was associated with one key in each component and the key that it was associated with was reversed over conditions. A range of different sounds were used: white noise, the sounds

of hens feeding (a sound similar to the sound of hens in a poultry shed), an alarm call and a male food call. It was expected that noise bias ( $\log q$ ) would be towards the keys associated with the food call, away from keys associated with white noise and the sounds of hens feeding, and mixture of bias towards and away from the keys associated with the chick alarm call. Based on the findings of McAdie, it was also expected that white noise would affect within-COD bias and local rates of responding differently from the other sounds.

## **Method**

### **Subjects**

The subjects were 6 Brown Shaver hens numbered 31 to 36. They had been flock-reared and were approximately two years and eight months old at the beginning of the experiment. All hens were weighed daily and at the end of each session they were given supplementary food (commercial laying pellets), as required to maintain them at approximately 80 % of their free feeding body weight. Four of the hens (Hens 31 – 33 & 36) had previously been trained to key peck during a university undergraduate laboratory. Three of these hens (Hen 31, 33 & 36) had previous experience responding on multiple-concurrent VI schedules of reinforcement and Hen 32 had experience responding in a discrimination experiment with two keys. The remaining 2 hens (Hen 34 & 35) were experimentally naive.

All hens were individually housed in metal grid cages measuring 30-cm wide  $\times$  45-cm long  $\times$  43-cm high. The hens were supplied with grit and vitamins weekly and water was freely available. They were maintained on a day/light cycle of 7 am – 6 pm and experimental sessions were scheduled for 7 days a week. All animals were treated in accordance with the animal ethics policies and procedures of the University of Waikato Animal Ethics Committee (Ref: 639). Hen 31 died, of peritonitis from an oviduct infection, after the completion of Condition 10.

### **Apparatus**

The experimental chamber was made of woodchip particle board (2-cm thick) and internally measured 58-cm wide  $\times$  42-cm long  $\times$  54-cm high. The floor consisted of a metal grid enclosed in an open steel tray (2-cm high). Figure 1.1 shows a photograph of the experimental chamber that was used throughout the experiment. The left frame shows an internal view with the response panel and the right frame shows the exterior.



*Figure 1.1.* Internal view of experimental chamber (left frame) showing the response panel, and exterior view (right frame).

There were three Perspex<sup>TM</sup> response keys situated on the right wall of the chamber (left frame in Figure 1.1) (termed the response panel) but only the left and right keys were active during the experiment. The two active keys were 3 cm in diameter, and positioned 12.5 cm apart 40 cm above the chamber floor. Each key could be separately backlit by either a red or green LED ('MARL' brand SX6 Midget Flange 28-V dc LED, from Farnell Electronics<sup>TM</sup>). A force of at least 0.04 N on the left key and of at least 0.08 N on the right key, as measured by a Paco Scientific force meter (model SE-8714), was required to operate these keys. Each effective key peck resulted in a 0.05-s audible beep, provided by a Piezo electronic sounder located behind the key.

The food magazine was located below the response panel and allowed 3 s to wheat, when the hopper was raised, through a magazine-access hole measuring 7-cm wide  $\times$  10-cm high  $\times$  7.5-cm deep and centred 12 cm above the chamber floor. A 24V/1-W white light bulb was located at the rear of the magazine and was turned on and illuminated the food when the hopper was raised. The key lights and magazine lights were the only sources of illumination within the chamber. The experimental chamber was enclosed in a sound-attenuating box made of particle board (2-cm thick) that was lined with polystyrene 4-cm thick. The dimensions of

the box were 65-cm wide × 100-cm long × 77-cm high. The sound attenuated box sat on top of a wooden bench 72 cm above the ground.

Two 4 inch dual cone circular speakers were mounted on the wall behind the response panel, 24 cm above the chamber floor. The speakers were 25 watt, 4 ohm “pro series SK14-4” speakers and were situated 15 cm apart and 3.5 cm from the edge of the wall. The sounds were amplified by a public address TOA-430M solid-state mixed power amplifier with a power output maximum of 45 watts rms, and frequency response of 1/- 3 dB (A), over the range of 50 – 15,000 Hz Amp. The sound dB (A) levels were measured daily using a sound level meter Amplaid SLM 13. At the end of each day, after all subjects had finished a session, the sound intensity was measured. The microphone attached to the sound meter was placed in the centre of the chamber on a wooden device so that the microphone was approximately 33 cm above the chamber floor (approximate hen-head height). Readings were taken with the chamber door closed and if the dB (A) level was above or below the required level it was corrected and the correction was noted in the data book. Background noise levels measured from within the chamber ranged from 45 - 55 dB (A).

It should be noted that the intensity of the sounds used in this and all the following experiments in this thesis was measured using the dB (A) scale. The sounds used included white noise, a chick alarm call, the sounds of hens in metal grid cages during feeding and a food call. These sounds are available on the DVD attached in Appendix 1a. The white noise was relayed by a noise generator (made by Med-PC™, model no. ANL-926) that produced broadband random frequencies between 0 and 20 kHz. All other sounds were relayed using Winamp Version 2.8. The food call was taken from a video, called ‘The food call’, downloaded from <http://www.youtube.com/watch?v=K27rJ1LwbKg>. The video was of an Aracauna rooster making a food call to a group of hens who came running to him upon hearing this call. The sound loop for the food call was 5-s long. The remaining sound samples (alarm call and sounds of hens feeding) were recorded using an Iriver IFP799 Mp3 recorder and were edited into a sound loop such that there was no discernable beginning or end. The chick alarm call was a recording of a one week-old hen chick that was taken to a room alone where she made repeated calls. This sound loop was 2-s long. The sound loop of the sounds of hens feeding was 57-s long. This sound was recorded in a room (4 m × 2 m) containing approximately 50 hens in metal grid cages, and was taken in the morning as the hens were fed. The sound recorder was placed on a table in the middle of the room and recording began when the experimenter entered the room holding a container of food and the food dispenser. On a

typical day, the hens reacted to seeing the food container by making a lot of clucking noises and the sound of the hens pushing up against the cages and banging can be heard.

The experiment was controlled by a Pentium III 997 MHz computer running Med-PC™ (Version IV) and was located in the same room as the experimental chamber.

### Procedure

A multiple-concurrent schedule of reinforcement procedure consisting of four components, each lasting 10 min, was used. A diagrammatic representation of the experimental procedure is presented in Figure 1.2. During Components 1 and 3 both keys were illuminated red and during Components 2 and 4 both keys were illuminated green. At the start of a session the hen was placed in the chamber and Component 1 began. The sound was not turned on in a session until the first response to the key associated with the sound (sound key) in Component 1, in that condition. Once the sound turned on it played continuously until a response was made to the other key (off key), or the component changed (see Figure 1.2).

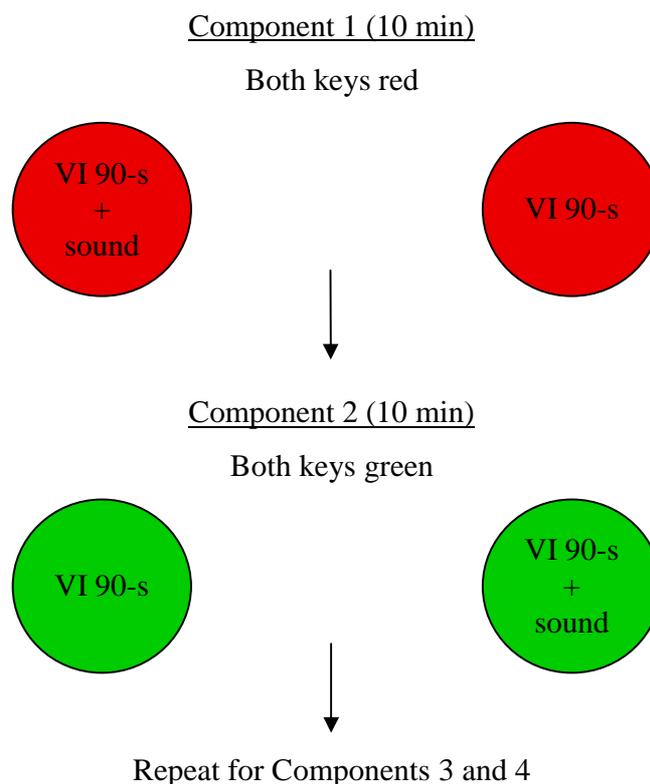


Figure 1.2. A diagrammatic representation of the experimental procedure for multiple-concurrent schedules with sound associated with one key in each component.

Independent concurrent VI 90-s VI 90-s schedules of reinforcement were in effect with a 3-s COD. The VI schedules were comprised of 15 intervals in random order from the arithmetic progressions of the form of  $j + kx$ ; where  $x = 0, 1, 2, \dots, 14$ ,  $j$  was equal to one fifteenth of the average VI length and  $k = 2j$ . The values of  $j$  were chosen to produce the appropriate mean interval in each schedule. When Component 1 was complete (i.e., 10 min had elapsed) Component 2 began with both keys changing to being lit green. This sequence was then repeated for Component 3 where both keys were lit red for 10 min followed by Component 4 with both keys lit green for 10 min. Across conditions, sound was associated with responding on one key in each component and these were the opposite keys across components. For example, the left key when the keys were red and right key when the keys were green. The alternate keys were not associated with sound. Sessions lasted for 40 min.

Conditions were changed when the data had reached both statistical and visual stability. Statistical stability was reached when the median of the proportion of responses for the last five sessions was within 0.05 of the median calculated for the five sessions prior. This had to occur on five, not necessarily consecutive, sessions. Thus, a minimum of 14 sessions was required for statistical stability. Once statistical stability had been reached, the graphs showing the logarithms of the response and time ratios plotted against sessions were visually examined. If these data paths were deemed to be not trending in any direction (as judged by two or more lab members) then the experimental condition was changed.

Before experimental conditions began the hens were trained to respond at a stable rate by gradually increasing the VI schedules and duration of the COD. The VI schedules were gradually increased from VI 30-s VI 30-s and the COD from 0 s to 3 s. Table 1.1 shows the experimental conditions, the sounds played, the decibel dB levels, and the side the noise key was on for the red and green components. In Conditions 1, 7, and 11, there were no sounds associated with either key throughout the sessions. Between Condition 1 and 2, some additional conditions were run with sound associated with one key but over these conditions the hens developed increasingly larger right key biases. The left key was tested and it was found that the force required for an effective peck was much greater than the right key. As a result the faulty left key was replaced. The data from these additional conditions are not reported. Between Conditions 7 and 8, a series of sessions were run with unequal reinforcement rates. These data are not reported. Between Conditions 10 and 11 there was a break of 2 years in which Experiment 2 was conducted. Hen 31 died during this period and did therefore not complete Conditions 11-13.

After Condition 1, the white noise was initially set at 90 dB. This was continued for six sessions and the sound level was then increased to 95 dB for six sessions. The sound level was then increased to 100 dB and when the behaviour was judged stable the condition was changed. In Condition 2, the sound associated with the left key in red and the right key in green was the sound of 'hens feeding' played at 100 dB. In Conditions 3 and 8, white noise at 100 dB was associated with the left key in the red components and associated with the right key in the green components. In Condition 4, this white noise was associated with the right key in the red and left key in the green component. In Conditions 5, 6, and 9, the white noise intensity was increased to 105 dB. The white noise was associated with the red/left and green/right keys in Conditions 5 and 8, and with the reversal of this in Condition 6. The sound used in Condition 10 was the alarm call played at 100 dB and this was associated with left key in the red and the right key in the green component. A food call, played at 100 dB, was associated with the red/left and green/right keys in Condition 12 and the opposite keys in Condition 13.

The data recorded for each hen for the red and green components were: the number of responses to the left and right keys, time (s) spent responding to the left and right keys, number of reinforcements to the left and right keys, and number of changeovers. Individual component data (components 1-4) were recorded in a separate file.

### **Data analysis.**

The raw data from the last five sessions of each condition for each hen are given in Appendix 1b. Data are described in sections and include only those taken from the last five sessions of each condition.

Responding, changeovers, and response rates from repeated conditions using the same sound (white noise), where the side of the sound key was the same across components, were averaged after analyses showed only small differences with no consistent trends across conditions or hens. For example, Conditions 3 and 8 both involved white noise at 100 dB and these data were averaged. These data were not averaged with the other white noise condition (Condition 4) with the same decibel level because the sound was associated with different keys in the components. Data from the three conditions without sound (Conditions 1, 7, & 11) were not pooled because a consistent trend was seen in the responding across subjects and so the individual conditions data are presented. For ease of analyses and interpretation, Table 1.2 shows the condition names, condition numbers, sound associated with the sound keys, decibel level of the sound, and the key associated with sound in the red and green components.

Table 1.1

*The order of experimental conditions, the types of sounds played in each condition, the decibel level dB (A), and the location of the noise key are shown in each column for each condition.*

<b>Condition Number</b>	<b>Sound</b>	<b>Decibel Level dB (A)</b>	<b>Noise Key in Red/Green</b>
1	None	-	-
2	Hens feeding	100	Left/right
3	White noise	100	Left/right
4	White noise	100	Right/left
5	White noise	105	Left/right
6	White noise	105	Right/left
7	None	-	-
8	White noise	100	Left/right
9	White noise	105	Right/left
10	Chick alarm call	100	Left/right
11	None	-	-
12	Food call	100	Left/right
13	Food call	100	Right/left

Table 1.2

*Condition name, condition number, the types of sounds played in each condition, the decibel level dB (A), and the location of the noise key are shown in each column for each condition. Conditions are shown for white noise (WN), the alarm call (AC), the sound of hens feeding (HF) the food call (FC) and for condition with no sound (NS).*

<b>Condition name</b>	<b>Condition Number</b>	<b>Sound</b>	<b>Decibel Level dB (A)</b>	<b>Noise Key in Red/Green</b>
<b>WN1</b>	Mean of 3 and 8	White noise	100	Left/right
<b>WN2</b>	4	White noise	100	Right/left
<b>WN3</b>	Mean of 6 and 9	White noise	105	Right/left
<b>WN4</b>	5	White noise	105	Left/right
<b>AC</b>	10	Chick alarm call	100	Left/right
<b>HF</b>	2	Hens feeding	100	Left/right
<b>FC1</b>	12	Food call	100	Left/right
<b>FC2</b>	13	Food call	100	Right/Left
<b>NS1</b>	1	None	-	-
<b>NS2</b>	7	None	-	-
<b>NS3</b>	11	None	-	-

## Results

### White Noise

Estimates of  $\log q$  (noise bias) were calculated (Equation 6) by combining data from response and time measures across Components 1 and 3 (red) and Components 2 and 4 (green) for each session.  $\log q$  was then calculated by taking half the absolute difference between these red and green combined data. These estimates of  $\log q$ , for both response and time allocation data, were then averaged over the last five sessions and these means are presented in Figure 1.3, which shows  $\log q$  from all white noise conditions (Conditions WN1-4) for each hen. Points below the line at zero indicate a bias away from the key associated with white noise and points above this line show a bias towards this key.

It is clear from Figure 1.3 that white noise biased the responding of the hens, but less noise bias was observed in the time allocated biases for most hens. Noise biases ( $\log q$ ) seen in the response data were away from the sound keys for 4 of the 6 subjects (Hens 32-35) in all conditions. Noise biases for the remaining 2 hens (Hens 31 & 36) were either away from the keys associated with white noise or very close to zero. The time allocation data did not show a consistent trend across hens or conditions, with noise bias both towards and away from white noise and generally close to zero for most hens. One exception is Hen 34 who showed the largest time allocation biases of the subject hens, away from the keys associated with sound. The mean absolute difference between the response and time allocation noise biases for all hens was 0.12 and 0.13 for white noise at 100 and 105 dB, respectively.

As can be seen in Figure 1.3, when white noise increased from 100 to 105 dB the noise biases from responding generally increased for 3 hens (Hens 32-34). The remaining 3 hens showed no consistent trend in noise biases across decibel levels. The mean response noise bias across hens was -0.13 and -0.18 for white noise at 100 and 105 dB, respectively.

A 3-s COD was in effect during all conditions and to analyse the effects of the onset of white noise the estimates of  $\log q$  (noise bias calculated in the same manner as above) from the response data after (post-COD) and during the COD (within-COD) were calculated. These estimates are presented in Figure 1.4 for each hen. Generally, the directions of these biases were the same for both post- and within-COD estimates and showed a similar trend to the noise biases from total responding seen in Figure 1.3, with some exceptions. Two hens (Hens 31 & 36) showed noise biases in the post-COD data towards the keys associated with white noise in two white noise conditions, opposite to their total response data that was away from the keys associated with white noise. There were, however, differences between the post- and within-

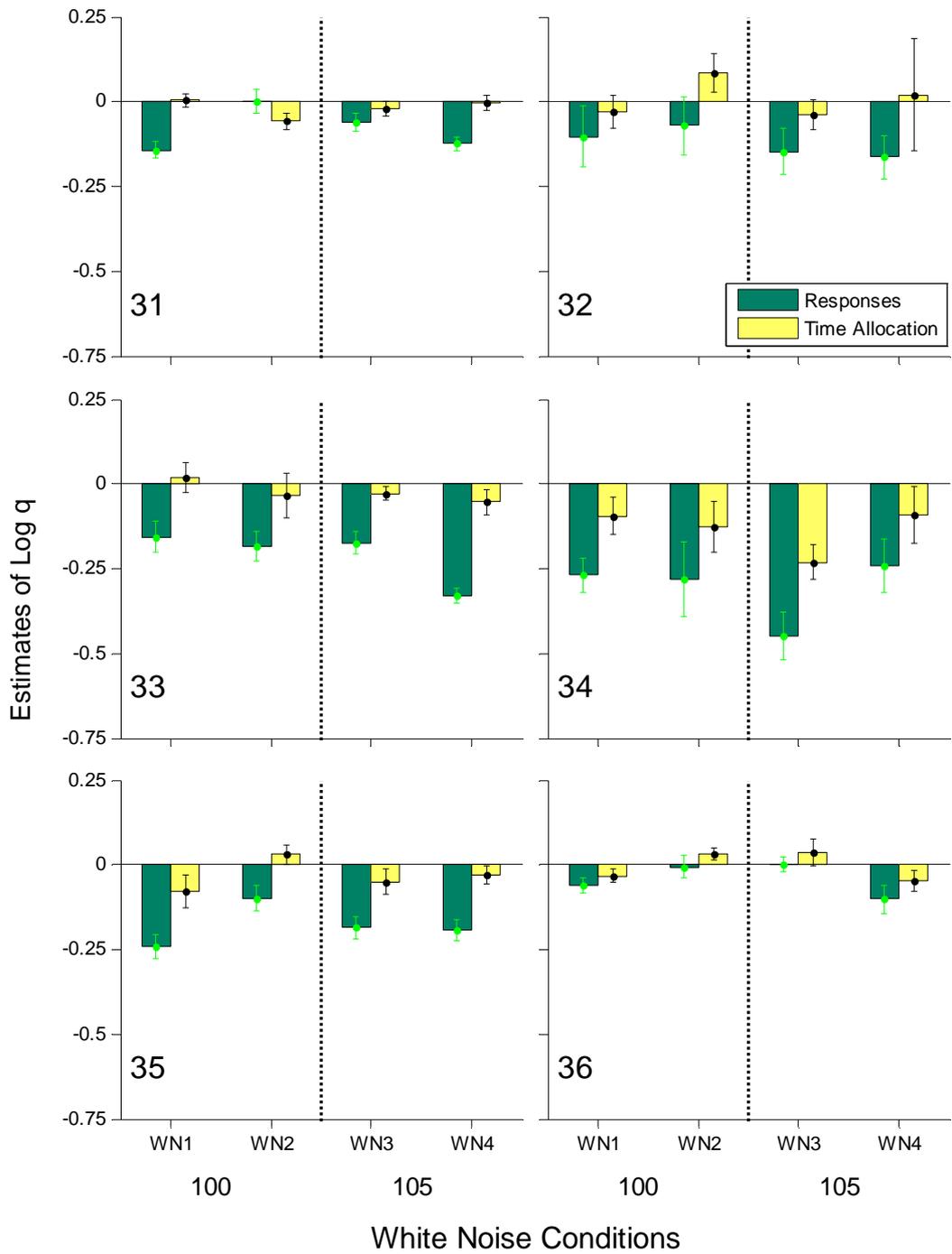


Figure 1.3. Estimates of  $\log q$  (noise bias) for responses and time allocation for white noise conditions at 100 and 105 dB (WN1-4), plotted for each hen. The error bars represent one standard deviation either side of the mean ratios

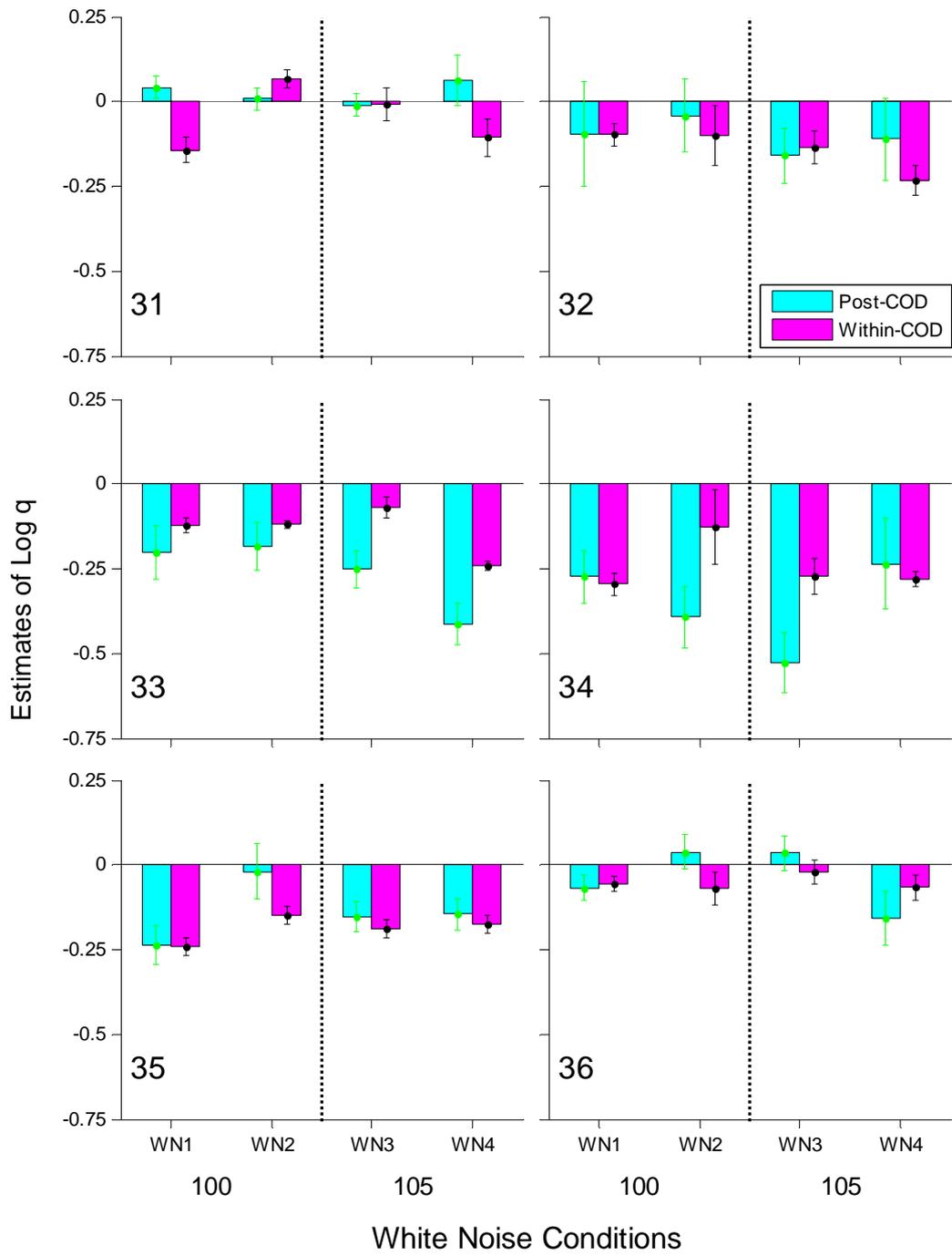


Figure 1.4. Estimates of  $\log q$  (noise bias) for responding post and within the COD across white noise conditions at 100 and 105 dB (WN1-4), plotted for each hen. The error bars represent one standard deviation either side of the mean ratios.

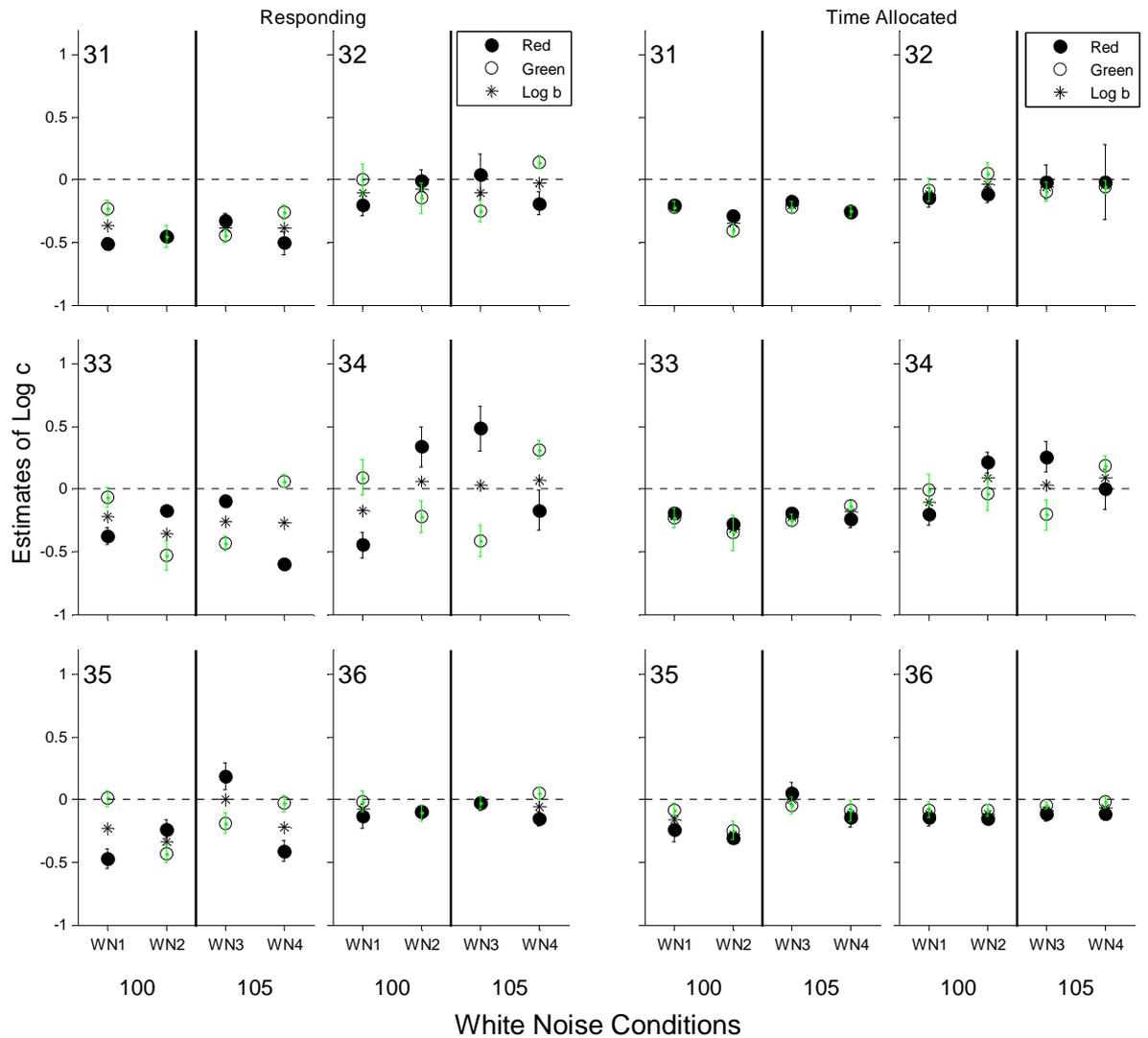
COD estimates in terms of their magnitude. For Hens 33 and 34, estimates of  $\log q$  for post-COD data tended to be larger than those from within the COD but Hens 31 and 35 showed the opposite, with generally larger values of  $\log q$  from the within-COD response data. The remaining 2 hens (Hens 32 & 36) showed a similar magnitude for both post- and within-COD estimate of  $\log q$ . The mean absolute difference between the post- and within-COD noise biases was 0.09 across all hens.

To examine key bias the estimates of  $\log c$  (Equation 1) are presented in Figure 1.5 for responding (left panel) and time allocation (right panel), for each hen. These were the mean ratios of responding taken to the left key logged (to the base 10) over the last five sessions. This figure also displays the estimates of  $\log b$  (key bias calculated using Equation 5) for each condition, indicated with an asterisk.  $\log b$  is the point halfway between the red and green components. The horizontal line at zero indicates where ratios would be expected to fall if the hens' behaviour showed no biases.

The data across the two panels in Figure 1.5 followed a similar pattern for all hens. This figure shows that the response and time estimates of  $\log c$  were predominantly towards the right key. A clear pattern can be seen where the hens showed more responding and spent more time responding to the right key in the red than the green component when it was not associated with white noise (WN1 & WN4), and still showed more responding towards the right key in the green than in the red component when it was not associated with white noise (WN2 & WN3). Thus, the hens distributed responses and time differently depending on which key the white noise was associated with.

The estimates of  $\log b$  shown in Figure 1.5 give an indication of key bias for conditions with white noise. If no key bias was shown  $\log b$  values should be zero. When  $\log b$  is below zero, the bias was towards the right key. This was the case for most of these white noise conditions. In three conditions (WN 2-4), this value was above zero but still small in magnitude for 1 hen (Hen 34). This plot also shows that key bias varied somewhat in size across hens and, for some hens, across conditions.

The mean number of changeovers (COs) per session in the red and green components across all white noise conditions are presented in Figure 1.6 for each hen. This figure shows that generally more COs occurred in the red component, regardless of condition. It also shows that 3 hens (Hens 33, 34, & 36) showed no systematic differences in the number of COs across conditions. However, the mean number of COs for the remaining 3 hens (Hens 31, 32, & 35) changed when the position of the sound key was reversed across conditions. These hens had a



*Figure 1.5.* Estimates of  $\log c$  for responses (left panel) and time allocation (right panel) in the red (filled circles) and green (unfilled circles) components for all white noise conditions (WN1-4), plotted for each hen. Estimates of  $\log b$  (key bias) were also plotted (asterisk) for each condition and hen. The error bars represent one standard deviation either side of the mean ratios.

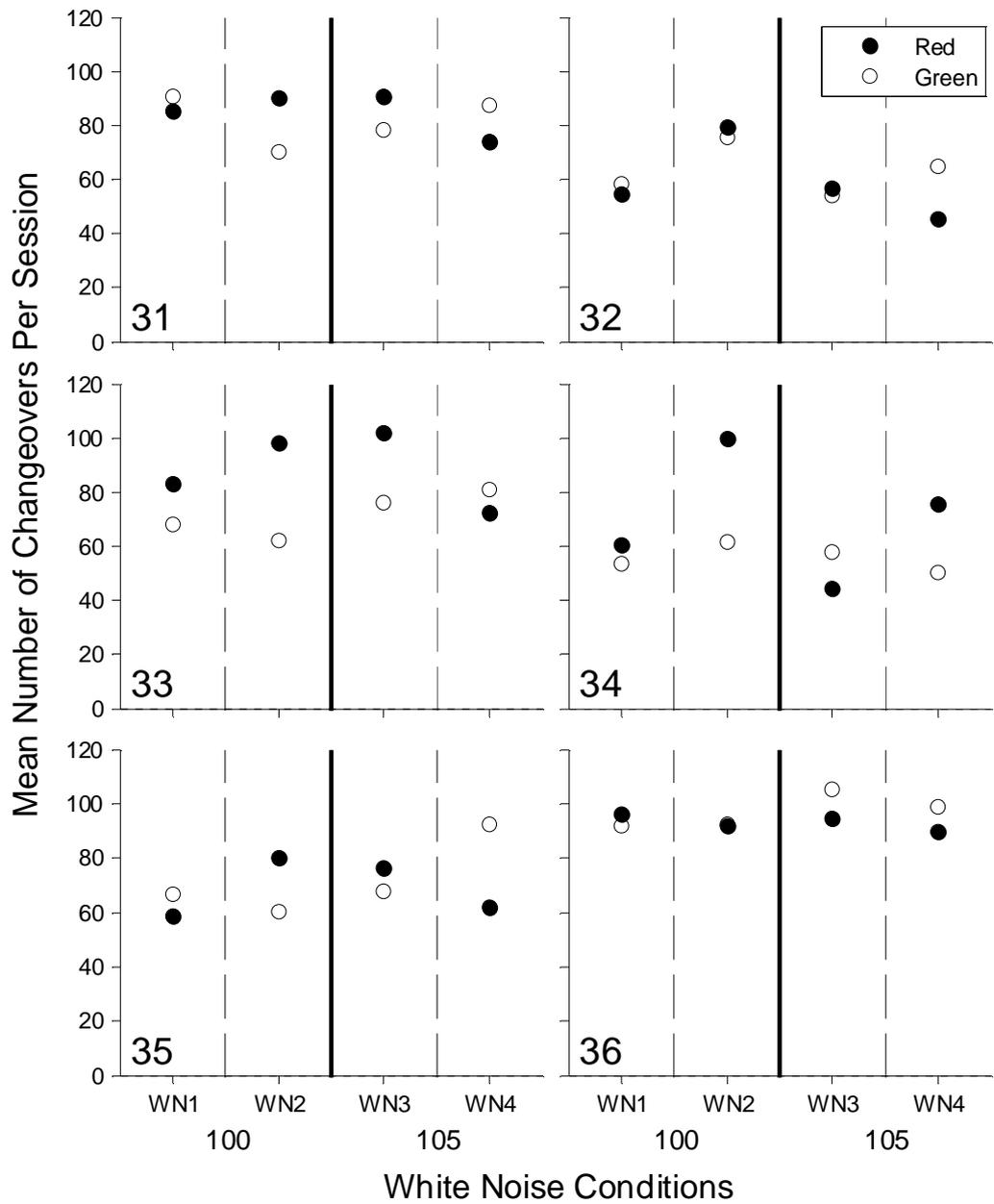


Figure 1.6. Mean number of changeovers (COs) per session in the red components (filled circles) and green components (unfilled circles), plotted for each hen for each white noise condition (WN1-4).

lower number of COs in components where the white noise was associated with the left key. For example, Hen 31 performed fewer COs in the red components when sound was on the red/left and the green/right key (Conditions WN1 and WN4). The opposite can be seen when the sound was associated with the red/right and green/left keys (Conditions WN2 & WN3) for this hen. This shows that there may have been a relationship between key bias and the number of COs for these hens. Across white noise conditions, the mean number of COs ranged from 45 to 105 and there were no systematic differences over decibel level across all hens.

The local response rates to the sound and no sound keys are presented in Figure 1.7, for each hen. Local response rates were calculated by dividing the number of responses on a key by the time spent on that key only. These response rates were calculated to the left and right key in each component (1-4) and then averaged across components for keys that were or were not associated with sound. For example, the data shown for the sound keys is the average rate of responding to the left key in the red components (1 & 3) and the right key in the green components (2 & 4). The local response rates for all hens (with the exception of Hen 31 in Condition WN2) were consistently lower on the sound keys compared to the no sound keys. Local response rates varied considerably across hens, ranging from 17 to 64 responses per min. There were no consistent trends in response rates across conditions and no consistent difference between response rates for white noise at 100 and 105 dB.

Overall, white noise, for both decibel levels, biased the behaviour of the hens away from the key associated with the noise. Across hens, time allocation noise biases ( $\log q$ ) tended to be smaller than those from responding. This means the hens divided their time between the keys more equally, but responded at a lower rate when white noise was associated with one key. The largest noise biases were seen in the post-COD response data, which followed similar trends to the total response data. The within-COD noise biases were smaller than the post-COD biases for 2 hens and in the opposite direction for 2 others. The largest biases were away from white noise at 105 dB. The number of COs per session showed no trend across conditions for 3 of the hens, and the remaining 3 hens showed a lower number of COs in components where sound was associated with the left key than in components where it was associated with the right key. Local rates of responding showed that 5 of 6 hens consistently responded at a lower rate to keys associated with white noise.

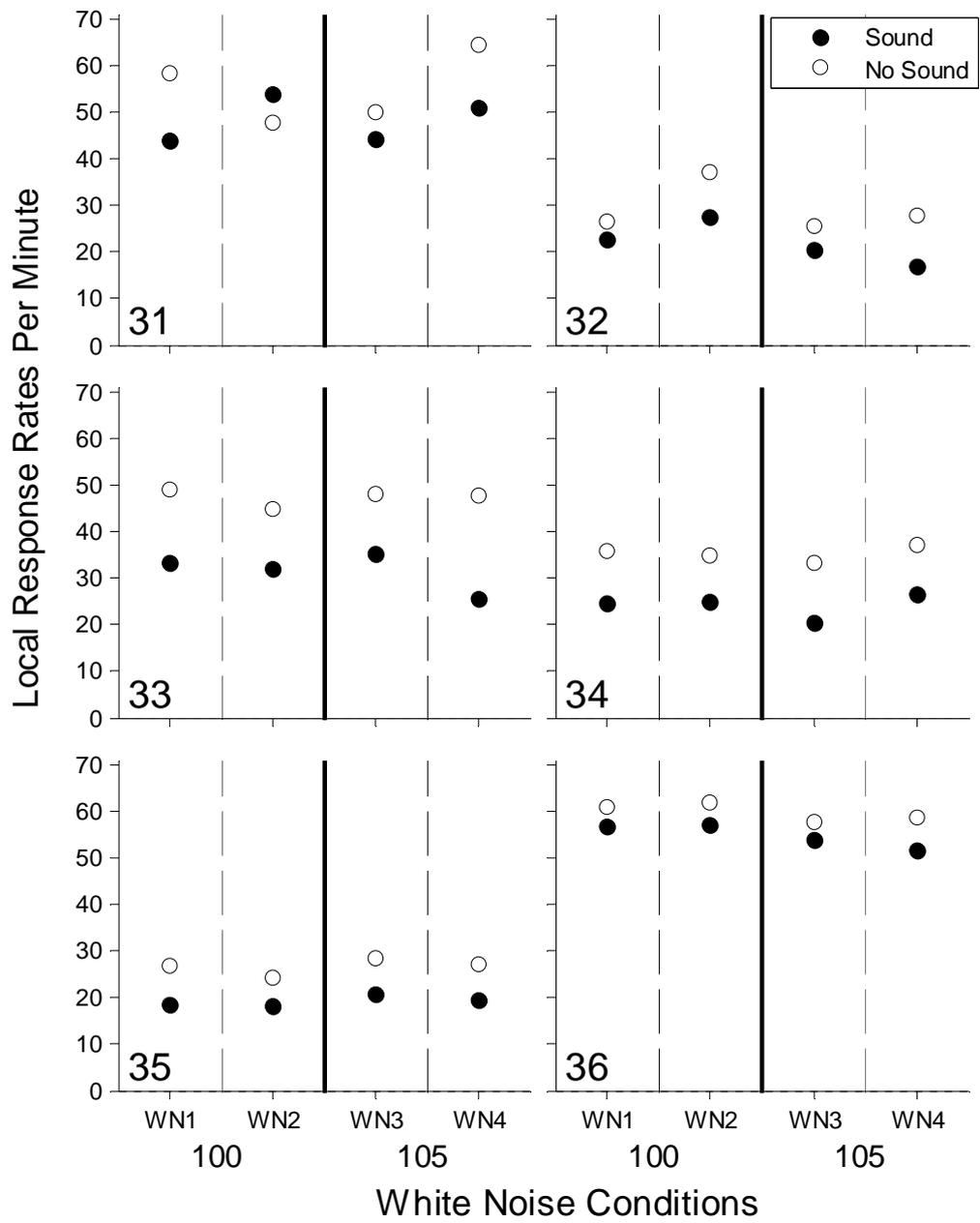


Figure 1.7. Local response rates (per min) to the keys associated with sound (filled circles) and those not associated with sound (unfilled circles), plotted for each hen for each white noise condition (WN1-4).

## Sound Conditions

The estimates of  $\log q$ , calculated in the same manner as outlined for white noise, for response and time allocation data are presented for each hen for the other sound conditions (sounds of hens feeding (HF), the chick alarm call (AC), and a food call (FC1 & FC2)) in Figure 1.8. Hen 31 died and did not complete conditions with the food call. Generally, smaller noise biases were observed in the response and time data for the sounds of hens feeding and the alarm call than for both food call conditions. This figure also shows that values of  $\log q$  for responding tended to be larger than those from time allocation for most hens. This difference was typically small with a mean absolute difference of 0.06 across conditions and subjects.

Generally, there were only small noise biases away from the keys associated with the sounds of hens feeding, where the largest response noise bias was -0.13 and the mean response bias across hens was -0.08. The time allocation noise biases for 4 hens (Hens 31, 32, 34, & 36) were also away from the keys associated with this sound, but towards it for 2 hens (Hens 33 & 35). However, most of these time allocation noise biases were also small, with the largest bias being 0.05.

When the alarm call was associated with the sound keys, noise biases for responding and time allocated to responding were close to zero for most hens, a similar result to the sounds of hens feeding.  $\log q$  estimates for the response data were away from the keys associated with the alarm call for 4 hens (Hens 32, & 34-36) and for Hens 31 and 33 they were equal to and very close to zero, respectively. The direction of the time allocation noise biases for all hens were the same as those seen in the response data, except for Hen 31 who showed a small time based noise bias towards the key associated with the alarm call. The largest noise bias shown across both measures for the alarm call was -0.08 and the mean noise bias across hens for the alarm call was -0.04. Estimates of  $\log q$  for responding were smaller for the alarm call than those of the sounds of hens feeding for all hens except Hen 35.

In the food call conditions, there were typically larger noise biases for all hens (especially in FC1) than were shown for the alarm call or sounds of hens feeding. Four of the 5 hens (Hens 32, & 34-36) showed both response and time allocation noise biases away from the food call in both food call conditions (FC1 & FC2). The largest of these noise biases was -0.32 and -0.26 for responding and time allocated, respectively. The remaining hen (Hen 33) also showed response noise biases away from the sound keys in both food call conditions. However, time-based noise biases for this hen were smaller and were towards the sound keys in the second food call condition (FC2). Most hens showed larger noise bias in the first (FC1)

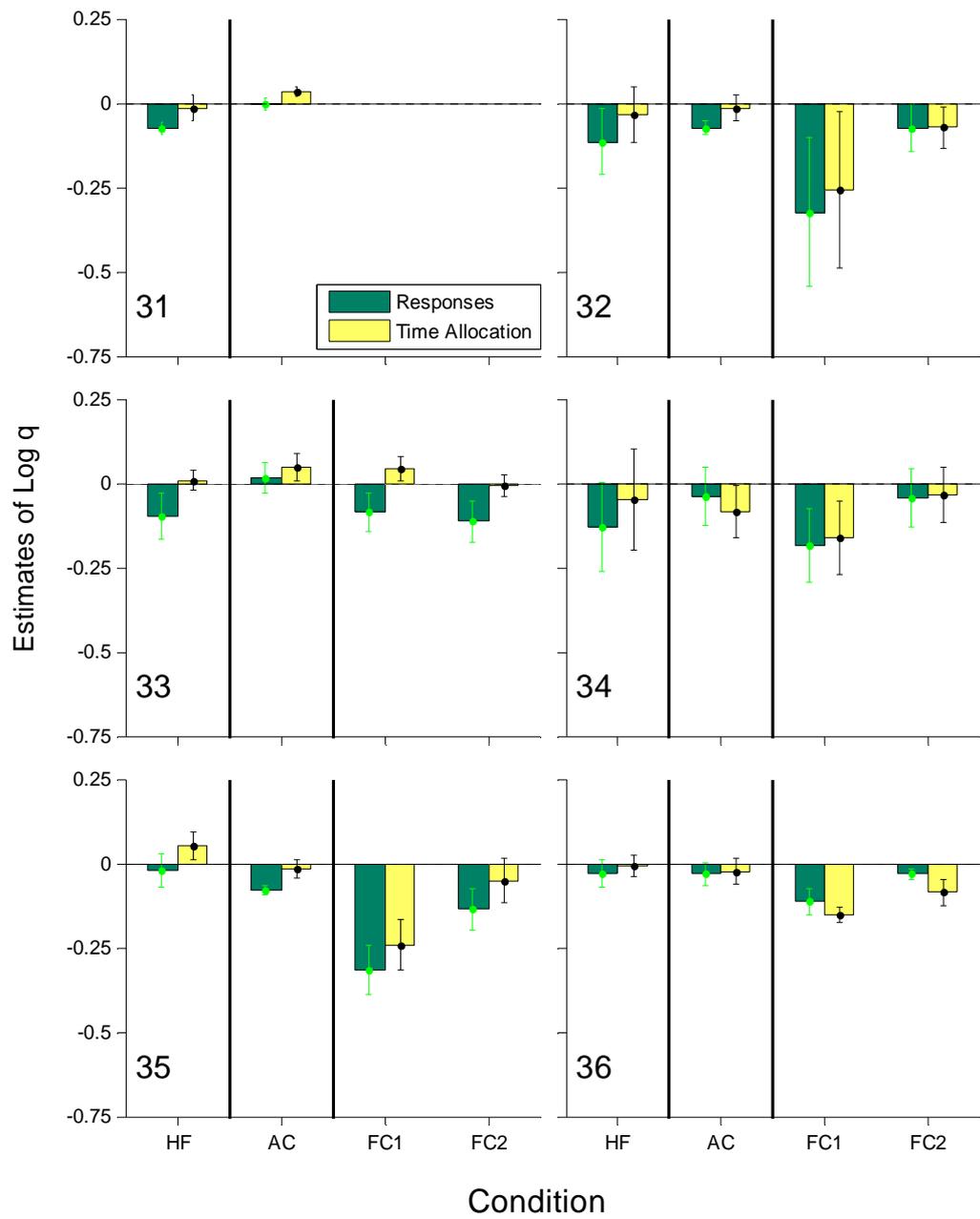


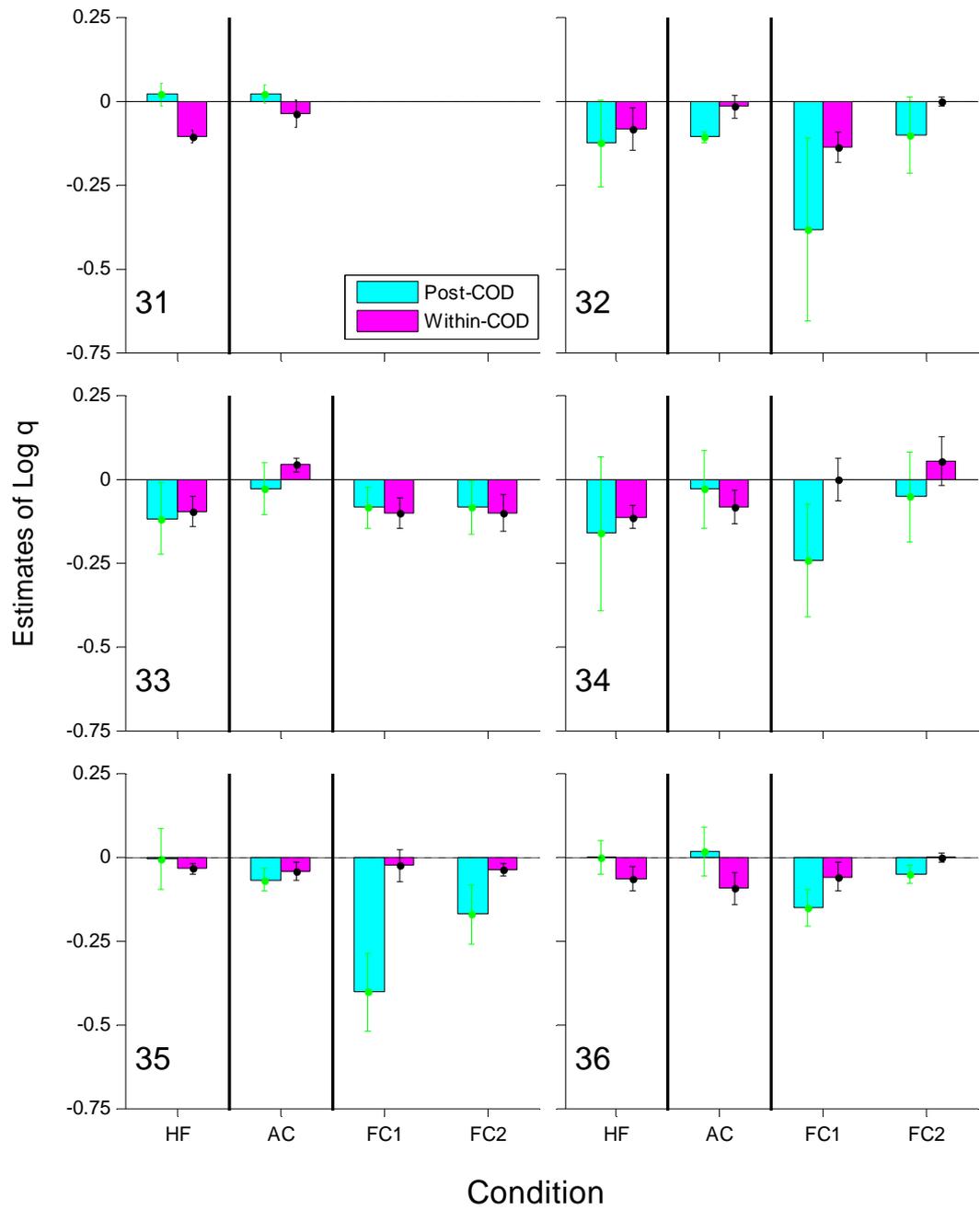
Figure 1.8. Estimates of  $\log q$  (noise bias) for responding and time allocation for conditions with the sounds of hens feeding (HF), alarm call (AC), and the food call (FC1 and FC2), plotted for each hen. The error bars represent one standard deviation either side of the mean ratios.

than the second food call condition (FC2), except Hen 33 whose response-based bias increased in the second (FC2). This was evident in the mean noise bias across conditions, which were -0.17 and -0.07 for the first and second food call conditions, respectively. For both food call conditions combined, the mean noise bias was -0.14.

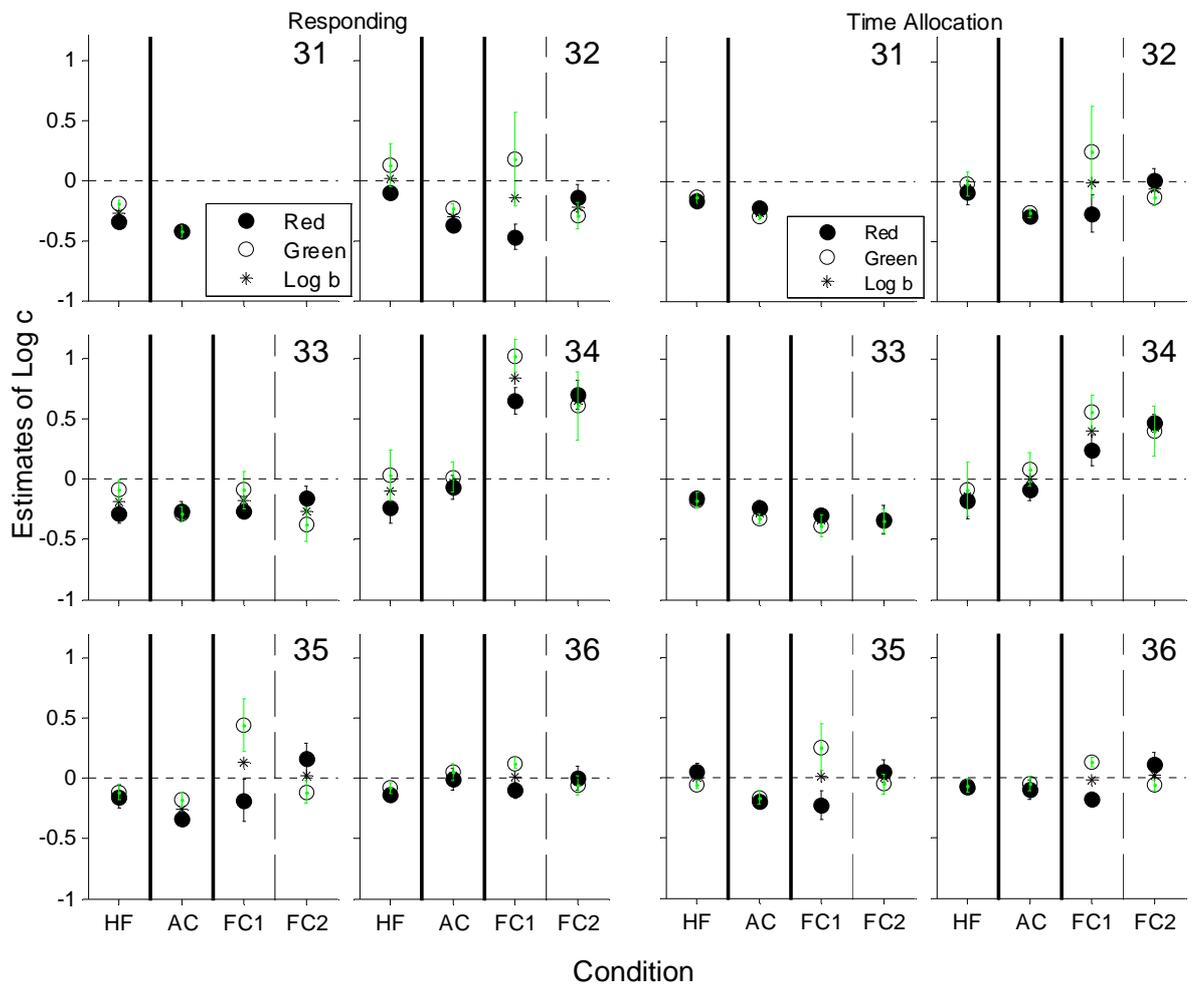
As with the white noise conditions, Figure 1.9 shows the differences between estimates of  $\log q$  (noise biases) from responding after and within the COD for the other sound conditions, for each hen. In general, the noise biases for both measures followed the same pattern as the total response data (Figure 1.8). Here, more hens showed larger noise biases in responding post the COD compared to the within-COD biases across conditions. The difference between these post- and within-COD noise biases was generally large for 3 hens (Hens 32, 34, & 35) and was largest in the food call conditions. For Hen 31, within-COD noise biases were away from the keys associated with sound while post-COD biases were towards the keys associated with sound. For Hen 33, post- and within-COD noise biases were generally similar across sounds. For Hen 36, within-COD biases were larger than post-COD biases for the sounds of hens feeding and the alarm call, with larger post-COD noise biases for the food call. For Hen 36, within-COD biases were larger than post-COD biases for the sounds of hens feeding and the alarm call, with larger post-COD noise biases for the food call.

Figure 1.10 shows the estimates of  $\log c$ , which were the logged (to the base 10) ratios of responses (left panel) and time allocation (right panel) in the red (filled circles) and green (unfilled circles) components, taken to the left key, plotted for each hen. Estimates of  $\log b$  (key bias calculated using Equation 5 in the same way as above) are also plotted in Figure 1.10 (asterisk). This figure shows that estimates of  $\log c$  followed a very similar pattern for the response and time allocation data for each hen and each sound. Across conditions, subjects predominantly showed response and time allocation key biases ( $\log b$ ) towards the right key except in conditions when the food call was played, when 2 hens (Hens 34 & 35) showed left key biases.

In general, the response and time allocated estimates of  $\log c$  for the sounds of hens feeding and the alarm call were towards the right key or close to zero and little difference was seen between responding in the red and green components. Estimates of  $\log b$  for the sounds of hens feeding and the alarm call gave biases all to the right key or close to zero for both response and time allocation data. When a food call was associated with the sound keys, Figure 1.10 shows that this sound biased the behaviour of the hens differently from the previous two sounds, and key bias also changed across food call conditions. In both food call



*Figure 1.9.* Estimates of  $\log q$  for responding post and within the COD for all sound conditions with the sounds of hens feeding (HF), alarm call (AC), and the food call (FC1 and FC2), plotted for each hen. The error bars represent one standard deviation either side of the mean ratios.



*Figure 1.10.* Estimates of  $\log c$  for responses (left) and time allocation (right) in the red (filled circles) and green (unfilled circles) components for conditions where the sounds of hens feeding (HF), the alarm call (AC) and the food call (FC1 & FC2) were associated with the sound keys, plotted for each hen. Estimates of  $\log b$  (key bias) were also plotted (asterisk) for each condition and hen. The error bars represent one standard deviation either side of the mean ratios.

conditions, estimates of  $\log b$  and  $\log c$  for response and time allocation varied in size and direction across hens. When conditions were reversed (from Condition FC1 to FC2), the direction of the time allocation and response estimates of  $\log c$  also reversed for all hens. Across the two food call conditions, Hens 33 and 32 showed estimates of  $\log b$  towards the right key while Hen 34 showed the opposite, key bias towards the left key. In both food call conditions, estimates of  $\log b$  for the remaining hens (Hens 35 & 36) were close to zero for both response and time allocated data.

The mean number of COs per session in the red and green components for all sound conditions are plotted in Figure 1.11, for each hen. Across conditions, the mean number of COs in both components showed no systematic changes for all hens except Hen 34. This hen performed more COs on average in the red component when the sound was on the left (Condition HF, AC and FC1) and when the sound key reversed more COs were seen in the green component (when the sound was again on the left). The lowest number of COs can be seen for the alarm call with the highest number generally shown for the sounds of hens feeding. Across the sound conditions, the mean number of COs ranged from 23 to 102.

The local rates of responding to the keys that either had sound or no sound are plotted in Figure 1.12, for each hen. Here, 3 hens (Hens 31, 33, & 35) consistently responded slower on the key associated with sound across conditions. This was also shown by Hen 32 except for the second food call condition (FC2) where this hen showed equal response rates to both sets of keys. The difference between response rates to the sound and no sound keys for these hens tended to be smaller than the differences seen with white noise. For the remaining hens (Hen 34 & 36), no consistent trend in local response rates was seen across conditions and response rates to both sets of keys were fairly similar. For most hens, the highest local response rates were seen for the sounds of hens feeding, with the lowest rates seen for the alarm call. Across these sound conditions, local response rates ranged from 8 to 76.

On the whole, noise biases varied substantially across these sound conditions. Noise biases for the response and time allocation data varied somewhat across hens but were generally smallest for the alarm call and largest for the food call. For 3 hens, noise biases from post-COD responding were generally larger than from within-COD responding and tended to follow the same pattern as the total response data. Key biases ( $\log b$ ) also varied across these conditions and most hens showed right key biases, except in both food call conditions where 2 hens showed left key biases. The mean number of COs and local response rates (per min) for these sounds showed no consistent trends across conditions or hens.

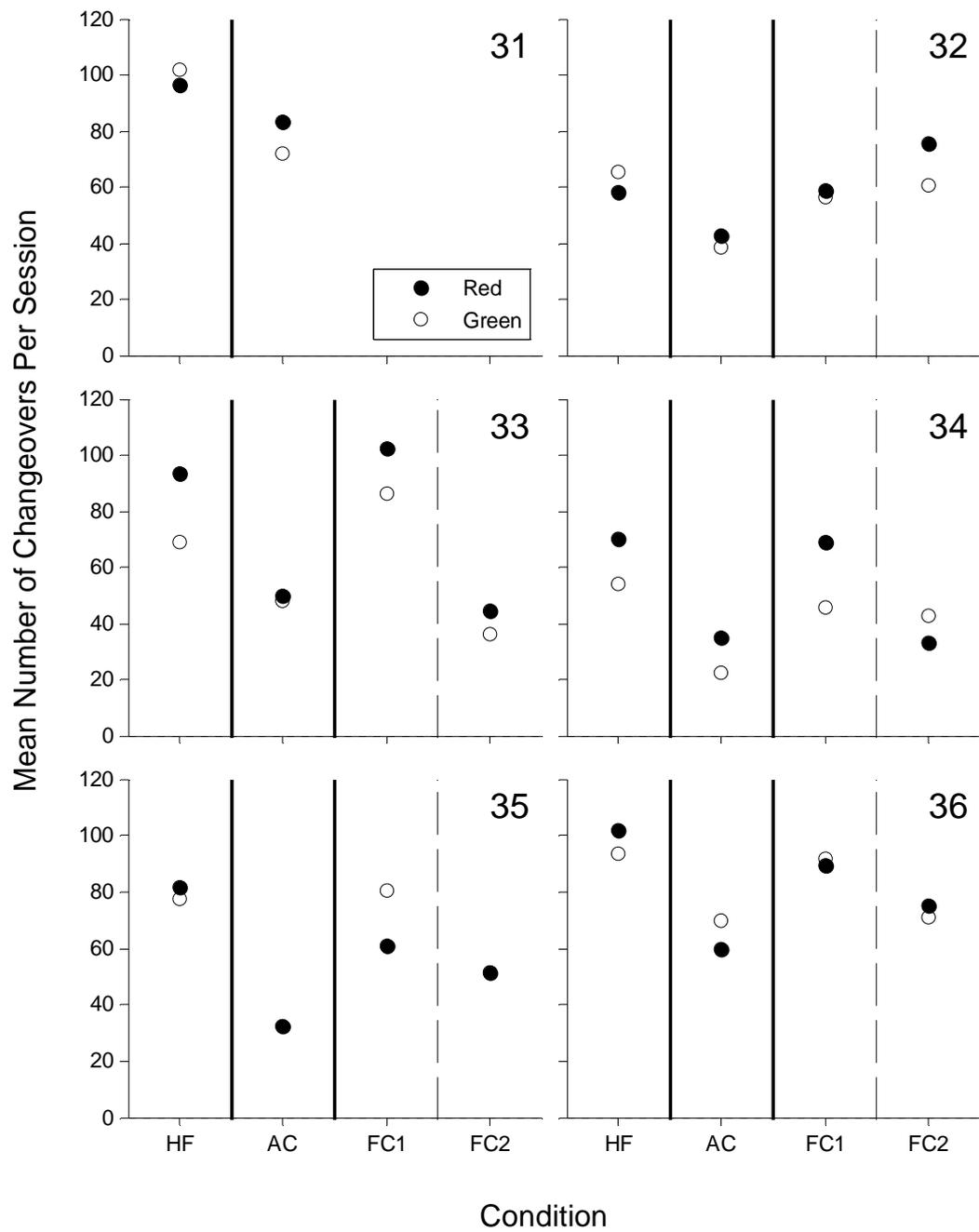


Figure 1.11. Mean number of changeovers (COs) per session in the red and green components across conditions with the sounds of hens feeding (HF), alarm call (AC), and the food call (FC1 and FC2), plotted for each hen.

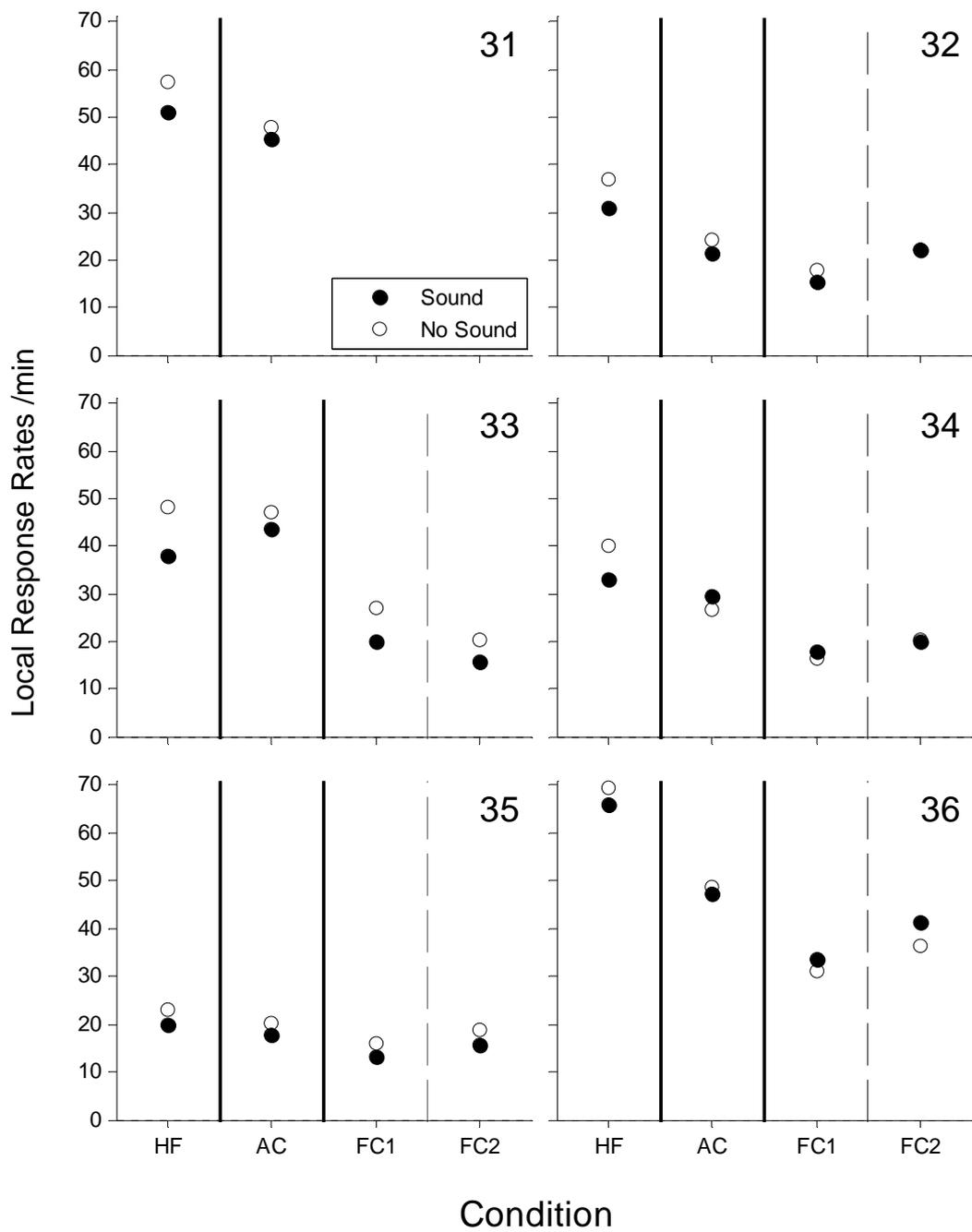


Figure 1.12. Local response rates (per min) to the sound (filled circles) and no sound keys (unfilled circles) in the red and green components across conditions with the sounds of hens feeding (HF), alarm call (AC), and the food call (FC1 and FC2), plotted for each hen.

## Conditions Without Sound

Data obtained in the conditions without sound (Conditions NS1, NS2, & NS3) can provide one measure of key/side bias ( $\log b$ ). These estimates of  $\log b$  are equivalent to the  $\log c$  estimates (overall bias) in these conditions. The point estimate logarithms of the ratios for the red (filled circles) and green (unfilled circles) components can be seen in Figure 1.13. Ratios of responding are presented in the left panel and time allocation in the right panel for all conditions without sound, for each hen. The horizontal line at zero indicates where the response ratios would be expected to fall if the hens' behaviour showed no biases.

Estimates of  $\log c$  for responses and time allocation followed a similar pattern for all conditions, for each hen. The data across conditions was also in the same direction for most hens and key bias decreased across conditions for both red and green components. One exception to this trend is the estimates of  $\log c$  for Hen 34, which increased again in the last condition without sound (NS3). This figure also shows that 4 hens (Hens 31-33 & 35) responded more and spent more time responding to the right key in both the red and green components for all three conditions, indicating a right key bias. Responding for the remaining 2 hens (Hens 34 & 36) showed biases towards the right key for the first two conditions without sound (NS1 & NS2). However, in the third condition without sound (NS3) biases for Hen 36 were close to zero for both measures and for Hen 34 were towards the left key for both measures.  $\log c$  for all response data ranged from 0.73 to -0.53 across all hens, and for the time allocation data ranged from 0.15 to -0.51.

The mean number of COs per session in the red and green components for all conditions without sound was examined and the data showed no consistent pattern across conditions for all hens, and so are not presented here. Generally, the mean number of COs in the red and green components was similar for most subjects, with few exceptions. The mean number of COs ranged from 24 to 106 across hens. Overall, the hens did not show a tendency to change keys in one component more than the other when sound was not associated with either key.

The local response rates in conditions without sound, to red/left and green/right keys and the green/left and red/right keys, were averaged across components (in the same way as above) and are presented in Appendix 1c. These local response rates followed no consistent trend across hens and no pattern was seen within conditions. However, the level of response rates to the keys did vary largely across hens with rates ranging from 17 to 86. All hens responded at consistent rates to both sets of keys.

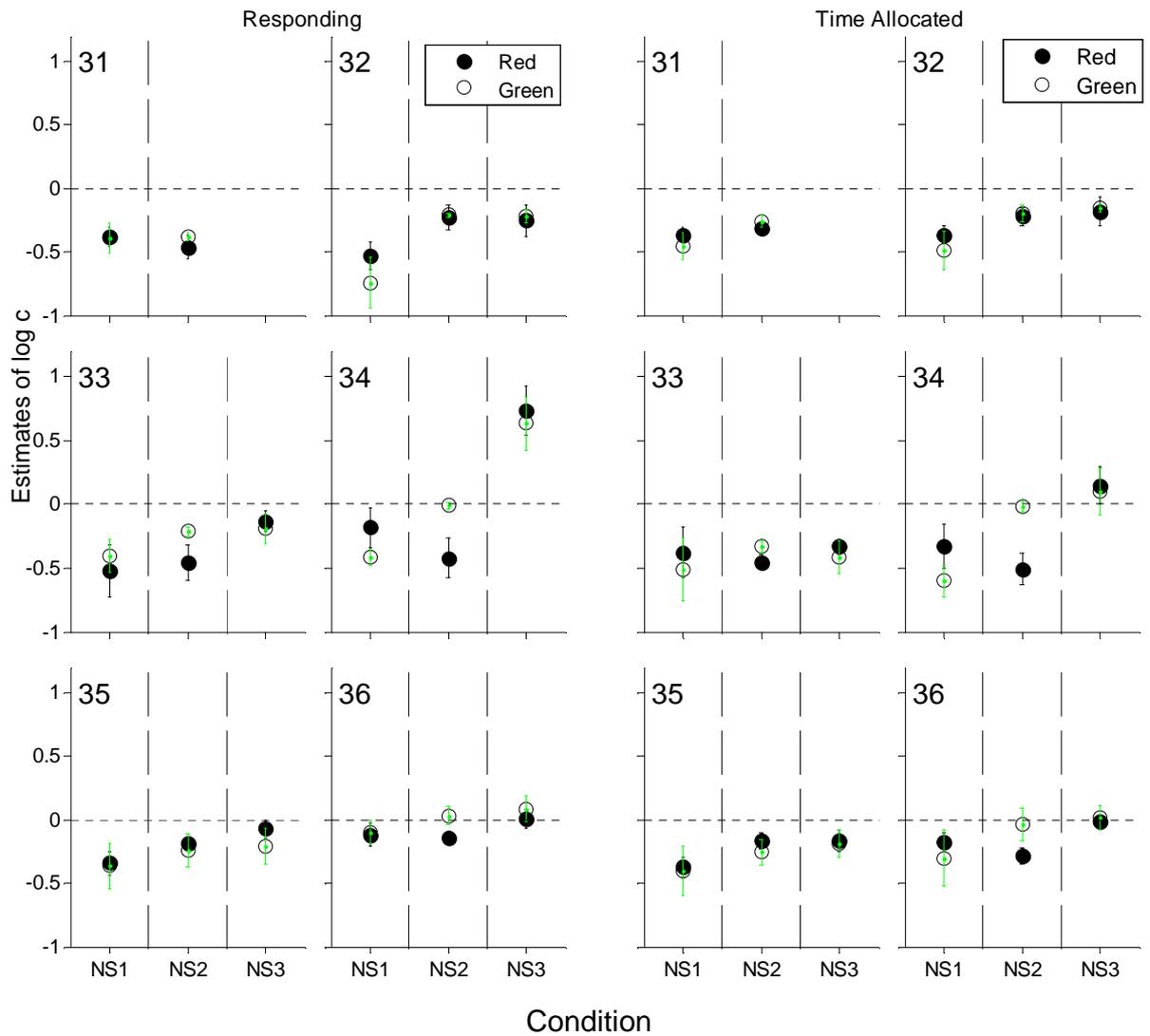


Figure 1.13. Estimates of  $\log c$  for responses (left) and time allocation (right) in the red (filled circles) and green (unfilled circles) components for conditions without sound (NS1-3), plotted for each hen. The error bars represent one standard deviation either side of the mean ratios.

Generally, in conditions where no sound was associated with the keys, there was a decrease in the magnitude of bias across conditions for all hens. There were also consistent right key biases shown for 5 of the 6 subjects. Although the mean number of COs and the local response rates varied in degree across hens, these measures showed no consistent patterns across conditions.

### **Comparison of Results**

Comparisons of the data from all conditions showed clear differences for some of the measures of behaviour reported here. For example, 3 hens showed the largest noise bias ( $\log q$ ) for white noise at 105 dB while for 3 hens a larger bias was shown away from the food call. The least bias was with the alarm call for 5 out of 6 subjects. Moreover, there were larger differences between the response and time allocation estimates of  $\log q$  for the white noise conditions than for the other sound conditions. For noise bias from responding within and post the COD, in general a larger bias was shown in the post-COD data for both white noise and other sound conditions. This difference between post- and within-COD responding was also larger when the degree of the noise bias from total responding was also large.

For the white noise conditions the hens tended to change keys less often in components where the sound was associated with the left key. However, this was not obvious for conditions with the other sounds, nor was any pattern seen in conditions where no sound was played. Generally, the average number of COs per session was lowest for the alarm call and highest for the sounds of hens feeding and white noise (regardless of decibel level). All hens tended to respond at lower rates to the keys associated with white noise, but no systematic effects were seen with the other sound conditions. Response rates for conditions without sound tended to be higher than in conditions with sound, with the lowest response rates seen for the first food call condition.

Different degrees of noise bias in the hens responding resulted from the different sounds, and so it was possible to rank each sound for each hen. These ranks ranged from the largest response bias ( $\log q$ ) away from the keys associated with sound (Rank 1) to the least bias (Rank 5), either zero or towards the keys associated with sound. Table 1.3 shows these rankings for all conditions with sound, for each hen. Here, it can be seen that for half of the hens the food call was ranked first, whereas for the other half white noise (at 105 dB) was ranked first. White noise at 105 dB was also ranked second for 3 hens and gave larger biases than white noise at 100 dB. The sound producing the least bias and the last rank most often was the alarm call.

Table 1.3.

*Ranks for each sound used, based on the noise biases from response data, ranging from largest bias away from the sound (1) to the least bias or bias towards the sound (5), for each subject.*

*Note: the \* indicates a positive noise bias, towards the sound key. The sounds shown are white noise at 100 dB (A)(WN100) and 105 dB (A) (WN105), an alarm call (AC), the sounds of hens feeding (HF) and a food call (FC). As Hen 31 died before completing the food call conditions, n/a is shown for Rank 5.*

<b>Hen</b>	<b>Rank 1</b>	<b>Rank 2</b>	<b>Rank 3</b>	<b>Rank 4</b>	<b>Rank 5</b>
31	WN 105	WN 100	HF	AC	n/a
32	FC	WN 105	HF	WN 100	AC
33	WN 105	WN 100	FC	HF	AC*
34	WN 105	WN 100	FC	HF	AC
35	FC	WN105	WN 100	AC	HF
36	FC	WN105	WN 100	HF	AC

In summary, the results presented here indicate that white noise at 100 and 105 dB and the food call gave the largest effects on the responding of the hens. The least effect of sound on behaviour was seen with the alarm call. Many of the hens also had right keys biases over the course of this experiment.

### **Discussion**

The performance of the hens in this experiment (using a multiple-concurrent schedule of reinforcement procedure) was biased by most sounds. This was also evidenced by McAdie (1998) and McAdie et al. (1993, 1996). The direction of this bias was mainly the same across hens but the degree of bias caused by the sounds varied across hens, showing that degrees of sound preferences were inconsistent. Generally, it was found that the sounds used in this experiment for these hens were either neutral (i.e., no noise bias) or not preferred but none of the sounds used here were shown to be preferred. As expected, the hens showed the largest noise bias away from the keys associated with white noise (when played at 105 dB). Within subjects, white noise also had a different effect on responding compared with other sounds, as shown by a difference in response and time allocation noise biases and lower local response rates to keys associated with white noise. It was expected that the sounds of hens feeding, which was similar to the sounds of hens in a commercial poultry shed used by McAdie et al. (1993, 1996), would bias the responding of the hens away from the sound keys. This was shown to be so but the biases were smaller than those with white noise. Although it was expected that the food call may be a sound preferred by hens, it was found here to bias the responding of most hens away from keys associated with it but generally to a lesser degree than white noise also. Generally, all hens showed very little bias away from the keys associated with the alarm call when a mixture of results was expected.

Using multiple-concurrent schedules McAdie (1998) found that results with white noise were different from those found with other sounds by McAdie et al. (1993). This led McAdie to suggest that white noise had different effects on behaviour to other sounds she examined. The present findings confirm the results of McAdie and others and showed that white noise affected behaviour differently to the other sounds. Here, noise biases from response and time allocation were different for white noise but this difference in magnitude was not seen for other sound conditions or in those without sound. Weardon and Burgess (1982) reported that studies using concurrent-schedules typically find measures of response and time allocation to be comparable (e.g., Bron, Foster, Sumpter and Temple, 2003; Foster, Sumpter, Temple, Flevill &

Poling, 2009; Temple, Scown & Foster, 1995). In the present experiment, there was little difference between response and time allocation noise biases in conditions with other sounds (excluding white noise) and in conditions without sound, which is consistent with this previous research. Given that the difference in response and time measures was not likely to be due to the procedure, as it was not seen with any sounds other than with white noise, it appears that white noise affected the behaviour of hens differently. When examining the effects of aversive stimuli the differences between response and time allocation measures may give another indication of aversiveness.

White noise played at 105 dB was generally the least preferred of all the sounds when compared to no sound, in that it showed the largest noise biases and the highest rank of 1 or 2 for most subjects. White noise also produced the largest mean noise bias and was ranked as the least preferred by 3 hens. Calculating the antilog of the white noise biases here suggests that the hens would generally prefer to work in silence 1.4 and 1.5 times more than working in the presence of white noise at 100 and 105 dB, respectively. This finding is consistent with McAdie (1998), who also found noise biases away from keys associated with white noise and the size of these biases increased with the decibel level. Another finding consistent with McAdie was that local rates of responding were lower on keys associated with white noise, compared to other sounds. It has been well documented that an aversive stimulus, such as electric shock, suppresses responding previously maintained by reinforcement (e.g., Azrin, 1956; de Villiers, 1980; Farley, 1980). McAdie reported that this suppressing effect suggested that white noise was, in effect, punishing key pecks and thus functioning as an aversive stimulus. It seems that for the hens used in the present experiment white noise also had a similar aversive effect.

The noise biases resulting from white noise found here tended to be larger than those found by McAdie (1998). Across hens and conditions using the same decibel level the response-based noise biases from McAdie's hens, analysed in the same way as this experiment, gave a mean noise bias of -0.05 for 100 dB and -0.08 for 105 dB. For white noise in the present experiment there was a mean noise bias of -0.13 for 100 dB and -0.18 for 105 dB. Both of these values were at least double those found by McAdie. It is unclear why these differences occurred as the same procedure and analyses were used.

There was a difference between the response and time allocated noise biases for white noise, in that the noise biases from the time data tended to be closer to zero, with larger biases shown in the response data. This is consistent with the results found by McAdie (1998). These

differences were also reflected in the local response rates where lower responding was seen towards the keys associated with white noise compared to conditions without sound. This finding indicates that when the white noise was on, the hens responded at a lower rate to the sound key but still spent a relatively similar amount of time on both keys. This was an unusual finding as the response data indicated that the hens responded more to the keys without sound, compared to those associated with white noise, but time allocation data showed very little effect of white noise. It was suggested by McAdie (1998), who observed a similar effect, that white noise may have reduced the reinforcing value of the VI schedule in effect, thus resulting in the lower local response rates and smaller biases in the time allocation data. This provides further evidence of the aversiveness of white noise.

For white noise, noise biases for post-COD responding in the presence of noise tended to be larger than the within-COD response biases and this was different from the findings of McAdie (1998). McAdie found generally larger noise biases in the within-COD data with white noise. Larger biases from post-COD compared to within-COD responding were also found with the food call. However, the within- and post-COD noise biases for the alarm call and the sounds of hens feeding were quite similar. In general, research using concurrent schedules that has examined COD data has found larger biases in the post-COD responding, when compared to the within-COD responding (e.g., Bron et al., 2003; Temple et al., 1995). Temple et al. found that behaviour during the COD was insensitive to reinforcement rate changes and their results supported the conclusions of Baum (1982), who suggested that within-COD data should not be included in analyses of choice behaviour. Bron et al. also found little sensitivity in the within-COD measures of bias resulting from different food types with possums. These findings are more similar to those found in the present experiment and it is unclear why McAdie's hens, which may have initially stopped responding at the onset of white noise, showed larger response biases during the COD.

The direction of the noise biases in responding shown here with the various sounds (food call, alarm call, and the sounds of hens feeding) were not all as expected. The food call was a sound that was expected to be preferred by the hens but estimates of noise bias indicated that it was not preferred by most hens when compared to silence. The food call also resulted in larger noise bias than white noise for 2 hens and was also a sound ranked the least preferred by 3 hens. Other research has found that male food calls attract hens (Gyger & Marler, 1988; Marler et al., 1986). However, Wilson and Evans (2010) found that hens were indifferent when presented with a video playback of food calling males and Evans and Evans (1999) found that

hens did not approach a speaker playing a male food call. It may be that the playback of the food call in the present experiment was not reproduced as a sound that hens recognise as signalling the presence of food. The food call was also not played at a decibel level representative of a live food call, or that used by other studies. For example, 70 dB (A) was used by Evans and Evans (1999) and 10 dB (A) above background noise (approximately 65 dB (A)) was used by Field et al. (2007). In addition, the hens used in this study had not had any previous experience with a male's food call, prior to this experiment, and this may be why they did not find it a positive sound. Conceivably, it may have been the frequency (Hz) or the rhythm of the sound that was not preferred for these hens because the food call is a sound that consists of only one repetitive pulse (Collias & Joos, 1953).

The sounds of hens feeding (Condition HF) biased the responding of 4 hens, but these noise biases tended to be smaller than those with white noise or the food call. This recording was used as it was deemed by this author to be a similar sound to the one (the sounds of hens in a commercial poultry shed) used by McAdie et al. (1993) and MacKenzie et al. (1993) and, therefore, was expected to give rise to larger noise biases. McAdie et al. found that their sound produced the largest bias in behaviour when compared to other sounds they used. In recalculating  $\log q$  from McAdie et al.'s data, in the same manner as the present experiment (using Equation 6), the mean noise bias for the sound of hens in a commercial poultry shed at 100 dB (A) was -0.17 across hens; whereas the mean bias shown here was -0.08. The results of McAdie et al. are more comparable to the present results of white noise at 105 dB. In addition, MacKenzie et al. played their sound at 90 dB (A) and still found that hens spent a significant proportion of time with this sound off while the louder intensity used here did not produce biases in responding under concurrent schedules. The sounds of hens feeding was also given a rank of 3 or 4, thus having only a small effect on the behaviour of the hens here. Thus, this sound was generally found to be only somewhat unpreferred to silence and noise biases were smaller than expected. One possibility is that the sound used in the present experiment was not as similar to the one used by McAdie et al. or MacKenzie et al. as hoped.

The alarm call proved to be the sound that had the least affect on responding in the present experiment. The noise biases resulting from this sound were very small, averaging only -0.04, and this sound was given the highest rank (most preferred compared to the other sounds) for 5 of 6 hens. It was expected that this sound would produce a neutral or perhaps even a preferred outcome. Hughes et al. (1982) and Collias (1952) both found increased vocalisations in hens when they were exposed to the playback of live chick alarm calls. However, this would

not necessarily indicate a preference for the sound. As previously mentioned, MacKenzie et al. (1993) found that half of their hens showed a neutral response to the playback of an alarm call, while the other half chose to spend the majority of their time with this sound off. This is somewhat similar to the results reported here. Moreover, the intensity of the alarm call used here (100 dB (A)) appears to have had less effect on the behaviour of the hens than found by MacKenzie et al, who used a lower intensity sound (90 dB (A)). It was also possible that had the subjects used here been broody hens, as used by Hughes et al. (1982), the outcomes may have been different. For a future experiment, it would be interesting to compare the responses of broody and non-broody hens to chick's alarm calls in order to confirm this theory. Overall, the findings here showed that the alarm call had little effect on the behaviour of the hens and appeared to be a sound they were indifferent to, even when played at a high intensity.

A right key bias was shown by all hens in this experiment and it is unclear how much this may have confounded the effects of sound on responding. As evidenced from the graphs plotting  $\log c$  (Figures 1.5 and 1.10), in most conditions there was a strong right key bias for responding in both the red and green components for many subjects. Although there were reversal conditions conducted for some sounds, it was clear that behaviour was not equivalent in these conditions. The evidence for this was shown in the estimates of  $\log c$ , which did not deviate as far from zero in the reversal conditions. The key bias also appeared to be related to the number of changeovers per session for 3 hens, where a lower number of COs occurred in components where white noise, and other sounds for some hens, was associated with the left key. Otherwise, the number of COs per session did not show any systematic differences between components across all conditions, as was found by McAdie (1991, 1998).

One disadvantage of playing sound in the multiple-concurrent schedules procedure was that the sound was played during the choice phase. This meant that the sound being played may have interfered with the hens' responses and this may in turn have affected which key the hens responded to. In the present experiment, the local rates of responding on the keys associated with white noise were lower than on keys that were not associated with white noise. It may have been that white noise turning on caused the hens to startle or freeze. This effect of sound on responding means that the sound may have been confounding response allocation in concurrent schedules, thus the procedure may not have reflected hens' preferences for sound. Using a procedure that allows the subject to choose between alternatives associated with stimuli that are not present during the choice phase would eliminate this problem. One such procedure is the concurrent-chains procedure, which could be used to assess preference for

different stimuli that are not present during the initial choice phase. It is also a procedure that can be easily applied to assess the effects of sound on choices.

In conclusion, this experiment showed that white noise and a food call had the greatest influence on the behaviour of the hens as these sounds resulted in the largest biases in responding. Based on the present findings, white noise was found to be aversive and no sound was preferred over the food call. The alarm call gave the least effect on the hens' responding, proving to be a stimulus they were indifferent to. The results reported here were generally in line with previous research. While the present experiment, which employed the multiple-concurrent procedure, was successful at showing the effects of sounds that were aversive, unpreferred and neutral, it did not identify any sound that was preferred by the hens. A disadvantage identified in using multiple-concurrent schedules here was that the presence of sound during the choice phase affected the rate of responding in the presence of white noise, thus possibly confounding response allocation measures in concurrent schedules. The concurrent-chains procedure could overcome this problem and is a procedure that has been used previously with domestic hens (Tannahill, 2004), and in the assessment of sound preferences (Otsuka et al., 2009). The next experiment used this procedure and assessed preferences for the same sounds using the same subjects, therefore any results could be compared back to those of the present experiment.



## EXPERIMENT 2

Multiple-concurrent schedules used in Experiment 1 allowed for the assessment of hens' preferences for different sounds. In Experiment 1, however, local response rates were shown to be lower on keys that were associated with white noise. This indicates that the presence of sound may have been interfering with responding. The concurrent-chains procedure overcomes this limitation by allowing the subject to choose between two schedules available in the initial-links that lead to another schedule during the terminal-link where the sound is present. In this arrangement sound is absent during the choice phase. Concurrent-chains can also be applied to assess the effects of sounds on choice behaviour. Therefore, the principal aim of this experiment was to assess preference for the same sounds used in Experiment 1, using the concurrent-chains procedure. This was done in order to compare the effects of sounds presented in the terminal-links on choices the hens make in the initial-links of the concurrent-chains procedure with the effects of sounds on responding found using multiple-concurrent schedules in Experiment 1.

The concurrent-chains procedure involves the simultaneous presentation of two schedules, termed the initial-links, one associated with each key. Instead of leading to a primary reinforcer on one key the initial-link schedule is followed by another schedule on that key, termed the terminal-link schedule, that must be completed in order for the subject to gain a primary reinforcer (Autor, 1960). Responding during the initial-link was suggested by Herrnstein (1964) and Autor (1960) as a measure of preference for the terminal-link schedule, and the terminal-link stimuli (i.e., different coloured keys) are generally regarded by them as conditioned reinforcers.

Concurrent-chains procedures have been extensively used to examine the effect of rate of, or delay to, reinforcement on animals' choices. Much of the research in this area has resulted in the development of mathematical models, typically derived from the GML (Baum, 1974), to account for behaviour under the concurrent-chains procedure. For example, Fantino (1969) conducted an experiment with pigeons where the overall rate of primary reinforcement was kept constant but the schedules in the initial- and terminal-links differed. It was found that the initial-link that led to the shorter terminal-link was preferred by the pigeons. This experiment by Fantino led Squires and Fantino (1971) to develop the *delay reduction theory* (DRT) to explain performance on concurrent-chain schedules. The DRT states that the terminal-link stimulus associated with the greatest reduction in time to primary reinforcement

would be preferred in the initial-links. Other models for behaviour under concurrent-chains also include the *hyperbolic value-added* model (Mazur, 2001) and the *contextual choice model* (Grace, 1994). Thus far, no consensus has been reached about which of these models best describes behaviour under concurrent-chains but one thing that seems clear is that the terminal-link stimuli are assumed by these authors to function as conditioned reinforcers.

Conditioned reinforcers have been widely studied particularly since the 1960's. A conditioned reinforcer is a previously neutral stimulus that has been paired with a primary reinforcer a number of times and has therefore gained the ability to strengthen responding. For example, the onset of a tone may indicate that food will follow thereafter and therefore the tone signals food. Conditioned reinforcers have been defined as signals to primary reinforcers and are considered by some to take on the properties of primary reinforcers (see Kelleher & Gollub, 1962, Shahan, 2010, and Williams, 1994, for reviews). More recently, researchers have moved away from the traditional views of conditioned reinforcement, suggesting that conditioned reinforcers 'signpost' or signal food, rather than function as reinforcers themselves (Davison & Baum, 2006; Shahan, 2010). This means that this 'signpost' or signal becomes a reliable predictor of reinforcement. To this end, conditioned reinforcers will be referred to as signals throughout this thesis.

The concurrent-chains procedure has been used extensively to examine preferences for these signals (Dunn & Spetch, 1990; Fantino, Freed, Preston & Williams, 1991; Grace & Savastano, 2000; Williams & Dunn, 1991). O'Daly, Meyer and Fantino (2005) used both concurrent and concurrent-chains schedules to assess the effects of the overall initial- and terminal-link durations (temporal context) on the value of conditioned reinforcers (signals). Although they found similar results using these two schedules, they suggested that they measured different effects; concurrent-chains assessed the relative strength of a stimulus as a signal while concurrent schedules assessed the signals value and were more effective at eliciting responding. Only a small number of studies have used concurrent-chains to assess preferences for other stimuli in the terminal-links, for example, the presence of other hens (Tannahill, 2004), electric shocks of various intensities (Rachlin, 1967; Schuster & Rachlin, 1968) and various sounds (Otsuka et al., 2009). Tannahill used equal initial- and terminal-link schedules in a modified concurrent-chains procedure to examine the effects of the presence of another hen on the subject hens' response ratios in the initial-links. In Tannahill's experiment, the terminal-links were a fixed delay where subject hens spent time next to another hen that was either dominant or subordinate to them. It was found that the hens' responding in the

initial-links was biased by the presence of conspecifics in the terminal-links and that social preferences varied across subjects.

In another study using concurrent-chains, Rachlin (1967) also used equal initial- and terminal-link schedules in a three key experiment assessing the effects of electric shock intensity on the behaviour of pigeons. During the experiment different intensities of shock, that were independent of responding, were associated with each terminal-link schedule. The findings showed that relative rates of responding in the initial-link were greatly affected by shock in the terminal-links, where pigeons responded relatively more on the key associated with the lowest shock intensity. These experiments showed that initial-link responding could be biased by other stimuli in the terminal-links, rather than just by reinforcement rate or delay differences.

To date, only one study has examined the effects of an auditory stimulus incorporating the use of the concurrent-chains procedure (Otsuka et al., 2009). After showing that six rats could discriminate between two different types of music, Otsuka et al. used a standard concurrent-chains procedure with VI 30-s schedules in the initial-links with a 2-s COD and equal Fixed Interval (FI) 7-s terminal-link schedules. In their experiments, music or a sound was played in each terminal-link and so a choice in the initial-links was always between terminal-links that both contained some type of auditory stimulus.

In their second experiment, Otsuka et al. (2009) played music by Bach in one terminal-link and from Stravinsky in the alternate terminal-link and white noise and a conspecific vocalisation were played in the terminal-links of their third experiment. The rats showed no preferences in the initial-links when pieces of music by Bach or Stravinsky were played in the terminal-links. Conversely, the ratio of terminal-link responding for 2 subjects was towards the terminal-link associated with music from Bach, for 1 subject it was towards the terminal-link associated with music from Stravinsky while the remaining rats showed equal ratios of responding to both music types. When white noise or the conspecific vocalisations were played in the terminal-links, all rats now showed equal rates of responding in both terminal-links. Responding in the initial-links, however, showed that most subjects preferred the initial-link key that led to the terminal-link with white noise. From this, Otsuka et al. concluded that music was a poor reinforcing stimulus for rats. The authors suspected that food may have been interfering with responding in the initial-links and to resolve this issue they conducted an extinction session. In this extinction session sounds were still played in the terminal-links but no food reinforcement was provided. After finding no difference between the previous session

and the extinction session, Otsuka et al. concluded that the concurrent-chains procedure was effective in detecting relative reinforcing or aversive properties of sound.

The concurrent-chains procedure has been used to assess preferences for stimuli in the terminal-link schedules, including auditory stimuli (Otsuka et al., 2009). Thus, it seems reasonable to conclude that it would be an effective procedure for examining domestic hens' preferences for different sounds. It is also a procedure that allows the assessment of preferences for stimuli associated with sound without the sound being played during the choice component. Therefore, the following experiment aimed to determine if responding in the initial-links could be biased by sound in the terminal-links and in a similar manner to that seen in Experiment 1. It was also the intention to assess whether the terminal-link responding would be affected by the presence of sounds.

The procedure used equal VI VI initial-link schedules and FI terminal-link schedules where a sound was associated with one terminal-link, across conditions. When using the concurrent-chains procedure, experimenters have often used FI schedules in the terminal-links (Davison, 1974; Killeen, 1970). The temporal pattern of an FI schedule was described by Dews (1978) as a 'break-and-run' pattern, where a pause is followed by a constant rate of responding. This was supported by Schneider (1969) and Sherman (1959, cited in Schneider, 1969), who suggested that under shorter FI schedules the typical scallop pattern is replaced by the 'break-and-run' pattern. Although this is the pattern expected in single FI schedules, it is unknown if this same pattern occurs during the FI of a terminal-link as few studies have reported on the pattern of responding in the terminal-links.

Independent initial-link schedules were used in the present experiment for ease of comparison with other data from Experiment 1. This means that the timing of the VI on one key was independent of the timing on the alternative key and although this may result in unequal obtained reinforcement rates, it is a common practice in concurrent-chains studies. It has also been shown that there are relatively little differences between overall results from dependent and independent initial-link schedules (Fantino & Royalty, 1987). A 3-s COD was also used in the initial-links of the present experiment in order to compare the responding within and post the COD directly with that seen in Experiment 1. Although this arrangement has not been typically employed in earlier studies using concurrent-chains (Alsop & Davison, 1988), it has been shown that using a COD may eliminate some terminal- and initial-link interactions, thus making GML analyses more straightforward (Davison, 1983).

O'Daly et al. (2005) found similar results when using concurrent and concurrent-chains schedules to assess the value of conditioned reinforcers (signals). Therefore, in the present experiment it was predicted that hens' responding in the initial-links would be biased by the presence of sound in the terminal-links and in the same direction as the biases found in Experiment 1. That is, generally away from most sounds with the largest degree of bias away from the terminal-link associated with white noise and the least amount of bias for the alarm call. Response biases from within-COD responding were expected to be smaller than those from the post-COD data, as no sound present meant that responding would be more typical of concurrent schedule post- and within-COD responding (Bron et al., 2003; Temple et al., 1995). For the terminal-links, it was predicted that sound would affect responding in the same way as Experiment 1. The rate of responding in the terminal-links with white noise was expected to be lower than those found with other sounds. It was also expected that a period of pausing would occur at the beginning of the FI schedule followed by a constant rate of responding until a reinforcer was delivered. It was the intention to compare any biases obtained in the initial- and terminal-links (calculated in the same manner as Experiment 1), with the noise biases found in Experiment 1.

## **Method**

### **Subjects**

The subjects were the same as in Experiment 1 and fell under the same ethical protocol (Ref: 639). Hen 31 died, of causes unrelated to the experiment, after the completion of Condition 6.

### **Apparatus**

The apparatus used was the same as in Experiment 1, with the following exceptions. The keys were now both backlit by a white LED ('MARL' brand SX6 Midget Flange 28-V dc LED, from Farnell Electronics™) and each key had one blue or yellow LED. The white noise was relayed by a white noise generator (made by Med-PC™, model no. ANL-912) that produced broadband random frequencies between 0 and 20 kHz. For Conditions 1-2 of the present experiment, the amplifier used was the same as used in Experiment 1 but this was replaced during Condition 3 to a Yamaha™ AX-892 Natural Sound Stereo Amplifier with a power output maximum of 115 watts rms, and frequency response of  $0 \pm 0.5$  dB, over the range of 20 – 20,000 Hz.

## Procedure

The experiment used a concurrent-chains procedure. At the start of each session the two keys were illuminated white and each key was associated with a VI 60-s schedule of reinforcement that was programmed to time independently. A 3-s COD was in effect during these initial-link schedules. This meant that if a reinforcer was due on one key a switch to that key would not result in a reinforcer until the first response that occurred after 3 s had elapsed. Once the timing of one VI had elapsed on a key in the initial-link, a response to that key caused the key colour to change and the terminal-link schedule began. A FI 10-s schedule of reinforcement was in effect during the terminal-link and one terminal-link was associated with sound across experimental condition. During the terminal-link the alternative key light was turned off and that key was inactive. The left key light changed to blue and right key light changed to yellow throughout all experimental conditions. The first response after the FI had elapsed caused the magazine to be raised for 3 s. After reinforcement, the initial-link schedules restarted with both keys lit white. Sessions lasted for 40 min and one session was conducted for each hen on a day. At the end of each day the sound dB was tested in the same manner as in Experiment 1.

Since the hens had previous experience responding on concurrent schedules of reinforcement, no training was required. Because two of the subject hens (Hens 31 & 33) had developed strong right key biases during Experiment 1, they were initially exposed to unequal schedules during the initial-links. The initial-link schedules for Hen 31 and 33 were VI 30-s left versus VI 90-s on the right key for four and nine sessions, respectively. The right key schedule was then increased to VI 120-s for 7 sessions and then finally to VI 150-s for eight sessions before both were made equal. Conditions were changed when the data from the initial-links had reached both statistical and visual stability, using the same criterion as in Experiment 1.

Table 2.1 shows the experimental conditions, the sounds played, and the decibel levels dB. In some conditions, a sound was associated with one of the terminal-link schedules. Because the subjects had previous experience with the sounds during Experiment 1, sounds were not introduced gradually. In Conditions 1, 5, 7, 12, and 15, there were no sounds associated with either terminal-link key throughout the sessions. This was done to obtain measures of key bias. In Conditions 2 and 11, the chick alarm call was associated with one terminal-link schedule only (the right yellow key in Condition 2 and the left blue key in

Table 2.1

*Table showing experimental conditions, the types of sounds played in each condition, the decibel level dB (A), and the terminal-link associated with noise in each condition are shown in each column for each subject in Experiment 2.*

<b>Condition number</b>	<b>Sound played</b>	<b>Decibel level dB (A)</b>	<b>Terminal-link with noise</b>
1	None	-	-
2	Chick alarm call	100	Right
3	White noise	100	Left
4	White noise	100	Right
5	None	-	-
6	White noise	105	Left
7	None	-	-
8	White noise	105	Right
9	Hens feeding	100	Left
10	Hens feeding	100	Right
11	Chick alarm call	100	Left
12	None	-	-
13	Food call	100	Left
14	Food call	100	Right
15	None	-	-

Condition 11), and was played at 100 dB. Once the hen entered the one of these terminal-links, the sound was played and remained on until the magazine was raised.

In Conditions 3, 4, 6 and 8, white noise was played during one terminal-link schedule. The noise was played at 100 dB in Conditions 3 and 4 in the left and right terminal-links, respectively. In Conditions 6 and 8, white noise was played at 105 dB, again in the left and right terminal-link schedules respectively. In Conditions 9 and 10, the sounds of hens feeding was played at 100 dB in the left (Condition 9) and then right terminal-links (Condition 10). The sound played in the terminal-link schedule in Conditions 13 and 14 was the food call and this was played at 100 dB in the left and right terminal-links, respectively.

The data recorded for each hen were; the number of responses to the left and right keys in the initial- and terminal-links, the proportion of responses to the left key in the initial-links, time (s) spent responding to the left and right keys in the initial and terminal-links, the number of reinforcements (terminal-link entries) on the left and right keys, the number of COs, and total session time.

### **Data analysis.**

The raw data from the last five sessions of each condition for each hen are shown in Appendix 2a. Data from the initial- and terminal-links were described separately below and include data taken from the last five sessions of all conditions. Responding across conditions that did not contain sound in the terminal-links (Conditions 1, 5, 7, 12, & 15) did not show any systematic changes across conditions or subjects and therefore these data were pooled. These pooled data are presented in the figures below and the data from the individual conditions without sound for each measure of behaviour are presented in figures that are shown in Appendices 2b to 2d.

## **Results**

### **Initial-link Behaviour**

#### **Noise bias for responses and time allocation.**

Estimates of  $\log q$  (noise bias) were calculated (Equation 6) by combining data from response and time allocated measures across the last five sessions from reversal conditions that had the same sound in one terminal-link.  $\log q$  was calculated by taking half the absolute difference between these combined data from reversal conditions. These estimates of  $\log q$ , for response and time allocation data, are presented in Figure 2.1 for all conditions where sound

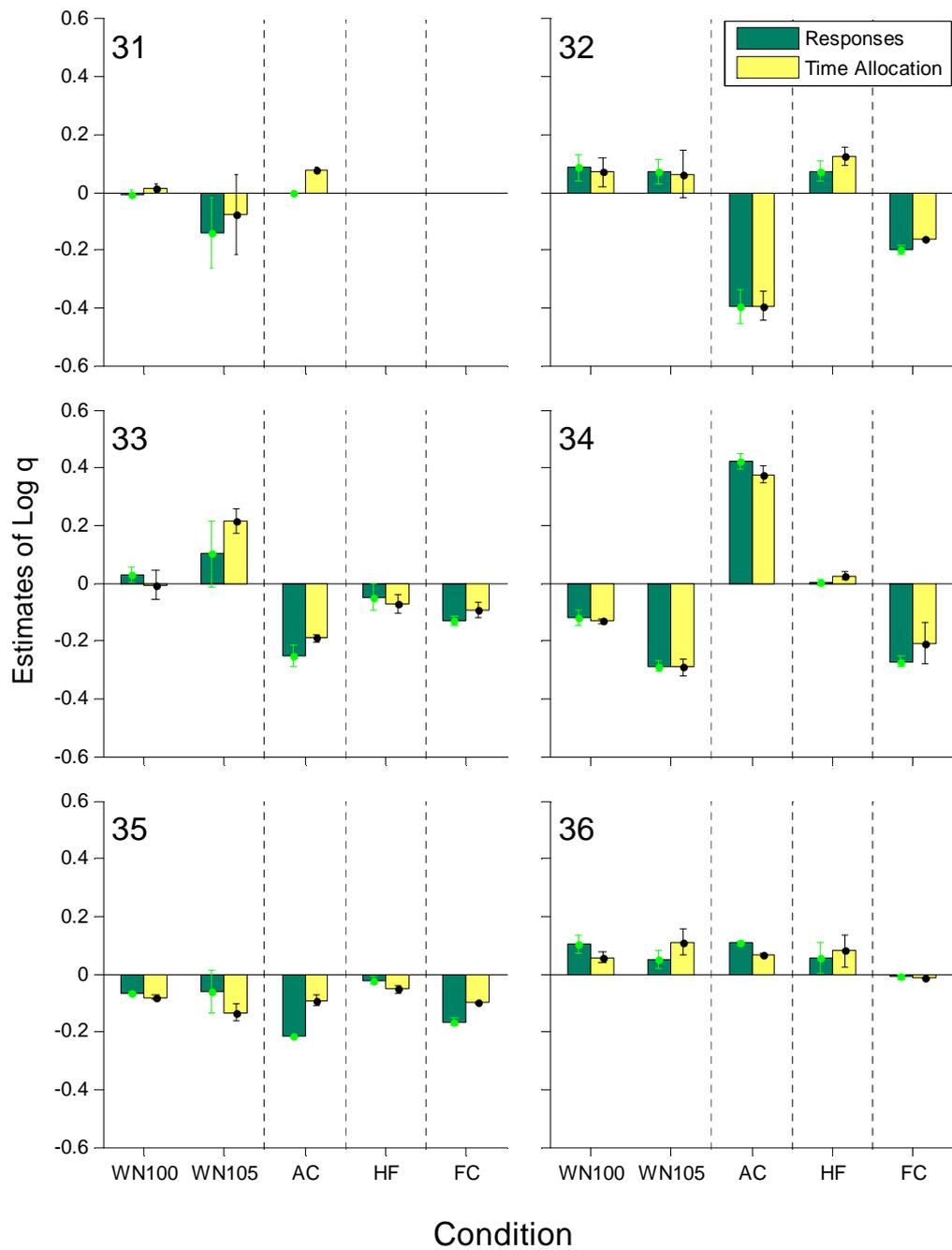


Figure 2.1. Estimates of  $\log q$  (noise bias) for responding and time allocation in the initial-links over conditions where white noise (WN100 and WN105), the alarm call (AC), sounds of hens feeding (HF), and the food call (FC) were played in one terminal-link, plotted for each hen. The error bars represent one standard deviation either side of the mean ratios.

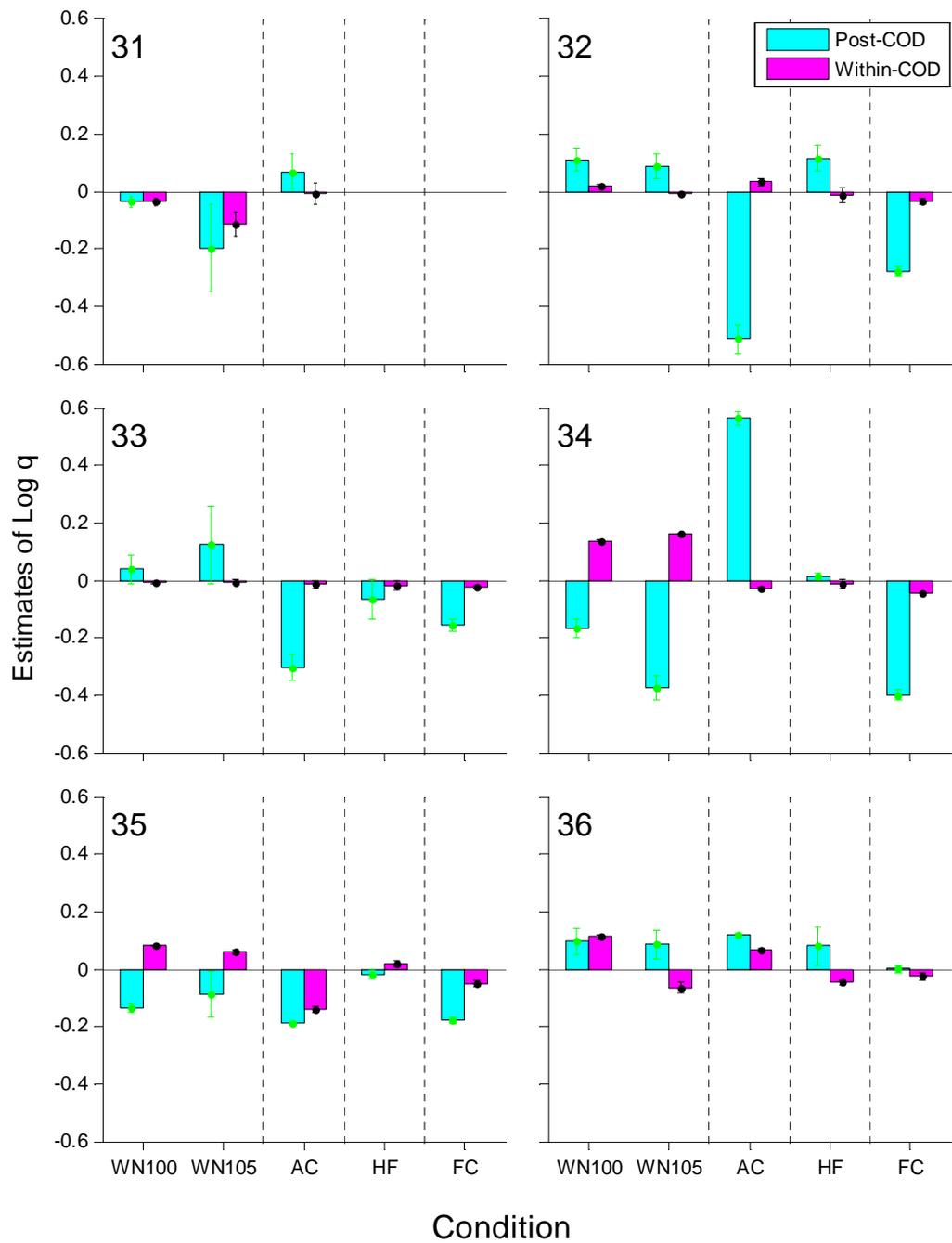
was played in one terminal-link (Conditions 2-4, 6, 8-11, 13, & 14), for each hen. Points below the line at zero indicate a bias away from the initial-link key associated with the terminal-link that produced sound and above the line at zero is towards this initial-link key. It is clear from Figure 2.1 that biases varied considerably across conditions and hens. The only sound that resulted in consistent biases across hens was the food call, where all hens showed varying degrees of bias away from the initial-link key leading to the terminal-link containing sound. Another consistency across subjects was that generally the differences between the response and time allocation data were small across subjects and conditions.

When one terminal-link contained white noise at 100 dB (WN100) initial-link noise bias differed across hens: Two hens (Hen 34 & 35) showed a bias away from the initial-link key leading to noise, while the others showed a bias towards it, although very small for Hens 31 and 33. The highest initial-link bias was 0.10 and the lowest was -0.12. When the white noise intensity increased to 105 dB (WN105), the size of the response and time allocation biases increased in magnitude for 3 hens (Hen 31, 33, & 34) and for the remaining hens the noise biases were similar in magnitude to those seen for white noise at 100 dB. The highest and lowest initial-link biases for white noise at 105 dB were 0.10 and -0.29, respectively.

Conditions where the alarm call (AC) was played during one terminal-link resulted in large noise biases in the initial-links for 2 hens but these were in opposite directions. Half of the hens (Hens 32, 33, & 35) showed biases away from the key leading to the terminal-link with sound, which were all larger in magnitude than those seen for white noise; the largest bias of these was -0.40. For the remaining subjects (Hens 31, 34, & 36), initial-link noise biases were towards the key leading to the terminal-link with the alarm call, and although 1 hen had response biases close to zero the other 2 hens showed large positive biases; the largest noise bias was 0.42 and represents a bias towards the key associated with sound. When the sounds of hens feeding (HF) was played in one terminal-link, noise biases in the initial-link were generally small across hens where the highest was 0.07 and the lowest was -0.05. The food call (FC) conditions resulted in negative estimates of  $\log q$  for all hens, where the largest of these was -0.27 and the smallest was -0.01.

#### **Noise bias for within- and post-COD responding.**

Figure 2.2 shows the estimates of  $\log q$  for initial-link responses post and within the COD. These were calculated in the same way as response and time allocation data, for



*Figure 2.2.* Estimates of  $\log q$  for responding post and within the COD in the initial-links over conditions when white noise (WN100 and WN105), the alarm call (AC), sounds of hens feeding (HF), and the food call (FC) were played in one terminal-link, plotted for each hen. The error bars represent one standard deviation either side of the mean ratios.

conditions where a sound was played in one terminal-link, and are plotted for each hen. Across hens and sounds this figure shows that post-COD response biases for 3 hens (Hen 31, 34, & 36) were larger than those from within the COD, and in some conditions much larger. For at least 1 subject in each condition, the within-COD biases were in the opposite direction to the post-COD biases. The post-COD biases followed the same trend as the total-response noise biases from the initial-link (Figure 2.1). In the white noise conditions (WN100 & WN105),  $\log q$  values for within-COD responding were generally larger than in the other sound conditions, which tended to be small.

### **Local response rates.**

The local response rates were calculated by dividing the number of responses on each initial-link key by the time spent on that key. These local response rates are presented in Figure 2.3 (white noise) and Figure 2.4 (no sound and other sounds), for each hen. These figures show the local response rates to the left and right initial-links keys when sound was played in either the left (L) or right (R) terminal-links, or no sound was played (NS). Across all conditions in Figure 2.3 and 2.4, 3 hens (Hens 31, 33, & 36) showed the same pattern of response rates. Response rates for the remaining hens changed across some sound conditions but there was no consistent pattern to these response rates. For all hens, there was generally no change in local response rates when the sound changed from the left to the right terminal-link. Local response rates in the initial-links of conditions without sound (NS) were comparable to those with sound.

### **Estimates of $\log c$ and reinforcers obtained.**

Estimates of  $\log c$  are presented in Figure 2.5 for conditions with white noise and in Figure 2.6 for conditions with no sound, or when other sounds were played in the left (L) and right (R) terminal-links, for each hen. These are logarithms of the mean ratios of responding over the last five sessions taken to the left key. These figures also display the logarithms of the ratio of reinforcers obtained, taken to the left key, indicated by an asterisk. The horizontal line at zero indicates where ratios would be expected to fall if the hens' behaviour showed no biases (i.e., no key bias) and if the same number of reinforcers was obtained on both keys. Points above the line indicate a bias towards or more reinforcers obtained on the left key.

As indicated from the noise bias estimates ( $\log q$  in Figure 2.1), hens reversed their response ratios when the condition reversed and sound was in the left and then the right terminal-link. What is of interest in Figures 2.5 and 2.6 was not the reversal of behaviour across reversal conditions but the degree to which ratios deviated from zero, which was where

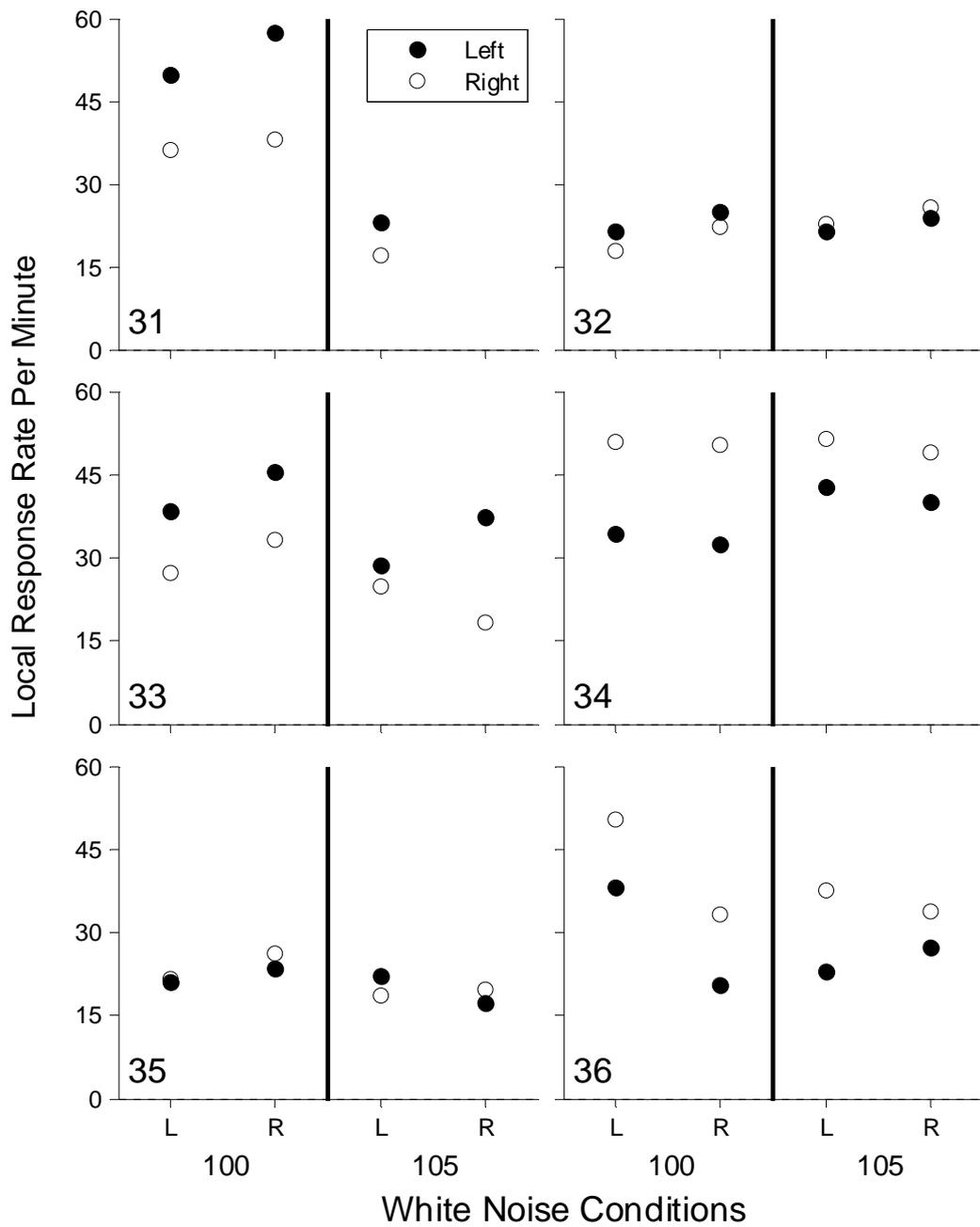


Figure 2.3. Local response rates (per min) on the left (filled circles) and on the right (unfilled circles) initial-link keys for conditions when white noise (at 100 and 105 dB) was played in either the left (L) or right (R) terminal-links, plotted for each hen.

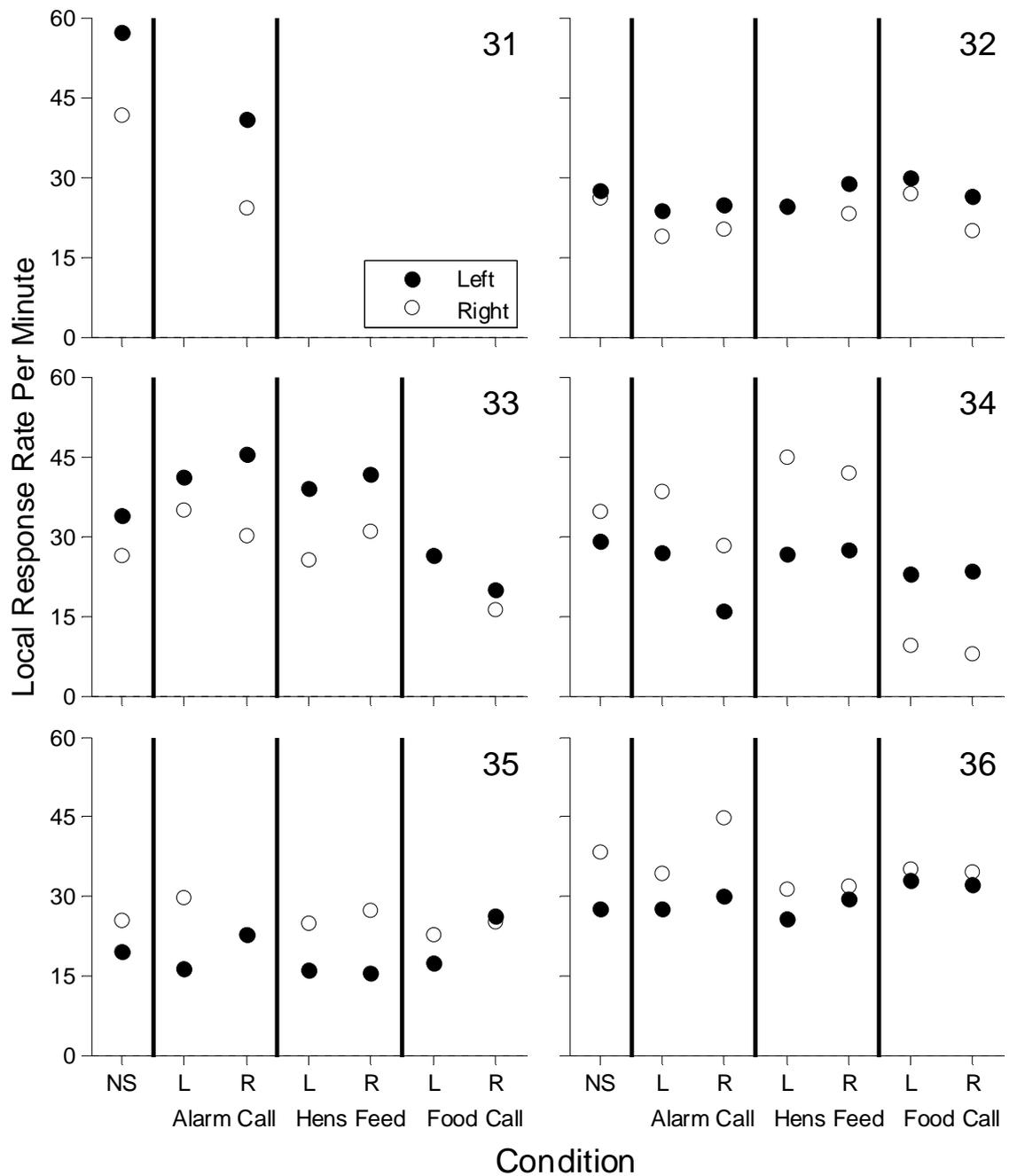


Figure 2.4. Local response rates (per min) on the left (filled circles) and on the right (unfilled circles) initial-link keys for conditions when no sound (NS), the alarm call, the sounds of hens feeding, and the food call were played in either the left (L) or right (R) terminal-links, plotted for each hen.

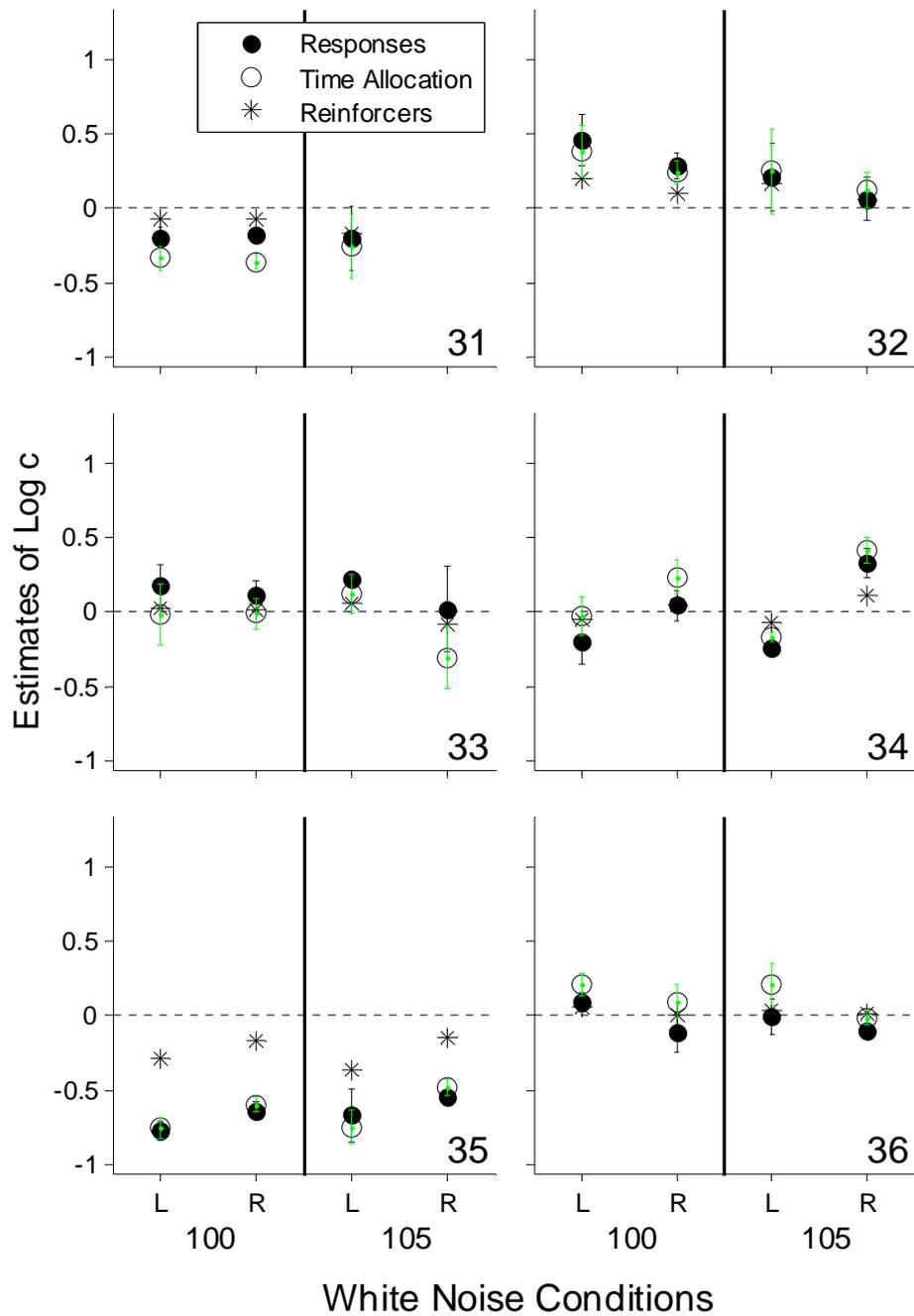


Figure 2.5. Estimates of  $\log c$  for responses (filled circles) and time allocation (unfilled circles), taken to the left key, in the initial-link for conditions when white noise was played in either the left (L) or right (R) terminal-link, plotted for each hen. The ratios of reinforcers obtained are indicated by an asterisk. The error bars represent one standard deviation either side of the mean ratios.

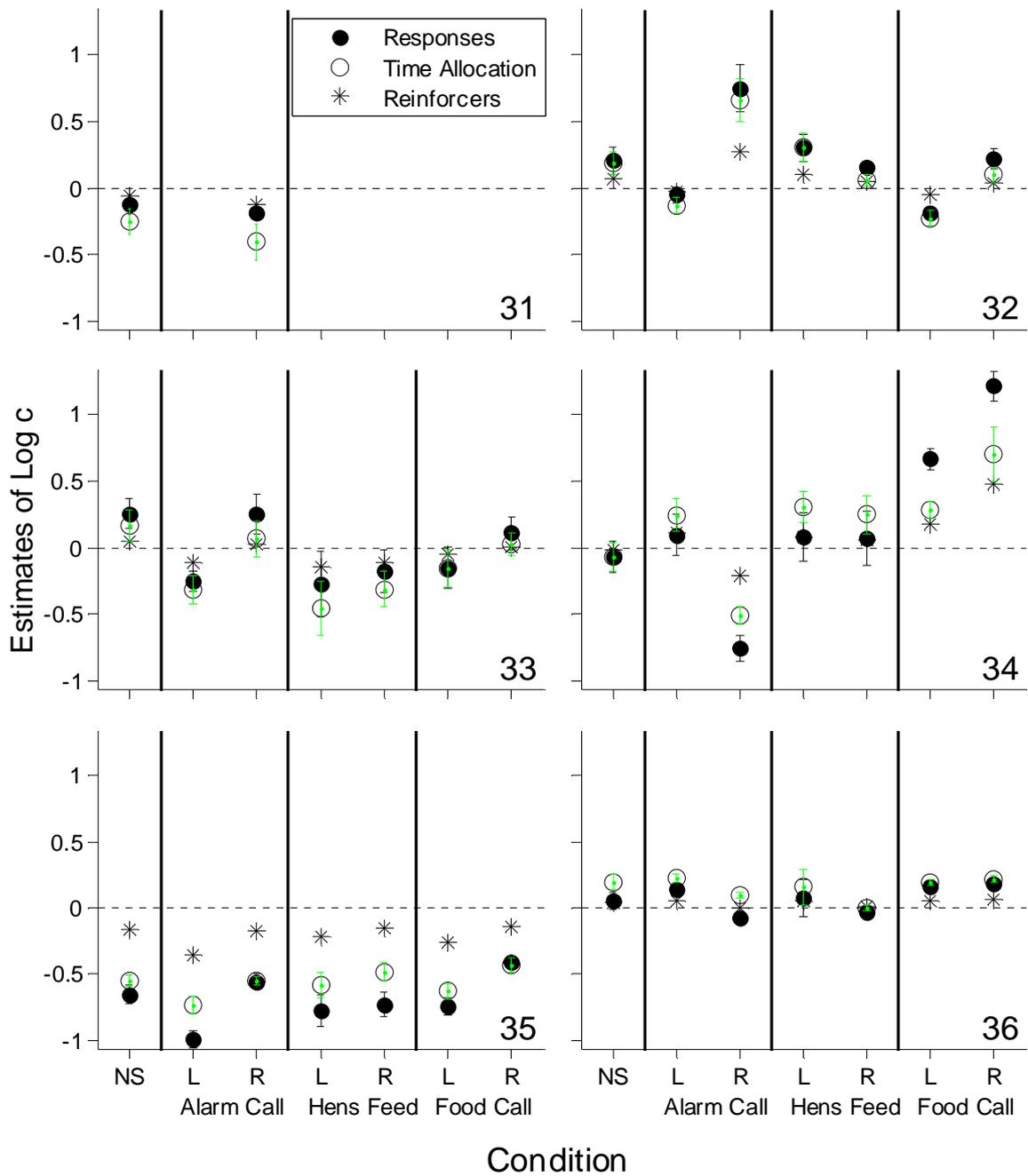


Figure 2.6. Estimates of  $\log c$  for responses (filled circles) and time allocation (unfilled circles), taken to the left key, in the initial-link for conditions when no sound (NS), or the alarm call, the sounds of hens feeding, and the food call were played in either the left (L) or right (R) terminal-link, plotted for each hen. The ratios of reinforcers obtained are indicated by an asterisk. The error bars represent one standard deviation either side of the mean ratios.

ratios would be expected to fall if no bias was shown. Both Hens 31 and 35 consistently showed right key biases across conditions. Hen 32 tended to favour the left key in some conditions while Hens 33 and 34 showed a mixture of key bias, either towards the left or towards the right keys. Hen 36 showed very little key bias across conditions.

Although the VI schedules operating in the initial-links were equal, the ratio of reinforcers obtained on both keys was not equal across conditions. For 3 hens (Hen 32, 34 & 35) the ratio of reinforcers obtained varied across all conditions. For Hen 31, more reinforcers were generally obtained on the right key and for the remaining hens (Hens 33 & 36) the numbers of reinforcers obtained on each key were relatively equal across conditions. The ratio of reinforcers obtained shows a similar pattern to the ratio of responses and time allocation (in terms of direction) across conditions. However, the ratio of responses and time allocation were always larger in magnitude than the ratio of reinforcers obtained.

### **Changeovers.**

The mean numbers of COs per session were examined and are shown in Appendix 2e (white noise conditions) and Appendix 2f (no sound and other sound conditions). All hens generally performed more changeovers when sound was associated with the right terminal-link but across conditions there was no consistent trend observed.

### **Sound ranks.**

In order to compare the hens' individual responses to the sounds across the two procedures, concurrent-chains and multiple-concurrent schedules (Experiment 1). The sounds were ranked based on the initial-link noise bias estimates ( $\log q$ ) from responding and these ranks are shown in Table 2.2, for each sound. The largest bias away from the sound was given a rank of 1 and the least bias or bias towards the sound was given a rank of 5. Four hens here showed initial-link biases towards the terminal-link associated with a sound, which were different sounds across hens, and these are indicated by an asterisk. Ranking sounds showed no consistent pattern across hens. For example, the alarm call was ranked first for 3 hens and was ranked last for the remaining 3 hens. The white noise conditions at 100 and 105 dB also showed inconsistent ranks among the hens. The food call was the only sound showing some degree of consistency, ranked 2<sup>nd</sup> for 4 hens and ranked 1<sup>st</sup> for the remaining hen.

### **Summary.**

In summary, conditions where sound was played in one terminal-link did not result in consistent noise biases ( $\log q$ ) in the initial-links across hens (Figure 2.1). Only one sound

Table 2.2.

Ranks for each sound, based on initial-link response biases ( $\log q$ ), ranging from largest bias away from (Rank 1) to the least bias or bias towards the initial-link key leading to sound (Rank 5), shown in each column for each subject. Note: the \* indicates a positive initial-link bias, towards the initial-link key leading to the sound. The sounds shown are white noise at 100 dB (WN100) and 105 dB (WN105), an alarm call (AC), the sounds of hens feeding (HF) and a food call (FC). As Hen 31 died before complete some conditions, an n/a is shown for some ranks.

<b>Hen</b>	<b>Rank 1</b>	<b>Rank 2</b>	<b>Rank 3</b>	<b>Rank 4</b>	<b>Rank 5</b>
31	WN 105	WN 100	AC	n/a	n/a
32	AC	FC	WN105*	HF*	WN100*
33	AC	FC	HF	WN100*	WN105*
34	WN105	FC	WN100	HF*	AC*
35	AC	FC	WN105	WN100	HF
36	FC	WN105*	HF*	WN100*	AC*

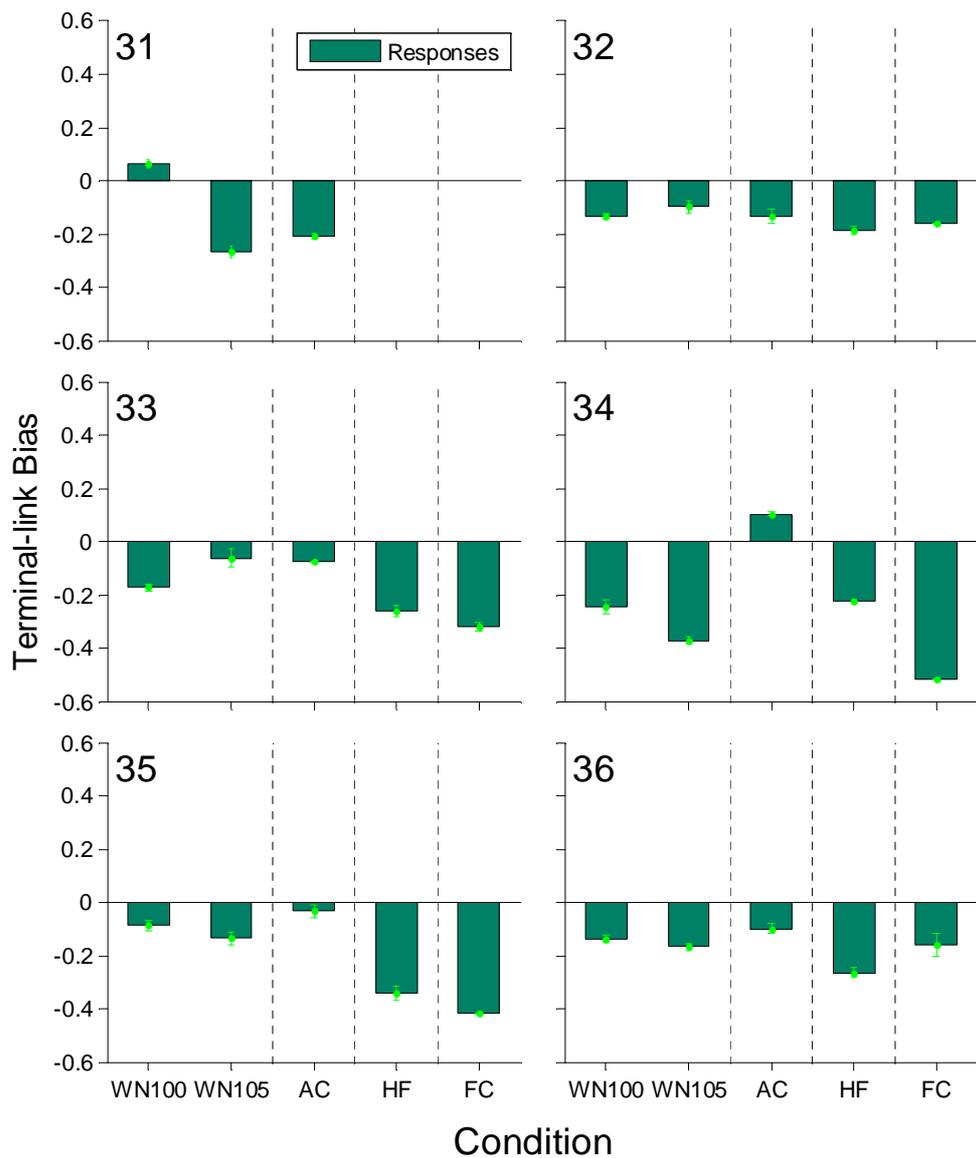
(food call) resulted in noise biases all in the same direction across subjects. The post-COD noise biases reflected those seen in the total response data and tended to be larger, and for 3 hens much larger, than the within-COD noise biases. Local rates of responding seen in the initial-links for individual hens were generally the same across all conditions and did not change when the conditions reversed. The estimates of  $\log c$  (Figures 2.5 and 2.6) showed that 2 hens had consistent right key biases, but that the direction of other hens' key biases varied across conditions or were very small. However, in spite of the key biases shown by some hens, noise biases ( $\log q$ ) were still observed. For all hens, the ratio of reinforcers obtained followed a similar pattern to the ratio of responses in the initial-links but were smaller in magnitude. Finally, the ranks given to each sound showed an inconsistent pattern across hens (Table 2.2) with the food call being the only sound to show a similar rank across hens.

### **Terminal-link Behaviour**

#### **Terminal-link bias.**

As sound was played during the terminal-link schedule (FI 10 s) it was possible to assess the effects of the sound playing on their rates of responding. Differences in responding in the left and right terminal-links can be seen using Equation 6 (typically a measure of noise bias). To calculate this bias, the number of responses in each terminal-link was first divided by the number of terminal-link entries on that side, because the number of entries was not equal. The number of responses per entry on the left was then divided by the number of responses per entry on the right, and then logged (to the base 10). This calculation gave a measure of the degree of difference between responding in the terminal-links across reversal conditions. In this thesis, this change in responding is termed 'terminal-link bias'. Figure 2.7 shows the terminal-link biases for conditions where a sound was played during one terminal-link, plotted for each hen. Points below the line shows that less responding occurred in the terminal-links with sound than in the terminal-link without sound and that this pattern reversed across reversal conditions.

Figure 2.7 shows that for all hens, and in most conditions, terminal-link bias was away from the terminal-link that contained sound. Across conditions where white noise was played in one terminal-link, all hens (except Hen 31 for WN100) showed a terminal-link bias away from the terminal-link with sound but the degree of this bias varied across subjects ranging from 0.06 to -0.37. For the alarm call, sounds of hens feeding, and the food call conditions all



*Figure 2.7.* Estimates of terminal-link bias from responding across reversal conditions that contained white noise (WN100 and WN105), the alarm call (AC), sounds of hens feeding (HF), and the food call (FC) in one terminal-link, plotted for each hen. The error bars represent one standard deviation either side of the mean response ratios.

hens showed a terminal-link bias away from the terminal-link associated with sound, with the exception of Hen 34 who showed a bias towards the terminal-link with the alarm call (AC). The terminal-link biases for the alarm call were generally smaller than for the other sounds, ranging from 0.10 to -0.21. For the sounds of hens feeding, terminal-link bias ranged from -0.18 to -0.34 and for the food call terminal-link biases were all negative and ranged from -0.16 to -0.52.

### **Local response rates.**

Figures 2.8 and 2.9 show the local response rates in the left (L) and right (R) terminal-links that contained white noise (Figure 2.8) and for conditions with no sound (NS), the alarm call, the sounds of hens feeding, and the food call (Figure 2.9), plotted for each hen. The response rates were calculated by dividing the total number of responses in a terminal-link by the total time spent in that terminal-link. Response rates in many conditions were lower in the terminal-link containing sound and this pattern remained when conditions reversed and sound was associated with the left and then the right terminal-link. An exception to this was the response rates for Hens 31 and 35, who had right key biases and predominantly responded at a higher rate in the right terminal-link regardless of condition. Figures 2.8 and 2.9 show that when any sound was playing in the terminal-link the hens tended to respond at a lower rate. This was so in 41 out of 54 cases. The mean local response rates in the terminal-links with and without sound (across subjects) are shown in Table 2.3. Except for the alarm call condition, it was clear that response rates were lower when the sound played during the terminal-link compared to when the terminal-link was without sound. The largest difference in mean local response rates between the terminal-links with and without sound was for the food call (a difference of 11) and the smallest difference was for the alarm call. For the terminal-links that contained sound, the lowest average rates were seen for the food call and the sounds of hens feeding. Response rates in the terminal-link without sound were comparable to the rates shown for conditions with no sound.

### **Terminal-link duration and entry pauses.**

Under FI schedules, a pause in responding is typically seen at the start of the schedule, after a reinforcer. As a FI schedule was used in the terminal-links of this experiment, it may be that the duration of this pause was affected by the presence of sound. Although the schedules in effect in both terminal-links were equal, if the hen paused for a long time or responded at a low rate the duration of terminal-link could be increased. In the present experiment a pause

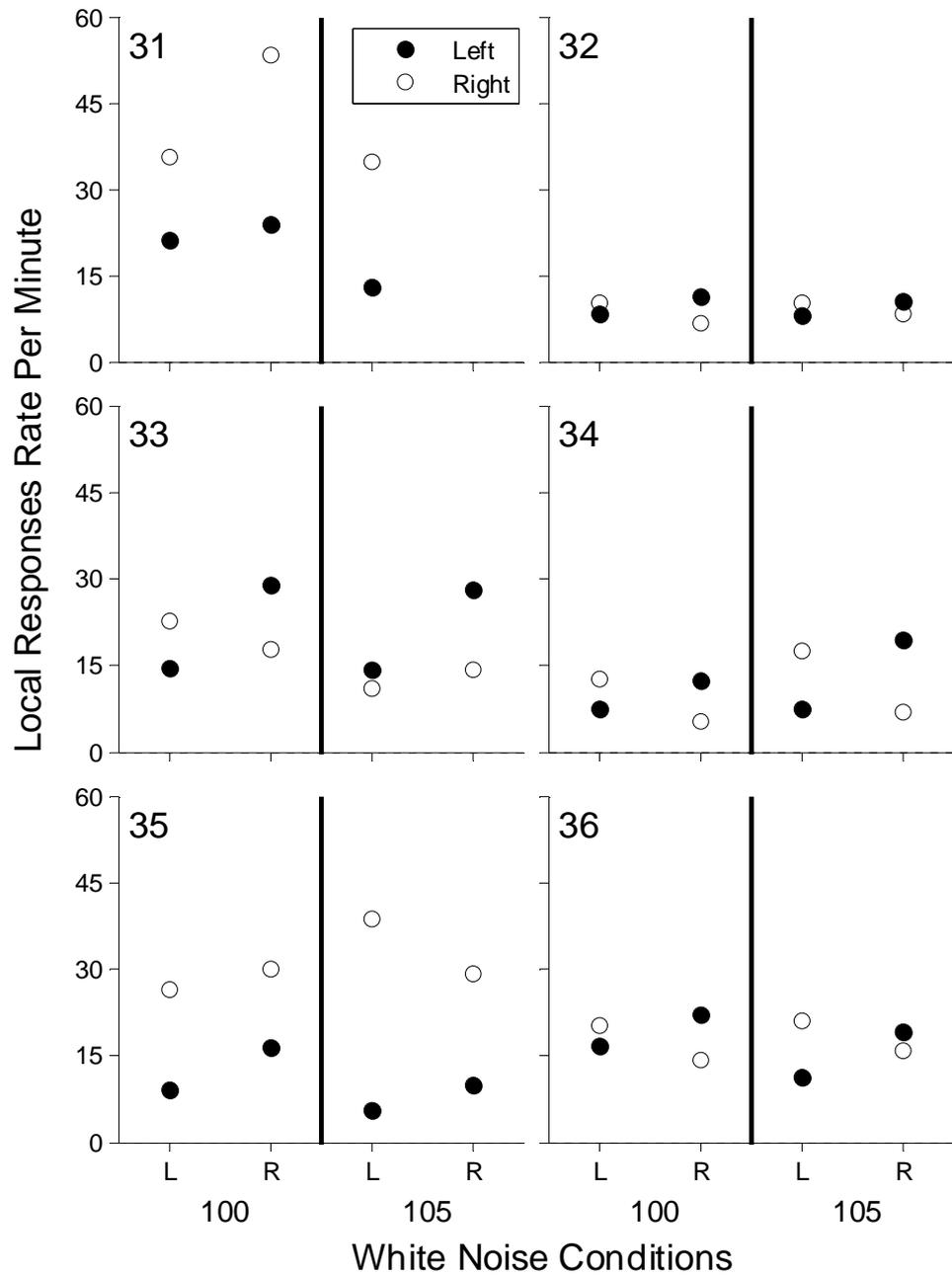


Figure 2.8. Local response rates (per min) on the left (filled circles) and on the right (unfilled circles) terminal-link keys for conditions when white noise (at 100 and 105 dB) was played in either the left (L) or right (R) terminal-links, plotted for each hen.

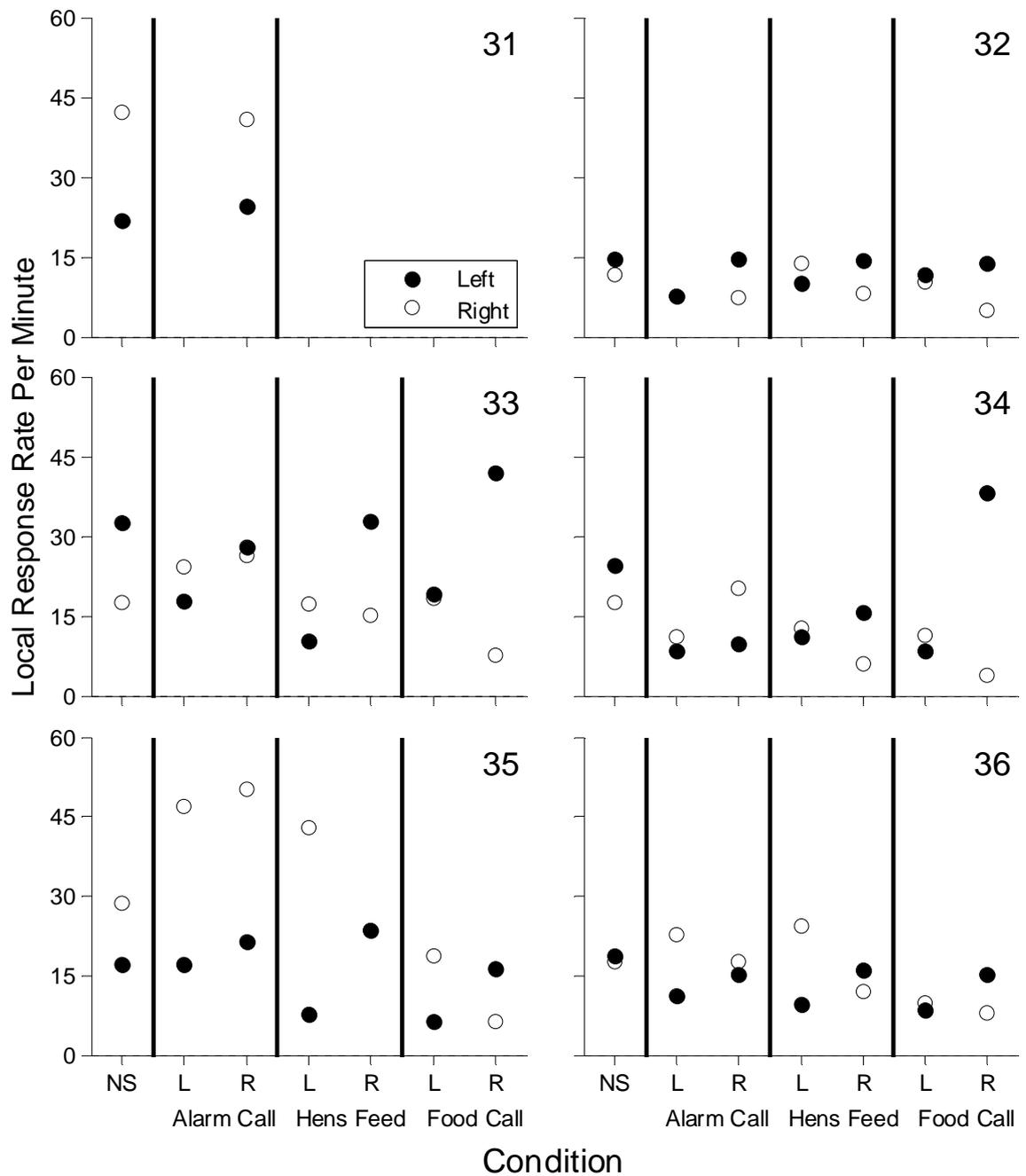


Figure 2.9. Local response rates (per min) on the left (filled circles) and on the right (unfilled circles) terminal-link keys for conditions when no sound (NS) or the alarm call, the sounds of hens feeding, and the food call were played in either the left (L) or right (R) terminal-links, plotted for each hen.

Table 2.3

*Table showing the mean local response rates (per min) in the terminal-links where a sound was or was not played, shown in each column for each condition.*

<b>Sound in terminal-link</b>	<b>Response rates (per min) in terminal-link with sound</b>	<b>Response rates (per min) in terminal-link without sound</b>
No sound	-	22 (left) 22 (right)
White noise 100 dB	17	20
White noise 105 dB	12	20
Alarm call	20	21
Hens feeding	11	21
Food call	8	19

that occurred after the terminal-link began is termed a 'terminal-link entry pause'. The mean terminal-link duration and terminal-link entry pauses are presented in Figure 2.10 and Figure 2.11 in conditions when sound was played in the left and the right terminal-link, for each hen. Figure 2.10 shows data from conditions with white noise and Figure 2.11 shows data from conditions with no sound, the alarm call, sounds of hens feeding, and the food call. The shaded area indicates the mean terminal-link entry pause, i.e., the pause that occurred before the first response in the terminal-link. The whole height of the bar shows the mean duration of the terminal-links (s). Given that a FI 10-s schedule was in effect, the mean duration of the terminal-links was expected to be slightly longer than 10 s, at the height indicated by the horizontal dotted line.

It is clear from Figures 2.10 and 2.11 that terminal-link entry pauses varied considerably across subjects and conditions. These figures also show that across reversal conditions hens paused for longer in the terminal-links that contained white noise in 6 out of 11 cases and in 8 out of 15 cases for the other sounds. For 2 hens (Hen 31 & 35), pauses were consistently longer in the left terminal-link across all conditions, except in both food call conditions Hen 35 gave longer pauses in the terminal-link that contained sound. Pausing for the remaining hens varied with many hens showing consistently longer pauses in the terminal-link containing sound across reversal conditions.

The duration of the terminal-links, also shown in Figures 2.10 and 2.11, varied somewhat across conditions and hens. Generally, the duration of the terminal-links was not equal across conditions but rather for most hens it often followed the same pattern as the entry pauses. For example, in the alarm call conditions Hen 32 paused for longer and spent longer in the terminal-links where sound was played. Another interesting aspect of these figures was that when a hen paused for a long time the terminal-link durations were frequently longer than the FI 10-s requirement. This was particularly evident in the food call conditions for all hens, especially when the sound was played in the right terminal-link.

The mean terminal-link durations and terminal-link entry pauses (across subjects) are presented in Table 2.4 for each condition. This table shows that in all conditions, except the alarm call, the mean terminal-link entry pauses were longer in terminal-links with sound than those without sound. The mean duration of the terminal-link was also always longer when the terminal-link contained sound, for all sounds. The biggest differences between terminal-links with and without sound can be seen for the food call, a difference of 3.7 s for the mean entry

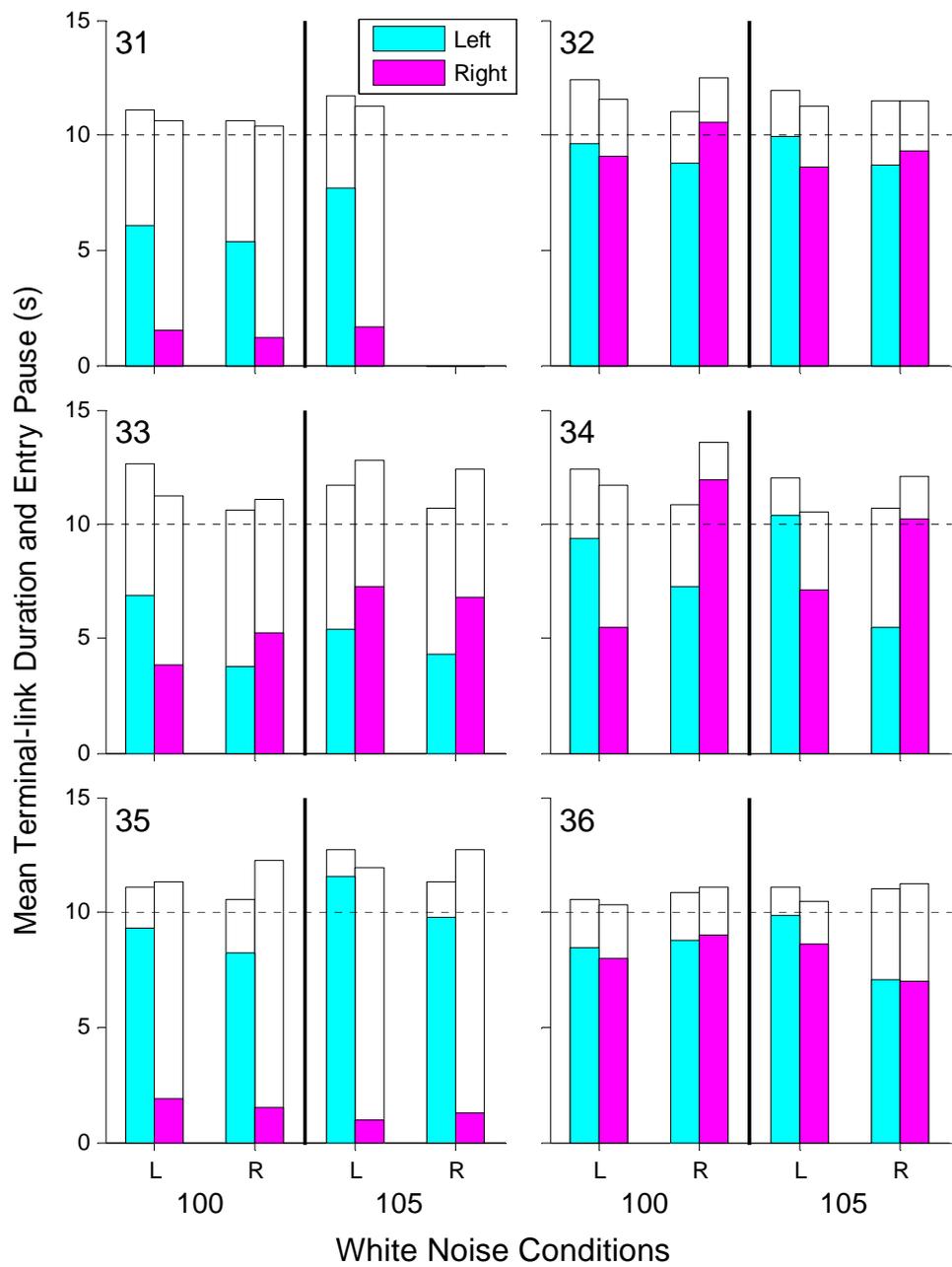


Figure 2.10. Mean terminal-link duration (full height of the bars) and terminal-link entry pause (s) (shaded bars) in the left and right terminal-links for conditions when white noise was played in the left (L) and the right (R) terminal-links, plotted for each hen.

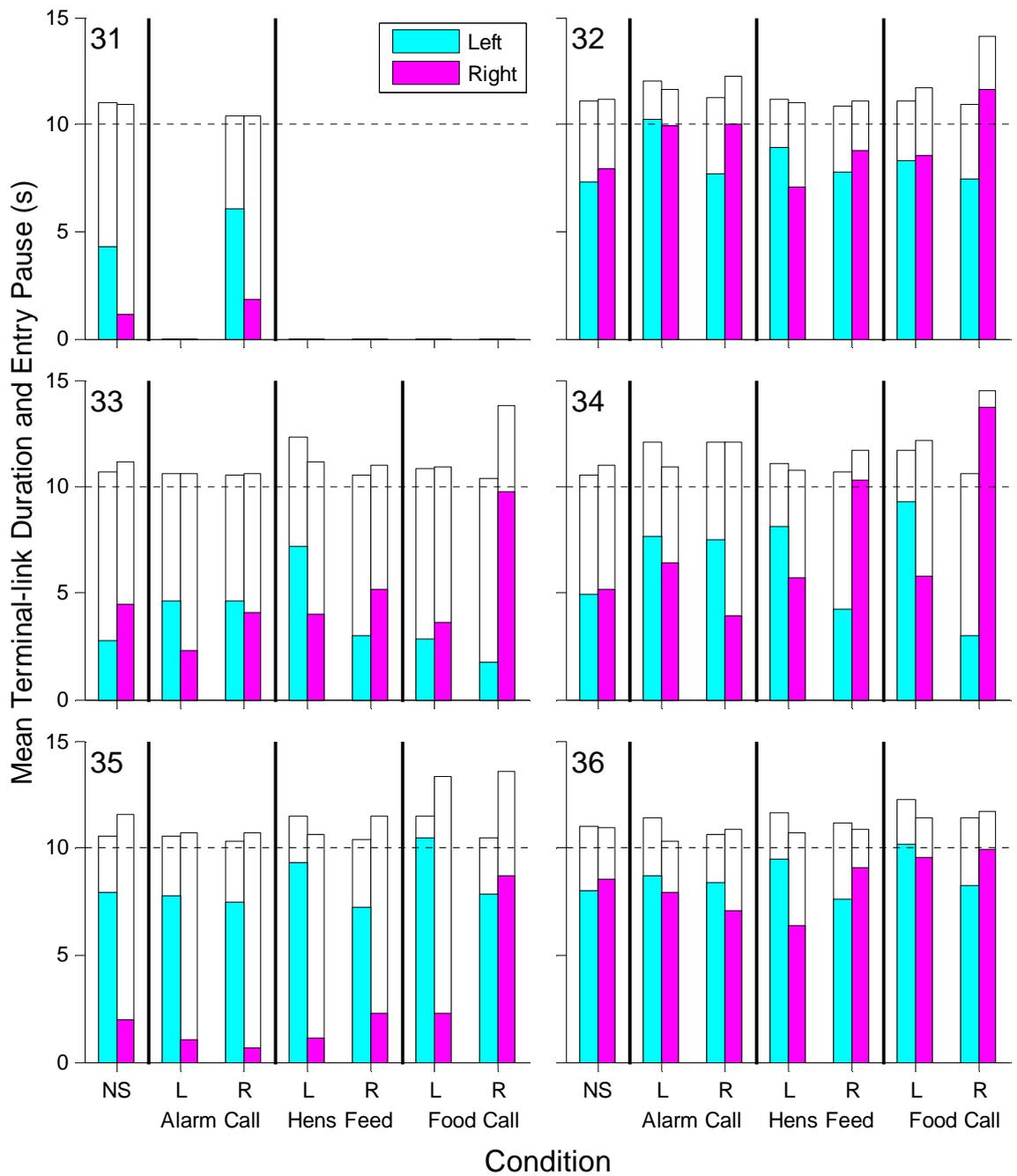


Figure 2.11. Mean terminal-link duration (full height of the bars) and terminal-link entry pause (s) (shaded bars) in the left and right terminal-links for conditions when no sound (NS) or the alarm call, the sounds of hens feeding, and the food call were played in the left (L) and the right (R) terminal-links, plotted for each hen.

Table 2.4

*Table showing the mean terminal-link entry pauses and the terminal-link durations for terminal-links with and without sound. These mean pauses and durations are shown in each column for all conditions.*

<b>Sound in terminal-link</b>	<b>Pause in terminal-link with sound (s)</b>	<b>Pause in terminal-link without sound (s)</b>	<b>Duration of terminal-link with sound (s)</b>	<b>Duration of terminal-link without sound (s)</b>
No sound	-	6.1 (left) 5.3 (right)	-	10.8 (left) 11.1 (right)
White noise 100 dB	7.4	6.0	11.8	10.9
White noise 105 dB	8.1	6.3	11.9	11.2
Alarm call	6.0	6.3	11.2	10.9
Hens feeding	7.9	5.4	11.4	10.8
Food call	9.5	5.8	12.5	11.3

pause duration and a difference of 1.2 s for the mean terminal-link duration. The food call resulted in the longest average terminal-link durations and entry pauses.

### **Summary.**

In summary, response rates in the terminal-links containing sound were lower than those where no sound was present and than those in conditions without sound. Figure 2.7 showed that in most conditions there was a terminal-link bias away from the terminal-link that contained sound. These biases were largest in conditions with the food call and white noise at 105 dB. Moreover, lower local response rates were shown in terminal-links that contained sound and this also reversed when the conditions reversed. Terminal-link entry pauses tended to be longer in those terminal-links that contained sound and these terminal-links were also generally longer in duration. The longest mean entry pauses and terminal-link durations were during the food call conditions.

### **Comparison of Initial- and Terminal-link Responding**

The sounds had effects on the ratios of responding in both the initial- and terminal-links. However, the direction of noise biases shown in the initial-links was sometimes opposite to the direction of any terminal-link biases. Only 1 hen (Hen 35) showed noise biases away from the initial-link key leading to sound and a terminal-link bias also away from this terminal-link, for all sound conditions. In 9 out of 28 cases hens showed lower response rates in the terminal-link with sound compared to the terminal-link without sound (which gave a negative terminal-link bias) and an initial-link bias towards this terminal-link. For example, Hen 36 responded at a lower rate in the terminal-links that contained white noise (at both intensities) but showed a small initial-link bias towards these terminal-links. Some subjects did also show initial- and terminal-link biases in the same direction, in 15 out of 28 cases. It was clear that for some subjects the effects of sound on responding were more obvious when the sound was present (terminal-link) than when it was absent (initial-link).

### **Comparison of Results with Those From Experiment 1**

Table 2.5 shows the estimates of noise bias ( $\log q$ ) from Experiments 1 and 2, for each sound and subject. In this table, positive noise biases, towards the sound keys, are represented in bold. There were only a few similarities in the degree and direction of the noise biases across experiments. In Experiment 1, all hens showed a bias away from the key associated with white noise at both decibel levels, whereas the initial-link biases in the present experiment

Table 2.5

*Comparison of noise bias estimates (log q) for each subject hen from Experiment 1(E1) and Experiment 2(E2). Bias estimates for each sound are shown in each column; white noise at 100 dB (WN100) and 105 dB (WN105), the alarm call (AC), the sounds of hens feeding (HF), and the food call (FC). Positive bias estimates (towards the key associated with sound) are shown in bold. Note: The cross indicates where no data are given because Hen 31 died.*

Hen	Sound Condition									
	WN100		WN105		AC		HF		FC	
	E1	E2	E1	E2	E1	E2	E1	E2	E1	E2
<b>31</b>	-0.071	-0.008	-0.091	-0.140	-0.001	-0.003	-0.075	+	+	+
<b>32</b>	-0.087	<b>0.086</b>	-0.155	<b>0.073</b>	-0.074	-0.397	-0.114	<b>0.074</b>	-0.198	-0.201
<b>33</b>	-0.169	<b>0.031</b>	-0.251	<b>0.102</b>	<b>0.014</b>	-0.252	-0.099	-0.048	-0.099	-0.131
<b>34</b>	-0.275	-0.121	-0.345	-0.287	-0.040	<b>0.423</b>	-0.132	<b>0.005</b>	-0.115	-0.272
<b>35</b>	-0.170	-0.064	-0.189	-0.061	-0.080	-0.217	-0.020	-0.022	-0.225	-0.165
<b>36</b>	-0.033	<b>0.102</b>	-0.050	<b>0.051</b>	-0.030	<b>0.108</b>	-0.030	<b>0.056</b>	-0.071	-0.010

were generally closer to zero or positive. The alarm call resulted in very small biases in Experiment 1 while in the present experiment large biases were seen that were both towards and away from the keys associated with sound, across subjects. Estimates of  $\log q$  in Experiment 1 for the sounds of hens feeding were all negative although for the present experiment values of  $\log q$  were positive for 3 hens, but these estimates were small across both experiments. The food call was the only sound where estimates of  $\log q$  across both experiments were all in the same direction, but these varied in degree. When comparing the response-based noise biases across the two experiments, out of 28 cases the direction of noise bias was the same in 13 cases, in opposite directions in 10 cases, and in the remaining 5 cases it was very close to zero in one experiment. Finally, when the hens did show a noise bias in the same direction across the two experiments, the magnitude of this bias was often larger in Experiment 1 than in the present experiment.

The lowest response rates shown in Experiment 1 were in the presence of white noise but in the present experiment the lowest rates in the presence of sound (terminal-links) were for the food call. The low response rates for the food call were a result of the longer terminal-link entry pauses that occurred for this sound.

### **Overall Summary**

Estimates of  $\log q$  (noise bias) shown in the initial-links of the present experiment were inconsistent across subjects and differed considerably, in both direction and magnitude, from the noise biases shown in Experiment 1. Terminal-link biases shown in the present experiment, however, were generally consistent across subjects and away from the terminal-links with sound. Although the local response rates in the initial-links did not differ across sounds, in the terminal-links rates of responding were lower when sound was present, for all sounds except the alarm call. In Experiment 1, the lowest local rates of responding were in the presence of white noise but in the present experiment they were lowest in the terminal-links containing the food call. The food call also gave the longest average terminal-link durations and entry pauses and was the only sound where all subjects showed noise biases in the same direction in the initial-link.

### **Discussion**

The concurrent-chains procedure used in the present experiment resulted in initial-link noise biases that varied substantially in direction and degree across conditions and hens. Contrary to predictions, responding in the initial-links was not biased in the same direction, or

to the same degree, by sounds that had also been used previously in Experiment 1 in a concurrent procedure. In Experiment 1, only 1 hen showed a positive noise for one sound only and here 4 hens (Hens 32-34 & 36) showed initial-link biases that were positive (towards the key leading to sound). It was expected that lower rates of responding would be seen in the terminal-links with white noise compared to the response rates in the terminal-links with the other sounds used, however, the lowest rates were shown in terminal-links with the food call. As predicted, a pause occurred at the start of the FI schedule in the terminal-links and unexpectedly, the duration of this pause was affected by the presence of sound and was longest for the food call.

### **Initial-link Behaviour**

Based on previous research with hens (McAdie, 1998; McAdie et al., 1993, 1996), the results of Experiment 1, and those of O'Daly et al. (2005) it was assumed that the direction of initial-link biases for any given sound would have been similar across hens. However, only for the food call sound did all hens show a preference in the initial-links away from the terminal-link associated with that sound. For the remaining sounds, half of the hens showed preferences towards the initial-link keys that led to a sound, and the other half of hens showed preference away from the initial-link keys that led to a sound. It is unclear why inconsistent preferences were shown here when the direction of preferences in Experiment 1 were more consistent across subjects. It may be that measures of choice using the concurrent-chains procedure were confounded by other variables (to be discussed later).

A possible reason for the hens' inconsistent preferences in the initial-links could be that sound in the terminal-links was less effective at biasing responding than it had been when present during the choice phase in Experiment 1. The inconsistent preferences found here were similar to those found using a similar procedure (Otsuka et al., 2009). Otsuka et al. found that rats' preferences in the initial-link differed across subjects when sound was associated with both terminal-links. However, Rachlin (1967) found that non-contingent electric shock in the terminal-links of a concurrent-chains procedure greatly influenced responding in the initial-links of all pigeons. This study by Rachlin showed that an aversive stimulus could bias the responding of all subjects in the same direction under a concurrent-chains procedure. The lack of common effects across hens seen here suggests that white noise used in the present experiment was not as aversive as an electric shock. For the hens in the present experiment

gaining access to food outweighed the effects of being in the presence of white noise, which was thought to be aversive.

There were differences observed between the noise biases of Experiment 1 and the present experiment. Here, 4 hens showed biases towards the initial-link keys that led to a terminal-link with sound in the concurrent-chains procedure but not in concurrent schedules. In Experiment 1, the direction of noise biases shown under concurrent schedules were more consistent across subjects. In addition, when the direction of noise bias across the two procedures was the same, biases from Experiment 1 tended to be larger than those from the present experiment. The two procedures gave different results and so it is difficult to draw conclusions about which procedure reflects the sound preferences of hens. The direction of noise biases in Experiment 1 were consistent across subjects and similar to previous findings using the same procedure (McAdie, 1998; McAdie et al., 1993, 1996). The biases were also predictable from other data on hens' reactions to different sounds (e.g., Mackenzie et al., 1993). Noise biases in the present experiment were inconsistent across subjects as were the results from Otsuka et al. (2009) and both sets of these results were difficult to interpret, for example, not as predictable for what is known about hens reactions to sounds. More confounds could also be identified in the concurrent-chains procedure than in concurrent schedules, such as unequal obtained reinforcement ratios and unequal durations of the terminal-links. Given all of these considerations concurrent schedules might be a more sensible procedure for future use when assessing sound preferences.

In the present experiment, although the scheduled ratios of reinforcement were programmed to be equal the obtained ratios of reinforcement were not always equal across conditions. This may have helped bias responding in the initial-links towards the key producing higher reinforcement rates. When using independent concurrent schedules, subjects can choose one key schedule exclusively by allocating all of their behaviour to that schedule. Thus, subjects may not always obtain equal rates of reinforcement. This finding is not uncommon when using independent schedules in the initial-links and as Snyderman (1983) points out "both the problem of terminal-link duration and that of unequal terminal-link entries are common to most concurrent-chains experiments" (p. 438). Killeen (1972) suggested that it is the obtained reinforcement ratio that influences subsequent behaviour, as opposed to the scheduled reinforcement ratios. This suggests that in the present experiment greater obtained reinforcers on one key may have led to greater choice for that alternative, thus causing key bias and confounding measures of noise bias. If this were the case then interpretations of  $\log q$ ,

which are based on the GML and which assume that obtained reinforcers are equal, must be made with caution.

Although the ratios of reinforcers obtained may have influenced the responding of hens, sound preferences were still evident. Initial-link biases tended to reverse when the sound in the terminal-link changed from one terminal-link to the other and this usually resulted in a reversal of the ratio of reinforcers obtained. Furthermore, in conditions without sound equal numbers of reinforcers were obtained on the left and right keys. If the number of obtained reinforcers were having a greater influence than sound on response ratios, then in conditions without sound it was likely that obtained reinforcers would have been unequal, and they were not. Further evidence of the effect of sound was that the ratios of responding tended to be larger than the ratios of reinforcers obtained. This effect is opposite to undermatching, which is a tendency to respond more on the alternative delivering the lower rate of reinforcement, which is typically seen in studies using concurrent schedules (Weardon & Burgess, 1982). Thus, it appears, from the present results, that sound had a greater effect on responding than the number of reinforcers obtained.

### **Terminal-link Behaviour**

In general, patterns of responding during the terminal-link FI schedules differed more systematically when sounds were present or absent than responding in the initial-links, and these patterns in the terminal-links reversed when conditions reversed. In most cases, local response rates were lower in terminal-links that contained sound and, unexpectedly, pauses in the terminal-links with sound were longer than in the terminal-links without sound, with the exception of the alarm call.

The effect of the presence of sound on the responding in the terminal-links was clear. All subjects displayed quite different rates of responding during each terminal-link FI schedule. The change in responding, when conditions reversed and the sound was in the left and then the right terminal-link, can be quantified. This can be thought of as terminal-link bias in the same way as when response rates differ in multiple schedules they can be referred to as bias (Barron & Davison, 1972). This measure (Figure 2.7) indicated that the hens responded more in the terminal-links without sound, in 26 out of 28 cases, and this pattern has reversed across reversal conditions. This was similar to the response rates from Experiment 1 but the initial-link noise biases were often towards the key that led to the terminal-links with sound. Thus, the effect of the presence of sound on responding in the terminal-links was similar to the effects shown in

Experiment 1. However, the noise biases across the two experiments were generally inconsistent. It appears that when performance under concurrent-chains and concurrent schedules is compared, responding in the terminal-links, rather than the initial-links, is comparable. It seems clear that choice was affected differently depending on whether sound was present (Experiment 1) or absent (initial-links of the present experiment) during the choice phase.

The local rates of responding in the terminal-links were lower in the presence of sound. When any sound was played in the terminal-links lower rates of responding occurred in 42 out of the 54 cases. Clearly, sound in the terminal-links slowed or delayed the hens responding. This finding was similar to Experiment 1, where lower local rates of responding were shown on keys associated with white noise compared to those without noise. Although in Experiment 1 response rates were lower on keys associated with the other sounds, the difference between responding to keys associated with and without sounds was only small. Reed and Yoshino (2008) found that when using concurrent schedules a brief loud tone suppressed responding in rats and this effect increased when the reinforcement schedule was lean. The authors concluded that the loud tone served as a punishing stimulus for the operant behaviour of these rats. In addition, Rutter et al. (1993) found that the sound of a conveyor belt suppressed responding of hens on a VR schedule and concluded that this sound was aversive to hens. McAdie (1998) found lower response rates when white noise was present during hens responding and suggested that hens find white noise aversive. As previously outlined, this suppression of responding is a common effect due to the presence of an aversive stimulus (e.g., Azrin, 1956). It appears that terminal-link responding was suppressed by sound in the present experiment, particularly by the presence of the food call but also by white noise and the sounds of hens feeding.

Another possible explanation for the lower responding in the presence of the sounds found here may be that the hens were engaging in 'other' behaviours. Evans and Evans (1999) found that hens fixated their heads downwards when played the recording of a food call. It has also been shown that hens were attracted to and moved towards a rooster's food call (Marler et al., 1986). It was possible that, during the food call conditions at least, the hens may have been engaging in foraging behaviours elicited by the food call rather than responding to the keys, thus reducing response rates. Unfortunately, it was not possible to confirm this hypothesis as the hens were not videoed in experimental sessions but it is recommended that future studies of this type do so.

In studies using FI schedules in the terminal-link, few have reported on the 'terminal-link entry pause'. This was the pause in responding after the FI terminal-link had been entered. In the present experiment, in 41 out of 54 cases, greater pauses occurred in the terminal-links with sound. It seems that these longer pauses were due to the presence of the sound as shorter pauses were observed in terminal-links without sound. Davison (1974) studied behaviour under single chain FI FI schedules and found that FI initial-link pauses consumed a greater proportion of the schedule than the FI terminal-link pauses. Davison suggested that the difference in pausing between the initial- and terminal-links was likely due to the primary reinforcement available in the terminal-links being more effective at maintaining responding. Upon inspection of Davison's data, the terminal-link entry pause was calculated to be a proportion of approximately 0.25 of the FI duration. These pause durations were considerably lower than the 0.5 proportion reported by Shull (1970; 1971) under single FI schedules. However, Shull's data are more comparable to pausing seen in the terminal-links without sound in the present experiment, where the entry pauses were an average proportion of 0.61 and 0.53 of the FI duration on the left and right keys, respectively. Conversely, in terminal-links with sound the proportion of pausing rose as high as 0.95 (food call), which was opposite to the findings of Davison. It seems that the sound had a suppressing effect on the hens' responses but it is unknown if the effect was due to the sound punishing responding, or if it was due to the hens engaging in other behaviours.

One unexpected result in this experiment, and a potential confound, was that in many conditions the terminal-link durations were longer than programmed. Under a FI 10-s schedule it was expected that the mean terminal-link durations would have been 10 to 11-s long. In the present experiment, the terminal-link durations were sometimes as long as 14.5 s in some conditions with sound. Davison (1974) found that in some FI initial-link schedules, of a two-link single chain, the duration of the initial-link was longer than the FI time when the FI value in one link was much higher than the value in the other link. These longer durations, seen in the present and in Davison's study, were due to lengthy pauses that exceeded the FI duration. This unforeseen result here meant that the terminal-link schedules were not always equal.

As previously stated, Fantino's delay reduction theory predicts that an animal will prefer the terminal-link that leads to a greater reduction in time to primary reinforcement (Fantino & Squires, 1971). In relation to the present experiment, longer terminal-link durations increased the delay to primary reinforcement and this may have decreased the value of the terminal-link. This in turn would mean that the hens were more likely to prefer the shorter

terminal-links, confounding the effects of the sound. However, there were very few cases where initial-link responding was biased away from the key leading to the longer terminal-links. For example, Hen 32 showed a noise bias towards the initial-link key leading to the terminal-link with white noise (100 dB) even when the duration of this link was always longer than the terminal-link without sound. Thus, it appears that responding in the initial-links was not biased away from keys associated with longer terminal-link durations. In order to avoid this problematic outcome a different schedule could be used in the terminal-links, such as a fixed-time (FT) schedule. Davison, Alsop and Denison (1988) found no preferences for FI or equally long FT terminal-link schedules, in a concurrent-chains procedure. Thus, FT terminal-links may be better for maintaining control of the experimental parameters without leading to differences in terminal-link duration or reinforcement rates across schedules.

### **General Results**

One surprising finding from this experiment was that the degree and direction of initial-link biases were in some cases inconsistent with responding in the terminal-links. In 9 out of 28 cases the biases in the initial-link were towards the keys leading to sound yet lower rates of responding were shown in the terminal-links with the same sound. Ostuka et al. (2009) also found that rats showed different response ratios in the initial- and terminal-links, when both terminal-links contained sound. When non-contingent shock was associated with the terminal-links, Rachlin (1967) found that response ratios in the initial-links differed in magnitude from those in the terminal-links but the direction of the ratios was consistent across both links. He suggested that shock had a greater effect in the initial-links because that was where the parameters were most responsive to the behaviour of the subject. This means that responding in the initial-links, rather than the terminal-links, could control the rate of shock delivered. This finding was unlike that of the present study, where some hens showed response ratios that were in opposite directions across the two links, i.e., initial-link bias was towards the key leading to sound and terminal-link bias was away from the terminal-link with sound. Few studies using concurrent-chains have reported on behaviour in the terminal-links and so it is difficult to make more comparisons. Furthermore, this effect was only seen in some cases and in other cases the initial- and terminal-link biases were in the same direction (15 out of 28). Hence, it is unclear how to interpret the differences in responding in the initial- and terminal-links and there are no models of performance under concurrent-chains that can account for this difference.

The estimates of noise bias from Experiment 1 and the present experiment showed that none of the sounds used were preferred by these hens. Although the food call was predicted to be a preferred sound it was not the case. As previously mentioned, this may have been because the subject hens used here did not have any previous experience with this sound and food, and so the food call was not meaningful. It may be possible to make a sound more meaningful by making it predictive of the arrival of food and then preference for this sound could be assessed. This may help to determine if a positive experience with sound is a necessary precondition for the sound to be preferred by hens.

It was possible that gaining access to food outweighed the effects of being in the presence of sounds that were thought to be aversive. As outlined, in the present experiment some hens continued to respond on the sound associated schedule in the initial-links even if their responding in the terminal-links was suppressed by the presence of sound. Otsuka et al. (2009) suggested that food reinforcement may have been masking the reinforcing effects of sound in their study but after conducting an extinction session the authors concluded that food did not interfere with rats' preferences for music. However, the use of one extinction session may not have been sufficient to discount the suggestion that food may mask the reinforcing effects of sound under concurrent-chains. Perhaps the preferences for sounds would be different if assessed using a procedure that does not rely on operant responding or food reinforcers, for example, a place conditioning procedure (to be described later).

To conclude, the present study showed that initial-link responding could be biased by the presence of sound in the terminal-links, but the directions of noise biases were inconsistent across subjects for all sounds except the food call. The direction and magnitude of noise biases here were also inconsistent with those seen under the concurrent schedule procedure for the same subjects (Experiment 1). Although both procedures showed that sound could bias responding away from schedules associated with sound, it may be more sensible to use concurrent schedules to assess sound preferences. This is because noise biases were more consistent across subjects and consistent with those from past research, and there were less confounds identified using concurrent schedules compared to using concurrent-chains. Some caution must be taken when arranging concurrent-chains procedures in that the presence of sound may cause longer pausing in the terminal-links, which can lead to increased terminal-link durations, and the use of independent schedules may lead to unequal obtained reinforcement ratios. An advantage of the concurrent-chains procedure was that sound was not present during the choice phase unlike in concurrent schedules. However, in both concurrent and concurrent-

chains procedures the food reinforcer may have masked the effects of sound on responding. It would be interesting to know how the results of Experiments 1 and 2 compare to those of another procedure where sound preferences are assessed where reinforcement is not available during the choice phase. Therefore, a place conditioning procedure was used in the next experiment to examine sound preferences.



### EXPERIMENT 3 <sup>1</sup>

Operant choice procedures, such as concurrent schedules and concurrent-chains, have the potential to measure hens' responses to both attractive and aversive sounds whilst providing a graded measure of preference. However, Experiment 1 showed that the presence of white noise suppressed responding, thus possibly confounding response allocation in concurrent schedules. Experiment 2, avoided this problem but in this experiment some hens gave initial-link biases towards the key leading to the terminal-link with sound and response rates in these terminal-links were generally lower than when sound was not present. This may mean that although the presence of sound suppresses responding, hens will still choose the key leading to sound in order to obtain food. Another procedure that can be applied to assess sound preferences is the *conditioned place preference* (CPP) procedure. This procedure allows for the assessment of the effects of sound on choices made in the absence of sound or reinforcers. CPP may also be useful for examining responses to both positive and negative sound stimuli and is a procedure that is both simple and quick compared to concurrent and concurrent-chains procedures.

The CPP procedure typically involves giving an animal a choice between spending time in two environments where one has been previously paired with a stimulus and the other has not. For example, the animal is placed in a chamber with two compartments that differ on some stimulus dimension, such as colour, and is allowed to move freely between the compartments for a set period of time. The animal is then confined to one compartment that is paired with an additional stimulus (e.g., injection of a drug). The animal is later confined in the alternate compartment where, typically, no additional stimulus is added. Both of these are the conditioning sessions. After the conditioning sessions, the animal is again allowed to move freely between the two compartments during the test sessions and a place preference is indicated if the animal chooses to spend more time in one compartment over the other. This procedure can also be used to assess the aversive effects of stimuli and is known as *conditioned place aversion* (CPA). The advantage of the CPP procedure is that it can be used to assess the conditioning effects of both positive and negative stimuli in a way that requires little training. It is also a suitable procedure for using with very young animals that would be difficult to test in operant procedures, such as concurrent schedules that require a lot of training and a large number sessions to obtain a stable performance.

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<sup>1</sup> This experiment has been published as Jones, A. R., Bizo, A L., & Foster, T. M. (2011). Domestic hen chicks' conditioned place preferences for sound. *Behavioural Processes*, In Press.

There are a number of ways in which the CPP procedure can be arranged. Studies have used either a 2- or 3-compartment apparatus to assess CPP (Tzschentke, 1998). In 2-compartment procedures trials begin by placing the animal centrally between the two compartments. With 3-compartment procedures, the third compartment is typically a middle compartment connecting the two main conditioning compartments and is used as a 'start' box. In their meta-analysis of CPP papers examining opiate and stimulant drugs with mice and rats, Bardo, Rowlett and Harris (1995) found larger effect sizes in studies using a 3-compartment, compared to a 2-compartment apparatus. The authors point out, however, that in most of the studies they reviewed the third (middle) compartment was novel during the test sessions and this may have resulted in larger effect sizes. In most 3-compartment studies time spent in the middle, or connecting, compartment has not been reported or included in analyses of CPP (e.g., Agmo & Berenfeld, 1990; Camacho, Sandoval & Parades, 2004; Meerts & Clark, 2007; Rubinov, Hagerbaumer & Juraska, 2009; Leri & Rizos, 2005; Robinet, Rowlett & Bardo, 1998). At least one study included time in this compartment as part of the time spent in the currently selected compartment (Hughes, Baker & Rettig, 1995).

Another element in the CPP procedure that varies across studies is the number and duration of conditioning and test-sessions. Tzschentke (2007) points out that CPP experiments usually involve test-sessions that are 15-20 min in duration but that the number of conditioning sessions can vary. Bardo et al. (1995) reported that for some drug types a longer conditioning duration resulted in larger effect sizes but no difference was found for the number of conditioning trials or the test duration (10-15 min compared with 20-30 min). CPP has also been assessed in two main ways; either conditioning one group and comparing the data with those from the control group (examining post-conditioning data only) or using pre-conditioning tests and comparing the data with those from post-conditioning sessions. Cunningham et al. (2003) reported that of the studies they reviewed relatively the same proportion used these two methods and they concluded that one method showed no advantage over the other. However, Bardo et al. found that most of the studies they reviewed that used pre-testing resulted in smaller effect sizes than those using post-conditioning data only.

CPP has been increasingly used in recent years (for a review see Tzschentke, 2007) to examine the conditioning effects of different drugs on mice or rats (e.g., morphine (Zheng et al., 2004), heroin (Leri & Rizos, 2005), and amphetamine (Robinet et al., 1998)). It has also been used to assess the effects of non-drug reinforcers, such as food (Lau, Bretau, Huang, Lin & Guo, 2006), mating activity (Camacho et al., 2004; Meerts & Clark, 2007), aggression and

sexual interactions (Meisel & Joppa, 1994), and access to a running wheel (Masaki & Nakajima, 2008) on place preference. Considering the variety of stimuli that have been studied using CPP, the procedure has the potential for examining the effects of many different phenomena, including sounds.

CPP procedures have been used with many different species (e.g., rats (Meerts & Clark, 2007), hamsters (Meisel & Joppa, 1994), mice (Fitchett, Barnard & Cassaday, 2006), and zebrafish (Lau et al., 2006)). However, to the authors knowledge there are only nine studies that have used CPP with avian subjects; four with Japanese Quail (Akins, Levens, Prather, Cooper & Fritz, 2004; Awaya & Wantanabe, 2003; Levens & Akins, 2001; Mace, Kraemer & Akins, 1997) and five with domestic hen chicks (Bronson et al., 1996; He, Bao, Li & Sui, 2010; He, Xiao & Sui, 2009; Hughes et al., 1995; Jiang, He, Wang & Sui, 2011). Of these studies, one has shown that food could produce a CPP in avian subjects (Mace et al.). In that study, Mace et al. assessed the conditioning effects of food on CPP in 12-day-old quails, while also establishing how many conditioning sessions were needed to produce a CPP. The quail chicks exhibited a CPP for food when they were conditioned with food in one compartment and no food or tainted food in the alternate compartment. This research found a clear CPP after four conditioning sessions but not after one conditioning session.

Bronson et al. (1996), using CPP with domestic hen chicks, examined the conditioning effects of different drugs using a 2-compartment apparatus. They found that all the drugs used (at particular doses) produced a CPP in the chicks. The authors noted that in the post-conditioning test sessions 30 % of drug treated chicks “froze”, i.e., didn’t move during the entire test session, compared to 5 % of non-treated chicks. They suspected that this may have been due to a place aversion but their following experiment failed to confirm this. The results lead the authors to conclude that the “freezing” was likely due to the effects of the drugs. Hughes et al. (1995) investigated the conditioning effects of cocaine using domestic hen chicks in a T-shaped apparatus. They paired red cards in one compartment with the injection of cocaine and found that the chamber associated with the red cards was initially preferred (in Test session 1) but that this preference declined over the following two test sessions. This decrease in preference was suggested to be a result of the extinction of the association between the red cards and cocaine. In three different studies, researchers used the same methods to assess CPP acquired through morphine injections (He et al., 2009, 2010; Jiang et al., 2011). These studies all used a 2-compartment apparatus with red and green floors and the experiment consisted of three parts; a 15-min pre-test, 6 conditioning sessions which alternated between

morphine and saline paired sessions, and a 15-min post-test session. All three of these studies found a clear CPP for the compartment paired with morphine injections. Thus, CPP was shown to be a successful procedure for use with domestic hen chicks.

Only one study was found that used the CPP procedure to assess the conditioning effects of sound with animals. Feduccia and Duvauchelle (2008), using a 2-compartment apparatus, assessed whether an auditory stimulus (music or white noise) would enhance the rewarding effects of MDMA (ecstasy) in rats. They found that white noise enhanced the conditioning effects of MDMA. The rats showed a CPP towards the compartment associated with both noise and MDMA but not towards the compartment associated with white noise or MDMA alone. This research showed that the CPP procedure can be effective for examining the conditioning effects of sound when combined with drugs and indicated that it may be a procedure that would be useful for exploring the effects of sound alone.

The above studies have shown that CPP is a procedure that can be used with domestic hen chicks. Due to availability and ease of handling domestic hen chicks, they were chosen as subjects for the present experiment. The present study aimed to assess CPP as a procedure for examining domestic hen chicks' responses to potentially positive and negative sounds. As part of this, the CPP procedure was also conducted with food, which normally results in a positive preference for the conditioning chamber, for comparison with the conditioning effects of sounds. The sounds were chosen to allow comparison with Experiments 1 and 2, and to see if it was possible to obtain positive place preferences. Three sounds were used; white noise, a rooster's food call, and domestic chick-sounds, that have been shown to be either aversive or attractive to hens in previous research. White noise has previously been shown to be aversive to hens in this thesis (Experiment 1 & 2) and other research (McAdie, 1998). Thus, it was selected as a sound that might result in a CPA. The food call was that of a rooster (the same as used in Experiment 1 and 2), that has been shown to function in the same way to a domestic hen food call (Wauters, Richard-Yris, Pierre, Lunel & Richard, 1999). Studies have also shown that food calls attract domestic hen chicks (Wauters & Richard-Yris, 2002) and are used as a separation call for a maternal hen and her chicks (Hughes et al., 1982). Chicks have been observed to spend more time near a speaker playing food calls than when no sound is played (Woodcock, Pajor & Latour, 2004) and they will follow a model emitting a maternal call rather than one emitting a tone burst (Fischer, 1976). Van Kampen and Bolhuis (1991) measured approach behaviour and found that chicks preferred a maternal call to an artificial sound when exposed to it during imprinting. Although the food call was not preferred by adult hens in

Experiment 1 and 2, for chicks it may be a sound that they have an innate preference for and so was selected as a possible preferred sound.

The domestic chick-sounds were a recording of the group of subject chicks used in this study making “peeps”, or pleasure notes, when in their aviary with no humans present. Studies have shown that chicks will move more quickly down a runway towards a conspecific compared to an empty box (Suarez & Gallop, 1983) and they will readily move towards each other when placed at opposite ends of a chamber (Vallortigara, Cailotto & Zanforlin, 1990) or towards video images of other chicks feeding (Clarke & Jones, 2001). After separation, chicks have also been shown to promptly emit pleasure notes upon being reunited with other chicks (Collias & Joos, 1953). It appears that chick-sounds attract domestic hen chicks and this was therefore selected as another possibly preferred sound. An alarm call was not used in the present experiment as there was no evidence to suggest it would be either preferred or aversive for domestic hen chicks. The alarm call resulted in very small noise biases in Experiment 1 and mixed results in Experiment 2 and was therefore it did not seem sensible to use in the present experiment.

A typical 3-compartment apparatus was used in the present study and the experiment involved four pre- and four post-conditioning test sessions. In order to reduce separation distress, a short duration test session (5 min) was used and had been successfully used in other chick studies using CPP (Bronson et al., 1996; Hughes et al., 1995). As the sessions were short, four were conducted to obtain a comparable sample of data but generally CPP studies use only one 20-min test session (Tzschentke, 1998). For the conditioning phase, three 15-min trials were conducted in each side compartment giving a total duration of 45 min for conditioning in each compartment. This was less than that used by Bronson et al. (2 hours) but more than used by Hughes et al. (30 min) and more than the minimum recommended by Bardo et al. (1995) (25-30 min).

The aim of the following experiment was to establish CPP as a method for assessing domestic hen chicks preferences for places previously paired with food or sound. If the food and the food call were attractive and white noise aversive, as previous research suggests, then it was expected that chicks would show a CPP towards the compartment associated with the food and the food call and away from the compartment associated with the white noise. Given that chicks are attracted to conspecifics, it was also predicted that chick-sounds would also result in a positive CPP.

## Method

### Subjects

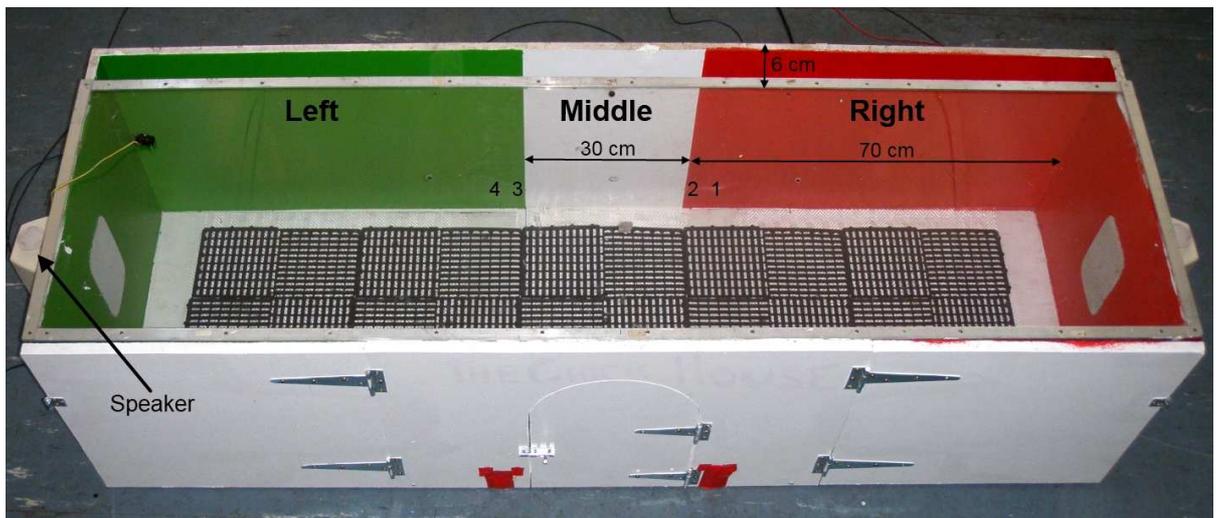
Thirty-seven, 1-day old Brown Shaver domestic hen chicks served as subjects. When the chicks were involved in experimental test sessions they were kept together in an aviary. They were provided with free access to NRM™ ‘chick starter + coxistac crumbles’ and water, and with a heat lamp providing warmth. For identification purposes, the chicks were individually marked with coloured pens and/or leg bands. All animals were treated in accordance with the animal ethics policies and procedures of the University of Waikato Animal Ethics Committee (Ref: 776).

### Apparatus

The experimental chamber had internal measurements of  $56 \times 170 \times 43$  cm and was made from particle board (2-cm thick). It had three hinged doors along one side and the roof was made of clear Perspex™, with a 6 cm gap for ventilation where the Perspex™ did not cover the box down the length of one side (as seen in Figure 3.1). The floor of the chamber was painted matte white and covered with clear plastic sheets with black rubber mats on top. The inside walls of the chamber were painted different colours. The walls of the middle compartment of the chamber were painted matte white and were 30-cm wide. The side and end walls of the left and right compartments of the chamber (each 70-cm long) were painted green and red, respectively. The paints used were Wattyl Colour Designer™ paints, purchased in New Zealand. The paint on the left compartment was ‘Nynke green’ with an RGB of 96, 139, 91 and the red used on the right compartment was ‘Firefly’ with an RGB of 170, 79, and 62.

Four infrared beams (as shown in Figure 3.1) located in the walls of the chamber (6 cm above the chamber floor) tracked the chicks’ movements from one compartment to another. Two of the beams were located exactly on the point where the middle compartment met the side compartments (beam 2 and beam 3). Another beam (1) was located 5 cm to the right of beam 2, and beam 4 was located 5 cm to the left of beam 3. Two speakers ( $12 \times 19 \times 20$  cm), which could produce tones between 70 and 20000 Hz with a maximum output of 120W, were attached to each end of the chamber and were covered with a white metal mesh speaker cover that was inserted into the chamber wall ( $12 \times 18$  cm).

A rectangle particle board divider, measuring  $30 \times 43 \times 54$  cm, was used to separate the compartments of the chamber during conditioning. Each side of the divider was painted the same colour as the compartment it faced (either red or green).



*Figure 3.1.* Diagram of experimental chamber showing chamber compartments, location of infrared beams and the hinged doors.

The experimental chamber was located in a room ( $480 \times 490 \times 260$  cm) that was illuminated by five fluorescent tube lights located around the ceiling. In the first week, the temperature of this room during baseline sessions was  $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$ , and for the remainder of the experiment it was kept at  $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Before sessions, a group of chicks were transported from the aviary to a small room adjacent to the experimental room. In this small room they were placed inside a sound-proofed, particle-board holding box ( $610 \times 460 \times 575$  cm). The box had an internal light and was lined with 4-cm thick polystyrene to help attenuate external sounds.

During experimental sessions, a Panasonic™ camcorder, model HDC-HS200, was used to digitally record each chick's behaviour in the experimental chamber. The camera was positioned directly above the chamber, 235-cm above the ground, and was centred so that the entire chamber was in view. The sounds were relayed using a media player and were amplified using a Yamaha™ stereo AX-300 amplifier. The amplifier was connected to the two speakers that were located at each end of the chamber. At the start of each conditioning session, the decibel level of the sounds was tested using an Amplaid SLM 13 decibel meter to ensure it remained close to 60 dB (A). The meter was placed in the centre of one compartment on a box so that it was approximately 7-cm above the ground and the sound was adjusted if needed.

The sounds used were Mp3 files of white noise, chick-sounds, and a food call (found in Appendix 1a). The "chick-sounds" recording was of the group of subject chicks making peeps

when in the aviary, with no humans present, and was recorded using an Iriver IFP799 Mp3 recorder. The “food call” was the same as used in Experiment 1 and 2. All sounds were edited such that there was no discernable beginning or end.

Data recorded for each session were: time spent in the left, middle and right compartments, number of visits to the left and right compartments, latency to first beam break and side chosen first. The experimental events were controlled and recorded by a Pentium III computer, located in the same room as the chamber, that had a 730MHz processor with 512MB of RAM, and was run using Med-PC™ (Version IV).

### **Procedure**

On Day 2 after hatching, the chicks were transported to the holding box and then to the experimental room for habituation training. Each chick was placed alone in the centre of the experimental chamber (middle in Figure 3.1) and had access to all three compartments with all doors closed for 1 min. Once all chicks had been exposed to the chamber, the group was returned to the aviary. This process was repeated for five consecutive days. After habituation training, the chicks were randomly assigned to one of four groups: Food, Food Call, Chick-sounds and White Noise groups.

There were three phases to this experiment: baseline (Phase 1), conditioning (Phase 2), and a repeat of baseline (Phase 3). Experimental sessions were conducted between the hours of 12:00 and 14:00. A session began once a chick had been placed in the chamber and the door was shut. Phase 1 consisted of four sessions conducted on alternating days where the food and food call groups began on the afternoon of the last habituation session (Day 6 after hatching) and the chick-sounds and white noise groups began the next day. In Phase 1, each chick was placed in the centre of the chamber where it had free access to all three compartments for 5 min. After 5 min had elapsed, the chick was removed from the chamber and returned to the holding box and the next subject was tested. After all chicks in the first group of the day had completed a session they were returned to the aviary and sessions for the other group were conducted. Sessions were always conducted first for chicks in the food and chick-sounds groups.

The time spent in the middle, left and right compartments, and the number of visits to the left and right compartments was recorded. Timing for the chick being in the middle compartment began when the session was started and paused when any beam was broken. The time spent in the left or right compartment began when a chick left the middle compartment

and broke a left (beam 3) or right beam (beam 2) and ended when the chick returned to the middle compartment. At the end of Phase 1, five chicks that had not moved in three or more (not necessarily consecutive) sessions were excluded from the remainder of the experiment and their data were not included in the analyses. To make the groups even numbered, 1 chick in the food group moved to the chick-sounds group, prior to conditioning.

In Phase 2 (conditioning), two conditioning trials were conducted daily (one in each compartment) on alternating days for 3 days. A trial lasted for 15 min and the two trials in a day were separated by 2 hours. For the food group, before each conditioning trial began food was removed from the aviary at least 12 hours in advance of an experimental session and was returned when the sessions ended. During conditioning trials in the experimental chamber, chicks in the food group were given free access to a combination of wheat, pellets and chick starter crumbles. For the sound groups, the chicks were continuously exposed to the sound in one side compartment for one of the two trials that day. The side compartment associated with the food or sound was counterbalanced across chicks in each group. During the first conditioning trial the chicks were first confined in the sound/food compartment before being confined in the no food/sound compartment and this reversed over the following two conditioning days. After the conditioning phase (Phase 2) was completed, Phase 3 began.

### **Data analysis.**

As a group design was used in the present study, the data were analysed in groups and this also made it easier to compare the data with other research of this type. The raw data from all pre- and post-conditioning test sessions for each subject can be found in Appendix 3a.

Thirty-two subjects completed conditioning but 5 of these did not move in any of the post-conditioning sessions. Although post-conditioning data were obtained for 27 subjects, many of these subjects also failed to move from the middle compartment in sessions of Phases 1 or 3 of the experiment.

## **Results**

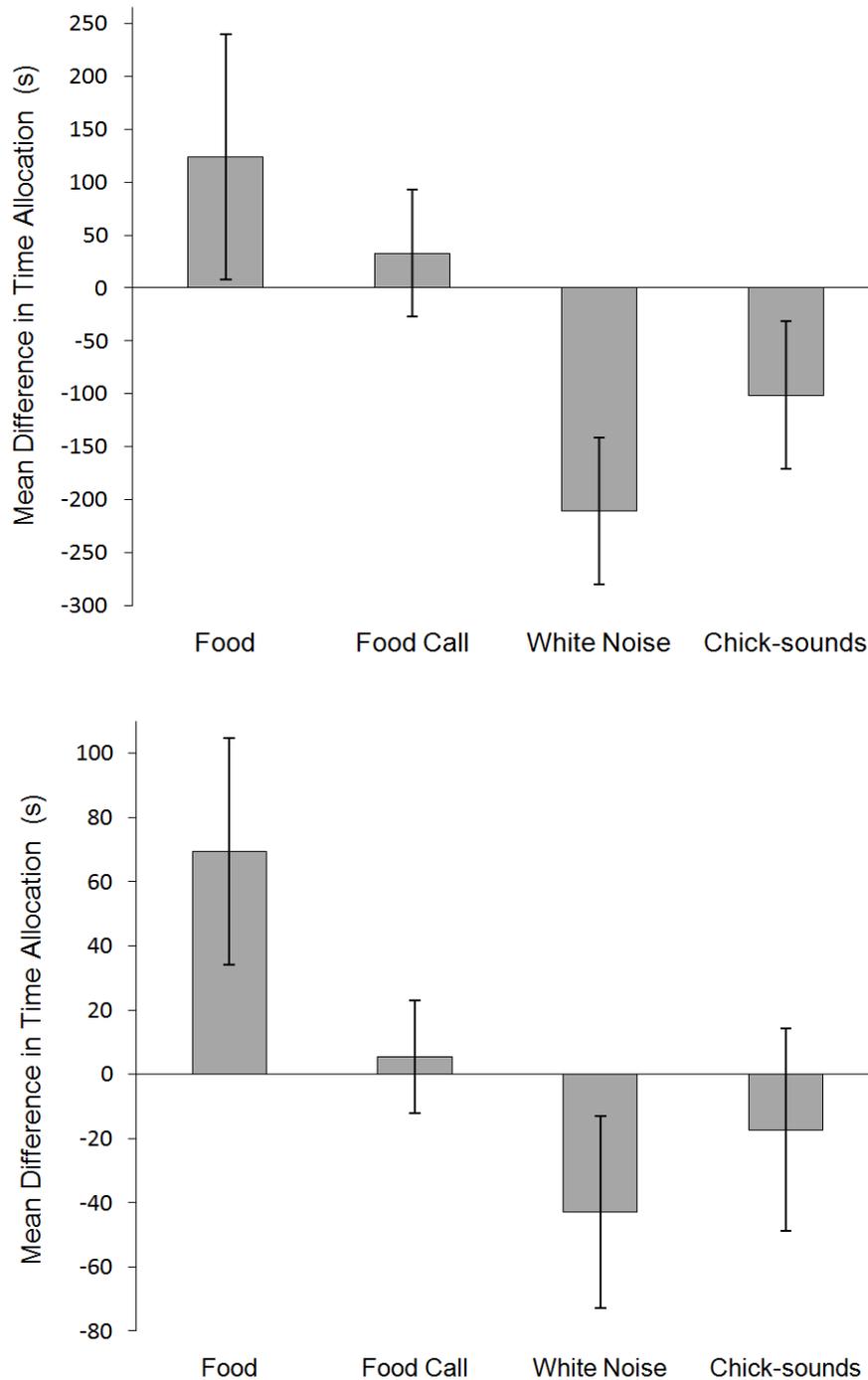
Across all subjects there was no movement from the middle compartment in 16 % of pre- and 40 % of post-conditioning sessions. The baseline data from Phase 1 also revealed that 22 chicks spent proportionally more time in the green compartment (left) compared to the red compartment (right), generally indicating a preference for the green (right) side. Of the five chicks that did not move from the middle compartment, three were in the white noise group, and one in each of the food and food call groups. From the video recordings it appears that 4 of

these chicks fell asleep, in that they sat with their head down and did not move for a considerable period, and 1 made distress calls continuously, across the post-conditioning test sessions. Of the chicks that did move between the compartments approximately 40 % also made continuous distress calls and close to 10 % appeared to have fallen asleep in a compartment after they had moved from the middle compartment.

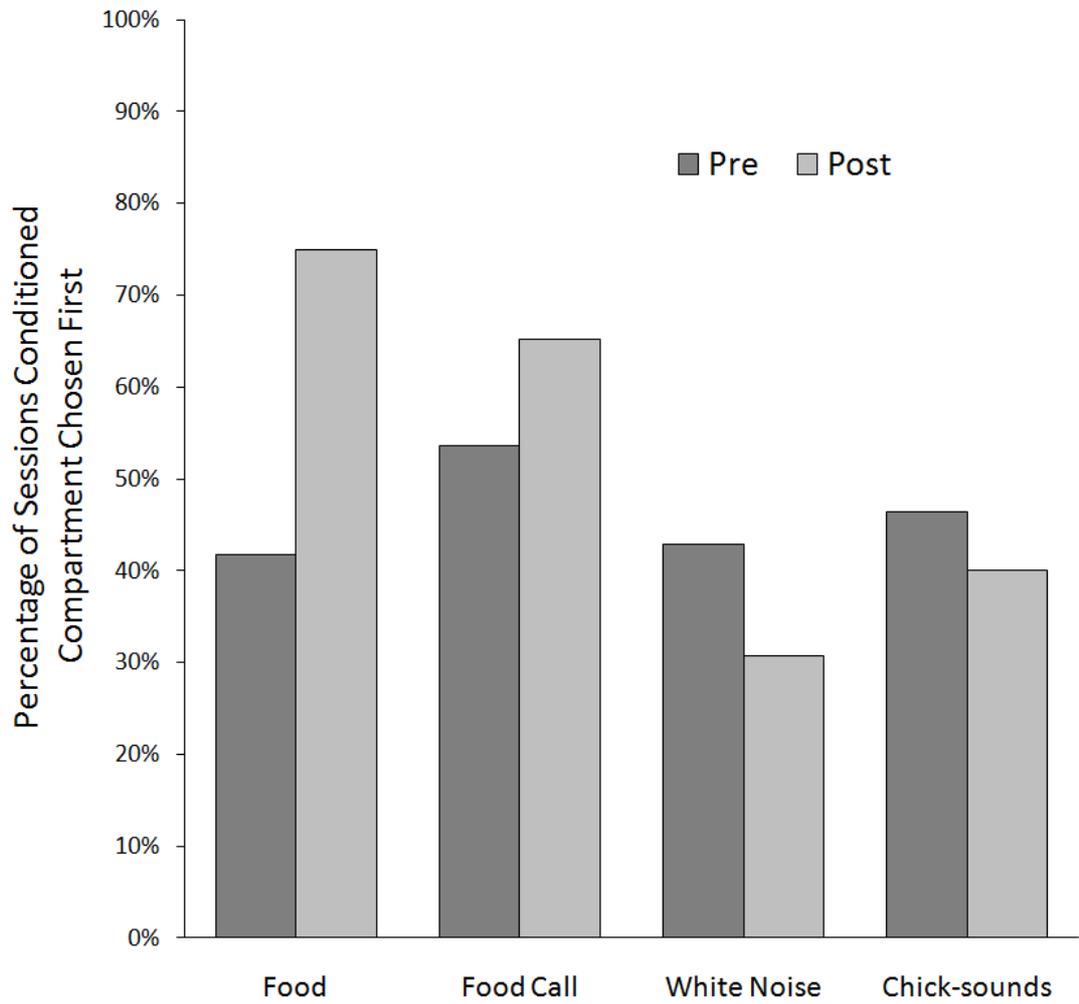
The mean  $\pm$  S.E.M difference in the time spent in the conditioned compartment in pre- and post-conditioning sessions (Phase 1 and Phase 3) is plotted in Figure 3.2, for each group. The top panel in Figure 3.2 shows difference scores calculated from the time spent in the conditioned compartment over all four pre- and post-conditioning sessions. There were positive differences for the food ( $M = 123$ ) and food call ( $M = 33$ ) groups, and negative differences for the white noise ( $M = -211$ ) and chick-sounds ( $M = -101$ ) groups. A one-way analysis of variance (ANOVA) showed that sound and food had a significant effect on the amount of time spent in the conditioned compartment ( $F(3, 28) = 3.3, p < 0.05, \eta^2 = 0.26$ ) and a Tukey's HSD post-hoc analysis showed that the time spent in the conditioned compartment after conditioning for the food group was significantly different from the white noise group (HSD = 4.10,  $p < 0.05$ ). Individual data for the differences in time spent in the conditioned compartment from pre- to post-conditioning test sessions can be found in Appendix 3b.

The bottom panel in Figure 3.2 depicts the mean difference in time based only on data from sessions in which the subject moved from the middle compartment in Phase 1 and Phase 3, in at least one session. For subjects in the food and food call groups, mean differences between time spent in the conditioned compartment pre- and post-conditioning were 69 s and 5 s, respectively. Subjects in the white noise and chick-sounds groups spent less time in the conditioned compartment post-conditioning, -43 s and -17 s less on average, respectively. However, a one-way ANOVA revealed that the effect of sound or food on the time spent in the conditioned compartment was not significant ( $F(3, 23) = 2.5, p > 0.05, \eta^2 = 0.24$ ).

The percentage of sessions where the conditioned compartment was chosen first for pre- and post-conditioning sessions (Phases 1 and 3), based on all sessions in which a choice was made (i.e., a chick moved from the middle compartment at least once), for all groups is plotted in Figure 3.3. These percentages increased from pre- to post-conditioning for the food and food call groups, and decreased for the white noise and chick-sounds groups. In Phase 1, percentages were relatively close to 50 % (range from 42 % to 54 %) for all groups. In Phase 3, they had a larger range [from 31 % (white noise) to 75 % (food)], with food call (65 %) and chick-sounds (40 %) in between.



*Figure 3.2.* Mean difference in time spent in the conditioned compartment from the pre- to post-conditioning sessions (Phases 1 and 3), for the food, food call, white noise and chick-sounds groups. The top panel shows this difference averaged across subjects. The bottom panel shows this differences calculated for sessions only where the subject moved from the middle compartment in both phases, again averaged across subjects. The error bars represent one standard error each side of the mean.

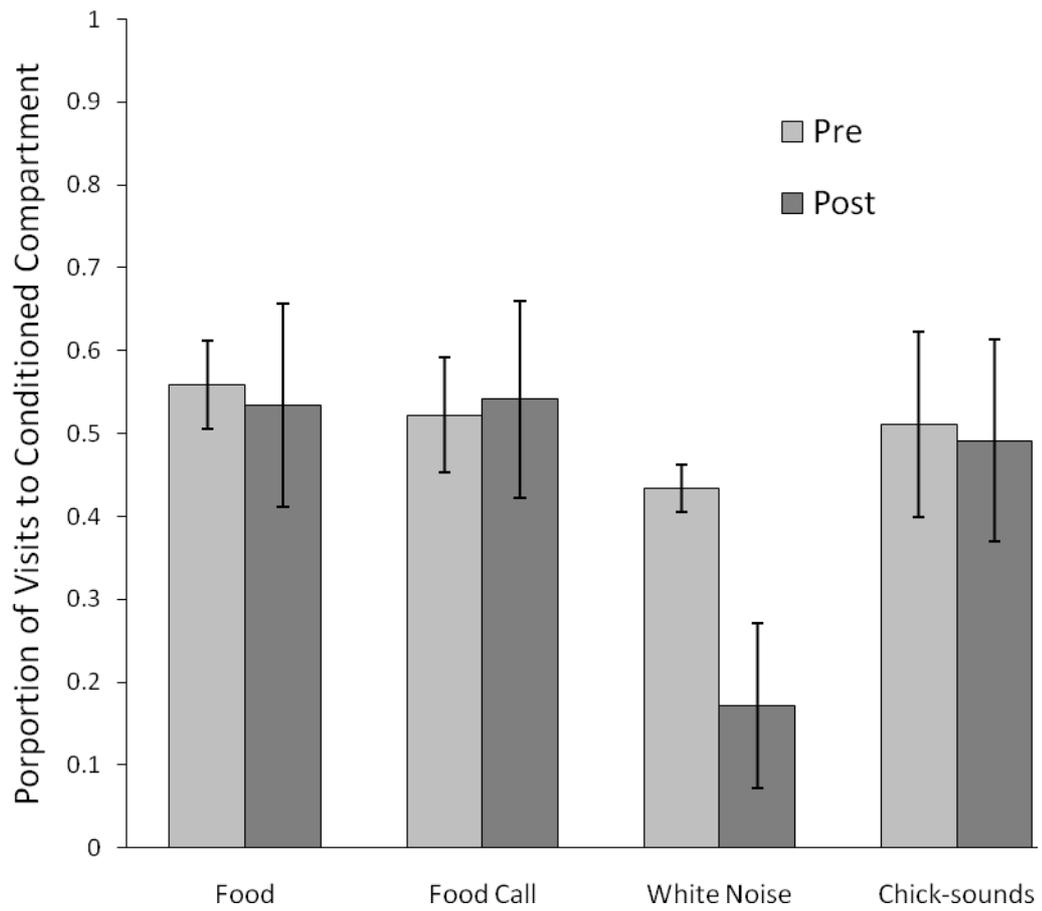


*Figure 3.3.* Percentage of sessions that the conditioned compartment was chosen first for the pre- and post-conditioning sessions (Phases 1 and 3), for the food, food call, white noise and chick-sounds groups.

Activity of the subjects showed that the total number of visits to both areas decreased for all groups from pre- to post-conditioning sessions. The proportion of visits to both compartments in pre- and post-conditioning sessions (Phases 1 and 3) are presented in Figure 3.4. Although there was a decrease in proportion of visits to the compartment conditioned with white noise, the proportion of visits were similar across pre- and post-conditioning for the other groups. A one-way repeated measures ANOVA showed no significant effect of pre- and post-test visits ( $F(1, 28) = 1.7, p > 0.05, \eta^2 = 0.06$ ), no main effect of group ( $F(3, 28) = 2.1, p > 0.05, \eta^2 = 0.18$ ), and no significant interaction ( $F(3, 28) = 1.3, p > 0.05, \eta^2 = 0.13$ ).

The mean number of seconds spent in the conditioned compartment across successive pre-conditioning (top panel) and post-conditioning (bottom panel) sessions are presented in Figure 3.5. For the pre-conditioning test sessions showed no consistent trend across sessions for any group. Across post-conditioning sessions, the food group increased their time spent in the conditioned compartment and there was a gradual increase for the white noise group. For the chick-sounds group there was a gradual decrease in time spent in the conditioned compartment while for the food call group time remained relatively stable across sessions. A two-way repeated measures ANOVA revealed no main effect of sessions ( $F(1, 28) = 2.7, p > 0.05, \eta^2 = 0.09$ ) and no main effect for conditioned groups ( $F(3, 28) = 2.0, p > 0.05, \eta^2 = 0.17$ ) but there was a significant interaction ( $F(3, 28) = 25.0, p < 0.05, \eta^2 = 0.47$ ). This interaction likely resulted from time spent in the conditioned compartment increasing across sessions for the food group.

In summary, post-conditioning test sessions showed that chicks spent significantly more time in the area associated with food and away from the area associated with white noise. These areas were also chosen more often after conditioning (food) and less often after conditioning (white noise). The difference in time spent in the conditioned area was not significant for the food call or the sounds of other chicks but chicks did choose to move towards these sides first across sessions more (food call) and less often (chick-sounds) after conditioning. The number of visits to the left and right compartments did not differ from pre- to post-conditioning and did not differ across groups. Across pre- and post-conditioning test sessions there was no significant difference between the groups or from pre- to post-tests.



*Figure 3.4.* Proportion of visits to the conditioned compartment for pre- and post-conditioning tests sessions, for the food, food call, white noise and chick-sounds groups.

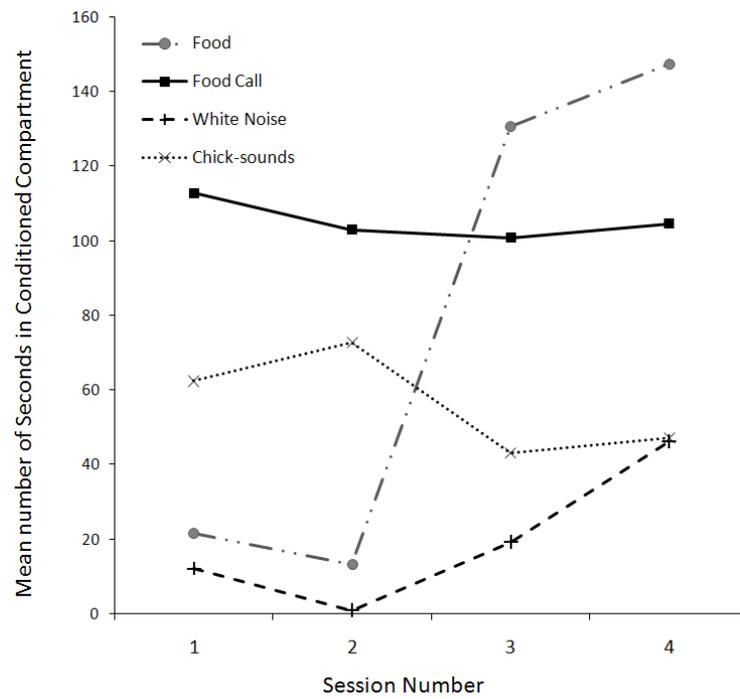
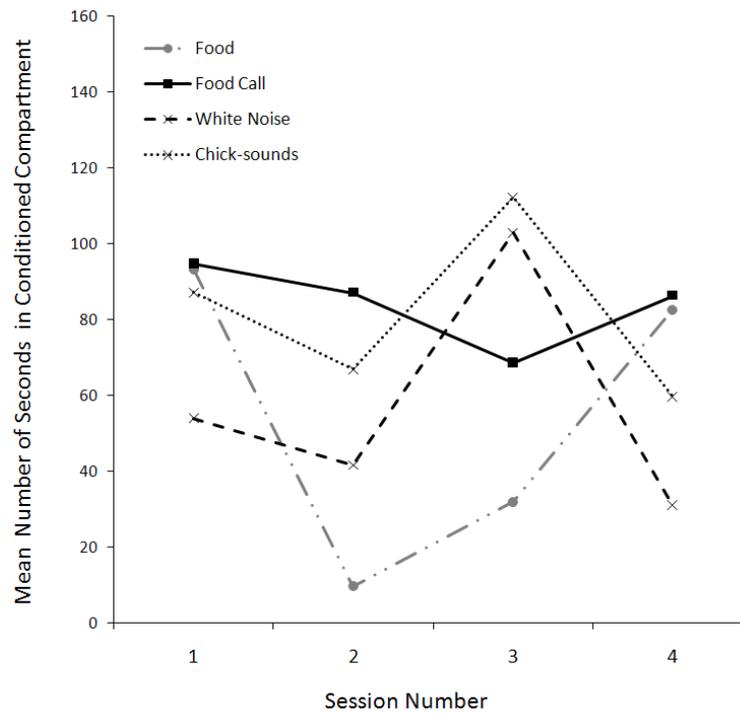


Figure 3.5. Mean number of seconds spent in the conditioned compartment across sessions for the food, food call, white noise and chick-sounds groups. The top panel shows data from the pre-conditioning test sessions and the bottom panel is data from post-conditioning test sessions.

## Discussion

Overall, the results showed a significant CPP for a place associated with food and avoidance of a place associated with white noise. This was similar to the percentage of sessions the conditioned side was chosen first. Additionally, the proportion of visits to the conditioned compartment did not differ across the food, food call and chick-sounds groups but was lower for the white noise group and much lower in the post-conditioning sessions. The time spent in the conditioned compartment across pre-conditioning test sessions also showed no differences across group or sessions and this was similar to the post-conditioning sessions for all groups except the food group, where time in the conditioned compartment increased over sessions.

When the CPP procedure was used to expose domestic hen chicks to different sounds and food, the chicks spent, on average, significantly more time in the compartment associated with food and less time in the compartment associated with white noise after conditioning. Although they also spent more time in the compartment associated with the food call and less time in the compartment associated with the chick-sounds post-conditioning, these differences were not significant. A place preference was shown towards the compartment associated with food and a place avoidance for the compartment associated with white noise. This is consistent with the data from Experiments 1 and 2 and also with the results of other research (McAdie, 1998). The effect size of 0.26 is a moderate effect size according to Ferguson (2009) and also shows that there was a clear effect of food or sound on the time spent in the conditioned compartment. These results suggest that the CPP procedure is a valid and reliable means of assessing domestic hen chicks' responses to a range of different stimuli, such as sounds or food.

As previously reported, the ability of food to condition a place preference with Japanese quail was examined (Mace et al., 1997). The quail spent 20 % more time in the compartment conditioned with food than would be expected by chance (50 %). The chicks in the food group in the present study spent 10 % (123 s) more time in the conditioned compartment during the post-conditioning sessions compared to the pre-conditioning sessions. This change in time spent in the conditioned side was lower than that reported by Mace et al. However, these differences may result because they used a 2-compartment apparatus, which effectively forces the animal to a side. In contrast, the present experiment used a 3-compartment apparatus that requires the animal to move actively from a central starting position to a side compartment of the apparatus in order to make a choice.

The present results also demonstrated that most chicks showed a place aversion for the compartment paired with white noise showing a 17.5 % decrease in time spent in the conditioned compartment from the pre- to post-conditioning sessions. This confirms the findings of Experiments 1 and 2 and those of McAdie (1998), when using concurrent-schedules, that white noise decreased rates of responding and biased hens behaviour away from keys associated with the noise. A confound identified in Experiments 1 and 2 was that the sound was present while preference was being assessed and may have influenced the measure of preference. Another limitation identified with using concurrent-schedules (as in the previous experiments of this thesis), was that the food reinforcers may have been interfering with responding during the preference assessment. In the present experiment, there were no food reinforcers or sound present during the preference assessment, thus eliminating the potential problem of food interfering with preference or the problem of any startle or freezing effects of sound during test sessions. More chicks in the white noise group, however, failed to move from the middle compartment compared to the other sounds and food. These findings suggest that white noise played at 60 dB (A) is an aversive stimulus for domestic hen chicks, even when not present at the time of testing and this has been shown in three different procedures.

Previous research has found that a food or hen call is a positive sound that attracts chicks (Fischer, 1976; van Kampen & Bolhuis, 1991; Woodcock et al., 2004). However, while the chicks in this experiment showed small positive preferences for the compartment associated with the food call this was not significant. Similarly, Lickliter (1989) found that quail chicks reared in a group did not show a preference for a maternal call, when the choice was between the call and four group mates and Snapp (1969) found that chicks did not approach a speaker playing food calls. The results of Experiments 1 and 2 showed that a food call was not preferred by adult hens and in some cases it reduced response rates when present. This is not consistent with the present results, as the chicks did not avoid the area associated with the food call but rather were indifferent to it. It appears that while the chicks in the present study may have found the food call somewhat positive (in that the side associated with it was chosen first more often after conditioning), it would appear that the association was not strong enough to result in a significant place preference. The chicks in the present study were artificially hatched and raised as a group; therefore they did not have any previous experience with calls by adult hens including food and maternal calls. Thus, the chicks and hens in this thesis did not previously learn to associate food calls with food rendering the food call a potentially weak

stimulus for conditioning a place preference or biasing responding towards a schedule associated with it.

The domestic hen chicks in the present experiment spent less time in the compartment associated with the chick-sounds (see Figure 3.2) and chose this compartment first less frequently post-conditioning (Figure 3.3). However, the differences between time spent in the conditioned compartment from pre- to post-conditioning were not significant. As previously mentioned, chicks have been shown to move towards the sight of other chicks (Suarez & Gallup, 1983; Vallortigara et al., 1990). They will also move towards video images of other chicks but not towards the sound alone of other chicks feeding (Clarke & Jones, 2001). The sound used in the present experiment of other chicks did not result in a CPP, which is consistent with the idea that the sounds of other chicks are not attractive.

The top and bottom panels of Figure 3.2 show that the effects of sound and food on CPP appear to be different if sessions where the chicks did not move from the middle compartment in the post-test sessions were either included (top panel) or excluded (bottom panel) from analyses. The difference in time spent in the conditioned compartment was significant only when the data from all sessions were included in the analyses. However, when the time spent in the middle compartment was excluded the pattern of results shown in the bottom panel mirrored those shown in the top panel of Figure 1. We would argue that when an animal failed to move from the middle compartment that they were still making a choice, albeit, not to move to one of the side compartments.

The present experiment reported two main measures to assess CPP; difference in time spent in each compartment and the percent of sessions where the conditioned side was chosen first. The findings here demonstrate that these two measures showed a similar change in pattern from the pre- to post-conditioning sessions. The measures of CPP using differences in time spent in the conditioned compartment (Figure 3.2) showed little differences between the pre- and post-conditioning data for the food call but the side chosen first data (Figure 3.3) showed an increase of 14 % in choosing the side associated with the food call. Additionally, the activity data (proportion of visits to the conditioned compartment) showed no significant differences from pre- to post-tests. This is consistent with the findings of Hughes et al. (1995), who also found no significant effect of activity, as recorded by the number of beam breaks. Generally, studies using CPP have used time as the measure of preference but using more than one measure may give a clearer picture of an animal's preferences.

Most commonly, CPP studies assessing the effects of drugs carry out conditioning sessions on alternate days (Tzschentke, 1998), as was used in the present study. However, these studies were mostly with mice or rats. Some of the studies using chicks (Bronson et al., 1996; Hughes et al., 1995) conducted conditioning sessions every day. It is possible that conducting conditioning sessions daily might work better with chicks. A shorter time between conditioning sessions and post-conditioning testing in the present study may have resulted in an increased effect of conditioning. It may also be that longer, or more frequent, conditioning sessions could have resulted in a significant CPP for all of the stimuli used here. Cunningham, Dickinson, Grahame, Okorn and McMullin (1999) found that mice showed an increase in preference for a place paired with cocaine when the conditioning trial durations were increased from 15 to 30 and 60 minutes, and Bardo et al. (1995) found that studies reported larger effect sizes, for some drug types examined, with the longer conditioning durations. This is an area that warrants further attention with the types of stimuli that were used in the present experiment. It is possible that longer conditioning sessions may have conditioned place preferences more effectively.

Other researchers have previously recognised that animals may fail to move about during post-conditioning test sessions and their chambers were only divided into 2 compartments. This way, a chick could be placed on a central line and any movement would then indicate a choice in one direction or the other. Although studies using a 3-compartment apparatus have yielded larger effect sizes (Bardo et al., 1995) we could find no study that systematically compared the results from a 2- and 3-compartment apparatus. In the present study, 5 of the 32 chicks failed to move from the middle compartment in all of their post-test sessions, and across all subjects in 40 % of the post-conditioning sessions there was no movement from the middle compartment of the chamber. This was significantly higher than the 5 % freezing observed by Bronson et al. (1996). One reason for the failure of the chicks to move in this experiment may be that they had not had long enough to adequately habituate to the apparatus and were too fearful to move about the chamber. The researcher observed that approximately 40 % of these chicks displayed continuous distress calls and distress may have caused them not to move. Alternatively, approximately 10 % of chicks appeared to actually fall asleep and so were relaxed in the environment.

The CPP procedure was shown here to be a successful method for use with domestic hen chicks, as shown by other research (e.g., Bronson et al., 1996; Hughes et al., 1995). It is also a procedure that could be used to assess preferences of domestic hen chicks for other

environmental stimuli that may affect their welfare, such as temperature, olfactory cues, or even social preferences (i.e., spending time with conspecifics). Because the CPP procedure is relatively simple procedure that requires less training than operant procedures, such as concurrent schedules, it can be used to assess sound preferences for other intensively farmed animals, such as domestic pigs, or possibly used to assess sound as a lure, to assist in pest control efforts, for example, with rats, ferrets, and stoats.

Overall, this experiment showed that it was possible to use the CPP procedure to assess preferences for places previously associated with negative or positive sounds or with food. It is also a procedure that could be effective for assessing the effects of sounds on the choice behaviour of domestic hens and the results could then be compared with those found in the previous two experiments. There was a similar pattern in the two main measures of place preference used here, differences in time spent in the conditioned compartment and percentage of sessions the conditioned compartment was chosen first. Place preferences were shown away from white noise and this was consistent with the preferences shown in Experiments 1 and 2 but results from the food call were not similar to these two experiments. Additionally, none of the sounds used in the present experiment resulted in a positive place preference. This is consistent with the results of Experiments 1 and 2, where also no sounds were preferred. As previously suggested, it may be possible to make a sound predictive of the arrival of food and in turn it may become a sound that is preferred.

## EXPERIMENT 4

Thus far in this thesis the various subjects have not shown a preference towards a schedule or an area associated with any of the sounds used here. They have, however, showed preferences away from schedules or areas associated with white noise. It may be possible to make a sound positive so that they prefer it to no sound. One way to make a stimulus positive may be by associating it with another positive stimulus, such as food, in an attempt to make it into a signal or conditioned reinforcer. By making a sound predictive of the arrival of food it could turn out that the value of this sound could be increased and that this sound would now be preferred.

Such associative conditioning is a form of classical conditioning. It is a way of increasing the value of a stimulus by associating it with a different positive stimulus. Food is a positive stimulus that can be used to increase the value of a range of neutral stimuli through association. There is a long history of research into procedures that are based on classical conditioning and one example of such a procedure is autoshaping. Autoshaping with birds, usually pigeons, occurs when the presentation of a key light is followed by reinforcement and through this association the bird comes to peck the lit key (Brown & Jenkins, 1968). Associative conditioning is also the basis of a procedure used to increase children's preferences for different flavours of food (for a review see Birch, McPhee, Steinberg & Sullivan, 1990). For example, Havermans and Jansen (2007) paired a preferred sweet taste with a neutral vegetable flavour and this was subsequently found to increase children's preferences for the previously neutral flavours. Thus, it may be that through the use of such associative conditioning with food that a sound that was previously neutral may come to be preferred.

Commonly, the value of such a signal is assessed from the animal's preference for it. Researchers have studied various factors that can affect the preference for such a signal. These factors include "the number of times the conditioned reinforcer [signal] has been paired with a primary reinforcer, the size and quality of the primary reinforcer, and the delay between the conditioned reinforcer and the delivery of the primary reinforcer" (Mazur, 1993, p.70). It has also been found, using concurrent-chains procedures, that a variable delay between the onset of the signal to the presentation of food was preferred to a fixed delay (Fantino, 1967; Rider, 1983). Furthermore, the delay between the onset of the signal to presentation of the primary reinforcer is a crucial factor in determining the signal's value and a variable delay results in a more preferred conditioned reinforcer (Mazur, 1993). Mazur suggests that to increase the

preference for the signal some of these delays should be very short, even if some are quite long. However, if the signal ceases to be paired with primary reinforcement, then its ability to act as a discriminative stimulus is weakened as it no longer serves to signal food. This, as Williams (1994) points out, is because when a stimulus is presented in the absence of a primary reinforcer the association between the two is weakened and eventually disappears.

Preference for a signal can be assessed out of the training context in which it was established as a signal. A study by O'Daly et al. (2005) established stimuli as conditioned reinforcers and then used transfer tests to assess the effects of temporal context (the overall initial- and terminal-link durations) on the value or preference for that signal. O'Daly trained pigeons on multiple-chain schedules where the terminal-link in both components was a FT 30-s schedule. In one component, the initial-link was a VI 10-s schedule, and in the other it was a VI 100-s schedule. DRT predicts that the stimuli associated with the VI 100-s initial-link would be preferred to one following the shorter VI 10-s schedule, because there was a greater reduction in time to primary reinforcement for the former alternative. The authors used three types of transfer tests to assess preference for the signal stimuli across test sessions. These transfer tests included: concurrent-chains schedules that commenced at the end of training, and concurrent-chain and concurrent probes that were presented in the blackout period between components of the multiple-chain procedure used during training. Each of these test procedures showed that pigeons generally preferred the terminal-link stimuli associated with the VI 100-s initial-link, as was predicted. This experiment indicates clearly that concurrent and concurrent-chains schedules can be used to assess preference for signals that were established as signals in a different setting such as during the multiple-chain schedules.

Thus, it is possible to create a signal, through associative conditioning, by making the stimulus predictive of the arrival of food and it is clear that the value of this signal can be affected by a number of factors. In order to create a sound that is preferred by hens, one must consider the delay between the onset of the signal and the primary reinforcer, the number of times they have been paired, and the context in which the signal is established. One way to establish a stimulus as a signal is by using concurrent-chains schedules, as used in Experiment 2. However, when using concurrent-chains temporal context can affect the value of the conditioned reinforcer (O'Daly et al., 2005) and so it would seem that establishing a stimulus as a signal in the simplest setting without the use of schedules of reinforcement, which could affect choice, may be a better option.

The aim of the present experiment was to create a preferred sound for hens by making a tone predictive of the arrival of food, thus creating a signal for food. This was to be done using a simple procedure without any response requirement. The intention was then to assess preference for this sound, using concurrent schedules of reinforcement.

Concurrent or concurrent-chains schedules have both been used when assessing preferences for a signal. O'Daly et al. (2005) showed that both procedures could be used in transfer tests to assess preferences for signals in a different setting from which the signal had been established. As previously mentioned, although O'Daly et al. found similar results when using concurrent and concurrent-chains probes, they suggested that these schedules were measuring different effects of a signal (conditioned reinforcer). Concurrent schedules have been effectively used to assess a range of different sounds on choice behaviour in this thesis (Experiment 1) and by others (McAdie, 1998; McAdie et al., 1993, 1996). This procedure also gave more consistent results across subjects in Experiment 1, unlike the inconsistent results obtained using concurrent-chains in Experiment 2. Comparison of Experiments 1 and 2 showed that when a sound was present, as opposed to absent, the effects of sound on choice behaviour were more evident using concurrent schedules. Hence, concurrent schedules appear to be a more suitable procedure than concurrent-chains for assessing preferences for signals and were therefore selected for use in the present experiment to assess preferences for a sound that had been associated with food.

As outlined above, research has shown that a variable delay between the onset of a signal until reinforcement delivery is preferred to a fixed one (Fantino, 1967; Rider, 1983) and Mazur (1993) suggested that some of these delays should be very short, even if some are quite long. To this end, in the present experiment the sound signalled food on a Variable-Time (VT) schedule. Rachlin (1976) noted that conditioned reinforcers (signals) are more valuable when the primary reinforcer occurs less frequently rather than more frequently. This means that the value of the signal decreases if primary reinforcement (food) is already provided at a high rate and so a variable Inter-Trial Interval (ITI) was used in the present experiment.

The effects of associative conditioning should be evident immediately following pairing. After establishing the stimulus in one terminal-link as a conditioned reinforcer O'Daly et al. (2005) conducted between 10 and 15 test sessions to assess preference for the stimulus and analysed data from only the first 5 test sessions. A pilot study conducted here also found very little change in response ratios after the first 10 training sessions. Thus only 10 training

sessions were used in the present experiment to assess the initial effects of associating a tone with food.

As outlined previously, MacKenzie et al. (1993) failed to find any consistent effects of a 3000 Hz pure tone at 90 dB (A) and on the location of the hens in a chamber where they could move to turn the sound off. When this tone increased in intensity to 100 dB (A), the hens still divided their time relatively evenly between the areas of the chamber. In another study, Muller (1987) used a titration procedure where the tone increased in intensity until automatically turned off or turned off by the hens, but the hens failed to learn to turn the tone off even when it reached 120 dB (A). Muller concluded that either the hens could not learn the procedure as used or that they did not find the loud tones aversive. Thus, these studies indicate that a pure tone does not appear to be an aversive stimulus for hens, even at high intensities, and thus it may be a neutral sound. Therefore, the present experiment used a pure 4000 Hz tone played at 80 dB (A). This frequency was chosen because it has been shown to be within the hearing ranges of hens (Temple et al., 1984). The hens were not exposed to the tone before the training (conditioning) began, in order to avoid any unconditioned stimulus pre-exposure effect.

In the present experiment, it was expected that hens would show a preference for the keys associated with the tone after the training phase was introduced. This would be shown by an increase in noise bias from responding towards the keys associated with the tone (calculated using Equation 6).

## **Method**

### **Subjects**

The subjects were 6 Brown Shaver hens numbered 111 to 116. They had been flock-reared and Hens 111 and 112 were approximately 2 years old at the beginning of the experiment and Hens 113 to 116 were approximately 4 years old. Hens 111 and 112 had previous experience responding on a single key with a continuous reinforcement schedule. The remaining hens (Hens 113 to 116) had previous experience responding on concurrent schedules of reinforcement and had been exposed to a 4000Hz pure tone (at 60 dB) during the pilot study from this experiment. All hens were weighed daily and at the end of each session they were given supplementary food (commercial laying pellets), if required to maintain them at approximately 80 % of their free feeding body weight. The housing of the hens was the same as that described in Experiment 1. All animals were treated in accordance with the animal

ethics policies and procedures of the University of Waikato Animal Ethics Committee (Ref: 763).

### **Apparatus**

The experimental chamber measured 45-cm wide × 61-cm long × 57-cm high and was made of 2-cm thick woodchip particle board. There was a hinged door along one side of the chamber. The floor consisted of a metal grid enclosed in an open steel tray (2-cm high). There were two Perspex™ response keys situated on one of the walls of the chamber (termed the response panel) that were 3 cm in diameter and positioned 40 cm above the floor with 20 cm between the two keys. Each key was backlit by a red or green LED ('MARL' brand SX6 Midget Flange 28-V dc LED, from Farnell Electronics™). An effective key peck required a force of at least 0.2 N and resulted in a 0.05-s audible beep, provided by a Piezo electronic sounder located behind the key.

The food magazine, filled with wheat, was located centrally between the two response keys and was operated for 3 s during reinforcement. A magazine-access hole, measuring 9-cm wide × 12-cm high × 5-cm deep and centred 12 cm above the chamber floor, allowed the subjects access to the wheat when the hopper was raised. A 24V/1-W white light bulb was located at the rear of the magazine and illuminated the food only when the hopper was raised. There was also a house light consisting of four white LEDs centrally located on the ceiling on of the chamber.

The experimental chamber was bolted to a concrete wall 110 cm above the ground. It was enclosed in a sound-attenuating box made of particle board (2-cm thick) that was lined with cardboard egg cartons on all of the walls except the concrete wall. There was a hinged door along one side of the sound-attenuating box (51-cm wide × 750-cm high × 120-cm long). There was an air space between the internal chamber and the egg cartons that was 37 cm on the right (to allow room for the magazine), 16 cm on the left, 7 cm at the top, and 8-cm below the chamber. There was no space between the concrete wall and the internal chamber or between the egg cartons on the hinged door and the internal chamber.

Two 4" dual cone circular speakers were mounted on the wall behind the response panel 24 cm above the chamber floor. The speakers were 40 watt, 4 ohm speakers that were 15 cm apart and 3.5 cm from the edge of the wall. The speakers were amplified by a public address Yamaha™ Natural Sound Stereo Amplifier AX-380 with a power output of 55 watts rms, and a frequency response of 0± 0.5 dB, over the range of 20 – 20,000 Hz.

The sound used was a pure tone at 4000 Hz that was relayed by a noise generator (Med-PC™, model no. ANL-912) that produced broadband random frequencies between 0 and 20 kHz. The sound dB levels were measured using a sound level meter Amplaid SLM 13. Background noise levels measured from within the chamber ranged from 45 - 55 dB. The experiment was controlled by a Pentium III 997 MHz computer running MED-PC™ (Version IV) and was located in the same room as the experimental chamber.

### **Procedure**

The experiment consisted of a baseline condition (preference assessment only) followed by conditions that included a training phase. During baseline sessions, the procedure used was the same as Experiment 1, a multiple-concurrent VI 90-s VI 90-s schedule of reinforcement and sessions lasted for 40 min. No sounds were associated with any keys during the baseline condition. Experimental sessions with training had two phases; a training phase for the first 20 min followed by a preference assessment phase lasting 20 min and are shown in a diagrammatic representation presented in Figure 4.1.

#### **Training phase.**

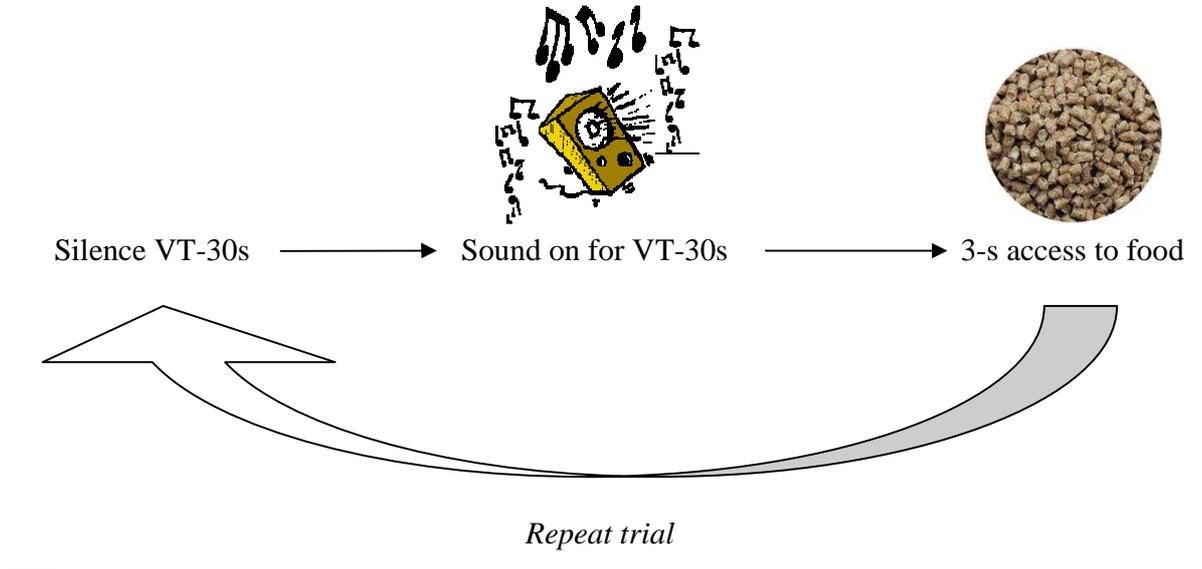
The training phase occurred in the same session before the preference assessment phase in Conditions 2 and 3. At the start of each session with a training phase the hen was placed in the experimental chamber and a trial began. During this time the house light was turned on and all of the keys were inoperative and were not illuminated; however, any responses made to the inoperative keys were recorded. At the start of a trial a period of silence (ITI), which averaged around 30 s, was followed by the sound of a tone that was played on a VT 30-s schedule (see Figure 4.1). Once the timing of the VT was complete the magazine was raised for 3 s and the tone was then turned off. The trial then restarted with a period of silence (ITI). During the ITI, the house lights remained on and there were no sounds played in the chamber. The training phase lasted for 20 min and consisted of approximately 20 trials, in other words the tone was paired with food on 20 occasions.

#### **Preference assessment phase.**

Once the training phase was complete the house lights were turned off and Component 1 of the preference assessment began, where both keys were lit red. The preference assessment used a multiple-concurrent VI 90-s VI 90-s schedule of reinforcement procedure. This procedure was the same as that outlined in Experiment 1 with one exception, each of the four components lasted for only 5 min, meaning that the total duration of the preference assessment

**Phase 1 - Tone training 20 min**

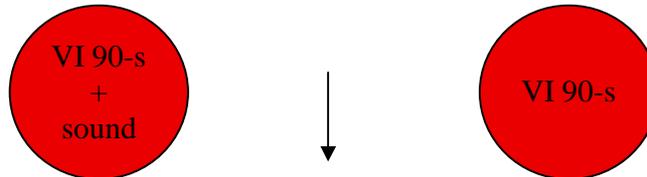
Key are unlit and house lights are on



**Phase 2 - Multiple concurrent schedules (preference assessment) 20 min**

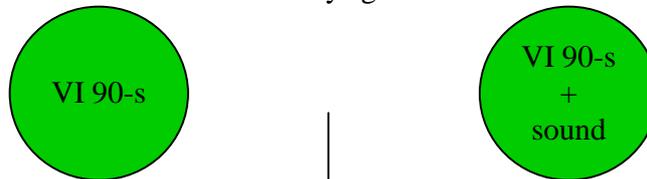
Component 1 (5 min)

Both keys red



Component 2 (5 min)

Both keys green



Repeat for Components 3 and 4

Figure 4.1. Diagrammatic representation of training conditions, with training phase and the preference assessment phase, of Experiment 4.

was 20 min. During Components 1 and 3 both keys were lit red and during Components 2 and 4 both keys were lit green. In the preference assessment, the pure tone was associated with the left key in Components 1 and 3 (red) and the right key in Components 2 and 4 (green). The sound played once a single response to the noise key turned the sound on and the sound was continuously played until a response was made to the other key, or the component changed. At the end of each day, the decibel level of the tone was tested to ensure it remained at 80 dB. The sound was tested in the same manner as outlined in Experiment 1.

### **Experimental conditions.**

Condition 1 was a baseline condition sessions in Condition 1 consisted of four 10 min components of the preference assessment only. In this condition, there were no sounds associated with either key (i.e., no training phase). Condition 1 was run until visual and statistical stability had been reached and assessed in the same manner as Experiment 1. In Condition 2, the 20 min training phase was introduced before the preference assessment and the pure tone was played during responding on the red/left and the green/right keys during the preference assessment. Condition 2 ran for 10 sessions. Condition 3 was the same as Condition 2 except the tone was turned off for both the training phase and the preference assessment. Condition 3 was conducted for 10 sessions.

Data recorded for the training phase were number of inoperative pecks to the left and right keys and number of reinforcers obtained. For the preference assessment phase the number of pecks to the left and right key, time spent responding to the left and right keys, number of changeovers, number of reinforcers on the left and right keys and total time were recorded.

### **Data analysis.**

Data from the last three sessions of Conditions 1 and 3 and the first three sessions of Condition 2 were analysed and the raw data are presented in Appendix 4a. Although the training (Condition 2) was run for 10 sessions, only the first three sessions are presented below because estimates of  $\log q$  (noise bias) did not change systematically after this point (see Appendix 4b).

## **Results**

Estimates of  $\log q$  were calculated, in the same manner as Experiment 1 (Equation 6), from the data in the preference assessment phase for only the first three sessions in the training

condition with sound (Condition 2). These estimates are presented in Figure 4.2 for responding and time allocation (left panel) and the responses post and within the COD (right panel) data. Points above the line at zero indicate a bias towards the keys associated with the tone. Figure 4.2 shows that response and time allocation estimates of  $\log q$  were generally similar in magnitude and direction for most hens. When training with sound began (Session 1), 3 hens (Hens 113, 114, & 116) showed a response noise bias towards the keys associated with the tone while the remaining 3 hens (Hens 111, 112, & 115) showed a noise bias away from the keys associated with the tone. Over the following two training sessions, the response and time allocation noise biases decreased in magnitude for 5 hens (Hens 111-113, 115, & 116), with these biases moving closer to zero. Noise biases were also examined across the four components within each training session with sound and no consistent changes were observed.

Estimates of  $\log q$  (noise bias) for responding post-COD generally showed the same pattern as the total response data. In Session 1 of training with sound, the post-COD biases were generally larger than those from within the COD. This difference, however, decreased over the following two training sessions. Although Hen 111 showed a large post-COD noise bias away from the key associated with the tone (training Session 1) her within-COD noise bias was in the opposite direction, towards the key associated with the tone. No other hens showed this pattern.

The local rates of responding are shown in Figure 4.3 for each hen. This figure shows response rates for the last three sessions of the baseline condition, for the first three sessions of the training condition with sound and for the last three sessions of Condition 3, training with no sound. Local rates of responding were calculated by dividing the number of responses on a key by the time spent on that key. The data shown were the average response rates to the left key in the red components (1 & 3) and the right key in the green components (2 & 4), and the right key in the red components (1 & 3) and the left key in the green components (2 & 4). The data were plotted this way to compare responding across conditions and assess any effects of the presence of sound on responding. In the training condition with sound, one set of keys was associated with the tone (the red/left & green/right keys) and the other set was not (red/right & green/left keys).

Local response rates to both sets of keys varied a great deal across conditions (Figure 4.3), with the highest rates generally seen in the baseline condition and the lowest in the training condition with no sound (Condition 3). Local response rates to the two sets of keys were generally similar across conditions for most hens. When training with sound was

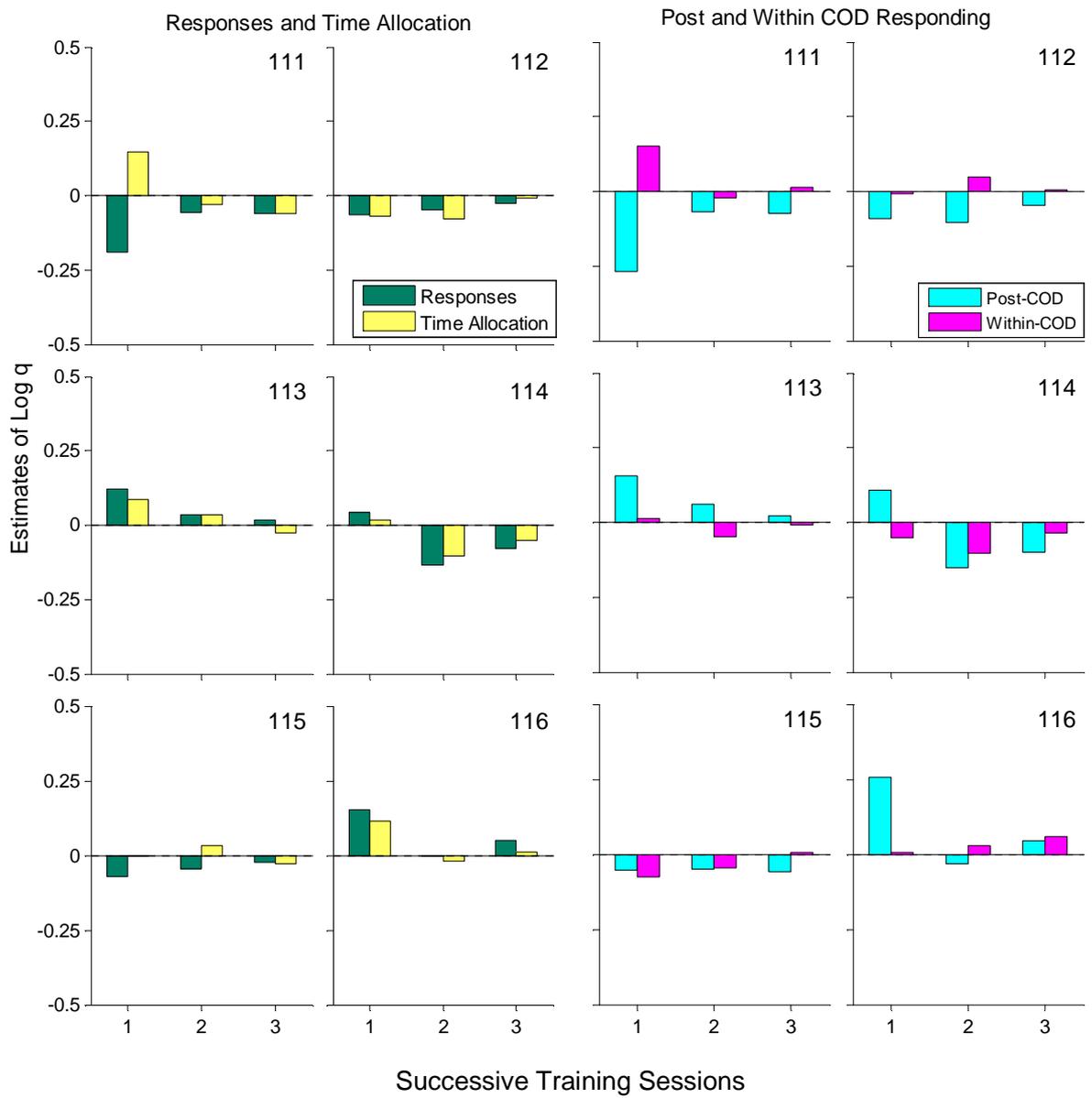


Figure 4.2. Estimates of  $\log q$  (noise bias) for the preference assessment phase for responses and time allocation (left panel) and responding post and within the COD (right panel), during the first three sessions (1-3) of training, plotted for each hen.

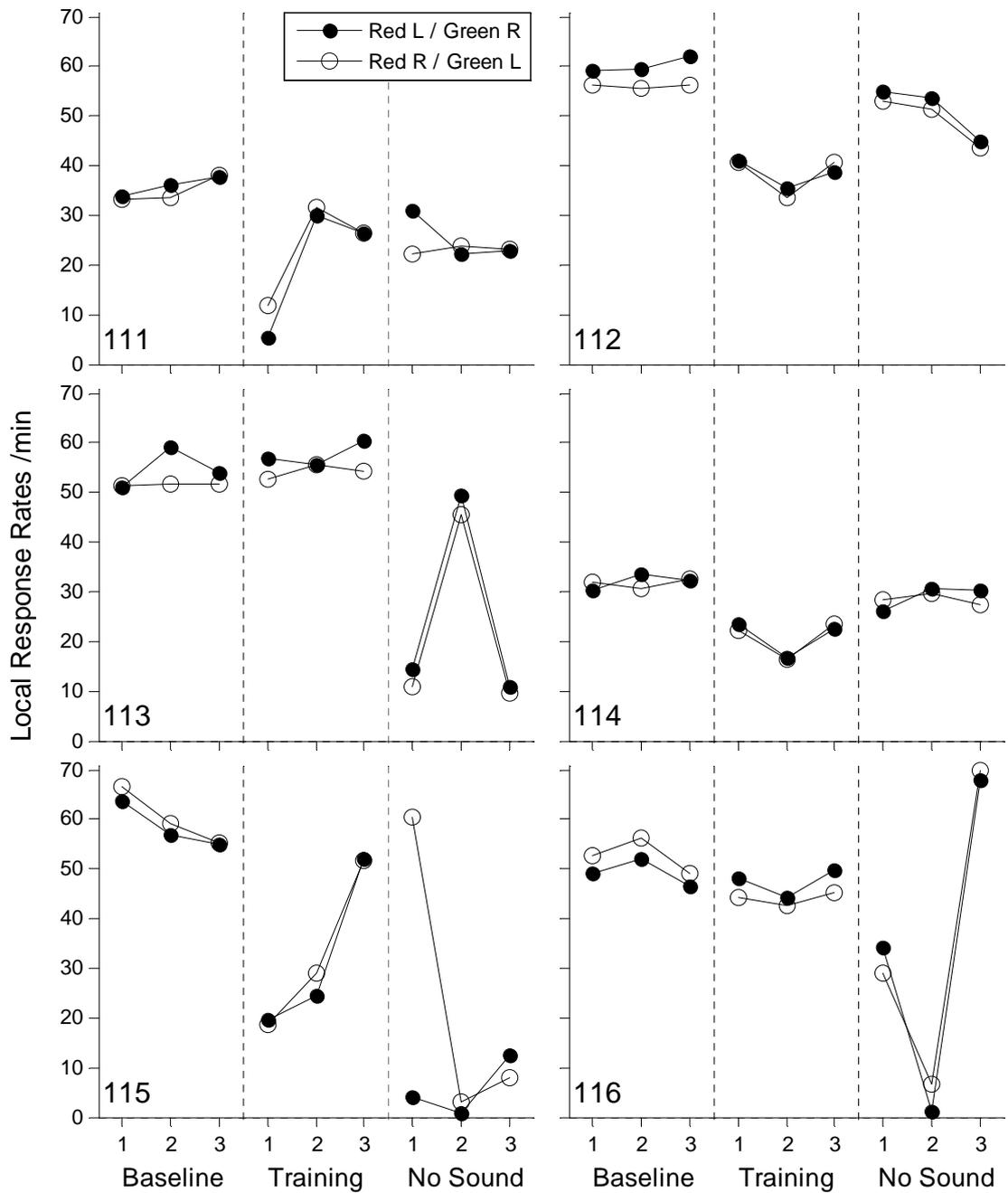
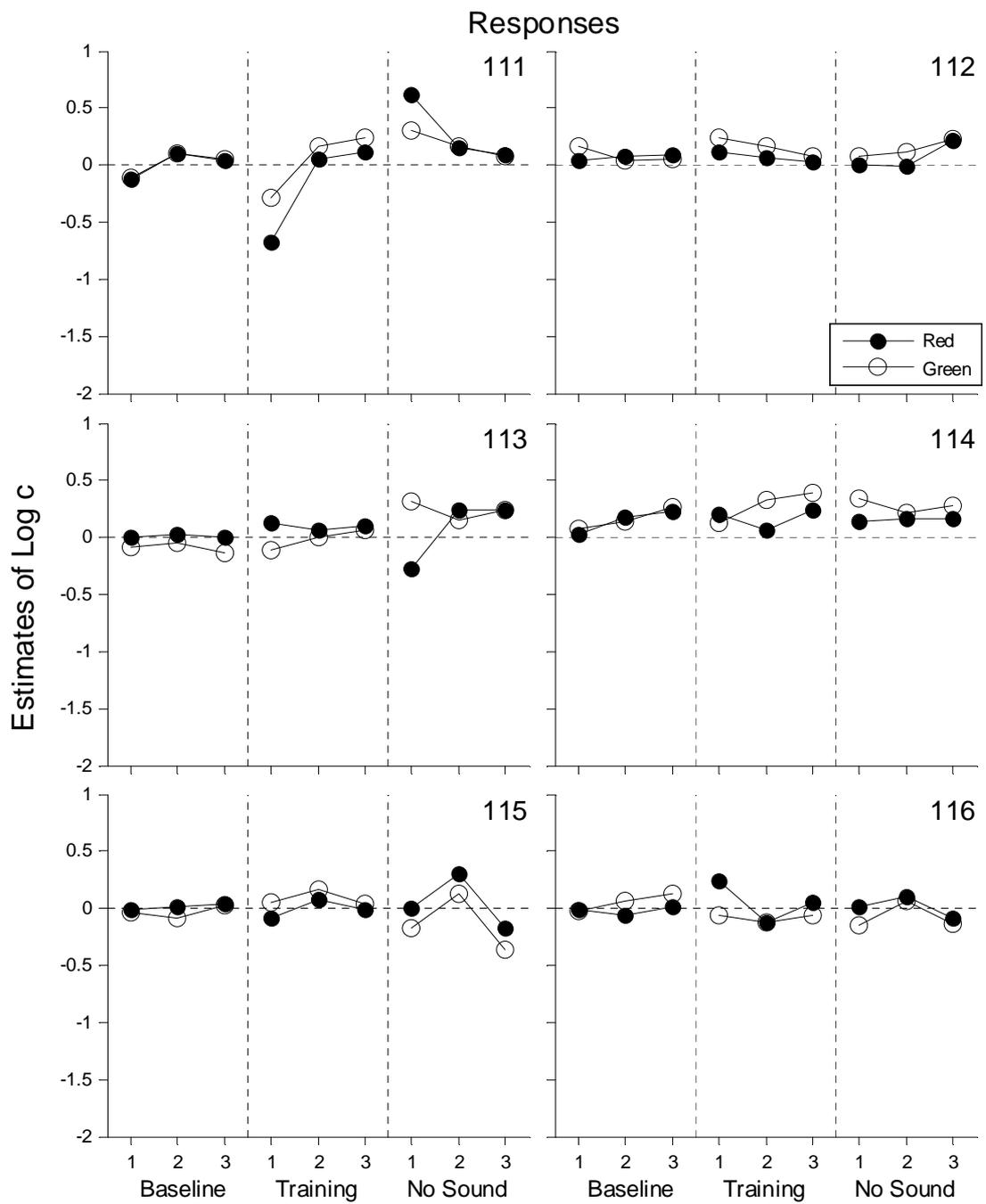


Figure 4.3. Local response rates (per min) to the red left (L) and green right (R) keys (filled circles) and red right (R) and green left (L) keys (unfilled circles) across baseline, training and no sound conditions, plotted for each hen.

introduced, response rates decreased for 4 hens (Hens 111, 112, 114, & 115) in the first session. Over the following two sessions the response rates increased again for 2 of these hens (Hens 111 & 115) and remained somewhat the same for the other 2 hens (Hens 112 & 114). Over all three training sessions with sound, local response rates remained stable for Hens 113 and 116, although they were lower than baseline for Hen 116. In the condition where no sound was played but the training phase still continued, local response rates varied considerably for 3 hens (Hens 113, 115, & 116). The response rates in this condition were generally lower than the previous two conditions and 2 hens (Hens 115 & 116) made as little as 8-20 responses in some of these sessions. For the remaining hens, response rates were more stable across the training with no sound condition and were either similar (Hen 111) or higher than (Hens 112 and 114) those rates shown in the training sessions.

To compare the responding during training with sound with that in Conditions 1 and 3 the estimates of  $\log c$  (Equation 1) are presented in Figure 4.4 (responses) and Figure 4.5 (time allocation) for all conditions, plotted for each hen. Any points below the line at zero indicate a bias to the right key and above is to the left key. These figures show that for 4 hens (Hens 111-114) log ratios of response and time allocation followed the same pattern and for Hens 115 and 116 they differed only in the condition with no sound. Across the three conditions, most hens showed only small key biases where estimates of  $\log c$  were generally close to zero in both the red and green components. It can also be seen that for most hens a change in  $\log c$  for responding and time allocation occurred from the baseline condition to Session 1 of the training condition with sound, where the difference between responding in the red and green components increased. Ratios of response and time allocation over the following two training sessions generally moved closer to zero and the difference between responding in the red and green components decreased, for most hens. Hen 114 was the only hen that showed an increase in the separation seen between responding (Figure 4.4) in the red and green components over these two training sessions. In the final condition, with no sound, estimates of  $\log c$  for responses were more variable than those from the previous conditions for 3 hens (Hens 111, 113, & 115). For the remaining 3 hens estimates of  $\log c$  were generally similar to those in the previous two conditions. Estimates of  $\log c$  for time allocation data (Figure 4.5) varied considerably across the three sessions for 3 hens (Hens 113, 115, & 116) in the training with no sound condition and were similar to baseline for the other hens.

Throughout the both training phases responses to the inoperative unlit keys were recorded and graphs of these data showed no consistent trends across subjects over the three



*Figure 4.4.* Estimates of  $\log c$  for responses in the red (filled circles) and green (unfilled circles) components across baseline, training and no sound conditions, plotted for each hen.

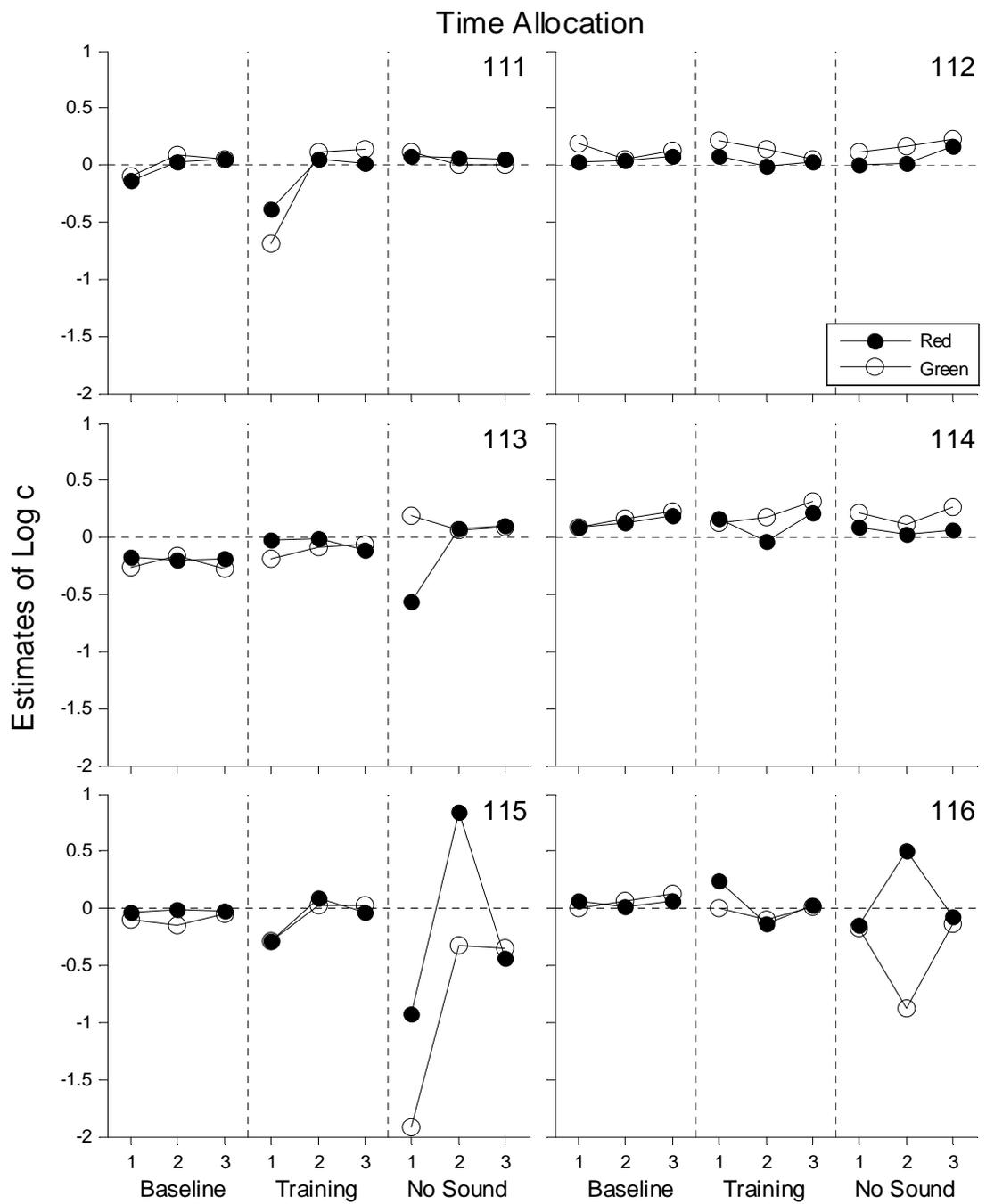


Figure 4.5. Estimates of  $\log c$  for time allocation in the red (filled circles) and green (unfilled circles) components across baseline, training and no sound conditions, plotted for each hen.

training sessions in Conditions 1 and 3. Generally, the number of these responses was very low and there were no consistent differences between the number of responses that occurred when the tone was or was not playing during the training phase with sound.

Overall, when training with sound was introduced for 3 hens (Hens 113, 114, & 116) noise bias ( $\log q$ ) was towards the keys associated with the tone while local response rates remained relatively the same. For the remaining 3 hens (Hens 111, 112, & 115), noise bias was away from the key associated with the tone and local response rates dropped considerably. When the sound was removed but training continued (Condition 3) the ratios of response and time allocation over the last three sessions varied considerably for most hens. The local rates of responding in the condition with no sound were lower than in the previous two conditions, for 3 hens, and remained similar to the training condition (Condition 2) for the 3 other hens.

### **Discussion**

In Condition 2 (training with sound), 3 hens showed a noise bias towards the keys associated with the tone after it had been associated with food in the training phase. These noise biases were evident only in the first session of this condition and were relatively small. The remaining 3 hens showed an increase in biases away from the key associated with the tone after training, and the degree of these biases were small for 2 of these hens. Although the training appears to have affected the noise bias of all hens, unfortunately, there were no common effects in the direction of the noise biases across hens after the tone training. One possibility for the failure of all hens to show a preference for the tone after training is that the sound training did not work, in other words the tone did not become a positive stimulus for these hens.

It is possible, however, that the preference assessment phase of each session was too long. Williams (1994) noted that a stimulus would lose its conditioned reinforcing value each time it was presented in the absence of a primary reinforcer. During the preference assessment phase after each training phase in each session the hens were exposed to the tone in the absence of food for long periods of time and consequently the signalling effects of the tone may have weakened. If this had been an issue then one would have expected that in the beginning of the first session of the preference assessment after the training with sound the hens would have shown a preference towards the keys associated with the tone. This effect would have then decreased over the duration of the first preference assessment session but this was not the case here. The cumulative responding over each of the 5 min components in the preference

assessment phase was inspected and there appeared to be no differences between responding across both red components and both green components in the first session after the training phase with sound (Appendix 4c). Thus, it seems unlikely that any positive association of the tone with food declined across one preference assessment phase.

It was assumed that the concurrent schedules procedure would have been able to reflect any preferences for a positive sound if a preference had been present, as this has been found by other research (O'Daly et al., 2005). Therefore, it seems that the training with sound procedure used in the present experiment did not establish the tone as a positive stimulus (or increase its value) for these hens. This indicates that there may have been problems with the way in which the training phase was arranged in the present experiment. Although O'Daly et al. (2005) assessed the value of a conditioned reinforcer (signal) using concurrent schedules, no effects were shown here but there were differences between the procedures used in their study and in the present one. One of the main differences was the way in which the stimuli (sounds here and key colour in their experiment) were established as signals. O'Daly et al. used multiple-chain schedules to establish the terminal-link stimuli as the signal. In that procedure the signal indicated a greater reduction in delay to reinforcement when compared to the alternative terminal-link. Here, food was always delivered in the presence of the tone and there were variable periods of silence between trials, hence the tone indicated a reduction in delay to reinforcement in only some trials (when a long ITI was followed by a short tone). Another difference between the present and O'Daly et al.'s experiment is that there was no response requirement throughout training used here, while in O'Daly et al.'s study responding was required in the terminal-link and so was the same context in which preference for the stimulus was assessed. It is possible that the context in which the signal was established in the training phase of the present experiment was too different from that in the preference assessment phase. Hence, context may have affected the way in which the hens responded to the sound, consequently affecting their preference for the tone.

Another element that may have affected the success of the tone training in the present experiment was that the hens may not have had enough exposure to the tone signalling food before a preference for it was assessed. In the training phase with sound, there were approximately 20 trials of the tone being associated with food before preference for the tone was assessed in any one session. This may not have been enough trials to establish the tone as a signal. O'Daly et al. (2005) conducted 20 training sessions before assessing preference for a signal and within each session the terminal-link stimuli was followed by food for 40 trials.

This makes a total of 800 trials where food followed the terminal-link stimuli before preference for it was assessed, a number far greater than used in the present study. Although training sessions continued for 10 sessions in the present study, making a total of 200 trials of food being associated with the tone, as the cumulative number of trials increased across sessions, preference for the tone did not increase.

As previously mentioned, research with hens has shown that they would not move to turn a pure tone on or off, either when it was delivered continuously or in pulses (MacKenzie et al., 1993). MacKenzie et al. did find, however, that hens would move to turn off other tape recorded sounds, showing that hens would learn to avoid other sounds. As previously outlined, Muller (1987) also found that hens did not learn to turn off a high intensity tone. It was suggested by Muller that the hens may not have found the tones to be aversive. From these two studies it was shown that tones, even very loud ones, did not seem to affect the behaviour of hens in that the tones were not avoided and this was one of the reasons it was selected for use in the present experiment. Although other research has showed that loud tones can suppress responding in rats (Reed & Yoshino, 2001, 2008), it may be that a pure tone is not a stimulus that affects the behaviour of hens (MacKenzie et al., 1993; Muller, 1987). A pure tone is not a naturally occurring sound. It is a steady tone without overtones that has different properties from naturally occurring sounds and this may be why hens do not react to it. A suggestion for future research of this type would be to use a sound that is known to affect the behaviour of hens, either positively or negatively, when attempting to alter the reinforcing value of the sound.

The present experiment employed a variable ITI to ensure that the frequency of primary reinforcement was not too high, as suggested by Rachlin (1976) to be a factor in determining the strength of a conditioned reinforcer. The period of silence in the ITI meant that the tone was signalling food and without the period of silence the tone would play continuously and therefore would no longer function as a signal. The length of the ITI used here, however, may have affected the success of conditioning. The use of a longer fixed ITI would have meant that the tone signalled a greater reduction in delay to reinforcement and the results may have been more conclusive.

In the present procedure the training phase in Conditions 2 and 3 can be thought of as a pre-feeding phase, as food was delivered non-contingently in this phase. It has been shown that response rates decrease when pre-feeding occurs (Grace, Schwendiman & Nevin, 1998; Plowright, Church, Behnke & Silverman, 2000). During the training with sound condition

there were no differences between the local rates of responding to keys associated and not associated with the tone but response rates to all keys decreased for most hens when training was introduced. Response ratios also became much more variable over time in Condition 3. Over the course of the experiment response rates continued to drop and were lowest in the last sessions of Condition 3 (training with no sound) as shown previously in Figures 4.3 and 4.4. However, it has been shown that pre-feeding does not affect choice behaviour (Temple, 1973). Ratios of responding and time allocated ( $\log c$ ) in Condition 3 were more variable for some hens but this was mainly due to the very low number of responses occurring; as low as 8-20 responses in an entire session for some hens. Thus, while the pre-feeding during the training phase in this experiment decreased the local rates of responding during the preference assessment, it was unlikely to have affected any choice behaviour in the preference assessment phase of Condition 2 and only appeared to affect the response ratios of some hens at the end of Condition 3.

Although in the present experiment there were 3 hens that increased their noise biases towards the keys associated with the tone, after it had been associated with food, this increase was only small and the remaining 3 hens showed the opposite, an increase in noise bias away from the keys associated with the tone. Generally, the present experiment was not successful in creating a tone as a positive or preferred sound for hens. There are a number of possible reasons for this outcome, including problems with the arrangement of the training phase. In spite of these problems it was thought that the procedure could work but one of the main issues that could be addressed was the use of a pure tone, as it may not be a stimulus that affects the behaviour of hens. Therefore, the next experiment the used other sounds, that have been shown to affect the behaviour of hens, as they may be more effective when attempting to increase the value of sounds by associating them with food.

## EXPERIMENT 5

Experiment 4 showed that only 3 hens' preferences shifted towards the keys associated with a tone that had been continuously associated with food. However, the magnitude of this shift was small. Thus, the experiment was not successful in changing the value of a 4000 Hz tone such that it would be preferred by hens. One suggestion for the failure to find an effect was that pure tones have not been shown to have an effect on hens' behaviour, even when played at very loud intensities (MacKenzie et al., 1993; Muller, 1987). It may be that a pure tone is not a sound that affects the behaviour of hens, even when it is likely to be at an aversive intensity or when it has been associated with food. As previously suggested, it may be better to examine sounds that have been shown to affect behaviour, such as white noise and a food call, and then determine if the effects of such sounds alter once they have been associated with food.

White noise and the food call have been shown, in this thesis, to be sounds that are not preferred by hens when compared to no sound. Experiment 1 showed that these sounds biased the responding of all hens when using concurrent schedules. In Experiment 2, the food call biased responding of all hens in the initial-links and the white noise biased responding of 3 hens. Because these experiments showed that white noise and the food call affected the behaviour of hens, which has not been shown for a pure tone, it may be possible to alter the preference or value of these sounds by associating them with food. In doing so, it may be possible to decrease the bias resulting from white noise and the food call by associating them with food in the same manner as in Experiment 4.

As discussed in Experiment 4, multiple-concurrent schedules have proved useful for assessing the effects of other sounds in this thesis and in research of others (McAdie, 1998; McAdie et al., 1993, 1996). Thus, multiple-concurrent schedules were used again in the present experiment to assess any effects that associating sounds with food has on the responding of hens. Also previously outlined, creating a positive association with a stimulus has been shown to increase preference for that stimulus, for example, when a key colour has been associated with a shorter delay to food (O'Daly et al., 2005) or a neutral flavour has been paired with a sweet one (Havermans & Jansen, 2007). Thus, it is possible to alter the effects of a stimulus by associating it with something that is positive or preferred, such as food.

The aim of the present experiment was to create a positive association with white noise and the food call by making these sounds predictive of the arrival of food. It was hoped that this would decrease the magnitude of noise bias ( $\log q$ ) shown away from the keys associated

with these sounds, compared to when there had been no association with food. It was also thought possible that the direction of  $\log q$ , during the conditions when sound was predictive of the arrival of food, would be towards the keys associated with sound. The experimental parameters were kept the same as those used in Experiment 4, in order to make comparisons across the two experiments. Given that the responding of these hens in Experiment 4 was stable after three sessions of training, three training sessions were conducted in the present experiment before the hens were returned to baseline conditions.

## **Method**

### **Subjects**

The subjects were the same as Experiment 4 and fell under the same ethical protocol (Ref: 763).

### **Apparatus**

The apparatus used was the same as Experiment 4 except that the food call (the same as used in Experiments 1 and 2) was relayed using a media player and the white noise was relayed using the noise generator from Experiment 4 (Med-PC™, model no. ANL-912).

### **Procedure**

The present experiment began two months after Experiment 4. The general procedure used was the same as in Experiment 4 with the following exceptions. Baseline conditions using multiple-concurrent schedules were conducted for 20 minutes. In these conditions, sound (at 100 dB) was always associated with responding on the red/left (Components 1 & 3) and green/right keys (Components 2 & 4). All baseline conditions were run to stability and assessed in the same manner as in the previous experiments. All training conditions were run for only three sessions. Data recorded were the same as in Experiment 4.

Table 5.1 shows the experimental conditions, the sounds played, and whether it was training or a baseline condition for all conditions. In Condition 1, white noise (100 dB) was associated with the red/left and green/right keys of a multiple-concurrent VI 90-s VI 90-s schedule of reinforcement. In Condition 2, training was introduced and white noise was associated with food, in the same manner as Experiment 4, during a 20-min training phase that occurred before the preference assessment. Sound was also played in the preference assessment components (on the red/left and green/right keys). Condition 3 was another baseline condition where the food call was played on the red/left and green/right keys and no

Table 5.1

*Experimental conditions, the sounds played in each condition, and whether it was a training or a baseline condition are shown in each column for each condition.*

<b>Condition Number</b>	<b>Sound Played</b>	<b>Training or Baseline</b>
1	White noise	Baseline
2	White noise	Training
3	Food call	Baseline
4	Food call	Training
5	Food call	Baseline
6	Food call	Training (no reinforcers)
7	Food call	Baseline
8	White noise	Baseline
9	White noise	Training (no reinforcers)
10	White noise	Baseline

training occurred before the preference assessment. Again, in Condition 4 training was introduced before the preference assessment and the food call was associated with food (as previously described) and also associated with the sound keys during the preference assessment. Condition 5 was a replication of Condition 3.

To examine the effects of sound alone during the training component, Condition 6 was a replication of Condition 4, except that no food was provided in the training phase. Instead, the sound played during training was on a VT 30-s schedule, with a variable ITI averaging around 30 s, and no food was provided. The training phase was still followed by a preference assessment phase where the food call was again associated with the sound keys. The remaining conditions followed the same pattern but instead of the food call white noise was played.

### **Data analysis.**

The data were analysed in the same manner as Experiment 4 and are presented from the last three sessions of each baseline condition and all three sessions from the training conditions.

## **Results**

To examine the effect of the presence of sound on responding of the hens the estimates of  $\log q$  (noise bias) (calculated in the same manner as Experiments 1 and 4, using Equation 6) are presented in Figure 5.1 for each white noise condition, plotted for each hen. Figure 5.2 shows these estimates for the food call conditions, plotted for each hen. Note that the scale for Hen 115 is different from the other subjects in both figures. In Figures 5.1 and 5.2 noise biases are shown for conditions when either white noise (5.1) or a food call (5.2) was played on the red/left and green/right keys in baseline but no training occurred before the preference assessment. A training phase occurred before the preference assessment in the training conditions with food (Training+food) and without food (Training-food). Points above the line at zero indicate a noise bias towards the keys associated with sound, and below zero indicate a bias away from the keys associated with sound.

Figures 5.1 and 5.2 show that for most subjects responding and time allocation estimates of  $\log q$  differed in magnitude and these estimates were often in opposite directions (e.g.,  $\log q$  for responding was negative and  $\log q$  for time allocated to responding was positive), especially for Hens 112 and 114. This indicates that more responses were on the key not associated with sound but more time was spent responding to keys associated with sound. For all hens, and in most conditions, response-based noise biases were away from the keys associated with white

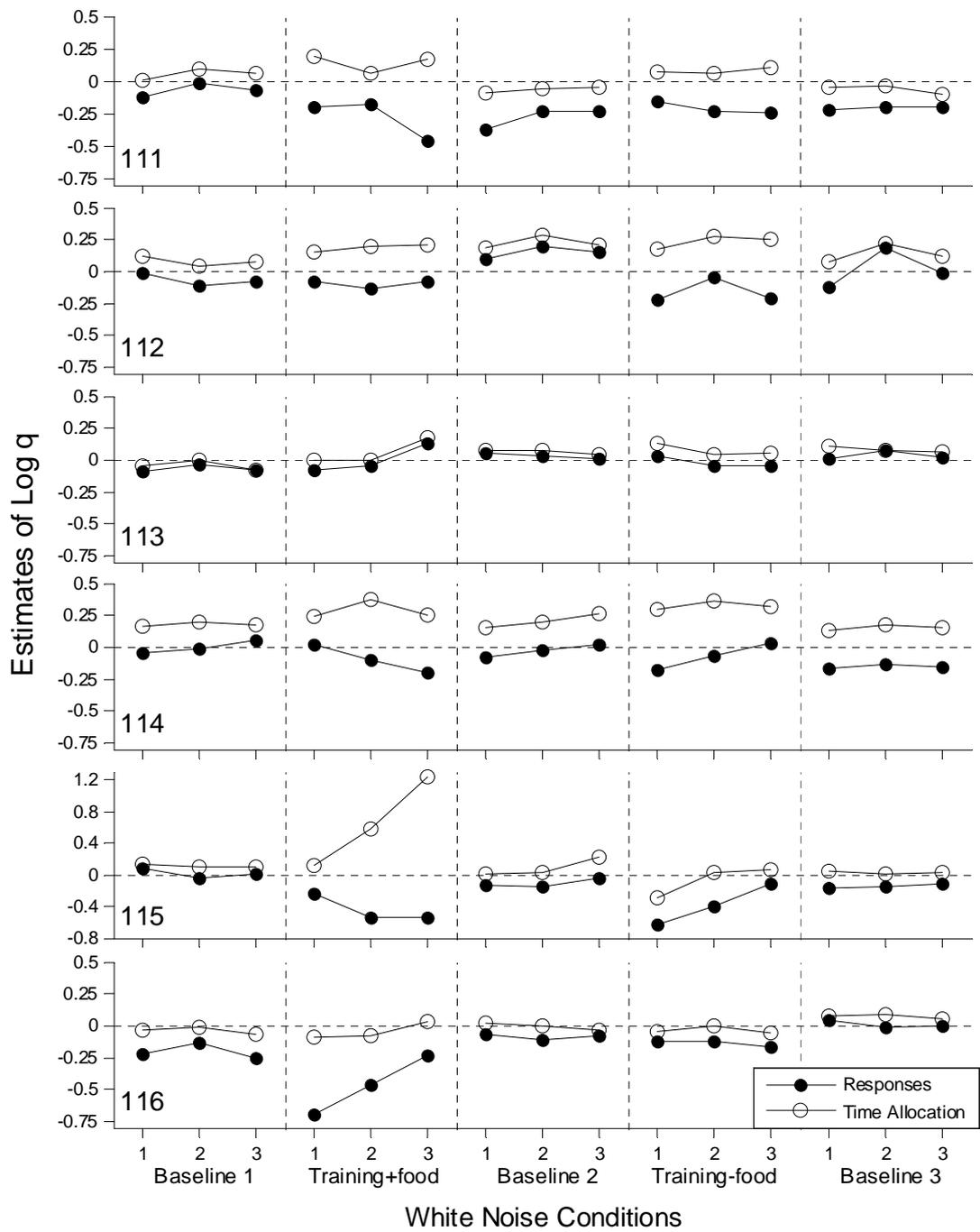


Figure 5.1. Estimates of  $\log q$  (noise bias) for responses (filled circles) and time allocation (unfilled circles) in the white noise conditions, plotted for each hen. Estimates are shown for each baseline condition and training conditions with and without food. Note that the scale plotted for Hen 115 is different from the other subjects.

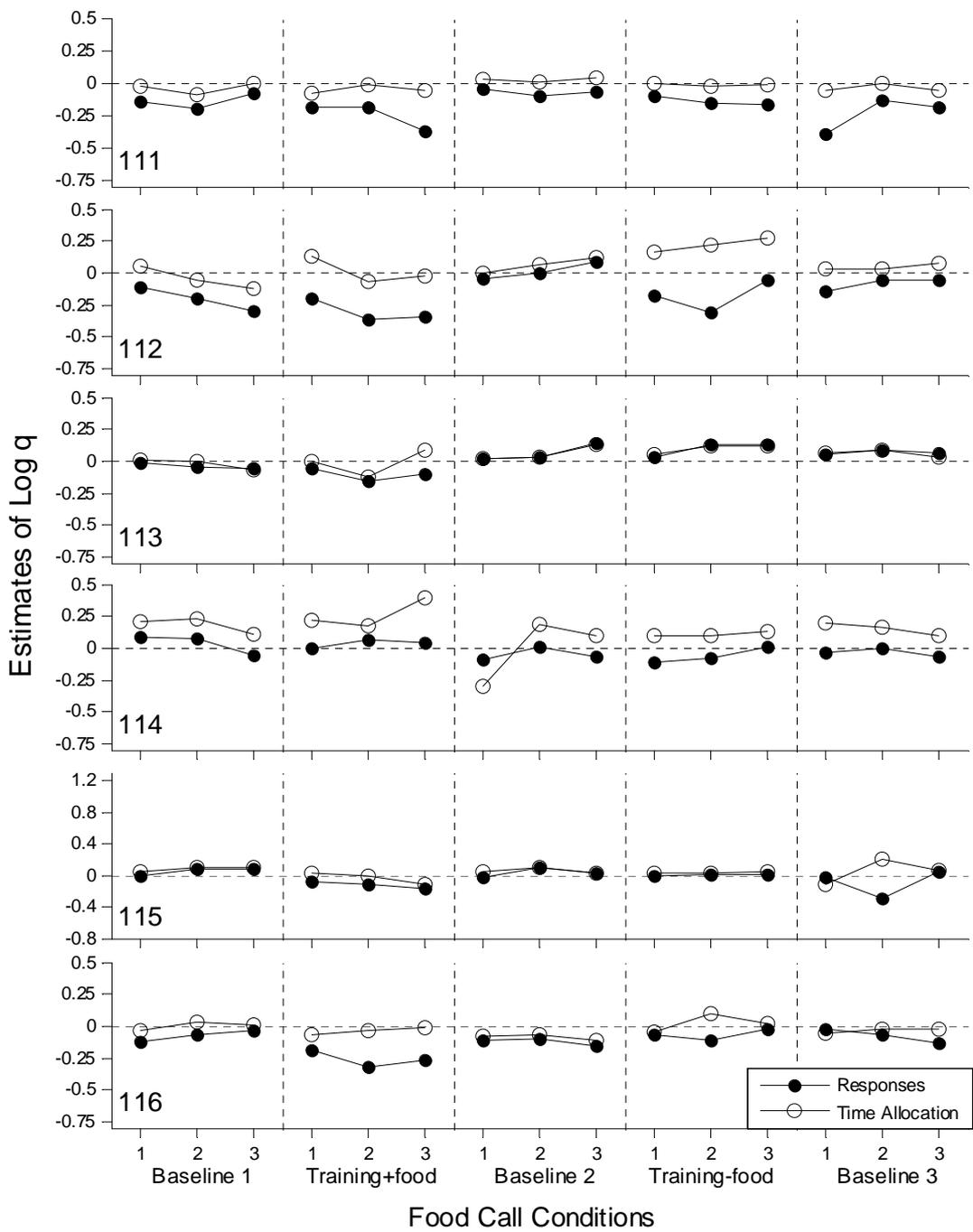


Figure 5.2. Estimates of  $\log q$  (noise bias) for responses (filled circles) and time allocation (unfilled circles) in the food call conditions, plotted for each hen. Estimates are shown for each baseline condition and training conditions with and without food. Note that the scale plotted for Hen 115 is different from the other subjects.

noise or the food call, while time allocated noise biases were either close to zero or towards the keys associated with sound. An exception to this was noise biases for Hen 113 whose response and time allocation biases were similar across conditions for both sounds. However, what is of most interest here is any changes in noise bias from baseline conditions to those where training occurred.

The time allocated noise biases across both sound conditions tended to be similar to biases in the baseline conditions for most hens, with only small changes seen for some hens. An exception to this was an increase in the time allocated noise bias during the training condition with food for Hen 115 (white noise) and for Hen 114 (food call), which was not seen in the training condition without food.

Across Figures 5.1 and 5.2, response-based noise biases in the training with food condition showed more change from the baseline conditions when white noise was associated with the sound keys, compared to when it was associated with the food call. When the training with food condition was introduced, there was an increase in response-based noise bias for white noise, 4 hens (112, 114, 115, & 116) showed an increase in response-based noise biases from Baseline 1 and for the food call 4 hens (Hens 111, 112, 114, & 115) showed an increase in response-based noise bias from Baseline 1. However, the degree of these increases was small for some subjects. When the training without food was introduced response-based noise biases for 2 hens (Hens 112 & 115) increased from Baseline 2 and this increase was also seen for 2 hens (Hens 111 & 112) for the food call. In the final condition (Baseline 3), for both sounds noise biases from responding were similar to Baseline 2, for most hens.

In the training condition with food, there was an increase in noise bias from Baseline 1 away from the sound keys in 9 out of 12 cases and the same was shown in 6 out of 12 cases for the training condition without food. Thus, the first training condition with food gave an increase in noise bias in more cases than did the training without food and the degree of this increase also tended to be greater when food was provided.

The estimates of  $\log q$  (noise bias) for responding post and within the COD for all conditions are presented in Figure 5.3 (white noise) and Figure 5.4 (food call), for each hen. Values of  $\log q$  were calculated in the same manner as above. Note that the scale for Hen 115 is different from the other subjects in both figures. As in Figures 5.1 and 5.2, noise biases are shown for baseline conditions for each sound and training conditions with (Training+food) and without food (Training-food). For Hen 115, estimates of  $\log q$  in the training condition with

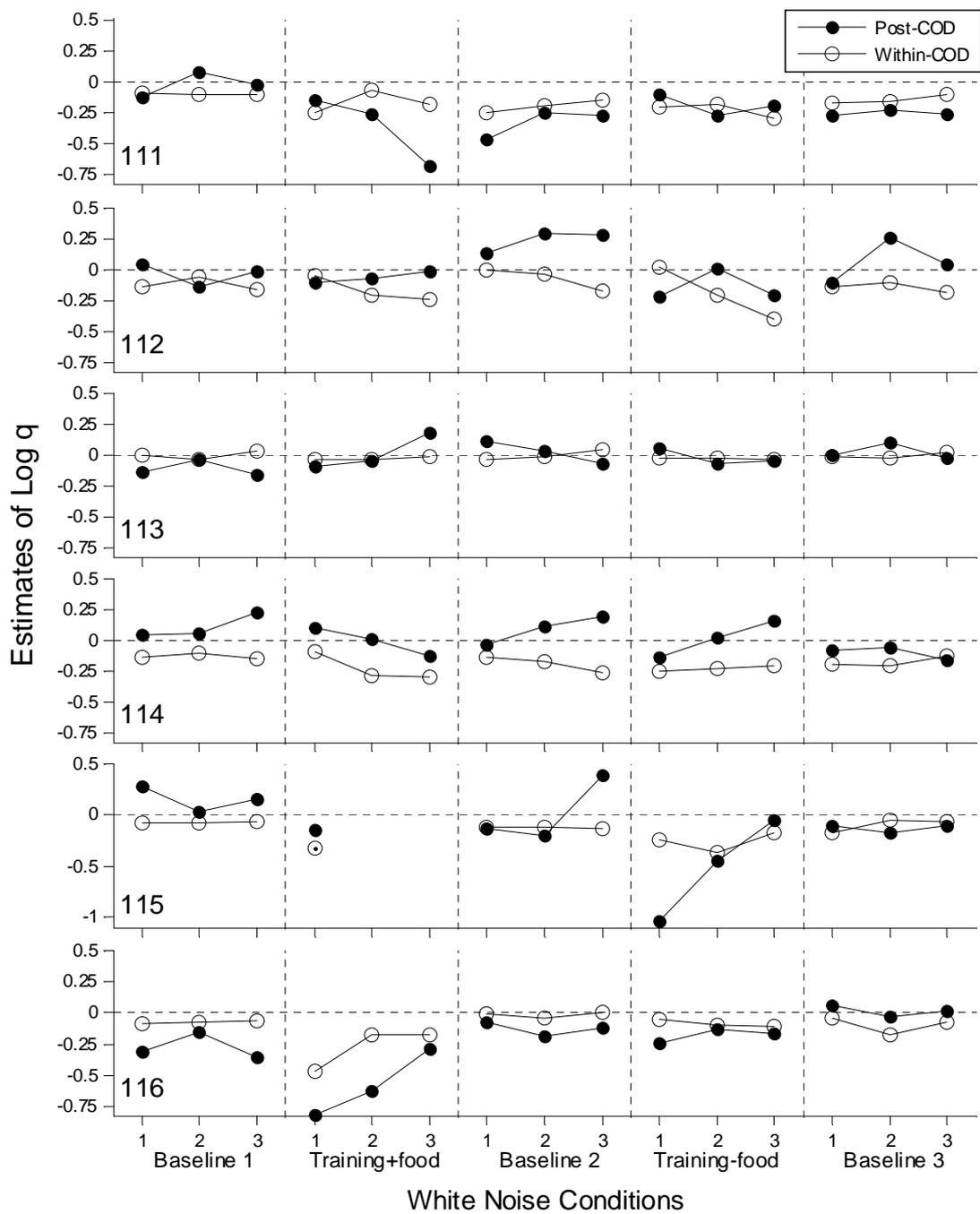


Figure 5.3. Estimates of  $\log q$  (noise bias) for responses post (filled circles) and within the COD (unfilled circles) in white noise conditions, plotted for each hen. Estimates are shown for each baseline condition and training conditions with and without food. Note that the scale plotted for Hen 115 is different from the other subjects.

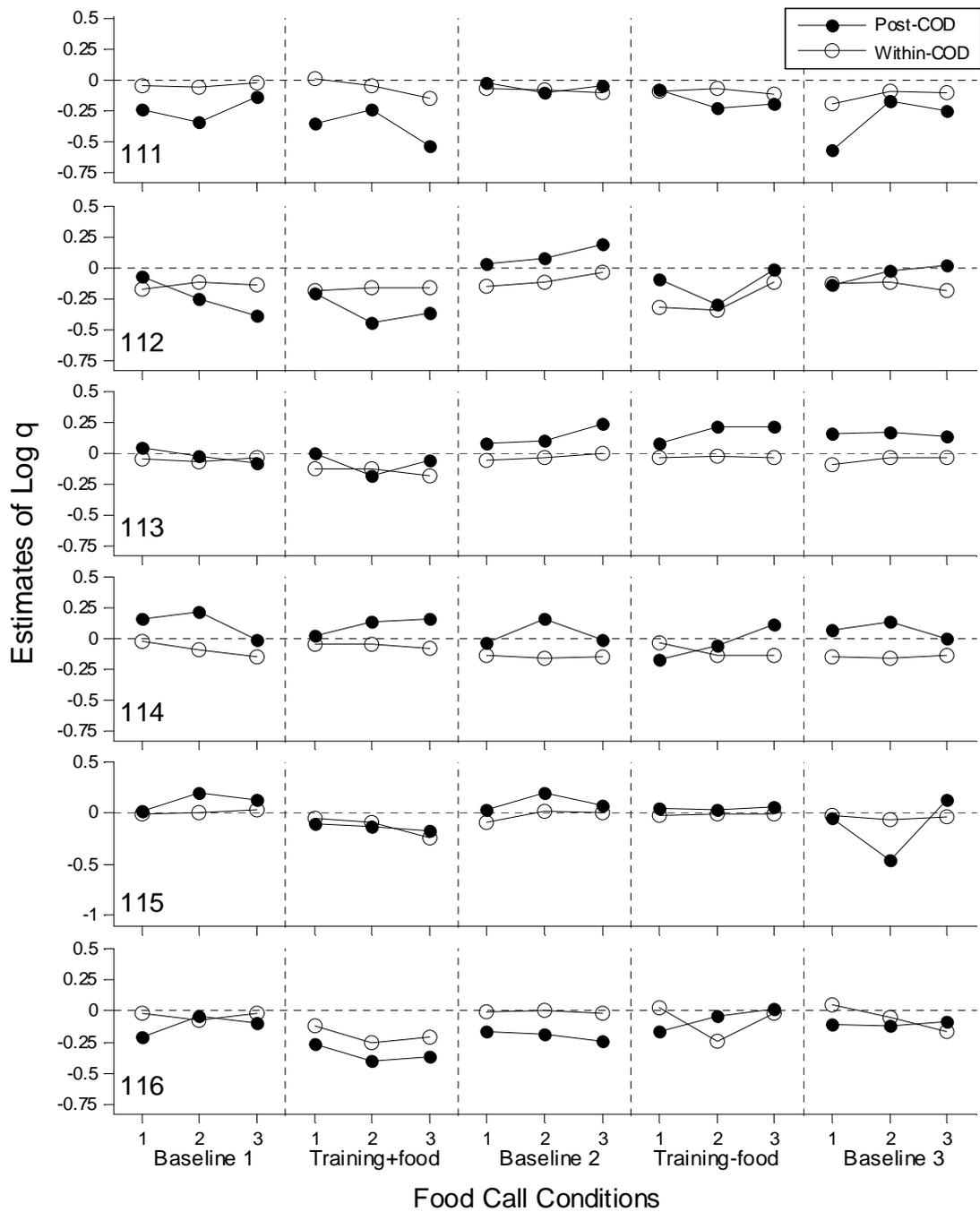


Figure 5.4. Estimates of  $\log q$  (noise bias) for responses post (filled circles) and within the COD (unfilled circles) in food call conditions, plotted for each hen. Estimates are shown for each baseline condition and training conditions with and without food. Note that the scale plotted for Hen 115 is different from the other subjects.

food are presented for only the first session of this condition because too few responses occurred in the following two sessions, therefore noise biases could not be calculated.

Figures 5.3 and 5.4 show that for 2 hens (Hen 112 & 114) noise biases ( $\log q$ ) for within-COD responding were generally larger than that from post-COD responding and these biases were in the opposite direction in some conditions. The post-COD noise biases generally followed the same trend as the total response data (Figures 5.1 & 5.2), for both sounds. For white noise, within-COD noise biases in both training conditions generally increased from baseline in 7 out of 12 cases, while for the food call this increase was seen in only 4 out of 12 cases. Increases in within-COD noise biases from baseline for the two training conditions occurred in 7 out of 12 cases for training with food and in 4 out of 12 cases for training without food.

Throughout training conditions with and without food, responses to the inoperative unlit keys were recorded and graphs of these data showed no consistent trends across subjects over the three training sessions. Generally, the number of these responses was very low and there were no consistent differences between the number of responses that occurred when the sound was and was not playing during the training phases.

Local rates of responding to the sound keys (red/left & green/right) and the no sound keys (red/right & green/left) are shown in Figure 5.5 for white noise and in Figure 5.6 for the food call, plotted for each hen. Local response rates were calculated by dividing the number of responses on a key by the time spent on that key only. The data shown for the sound keys was the average rate of responding to the left key in the red components (1 & 3) and the right key in the green components (2 & 4). Figures 5.5 and 5.6 show that the local rates of responding for both sounds varied across conditions but that they were lower on the sound keys for all hens, with the exception of Hen 113, who showed similar response rates to all keys for both sounds. The differences between responding to the sound and no sound keys were also larger for white noise than for the food call, for all hens. With white noise, in both training conditions the difference between response rates to the sound and no sound keys tended to increase from baseline (9 out of 12 cases) and also increased for the food call but in fewer cases (4 out of 12 cases). For both sounds, across training sessions with food the local rates of responding to all keys decreased from Baseline 1 for most hens (10 out of 12 cases) and a larger decrease in rate was seen on the sound keys. For training with no sound, there was again a decrease in local response rates to the sound and no sound keys from Baseline 2 but only for some hens (7 out of 12 cases).

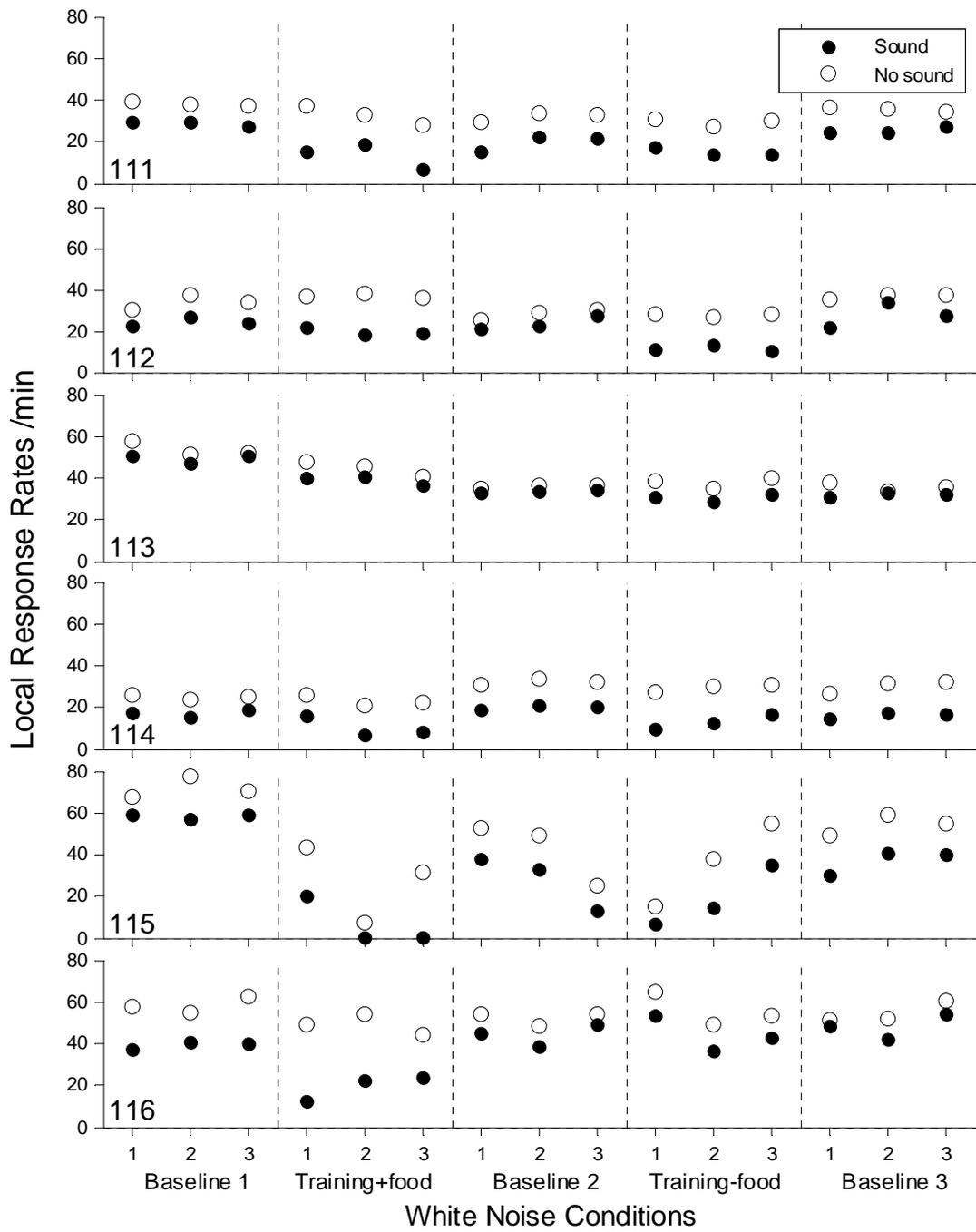


Figure 5.5. Local response rates (per min) to the sound keys (filled circles) and no sound keys (unfilled circles) in the white noise conditions, plotted for each hen. Response rates are shown for each baseline condition and training conditions with and without food.

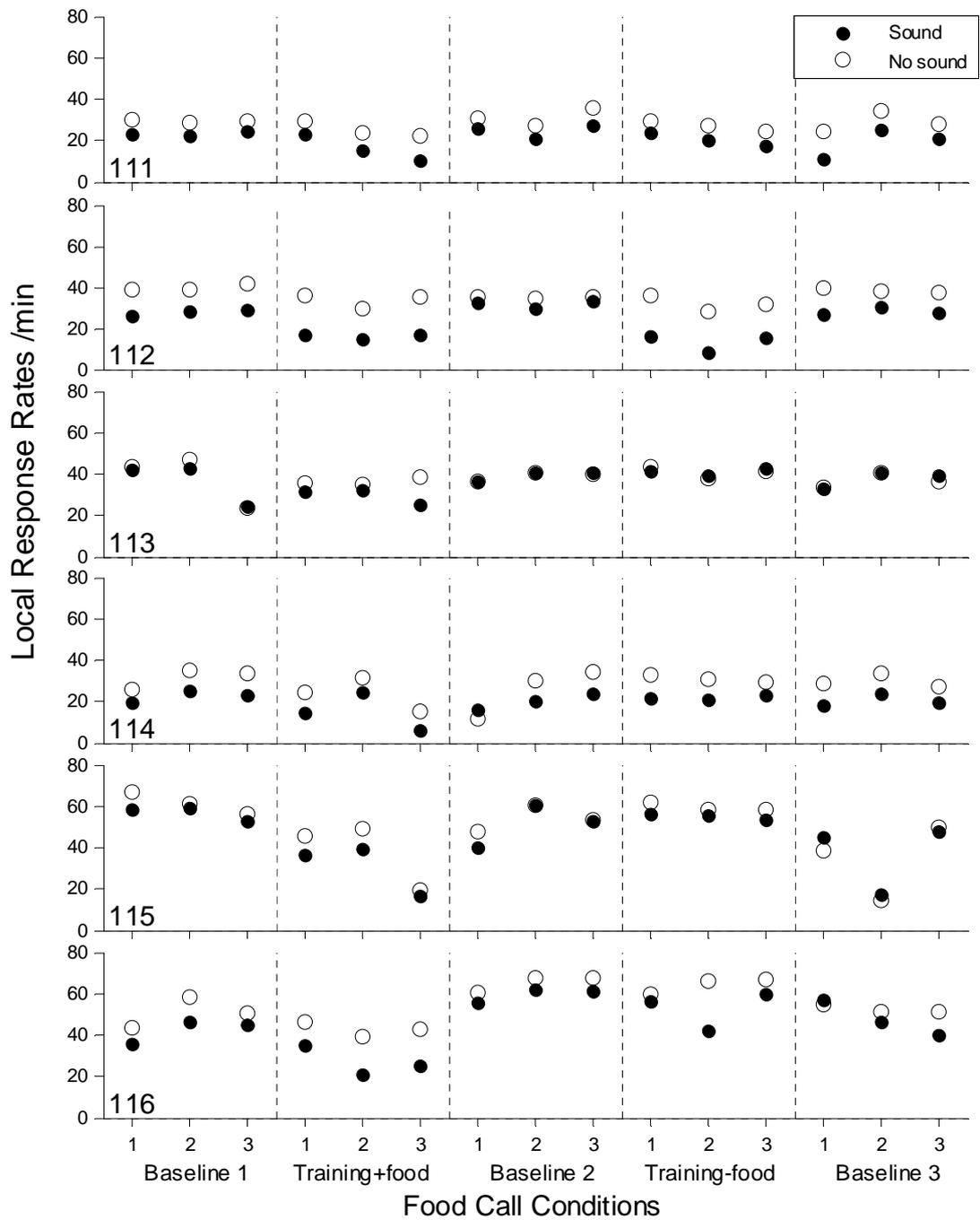


Figure 5.6. Local response rates (per min) to the sound keys (filled circles) and no sound keys (unfilled circles) in the food call conditions, plotted for each hen. Response rates are shown for each baseline condition and training conditions with and without food.

The estimates of  $\log b$  (key bias) across conditions are presented in Appendix 5a (white noise) and Appendix 5b (food call). These figures showed that there were no consistent trends seen across conditions for both sounds and there were also no systematic differences in  $\log b$  between the baseline and training conditions. The mean number of COs in the red and green components was also inspected and are shown in Appendix 5c (white noise) and Appendix 5d (food call). The changeover data followed a similar trend to that seen for the local rates of responding where for most hens the mean number of COs dropped in both components when training with and without food was introduced (18 out of 24 cases across both sounds).

In summary, when either training condition was introduced there was generally an increase in response-based noise bias ( $\log q$ ) away from the keys associated with sound. Time allocation noise biases showed the opposite effect in 13 of 24 cases, with an increase in noise bias from baseline, towards the keys associated with sound. Noise biases for post- and within-COD responding also tended to increase compared to baseline for both training conditions, with more responding towards the key not associated with sound for both white noise and the food call. The increases in noise bias, however, were larger when food was used during training than when it was not, for both sounds. The local rates of responding on all keys in both training conditions generally decreased compared to baseline with larger decreases to the keys associated with sound and this was so in more cases for white noise than for the food call. The mean number of COs in training conditions also decreased from baseline, following a similar pattern to the local response rates but no systematic differences were seen across components.

## Discussion

It was the aim of this experiment to see if the training procedure would increase the reinforcing value of white noise and a food call for hens. This would have been shown by a decrease in noise bias away from keys associated with these sounds, after they had been associated with food. The overall findings here showed that for most hens when training was introduced, regardless of whether it included food or not, there was an increase in the response-based noise bias away from the keys associated with white noise and the food call. Thus, it appears that an opposite result was found to that expected. It is not clear why this occurred but some possible explanations will be discussed below.

Although the training used in the present experiment increased response-based noise biases away from keys associated with sound, training appeared to have only a small effect on the time spent responding. This resulted from most hens showing lower local rates of

responding to keys associated with white noise and the food call than to keys not associated with sound, during both training conditions. The lower response rate indicates that sound slowed the hens' responding. As previously outlined, it has been well documented that an aversive stimulus, such as electric shock, suppresses responding previously maintained by reinforcement (e.g., Azrin, 1956; de Villiers, 1980; Farley, 1980). The reduced rates of responding seen in the present experiment may then be an indication that for these hens both sounds were initially aversive during baseline conditions and that the noise biases indicated that the training in this experiment increased the aversiveness of these sounds.

One consideration here is that during training conditions the hens were exposed to both the sounds for almost double the amount of time in a session than they were during the baseline conditions. In baseline conditions, the sound was on for approximately half of the session duration, a total of 10 min. During the training phase, sound was on for an additional 10 min (approximately), making a total duration of exposure to sound for 20 min in a whole session. The increase in exposure to sound could have resulted in the increased aversiveness of sounds for these hens. Although in Experiment 1 the sound was also on for approximately 20 min (of a 40 min session), it may not be the total duration of exposure time that affected the hens response to the sounds here but rather the increase in exposure time compared to baseline conditions. One possible way to resolve this issue would be to conduct 40 min baseline sessions that would then have a comparable duration of exposure to sound with the training conditions. One could then compare the results to determine if the total exposure to sound affected the hens' noise biases.

It has been well documented that animals prefer a signalled to an unsignalled presentation of an aversive stimulus, usually shock (for a review, see Badia, Harsh & Abbott, 1979). If the sounds used in the present experiment were aversive for these hens then it may be that unsignalled / unpredictable exposure to this possibly aversive stimulus decreased the preference for it and increased its aversiveness. During the training phase, regardless of whether reinforcement was provided or not, a variable ITI was used between presentations of the sound. This meant that the onset of sound was unpredictable in this situation. Alternatively, during the preference assessment phase the hens were able to control when the sound was on and off by responding to the keys associated or not associated with sound. In the preference assessment, the onset of sound was now predictable and under the hens' "control". It is possible that for these hens being exposed to an aversive and unpredictable stimulus

increased the aversiveness of that stimulus, resulting in less responding and so a large response-based noise bias away from keys associated with that stimulus.

Other people have argued that if sounds are aversive then they may be affecting the welfare of animals exposed to these aversive sounds. Zimmerman and Koene (1997) found that loss of predictability and controllability impaired the welfare of hens, as measured by an increase in gack-calls. Unpredictability of aversive events can also result in physical abnormalities, such as stress-induced gastric ulcers in rats (Seligman & Meyer, 1970; Weiss, 1972). In their review, Bassett and Buchanan-Smith (2007) suggested that, compared to never having predictability, the loss of predictability of an aversive event may have a detrimental effect on an animal's welfare, as shown by an increase in measures taken to indicate the presence of stress. In the context of the present experiment, during the training phase the hens no longer had control over when the sound was delivered, thus there was a loss of predictability and control. One study showed that when rhesus monkeys lost control of the presentation of an aversive auditory stimulus, which was previously controlled by lever presses, this loss of control, compared to never having had control, increased plasma cortisol levels in the monkeys blood (taken to indicate the presence of stress) (Hanson, Larsen & Snowdon, 1976). There was also an increase in aggressive behaviour shown by the monkeys. The increased suppression of responding to keys associated with both sounds during the training conditions indicates increased aversiveness of these sounds. Thus, it appears that the training phase in the present experiment may have been itself aversive for these hens but without any additional behavioural or physical measures taken to indicate such changes this can only be a supposition. It is recommended that future experiments assessing the effects of aversive stimuli that are unpredictable, or where predictability is taken away, use additional measures that may show a negative impact on welfare. For example, these measures could include physiological indicators, as used by Hanson et al., or behavioural indicators of diminished welfare (i.e., frequency of abnormal behaviours) (Keeling & Jensen, 2009).

One unusual finding in the present experiment was that bias resulting from time allocation for 4 hens in the white noise conditions and 2 hens in the food call conditions (in 13 out of 24 cases for both sounds) increased towards the keys associated with sound when the training conditions were introduced. Hence, these animals increased the amount of time they were in the presence of the sound but they responded at a much slower rate when the sound was playing. One idea that could help explain this outcome is that the training phase may have taught the hens not to respond in the presence of sound. Given that during training with food

the hens were non-contingently provided with the food, when the sound was then played in the preference assessment phase food was provided contingently on responding to the keys. It is possible that these hens learnt not to respond in the presence of sound and that food would be provided non-contingently. However, this seems unlikely as the same procedure was used in Experiment 4 and this effect was not observed in time allocation biases there, or in the local rates of responding. Furthermore, if the hens learnt that the presence of sound resulted in non-contingent food this effect would not have been seen in training conditions without food, and in some cases it was observed. Consequently, it is not clear why there was an increase in time allocation biases towards the keys associated with sound for some hens and this is an area that warrants further investigation.

In the present experiment there was a greater increase in the degree of noise bias away from the sound keys in training with food compared to training without food. The training without food condition was conducted to separate the effects of sound from food. Given that it seems unlikely that the presence of food would increase the aversiveness of a sound there must be another explanation for the greater increase in noise bias with food than without food. The order of training conditions and previous experience may have played a part. After having already experienced a training phase, albeit with food provided, the second training phase without food may have had less impact on the hen's responding. As the training condition without food was conducted in an attempt to help understand and to compare with the results from the first training condition (with food), counterbalancing was not possible. It is also possible that the hens' previous experience to the unpredictable presentation of sound and to the increased total duration of exposure to these sounds reduced the effects of sound on responding. However, there is no way to confirm these suggestions without replicating these conditions in a different order.

As previously outlined in Experiment 4, there were some possible problems with the way in which the training phase was arranged in the present and the previous experiment. Some ways to overcome these problems in future experiments could be to arrange the training phase differently, such as associating the sound with food for a larger number of trials and using a longer ITI. With a longer ITI the sound would then signal a greater reduction in the delay to reinforcement, which according to DRT would increase its value (Squires & Fantino, 1971). Alternatively, it may be better to establish the sound stimuli as a signal using a procedure that relies on the animal making responses, such as in multiple-chain schedules. Concurrent or concurrent-chains schedules could then be used as probes to assess preference

for the sound in a similar way to those used by O'Daly et al. (2005), which would avoid the possibility of extinguishing any conditioned effects. In this way, there is less exposure to the stimulus in the absence of food. The use of probes would also mean that the context in which the signal was established and the assessment of preference for the stimulus are similar. Thus, probes may better aid in assessing preferences for a signal without affecting its value or signalling ability in the process and would keep the context the same as when the signal was established.

In conclusion, the original intention of the present experiment was to increase the reinforcing value of sounds that were previously not preferred by associating them with food. However, this was not successful. The training phase used in the present experiment generally increased response-based noise biases away from the keys associated with sound, rather than towards them. This may indicate that the training in fact decreased the value of the sound and increased its aversiveness. One possible explanation for this outcome was that during training the hens were exposed to an unpredictable sound that they no longer had control over. It is suggested that future experiments of this type also include measures that may indicate any negative effects on welfare, such as indicators of stress within blood or abnormal behaviours, which can then be compared with the effects of sounds on responding.



## GENERAL DISCUSSION

The series of experiments presented in this thesis attempted to examine the effects of different sounds on the choice behaviour of domestic fowl using a range of procedures. These included concurrent and concurrent-chains schedules and the conditioned place preference procedure. It was clear the different procedures gave conflicting conclusions about the hens' preferences for sounds. Only the food call and white noise were consistently not preferred by hens and white noise was also not preferred by domestic hen chicks. Otherwise, measures of preference for the different sounds used were inconsistent across hens. Nevertheless, the procedures used here all gave some information about the preferences of domestic fowl for the sounds used but the results were not always consistent across animals.

Although there is a body of research investigating animals' responses to sounds, there have been only a few studies that have made use of concurrent schedules to assess animals' preferences for sounds. The results of three studies by McAdie and other co-workers using concurrent schedules (McAdie, 1998; McAdie et al., 1993, 1996) were consistent with those found here. In Experiment 1, when using concurrent schedules the results were consistent across hens where all sounds biased their responding away from keys associated with sound and these biases were largest for white noise and the food call. This result was the same as that shown in Experiment 5, also using concurrent schedules, white noise, and the food call, but with a different group of subjects. However, the results found here using concurrent and concurrent-chains schedules were consistent for only the food call, which showed the same effects on responding (Experiments 1, 2, & 5). Thus, the results across the two procedures were quite different.

Concurrent-chains schedules have been used by only one other study to assess animals' preferences for sounds (Otsuka et al., 2009). Otsuka et al.'s (2009) data were inconsistent across subjects (rats), but this was similar to the data from concurrent-chains schedules used here with hens (Experiment 2). Under concurrent-chains the noise bias in the initial-links, resulting from sound in the terminal-links, varied considerably across hens for all sounds except the food call. In contrast to the results found using concurrent schedules, some hens showed initial-link biases towards the key leading to sound. It is unclear why the results across the two procedures were contradictory and comparisons of these procedures are problematic.

Three different procedures were used in this thesis and there were some consistencies and inconsistencies across their results. Under the CPP procedure (Experiment 3) an area

associated with white noise was avoided by domestic hen chicks and this was consistent with the results under concurrent schedules using adult hens (Experiments 1 & 5). However, for the food call the results from both concurrent and concurrent-chains schedules were inconsistent with those found using the CPP procedure. Thus, there were no commonalities across all three procedures used in this thesis but some of the inconsistencies may be explained.

Under concurrent schedules hens' responding was biased or slowed by the presence of white noise (Experiment 1). Thus, white noise was suggested to be aversive to these hens. From this, it was assumed that white noise would also bias the responding of the same hens away from the initial-link key leading to sound under a concurrent-chains procedure (Experiment 2). However, this was not the case. Examination of the noise biases ( $\log q$ ) from responding during both of the concurrent and concurrent-chains experiments (Figures 1.3 & 2.1) revealed that for 3 hens (Hen 31, 34, & 35) the direction of noise bias resulting from white noise was the same, and were opposite for the remaining 3 hens (Hen 32, 33, & 36). Hence, no consistent effects were seen across experiments. As previously discussed, O'Daly et al. (2005) suggested that when assessing preference for signals (conditioned reinforcers) concurrent and concurrent-chains schedules were measuring different effects of the signal stimuli. They stated that concurrent-chains assessed the relative strength of a stimulus as a signal, while under concurrent schedules the signal stimulus was more effective at eliciting responding. Adding to this, Grace and Savastano (1997) stated that initial-link stimuli had weaker control over responding than concurrent schedule stimuli that were immediately followed by food. This can be taken to mean that in the present experiments the presence of sound in concurrent schedules was a more effective stimulus at biasing behaviour than when it was present in the terminal-links of a concurrent-chains procedure. Although O'Daly et al. found that preferences for a signal stimulus were the same when using concurrent and concurrent-chains probes, inconsistent results were found here using these two schedules. This difference in results may be related to the fact that O'Daly et al. were measuring preference for a key colour which had been established as a signal, while in the present experiments preference was measured for sounds that had not been established as signals. It seems from the results presented here that the presence of sound in concurrent schedules and in the terminal-link of concurrent-chains schedules had different effects on preference and that these two procedures were measuring different effects of sound.

The purpose of adding sound to the concurrent and concurrent-chains schedules was to see if the presence of sound had the same effects on behaviour. However, these two procedures

delivered different results. If O'Daly et al.'s (2005) assumption is correct (when assessing preference these two procedures measure different effects of a stimulus), then each of these procedures may be better suited for assessing different stimuli. Continuous white noise was shown to be an aversive stimulus for hens in concurrent schedules, in this thesis and in McAdie (1998), as the noise suppressed their responding when it was present. When white noise was used in the concurrent-chains procedure, initial-link preferences for schedules associated with white noise were inconsistent across hens and this result was inconsistent with what is known about white noise. The consistent results found using concurrent schedules demonstrates that they may be more suitable than concurrent-chains for assessing preferences for negative or aversive continuous sounds. If it is assumed that aversive sounds have a negative impact on an animal's welfare then concurrent schedules may be a promising procedure for identifying these aversive sounds.

Concurrent-chains procedures have been used extensively to assess animals' preferences for conditioned reinforcers (signals) (e.g., Dunn & Spetch, 1990; Grace & Savastano, 2000; Preston & Fantino, 1991; Williams & Dunn, 1991). In these experiments, the stimuli (key colour) in one terminal-link leads to a better overall outcome than the alternate terminal-link and this stimuli is therefore established as a signal. Concurrent-chains were used in Experiment 2 but with equal initial- and equal terminal-links, thus the overall outcomes were the same on both chains. Although some hens showed preferences towards the initial-link key leading to sound when they had shown the opposite in Experiment 1 (a bias away from the key leading to sound), this effect was not consistent and shown in only 10 out of 28 cases. It may be that in these cases the sound added 'extra' value to the terminal-link when it was present, therefore acting as signal, but this was not a consistent effect across subjects. Otsuka et al. (2009) used a similar concurrent-chains procedure to that used here and also found inconsistencies across the response ratios in the initial- and terminal-links. It appears from the results presented here, and those of Otsuka et al., that concurrent-chains are not suitable for assessing sound preferences. However, concurrent-chains are evidently useful for assessing preferences for signals. It is clear that none of the sounds used in this thesis were already signals for hens, even the food call, but some sounds that animals are exposed to do signal a forthcoming event (i.e., the sound acts as a conditioned reinforcer). Thus, concurrent-chains may be more suited to assessing preferences for sounds that already function as a signal. Identifying these sound signals means that they could be used to attract an animal, which could assist in pest control efforts such as luring pest species (e.g., ferrets, rabbits, or possums).

It was argued earlier that concurrent schedules have the advantage over other preference procedures because they allow for the differentiation between the degrees of preference for different stimuli and make it possible to quantify the effects of sounds on choice behaviour. While concurrent-chains can also be used in this way, as this thesis has showed they are not suited to assessing animals preferences for sounds. Concurrent schedules can be used to assess sounds that may be aversive or preferred and when used here (Experiments 1 & 5) generally gave consistent results across subjects and experiments. Because the size of an animal's preference for one alternative over another can be quantified using concurrent schedules, this procedure has the advantage over some other procedures. This includes such procedures as escape or avoidance procedures, which as previously noted are normally used to assess sounds that animals avoid or find aversive and it is unclear which measures of behaviour (latency or avoidance distance) should be taken to indicate greater aversiveness. Furthermore, procedures that rely on measuring an animal's time spent near a sound source can be used only for sounds that attract and T- or Y-maze procedures can be practically problematic when giving the animal a choice between some types of stimuli (e.g., sounds, lighting, or temperature). Concurrent schedules have advantages over using other procedures and have been shown here to be useful for assessing domestic hens' preferences for different sounds.

Adult hens showed a preference away from a food call under concurrent (Experiments 1 & 5) and concurrent-chains schedules (Experiments 2) while domestic hen chicks spent more time in the area associated with the food call and choose this area first more often. The difference in these two findings may be a result of learning. Fisher (1976) found that domestic hen chicks showed an unlearned response to a food call and this may be why it was preferred by the chicks. Research into the effects of food calling has examined the responses of domestic hens that have been housed with males, thus the hens have had continued exposure to food calls (e.g., Evans & Evans, 1999; Gyger & Marler, 1988; Marler et al., 1986; van Kampen, 1994). One possible explanation for the lack of preference shown by adult hens for a food call is that the 'unlearned' preference in chicks may disappear in adult hens without continued exposure to the sound. Further research comparing the responses adult hens that have not been exposed to food calls with those that have had a lot of experience with food calls could confirm this.

The conditioned place preference procedure (CPP) was used in Experiment 3 to assess domestic hen chicks' preferences for places associated with sounds. Domestic hen chicks were chosen because they have been used in previous research with CPP and were easier to handle when conducting the CPP procedure. There were some advantages to using this procedure,

over using concurrent and concurrent-chains schedules, which included the assessment of preference in the absence of sound and in the absence of reinforcement. The results for white noise using the CPP procedure were consistent with those using concurrent schedules (Experiments 1 & 5). However, the food call gave different results from the concurrent (Experiments 1 & 5) and concurrent-chains schedules (Experiments 2). This may have been due to the use of different subjects (i.e., chicks vs. adult hens), rather than the different procedures. Previous researchers showed that the CPP procedure can be used with domestic hen chicks to assess the effects of the injection of drugs on their place preferences (Bronson et al., 1996; He et al., 2009, 2010; Hughes et al., 1995; Jiang et al., 2011). The results found in Experiment 3 are consistent with previous research and suggest the procedure can be used to assess the effects of sound and food on CPP by domestic hen chicks. CPP is a quick and easy procedure to conduct compared to concurrent and concurrent-chains procedures. It also has the potential for use in assessing a wide range of stimuli, such as temperature, sounds, or lighting that would otherwise be difficult to assess.

In Experiments 4 and 5 an attempt was made to increase hens' preference for sounds by associating those sounds with food. It has already been mentioned that although these experiments were not successful in their attempts there are some reasons, as previously discussed, for the results found. In Experiment 4, had the training phase been arranged differently (see Experiment 4 discussion) then preference towards the tone may have been shown by all hens and to a greater degree. In Experiment 5, hens did not increase their preference for keys associated with white noise or a food call that had been previously associated with food. Instead, response-based noise bias resulting from the presence of these sounds appeared to increase and it was argued that this may be due to both the aversive nature of the sounds and the unpredictability and uncontrollability of the sound when presented in the training phase. As discussed previously, this explanation was supported by research on the effects of predictability (Bassett & Buchanan-Smith, 2007) and by research examining the effects of loss of control of an aversive auditory stimulus (Hanson et al., 1976).

The results of Experiments 4 and 5 clearly showed that the training procedure did not result in the sound being positively associated with food. It may still be possible to increase the value of a tone and decrease the aversiveness of white noise or a food call by using a different procedure to associate sound with food. One possibility would be to use concurrent-chains to establish the sound as a signal. This procedure has been extensively used to establish different key colours as signals (e.g., Dunn & Spetch, 1990; Fantino et al., 1991; Grace & Savastano,

2000; Williams & Dunn, 1991) and has been used to assess preferences for sounds also (Otsuka et al., 2009). Although concurrent chains were used in Experiment 2, the procedure was not successful and none of the sounds assessed had been previously established as signals. It may be possible to replicate the procedure used by O'Daly et al. (2005) and a sound could be associated with the terminal-link leading to the greater reduction in delay to reinforcement. This way, the value of the sound would be changed by making it predictive of a better outcome. By replicating O'Daly et al.'s procedure, preference for the sound could be assessed using transfer tests, such as concurrent or concurrent-chain probes, or concurrent-chains, and the results could be compared back to those found here.

None of the sounds used throughout this thesis were preferred by hens. It may be that a positive experience with sound is a necessary precondition for hens to show a preference towards it. When an attempt was made to associate sounds with food (Experiments 4 & 5), it was not successful. It is well known that humans have preferences for different types of music and research has shown that Java Sparrows also show music preferences (Watanabe & Nemoto, 1998). However, in studies assessing animals' preferences for sounds most animals have preferred sounds that function as a type of signal. For example, these have included signals for food (Moffat & Hogan, 1992), or a signal for mating (e.g., with crickets (Tregenza, Simmons, Wedell & Zuk, 2006), and toads (Marquez, Bosch & Eekhout, 2008)). There is large body of research investigating imprinting in domestic hen chicks, and many of these studies involve imprinting auditory stimuli (e.g., Gvoryahu, Cunningham & van Tienhoven, 1989; Park & Balaban, 1991; Russock & Hale, 1979; van Kampen & Bolhuis, 1991). Imprinting occurs when a young animal is exposed to an object during a critical learning phase and that they will then follow or prefer (for a review, see Bolhuis, 1991). Auditory imprinting typically involves exposing a chick to an object that is paired with a sound within the first 24 hrs after hatching (e.g., van Kampen & Bolhuis, 1991). This means that auditory imprinting involves a particular type of experience with sound that may enhance preference for it. Hence, most researchers have examined imprinted sounds rather than assessing chicks' preferences for sounds that they have no experience of. It appears that for many animals, including domestic fowl, sounds that are preferred are ones that function as signals or have previously been associated with a positive stimulus.

Throughout this thesis, white noise has been shown to be a sound that is not preferred across three different procedures (Experiments 1, 2, & 3). Furthermore, when an attempt was made to decrease the noise bias resulting from white noise by associating white noise with food

(Experiment 5) surprisingly, the noise bias increased, i.e., preference for keys associated with white noise decreased. Results from Experiment 5 suggested that the aversiveness of white noise increased when its presence became unpredictable and was no longer “controlled” by the hens. It was suggested in this thesis, and also by McAdie (1998), that white noise was an aversive stimulus for hens because it slowed responding when present and this suppression has been suggested to indicate an aversive stimulus (Rushen, 1996). However, further research is needed to determine if white noise acts as a punisher. This could be done by establishing stable responding to keys and then examining the effects of making white noise contingent on responding to these keys. As previously outlined, the suppression effect on responding caused by an aversive stimulus has been widely researched and the effects of white noise here are similar to those found using an aversive stimulus such as electric shock (Azrin, 1956; de Villiers, 1980) or a loud tone (Reed & Yoshino, 2001, 2008). Other animals, such as pigs (Hutson et al., 2000), rats (Hughes & Bardo, 1981), mice, (Barnes & Kish, 1957), and sparrows (Dobson & Petrinovich, 1973; Watanabe & Nemoto, 1998) have also preferred other sounds to white noise or avoided white noise. Alternatively, white noise has been shown to help improve cognitive performance in people with ADHD (Soderlund, Sikstrom & Smart, 2007) and improve working memory in monkeys (Carlson, Rama, Artchakov & Linnankoski, 1997). It is clear that white noise has different effects on different animals but avoidance of an area associated with white noise and the reduced response rates and noise biases in responding away from keys associated with white noise found in this thesis and those of McAdie offer strong support that it is an aversive stimulus for domestic fowl.

In a natural setting, hens will readily move towards a food calling male where the sound signals the presence of food (Marler et al., 1986). Surprisingly, the adult hens in this thesis (Experiments 1, 2, & 5) preferred the absence of a food call to its presence, as shown by response-based noise biases away from the keys associated with the food call. Similar noise biases were shown away from keys associated with white noise and the food call in Experiments 1, 2, and 5 but the degree of noise bias was usually smaller for the food call than for white noise. Contrary to this, using the CPP procedure (Experiment 3) the area associated with the food call was chosen first more often by chicks after conditioning and more time was spent in this area. However, a significant CPP was not found but it was not significant away from the area associated with sound either. One unusual finding in Experiment 5 that was not found in Experiment 1 was that hens showed lower rates of responding in the presence of the food call when they were responding in a concurrent procedure. This would suggest that for

the hens in that experiment, the food call may have been an aversive stimulus in that it reduced their response rates in a similar way to white noise. It is unclear why this difference occurred as the main difference between Experiments 1 and 5 was that the session length was longer. Although the food call was expected to be a preferred sound, it was likely that as the hens did not have previous experience with food calls being associated with food meant that they had not learned to respond to it as a signal for food. All hens in this thesis were raised from day-olds with no experience of roosters or food calls, suggesting that responding to the food call may be learned rather than innate. However, when the food call was associated with food in Experiment 5, preference for it did not increase. This was likely a result of the training phase not being arranged in such a manner that the hens could learn to associate sound with the food. It is suggested that further investigation of adult hens' preferences for a food call without prior experience of food calls is warranted.

The sounds used throughout this thesis have clearly resulted in different effects on the choices made by both hens and chicks. As outlined in the introduction, animals can either show indifference, preference for, or a preference away from different kinds of sounds. There were no sounds used in this thesis that were preferred or can be considered positive for hens. Some of the sounds used here (white noise & food call), however, can be considered aversive to the subjects. The alarm call and the sounds of hens feeding were sounds that could not be considered aversive but perhaps are sounds that are just not preferred. The sounds of hens feeding resulted in generally small noise biases ( $\log q$ ) away from the keys they were associated with in concurrent and concurrent-chains schedules (Experiments 1 & 2). The alarm call also resulted in small noise biases in concurrent schedules but resulted in biases that were both towards and away from keys leading to this sound in concurrent-chains schedules. However, these sounds did not affect the rates of responding when they were present, in either experiment, and can therefore not be considered aversive. In general, the different sounds used throughout this thesis had different effects on the hens responding and could be considered neutral, not preferable, or aversive. This was similar to the results of McAdie et al. (1993) using a range of different sounds.

Intensively farmed animals, such as domestic fowl, are often exposed to a range of continuous noises and this has been shown to have a negative impact on animal health (Algers, Ekesbo & Stromberg, 1978). Assessing the sound preferences of animals could aid in identifying sounds that may be aversive or sounds that may help to improve animal welfare. Few studies have examined the effects of sounds as a stressor or as potential environmental

enrichment for domestic fowl. Avoiding exposing domestic fowl to excessive or aversive noises could help improve their welfare and may also aid in improving productivity (Voslarova et al., 2011). As previously noted, it has been reported that playing a radio helped to decrease aggression and increase egg laying in chickens (Jones & Rayner, 1999). A recent study showed that exposure to intermittent noises resulted in a lower body weight in broiler chickens during their fattening period (Voslarova et al., 2011). Given the potential benefits of exposing domestic fowl to certain sounds, or removing exposure to aversive sounds, it is important to establish which sounds domestic fowl prefer or find aversive.

In summary, the series of experiments in this thesis have added to the knowledge of the use of concurrent and concurrent-chains schedules and the conditioned place preference procedure for assessing animals' preferences for sounds. Some of these procedures were effective for identifying aversive or negative sounds (concurrent schedules and CPP) while the results of the concurrent-chains procedure have been inconclusive and the data were difficult to interpret. The conditioned place preference procedure is a promising procedure for assessing the effects of a range of stimuli, such as sounds, lighting, or temperature, and with some refinement could be used with a range of different species. It is also a procedure that would be useful for investigating the affective properties of sounds. It must be acknowledged, however, that for domestic fowl preferences have not been shown towards sounds, in this thesis or by others (e.g., MacKenzie et al., 1993; McAdie et al., 1993). However, the hens did show preferences away from sounds that were aversive. Thus, it seems clear from the findings of this thesis that concurrent schedules and the CPP procedure at least could be used with a range of species to assess their preferences for various environmental stimuli and may provide information with the potential to improve animal welfare.



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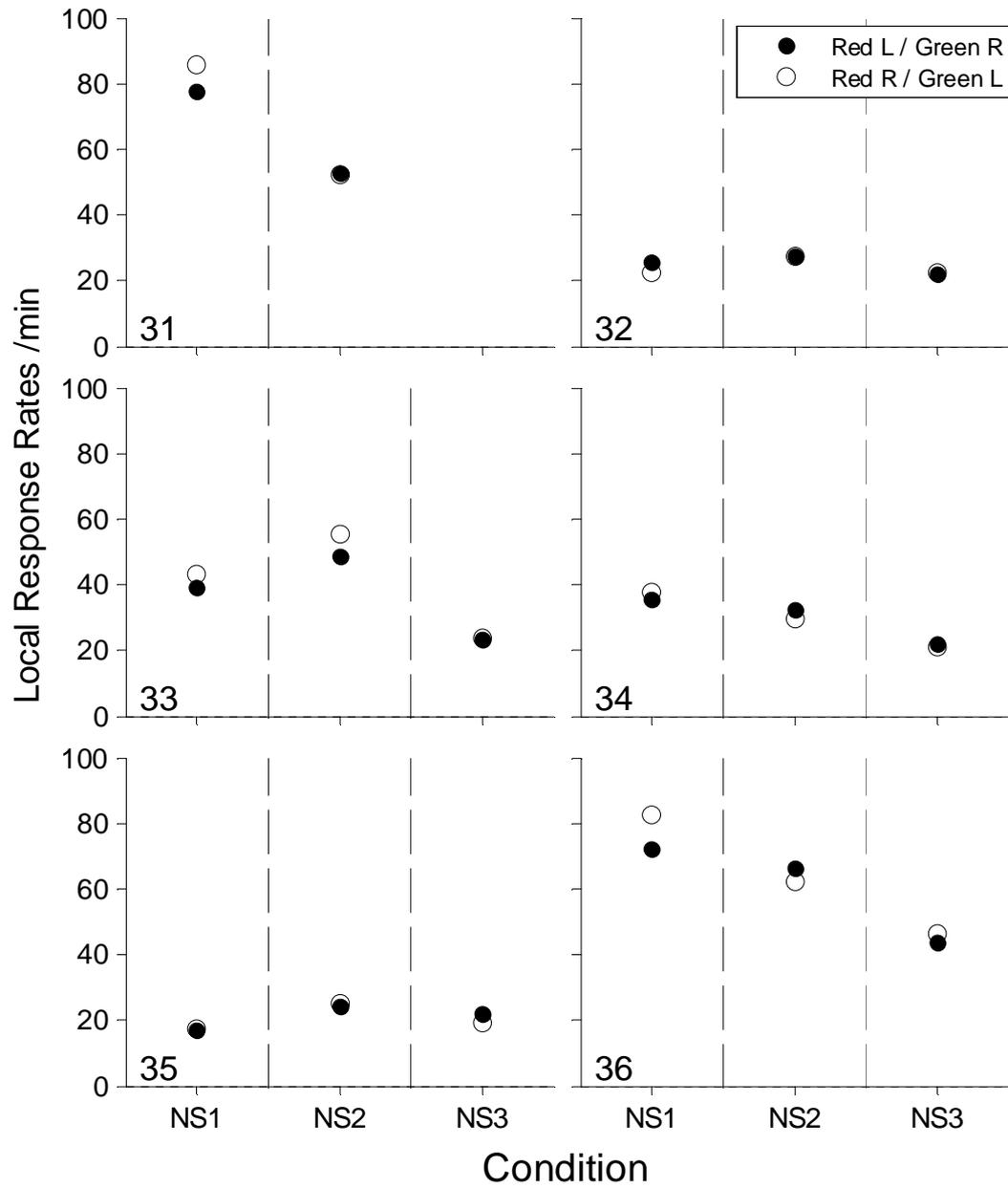
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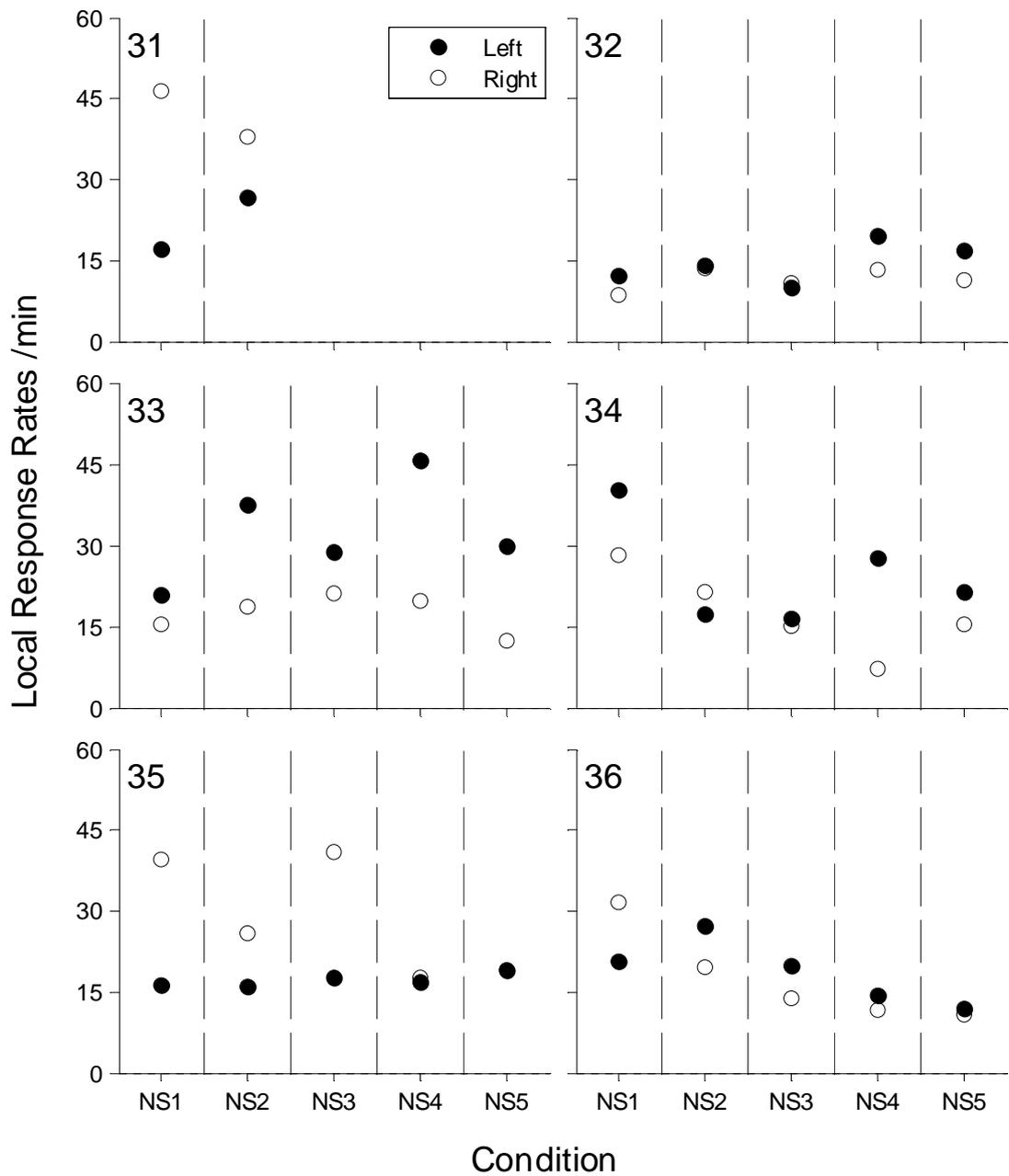
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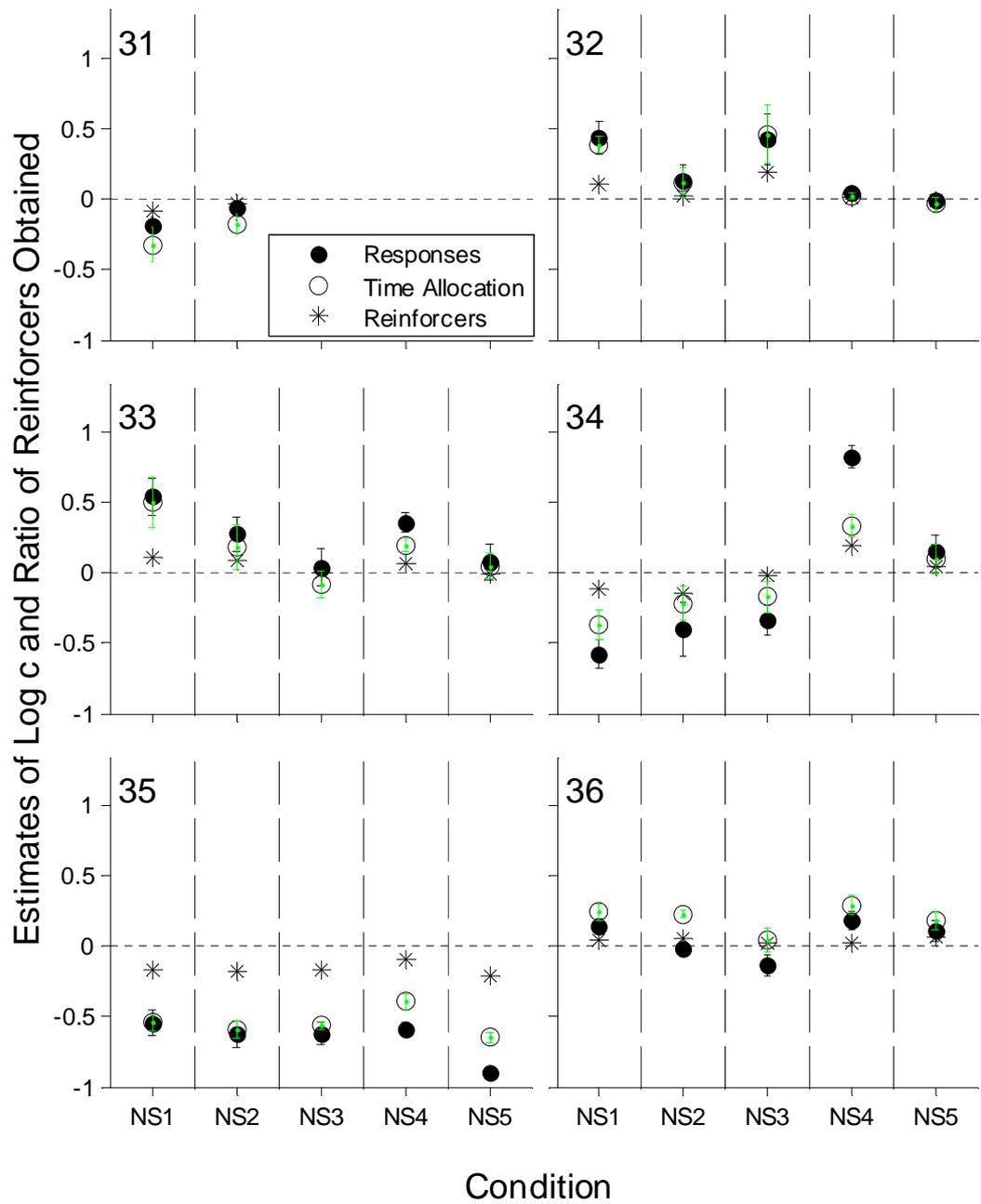
## APPENDICES



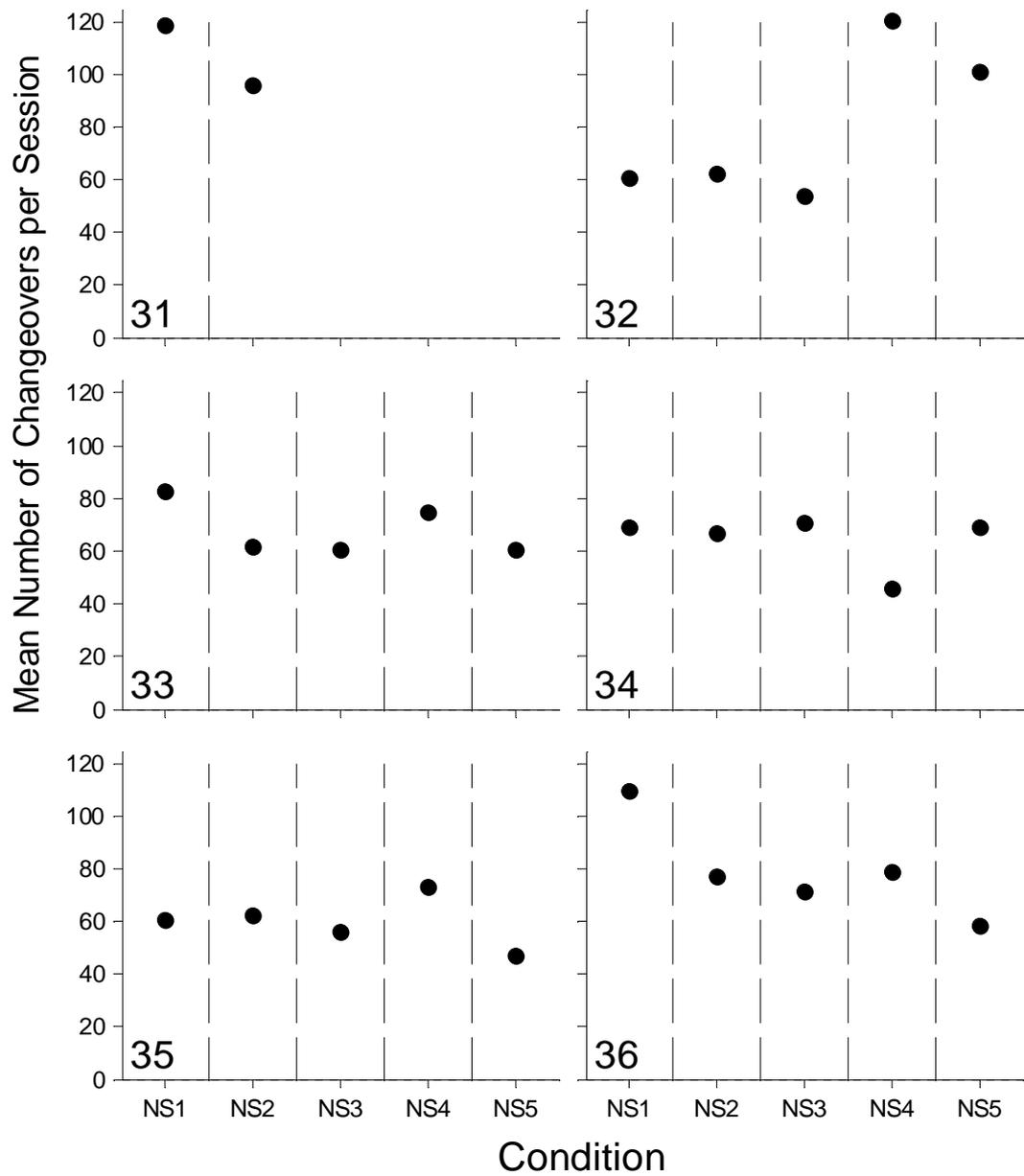
*Appendix 1c.* Figure showing the local rate of responding (per min) to the red left (L) and green right (R) keys and the red right (R) and green left (L) keys, averaged across components, in the conditions with no sound (NS1-3) for Experiment 1, plotted for each hen.



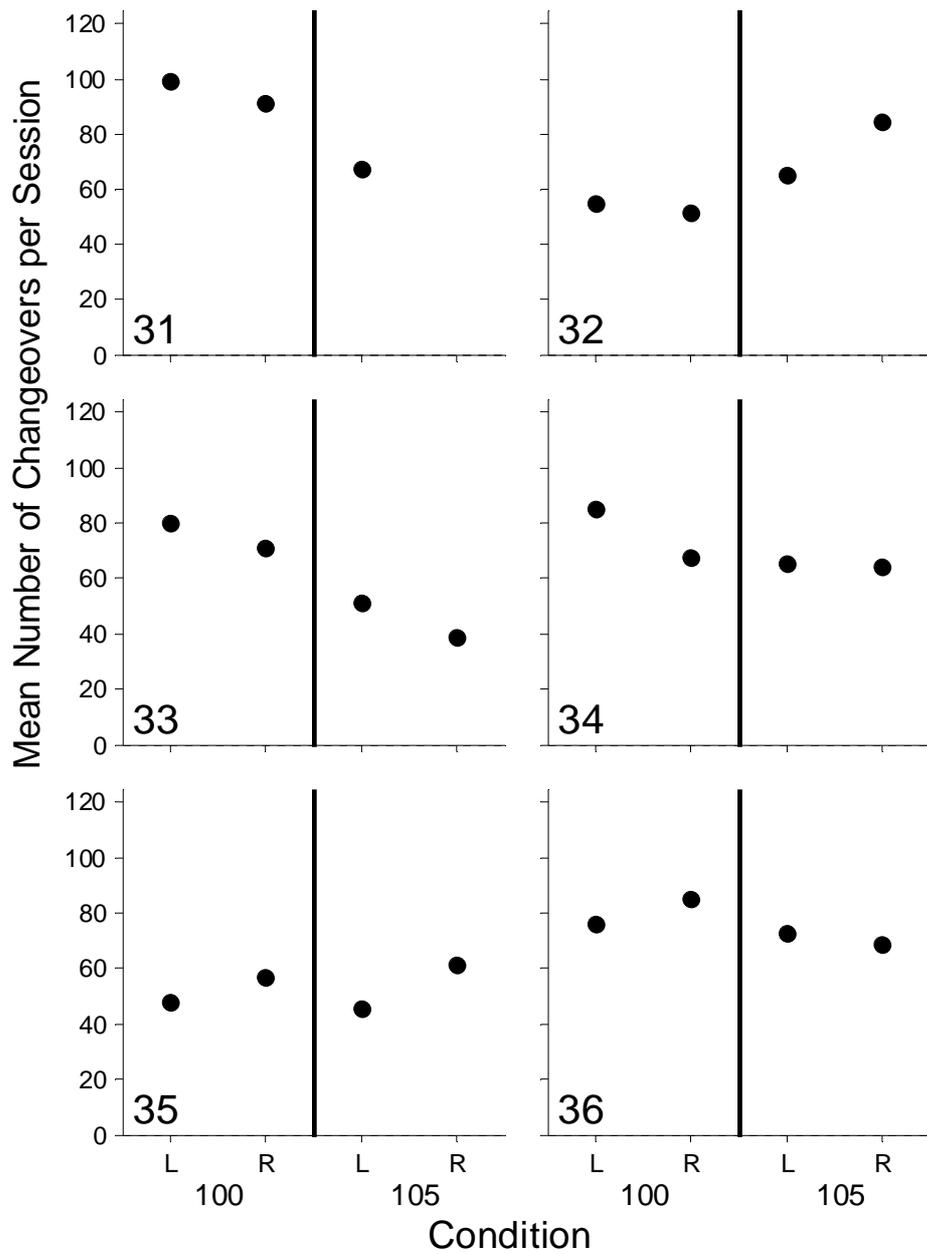
Appendix 2b. Figure showing the local rate of responding (per min) to the left and right initial-link keys in the conditions with no sound (NS1-5) for Experiment 2, plotted for each hen.



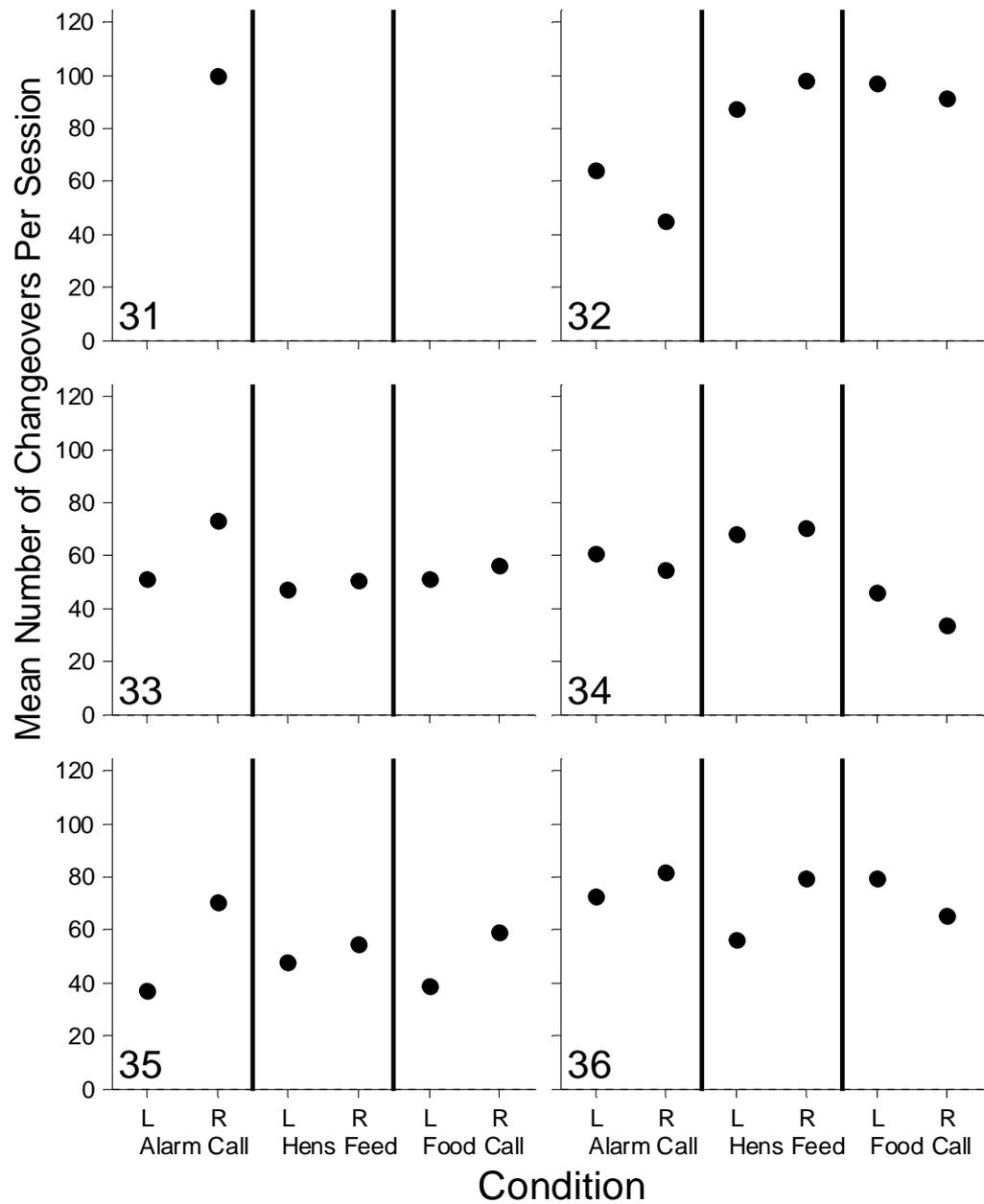
Appendix 2c. Estimates of  $\log c$  for responses (filled circles) and time allocation (unfilled circles), taken to the left key, in the initial-link for conditions with no sound (NS1-5), plotted for each hen in Experiment 2. The ratios of reinforcers obtained are indicated by an asterisk. The error bars represent one standard deviation either side of the mean ratios.



*Appendix 2d.* Mean number of changeovers per session for conditions with no sound (NS1-5), plotted for each hen in Experiment 2.



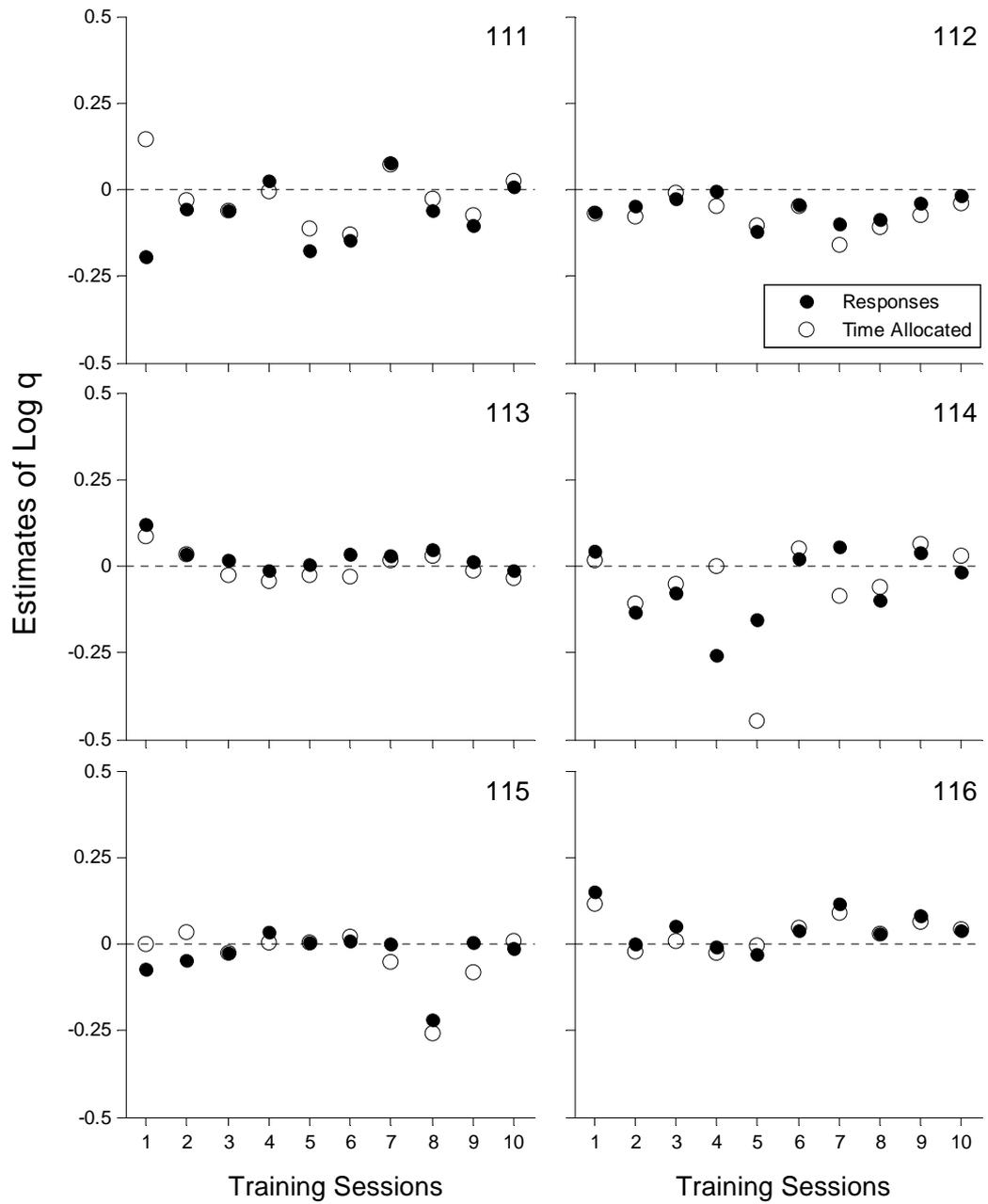
Appendix 2e. Mean number of changeovers per session for conditions when white noise at 100 and 105 dB was associated with either the Left (L) or Right (R) terminal-link, plotted for each hen in Experiment 2.



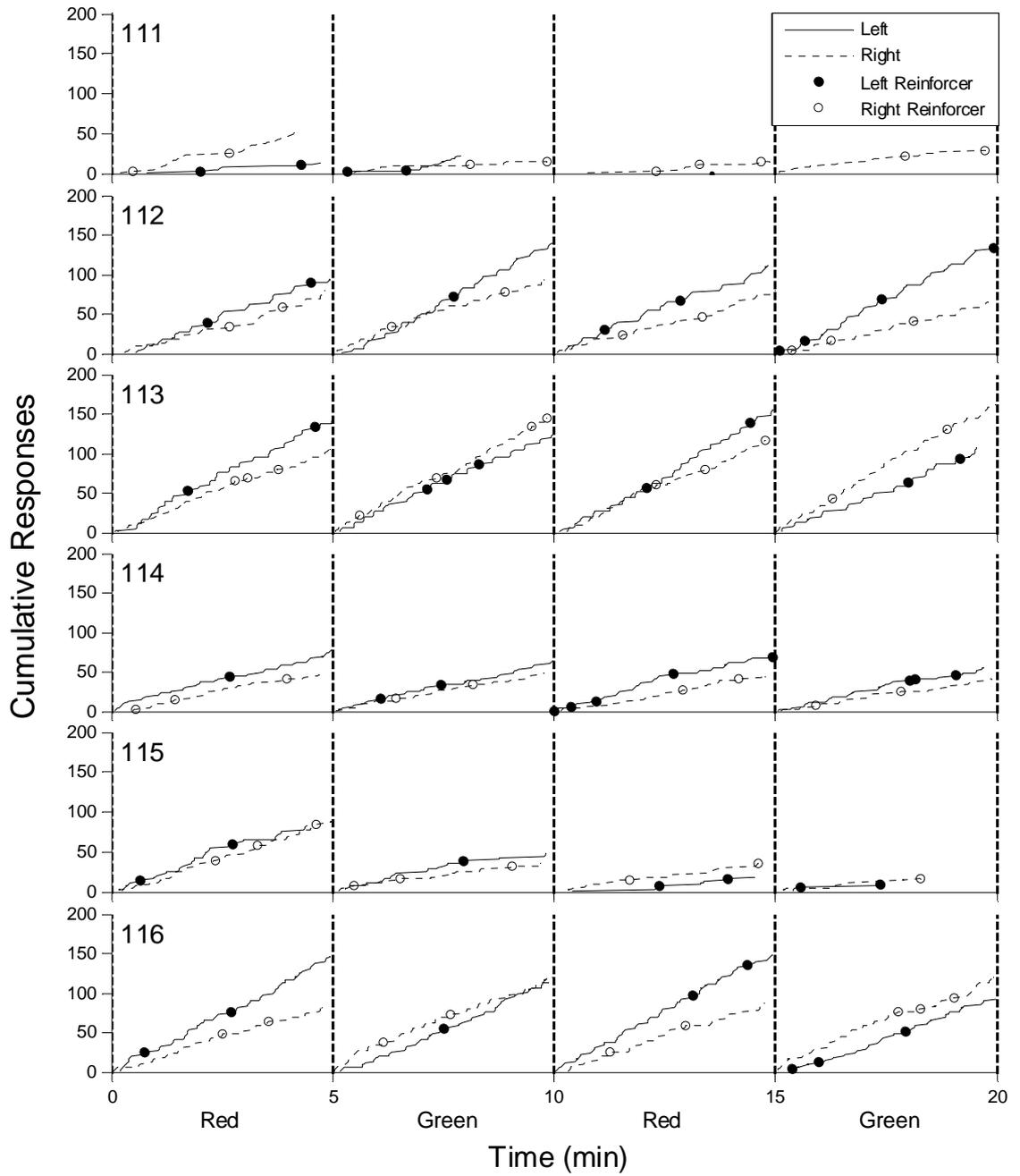
*Appendix 2f.* Mean number of changeovers per session for conditions when either the alarm call, sounds of hens feeding or the food call was associated with either the Left (L) or Right (R) terminal-link, plotted for each hen in Experiment 2.

<b>Food</b>		<b>Food Call</b>	
<b>Subject</b>	<b>Difference in Time Spent in Conditioned Compartment</b>	<b>Subject</b>	<b>Difference in Time Spent in Conditioned Compartment</b>
1	-301	9	-124
2	390	10	-146
3	423	11	-20
4	610	12	-50
5	-211	13	213
6	-94	14	313
7	165	15	144
8	7	16	-67
<b>White Noise</b>		<b>Chick-sounds</b>	
<b>Subject</b>	<b>Difference in Time Spent in Conditioned Compartment</b>	<b>Subject</b>	<b>Difference in Time Spent in Conditioned Compartment</b>
17	-155	25	22
18	-500	26	-295
19	-139	27	176
20	80	28	54
21	-420	29	-409
22	-343	30	-232
23	-169	31	-107
24	-39	32	-21

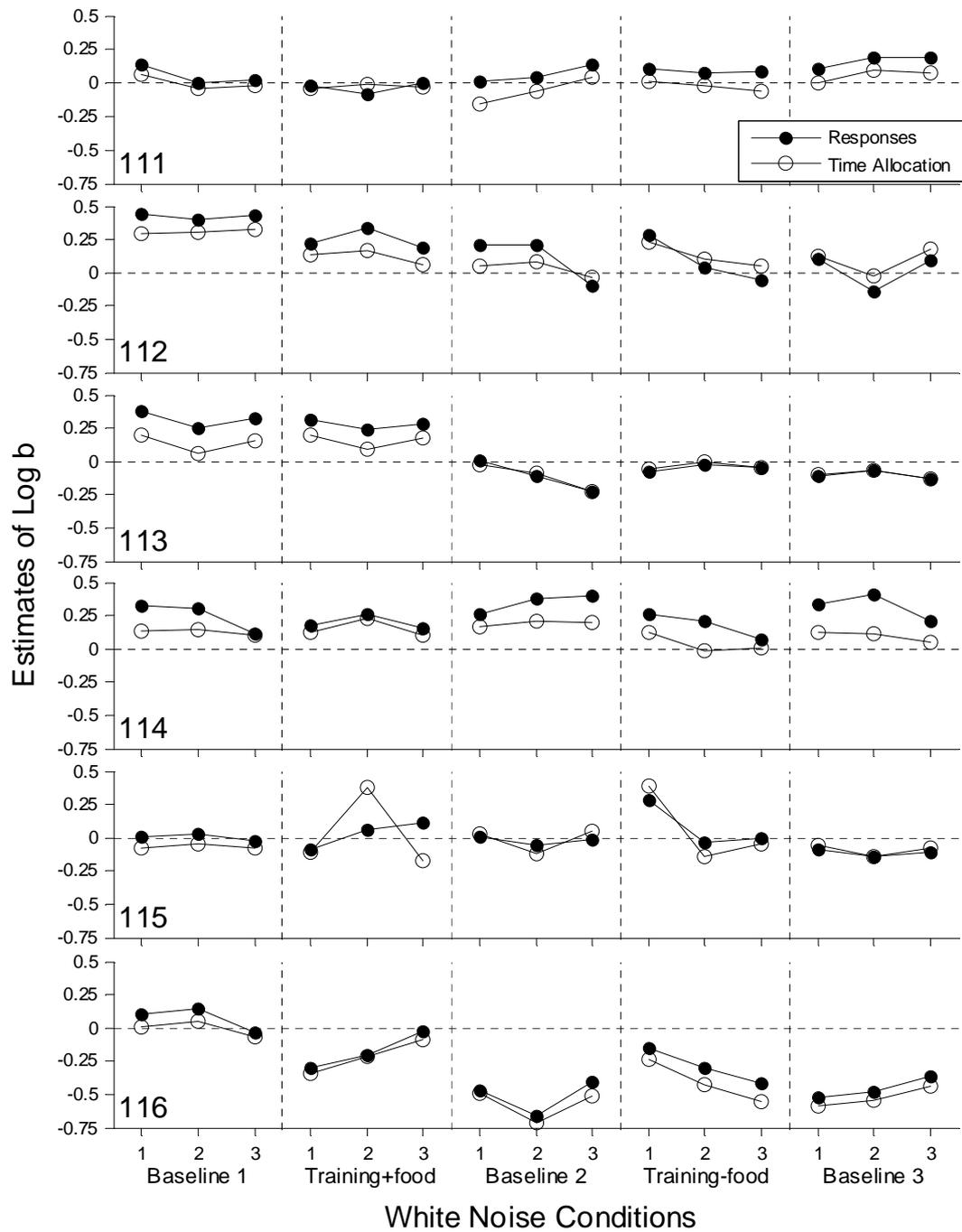
*Appendix 3b.* Difference in time (s) spent in the conditioned compartment from pre- to post-conditioning sessions for individual subjects in each group of Experiment 3; food, food call, white noise and chick-sounds.



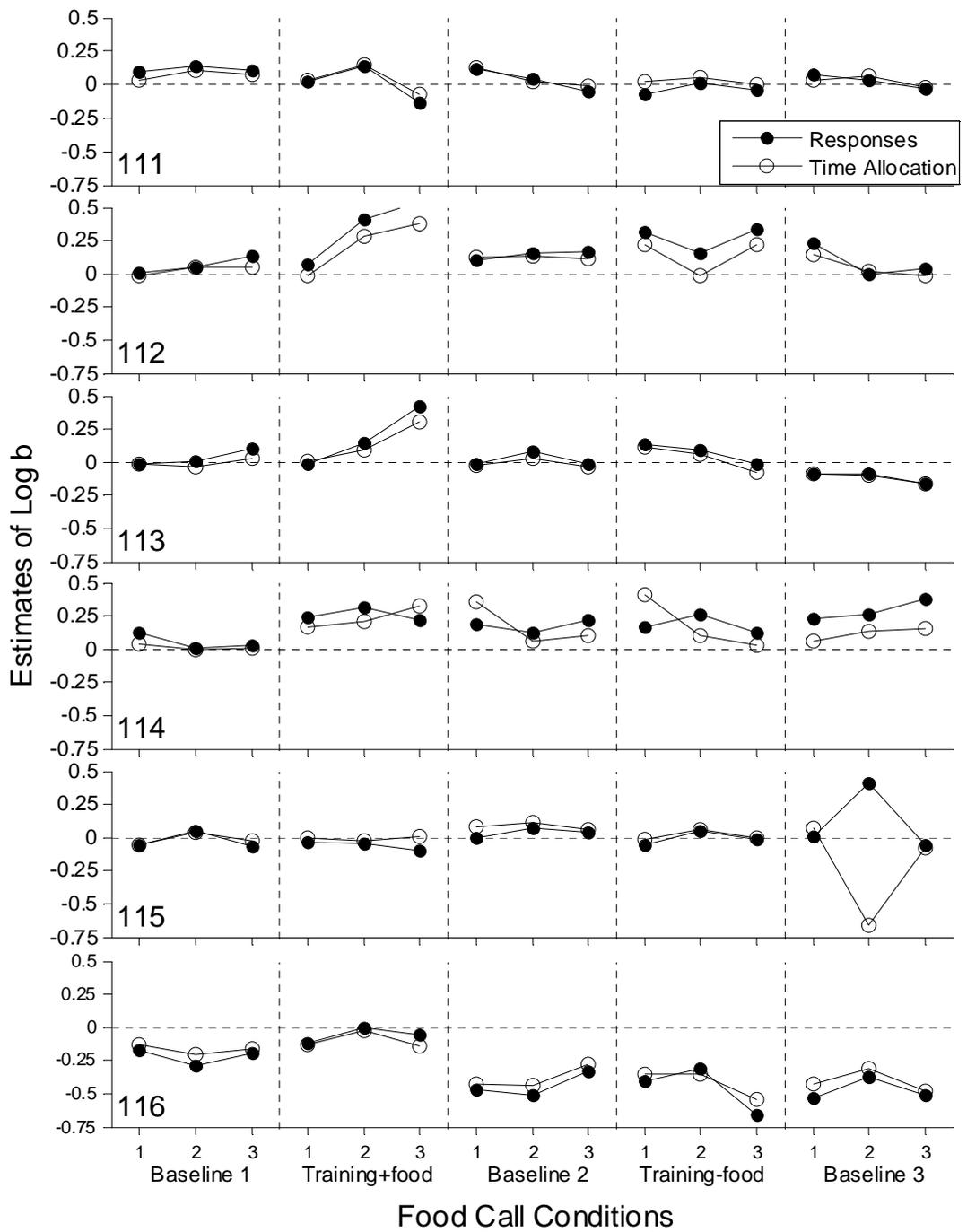
*Appendix 4b.* Estimates of  $\log q$  for responses (filled circles) and time allocated (unfilled circles) taken to the left key, plotted for all 10 training sessions with a tone, for each hen in Experiment 4.



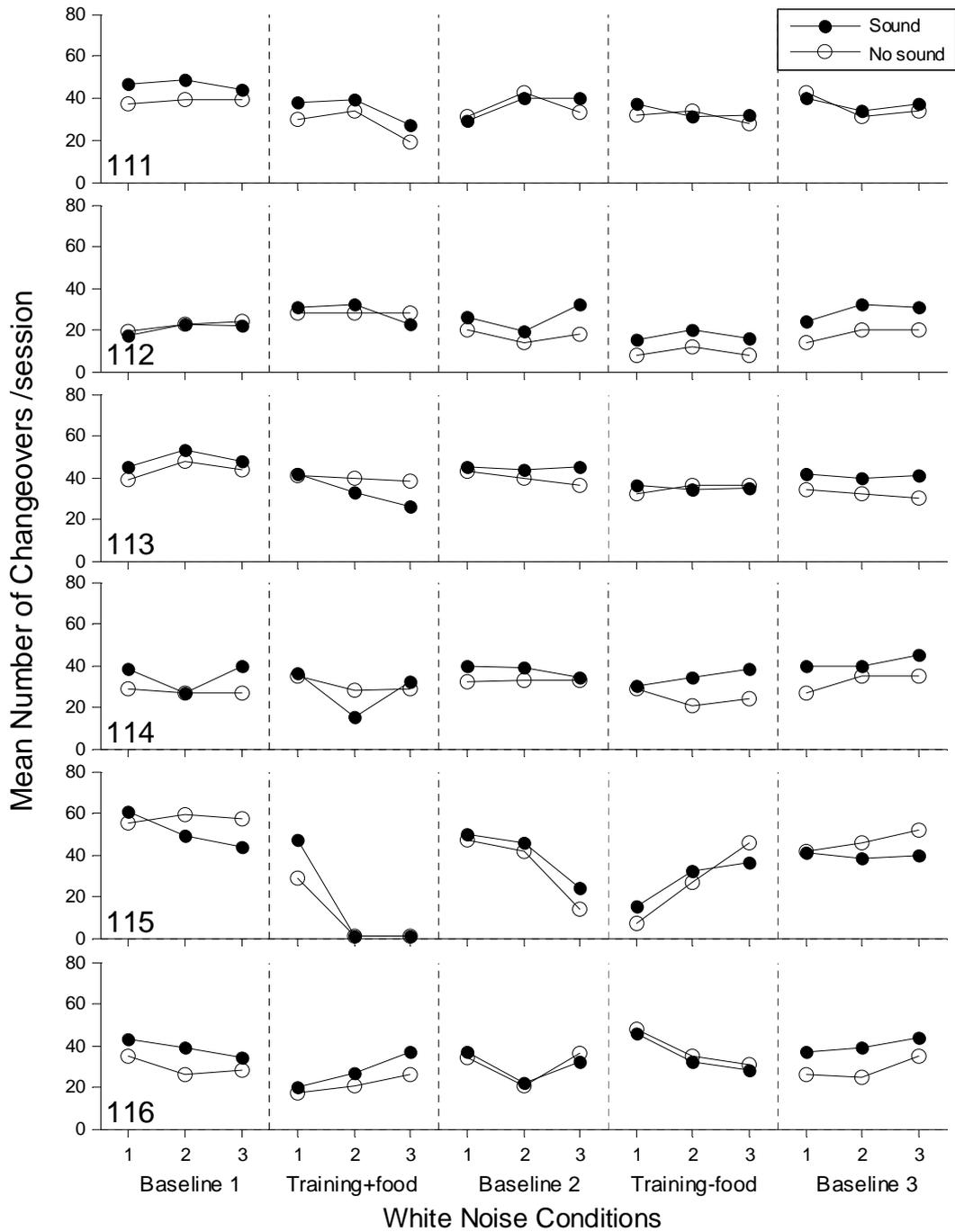
*Appendix 4c.* Cumulative responding over time to the left (filled circles) and right keys (unfilled circles) across the four 5 min components, plotted for each hen. These data are from the first session of the preference assessment, after the first training phase, in Condition 2 of Experiment 4.



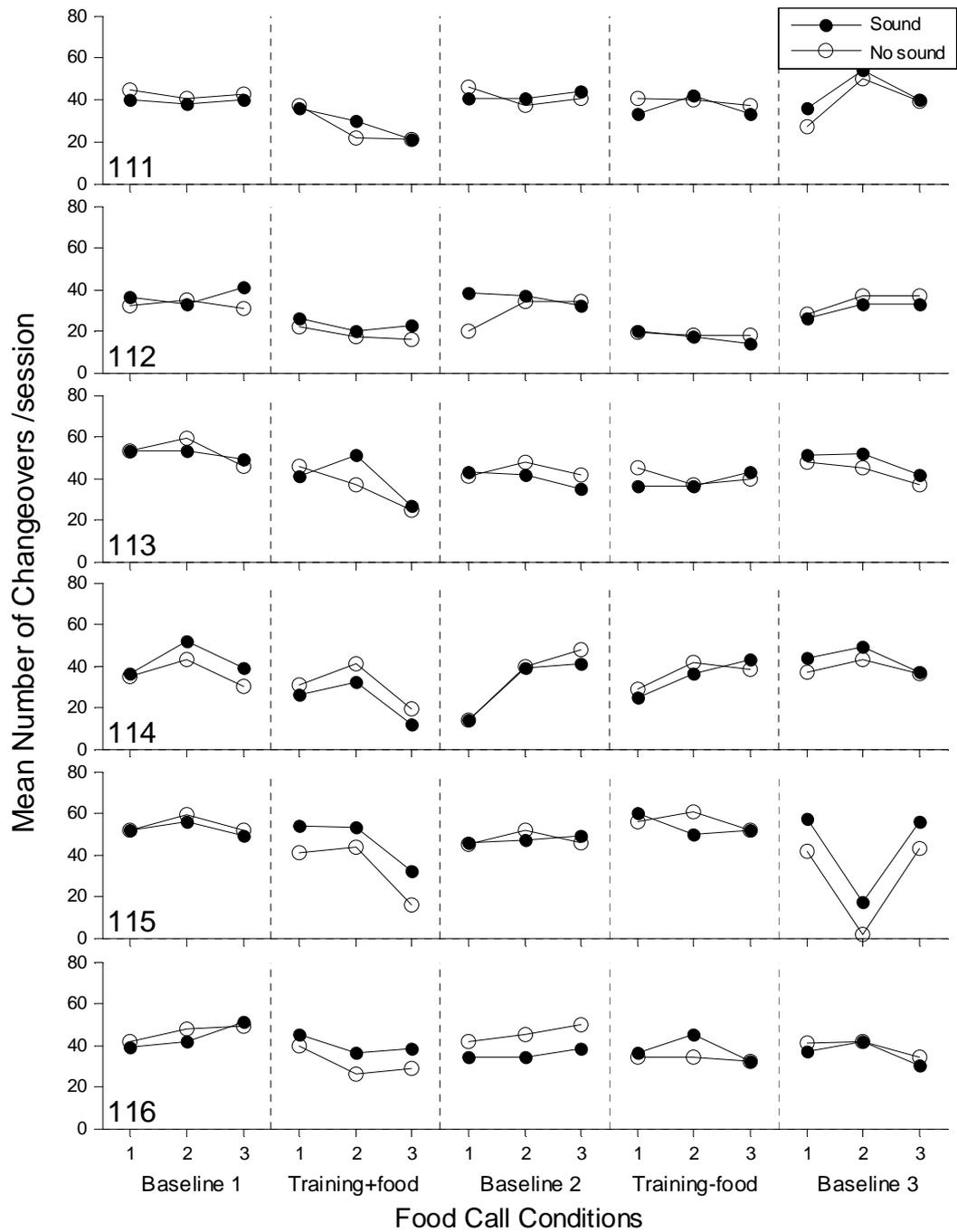
*Appendix 5b.* Estimates of  $\log b$  for responses (filled circles) and time allocated (unfilled circles) taken to the left key, plotted for all white noise conditions for each hen. Estimates of  $\log b$  are shown for the last three sessions of each baseline condition and each of the three training sessions with and without food.



*Appendix 5c.* Estimates of  $\log b$  for responses (filled circles) and time allocated (unfilled circles) taken to the left key, plotted for all food call conditions for each hen. Estimates of  $\log b$  are shown for the last three sessions of each baseline condition and each of the three training sessions with and without food.



Appendix 5d. Mean number of changeovers per session for conditions during the red (filled circles) and green (unfilled circles) components for conditions with white noise, plotted for each hen. Mean changeovers are shown for each baseline condition and training conditions with and without food.



Appendix 5e. Mean number of changeovers per session for conditions during the red (filled circles) and green (unfilled circles) components for conditions with the food call, plotted for each hen. Mean changeovers are shown for each baseline condition and training conditions with and without food.