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### Domestic hen chicks' conditioned place preferences for sound

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#### ABSTRACT

Food and sounds (white noise, a food call and the sound of other chicks) were used in an attempt to establish conditioned place preferences with domestic hen chicks. Thirty-two chicks were randomly allocated to one of the 4 groups, and exposed to a 3-compartment apparatus to establish a baseline of their movements across 4 15-min sessions. They were then confined to one compartment and provided with free access to food or exposed to one sound for 15 min and then they were confined to the alternate compartment with no food or sound for 15 min. This process was repeated 3 times. Post-conditioning test sessions showed a conditioned place preference towards the area associated with food and away from the area associated with white noise. After conditioning, chicks showed no preference for spending time in the side associated with the food call or the sounds of other chicks; however, they entered a compartment first more often when it was associated with the food call and less often when it was associated with chick-sounds. Overall, these results showed that it was possible to use the conditioned place preference procedure to assess the effects of sounds and that the procedure has potential use for assessing other environmental stimuli.

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For domestic fowl, sound is a means of communicating information about the location of food and the presence of predators and it is also used to attract the attention of young chicks (Gyger et al., 1987; Wauters and Richard-Yris, 2002; Woodcock et al., 2004). Research has shown that some sounds can have a negative impact on animal health across a variety of contexts and species (see Algers et al., 1978).

A range of procedures have been used to assess the responses of domestic fowl to sounds. Physiological measures have been used to capture the negative effects of sound on hen welfare. For example, it was found that exposure to slaughterhouse sounds for 10 min (at 80 and 100 dB (A)) increased hens' plasma corticosterone levels (Chloupek et al., 2009), and the sounds of vehicles (90 dB (A)) increased the ratios of heterophil to lymphocyte within the blood of hens (Campo et al., 2005). Other studies have examined whether sounds will attract the hens to stay near them or to move towards. For example, quail and domestic hen chicks spent more time near a speaker playing maternal calls (Park and Balaban, 1991), and male food calls attracted hens to move towards them (Marler et al., 1986). The number, latency or speed of approaches, or the time spent near the sound source have also been used to measure the effects of auditory stimuli on imprinting in domestic hen chicks (e.g., Gvaryahu et al., 1989; Russock and Hale, 1979; White and del Rio Pesado, 1983; van Kampen and Bolhuis, 1991). The above procedures have neither been used to examine responses to potentially aversive sounds nor to compare responses to potentially aversive sounds with those to potentially attractive sounds. A disadvantage of this procedure is that if it were to be used with aversive sound the animals cannot escape or avoid them during testing.

Choice studies have assessed the effects of both positive and negative sounds. Using a T-maze procedure Kent (1993) gave chicks a simultaneous choice between two sounds, each played at one end of the T-maze, and found they preferred maternal clucks played at frequencies close to a normal cluck. Mackenzie et al. (1993) used a procedure that allowed hens to move from one end of a chamber to another to turn a sound on or off. The most aversive sounds were a dog bark and the sounds of hens in a commercial poultry shed (both played at 90 dB (A)), that the hens choose to keep off the majority of the time. Sumpter et al. (2002) point out, however, a problem with such discrete-trial choice procedures is that animals are likely to select the same choice on nearly every trial and as a consequence will no longer be exposed to the less-preferred alternative.

Operant choice procedures, such as concurrent schedules, have the potential to measure hens' responses to both attractive and aversive sounds. McAdie (1998) and McAdie et al. (1993) used multiple concurrent schedules to assess hens' preferences between the absence and presence of various sounds. The procedure used allowed the animal to choose between two keys to peck, both of which periodically gave access to food, with responding on one key

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associated with a sound being played. These studies suggested the hens' preferences were for the absence of sound over white noise (McAdie, 1998) and over the sounds of hens in a commercial poultry shed at feeding time (McAdie et al., 1993) when response allocation was examined. However, the hens behaved differently in the presence of these two sounds. Hens responded more slowly in the presence of white noise than they did with no sound but responded at similar rates in no sound and in the presence of the poultry shed noise. Thus, the presence of white noise seemed to suppress responding while the poultry shed sound did not. McAdie acknowledged this as a potential limitation of using concurrent schedules to assess preferences, that is, different sounds might have different effects and thus the measure of preference could be confounded if sound differentially affected the behaviour that was being used to measure it. One way to overcome this would be to find a procedure that allows for the assessment of responses to both positive and negative sound stimuli in the absence of the sound itself. One such procedure is the conditioned place preference (CPP) procedure.

The CPP procedure typically involves giving an animal a choice between spending time in two compartments where one has been previously paired with a particular stimulus (e.g., injection of a drug) and the other has not (for a review see Tzschentke, 2007). A CPP is indicated if the animal spends more time in one compartment over the other in the absence of the stimuli but after exposure to them. An 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 procedures such as concurrent schedules which require a lot of training and a large number of sessions to obtain stable performance. A further advantage the CPP procedure has over other procedures is that it avoids the problem of unwanted interference from the presence of the stimulus, such as the effects of drugs or sounds, as the stimulus is not present at the time of testing.

CPP has been used to examine the conditioning effects of different drugs on mice or rats (e.g., morphine (Zheng et al., 2004), heroin (Leri and 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 et al., 2006), mating activity (Camacho et al., 2004; Gutiérrez and Domjan, 2011; Meerts and Clark, 2007), aggression and sexual interactions (Meisel and Joppa, 1994), and access to a running wheel (Masaki and 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 stimuli, including sounds.

CPP procedures have been used with many different species (e.g., rats (Meerts and Clark, 2007), hamsters (Meisel and Joppa, 1994), mice (Fitchett et al., 2006), and zebrafish (Lau et al., 2006)). To the authors knowledge there are only a handful of studies that have used CPP with avian subjects; such as Japanese Quail (e.g., Akins et al., 2004; Awaya and Wantanabe, 2003; Gutiérrez and Domjan, 2011; Levens and Akins, 2001; Mace et al., 1997) and domestic hen chicks (e.g., Bronson et al., 1996; He et al., 2009, 2010; Hughes et al., 1995; Jiang et al., 2011). Of these studies, one showed that food could produce a CPP in avian subjects (Mace et al.). Mace et al. found that 12-day-old quails exhibited a CPP for food when conditioned with food in one compartment and no or tainted food in the alternate compartment.

Bronson et al. (1996) examined the conditioning effects of different drugs on CPP in domestic hen chicks. 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" compared to 5% of non-treated chicks and they concluded that the "freezing" was likely due to the previous effects of the drugs. Hughes et al. (1995) investigated the conditioning effects of cocaine using domestic hen chicks. They found that the compartment previously associated with cocaine was initially preferred (in Test session 1) but that this preference declined over the following two test sessions suggesting extinction of the association between the compartment and cocaine. A clear CPP was demonstrated by domestic hen chicks for a compartment previously paired with morphine (He et al., 2009, 2010; Jiang et al., 2011).

Two recent studies have used sound in the context of testing CPP with rats. Feduccia and Duvauchelle (2008) assessed whether an auditory stimulus (music or white noise) would enhance the rewarding effects of MDMA (ecstasy) in rats. 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. Polston and Glick (2011) used music as the contextual cue to assess the effect of cocaine on CPP. They found that the rats showed a preference for the compartment with the music that had been associated with cocaine. These two studies showed that the CPP procedure can be effective when using sound paired with drugs and indicated that it may be a procedure that would be useful for exploring the effects of sound alone.

The present study attempted 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 in order to compare the effects of food on place preferences with those of sounds. Three sounds were used; white noise, a rooster's food call, and domestic hen chick-sounds. White noise has previously been shown to be aversive to hens (McAdie, 1998). Food calls have previously been shown to attract domestic hen chicks (Wauters and Richard-Yris, 2002) and chicks will spend more time near a speaker playing food calls than when no sound is played (Woodcock et al., 2004). Chicks also prefer a maternal call to an artificial sound when exposed to it during imprinting (van Kampen and Bolhuis, 1991). The domestic hen chick-sounds used here were a recording of the group of subject chicks used in this study making "peeps", or pleasure notes, when in their aviary. Studies have shown that chicks will move more quickly down a runway towards a conspecific compared to an empty box (Suarez and Gallup, 1983) and will readily move towards each other when placed at opposite ends of a chamber (Vallortigara et al., 1990) or towards video images of other chicks feeding (Clarke and Jones, 2001).

The aim of the present experiment was to establish whether CPP would serve 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.

Both 2- and 3-compartment apparatus have been used to assess CPP (Tzschentke, 1998). Bardo et al. (1995) found larger effect sizes in their meta-analysis of drug studies using a 3-compartment compared to a 2-compartment apparatus. The present experiment used a 3-compartment apparatus. Short duration test sessions (5 min) were used as has been successfully used in other CPP studies with chicks (Bronson et al., 1996; Hughes et al., 1995). These tests were kept short in an attempt to reduce any separation distress. Generally, CPP studies use one 20-min test session (Tzschentke, 1998) but as the present test sessions were short, four pre- and four post-conditioning sessions were conducted to obtain a comparable sample of behaviour. Bardo et al. (1995) recommend a minimum around 25-30 min for the conditioning phase in CPP studies. Bronson et al. used a total of 2 h for conditioning while Hughes et al. used 30 min. The present study used three 15-min conditioning trials in each side compartment giving a total duration of 45 min in each compartment.

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