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Examining affective structure in chickens: valence, intensity, persistence and generalization measured using a Conditioned Place Preference Test

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ABSTRACT

When measuring animals' valenced behavioural responses to stimuli, the Conditioned Place Preference (CPP) test goes a step further than many approach-based and avoidance-based tests by establishing whether a learned preference for, or aversion to, the location in which the stimulus was encountered can be generated. We designed a novel, four-chambered CPP test to extend the capability of the usual CPP paradigm to provide information on four key features of animals' affective responses: valence, scale, persistence and generalization. Using this test, we investigated the affective responses of domestic chickens (*Gallus gallus domesticus*) to four potentially aversive stimuli: 1. Puffs of air; 2. Sight of (robotic) snake; 3. Sprays of water; 4. Sound of conspecific alarm calls. We found conditioned avoidance of locations associated with the air puffs and water sprays (Friedman's $\chi^2_{(3)} = 13.323$ $p > .005$; $\chi^2_{(3)} = 14.235$ $p > .005$), but not with the snake and alarm calls. The scale of the learned avoidance was similar for the air puff and water spray stimuli, but persistence and generalization differed. We conclude that the four chambered CPP test can have a valuable role to play in making multi-feature measurements of stimulus-generated affective responses, and we highlight the value of such measurements for improving our understanding of the structure of affect in chickens and other animals.

1. Introduction

Decades of research have revealed many of the preferences of domestic laying hens and broilers (*Gallus gallus domesticus*) for different environments and resources and their motivation to gain access to these, and this information has been invaluable for the development and design of housing and husbandry systems to improve welfare (e.g. Cooper and Appleby, 2003; Dawkins, 1983; Hughes and Black, 1973; Nicol, 1986; Olsson and Keeling, 2002). Less studied to date, however, are these birds' affective responses to the variety of discrete stimuli and events that they may encounter during their daily lives, many of which may be aversive to them (e.g. Bertolus et al., 2015; Cooper et al., 1998; Pajor et al., 2000; Rutter and Duncan, 1992). For example, on farm, including free-range farms, chickens can experience a range of potentially punishing events (i.e. events that they would avoid if possible), including the sudden onset of loud noises or bright lights, flickering lights, rain, wind and encounters with aggressive conspecifics or predators (e.g. Kristensen et al., 2007; McAdie et al., 1993). In recent years a number of emotion theorists have proposed that animals' long-term affective states or "moods" represent integrations of both the reward and punishment experiences of their day-to-day lives, not only as a result of encountering preferred and non-preferred resources and environments, but also from their experiences of more briefly encountered, discrete stimuli and events such as those listed above (Eldar et al., 2016; Mendl et al., 2010; Nettle and Bateson, 2012). If this is correct, it is important to find out whether and to what extent different, short-term events and stimuli are perceived by animals as being punishing or rewarding, as each of these events may contribute significantly to their long-term affective states and welfare. The experiments reported in this paper consider this issue for chickens in particular.

In addition to immediate concern for the welfare of farmed chickens, these animals' evaluations of potentially punishing stimuli and events are valuable to study because of the role such information can play in furthering our knowledge and understanding of the affective states of these animals and how they are structured. For example, it is well known that many animals, including chickens, find the taste of quinine aversive, as evidenced by their behavioural responses to ingesting it and by its capacity to act as an instrumental punisher (e.g. see Dwyer, 2011; Sherwin et al., 2002). And this knowledge can be used in the design of experiments that investigate the multiple components of negative affective responses, and their effects on learning and

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behavioural decision-making (e.g. Harlander-Matauschek et al., 2009; Steiner et al., 2001). However, our understanding of the many other sorts of discrete stimuli that domestic chickens experience as punishing is far from complete, with the consequence that researchers are sometimes left having to make guesses and assumptions about the aversiveness of particular stimuli, rather than basing their studies on empirical evidence. For example, in a study of anticipatory behaviour, Zimmerman et al. (2011) proposed the explicit assumption that sprays of water would be perceived as aversive (negative) by hens.

1.1. The structure of affective states

Despite a dramatic increase in research interest in the topic of animal affect in recent years (for examples of recent reviews see: Bliss-Moreau, 2017; Gygax, 2017, Paul & Mendl in press, Perry and Baciadonna, 2017; Weary et al., 2017), important questions remain about the structure and function of affective states in a wide range of species, including birds such as the domestic chicken. Punishing or aversive stimuli can vary in their severity, frequency, and the nature of their consequences. For example, a negative affective state can be produced by a physical stimulus that has a direct effect on the animal, whether that is a severe injury or a brief disruption of physiological homeostasis. A negative affective state might also arise from exposure to stimuli that have meaning for the animal (e.g. visual or auditory stimuli that predict the advance of a predator), yet have no direct physical effects. Whether and how animals' responses to these types of stimuli differ, and how their consequent affective responses might vary, is not well understood. Certainly, some punishers are stronger and have more intense effects than others. But it is also possible that the affective consequences of different types of punisher differ in ways that go beyond strength or intensity. For example, some punishers may have mild yet long lasting effects, while others might have a powerful but only brief impact. In other words, the structure of the resulting affective states may vary according to more than one dimension, and different types of punisher may influence these dimensions differentially.

Anderson and Adolphs (2014) proposed an influential framework for studying the multi-faceted structure of affective states and responses in a wide range of non-human animals. They suggested that many animal vertebrates and even some invertebrate species can be shown to possess four "emotion primitives" – basic building blocks of what we call "emotion" in humans. They argued that in addition to the two commonly discussed dimensions of affect, "valence" (positivity vs negativity of response) and "scalability" (magnitude or intensity of response), two further properties, "persistence" and "generalization", should also be regarded as defining features of affective (emotion-like) states in animals. Persistence represents the extent to which affective responses endure over time following their initial triggering. Examples of this in humans are commonplace, with states such as anxiety and depression sometimes long out-lasting the event or events that triggered them (e.g. see Charney et al., 1998). But Anderson and Adolphs (2014) point out that this sort of behavioural and physiological persistence of response can also be seen in a wide variety of animal species. For example, pigs exposed to brief bouts of social isolation, restraint and loud noise while away from their home pens show reduced activity levels once returned to their home pens (Reimert et al., 2017). And even in *Drosophila*, noxious air puffs promote a persistent, elevated motor activity (Lebestky et al., 2009). Generalization concerns the tendency for stimuli similar to a primary emotive stimulus to have a capacity to arouse equivalent (albeit often less intense) affective responses in a likewise manner. This fourth feature of affective responses can also be seen in a range of animals, both in the form of generalized instrumental and classically conditioned responses (e.g. in rodents – McLaren and Mackintosh, 2002), and more recently in judgement bias tests in which affective state manipulations are seen to influence subject animals' responses to novel and ambiguous stimuli (e.g. Harding et al., 2004; Mendl et al., 2009).

It is possible to conduct a range of behavioural tests to assess a variety of aspects of both the valence and the scale of an animal's response to a stimulus and thereby to establish whether, and how much of, a positive or negative state has been induced. Such tests include approach-avoidance tests, preference tests, consumer demand tests, cognitive bias tests and progressive ratio tests (e.g. Dawkins, 1990; Duncan, 1978; Harding et al., 2004; Hodos, 1961; Mendl et al., 2009). Tests for the persistence and generalization of affective responses are less common, however (Anderson and Adolphs, 2014), although the process of generalization has been the subject of research in the field of animal-human (stock person) interactions for a number of years (e.g. see Brajon et al., 2015; Breuer et al., 2003). To better understand how different types of stimuli differentially and interactively influence all four of these "emotion primitives", an experimental approach is needed which is able to assess all of these facets of affect within a single, unified paradigm. We propose that a modified version of a conditioned place preference test has utility in this regard.

1.2. The Conditioned Place Preference Test

The method that was developed for use in the present experiments to assess the affective valence, scale, persistence and generalization of domestic chickens' responses to a range of potential punishers was the Conditioned Place Preference (CPP) Test (also sometimes known as the Conditioned Place Aversion Test when punishers are studied – e.g. Wang et al., 2017). CPP Tests were originally designed and used within the discipline of psychopharmacology and have been employed extensively to investigate the psycho-affective properties of a range of drugs including opiates, benzodiazepines and selective serotonin reuptake inhibitors (for reviews see e.g. Bardo and Bevins, 2000; Tzschentke, 2007). They are based on the principle of classical conditioning and the observation that many animals readily develop conditioned associations between the features of a location (e.g. in distinctively coloured or patterned chambers of an experimental testing box) and the discrete stimuli that they experience while there. In most CPP experiments, a two-chambered apparatus is used, in which one chamber or compartment of a test box is paired with a stimulus (e.g. provision of a food or injection of morphine) while the adjacent compartment is paired either with no stimulus, or a sham control (e.g. injection of saline). When subsequently given the choice to spend time in the chamber that was previously paired with the stimulus, or the one that was not, an animal's preference for the stimulus-paired location is interpreted as an indication that the original, unconditioned stimulus had been perceived by the animal to be relatively rewarding (indicating positive affective valence), or vice versa in the case of a punishing stimulus (indicating negative affective valence). To avoid possible confounds resulting from animals that have pre-existing preferences for the coloured or patterned location cues (i.e. the discriminative stimuli, which should ideally be affectively neutral themselves), associative pairings are generally counterbalanced between subjects, and the outcome measures used are based, not on absolute preferences, but on changes in preference occurring between the pre- and post-conditioning phases.

Although CPP tests have predominantly been employed for neurological and psycho-pharmacological research in rodents (see Tzschentke, 2007), they have also had some use in farm animals in recent years (e.g. de Jonge et al., 2008), including chickens and chicks (Buckley et al., 2012; Dixon et al., 2013; Jones et al., 2012; Nasr et al., 2013). In the present experiments, we sought to make use of the CPP paradigm to find out whether four potentially punishing stimuli can be said to generate negative affective states in chickens, and to attempt to extend the usefulness of the CPP test making measurements of all four facets of affective responses outlined by Anderson and Adolphs (2014) Traditionally, the CPP test is used to measure the relative valence of an animal's affective response to a stimulus and its control, and a measure of scale or intensity can also be obtained from the proportional amount