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Positive affective state induced by opioid analgesia in laying hens with bone fractures

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ABSTRACT

Laying hens with keel fractures ($n = 35$) and control hens with no fractures ($n = 12$) were trained to associate the colour and position of an environment with the effects of either a subcutaneous injection of an opioid analgesic drug (butorphanol) or a subcutaneous injection of saline in a conditioned place preference experiment. Each hen experienced 12 post-injection 30-min exposures to a specified environment over a period of 3 days to allow an association to form. After a 24 h drug clearance period the procedure was repeated. Hens that had initially been given butorphanol and experienced its effects in an environment of one colour, were now given saline paired with the environment of the alternative colour. Similarly, hens that had initially been given saline now received butorphanol. Following this sequential conditioning procedure all hens made 7 choices in a drug-free state between the two coloured environments previously experienced, using a T-maze preference test. Choice data were analysed using a random effect logistic regression model that accounted for bird identity, batch and fracture status. Birds with healed keel fractures preferred the environment where they had experienced the drug, a significantly different pattern of choice from the non-fractured birds that exhibited no preference. That the conditioned place preference was shown only by the fractured birds suggests it was the analgesic properties of butorphanol that were rewarding, and provides further evidence that healed keel fractures are a source of chronic pain.

1. Introduction

Animal welfare researchers have for many years used preference and willingness-to-work tests to establish what features of the environment animals find rewarding (Mason et al., 1998; Abeyesinghe et al., 2001; Kirkden and Pajor, 2006; Nicol et al., 2009, 2011). Studies of animal emotion show that the acquisition of reward, or the termination of punishment, both result in a positively-valenced affective state (Panksepp, 2005; Rolls, 2005) so animals' preferences can provide information relevant to their welfare state. Because preference and willingness-to-work tests depend on an animal's evaluation of its external environment, they have largely been utilised to assist in the design of animal housing and management systems (Fraser and Nicol, 2011). However, similar tests can also be used to assess whether changes in internal physiological or neural processes, such as may occur following administration of a nociceptive stimulus or a drug, are experienced as attractive or aversive. In these cases, affective state can be inferred if, via associative learning, a neutral aspect of the environment becomes conditioned with the internal effects of the procedure. As the relevant association forms, the animal's preference or willingness-to-work to

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gain access to (a conditioned place preference CPP), or avoid (a conditioned place avoidance CPA), the previously neutral location or stimulus can be assessed as a proxy measure of the affective state resulting from the procedure.

This methodology has been widely used to assess the effects of drugs (for reviews see Tzschentke, 1998, 2007). It holds particular promise for distinguishing situations in which animal nociceptive responses are accompanied by negative affective states indicating pain, although so far it has rarely been used in this context. In mammals, evidence that nociceptive responses are accompanied by negative affective states has come from studies of laboratory rodents showing that injection of formalin into a paw (Wang et al., 2009) or foot shock (Cain et al., 2004) result in CPA. The CPA tests used with rodents rely on experimenters knowing with some certainty that any negative affective state will be experienced almost immediately following the injection or shock procedure. It is more difficult to measure how the affective state of farmed, laboratory or companion animals is influenced by some of the chronic clinical pathologies which they routinely experience as no single (timed) event produces the pathology which can be directly linked with a neutral location or stimulus. Fractures in laying hens generally heal within 35 days (Richards et al., 2011) but the resultant callus formation at the site of fracture is permanent and may be associated with ongoing pain. In the current study we therefore developed a protocol to assess whether negative affective state induced by healed fractures was alleviated by the provision of a relevant analgesic drug, given at a specified point in time.

We studied laying hens with keel fractures as this is a highly prevalent problem in commercial egg production. In furnished (colony) cage flocks, an average of 36% hens per flock sustained a fracture during the laying period (Wilkins et al., 2011). Among a variety of non-cage aviary and free-range systems, average prevalence varied from a low of 45% in organic flocks kept in mobile housing, to a high of 86% in free-range flocks where aerial perches were suspended in the indoor house (Wilkins et al., 2011). Birds with healed fractures show impaired mobility (Nasr et al., 2012a; Richards et al., 2011), an effect partially mitigated by treatment with the NSAID meloxicam (Nasr et al., 2013a) and, to a greater degree, the opioid butorphanol (Nasr et al., 2012b). Instead of using a CPA test, as used to assess acute pain in rodents, we developed a method to assess whether hens formed a CPP for butorphanol (the drug that had the greatest effect in restoring mobility). CPP procedures are widely used to examine the general rewarding properties of drugs (Tzschentke, 1998, 2007) but have not previously been used to assess affective states associated with analgesia. To distinguish affective states that might arise due to analgesic and general rewarding properties of the drug we compared the CPP of fractured and non-fractured hens.

2. Materials and methods

2.1. Subjects and housing

48 Lohmann brown laying hens aged approximately 40 weeks, were selected from each of two different free-range farms (Batch 1 and Batch 2). At the farm, birds were examined by a validated method of palpation of the keel to detect any abnormalities or fractures (Wilkins et al., 2004). Batch 1 comprised 24 birds with detectable healed keel fractures of varying severity, whilst Batch 2 comprised 12 birds with detectable healed keel fractures and 12 birds we suspected had no fractures. In all cases, the fracture status diagnosed by palpation was later confirmed by dissection at the end of the experiment.

The birds were transported to the experimental housing in their respective batch (day 0) and individually identified via coloured leg tags. Birds from each batch were housed together in a floor pen (3 m × 3.5 m) for 3 weeks until they were calm to handle. Hens were provided with wood shavings bedding, a nest box, *ad libitum* layers' mash from two suspended poultry feeders and water from two suspended poultry drinkers. The lighting programme was 14 h light: 10 h dark and ambient temperature ranged between 19 and 21 °C.

The experiment was carried out under Home Office licence. For habituation purposes, a tunnel (1.79 m length) which would be used later in the CPP procedure was placed in the home pen during week 2. Initially, the hens could explore the tunnel freely. Between days 25 and 29 the tunnel was connected to a start box (35 cm × 37 cm × 45 cm, $L \times W \times H$) to form a T-maze and hens were individually placed in the start box 6 times a day (to conduct training trials) for 5 days. On each day, by means of blocking one of the tunnel exits, hens were forced to go to the right on 3 trials and to the left on the other 3 trials. After these 30 trials, all hens walked through the tunnel without showing fearful behaviour or stopping.

2.2. Conditioning phase 1 and 2

The aim of conditioning phases 1 and 2 was to establish associations between particular coloured environments and the effects of saline or butorphanol. Because of the numbers of birds it was not possible to perform conditioning trials for all birds on the same day. For each batch, birds were therefore divided into two groups, with 12 birds subjected to a first phase of conditioning between days 30 and 32 (Group A) and the other 12 birds subjected to this first phase between days 32 and 34 (Group B). The first phase of conditioning began in the home pen where half the hens were injected subcutaneously (s/c) with butorphanol (Torbugesic 1% w/v solution for injection) at a dose of 2 mg/kg and the other half of the hens were injected with an equivalent volume of saline. Hens were kept in the home pen for a period of 15 min to allow time for active effects of the drug to be experienced (Sladky et al., 2006; Singh et al., 2011). After this 15 min holding period, hens were transferred to the experimental room pen and placed in the start box of the T-maze which connected two experimental pens. Only one arm of the T-maze was open so that hens had to walk in a specified direction (either left or right) to a specified 1.5 m × 1.2 m coloured environment (either orange walls and shavings or blue walls and shavings) where they remained for 30 min with *ad libitum* uncoloured food and water. At the end of the 30 min exposure, the hens were placed back in the start box of the T-maze and the hens walked again to the available