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In search of stress odours across species: Behavioural responses of rats to faeces from chickens and rats subjected to various types of stressful events

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ABSTRACT

Stressed animals have an increased risk of health and welfare problems, thus methods for easy and early stress detection are important for appropriate animal management. Using the ability of rats to distinguish between faeces odours from stressed and non-stressed conspecifics, we investigated whether rats could detect stress status in another species (the chicken), which would suggest a commonality in odorous stress signatures across species. We carried out four experiments to investigate the existence of stress-specific odours. In the first experiment using a T-maze, male Brown Norway (BN) rats (n = 12) were found to sniff the faeces samples from stressed rats and chickens less relative to the samples from non-stressed individuals ($P < 0.05$). In the second experiment, where odours were presented in an arena one at a time, male BN rats (n = 16) sniffed faeces samples from stressed rats and chickens for longer than those from non-stressed controls ($P < 0.05$). Within each test, the same responses to stress odours were seen independent of species of origin. This suggests that both in rats and chickens stress gives rise to specific volatile organic compounds (VOCs). In a third experiment, faeces from chickens, which had been stressed or non-stressed at hatching and subsequently exposed or not to acute stress at two weeks of age were tested on male BN rats (n = 18). These rats were also tested with faeces from non-stressed and acutely stressed rats as well as herb odour (1-hexanol) used as control. Number of freezing episodes was higher when rats were exposed to any of the samples originating from stressed individuals compared to that observed with herb odour ($P < 0.05$). Also, defensive burying was more likely to occur when rats were exposed to faeces from chickens stressed at hatching ($P < 0.05$). Finally, a fourth trial analysed faecal samples from non-stressed and acutely stressed rats using gas chromatography coupled with mass spectrometry (GC–MS), and identified ten VOCs potentially involved in the distinctive smell detectable in faeces from acutely stressed rats. These findings confirm the existence of stress-specific odours in rats and indicate that, although not necessarily identical, a similar type of odour may be present in stressed poultry. In addition, this odour could be detected by rats in chicken faeces collected almost two weeks after the birds had been exposed to a stressful event. Our results suggest that patterns of VOCs may have the potential to be used as a tool for early, non-invasive screening of stress status in animals.

1. Introduction

In some species, exposure to a stressful situation may give rise to specific body odours. Rats and mice give off specific odorous alarm signals (Zalaquett and Thiessen, 1991; Brechbühl et al., 2013; Inagaki et al., 2014), and rats have been shown capable of distinguishing between stressed and non-stressed conspecifics based on their odours (Valenta and Rigby, 1968; Mackay-Sim and Laing, 1980). Stress odours may depend on the type of stress experienced, where acute stress can give rise to specific odours thought to signal danger to conspecifics, as seen in mice (Brechbühl et al., 2013), whereas chronic stress odours may arise from lasting changes in the hormonal or metabolic state of the animal. As most work in this area have been carried out in mammals, particularly in rodents, we aimed to address the question whether birds, here represented by the domestic chicken, also exhibit a change in odour when stressed.

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We decided to use rats as biological sensors of potential odour changes in the faeces from chickens that either had or had not been exposed to stressful events in early life. Rats, and not chickens, were chosen as the subject of behavioural study, as it is well-established that rats have very sensitive olfactory perception, and can distinguish between odours, which differ only slightly (e.g. Clarin et al., 2010). Also, even though faeces contain hundreds of different volatile molecules, rats can discriminate between different physiological states (oestrus vs dioestrus), even in other species (fox and horse; Rampin et al., 2006). They may thus be able also to detect potential differences in smell between stressed and non-stressed individuals of species other than rats.

Although chickens were not used in the present study for the purpose of odour detection, the existence of stress odours in avian species would nevertheless be more likely if birds are able to detect such odour changes themselves. Birds have a more acute sense of olfaction than previously thought. Petrels and albatrosses, for example, have been found to use odours when foraging for krill at sea (Nevitt et al., 2004). Jungle-fowl have individual odours emanating from their uropygial gland secretions (Karlsson et al., 2010), and chickens use olfaction in their social interactions to a surprising extent (Krause et al., 2016). For example, week-old chicks appear to find cat odour aversive (Fluck et al., 1996), showing that they are able to detect and distinguish between odours. Also, exposure to certain odours whilst still in the egg influence the feeding behaviour of young chickens once hatched (Bertin et al., 2010). These findings indicate that potential changes in body odours in birds caused by stress could be perceived by their conspecifics.

Some, although sparse, evidence exists as to the existence of avian odours indicative of stress. Changes in the odour of bird excreta (henceforth referred to as faeces) caused by infection have been found in ducks, as mice were able to distinguish faeces from mallards infected with avian virus from that of non-infected birds (Kimball et al., 2013). Tullo et al. (2017) found differences in the composition of air samples taken from boxes of broilers with or without coccidiosis. Krause et al. (2016) suggest that olfactory cues are more reliable in signalling health status than visual signals, which often becomes apparent with a certain delay. However, it may be that such odour changes are associated with the stress response induced by the burden of being sick rather than the disease itself. If stress-induced odours exist in birds, monitoring of these odours could potentially be used for the detection of stress in poultry production where easy, non-invasive methods to measure stress are lacking.

The experiments described here form part of a larger project investigating the long-term consequences of exposure to stressful events in early life on the health and behaviour of production animals, including chickens. The overall aim of the four experiments reported in this article was to investigate olfactory-based, non-invasive indicators of stress across two very different species. This was done by studying the behavioural response of rats to faeces from stressed and non-stressed chickens, and comparing this to their response to faeces from stressed and non-stressed conspecifics (Experiments 1–3). The faeces used to detect stress odours were collected from animals immediately or several days after the stressful experience. Faeces from acutely stressed rats have a particular odour, detectable by humans, that differs from that of non-stressed rat faeces. We therefore also carried out analyses of the volatile organic compounds (VOCs) of acutely stressed and non-stressed rat faeces using mass spectrometry combined with gas chromatography (GC–MS) to investigate if specific stress molecules could be identified (Experiment 4). Each experiment is presented separately below with a short introduction followed by methods and results. For ease of reading, the results are briefly discussed when presented, and a broader discussion of the combined results is given at the end. All procedures used in the experiments reported in this article were approved by the local ethics committee (Comité d'éthique appliqué à l'expérimentation animale; permissions no.11/013, 12/154 and 01730.02) and carried out in accordance with current European legislation (EU Directive 2010/63/EU) and ethical guidelines (Sherwin et al., 2003).

2. Experiment 1: simultaneous exposure to odours from stressed and non-stressed chickens and rats

In order to compare the behavioural responses of rats to faeces from two different species, rats were exposed in a T-maze to faeces from either stressed and non-stressed chickens or faeces from stressed and non-stressed rats. We hypothesised that the expected differences in response to stressed and non-stressed rat faeces would be similar to that seen with the chicken faeces. The behavioural response measured were those used as standard in T-maze trials, and were not meant to measure any stress potentially experienced by the rat as a consequence of the odour exposure.

2.1. Materials and methods

2.1.1. Animals and housing

We obtained male, 7-week-old Brown Norway rats (n = 12) from a commercial breeder (Janvier-Labs.com, France) and housed them in groups of three in conventional, multi-rack, plastic cages covered by a metal grill, with free access to commercial rat pellets and tap water. Cage cleaning was carried out weekly. The rats were accustomed to and kept in an inverse light cycle (12 h dark per 24 h starting at 07:00 h), and all handling of the rats were carried out in red lighting during the dark period when the rats are naturally active.

2.1.2. Faeces samples

The chicken faeces were collected from 12-day-old male and female broiler chickens, which had been exposed to one of two treatments post-hatch in a previous study (Koch et al., 2015). Briefly, to mimic suboptimal conditions of broiler chick transport from hatchery to farm, newly-hatched chicks (n = 48) were deprived of feed and water and put in a transportation box whilst undergoing irregular movement and variable room temperature for 24 h following hatching (Stress group). When removed from the transportation box, the birds were checked for signs of injury or disease (none were found), and moved into the experimental rearing facility and placed in 6 pens (LxW: 1 m x 1 m) of 8 birds. Another group of newly-hatched chicks (n = 48) from the same cohort were placed directly in the experimental rearing facility, also in 6 pens of 8 birds each, immediately after being withdrawn from the hatcher (non-stressed Control group). Fresh chicken faeces without caeca content were collected at 12 days of age from 6 pens per group as follows: collecting devices consisting of a box (LxWxH: 55 cm × 40 cm × 5 cm) covered with wire mesh were present on the cage-floor of each pen of all groups of chickens (from day 0). In the morning on day 12, aluminium trays were placed under each box, and faeces samples were collected for 2 h and stored at −80 °C in separate containers for each pen. Prior to testing, the samples were removed from the freezer, and all samples from each treatment (Stress or Control) were mixed within treatment. The two mixtures were divided into 0.5–1.0 g samples placed onto cotton disks, each wrapped in tinfoil and stored at −20 °C until 30 min before use, where the samples were left at room temperature to defrost.

The rat faeces were collected from 24 male Wistar rats taking part in another experiment (see Raynaud et al., 2015). At the time of faeces collection, half of these rats had from 8 weeks of age been subjected for 17 days to a chronic variable stress schedule consisting of daily exposure to mild, unpredictable stress of a social, physical or dietary nature, which is a validated method commonly used to induce chronic stress in rats (Willner, 2005). The other 12 rats were non-stressed controls. Fresh faecal pellets were collected from the cage floor and stored for individual rats in glass tubes at –80 °C. Prior to testing, the tubes were removed from the freezer, and samples were prepared by