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Unpredictable and repeated negative stimuli increased emotional reactivity in male quail

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ABSTRACT

Chronic stress is considered detrimental for an individual as it is a long-lasting negative emotional state, without or with a limited habituation. The reactivity and sensitivity of animals to stressors depend on the animal's organismic characteristics such as sex. In poultry, the studies dealing with chronic stress were mainly performed on females. Our study then focused on male quail and aimed at assessing the effect of unpredictable and repeated negative stimuli (URNS) on their general activity, tonic immobility, emotional reactivity to a human, a novel object, novel foods or when restrained in a crush cage, as well as on their plasma corticosterone concentrations.

Quail were then left undisturbed or submitted to URNS during 6 weeks (n = 12 in each group). The results indicated that disturbed males spent a greater proportion of time far from the human than control ones (on the 1st test, 44.23 ± 5.28 *vs* 21.80 ± 6.12%, P = 0.01), probably to avoid the human. Disturbed males pecked at the novel object latter (P = 0.03) and less (P = 0.05) than controls. When restrained in a crush cage, disturbed quail were more often motionless than controls (13.49 ± 4.57 *vs* 4.76 ± 2.11%, P = 0.05). Disturbed quail ate a greater cumulative amount of mealworms (P = 0.05) or ate red maggots with a shorter latency compared to controls (P < 0.05 for the 2nd and 3rd red maggots, on the 2nd test). Concerning the general activity, disturbed males only showed less stereotypic behaviour than control ones after 14 days with URNS (1.4 ± 0.4 *vs* 4.9 ± 1.2% respectively, P = 0.03), but this difference disappeared after 34 days with URNS (P > 0.1). More importantly, stereotypic behaviours represented less than 5% of the time budget of both groups. URNS had however no effect on tonic immobility, general activity and corticosterone concentrations.

This study demonstrated that URNS induced an increase of male quail's emotional reactivity to a human (when tested for the 1st time), to a novel object and when restrained in a crush cage. URNS also altered male quail's feeding behaviour. Disturbed quail were more interested by the novel foods than control quail presumably because they considered them as positive rewards after repeated exposures and they used them as a way to compensate their negative emotional state. All in all, our results demonstrated that URNS induced a chronic stress state in male quail that can have negative consequences on their welfare.

1. Introduction

Stress is known to impair animal welfare (Moberg and Mench, 2000). The reactivity and sensitivity of animals to stressors depend on the stressor itself – its type (psychogenic or immune stressors) and its characteristics (controllability, predictability, ambiguity,

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chronicity, intermittence, intensity, *etc.*) – and also depend on its significance as appraised by the animal (Lazarus, 1993; Anisman and Matheson, 2005). Individual variability is then possible as this appraisal is influenced by the animal's organismic characteristics (genetic, age, sex), previous experiences and personality (Anisman and Matheson, 2005). The definition of stress that is used in this paper digress from the classical homeostatic definition (Moberg, 1987) to adopt a psychological approach (Lazarus, 1993). Acute stress is induced by a short-term but intense stimulus, perceived by the individual as negative. It is generally considered as beneficial as it makes the individual respond quickly to a potential threat, thus allowing a homeostasis recovery and increasing the individual's chances of survival (Awerman and Romero, 2010). Chronic stress can be described as a long-lasting negative emotional state induced by a prolonged or repeated exposure to negative stimuli (Destrez et al., 2013b), without or with limited habituation. Actually, if the animal habituates to the stressors, then it will stop responding to them and chronic stress will not appear. Chronic stress involved accumulative biological costs (Moberg and Mench, 2000) explaining why it is considered as detrimental for the animal (Awerman and Romero, 2010). The negative stimuli used to induce chronic stress can be very stressful, like intense electric foot shock, cold water immersion, and/or restraint for long periods (Katz et al., 1981; Katz, 1982), but the stressors can also be of mild intensity, diverse, and applied randomly in order to avoid habituation (see Willner, 1997 for review). These unpredictable and repeated negative stimuli (URNS) induced various negative consequences for emotional reactivity (Destrez et al., 2013a), cognition (Destrez et al., 2013b), feeding behaviour (Dallman et al., 2003; Favreau-Peigné et al., 2014), immunity (Cremaschi et al., 2000), and reproduction (Dalla et al., 2005; Cyr and Romero, 2007).

In poultry, and more specifically in quail, most of the studies dealing with chronic stress were performed on females. They showed that URNS reduced body weight (Calandreau et al., 2011a), improved spatial learning and memory (Calandreau et al., 2011a; Laurence et al., 2012), as well as increased their emotional reactivity when humans are present (Laurence et al., 2014) and when restrained in a crush cage (Favreau-Peigné et al., 2014). Further to these studies, recent research focused on the influence of the female quail's genetic background on their sensitivity to URNS. They demonstrated that female quail selected for a high fearfulness (*i.e.* Long Tonic Immobility; see Jones et al., 1991 for a full description) had lower plasma corticosterone levels (Calandreau et al., 2011b), higher emotional reactivity (in open field tests (Calandreau et al., 2011b; Laurence et al., 2012), to human (Favreau-Peigné et al., 2014), to a novel object (Favreau-Peigné et al., 2014)) after exposure to URNS, compared to quail selected for a low inherent fearfulness (*i.e.* Short Tonic Immobility). The genetic background of female quail also influenced the effect of chronic stress on their feeding behaviour (Favreau-Peigné et al., 2014). To our knowledge, in poultry, there was no study dealing with the consequences of URNS in males and only scarce studies noticed the influence of sex on the susceptibility to chronic stress in these species. For instance, chronic daily restraints induced negative consequences on the immunity that were worse in male than in female quail (Nazar and Marin, 2011). Farming systems for meat production can expose males to unpredictable and repeated negative stimuli such as sudden noises, human handlings and actions in the animals' environment, novel feed, high stocking density, which can lead to welfare issues due to an accumulation of negative emotional experiences. Thus, our study dealt with chronic stress in male quail focusing on the consequences of URNS on their general activity, plasma corticosterone and emotional reactivity. As emotional reactivity is a complex and multi-dimensional trait (Ramos and Mormède, 1997), it was assessed through different behavioural tests involving fear-eliciting stimuli based on novelty, restraint, human presence and an unlearned fear response (*i.e.* tonic immobility). We hypothesized that the URNS procedure will induce an altered general activity, a greater corticosterone response, and a greater emotional reactivity towards fear-eliciting events in male quail, thus leading to the conclusion that they will be experiencing a chronic stress state.

2. Material and methods

The study was conducted indoors at the Pôle d'Expérimentation Avicole de l'INRA Centre Val de Loire (UE 1295, Nouzilly, France) between February and April 2010.

2.1. Ethics statement

In 2010, when this study was carried out, approval from an ethics committee was not obligatory in France and there were no Institutional Animal Care and Use Committee at the time the study was conducted. However, this experiment including all animal care procedures were conducted in accordance with the guidelines set by the European Communities Council Directive (86/609/EEC) and with the French legislation on animal research. This study complied with the principle of the 3Rs: i/the use of animals cannot be avoided as the aim of the study was to investigate chronic stress in farm animals so their behavioural and physiological responses to URNS needed to be observed; ii/the number of animals used was the minimum required to obtain statistically relevant data while taking into account the potential variability of the behavioural responses; iii/the URNS procedure involved negative stimuli that were aversive but not harmful, and considered of mild intensity in the literature; iv/the housing condition allowed social interactions and provided enrichment (turf on the ground, radio music); v/the animals could not be used for other studies, so they were humanely euthanized by cervical dislocation at the end of the experiment.

2.2. Animals and housing

From the day of hatching to day 16, Japanese quail chicks (*Coturnix japonica*) were reared in communal pens under the same conditions: they were maintained under a temperature of 40 °C and under continuous dim light (to help them finding the feed troughs) from the day of hatching to day 6, and then, from day 7 to day 16, the temperature and the photoperiod were gradually adjusted to 24 °C and a 12:12 h light/dark schedule (light on at 08:00 h) respectively. Chicks were wing-banded at 3 days of age. On the 16th day after hatching, chicks were sexed, and then, only twenty-four male quail were weighed and tested for their tonic immobility before being transferred in single home cages ($35 \times 50 \times 21$ cm; Fig. 1), placed in two different rooms depending on their experimental groups (n = 12 in the 1st room with disturbed quail and n = 12 in the 2nd room with control quail). In these rooms, the temperature and light conditions were similar (*i.e.* 24 ± 2 °C and a 12:12 h light/dark schedule). Water and food were available *ad libitum* (except during the food deprivation stressor).

Each individual cage contained a plastic square mesh on the whole surface of the ground and a rectangle of synthetic turf on a quarter of the ground. Cages were separated laterally by opaque walls (Fig. 1); then, visual and tactile social contacts between quail were only allowed in front of the cage, when quail put their head out of the cage. A hiding place was built in the right corner, in the back side of the cage, with opaque plastic wall placed on the three sides of the cage. This hiding place did not enable the quail to escape from the URNS (or the human) but was provided to the animal as a safe place (confined, with a roof). Radio music was broadcasted in the room of both groups in order to enrich their environment.