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Human handling and presentation of a novel object evoke independent dimensions of fear in Japanese quail

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ABSTRACT

Fear is a concept comprising several dimensions, but the nature of these dimensions and the relationships between them remain elusive. To investigate these dimensions in birds, we have used two genetic lines of quail divergently selected on tonic immobility duration, a behavioural index of fear. These two lines differ in their behavioural response to some, but not all, fear-inducing situations. In the present study, we investigated the contribution of human intervention in the differentiation between the two lines. To do this, fear responses towards a novel object were compared between lines in three conditions: (1) in the home cage without any human intervention, (2) in the home cage after human handling and (3) after placement in a novel environment by human handling. Fear behaviour differed between lines after human handling, with or without placement in a novel environment, but presentation of a novel object in the home cage without any human intervention induced similar fear responses in the two lines of quail. These results lead us to suggest that in quail, human intervention evokes a dimension of fear that differs from that evoked by sudden presentation of a novel object, in that these two dimensions may be selected independently.

1. Introduction

Genetic selection on fear behaviour has proved useful not only to obtain animal models helping to understand the mechanisms of fear behaviour, but also to reduce the expression of undesirable fear behaviour in farm species (Landgraf and Wigger, 2002; Faure and Mills, 1998). The success of such experiments has strengthened the initial assumption that selection on a single behavioural fear trait affected fearfulness in general, *i.e.* the propensity to be more or less easily frightened (Faure et al., 2006; Broadhurst, 1975; Landgraf and Wigger, 2002; Jones, 1996). In this regard, fear has traditionally been considered as a unitary concept. However, this view has recently been challenged by data in birds and mammals, which have uncovered the complexity of this seemingly simple concept (see for example Russell, 2003; Campler et al., 2009; López-Aumatell et al., 2009). In birds, evidence notably came from two lines of Japanese quail that have been divergently selected for over 40 generations on tonic immobility duration, a behavioural index of fear, and that are considered to differ in their inherent fearfulness (Mills and Faure, 1991; Faure et al., 2006). In a number of classical behavioural tests, including the 'open-field', the 'capture' and the 'hole-in-the-wall' box tests, quail of the long tonic immobility line (LTI) exhibit consistently higher levels of fear than quail of the short tonic immobility line (STI) (Faure et al., 2006). However, quail of both lines have been shown to display similar fear reactions when presented with a novel object or a novel sound in their home cage (Valance et al., 2007; Richard et al., 2008; Saint-Dizier et al., 2008), suggesting that novelty-induced fear responses have not been affected by selection on tonic immobility. Thus it appears that genetic selection on a behavioural fear trait does not necessarily affect all dimensions of fear.

Although the multidimensional nature of fear has been recognised by authors from various backgrounds, there is no consensus about what the underlying dimensions may actually be in practice (Russell, 2003). In the present study, we used the term 'dimensions' to name distinct emotions that share the definition of fear as an emotion triggered by the perception of a danger (Jones, 1996). Thus, we considered that the concept of fear comprised several emotions and that distinct dimensions of fear might be evoked independently and should differ in their genetic substrate. In an attempt to clarify these concepts, we sought to experimentally characterise the dimensions of fear that were affected by genetic selection in the STI and LTI lines of quail. We hypothesised that the choice of the criterion used for selecting the individuals, the duration of tonic immobility, may have played a critical role in determining the outcoming phenotype. Tonic immobility is an unlearned fear response characterised by profound motor inhibition (Mills and Faure, 1991)

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and it is experimentally induced by physical restraint in a sound-proof room. Thus, two major characteristics of the selection test are placement in a novel environment and obvious human intervention. Both of these factors, or at least one of them (human intervention), were present in the fear tests in which LTI quail have been shown to display higher levels of fear than STI quail, notably the open-field and 'hole-in-the-wall' box tests as well as restraint in a 'crush cage' and capture by a human (Jones et al., 1991, 1994; Mills and Faure, 2000). In contrast, in the home cage and in the absence of human interference, presentation of a novel object or of a novel sound induced similar behavioural fear reactions in the two lines of quail (Valance et al., 2007; Saint-Dizier et al., 2008; Richard et al., 2008). These observations have led us to suggest that human intervention, combined or not with exposure to a novel environment, might be a critical factor in revealing the differences in behaviour between STI and LTI quail. In other words, we hypothesised that selection on tonic immobility duration affected a dimension of fear evoked by human intervention, but not the dimension evoked by a novel object.

In the present experiments we sought to investigate the contribution of human handling (in the form of a single, brief manipulation of the quail by the experimenter) and placement in a novel environment in the behavioural differentiation between STI and LTI quail. To do this, two experiments with quail from the same hatches were carried out in parallel. The quail were subjected to three variants of the novel object test: in the home cage without any human intervention in the cage, in the home cage after human handling (Experiment 1) and after placement in a novel environment by human handling (Experiment 2). We hypothesised that the behavioural responses of STI and LTI quail induced by presentation of a novel object after human handling and placement in a novel environment might differ, whereas STI and LTI quail should present similar fear responses when the novel object is presented in the home cage without any human intervention.

2. Experiment 1: testing in the home cage with or without human handling

2.1. Materials and methods

2.1.1. Subjects

Japanese quail (*Coturnix japonica*) of the 43rd generation of the LTI and STI lines selected and maintained at the Pôle Expérimental Avicole de Tours, France (Mills and Faure, 1991), were used in the present experiment. Tonic immobility duration data are not available for the individuals used in this experiment, but at the 42nd generation, the mean duration of tonic immobility (±standard deviation) was 241 (±82) s in the LTI line and 12 (±18) s in the STI line. On the day of hatching, chicks of both lines were wing-banded and transferred to communal floor pens, where they were reared in single line groups under continuous illumination for three weeks. On the 21st day after hatching, the chicks were sexed on the basis of plumage colour and transferred to communal floor pens, where they were reared in single line, single sex groups under a 16:8 h light:dark schedule, so that all quail were sexually mature at the time of testing (six weeks). Unless otherwise specified, food and water were freely available at all times. The birds were treated according to the European Union's Council Directive of November 24, 1986 (86/609/EEC) throughout. All procedures described here fully comply with French legislation on research involving animals.

2.1.2. Testing apparatus and procedure

Adult LTI and STI male quail were randomly assigned to one of the two experimental groups: (I) testing in the home cage with minimal human intervention ($n = 16$ per line), each quail being tested

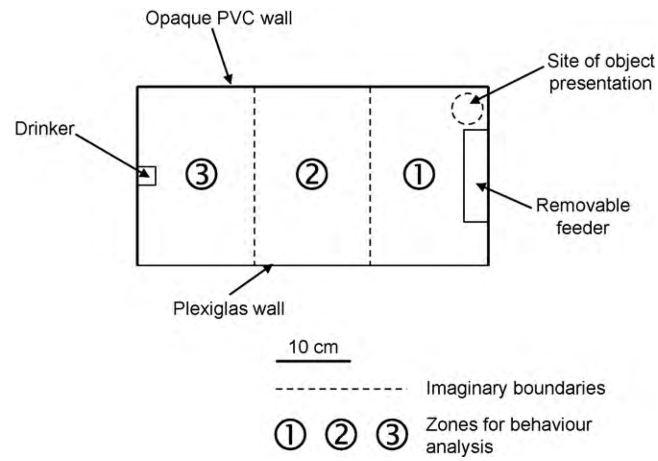

Fig. 1. Diagram of an experimental cage viewed from the top with roof removed, showing the site at which the novel object was dropped as well as the boundaries of the three zones used for behaviour analysis.

twice: with (HC-OBJ) or without (HC-CTRL) presentation of a novel object. (II) human handling and testing in the home cage ($n = 16$ per line), each quail being tested twice: with (HHC-OBJ) or without (HHC-CTRL) presentation of a novel object.

One week before testing, all quail were transferred to individual PVC cages (length = 42 cm; width = 24 cm; height = 25 cm; Fig. 1) with wood-shavings on the floor, an opaque PVC roof and a front wall made of clear Perspex. These individual cages were designed to allow presentation and withdrawal of a novel object from a distance and observation of the quail with minimal disturbance, the experimenter remaining out of sight of the bird during the procedure (see Richard et al., 2008 for details). The room was maintained at 20 °C under a 16:8 h light:dark photoperiod. During the week preceding testing, the food trough of every cage was removed daily for 2 h. The return of the feeder in the cage after a short food deprivation was used to attract the quail momentarily to the part of the cage where the object would be dropped on the testing day.

On the day of testing, the food trough of every cage was removed for 40 min. At the end of this 40-min period, quail from group (II) were taken out of their individual cages, carried by hand out of the testing room and returned to their home cages (HHC). The duration of this manipulation was also approximately 40 s. The quail were then left undisturbed for 1 min, after which the novel object test began. Quail from group (I) were left undisturbed between the removal of the food trough and the novel object test (HC).

In the 'object' situation (HHC-OBJ, HC-OBJ), when the food trough was replaced in the cage, as soon as the quail pecked at the food, a multicoloured cylinder (outer diameter = 4 cm; height = 21 cm, covered with 2-cm wide horizontal stripes of blue, yellow, grey, red, black and white tape) was dropped into the cage near to the food trough (Fig. 1). If a quail failed to peck at the food, the multicoloured cylinder was dropped into the cage 60 s after the return of the food trough. The object was withdrawn 5 min after being dropped. A video recording was made of the behaviour of the quail during the object presentation. In the control situation (HHC-CTRL, HC-CTRL), the behaviour of the quail was recorded for 5 min, starting as soon as a quail pecked at the food after the return of the food trough. If a quail failed to peck at the food, the video recording started 60 s after the return of the food trough, without further disturbance.

Each quail was tested twice: once in the control situation (HHC-CTRL and HC-CTRL) and once in the 'object' situation (HHC-OBJ and HC-OBJ). To avoid an effect of testing order, half of the quail of these groups were tested first in the 'object' situation and then in the control situation; the other half were tested in the reverse