Estimations du volume de l'arcopallium / PoA

Pour l'étude d'activation neuronale, la mesure de la densité de neurones activés au sein de l'arcopallium / PoA a nécessité une évaluation de la surface occupée par cette région sur chacune des coupes, ce qui nous a permis d'estimer le volume de l'arcopallium / PoA chez les cailles expérimentales. Nous avons ainsi observé une différence significative entre les deux lignées, l'arcopallium / PoA des cailles STI présentant un volume plus important que celui des cailles LTI ($p<0.01$, test t pour données non appariées, voir figure). Ces résultats confirment ceux obtenus lors d'une précédente étude morphologique de l'arcopallium chez les deux lignées (Richard, 2000, Richard *et al.,* 2005). De plus, nous avons cherché à savoir si cette différence de volume affectait la partie antérieure (AA) et/ou la partie postérieure (AP) de l'arcopallium / PoA. Nous avons observé que les cailles STI présentaient un AP plus volumineux que les cailles LTI ($p<0.05$, test t pour données non appariées, voir figure) alors que n'avons pas pu mettre en évidence de différence de volume de l'AA entre les deux lignées ($p > 0.1$, test t pour données non appariées, voir figure). Le fait que les cailles exprimant des comportements de peur réduits (cailles de la lignée STI) présentent aussi un volume de l'AP plus important que les cailles présentant des comportements de peur exacerbés (LTI), nous a conduit à suggérer l'existence d'une sous-région au sein de l'AP qui aurait un rôle inhibiteur sur les comportements de peur. Ce point sera développé dans la discussion générale en tenant compte des résultats du chapitre 1.

Commentaires sur le Chapitre 2

Dans l'expérience présentée dans ce deuxième chapitre, nous avons observé une activation neuronale dans le noyau paraventriculaire de l'hypothalamus (PVN) en réponse à la présentation d'un objet nouveau dans la cage d'élevage, chez les cailles LTI. **A la suite de la discussion sur ce résultat au sein de l'article, nous concluons que le PVN des oiseaux semble pouvoir jouer un rôle comparable au PVN des mammifères dans le contrôle de l'axe corticotrope.**

De plus, notre étude a montré que les cailles des lignées LTI et STI présentent des différences d'activation neuronale dans l'arcopallium / PoA et le noyau de la strie terminale (BST), indépendamment de la présentation de l'objet nouveau. **Ce résultat suggère que le fonctionnement basal des structures concernées diffère entre les deux lignées. Les cailles des lignées LTI et STI présentant des différences comportementales extrêmes dans de nombreuses situations de peur, nos résultats suggèrent que l'arcopallium / PoA et le BST pourraient être impliqués dans l'expression des comportements de peur de ces cailles.** L'originalité de cette étude se trouve dans l'observation de spécificités fonctionnelles dans les sous-régions de l'arcopallium / PoA et du BST. Ainsi, nous avons observé des différences entre les deux lignées de cailles dans la partie latérale du BST (BSTL), la partie caudale du noyau taeniae de l'amygdale (TnA) et l'arcopallium antérieur (AA). Les résultats obtenus sur l'AA confirment ceux obtenus au cours de notre étude lésionnelle, à savoir que cette sous-région semble jouer un rôle dans le contrôle des réactions de peur chez l'oiseau, contrairement à ce que des études neuroanatomiques antérieures avaient laissé suggérer (Zeier et Karten, 1971 ; Davies *et al.,* 1997a). Il serait désormais intéressant de tester, par des études fonctionnelles, les hypothèses d'implication du BSTL ou de la partie caudale du TnA.

La discussion concernant les résultats principaux de cette expérience sera reprise dans la discussion générale. Nous avons choisi ici de commenter certains points qui n'ont pas (ou peu) été discutés dans l'article mais qui pourront être utile aux réflexions de la discussion générale.

Différences d'activation neuronale entre les cailles STI et LTI :

Dans cette étude, nous avons observé des différences d'activité neuronale entre les cailles STI et LTI mais nous n'avons pas discuté du sens de variation de ces différences au

sein de l'article. Par exemple, nous avons observé une densité plus importante de neurones activés dans le BSTL chez les cailles LTI que chez les cailles STI. Les cailles LTI présentant de façon générale des comportements de peur exagérés, il serait tentant de conclure que le BSTL pourrait jouer un rôle activateur dans l'expression des comportements de peur. A contrario, l'observation d'une activation neuronale supérieure chez les STI que chez les LTI dans la partie caudale du TnA pourrait laisser penser que cette structure joue un rôle inhibiteur sur les comportements de peur. Cependant, nous ne possédons pas d'information quant à la nature chimique exacte de l'ensemble des neurotransmetteurs présents dans les neurones activés. Il est donc possible que l'activation neuronale observée concerne des neurones au rôle activateur ou inhibiteur. Plus généralement, la nature des circuits contrôlés par ces neurones détermine, *in fine*, l'effet sur les comportements de l'activation de ces neurones (activateurs ou inhibiteurs). Ainsi le sens de variation des différences observées sur les densités de neurones activés entre les cailles LTI et STI ne nous a pas semblé très informatif, l'essentiel de l'information se trouvant dans l'existence de différences entre les deux lignées.

La protéine Fos comme marqueur d'activation neuronale :

Il est important de noter que l'observation d'une activation neuronale, associée à des différences comportementales, ne démontre pas à elle seule une implication directe dans l'expression des comportements étudiés, mais elle nous permet toutefois de suggérer une telle implication. Par ailleurs, il peut sembler surprenant de ne pas avoir observé d'augmentation de l'activation neuronale en réponse au test de l'objet nouveau dans d'autres structures que le PVN, telles que par exemple l'arcopallium / PoA, qui est pourtant connu pour son implication dans l'expression des comportements de peur chez l'oiseau (Phillips et Youngren, 1971 ; Lowndes et Davies, 1995). Mais, l'absence d'activation neuronale en réponse à l'objet dans l'arcopallium / PoA observée dans cette étude n'exclut pas une implication de cette région dans le contrôle des comportements de peur. En effet, Fos n'est pas un marqueur universel d'activation neuronale et il est possible que l'activation des circuits de la peur au sein de l'arcopallium / PoA fasse intervenir des chaines de signalisation cellulaire autres que celles incluant la protéine Fos (Hoffman, 2002). De plus, au cours de notre étude, nous avons observé une relativement forte variabilité interindividuelle dans les densités de neurones marqués. Il est donc possible que cette variabilité ait masqué l'activation de certaines structures.

Discussion du test de l'objet nouveau utilisé dans cette étude :

Une autre hypothèse pouvant expliquer que l'augmentation de l'activation neuronale ne s'observe que dans une seule structure, serait que le test de l'objet nouveau n'est pas un test adapté à l'étude des réponses de peur. Cette hypothèse est soutenue par le fait que les cailles des lignées STI et LTI montrent des réponses comportementales et une augmentation du taux de corticostérone plasmatique similaire dans ce test (voir résultats de cette étude et Richard *et al*., 2007b). Cependant, nous avons choisi d'utiliser ce test car il semblait être adapté à une étude de l'activation neuronale associée à l'expression des comportements de peur. En effet, d'une part, la plupart des tests de peur utilisés chez les oiseaux induisent un changement d'environnement, susceptible d'engendrer une activation neuronale dans des régions non spécifiques de la peur, liée par exemple à l'exploration. Dans le test de l'objet nouveau, la stimulation effrayante est caractérisée par la nouveauté de l'objet et la soudaineté de la présentation, soit un nombre de facteurs plus limité que la plupart des autres tests de peur disponibles chez l'oiseau, permettant ainsi d'étudier les réponses neurobiologiques des cailles de façon plus ciblée. D'autre part, ce test induit chez les cailles des réponses comportementales et physiologiques caractéristiques d'un état de peur. En effet, nous avons pu observer, dans la présente étude, des comportements d'évitement (mesurés par le temps passé près *vs*. loin de l'objet) et des tentatives de fuite (temps passé à faire des mouvements de va-et-vient). Une précédente étude a par ailleurs démontré une augmentation du taux de corticostérone plasmatique en réponse à ce test (Richard *et al*., 2007b). Enfin, nous avons observé chez les cailles LTI une augmentation de l'activation neuronale dans le PVN en réponse à ce test, probablement en relation avec l'activation de l'axe corticotrope. Nous concluons donc que le test de l'objet nouveau induit effectivement des réponses de peur chez les cailles. Mais une question reste en suspens : pourquoi ce test n'engendre-t-il pas de différences comportementales entre les cailles les cailles STI et LTI, alors qu'elles se différencient dans la plupart des tests de peur (tels que les tests d'open field, d'émergence…) ? Pour répondre à cette question nous avons, dans une troisième étude, cherché à mieux caractériser les réponses comportementales des cailles des deux lignées face à la présentation d'un objet nouveau dans leur cage d'élevage. Cette étude nous permettra de mieux comprendre la notion du terme « peur ».

CHAPITRE 3

Caractérisation des réponses comportementales des lignées de cailles STI et LTI dans le test d'objet nouveau

Article accepté dans

Applied Animal Behaviour Science

CHAPITRE 3 :
Caractérisation des réponses comportementales des lignées de cailles STI et LTI dans le test d'objet nouveau.

Au cours de l'étude présentée dans ce troisième chapitre nous avons cherché à comprendre l'absence de différence de réponses comportementales entre les deux lignées dans le test d'objet nouveau, observée au cours du deuxième chapitre. Lors de l'étude d'activation neuronale, nous avons justifié notre choix d'employer une situation de test bien particulière qui impliquait l'absence d'un changement d'environnement pour les cailles tout en induisant une stimulation forte et durable. Nous avons ainsi choisi le test d'introduction soudaine d'un objet nouveau dans la cage d'élevage. Les caractéristiques de l'objet (taille et visibilité) et le mode d'introduction (soudaineté) rendent la stimulation forte alors que la présentation répétée de l'objet (5 fois 5 minutes sur une période de 30 minutes) permettent de prolonger la stimulation dans le temps. Ce test induit des réactions comportementales caractéristiques d'un état de peur (évitement et tentatives de fuite) chez les cailles des deux lignées. Ces réponses, observées au cours de la première présentation de l'objet, étaient toujours présentes lors de la dernière ($5^{ème}$) présentation. De plus, la première présentation de l'objet nouveau induit une augmentation de la corticostéronémie, chez les deux lignées de cailles (Richard *et al.,* 2007b). Cependant, nous n'avons pas visualisé de différences comportementales ou physiologiques entre les deux lignées de cailles pourtant connues pour se différencier dans de nombreux tests de peur (Mills et Faure, 1991 ; Jones *et al*., 1991 ; 1994). Nous nous sommes alors demandés si les caractéristiques du test d'objet nouveau pouvaient être à l'origine de l'absence de différence observée entre les deux lignées dans ce test. Nous avons supposé que la petite taille de la cage limitant le répertoire comportemental des cailles, ne permettait pas d'observer d'éventuelles différences de stratégies de réponses entre les deux lignées. Plus précisément, le dispositif utilisé ne laisse pas aux cailles la possibilité d'échapper à la situation. Or, la peur de la nouveauté se caractérise principalement par des réponses de fuite et d'évitement (Bronson, 1968 ; Jones, 1987b). Ainsi en laissant davantage la possibilité aux cailles des deux lignées de fuir, celles-ci pourraient manifester des différences dans leurs réponses comportementales. Pour tester cela, nous avons agrandi la cage de test et y avons ajouté une zone à partir de laquelle l'objet n'était pas visible. **Cette étude est présentée dans les pages suivantes sous la forme d'un article qui a été accepté dans Applied Animal Behaviour Science.**

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Selection for tonic immobility duration does not affect the response to novelty in quail

Hélène Saint-Dizier, Christine Leterrier, Frédéric Lévy, Sabine Richard *

Equipe Comportement, Neurobiologie et Adaptation, Unité de Physiologie de la Reproduction et des Comportements, UMR85 INRA-CNRS-Université de Tours-Haras Nationaux, F-37380 Nouzilly, France

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Abstract

Genetic selection on a single fear test, the tonic immobility test, seems to result in selection on fearfulness, i.e. the propensity to exhibit fear responses, whatever the fear tests used. However, the conception of fear as a single variable has been challenged by the recognition that fear is multidimensional. This study was designed to test whether genetic selection on a classic index of fear in birds, tonic immobility duration, is accompanied by changes in the response to a single dimension of fear – novelty.

Two lines of quail divergently selected for long (LTI) or short (STI) duration of tonic immobility were exposed to a novel object in their home cage. Quail of both lines showed typical fear reactions in response to novelty but there was no difference between lines. We conclude that genetic selection for tonic immobility duration does not affect all dimensions of fear, notably not novelty. Further studies are needed to investigate the dimensions of fear on which the two lines of quail could have been selected.

Keywords: Fear behaviour; Avoidance behaviour; Birds; Emotions; *Coturnix japonica*

1. Introduction

It is generally accepted that some animals are able to feel emotions, but the nature of the emotions felt is still a matter of controversy (Panksepp, 2005; Paul et al., 2005; Watt, 2005; Dawkins, 2006). Most investigations on animal emotions have been undertaken in mammals, and little is known in other classes of vertebrates. However, the understanding of emotions in animals

* Corresponding author. Tel.: +33 247427657; fax: +33 247427743.
E-mail address: Sabine.Richard@tours.inra.fr (S. Richard).

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has become a major issue today because assessment of animal welfare relies in part on the characterisation of an individual's emotional state (Désiré et al., 2002; Dawkins, 2006; Boissy et al., 2007). Moreover, emotional responses may compromise farm animal welfare. Thus, fear, an adaptive emotional response that is induced by the perception of any actual or perceived danger (Boissy, 1995; Jones, 1996), may induce maladaptive behaviour under intensive rearing conditions. For example, excessive levels of fear in poultry may induce panic responses, leading to some animals suffering from severe injuries or even dying (Mills and Faure, 1990; Jones, 1996; Faure et al., 2003; Sanotra and Weeks, 2004). These problems have prompted investigations on fear in birds.

There are good correlations between several behavioural tests used to study fear in birds, suggesting that these tests induce the same state in animals and that fear might be considered as a single underlying variable (Jones, 1996). The tonic immobility test, measuring the duration of motor inhibition induced by manual restraint, has become widely used to study fear in birds (Jones and Mills, 1983; Mills and Faure, 1986). Tonic immobility is an innate anti-predator behaviour with wide phylogenetic distribution (e.g. lizards: Prestrude and Crawford, 1970; birds: Jones, 1986; rats: McGraw and Klemm, 1973). It is one of the last fear reactions that prey may exhibit when captured by a predator, immobility being likely to minimise the probability of further attack (Gallup et al., 1971). This behaviour has been used in a genetic selection program on fear responses in quail. A number of classic tests show that Japanese quail selected for long (LTI) or short (STI) duration of tonic immobility (Mills and Faure, 1991) display divergent levels of fear: compared to STI quail, LTI quail freeze more, vocalise and move less in an open field, emerge later from a ''hole-in-the-wall'' box (Jones et al., 1991), and struggle less during restraint in a ''crush cage'' (Jones et al., 1994). Moreover, a quantitative trait loci (QTL) study, undertaken on a F2 cross between the STI and LTI lines, has suggested that tonic immobility and open-field behaviour might be under the influence of a common genome region (Beaumont et al., 2005). Thus, it has been suggested that genetic selection using a single fear test, tonic immobility, results in selection for fearfulness, i.e. the propensity to exhibit fear responses, whatever the fear test used (Jones et al., 1991; Mills and Faure, 1991).

However, the conception of fear as a single variable has been challenged by the recognition that fear is multidimensional (Archer, 1979; Thomson, 1979; Boissy, 1995; Ramos and Mormède, 1998; Désiré et al., 2002): very diverse events may generate fear responses, and it is likely that these events are not perceived equally by animals and generate diverse internal states. For example, fear of predators may not have the same significance for the animal as fear of a novel environment. The multidimensional aspect of fear has seldom been taken into account in studies on fear in birds, the majority of tests used involving several dimensions of fear. For example, in both the tonic immobility test and the open-field test, the bird experiences capture by a human, transfer to a novel environment, and in most cases social isolation. Each of these three factors is known to induce fear, but it is very likely that they are perceived and processed differently by the bird. In line with this hypothesis, several authors have highlighted the weaknesses of the correlations found between various fear tests, notably in Japanese quail (Archer, 1973; Mignon-Grasteau et al., 2003; Miller et al., 2005). Therefore, in order to understand better the determinants of fear in birds, fear tests need to be re-evaluated, taking into account their multidimensional nature.

The present experiment was designed to test whether genetic selection using a classic index of fear in birds, tonic immobility duration, is accompanied by changes in the response to a single dimension of fear: novelty. Novelty was chosen as the first dimension to be investigated because it is one of the main factors known to evoke fear responses in birds, as in mammals (Bronson,

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1968; Boissy, 1995; Jones, 1996). The experiment consisted in comparing the behaviour of STI and LTI quail in response to the introduction, at a distance, of a novel object into their home cage, minimising the influence of other dimensions. Because predator-like stimuli are known to induce fear intrinsically, independently of their novelty (Boissy, 1995), we chose to use a novel object that had no *a priori* biological value. It was expected that quail of both lines would exhibit fear responses. Moreover, based on evidence in the literature that STI and LTI quail consistently differ in their fear behaviour (Jones et al., 1991, 1994; Mills and Faure, 1991), we predicted that LTI quail would present more intense fear responses than STI quail.

2. Materials and methods

2.1. Subjects

The experiment involved 40th generation Japanese quail (*Coturnix japonica*) selected for long tonic immobility (LTI, $n = 18$) and short tonic immobility duration (STI, $n = 18$), selected and maintained at the Unité de Recherches Avicoles, Nouzilly, France (Mills and Faure, 1991). The selection process has been continued since the foundation of the lines and at the 40th generation, the mean duration of tonic immobility (±standard deviation), measured as in Mills and Faure (1991), was 228 s (±80) in the LTI line and 14 s (±35) in the STI line. In this experiment, male and female chicks of both lines were wing-banded on the day of hatching and transferred to a communal floor pen maintained at approximately 40 °C by continuous illumination with commercial brooder lamps. Over the second and third weeks after hatching, the temperature was gradually reduced to 20 °C and then stabilised. On the 21st day after hatching, the photoperiod was adjusted to a 16:8 h light:dark schedule. Standard food and water were freely available at all times, unless otherwise specified.

Throughout the experiment the birds were treated according to the European Communities Council Directive of November 24, 1986 (86/609/EEC). All procedures described here fully comply with French legislation on research involving animals.

2.2. Testing apparatus and procedure

At the 8th week of age, 36 adult males were transferred to a testing room maintained at 20 °C and under a 16:8 h light:dark photoperiod. The choice to study quail of one sex was made to reduce interindividual variability and males were chosen to avoid the changes in behaviour provoked in females by laying, which may occur at rather unpredictable times. All quail were housed individually in PVC cages (see Fig. 1 for measurements) with wood-shavings on the floor and an opaque PVC roof. The front wall of each cage was made of clear plexiglas so that the quail could be observed with a video camera. The cages were designed in such a way that an object could be introduced into the corner of the cage near the food trough via a

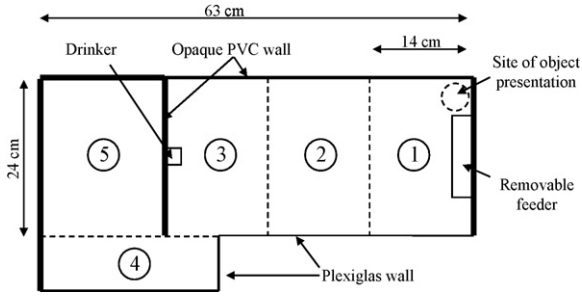

Fig. 1. Diagram of an experimental cage viewed from the top with roof removed, showing the site where the novel object was dropped and the five zones used for behaviour analysis.

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cylindrical PVC chimney in the roof. The object consisted of a 21 cm × 4 cm multicoloured cylinder covered with 2-cm horizontal strips of blue, yellow, grey, red, black and white tape, similar to that used by Jones (1987). The object was suspended by a string so that its base remained 5–6 cm above the floor. This apparatus allowed the object to be introduced into the cage and removed from a distance, with minimal disturbance to the quail, the experimenter remaining out of sight of the bird during the procedure. The birds also had access to a compartment from which they could not see the object (Fig. 1: zone 5).

All quail were tested in two situations: the object situation and the control situation. To control for an effect of testing order, half of the quail of each line were tested first in the object situation (O1) and then in the control situation (C2), and the other half were tested in the reverse order (C1 and O2). The first test was performed 7 days after the quail had been transferred into individual cages, and the second 2 days later. The entire experimental procedure was performed blind with respect to the line. On test days, the food trough of every cage was removed for 40 min before the test. Preliminary studies have shown that a longer period of food deprivation induced a strong motivation to peck at the food, which strongly reduced the behavioural repertoire of the birds. To habituate the quail to the procedure, the food trough was removed from each cage for 2 h every day, during the week preceding testing and between the two tests. In the object situation, the food trough was replaced and the object was dropped into the cage as soon as the quail pecked at the food. If a quail failed to peck at the food, the object was dropped 60 s after the return of the food trough. The object was withdrawn after 5 min and the quail was left undisturbed for 1 min. The object presentation procedure was then repeated four more times at 1-min intervals, without waiting for the quail to peck in the food trough (Fig. 2). Video recording of quail behaviour was used in order to minimise disturbance from the experimenter. In the control situation, the food trough was replaced and the behaviour of each quail was videoed without disturbance for 30 min from the moment they pecked at the food. If a quail failed to peck at the food, the video recording started 60 s after the return of the food trough.

2.3. Behaviour analysis

To analyse the locomotor behaviour of the quail, the cage was subdivided into five zones: zone 1 included the food trough and the site of object presentation; zones 2 and 3 were intermediate zones; zone 4 was the furthest area from which the quail could see the object, and zone 5 was the area from which the quail could not see the object (Fig. 1). The amount of time spent in zones 1, 4 and 5 were recorded. The number of quail which displayed freezing and the time spent pacing were also recorded. Freezing behaviour was defined as complete immobility, occurring suddenly with an abrupt interruption of ongoing activities and lasting for more than 3 s, sometimes including very small head movements such as those associated with breathing. Pacing was defined as repetitive movements fixed in form and orientation, including jumps and head movements.

The behaviour of quail in the object situation was analysed during the first and fifth presentations of the object and their behaviour in the control situation was analysed at corresponding time windows. All observations were made by a single experimenter, who did not know the line identity of the quail, using The Observer 3.0 software (Noldus Information Technology, The Netherlands, 1993).

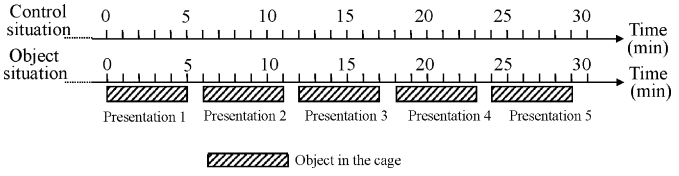

Fig. 2. Diagram illustrating the time-course of the test procedure in the object situation and in the control situation, starting when a quail resumed pecking at the food after the return of the food trough. The procedure during the object situation consisted in five 5-min object presentations separated by 1-min breaks. Each quail was tested in both situations.

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2.4. Statistical analysis

Because the data were not normally distributed, nonparametric statistical analyses were performed using Statview 5.0 software (SAS Institute Inc., USA, 1992–1998). In all tests, $p < 0.05$ was considered as significant. Mann–Whitney tests were performed to check a possible effect of testing order in each situation: quail tested on the first and second day of testing in the control situation were compared (C1 vs. C2), and the same comparison was made for the object situation (O1 vs. O2). The behavioural parameters recorded in the two situations were compared (object vs. control) within each line (STI and LTI) using Wilcoxon tests (paired comparisons). To evaluate the line effect on the response to the object, we used a compound variable reflecting the difference between the two situations (object–control) for each variable investigated. These compound variables were compared between STI and LTI quail using Mann–Whitney tests. Changes in the variables between the first and fifth object presentations were assessed within each experimental group using Wilcoxon tests. The occurrence of freezing behaviour was compared between situations (control vs. object) as well as between presentations (first vs. fifth) using a MacNemar test (paired comparisons). The effect of line on the occurrence of freezing behaviour was tested using a Fisher's exact test. For all comparisons, the degree of freedom was 1. Box-plot diagrams were used to illustrate the results. Values illustrated by a box-plot are the median, upper and lower quartiles, 10th and 90th percentiles.

3. Results

3.1. Effect of testing order

No difference was found in the control situation between quail tested on the first day of testing (C1) and those tested on the second day (C2), ($p > 0.05$ for each variable, Mann–Whitney tests). Similarly, no difference was found between the 2 days of tests in the object situation (O1 vs. O2, $p > 0.05$ for each variable). Therefore, the data from the two test days (in either the control or the object situation) were combined.

3.2. Behaviour during the first object presentation

The time spent in zone 1 (where the object was dropped) was significantly shorter in the object situation than in the control situation in both lines ($p < 0.01$ for each line, Wilcoxon tests Fig. 3A). The time spent in zone 4 (area furthest away from the object from which the quail could still see the object) was significantly longer in the object situation than in the control situation in the STI line ($p = 0.02$, Fig. 3A), and the same trend was observed in the LTI line ($p = 0.06$, Fig. 3A). The time spent in zone 5 (from which the quail could not see the object) did not differ significantly between the two situations in either line ($p > 0.1$ for each line, Fig. 3A). The time spent pacing was significantly longer in the object situation than in the control situation in both lines ($p < 0.001$ for each line, Fig. 4A). The number of animals displaying freezing did not differ significantly between the two situations in either line (control situation vs. object situation: LTI, 1 vs. 6 quail; STI, 0 vs. 4 quail; $p > 0.05$ for each line, MacNemar tests).

3.3. Behaviour during the fifth object presentation

There was no significant difference between the two situations (control vs. object) for the time spent in zones 1, 4 and 5 in either line during the fifth object presentation ($p > 0.1$ for each comparison, Wilcoxon tests, Fig. 3B). The time spent pacing was significantly longer in the object situation than in the control situation in both lines ($p < 0.01$ for each line, Fig. 4B). The number of

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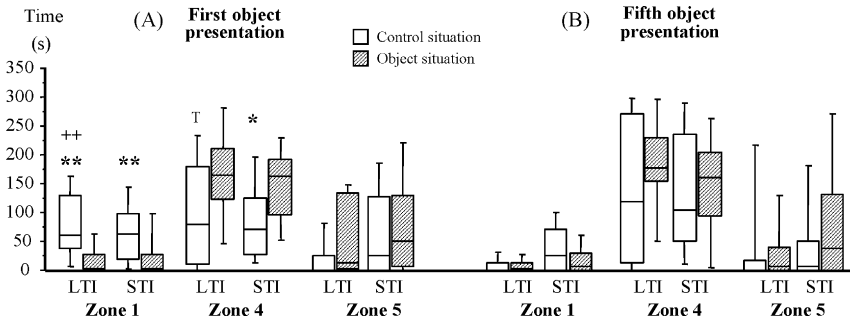

Fig. 3. Box-plot diagrams illustrating the time spent in three different zones of the cage by quail selected for long (LTI) or short tonic immobility duration (STI) during the first (A) and the fifth (B) presentations of a novel object (hatched bars) and during a control situation (open bars). Each quail was tested in both situations (object and control). Zone 1: area of the cage including the food trough and the site of object presentation. Zone 4: area of the cage furthest away from the object from which the quail could see the object. Zone 5: area of the cage from which the quail could not see the object; $n = 18$ for each bar. Comparison between control and object situations, $^{**}p < 0.01$; $^{*}p < 0.05$; T: $p < 0.1$ (Wilcoxon test). Comparison between the first and fifth presentations, $^{++}p < 0.01$ (Wilcoxon test).

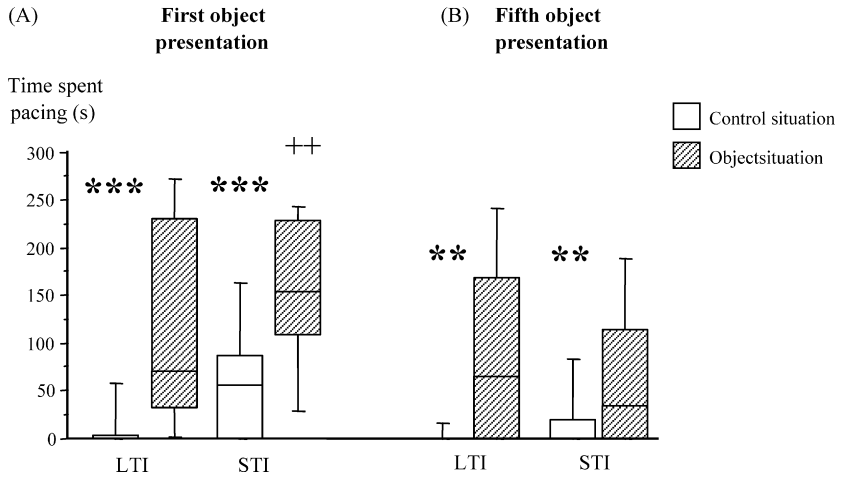

Fig. 4. Box-blot diagrams illustrating the time spent pacing by quail selected for long (LTI) or short tonic immobility duration (STI) during the first (A) and the fifth (B) presentations of a novel object (hatched bars) and during a control situation (open bars); $n = 18$ for each bar. Comparison between control and object situations, $^{***}p < 0.001$; $^{**}p < 0.01$ (Wilcoxon test). Comparison between the first and fifth presentations, $^{++}p < 0.01$ (Wilcoxon test).

quail displaying freezing was significantly higher in the object situation (LTI = 6; STI = 8) than in the control situation (LTI = 0; STI = 0) in both lines ($p < 0.05$ for each line, MacNemar tests).

3.4. Effect of the line

There was no significant difference between the two lines in the compound variable reflecting the difference between the two situations (object–control), whatever the variable involved ($p > 0.05$ for each variable, Mann–Whitney and Fisher's exact tests).

The only differences observed between the two lines were in the behavioural changes over time: during the object situation, STI quail showed a significant decrease in the time spent pacing

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between the first and fifth object presentations ($p < 0.01$, Wilcoxon test, Fig. 4), while this pattern was not observed in LTI quail. In the object situation, there was no other significant change between the first and fifth object presentations. In the control situation, LTI line quail showed a significant decrease in the time spent in zone 1 (where the food trough was located) between the first and fifth periods of observation ($p < 0.01$, Fig. 3), while this pattern was not observed in STI line quail. In the control situation, there were no other significant changes in any other variable between the first and fifth periods of observation.

4. Discussion

Japanese quail of two lines (STI and LTI) divergently selected for tonic immobility duration, a classic index of fear, did not differ in their behavioural response to novelty, the introduction of a novel object into the home cage inducing similar reactions in the two lines. Quail of both lines spent significantly less time in the zone where the object was introduced, in the object situation than in the control situation. In the object situation, they also spent more time in the part of the cage furthest away from but still in sight of the object. Quail of both lines also exhibited more pacing behaviour in the object than in the control situations. Finally, the number of quail displaying freezing in the object situation was similar in the two lines. Thus, none of the measured variables differed significantly between the STI and LTI lines. The only difference between the two lines was that STI quail exhibited a slightly more pronounced habituation, with reduced pacing behaviour between the first and fifth object presentations, while no sign of habituation was observed in the LTI line with this variable. However, this observation was not sufficient to conclude that the two lines of quail differed in their response to novelty.

Up to now, the behaviour of STI and LTI quail has been shown to differ in many fear tests (Jones et al., 1991, 1994; Mills and Faure, 1991). However, presentation of a novel object is not the first test in which no difference between lines has been observed: a previous study by Valance et al. (2007) found that the behaviour of STI and LTI quail did not differ after exposure to a novel sound. Evidence that STI and LTI quail do not differ in response to presentation of either a novel object or a novel sound indicates that genetic selection for tonic immobility duration is not accompanied by changes in response to novelty, although more novelty tests would be necessary to validate this statement. We conclude that reaction to novelty is to a very little, if any, degree influenced by genetic selection on a classic fear test: the tonic immobility test.

Since STI and LTI quail are known to differ in many fear tests, the present results could suggest that the introduction of a novel object into a quail's home cage is not an appropriate fear test. However, the test used does induce typical fear responses. For example, the number of quail displaying freezing was significantly higher in the object than in the control situation. Freezing is a classic index of fear in birds, as in mammals (Duncan, 1985; Boissy, 1995). In addition, quail exhibited significantly more pacing behaviour in the object than in the control situation. Pacing in a closed space is usually interpreted as an attempt to escape (Murphy, 1978). Finally, avoidance reactions were observed during the first presentation, indicated by a decrease in the time spent in the zone where the object was introduced and an increase in the time spent in the zone furthest away from the object. These results are in line with several studies showing that novelty induces typical fear reactions in birds (Bronson, 1968; Jones, 1996), including quail (Richard-Yris et al., 2005). We conclude that the introduction of a novel object into the home cage is a good way to induce fear reactions in quail.

It may also be argued that the introduction of a novel object induced such high fear levels that the behavioural responses of all quail reached a "ceiling" that was similar in both lines.

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However, at the behavioural level, results show that fear was not at its highest level, at least during the fifth presentation, when the avoidance reactions (as indicated by the time spent in zones 1 and 4) observed in the first presentation were not maintained in either line. In addition, pacing in the object situation decreased significantly between the first and fifth presentations of the novel object in STI quail. Finally, at the endocrine level, recent data indicate that a novel object induces a lower rise in plasma corticosterone levels than restraint in a ''crush cage'' (Hazard et al., 2005; Richard et al., 2007), suggesting that quail do not perceive a novel object as the most frightening situation. Even though a potential ''ceiling'' effect was not tested in the present experiment, we consider the introduction of a novel object into a quail's home cage as a mild fear test since it induces fear reactions which are typical but not as strong as in other fear-inducing situations. It therefore appears unlikely that the similar reaction of STI and LTI quail to this test could be accounted for by a ''ceiling'' effect.

Another argument that might explain the absence of difference between the two lines is that a small cage limited their behavioural repertoire, masking potential behavioural differences between lines. It is known that repetitive or stereotypic behaviour, such as pacing, is often displayed by animals in small or under-stimulating environments (Mason and Latham, 2004). However, in the present study we observed a variety of fear behaviour, including avoidance of the novel object, freezing and an increase in pacing that was interpreted as an attempt to escape (Murphy, 1978). Moreover, the cages provided a hiding area where quail could avoid seeing the object (zone 5), and quail of both lines did not use this area more in the object situation than in the control situation. These results suggest that quail controlled the situation by maintaining visual contact with the object. As a whole, the quail's behavioural repertoire proved to be diverse, and we conclude that the absence of difference between the two lines was not induced by specific characteristics of the cage.

The fact that divergent selection for tonic immobility duration, a classic index of fear, was not accompanied by differences between STI and LTI quail in their response to novelty, a typical dimension of fear (Bronson, 1968; Boissy, 1995; Jones, 1996), raises the question about the dimensions of fear on which the two lines of quail could have been selected. In other words, if the STI and LTI lines were selected on aspects of fear other than novelty, what were they? During the tonic immobility test, birds are exposed to at least three factors of fear: (1) physical transfer by a human, which might simulate an attack by a predator, (2) social isolation and (3) exposure to a novel environment. The main differences with the introduction of a novel object are human intervention and a change in the physical and social environment. Similarly, in three tests that have previously been used to compare STI and LTI quail (Jones et al., 1991, 1994), namely, the open-field test, the emergence test and the restraint test in a ''crush cage'', birds are also exposed to capture by a human and transfer to a novel environment, whereas in the present investigation, the object was introduced remotely with minimum human interference and without any change in environment. In previous studies using a novel object as a fear stimulus and showing different behavioural responses in STI and LTI quail, human intervention was also involved, since the object was introduced into the cage by a human hand (Richard-Yris et al., 2005). By contrast, when a novel sound was produced in the home cage without any human intervention, STI and LTI quail showed similar behavioural responses (Valance et al., 2007). This suggests that the absence of difference between the two quail lines when presented with a novel object was due to the absence of two crucial factors in the tonic immobility test: human intervention and a change in the physical and social environment. It could also suggest that these were the only aspects of fear being selected in the STI and LTI lines. Additional studies are nevertheless necessary to test the respective influences of human

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transfer and a change in the physical and social environment on the behaviour of STI and LTI quail in response to the introduction of a novel object.

5. Conclusion

Genetic selection on tonic immobility does not allow selection on all aspects of fear. These results confirm that the concept of fear comprises several independent variables and that a single fear test, such as the novel object test or the tonic immobility test, may not reflect all aspects of fear. Thus, the aspects of fear involved in behavioural tests should be defined precisely and their interactions analysed, rather than treating fear as a one-dimensional concept. A better knowledge of the components of fear will help in understanding the way animals perceive their environment and in designing farming practices that do not compromise animal welfare.

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Commentaires sur le Chapitre 3

Dans l'expérience présentée dans ce troisième chapitre, les cailles des deux lignées montrent un évitement de l'objet et utilisent la possibilité de s'éloigner davantage (augmentation du temps passé dans la nouvelle zone la plus éloignée de l'objet et d'où l'objet est toujours visible). Elles présentent aussi des comportements de freezing. Notons que la présence de freezing dans ce nouveau dispositif (comportement qui ne s'observait pas dans la petite cage sans extension) peut être mise en relation avec l'agrandissement de la taille de la cage. En effet, en milieu naturel, le comportement de freezing s'observe lorsque l'oiseau se trouve loin de son prédateur (Gallup, 1974). Enfin, les cailles montrent aussi une augmentation du temps passé à faire des mouvements de va-et-vient le long de la paroi de la cage en réponse à l'objet. Ce comportement, qui est aussi exprimé en situation témoin en proportion moindre, peut être décrit comme un comportement de marche stéréotypée et pourrait traduire une réponse de l'animal placé dans un environnement étroit et non stimulant (Mason et Latham, 2004). Mais ce comportement s'exprime de façon plus élevée en réponse à l'objet nouveau. Nous avons donc conclu que ce comportement pouvait être interprété comme des tentatives de fuite (Murphy, 1977) en réponse à la présentation de l'objet, alors que l'établissement de ce comportement en faible proportion en situation témoin, serait dû aux conditions expérimentales, et notamment au maintien des animaux en isolement social pendant une semaine (Mason et Latham, 2004). Par ailleurs, dans cette étude, l'environnement de la cage est agrandi et diversifié par un espace où l'objet n'est plus visible. Dans ce nouveau dispositif, les cailles montrent un répertoire comportemental varié (comportement alimentaire, bain de poussière, déplacement, évitement, freezing, …) et n'utilisent pas la possibilité de ne pas voir l'objet : le temps de présence dans la zone d'où l'objet n'est pas visible est similaire entre la situation témoin et la situation de test avec l'objet. Nous pouvons envisager deux hypothèses pour expliquer ce fait : le contrôle de la situation par les cailles passerait par le maintien d'un contact visuel avec le stimulus effrayant et / ou le maintien d'un contact visuel avec l'objet reflèterait le conflit de motivation entre la fuite et l'exploration de l'objet (Murphy, 1977 ; 1978). Quoiqu'il en soit, l'ensemble des réponses comportementales des cailles des deux lignées dans ce nouveau dispositif sont caractéristiques d'un état de peur mais restent similaires, à l'image de ce qui avait été observé dans le test d'objet nouveau utilisé dans l'étude précédente. **Ainsi, la modification de l'environnement de test (augmentation de la taille de la cage et ajout d'une zone d'où l'objet n'est plus visible) n'a pas induit de**

différence comportementale entre les deux lignées de cailles. Les caractéristiques de la cage ne sont donc pas à l'origine de l'absence de différence entre les deux lignées.

Une telle absence de différence comportementale entre les deux lignées de cailles a aussi été observée au cours d'une étude récente impliquant la présentation d'un son nouveau dans la cage d'élevage (Valance *et al.,* 2007). Ces résultats nous ont amenés à rechercher plusieurs hypothèses pouvant expliquer l'absence de différence dans ces deux tests. Ces hypothèses ont été discutées au sein de l'article présenté dans ce chapitre mais celle qui semble la plus probable concerne les singularités des tests d'objet / de son nouveau par rapport aux autres tests précédemment utilisés pour comparer les lignées STI et LTI. En effet, la différence majeure est que la présentation de l'objet nouveau (ou d'un son nouveau) est réalisée dans la cage d'élevage alors que les autres tests impliquent une manipulation par l'homme et un changement d'environnement (tests d'immobilité tonique, d'open field, d'émergence par exemple). Or, ces deux aspects (manipulation par l'homme et changement d'environnement) sont des composantes essentielles du test d'immobilité tonique, qui est à la base de la sélection des deux lignées de cailles. Cette observation ouvre de nouvelles perspectives de recherche en relation avec le caractère multidimensionnel de la notion de peur, qui est rarement pris en compte dans les études menées chez les oiseaux. **Nous pouvons ainsi supposer que la réactivité à la nouveauté (aspect essentiel dans le test d'objet nouveau) est une dimension de la peur qui n'est pas déterminante pour la sélection des cailles STI / LTI puisque les deux lignées ne se différencient pas dans ce test. Le changement d'environnement et/ou la manipulation par l'homme pourraient, par contre, être des dimensions de la peur qui seraient à l'origine des différences entre les deux lignées.** Cette hypothèse nécessite cependant d'être confirmée par des tests supplémentaires ne faisant intervenir qu'une seule des dimensions à la fois. Ces tests pourraient nous permettre non seulement de préciser les dimensions de la peur sur lesquelles les lignées ont été sélectionnées, mais aussi de montrer que différentes réactions émotionnelles regroupées sous le terme de peur sont à prendre en compte pour l'étude des mécanismes liés à la peur. Si tel est le cas, un test de peur donné tel que le test de l'objet nouveau, induirait des réponses liées à certains aspects de la peur. Un tel test est-il alors adapté à l'étude des mécanismes neurobiologiques contrôlant les réponses de peur ? Et plus généralement, existe-t-il un test de peur plus adapté que les autres pour étudier ces mécanismes ? Nous tenterons d'apporter des éléments de réponse à cette question dans la discussion générale de ce mémoire.