Implication différentielle de sous-régions de l'arcopallium / amygdale palliale postérieure dans le contrôle des comportements de peur chez l'oiseau

Article soumis dans
Behavioral Neuroscience

CHAPITRE 1 :

Implication différentielle de sous-régions de l'arcopallium / amygdale palliale postérieure dans le contrôle des comportements de peur chez l'oiseau.

Notre première approche expérimentale a ciblé la seule région du cerveau d'oiseau initialement connue pour être impliquée dans l'expression des comportements de peur : l'arcopallium / amygdale palliale postérieure (PoA). Différentes études fonctionnelles ont en effet démontré l'implication de cette région, souvent comparée à l'amygdale des mammifères (Zeier et Karten, 1971 ; Veenman *et al.,* 1995 ; Reiner *et al.,* 2004 ; Atoji *et al*., 2006), dans le contrôle central des comportements de peur chez les oiseaux. Ainsi, des lésions de l'arcopallium / PoA provoquent une diminution des manifestions comportementales de la peur chez les oiseaux (Phillips et Youngren, 1986 ; Lowndes et Davies, 1995) alors que des stimulations électriques les augmentent (Phillips et Youngren, 1971, Andrew et Oades, 1973). De plus, des études neuroanatomiques ont suggéré que cette région se subdivise fonctionnellement en deux principales sous-régions. En effet, ces études montrent que la partie postérieure de l'arcopallium / PoA projette vers de nombreuses structures considérées comme « limbiques », telles que le noyau du lit de la strie terminale et l'hypothalamus, suggérant une implication possible dans l'expression des comportements de peur (Zeier et Karten, 1971 ; Davies *et al.,* 1997a). A contrario, la partie antérieure de l'arcopallium projette majoritairement vers des structures considérées comme « sensorimotrices » telles que le lobe optique, la formation réticulée latérale et le noyau du pont latéral, supposant un rôle plus général dans le contrôle des mouvements (Zeier et Karten, 1971 ; Davies *et al*., 1997a). Compte tenue de l'absence de données fonctionnelles venues valider cette hypothèse de subdivision fonctionnelle de l'arcopallium / PoA, les délimitations des subdivisions internes de l'arcopallium / PoA restent sujettes à controverse. **L'objectif de notre première étude fut d'évaluer les rôles spécifiques de la partie antérieure et de la partie postérieure de l'arcopallium / PoA dans l'expression des comportements de peur chez l'oiseau. Pour cela, nous avons utilisé une approche lésionnelle et évalué les conséquences de cette inactivation sur les comportements de peur. Compte tenu des données neuroanatomiques présentées ci-dessus, nous avons fait l'hypothèse que les lésions de la partie antérieure et de la partie postérieure devraient entraîner des conséquences différentes sur les réponses de peur.**

Nous avons choisi d'utiliser des lésions électrolytiques plutôt que chimiques de façon à léser tous les neurones de la sous-région visée. En effet, lors d'une étude lésionnelle réalisée chez le perroquet, des lésions à l'acide kaïnique n'ont pas affecté tous les neurones de la région visée contrairement à des lésions électrolytiques (Heaton et Brauth, 2000).

Nous avons aussi choisi de tester les animaux dans différents tests de peur. En effet, comme nous l'avons vu dans l'introduction, les comportements de peur de l'oiseau peuvent être induits par diverses situations et il est possible que certaines structures soient davantage sollicitées dans certaines situations de peur que dans d'autres. Nous avons choisi d'utiliser le test de présentation d'un objet nouveau pour tester l'effet des lésions sur les réponses face à la nouveauté dans un environnement familier ; le test d'immobilité tonique, quant à lui, mime la rencontre avec un prédateur ; enfin, les tests d'open-field et d'émergence, permettent de tester les réponses induites par le placement en environnement nouveau, avec ou sans abri potentiel (voir introduction générale).

Pour finir, nous avons utilisé dans cette étude des cailles de la lignée LTI. En effet, nous avons choisi d'utiliser des cailles montrant, par nature, des comportements de peur exacerbés, car d'après la littérature, nous nous attendions à ce que les lésions de l'arcopallium / PoA entraînent une diminution des comportements de peur. De plus, une précédente étude avait montré que des lésions de l'arcopallium / PoA entraînaient une diminution des comportements de peur dans les tests d'immobilité tonique, d'open field et d'émergence chez les cailles de la lignée LTI, mais pas chez les cailles STI (Davies *et al.,* 1997b).

Cette étude est exposée sous forme d'un article qui a été soumis à la revue Behavioral Neuroscience. La réalisation de cette étude a été précédée d'une mise au point méthodologique qui sera présentée dans les pages suivantes.

Chapitre 1, Etude préliminaire :
Mise au point d'une méthode d'anesthésie profonde chez les cailles de la lignée LTI

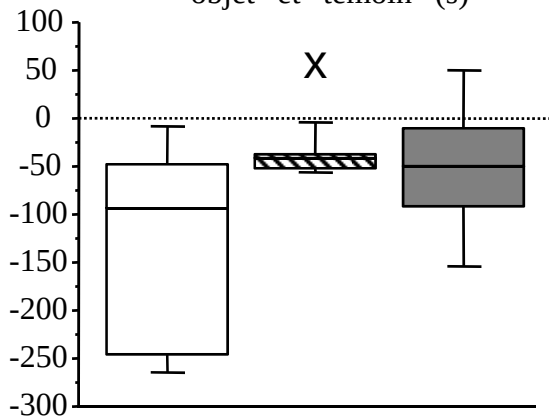
Réaliser des lésions électrolytiques dans le cerveau d'un animal requiert une intervention chirurgicale sur des individus profondément anesthésiés, placés dans un appareil stéréotaxique. Une première étape a donc consisté à trouver le dosage d'anesthésiant (mélange de xylazine et de kétamine) adéquat car il existe peu de données dans ce domaine chez l'oiseau (Pionneau, 1985). Les tout premiers essais, réalisés sur des cailles de la lignée LTI, ont montré qu'il existait une forte variabilité interindividuelle et que de nombreux facteurs environnementaux et physiologiques intervenaient dans cette variabilité. Nous avons par exemple observé de fortes différences de sensibilité à la température ambiante avant, pendant et après l'opération chirurgicale. Nous avons aussi pu observer que le dosage utilisé ne pouvait être directement proportionnel aux poids des cailles, les cailles de poids élevé montrant généralement une sensibilité au mélange anesthésique beaucoup plus forte que les cailles de poids moyen. Ces travaux par essais et erreurs nous ont permis de définir l'environnement le mieux adapté aux cailles les plus sensibles : température légèrement au-dessus de celle utilisée en condition d'élevage (entre 20 et 25°C) pendant les 24h qui précédaient et qui suivaient l'opération, bouillottes chauffantes pendant l'opération et jusqu'à 2h après le réveil ; retrait de l'eau et de l'aliment 2h avant l'opération. Nous avons aussi adapté le dosage d'anesthésiant en fonction des réactions de chaque caille : nous réalisions une première injection (1,25 ml/kg) qui permettait d'anesthésier profondément certains individus mais pas tous, que nous complétions par une seconde injection (1.25 ml/kg) suivant les réactions de la caille, 10 à 15 min après la première injection.

A la suite de ces mises au point, nous avons réalisé les premières opérations chirurgicales sur des individus SHAM (anesthésiés et opérés mais non lésés) puis comparé leur comportements à des individus témoins (ne subissant aucun traitement particulier), une semaine après ce protocole opératoire. Au cours des premières observations, il nous a semblé que les individus SHAM présentaient moins de comportements de peur que les individus témoins. Cette observation nous a conduit à suggérer que l'anesthésie et/ou l'opération en elle-même pourraient être à l'origine de cette réduction des comportements de peur. Pour tester cette hypothèse, nous avons comparé les réactions de cailles anesthésiées (mais non opérées), de cailles opérées mais non lésées (SHAM) et de cailles

Figures Chapitre 1:

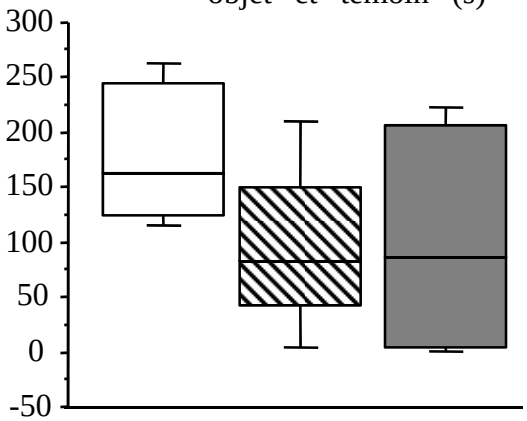
A

Différence pour la variable “temps passé dans la zone de présentation” entre les situations “objet” et “témoin” (s)



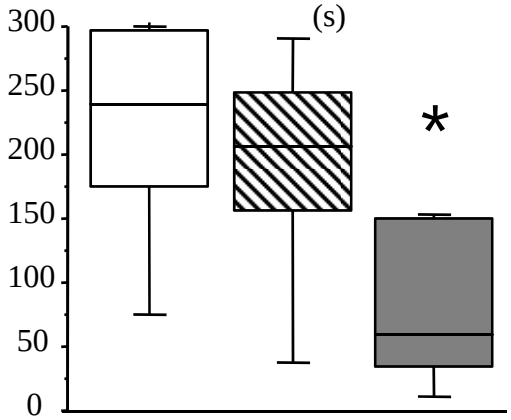
B

Différence pour la variable “temps passé à essayer de s’échapper” entre les situations “objet” et “témoin” (s)



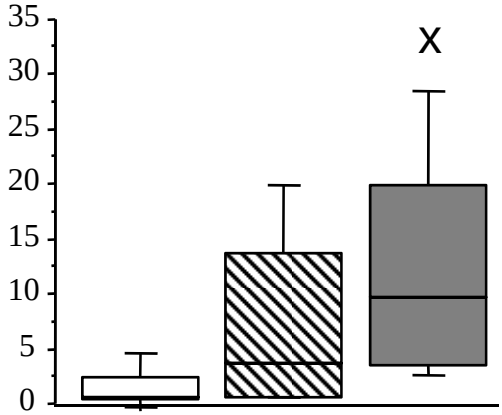
C

Latence de sortie de la zone centrale dans le test d’open field (s)



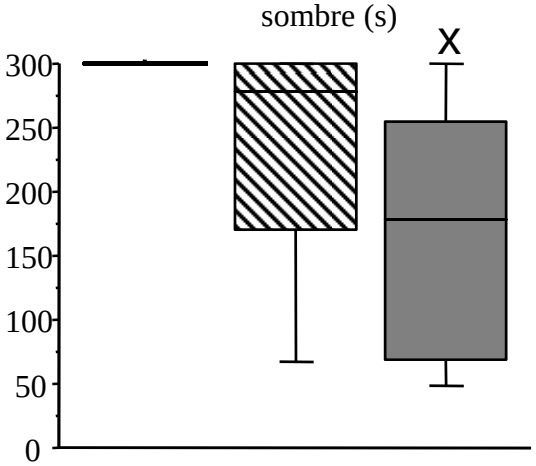
D

Nombre de changements de zones dans le test d’open field



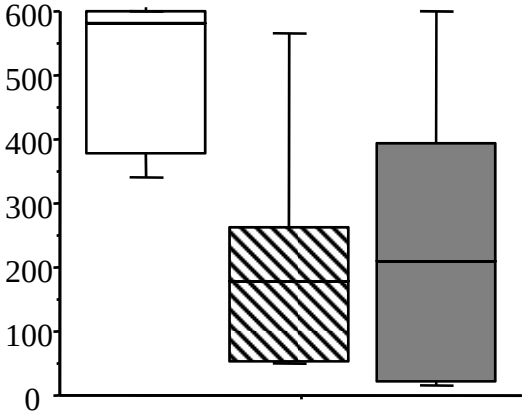
E

Latence d’émergence du compartiment sombre (s)



F

Durée d’immobilité tonique (s)



Témoin (n=5)

Anesthésié (n=6)

SHAM (n=9)

X Différents du groupe Témoin

* Différents des deux autres groupes

témoins non manipulées dans les quatre tests de peur sélectionnés pour l'étude à suivre : test de présentation d'un objet nouveau, test d'émergence, test d'open-field et test d'immobilité tonique. Les cailles étaient testées une semaine après les traitements.

Dans le test d'objet nouveau, nous avons observé chaque caille dans deux situations : lors de l'introduction soudaine d'un objet pendant 5 min dans la cage d'élevage et lors d'une situation témoin (sans présentation de l'objet). Nous avons évalué l'évitement de l'objet en mesurant le temps passé dans la zone de présentation de l'objet dans les deux situations. Nous avons aussi enregistré le temps passé à réaliser des déplacements indiquant des tentatives de fuite. Les analyses statistiques ont été réalisées sur le changement de comportement induit par la présentation de l'objet (différence « objet » moins « témoin »). Dans ce test, le traitement préalable a influencé le comportement de peur des cailles, les individus anesthésiés passant moins de temps à éviter l'objet que les cailles témoins (test de Kruskal-Wallis suivi de tests de Mann-Whitney, $p < 0,05$; voir figures chapitre 1 A et B). Dans le test d'open-field, nous avons pris en compte deux mesures de l'exploration de l'environnement qui sont la latence à sortir de la zone centrale (où les cailles étaient placées en début de test) et le nombre de zones traversées au cours du test pendant 5 min. Dans ce test, le traitement préalable a influencé le comportement de peur des cailles, les individus SHAM sortant plus vite de la zone centrale que les cailles témoins et anesthésiées, et traversant plus de zones que les cailles témoins (tests de Kruskal-Wallis suivi de tests de Mann-Whitney, $p < 0,05$; voir figures C et D). Dans le test d'émergence, nous avons placé les cailles dans un compartiment obscur et mesuré la latence d'émergence de ce compartiment pour aller vers un compartiment éclairé et ouvert. Nous avons observé que le traitement préalable influençait le comportement de peur des cailles, les individus SHAM émergeant plus rapidement que les cailles témoins (test de Kruskal-Wallis suivi de tests de Mann- Whitney, $p < 0,05$; voir figure E). Enfin, les cailles SHAM et anesthésiées ont eu tendance à passer moins de temps en immobilité tonique que les cailles témoins (test de Kruskall-Wallis, $0.05 < p < 0.1$ suivi de tests de Mann-Whitney, $p < 0,05$; voir figure F).

Ainsi, les résultats montrent que les cailles SHAM (anesthésiées et opérées) présentent significativement moins de comportements de peur que les cailles témoins sur certaines variables prises en compte. Les cailles anesthésiées, quant à elles, se différencient des cailles témoins de façon moins flagrante, mais montrent tout de même moins de

comportements de peur que les cailles témoins sur une variable du test de l'objet nouveau. Nous avons conclu que le protocole opératoire avait un effet sur les cailles. Plusieurs hypothèses peuvent être avancées pour tenter d'expliquer ces résultats. Il est vraisemblable que les nombreuses manipulations des cailles par les expérimentateurs au cours du protocole aient pu modifier les comportements des cailles dans les tests de peur. Il a par exemple été montré que des manipulations par l'homme répétées diminuaient les réponses de peur ultérieures de poussins domestiques dans différents tests de peur (Jones et Faure, 1981). Un éventuel effet physiologique à long terme du mélange anesthésique, se prolongeant plus d'une semaine après son administration, nous semble moins plausible. En effet, si à court terme il est connu que la kétamine entraîne une diminution des comportements de peur chez les mammifères (Pietersen *et al.,* 2006), à notre connaissance aucun effet à long terme sur les émotions n'a été décrit après une injection aiguë de kétamine. Enfin, nous ne pouvons pas exclure un éventuel effet de l'opération en elle-même. En effet, les cailles SHAM ont montré moins de comportements de peur que les cailles anesthésiées sur une variable du test d'open field.

Nous avons donc conclu que le protocole chirurgical, indépendamment de l'application de lésions électrolytiques, diminuait les réponses de peur des cailles. Ces résultats nous ont conduit à utiliser comme groupe « témoin » dans l'expérience de lésions de l'arcopallium / PoA, un groupe de cailles opérées mais non lésées (SHAM) pour comparer leurs comportements à ceux de cailles lésées. Notons que si ces cailles SHAM montrent moins de comportements de peur que des cailles témoins, s'agissant de cailles LTI, leur niveau de peur reste élevé en comparaison de cailles non sélectionnées. Par exemple, des cailles non sélectionnées montrent en moyenne des durées d'immobilité tonique proche de 80 s alors que les cailles SHAM ont montré une durée moyenne de 249 s dans le test d'immobilité tonique.

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Subdivisions of the arcopallium / posterior pallial amygdala complex are differentially involved in the control of fear behavior in the Japanese quail.

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Abstract

Growing evidence suggests that the arcopallium / posterior pallial amygdala plays a major role in the control of fear behavior in birds. This brain region comprises several subdivisions, but no direct evidence is available about its functional parcellation. The aim of the present study was to investigate the relative involvement of two subdivisions of the arcopallium / posterior pallial amygdala complex in four classical tests of fear in quail: the presentation of a novel object, the 'hole-in-the-wall', 'open-field' and tonic immobility tests. Bilateral electrolytic lesions damaging the posterior part of the arcopallium / posterior pallial amygdala resulted in an increase in fear behavior in the 'open-field' test, whereas quail with lesions damaging the anterior part of the arcopallium displayed a decrease in an overall fear score, compared to quail with bilateral nidopallium or sham lesions. The differential involvement of the anterior and posterior parts of the arcopallium / posterior pallial amygdala in fear behavior is discussed in view of the known connections from the arcopallium / posterior pallial amygdala complex towards brain regions considered to be limbic in nature.

Keywords:
Avian brain; Emotions; Birds; Archistriatum; Amygdala.

A major challenge of behavioral neuroscience is to understand the central control of emotions in vertebrates. Most investigations of the central control of emotions in vertebrates have been carried out in mammals, notably in the study of fear, an adaptive emotional response that is induced by the perception of a danger (Jones, 1996). However, knowledge of the central control of fear reactions in birds is important for a better understanding of the phylogenetic continuity of emotions. Moreover, growing concern for animal welfare has motivated research into the neural mechanisms controlling emotions, in particular fear reactions in birds. A variety of reports have suggested that a large region of the basolateral caudal telencephalon, originally called the archistriatum (Kuenzel & Masson, 1988), including the arcopallium together with the posterior pallial amygdala (PoA; Reiner *et al.,* 2004), is involved in the expression of fear reactions in birds and could be at least partially homologous with the mammalian amygdala (Zeier & Karten, 1971; Davies, Csillag, Székely & Kabai, 1997). Lesions of the arcopallium / PoA have been reported to reduce escape and fear behavior in mallards (Phillips, 1964) and domestic chicks (Phillips & Youngren, 1986; Lowndes & Davies, 1996). In contrast, electrical stimulation of the arcopallium / PoA has been demonstrated to elicit fear and escape responses in mallards (Phillips, 1964), pigeons (Goodman & Brown, 1966) and chickens (Phillips & Youngren, 1971). Therefore, the arcopallium / PoA appears to play an important role in the control of fear reactions in birds.

The arcopallium / PoA is a large, heterogeneous brain region and the contribution of its individual subdivisions to the control of fear behavior is unclear. The internal organization of the arcopallium / PoA has been the subject of a number of neuroanatomy studies, but little information is available about its functional parcellation. Zeier and Karten (1971) and Davies *et al.* (1997) have suggested that, in the pigeon and domestic chick respectively, the posterior part of the arcopallium / PoA may have limbic functions, because it gives rise to efferents terminating in areas of the brain considered to be limbic in nature, such as the hippocampal formation, septal area, medial striatum, nucleus accumbens, ventral paleostriatum, dorsomedial thalamus and hypothalamus. In contrast, the anterior part of the arcopallium is considered to be non-limbic, because it mainly gives rise to sensory, somatosensory and motor telencephalofugal efferents (Zeier & Karten, 1971; Davies *et al.,* 1997). To our knowledge, the involvement of individual arcopallium / PoA subdivisions in the control of fear behavior in birds has not been investigated directly. Therefore, the aim of the present study was to investigate the role of individual subdivisions of the arcopallium / PoA complex, in the expression of fear behavior in quail.

To this end, the effects of lesions of the anterior arcopallium (Reiner *et al.,* 2004) *versus* the posterior part of the arcopallium / PoA (including the dorsal, intermediate and medial arcopallium and PoA of Reiner *et al.,* 2004) were investigated in four behavioral tests of fear: the presentation of a novel object in the home cage, the 'hole-in-the-wall', 'open-field' and tonic immobility tests. A variety of behavioral tests were used, because different stimuli may evoke different fear responses relying on different neuronal circuits.

Materials and methods

Subjects

Japanese quail (*Coturnix japonica)* of the 42nd and 43rd generations of the Long Tonic Immobility duration (LTI) line, selected and maintained at the Unité Expérimentale Avicole, INRA, Nouzilly, France (Mills & Faure, 1991), were used in the present experiment. The genetic selection for long tonic immobility has resulted in LTI quail showing high levels of fear behavior in a variety of tests (Faure *et al.,* 2006). Quail expressing high amount of fear behavior were used to minimize the chance of a 'floor' effect masking any lesion-induced reductions in fear behavior.

On the day of hatching, male and female chicks were transferred to a communal floor pen maintained at approximately 40°C by continuous illumination with commercial brooder lamps. During the second and third weeks after hatching, the ambient temperature was gradually reduced to 20°C. On the 21st day after hatching, male and female quail were separated and 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. The quail were treated according to the European Communities Council Directive of November 24, 1986 (86/609/EEC) throughout. All procedures described here comply with French legislation on research involving animals and were approved by a local ethics committee (Comité Régional d'Ethique pour l'Expérimentation Animale, Centre-Limousin, file no. CL2006_051).

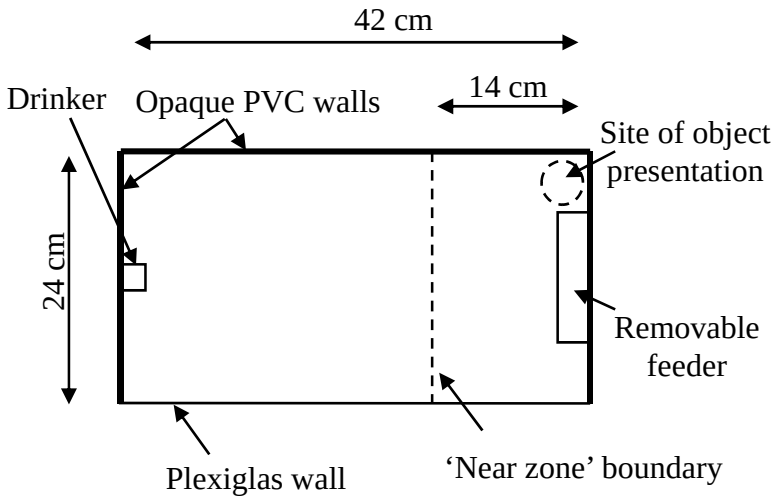
Surgical procedure

At five weeks of age and at least 24 h before surgery, 39 adult males were transferred to a holding room maintained at 20°C with a 16:8 h light: dark photoperiod. A single sex was employed in these experiments, to reduce inter-individual variability and males were chosen to avoid the changes in behavior in females related to laying, which may occur unpredictably. The quail were housed individually in wooden cages, with wood-

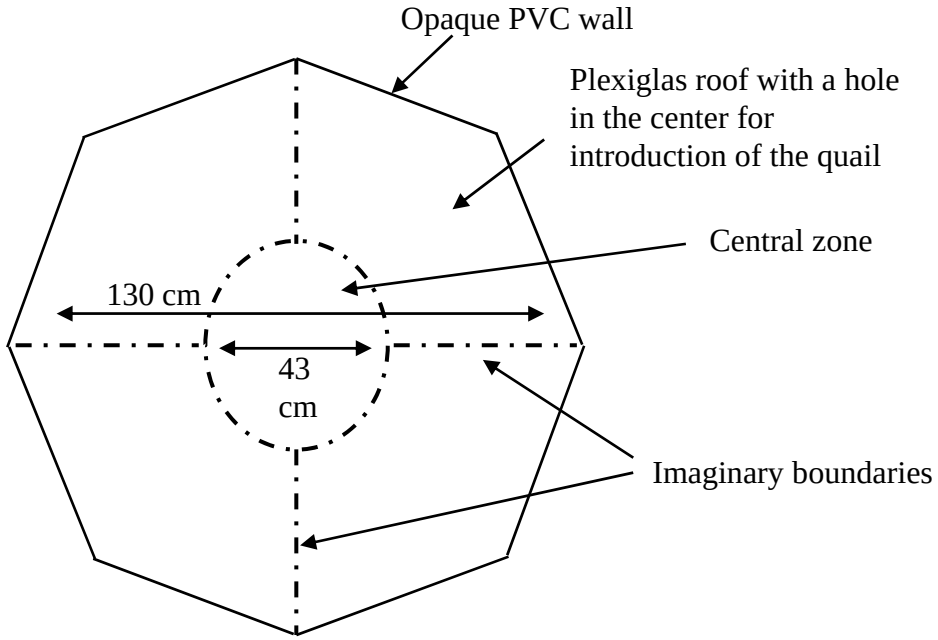
shavings on the floor. Their food and water were removed 2 h before surgery. Each quail was weighed and then anesthetized by intramuscular injection of 2.5 ml/kg of a mixture of ketamine (93.75 mg/kg; Imalgene, Merial SAS, Lyon, France) and xylazine (6.25 mg/kg; Paxman, Virbac France SAS, Carros, France). Immediately prior to surgery, each quail was placed in a small animal stereotaxic instrument, with a horizontal bar placed at the angle between the upper and lower beak, 8 mm anterior and 8 mm below the ear bars, to give an orientation similar to that used by Baylé *et al.* (1974) in the preparation of their stereotaxic atlas of the quail brain. The body temperature of the quail was maintained during surgery with the aid of a thermostatically controlled heated underblanket. A midline sagittal incision was made through the scalp, which was then retracted. A hole was drilled through the cranium and the dura mater incised. A stainless steel electrode with an insulated shaft was introduced into the telencephalon under stereotaxic control. Quail were given one of the following treatments: bilateral lesions of the 1) anterior part of the arcopallium (AA), 2) posterior part of the arcopallium and PoA (AP), and 3) nidopallium (NIDO). The nidopallium was chosen as a control region for the effect of brain damage *per* se, because it is located above the arcopallium / PoA and does not appear to be involved in fear behavior (Lowndes & Davies, 1996). Electrolytic lesions were made by passing a constant current (3500 Lesion Making Device, UGO Basil, Comerio, Italy) of 1 mA through the uninsulated tip (0.5mm) of the electrode for 15 s. For each hemisphere of AA quail, electrode penetrations were made 2.5 mm (1 lesion site) and 1.5 mm (2 lesion sites) rostral to the parieto-occipital suture. These three electrode penetrations were placed 4.5 mm, 4.25 mm and 5.25 mm from the midline respectively and 4.5 mm, 4.5 mm and 3.5 mm below the surface of the brain respectively (the lesion sites lay between levels A7.0 and A6.0 according to the atlas of the quail brain by Baylé, Ramade & Olivier, 1974). For each hemisphere of AP quail, electrode penetrations were made 1.5 mm (1 lesion site) and 0.5 mm (2 lesion sites) rostral to the parieto-occipital suture. These electrode penetrations were placed 4.5 mm, 4.25 mm and 5.25 mm from the midline respectively and 4.5 mm, 4.5 mm and 3.5 mm below the surface of the brain respectively (the lesion sites lay between levels A6.0 and A4.5 in the Baylé *et al.,* 1974 atlas of the quail brain). For NIDO quail, bilateral electrode penetrations were placed 1.5 mm (1 lesion site) and 0.5 mm (2 lesion sites) rostral to the parieto-occipital suture. These three electrode penetrations were placed 3.5 mm, 3.25 mm and 4.25 mm from the midline respectively and 2 mm, 2 mm and 1 mm below the surface of the brain respectively (the lesion sites lay between levels A7.0 and A6.0 in the Baylé *et al.,* 1974 atlas). Sham quail received similar electrode penetrations to

Figure 1. A: Diagram of a cage in which the quail were housed after surgery and where the novel object test was performed (viewed from above). The diagram shows the site where the novel object was dropped into the cage and the limit of the zone near to the object (dashed line). B: Diagram of the 'open-field' test arena (viewed from above), showing the 5 zones.

A



B



those of AA, AP or NIDO quail, but no current was passed through the electrode. After the electrode was withdrawn from the last site in each hemisphere, the hole in the cranium was sealed with gelatin sponge (Pangen 2, Laboratoires URGO SA, Chenove, France) and the overlying scalp incision was closed with sutures. All quail then received an intramuscular injection of amoxicillin (300 mg; Duphamox, Fort Dodge Santé Animale, Tours, France) and were placed individually in a small cardboard box with wood shavings on the floor and a hot-water bottle, until they had recovered from the anesthesia. The quail were then housed individually in PVC cages (see Figure 1A for measurements) with wood-shavings on the floor and food and water available *ad libitum*. They were left undisturbed in these cages until the behavioral tests were performed. Each quail was then coded so that all subsequent procedures were performed by an experimenter 'blind' to its previous treatment.

Behavioral testing

All quail were tested one week after surgery in the following order: using the novel object and associated control tests, the 'hole-in-the-wall', 'open-field' and tonic immobility tests. The behavior of each quail was videoed during all except the tonic immobility test, with the experimenter remaining out of sight of the quail during recording.

Novel object test

All quail were tested in their home cage in two situations: with the novel object and without it. To avoid any effect of test order, half of the quail were tested first with the novel object and then in a control test without it, while the remainder of the quail were tested in the opposite order. The first test was performed seven days after surgery and the second test, at the same time on the following day. The test procedure has been described in detail previously (Richard *et al*., 2007), but briefly, on test days food was removed from each cage 40 min before the test. To habituate the quail to this procedure, the food trough was removed from each cage for 2 h every day, during the week preceding testing. In the novel object test, the food trough was replaced and the object was dropped into the cage immediately when the quail pecked at the food. The object, a 21x4 cm multicolored cylinder covered with 2-cm horizontal stripes of colored tape, was introduced into the cage near the food trough and then withdrawn 5 min later. In the control test without the novel object, the food trough was replaced and the quail was left undisturbed for 5 min. The time spent in the zone near the novel object (see fig. 1A) and the time spent pacing were

recorded. Similar measurements were made in the control test without the novel object. Behavioral measurements were made using The Observer 3.0 software (Noldus Information Technology, The Netherlands, 1993). For each quail, the change in the time spent near the object or in the time spent pacing induced by presentation of the object was calculated as the difference in the expression of the two parameters measured between the test with the novel object and that without it.

'Hole-in-the-wall' test

One hour after the second part of the novel object test, each quail was tested in the 'hole-in-the-wall' test in a separate room. This test is a classical test of fear in birds, based on the premise that timid birds will take longer than less fearful ones to emerge from a dark sheltered area into a brightly lit, unfamiliar and therefore potentially frightening one (Jones, 1996). The apparatus consisted of a wooden box (length = 70cm, width = 27 cm; height = 29 cm) with two compartments of equal size: a dark compartment with a wooden lid and a brightly lit compartment, with a wire mesh lid. A guillotine trapdoor covered a 17 x 10 cm opening in the middle of the wooden wall separating the two compartments. The floor of the two compartments was covered with white paper, which was replaced between tests. Each quail was carried by hand from its home cage, placed in the dark compartment and allowed a 1 min acclimatization period before the door was raised, to give access to the brightly lit compartment. The brightly lit compartment was then videoed from above for five minutes and then the quail was replaced in its home cage. The latency for the quail to poke its head through the door into the light compartment was measured (see Jones, 1996, for details of the 'hole-in-the-wall' test).

'Open-field' test

The 'open-field' test was performed between one and three hours after the 'hole-in-the-wall' box test in the same room. The 'open-field' was a 130 cm diameter octogonal arena with beige PVC walls, a white painted wooden floor and a Plexiglas lid. To aid analysis of the locomotor activity of the quail, the floor of the 'open-field' was divided into 5 zones (see fig. 1B). Each quail was carried by hand from its home cage and placed at the center of the 'open-field'. The behavior of each quail was videoed from above for 5 min after the beginning of the test. The quail were then returned to their home cage and the floor of the arena was cleaned. The latency to leave the central zone (43 cm diameter) and the number of zone boundaries crossed were recorded, as described by Jones (1996).

Tonic immobility test

The tonic immobility test was performed between one and three hours after the end of the 'open-field' test, in the same room. A detailed account of the tonic immobility test procedure has been published previously (Mills & Faure, 1991). Briefly, the experimenter placed each quail on its back in a U-shaped wooden cradle covered with a cloth and restrained it for 10 s (with one hand on the sternum and one hand lightly cupping the head of the quail). The observer sat nearby in full view of the bird but remained silent and as motionless as possible. If an attempt at induction of tonic immobility was unsuccessful (*i.e.* if the quail righted itself less than 10 s after the end of restraint), the experimenter immediately reattempted to induce tonic immobility. In the present experiment, tonic immobility was expressed by all quail after a maximum of two attempts at induction. The observer recorded the duration of tonic immobility, *i.e.* the time until the bird righted itself after the end of restraint. If a quail failed to right itself after 10 min, the test was terminated and a tonic immobility duration of 600 s was assigned to it.

Overall fear score

To provide a generalized view of the effect of the lesions on fear behavior, an overall fear score was calculated. Quail were ranked according to their behavioral responses in each test and a score for each test was calculated from the sum of the ranks of the recorded behavioral variables in that test. The overall fear score for each quail was calculated as the sum of scores for that quail in each behavioral test (sum of the four scores). Thus, quail displaying the least pacing and standing nearest the object in the test of the novel object, emerging fastest in the 'hole-in-the-wall' test, leaving the central zone fastest and crossing the highest number of zones in the 'open-field' test and righting themselves fastest in the tonic immobility test, received the lowest overall fear score. The score theoretically ranged from 4 (because there were 4 tests) to 112 (= 4 x 28 quail included in the analysis).

Histology

At the completion of the behavioral tests, the quail were weighed and given a lethal injection of sodium pentobarbital (360 mg/kg, i.p.; Pentobarbital Sodique, Sanofi Santé Animale, France). Their brains were removed and fixed by immersion overnight in 4% paraformaldehyde in phosphate buffered saline (PBS; 0.1M, pH=7.4) at 4°C. The brains

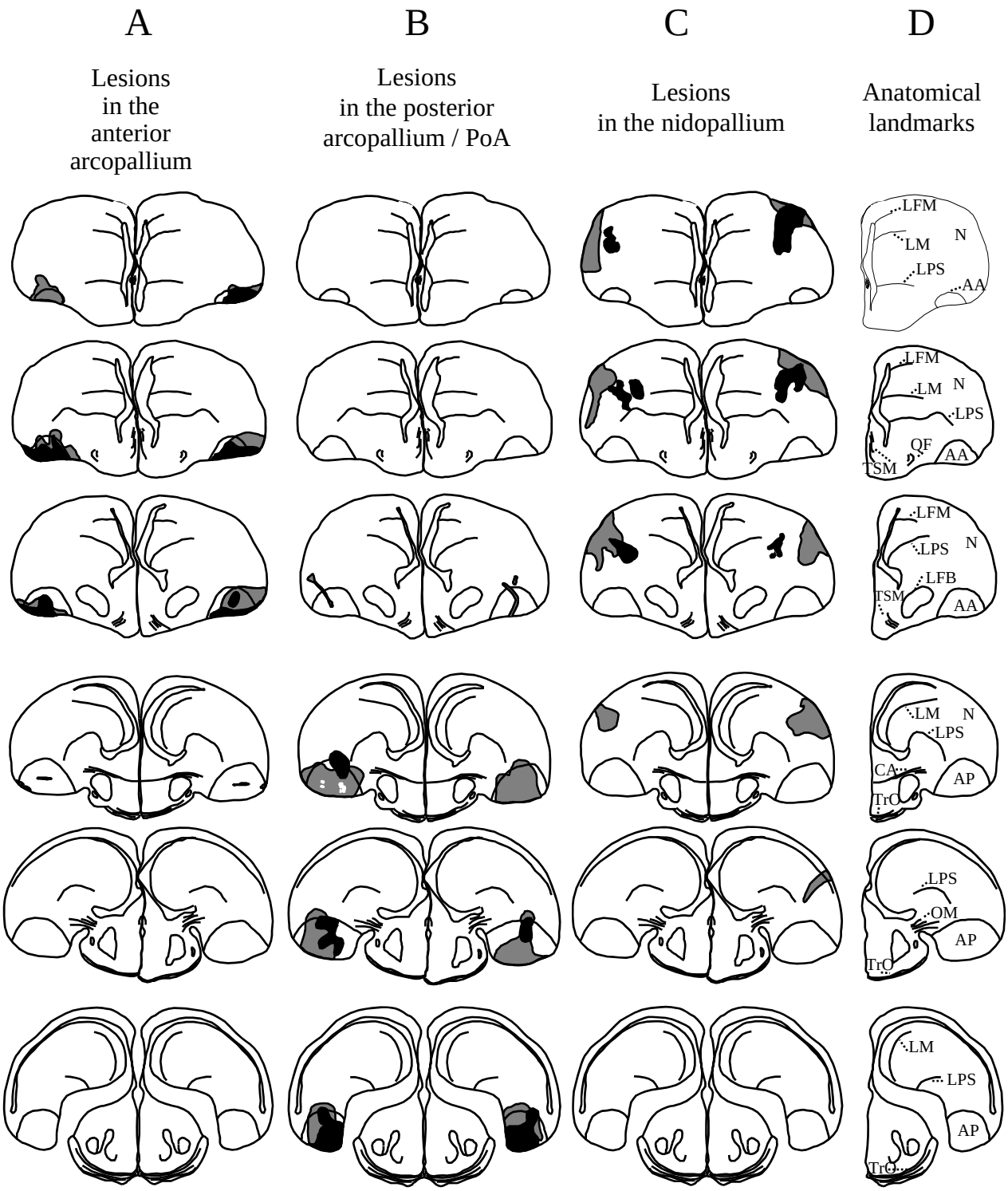
were then cryoprotected by immersion in 30% sucrose in PBS for 24 hours at 4°C and frozen on dry ice. Serial coronal sections (50µm) were cut using a cryostat at -20°C. The sections were stored in PBS at + 4°C. Every fourth section was collected onto glass slides and stained with cresyl violet. The sections were viewed in a light microscope and the lesion sites examined. The borders of the arcopallium / PoA complex as defined by Reiner *et al*. (2004) were determined with the aid of atlases of the domestic chick (Kuenzel & Mason, 1988) and Japanese quail (Baylé *et al.,* 1974) brain. The outlines of the sections and the lesion sites within each section were drawn with the aid of a *camera lucida* drawing attachment. These drawings were then used to determine the accuracy of placement and the extent of the lesions in each brain. To evaluate the relative extent of the lesion within the arcopallium / PoA, the surface areas of the lesion and the arcopallium / PoA were estimated for each section in which they were present. Then, a ratio between the total surface areas of the lesion and the anterior or posterior arcopallium / PoA was calculated. Quail that had received a small lesion on one side of the target region (< 10% of the area of that region) and quail that had received a large lesion on one side in the non-target part of the arcopallium / PoA complex (> 20% of the area of that region), were removed from the analysis. Moreover, the total surface areas of the lesions were measured in all quail, including NIDO quail.

Statistical analysis

The behavioral data were not normally distributed and therefore, nonparametric statistical analyses were performed using Statview 5.0 software (SAS Institute Inc., USA, 1992-1998). To investigate whether surgery had any deleterious effect on the general health of the quail, the weight gain during the experiment (weight at the end minus weight at the beginning of the experiment) was compared across treatment groups, using the Kruskall-Wallis test. The total extent of the lesions, the variables for each test of fear and the overall fear score were compared across treatment groups using Kruskall-Wallis tests followed by Mann-Whitney tests when appropriate. For all tests, $p<0.05$ was accepted as significant.

Figure 2: A series of charts illustrating the extent of the lesions in A: the anterior part of the arcopallium, B: the posterior part of the arcopallium / PoA and C: the nidopallium, in quail with the smallest (black) and largest (grey) lesions. D: Schematic drawings of coronal sections of the quail brain illustrating the localization of the anterior and posterior parts of the arcopallium and other anatomical landmarks.

AA, Anterior Arcopallium; AP, Posterior Arcopallium + posterior pallial amygdala; CA, Commissura Anterior; LFB, Lateral Forebrain Bundle; LFM, Lamina Frontalis Suprema; LM, Lamina Mesopallialis; LPS, Lamina Pallio-Subpallialis; N, Nidopallium; OM, Tractus Occipitomesencephalicus; QF, Tractus Quintofrontalis; TrO, Tractus Opticus; TSM, Tractus Septopallio-Mesencephalicus.



Results

Histology

After histological analysis of the lesions sites, one AA quail was removed from the analysis because it received too small a lesion and one quail was also removed because it received too large a lesion in the posterior arcopallium. In the AA quail (n = 7), a mean of 39.2 % of the left and 41.5 % of the right anterior arcopallium were destroyed, 3.3% of the left posterior arcopallium and 3.3% of the right posterior arcopallium were lesioned (Figs. 2A & 3A).

Two AP quail were removed from the statistical analysis because they received too small lesions in the posterior arcopallium and two quail were removed because they received too large lesions in the anterior arcopallium. In AP quail (n = 5), 49.5 % of the left and 40.0 % of the right posterior arcopallium were lesioned, 4.1% of the left anterior arcopallium and 4.9% of the right anterior arcopallium, were lesioned (Figs. 2B & 3B).

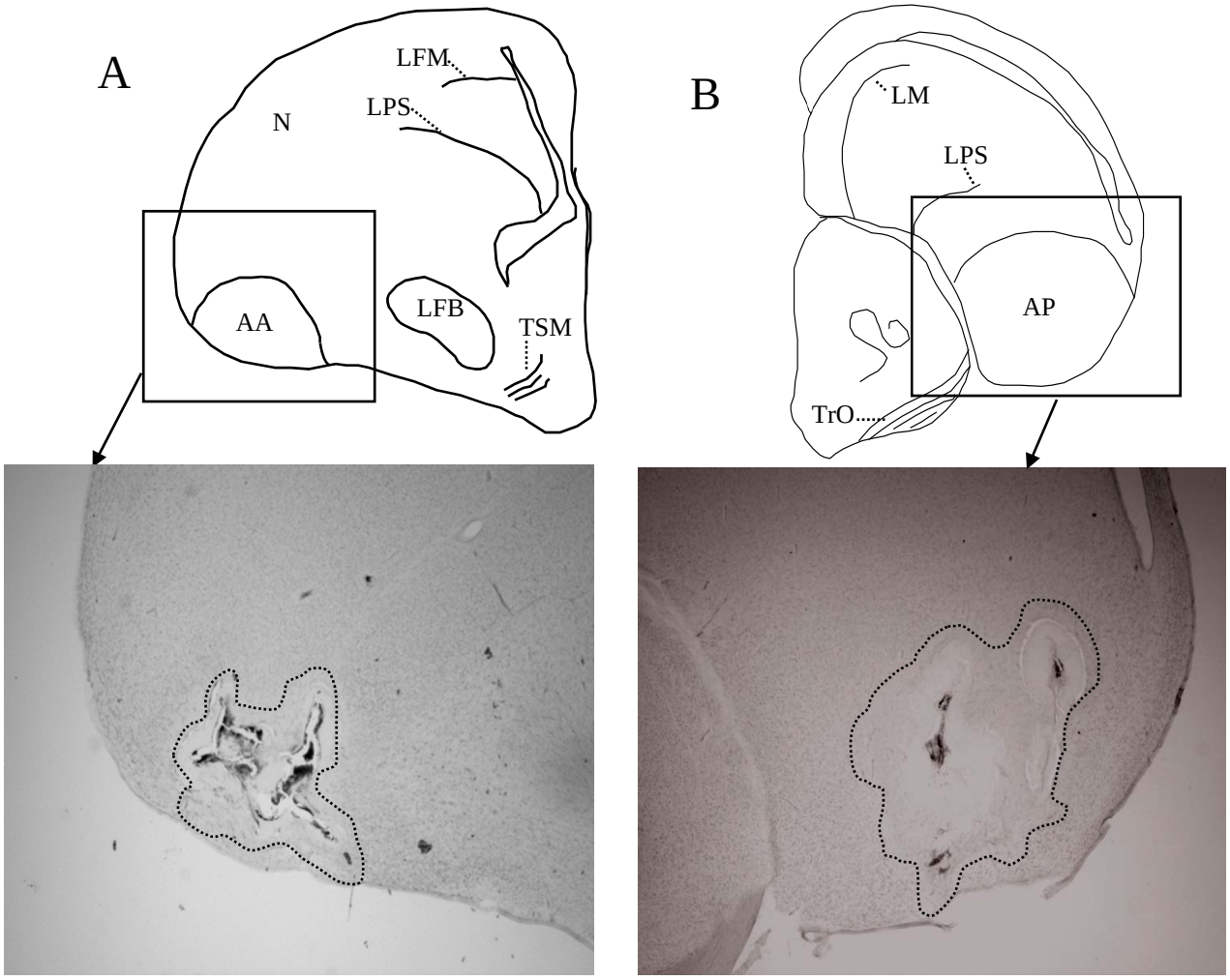
In NIDO quail (n=7), the lesions affected the dorsolateral nidopallium (Figs. 2 C & 3C). The extent of tissue damaged was similar to that of AA and AP quail (mean ± SEM: NIDO = 3.2 ± 0.7 mm^3; AA = 2.1 ± 0.9 mm^3; AP = 3.5 ± 1.6 mm^3; H = 4.54, p = 0.104, Kruskall-Wallis test).

Behavior

All quail awoke from the anesthetic about two hours after surgery ended and recovered normal locomotor activity and feeding behavior within a maximum of 36 hours. There was no significant effect of treatment on weight gain during the experiment (H = 4.77, p>0.05, median weight gain for all groups = 11.5 [3.5; 16.5] g), indicating that all quail displayed similar food intake / expenditure. Neither was there any difference in behavior between SHAM and NIDO quail in any of the behavioral variables investigated.

There was a significant effect of treatment on the latency to leave the central zone in the 'open-field' test (H = 10.68, p<0.05, Fig. 4A). AP quail showed a significantly longer latency to leave the central zone than SHAM (z = 2.1, p<0.05), NIDO (z = 2.2, p<0.05) and AA quail (z = 2.68; p<0.01). The latency for AA quail to leave the central zone tended to be shorter than that of SHAM quail (z = 1.81, p = 0.07), but it did not differ significantly from that of NIDO quail (z = 0.19). There was no significant effect of treatment on the number of zone boundaries crossed in the 'open-field' test (H= 5.53, p> 0.05, Fig 4B).

Figure 3: Photographs of lesions in one cerebral hemisphere representative of quail with lesions in A: the anterior arcopallium, B: the posterior arcopallium + posterior pallial amygdala and C: the nidopallium.
AA, Anterior Arcopallium; AP, Posterior Arcopallium + posterior pallial amygdala; LFB, Lateral Forebrain Bundle; LFM, Lamina Frontalis Suprema; LM, Lamina mesopallialis; LPS, Lamina Pallio-Subpallialis; N, Nidopallium; QF, Tractus Quintofrontalis; TrO, Tractus Opticus; TSM, Tractus Septopallio-Mesencephalicus.



There was no significant effect of treatment on the time spent in the zone near the object in the 'novel object' test, nor in the difference between the time spent pacing in the control and 'novel object' tests ($H < 3.66$, $p > 0.05$ for both measures; Figs 4C,D). Neither was there any significant effect of treatment on the latency to emerge from the dark compartment in the 'hole-in-the-wall' test ($H = 5.57$, $p > 0.05$; Fig. 4E) or in the duration of tonic immobility ($H = 3.77$, $p > 0.05$; Fig. 4F).

There was a significant effect of treatment on the overall fear score ($H = 8.9$, $p < 0.05$; Fig. 5). AA quail showed a significantly lower score than SHAM ($z = 2.07$, $p < 0.05$), NIDO quail ($z = 1.98$, $p < 0.05$) or AP quail ($z = 2.52$; $p < 0.05$). The overall fear score of AP quail did not differ significantly from that of SHAM or NIDO quail ($z = 1.54$, $p > 0.05$ and $z = 0.98$, $p > 0.05$ respectively).

Figure 4: Box-plot diagrams illustrating the effect of anterior arcopallium lesions and posterior arcopallium + posterior pallial amygdala lesions on A) the latency to leave the central zone in the 'open-field' test, B) the number of zone boundaries crossed in the 'open-field' test, C) the difference in the time spent in the 'near zone' between the object and control trials in the novel object test, D) the difference in time spent pacing between the object and control trials in the novel object test, E) the latency to emerge from the dark compartment in the 'hole-in-the-wall' test, F) the duration of tonic immobility.

AA, bilateral lesions of the anterior arcopallium; AP, bilateral lesions of the posterior arcopallium + posterior pallial amygdala; NIDO, bilateral lesions of the nidopallium; SHAM, bilateral electrode penetrations of the telencephalon without electrolytic lesions. Values illustrated by a box-plot are the median, upper and lower quartiles, 10th and 90th percentiles. *= p<0.05, **= p<0.01.

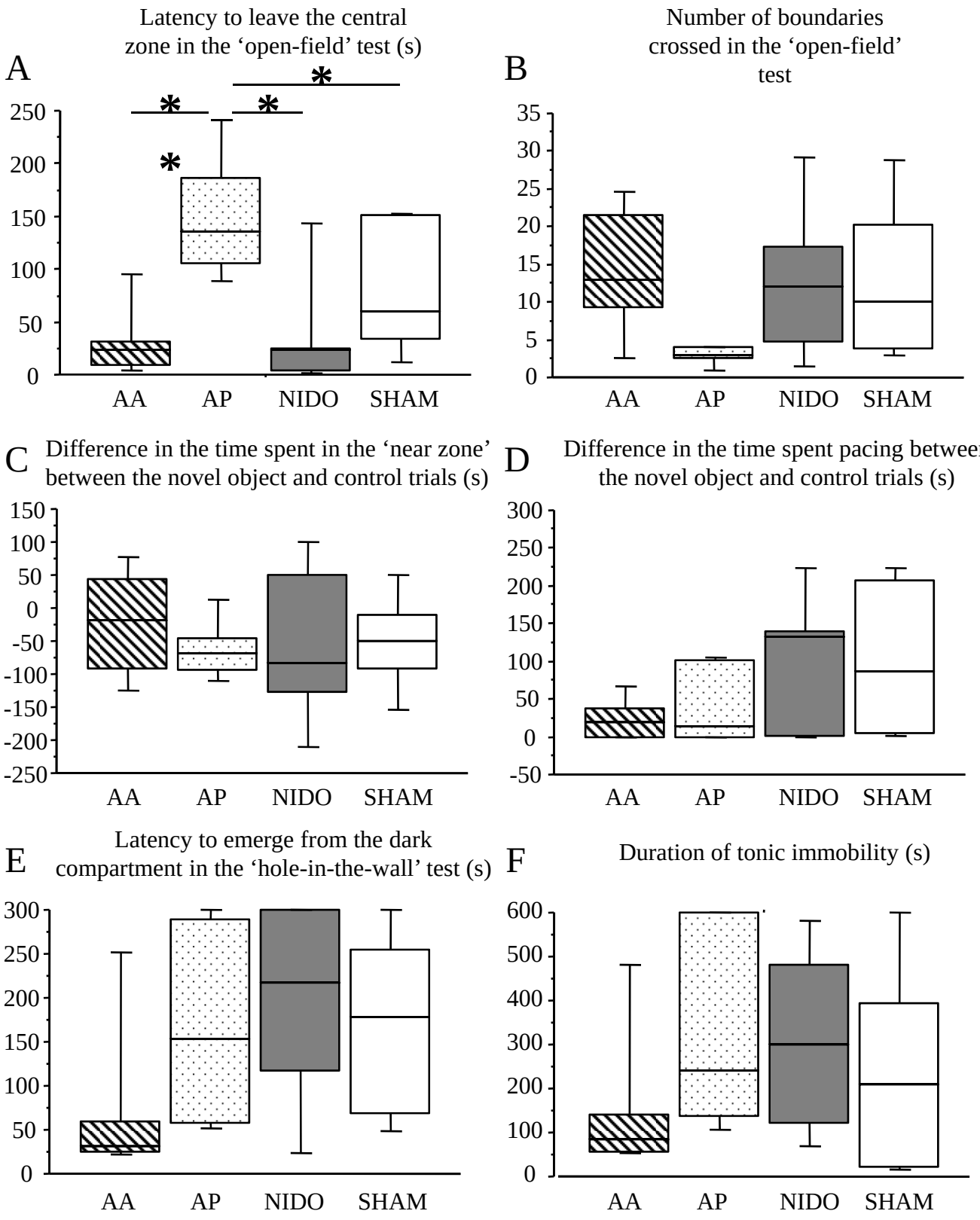
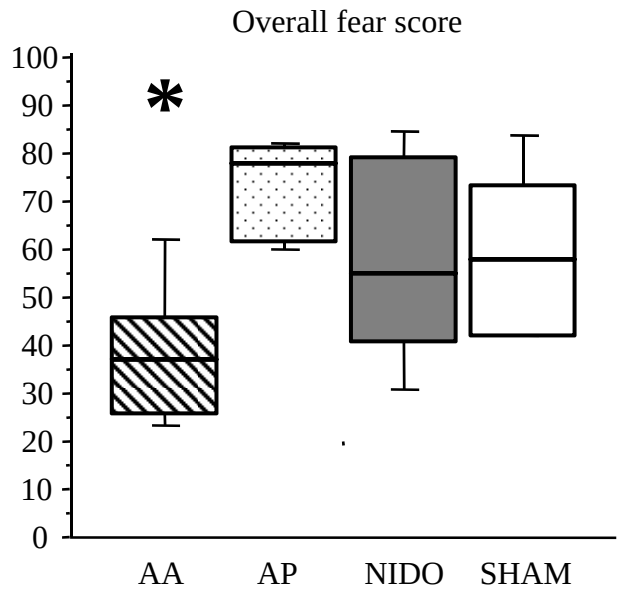


Figure 5: Box-plot diagrams illustrating the effect of anterior arcopallium lesions and posterior arcopallium + posterior pallial amygdala lesions on the overall fear score derived from the novel object, 'hole-in-the-wall' , 'open field' and tonic immobility tests. The score theoretically ranged from 4 to 112 (see text for details).

AA, bilateral lesions of the anterior arcopallium; AP, bilateral lesions of the posterior part of the arcopallium + posterior pallial amygdala; NIDO, bilateral lesions of the nidopallium and SHAM, bilateral electrode penetrations of the telencephalon without electrolytic lesions.

Values illustrated by a box-plot are the median, upper and lower quartiles, 10th and 90th percentiles. * = $p<0.05$ compared to SHAM, NIDO and AP quail.



Discussion

The results of the present study indicate a differential involvement of the posterior arcopallium / PoA and the anterior arcopallium in the control of fear behavior in Japanese quail. Electrolytic lesions of the posterior arcopallium / PoA significantly increased a fear response in the 'open-field' test. In contrast, lesions damaging the anterior arcopallium did not significantly affect fear responses in any of the four individual tests of fear investigated, but they did significantly reduce the overall fear score. Nidopallium lesions of similar size to those of the anterior or posterior arcopallium / PoA did not affect quail fear behavior in any of the tests investigated and therefore, the effects of lesions of the anterior and posterior arcopallium / PoA are unlikely to be due to brain damage *per se*.

In the present study, electrolytic lesions damaging the posterior part of the arcopallium / PoA induced an increase in fear behavior (as indicated by an increased latency to leave the central zone) in the 'open-field' test, in comparison to quail with anterior arcopallium, nidopallium or sham lesions. Zeier & Karten (1971) and Davies *et al*. (1997) have previously suggested that the posterior part of the arcopallium / PoA has limbic functions and may be partly homologous to the mammalian amygdala, which is known to be critical for the processing of fear responses in mammals (for review, see LeDoux, 2000). The results of the present study are, to our knowledge, the first to directly implicate the posterior arcopallium / PoA in avian fear behavior. However, it is perhaps surprising that lesions of this region induced an increase in fear in the 'open field' test, because lesioning the entire arcopallium / PoA has been demonstrated to decrease fear in 'open-field' tests in domestic chicks (Phillips & Youngren, 1986; Lowndes & Davies, 1996). Lesions of the entire arcopallium / PoA have also been reported to reduce avian fear behavior in a variety of other tests (*e.g*. Dafters, 1975; Martin, Delanerolle & Phillips, 1979; Phillips, 1964). However, Maser et al. (1973) reported an increase in fear behavior in the tonic immobility test after partial lesions of the arcopallium in chickens. These lesions mainly affected a restricted portion of the ventral intermediate arcopallium, which was also damaged in quail receiving AP lesions in the present study. Therefore, the posterior arcopallium / PoA may contain a small population of neurons with an inhibitory action on the expression of fear behaviour: lesioning such an inhibitory region, while leaving the rest of the arcopallium / PoA complex intact, would increase fear responses, as observed in the present study.

The results of the current study also provide the first direct demonstration that the anterior arcopallium plays a role in the control of fear behavior in birds. Quail with lesions

damaging the anterior arcopallium displayed a decrease in the overall fear score compared to quail with posterior arcopallium / PoA, nidopallium or sham lesions. A decrease in fear behavior after lesions of the entire arcopallium / PoA has been widely reported in the literature (Phillips, 1964; Dafters, 1975; Martin *et al*., 1979; Phillips & Youngren, 1986; Lowndes & Davies, 1996). However, the involvement of the anterior arcopallium alone in fear behavior is somewhat surprising, since it appears to be mainly connected to brain structures involved in the somatic sensorimotor system (Zeier & Karten, 1971; Davies *et al.*, 1997). However, Davies et al. (1997) have also shown that the anterior arcopallium of the domestic chicks has widespread projections to structures that are traditionally considered to be limbic in nature, such as the medial striatum (formerly named lobus parolfactorius), hippocampal formation, nucleus accumbens and lateral part of the bed nucleus of the stria terminalis. Davies *et al.* (1997) described that it is the ventral part of the anterior arcopallium that contributes most strongly to this limbic projection. In the current experiment, lesions of the anterior arcopallium always affected its ventral part (see Figure 2), potentially disrupting the limbic projection and thus, decreasing fear behavior.

Comparison of the effects of lesions on the fear responses observed in the variety of tests employed in the current experiment supports the possibility that different fear responses are subserved by different neural circuits. Although lesions of the anterior arcopallium did not significantly affect fear responses in any of the individual tests investigated, they significantly decreased the overall fear score and within each individual test, lesioned quail displayed numerically less fear behavior than quail with posterior arcopallium / PoA, nidopallium or sham lesions (see Figs. 4 & 5). Thus, it appears that the anterior arcopallium is involved in aspects of fear common to all of the tests employed. In contrast, lesions damaging the posterior arcopallium / PoA affected fear responses in one specific test, the 'open-field' test, but not in the novel object, 'hole-in-the-wall' or tonic immobility tests. In view of the extensive projections of the posterior arcopallium / PoA to brain regions considered to be limbic (Zeier & Karten, 1971; Davies *et al*., 1997), lesions of this structure were expected to affect fear behavior in all of the different tests used, but the relatively small number of individuals and relatively small lesion sizes might explain their lack of effect in some of the tests. However, a dissociation between the effects of lesions in individuals tests has previously been demonstrated: lesions of the entire arcopallium / PoA in domestic chicks reduced fear in the 'open-field' but not the fear responses to a novel object (Lowndes & Davies, 1996). Therefore, the posterior arcopallium / PoA may be involved in some, but not all fear responses. It appears to be

particularly involved in the behavioral responses induced by placement in a novel environment, a principal characteristic of the 'open-field' test. Such a restricted role in fear behavior is consistent with the notion that fear is a multidimensional concept (Boissy, 1995; Désiré, Boissy & Veissier, 2002; Russell, 2003). Thus, different brains areas may be required to elicit different aspects of fear behavior. A selective involvement of individual brain regions in different fear responses has also been observed in rats. For example, it has been suggested that the bed nucleus of the stria terminalis may preferentially mediate the expression of unconditioned fear, whereas the central nucleus of the amygdala may preferentially mediate the expression of conditioned fear (Fendt, Endres & Apfelbach, 2003; LeDoux, 2000). Therefore, it appears reasonable to assume that different aspects of fear may be controlled by different fear circuits in both the avian and mammalian brains, although the existence of a one-to-one relationship between a behavior and a brain region is probably over-simplistic (Walker, Toufexis & Davis, 2003). The results of the present study suggest that the posterior arcopallium / PoA controls a specific aspect of fear whereas the anterior arcopallium is involved in aspects of fear common to the tests employed in the current experiment.

The differential involvement of the anterior and posterior parts of the arcopallium / PoA in fear behavior observed in the present study, constitutes a first step towards a better understanding of the neural circuits subserving fear in the avian brain. First, the posterior arcopallium / PoA may cause inhibition of fear responses in the 'open-field' test via its projections to others structures considered to be limbic in nature, such as the hypothalamus, dorsomedial thalamus, hippocampal complex and medial striatum (Zeier & Karten, 1971; Davies *et al.,* 1997; Atoji, Saito & Wild, 2006). This inhibitory action might be mediated by GABA, the main inhibitory neurotransmitter in vertebrates, since mRNA for its synthetic enzyme glutamic acid decarboxylase is expressed in the posterior arcopallium / PoA of domestic chicks (Sun et al., 2005), probably in local circuit neurons. Even if GABAergic local circuit neurons are scarce in the posterior arcopallium / PoA, they might constitute a local inhibitory network involved in the control of fear behavior. Second, it is possible that the anterior arcopallium may facilitate behavioral expressions of fear via its projections to limbic brain areas such as the medial striatum, hippocampal formation, nucleus accumbens and bed nucleus of the stria terminalis (Davies *et al*., 1997). Finally, the fact that the anterior arcopallium projects to the posterior arcopallium / PoA (Davies *et al.,* 1997) suggests that it can influence the function of the posterior arcopallium / PoA.

Additional studies will be necessary to elucidate the precise role of the arcopallium and PoA in the expression of fear behavior in birds. This heterogeneous brain area processes multimodal information and may, to some extent, be compared to parts of the mammalian cortex (Zeier & Karten, 1971; Davies *et al.,* 1997; Reiner *et al*., 2004; Atoji *et al.,* 2006). Several reports have described its involvement in complex cognitive tasks such as passive avoidance learning, filial imprinting and food learning (Lowndes & Davies, 1996; Lowndes, Davies &Johnson, 1994; Aoki, Csillag & Matsushima, 2006; Aoki, Suzuki, Izawa, Csillag & Matsushima, 2006). Therefore, it is not surprising that the involvement of the arcopallium / PoA region in fear behavior is complex. Such a cerebral complexity mirrors the complexity of fear itself and its behavioral expression.

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References

Aoki, N., Csillag, A., & Matsushima, T. (2006). Localized lesions of arcopallium intermedium of the lateral forebrain caused a handling-cost aversion in the domestic chick performing a binary choice task. *European Journal of Neuroscience, 24*(8), 2314-2326.

Aoki, N., Suzuki, R., Izawa, E., Csillag, A., & Matsushima, T. (2006). Localized lesions of ventral striatum, but not arcopallium, enhanced impulsiveness in choices based on anticipated spatial proximity of food rewards in domestic chicks. *Behavioural Brain Research, 168*, 1-12.

Atoji, Y., Saito, S., & Wild, J. (2006). Fiber connections of the compact division of the posterior pallial amygdala and lateral part of the bed nucleus of the stria terminalis in the pigeon (*Columba livia*). *Journal of Comparative Neurology, 499*, 161-182.

Baylé, J.-D., Ramade, F., & Olivier, J. (1974). Stereotaxic topography of the brain of the quail (*Coturnix coturnix japonica*). *Journal of Physiology, 68*, 219-241.

Boissy, A. (1995). Fear and fearfulness in animals. T*he Quarterly Review of Biology, 70*, 165-191.

Dafters, R. (1975). Active avoidance behavior following archistriatal lesions in pigeons. *Journal of Comparative Physiological Psychology, 89*, 1169-1179.

Davies, D. C., Csillag, A., Székely, A. D., & Kabai, P. (1997). Efferent connections of the domestic chick archistriatum: a phaseolus lectin anterograde tracing study. *Journal of Comparative Neurology, 389*, 679-693.

Desire, L., Boissy, A., & Veissier, I. (2002). Emotions in farm animals: a new approach to animal welfare in applied ethology. *Behavioural Processes, 60*, 165-180.

Faure, J. M., Arnould, C., Beaumont, C., Guémené, D., Leterrier, C., Mills, A. D., et al. (2006). Consequences of selection for fear in Japanese quail. *Archiv für Geflügelkunde, 70*, 216-222.

Fendt, M., Endres, T., & Apfelbach, R. (2003). Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks freezing induced by trimethylthiazoline, a component of fox feces. *Journal of Neuroscience, 23*, 23-28.

Goodman, I. J., & Brown, J. L. (1966). Stimulation of positively and negatively reinforcing sites in the avian brain. *Life Sci, 5*(8), 693-704.

Jones, R. B. (1996). Fear and adaptability in poultry : insights, implications and imperatives. *World's Poultry Science Journal, 52*, 131-174.

Kuenzel, W. J., & Masson, M. (1988). *A stereotaxic atlas of the brain of the chick (Gallus gallus domesticus)*. Baltimore, MD: John Hopkins University Press.

LeDoux, J. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience, 23*, 155-184.

Lowndes, M., & Davies, D. C. (1996). The effect of archistriatal lesions on 'open field' and fear/avoidance behaviour in the domestic chick. *Behavioural Brain Research, 72*(1-2), 25-32.

Lowndes, M., Davies, D. C., & Johnson, M. H. (1994). Archistriatal lesions impair the acquisition of filial preferences during imprinting in the domestic chick. *European Journal of Neuroscience, 6*, 1143-1148.

Martin, J. T., Delanerolle, N., & Phillips, R. E. (1979). Avian archistriatal control of fear-motivated behavior and adrenocortical function. *Behavioural Processes, 4*(4), 283-293.

Maser, J., Klara, J., & Gallup, G. G. (1973). Archistriatal lesions enhance tonic immobility in the chicken (*Gallus Gallus*). *Physiology & Behavior, 11*, 729-733.

Mills, A. D., & Faure, J. M. (1991). Divergent Selection for Duration of Tonic Immobility and Social Reinstatement Behavior in Japanese Quail (*Coturnix coturnix japonica*) Chicks. *Journal of Comparative Psychology, 105*, 25-38.

Phillips, R. (1964). "Wildness" in the Mallard duck: effects of brain lesions and stimulation on "escape behavior" and reproduction. *J Comp Neurol, 122*, 139-156.

Phillips, R., & Youngren, O. (1971). Brain stimulation and species-typical behaviour: activities evoked by electrical stimulation of the brains of chickens (*Gallus Gallus*). *Animal Behaviour, 19*, 757-779.

Phillips, R., & Youngren, O. (1986). Unilateral kainic acid lesions reveal dominance of right archistriatum in avian fear behavior. *Brain Research, 377*, 216-220.

Reiner, A., Perkel, D. J., Bruce, L. L., Butler, A. B., Csillag, A., Kuenzel, W., et al. (2004). Revised nomenclature for avian telencephalon and some related brainstem nuclei. *Journal of Comparative Neurology, 473*, 377-414.

Richard, S., Wacrenier-Ceré, N., Hazard, D., Saint-Dizier, H., Arnould, C., & Faure, J. M. (2007). Behavioural and endocrine fear responses in Japanese quail upon presentation of a novel object in the home cage. *Behavioural Processes,* doi: 10.1016/j.beproc.2007.07.005.

Russell, J. A. (2003). Core affect and the psychological construction of emotion. *Psychological Review, 110*, 145-172.

Sun, Z., Wang, H. B., Laverghetta, A., Yamamoto, K., & Reiner, A. (2005). The distribution and cellular localization of glutamic acid decarboxylase-65 (GAD65) mRNA in the forebrain and midbrain of domestic chick. *Journal of Chemical Neuroanatomy, 29*(4), 265-281.

Walker, D. L., Toufexis, D. J., & Davis, M. (2003). Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *European Journal of Pharmacology, 463*(1-3), 199-216.

Zeier, H., & Karten, H. (1971). The archistriatum of the pigeon: organization of afferent and efferent connections. *Brain Research, 31*, 313-326.

Commentaires sur le Chapitre 1

L'expérience présentée dans ce chapitre a permis de montrer l'existence d'une implication différentielle de deux sous-régions de l'arcopallium / amygdale palliale postérieure (PoA) dans le contrôle des comportements de peur chez les oiseaux. En effet, alors que des lésions de la partie antérieure de l'arcopallium (AA) ont entraîné une diminution des comportements de peur de façon globale dans différents tests, des lésions de la partie postérieure (AP) ont entraîné une augmentation des comportements de peur exprimés dans l'open-field uniquement. **Les différences observées entre l'implication de l'AA et de l'AP dans le contrôle des comportements de peur se situent donc à deux niveaux : d'une part, les deux sous-régions semblent jouer des rôles opposés et d'autre part, alors que l'AP semble être sollicité dans des situations de peur spécifiques, l'AA semble jouer un rôle plus général dans les comportements de peur.**

AA et AP, des rôles opposés ?

Le résultat le plus inattendu de cette étude fut d'observer une augmentation des réponses de peur des cailles après lésion de l'AP. En effet, des lésions affectant l'ensemble de l'arcopallium / PoA induisent généralement une diminution des réactions de peur (Phillips, 1964 ; Phillips et Youngren, 1986 ; Lowndes et Davies, 1995). Cependant, dans les années 70, Maser *et al*. (1973) avaient déjà observé une augmentation des comportements de peur (augmentation de la durée d'immobilité tonique) après lésion partielle de l'arcopallium / PoA chez le poulet. Ces lésions partielles semblaient affecter principalement un petit territoire qui était aussi affecté par les lésions que nous avons effectuées dans le groupe AP (partie ventrale et centrale de l'AP). Nos résultats, ainsi que ceux de Maser, nous conduisent à suggérer qu'il puisse exister une sous-région inhibitrice des comportements de peur au sein de l'AP. L'hypothèse d'une sous-région inhibitrice au sein de l'arcopallium / PoA avait déjà été émise à la suite d'une étude réalisée au laboratoire quelques années auparavant (Richard, 2000 ; Richard *et al*., 2005). En effet, une étude morphologique avait montré que le volume de l'arcopallium / PoA était plus important chez les cailles de la lignée STI que chez les cailles LTI. Il avait été supposé que, puisque les cailles de la lignée STI montrent en général des comportements de peur réduits, la supériorité de volume de l'arcopallium / PoA chez ces cailles était peut-être liée au développement préférentiel d'une région ayant un rôle inhibiteur sur les comportements de peur (Richard, 2000). Cependant, mis à part la présence de GABA, neurotransmetteur à

action inhibitrice, au sein de l'arcopallium / PoA (Sun *et al.,* 2005), les éléments confortant l'hypothèse d'une éventuelle action inhibitrice de l'AP sur les comportements de peur, sont peu nombreux. De nouvelles études de neuroanatomie fonctionnelle ciblées sur cette région seront nécessaires pour tester cette hypothèse.

Au cours de cette étude nous avons été surpris de découvrir l'implication de la partie antérieure de l'arcopallium (l'AA) dans l'expression des comportements de peur. En effet, compte tenu des principales connexions de l'AA, différents auteurs ont supposé que cette structure était probablement davantage impliquée dans le contrôle de fonctions sensorimotrices que dans le contrôle de fonctions émotionnelles (Zeier et Karten, 1971 ; Davies *et al.,* 1997a). Cependant, Davies *et al*. (1997a) ont décrit des projections de la partie ventrale de l'AA vers le noyau du lit de la strie terminale et le noyau accumens, structures traditionnellement considérées comme « limbiques ». Or dans notre étude, les lésions de l'AA affectaient systématiquement ce territoire ventral. Ces données nous conduisent à suggérer que la partie ventrale de l'AA puisse contenir une population de neurones impliqués dans le contrôle des comportements de peur.

Ainsi compte tenu des résultats obtenus sur l'AP et l'AA, il serait intéressant de poursuivre la spécification des limites de sous-régions de l'arcopallium / PoA responsables du contrôle des réponses de peur. Notre étude a en effet permis de délimiter deux larges sous-régions impliquées, mais d'autres investigations devront affiner l'identification des limites entre les différentes sous-régions de l'arcopallium. Pour cela, l'utilisation d'un marqueur d'activation neuronale permettrait de repérer plus précisément des populations de neurones activés en réponse à une situation effrayante. Une telle étude sera présentée dans le chapitre 2.

AA et AP, implication globale vs. implication spécifique dans les comportements de peur ?

Dans cette étude, l'effet de l'inactivation de deux sous-régions de l'arcopallium / PoA a été étudié dans des tests de peur variés, permettant de visualiser les implications respectives des sous-régions dans différents aspects de la peur. Le fait que les lésions de l'AA induisent une légère diminution des comportements de peur dans tous les tests, laisse entendre que cette région serait modérément impliquée dans l'activation d'un large spectre de comportements de peur chez l'oiseau. En effet, la diminution des comportements de peur induite par la lésion n'est significative pour aucun des tests considérés isolément, mais elle est significative lorsqu'on considère un score global de peur, qui prend en compte les réactions de peur des individus au cours des quatre tests de peur. Ce résultat indique

que l'AA semble participer à la modulation de comportements de peur variés. A l'inverse, l'inactivation de l'AP n'a eu d'effet que sur les comportements de peur des oiseaux dans le test d'open-field. Il est donc possible que cette sous-région ne soit impliquée que dans certains aspects de la peur. Dans le test d'open-field, les stimuli inducteurs de peur étant une manipulation par l'expérimentateur et un placement dans un environnement nouveau sans possibilité d'échappement (voir introduction générale), il est probable que les aspects de la peur concernés par l'AP soient liés à ces stimuli. Ces résultats suggèrent que l'AA et l'AP jouent des rôles distincts dans le contrôle des comportements de peur, l'AA semblant faciliter l'expression des comportements de peur en général, alors que l'AP semble exercer une action inhibitrice focalisée sur certains comportements de peur. Il est cependant nécessaire de compléter ces données par des études approfondies de l'implication de chaque sous-région de l'arcopallium / PoA avec l'emploi de tests induisant des aspects spécifiques de la peur. De plus, pour améliorer la compréhension des liens existant entre ces sous-régions et les comportements de peur, il apparaît aussi nécessaire d'approfondir la réflexion sur le concept de peur. Les diverses situations expérimentales utilisées semblent susciter divers aspects de la peur, potentiellement contrôlés par des circuits neuronaux différents. Ces différents aspects peuvent-ils être globalisés dans une seule et même unité, « la peur », ou correspondent-ils à « différentes peurs » ? Ces questions seront abordées dans le chapitre 3 et la discussion générale de ce mémoire.