

# Evolutionary coherence of the mammalian amygdala

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Despite great interest in the role of the amygdala in animal and human behaviour, its very existence as a structurally and functionally unified brain component has been questioned, on the grounds that cell groups within it display divergent pharmacological and connective characteristics. We argue that the question of whether particular brain nuclei constitute a valid structural and functional unit is inherently an evolutionary question, and we present a method for answering it. The method involves phylogenetic analysis of comparative data to determine whether or not separate regions of the putative brain structure show statistically correlated evolution. We find that, in three separate groups of mammals (primates and two groups of insectivores), evolutionary changes in the volumes of amygdala components are strongly correlated, even after controlling for volumetric change in a wide range of limbic and other brain structures. This allows us to reject the strong claim that the amygdala is neither a structural nor a functional unit, and demonstrates the importance of evolutionary analysis in resolving such issues in systems neuroscience.

**Keywords:** amygdala; brain evolution; primates; insectivores

## 1. INTRODUCTION

Neuroscience has traditionally delineated brain systems by grouping together components that have obvious structural and functional connections. This process of delineation is partially subjective, however, as exemplified by recent debate about the amygdala. It has been argued that the term ‘amygdala’ combines cell groups arbitrarily, and that the amygdala is neither a structural nor a functional unit (Swanson–Petrovich hypothesis; Swanson & Petrovich 1998). This argument is based on the following facts: cell groups within the amygdala are derived from different regions, these groups have unique patterns of connectivity, and they can be distinguished by the distributions of neurotransmitters within each grouping. Four nuclear groups were identified (Swanson & Petrovich 1998): (i) the central nucleus (autonomic); (ii) the medial nucleus (accessory olfactory system); (iii) the cortical and basomedial nuclei (main olfactory system); and (iv) the lateral and basolateral nuclei (frontotemporal cortical system). Despite these differences, the presence of dense intra-amygdala connections may imply close integration of the activities of the different nuclei (e.g. Aggleton 1985; Pitkanen 2000). The problem lies in deciding what constitutes sufficient evidence to conclude that the amygdala does, or does not, exist as a structurally and functionally integrated entity, hence breaking the cycle of claim and counter-claim based on affinities among nuclei and differences between them, respectively. We suggest that the crux of the matter is how such nuclei evolved. Like all complex biological traits that function conspicuously well, neural systems exist by virtue of the fact that they evolved

by the process of natural selection (Young *et al.* 2000). Therefore, the question of whether particular components constitute parts of a unified neural system or structure is essentially an evolutionary question: did they evolve together, in a coordinated fashion, and in a way that cannot be attributed merely to their integration within a larger more global system (e.g. the limbic system, or even the brain as a whole)? In order to answer this question, we apply a method for detecting correlated trait evolution to comparative data on the volumes of amygdala components in two orders of mammals (primates and insectivores). Previously we reported that two amygdala components (the centromedial and corticobasolateral nuclear groups) showed correlated variation after accounting for variation in the size of other major brain structures (Barton & Aggleton 2000; Barton & Harvey 2000). A critical test of the Swanson–Petrovich hypothesis, however, would require: (i) a more fine-grained analysis of amygdaloid and associated limbic regions, and (ii) analysis of several separate taxonomic groups. With regard to (i) it is particularly important to differentiate corticobasolateral components that, in common with the centromedial amygdala, have significant olfactory inputs from those that do not. We achieve this by separate analyses of the basal nucleus. We also distinguish between separate palaeocortical components where the data allow. With regard to (ii), we perform separate analyses of three taxa: primates and two groups of insectivores.

## 2. METHODS

We tested for correlated volumetric evolution of brain components using the method of independent contrasts (Felsenstein 1985; Harvey & Pagel 1991). This method estimates the quantitative evolutionary change in character states at each node of a

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phylogenetic tree, based on the values of the character states in extant species. It is then possible to evaluate the relationship between the changes in two or more characters, i.e. whether they exhibit correlated evolution. We used the version of this general method implemented in the computer program CAIC (Purvis & Rambaut 1995). Because CAIC standardizes the change estimates or contrasts according to the amount of time since the evolutionary divergence of the taxa compared, the output values should have homogenous variances and be suitable for analysis by standard linear methods such as regression. However, before proceeding with the analysis, we checked that the contrast values met this and other assumptions (Purvis & Rambaut 1995). A phylogenetic tree for primates, including branch lengths, was taken from the literature (Purvis 1995), whilst the tree for the insectivores was constructed by one of us (Grenyer & Purvis 2003) using similar techniques to those used by Purvis (1995). Because the phylogenetic relationships of insectivores in the subfamily crocidurinae are largely unknown, we excluded these from independent-contrasts analysis and performed analyses on the raw species data. Although treating comparative data in this way (without controlling for phylogenetic relationships) is questionable, the results can be treated as an indication of whether the patterns are similar to those in other taxonomic groups.

The data subjected to comparative analysis consist of volumes of amygdala components in 43 non-human primate species and 50 insectivore species (including 13 species of crocidurine shrews, subfamily crocidurinae) (Stephan *et al.* 1981, 1987, 1991). The species used are listed in electronic Appendix A, available on The Royal Society's Publications Web site. These data, produced by a single research group using uniform methods and consistent demarcation criteria across species, consist of measurements of a centromedial group (central and medial nuclei and anterior amygdaloid area) and a corticobasolateral group of nuclei (cortical, lateral and basal nuclei), together with other limbic and non-limbic brain structures. According to the hypothesis that the amygdala consists of four unrelated groups of nuclei, neither the central nor the medial nucleus (together comprising the centromedial group) has any special connection with any of the components of the corticobasolateral group (Swanson & Petrovich 1998). Thus, the hypothesis predicts that evolutionary changes in the centromedial and corticobasolateral groups should be uncorrelated once changes in other brain structures have been taken into account. However, these groupings are rather heterogeneous. Data are also available for a more homogeneous component of the corticobasolateral group, the basal nucleus (Stephan *et al.* 1987), allowing a more fine-grained comparison with the centromedial amygdala. Volumes were also obtained from the same sources for other structures that have important connections with amygdaloid components: non-amygdaloid palaeocortex, olfactory bulb, accessory olfactory bulb, nucleus of the lateral olfactory tract (sufficient data for primates only), striatum and neocortex. In primates, subregions of the non-amygdaloid palaeocortex were not differentiated, hence palaeocortex volume measurements include olfactory cortices, olfactory tubercle, substantia innominata, the lateral olfactory tract and anterior olfactory nucleus (Stephan *et al.* 1981, 1987). In insectivores, however, separate measurements of olfactory cortices and substantia innominata were available (Stephan *et al.* 1991). These different datasets were analysed separately.

In the first set of analyses, we tested for correlated evolution among amygdala components after removing the effects of evolutionary change in the volume of the rest of the telencephalon.

We did this by regressing amygdala component volumes on the volume of the rest of the telencephalon, taking residuals and then correlating the residuals. This is analytically equivalent to calculating a partial correlation, but allows us to set regressions through the origin, as required in the method of independent contrasts (Harvey & Pagel 1991; Purvis 1995). In analyses not presented here, we found that using total brain size, rather than just the telencephalon, made no difference to the conclusions. In the second set of analyses, we used multiple regression to test for correlated evolution between amygdala components and a range of other brain structures, including palaeocortical structures with direct amygdala connections. Reported correlations are positive unless stated otherwise.

### 3. RESULTS

In both primates and insectivores, centromedial and corticobasolateral amygdala volumes show strongly and positively correlated evolution after statistically removing the effects of telencephalon volume (correlations between centromedial and corticobasolateral volumes, with telencephalon volume partialled out: primates,  $r = 0.61$ ,  $n = 40$ ,  $p < 0.0001$ ; insectivores,  $r = 0.59$ ,  $n = 33$ ,  $p = 0.0002$ ). In crocidurine shrews, where the lack of phylogenetic information precludes the use of the independent-contrasts method, analysis based on species values shows a similar relationship ( $r = 0.98$ ,  $n = 13$ ,  $p < 0.0001$ ). Crucially, significant correlations were also obtained when the basal nucleus, rather than the whole corticobasolateral complex, was tested against the centromedial complex (primates,  $r = 0.43$ ,  $n = 40$ ,  $p = 0.006$ ; insectivores,  $r = 0.75$ ,  $n = 23$ ,  $p < 0.0001$ ; crocidurine shrews,  $r = 0.74$ ,  $n = 11$ ,  $p = 0.01$ ).

Multiple regressions of each amygdala component on a range of palaeocortical and other brain structures show that the strongest and most consistent evolutionary relationships in both primates and insectivores are those between amygdala components (tables 1 and 2). For crocidurine shrews, the comparatively large number of independent variables (eight brain-structure volumes) relative to the smaller number of cases ( $n = 13$  species) resulted in non-significant regression coefficients for each brain structure, even though the overall regression was highly significant ( $p < 0.0001$ ). Therefore a stepwise procedure was used to select the most relevant independent variables. Using this procedure, centromedial amygdala volume showed a strong positive correlation with corticobasolateral amygdala volume ( $F = 494.7$ ,  $p < 0.0001$ ) and vice versa ( $F = 15.2$ ,  $p < 0.01$ ). Again, similar results were obtained when the basal nucleus, rather than the whole corticobasolateral complex, was tested against the centromedial complex. In primates, the partial regression coefficient, controlling for all other variables, was significant ( $t = 2.6$ ,  $p = 0.01$ ). In insectivores, stepwise selection of variables was necessary and identified centromedial volume as the strongest correlate of basal-nucleus volume ( $F = 190.1$ ,  $p < 0.0001$ ). Small sample size precluded multiple regression analysis of the basal nucleus in crocidurine shrews.

Tables 1 and 2 reveal that, once the strong intra-amygdala relationships are taken into account, amygdala components are additionally correlated with other brain structures, and that these correlations differ for the two

Table 1. Multiple regression analyses of the evolutionary relationships between sizes of amygdala components and other brain structures in primates for centromedial amygdala and corticobasolateral amygdala.

(Both regressions are based on 40 independent-contrast values for each variable, computed from 43 species values. Two-tailed tests: \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .)

	standardized coefficient	<i>t</i> value	<i>p</i> value
centromedial amygdala: $r^2 = 0.90$ , d.f. = 7,33, $p < 0.0001$			
olfactory bulb	-0.14	-1.33	0.19
accessory olfactory bulb	-0.07	-0.56	0.58
nucleus of lateral olfactory tract	-0.20	-1.42	0.16
striatum	0.04	0.12	0.90
neocortex	-0.35	-1.21	0.24
palaeocortex	0.61	2.81	0.008**
corticobasolateral amygdala	0.77	3.90	0.0005***
corticobasolateral amygdala: $r^2 = 0.95$ , d.f. = 7,33, $p < 0.0001$			
olfactory bulb	0.09	1.14	0.26
accessory olfactory bulb	-0.02	-0.23	0.82
nucleus of lateral olfactory tract	0.14	1.33	0.19
striatum	-0.22	-1.09	0.29
neocortex	0.37	1.82	0.08
palaeocortex	-0.05	-0.25	0.80
centromedial amygdala	0.41	3.90	0.0005***

Table 2. Multiple regression analyses of the evolutionary relationships between sizes of amygdala components and other brain structures in insectivores for centromedial amygdala and corticobasolateral amygdala.

(Both regressions are based on 33 independent-contrast values for each variable, computed from 37 species values. Other details as for table 1.)

	standardized coefficient	<i>t</i> value	<i>p</i> value
centromedial amygdala: $r^2 = 0.97$ , d.f. = 7,26, $p < 0.0001$			
olfactory bulb	0.02	0.06	0.95
accessory olfactory bulb	0.09	1.32	0.20
striatum	-0.17	-0.76	0.46
neocortex	0.21	1.04	0.31
olfactory cortex	0.38	1.26	0.22
substantia innominata	-0.01	-0.17	0.86
corticobasolateral amygdala	0.52	2.99	0.006**
corticobasolateral amygdala: $r^2 = 0.97$ , d.f. = 9,24, $p < 0.0001$			
olfactory bulb	0.54	1.85	0.07
accessory olfactory bulb	-0.03	-0.46	0.65
striatum	0.12	0.54	0.60
neocortex	0.27	1.30	0.20
olfactory cortex	-0.36	-1.23	0.23
substantia innominata	-0.02	-0.20	0.84
centromedial amygdala	0.49	2.99	0.006**

amygdala components in accord with functional and connective differences (Swanson & Petrovich 1998). In primates, centromedial volume correlates with palaeocortex volume (table 1). In some primate species the accessory olfactory bulb is lacking or vestigial so that volumes are given as zero (Stephan *et al.* 1987). Exclusion of these species revealed significant positive correlations between centromedial amygdala and striatum ( $t = 3.26$ ,  $p = 0.004$ ), and between corticobasolateral amygdala and both neocortex ( $t = 4.03$ ,  $p = 0.0006$ ) and nucleus of the lateral olfactory tract ( $t = 2.76$ ,  $p = 0.012$ ). The basal nucleus on its own also correlated with neocortex ( $t = 2.49$ ,  $p = 0.02$ ), and correlated *negatively* with the nucleus of the lateral

olfactory tract, reflecting the lack of olfactory projections to the basal nucleus. Stepwise variable selection did not change these results. Amongst insectivores, simultaneous multiple regressions revealed no correlates of amygdala-component volume in addition to the reciprocal amygdala component (table 2). However, additional correlates were identified using stepwise variable selection. For the centromedial amygdala these were olfactory cortex ( $F = 11.2$ ,  $p < 0.01$ ) and accessory olfactory bulb ( $F = 5.2$ ,  $p < 0.05$ ). Corticobasolateral amygdala correlated additionally with striatum ( $F = 11.2$ ,  $p < 0.01$ ). These evolutionary relationships among amygdala components and other structures are summarized visually in figure 1.

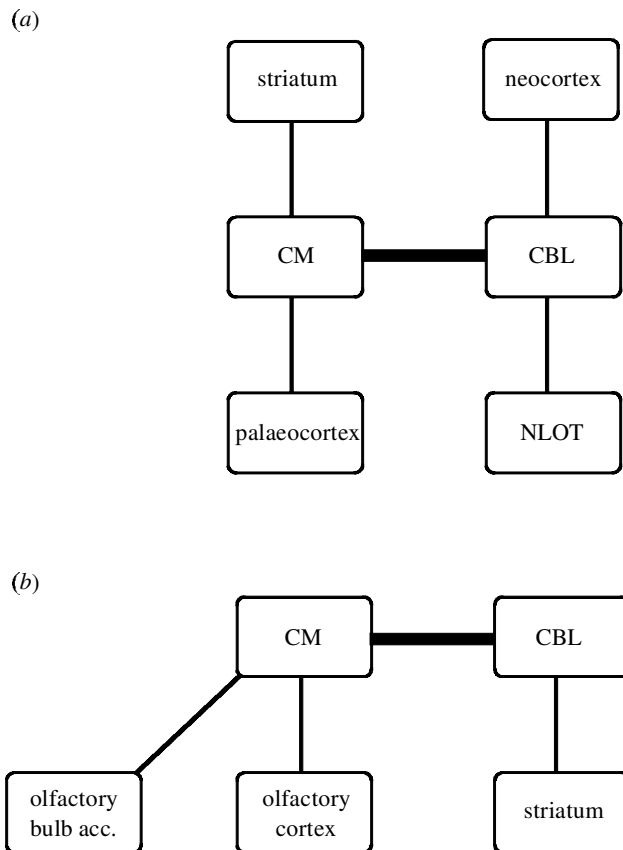


Figure 1. Summarized patterns of correlated volumetric evolution among amygdala components and related brain structures for (a) primates and (b) insectivores. Significant positive partial correlations between amygdala components and other structures, controlling for variation in a range of structures (see tables 1 and 2 and § 3), are indicated by connecting lines. Heavier lines are used to connect amygdala components, indicating the stronger evolutionary relationships that these components have with each other than with other structures. CM, centromedial amygdala; CBL, corticobasolateral amygdala; NLOT, nucleus of the lateral olfactory tract; olfactory bulb acc., accessory olfactory bulb.

#### 4. DISCUSSION

Our results show clearly that the two amygdala components evolved together, independently of changes in other brain structures, within each of the taxonomic groups investigated. This pattern was found even when the more homogeneous basal nucleus was differentiated from the rest of the corticobasolateral group. The only plausible explanation for such strongly and consistently correlated volumetric evolution is that there exist primary structural and functional links between nuclei in the separate components. Whilst refuting the strong claim that these nuclei are parts of entirely separate brain systems in mammals (Swanson & Petrovich 1998), this does not deny that there are important differences between them in their pharmacological properties, connections and functions. Indeed, the analyses show particularly consistent links between olfactory cortices and the centromedial group of nuclei. These links accord with the connections of piriform cortices and other olfactory structures, which innervate the superficial nuclei of the amygdala, including the molecular layer adjacent to the medial nucleus (Turner *et*

*al.* 1978; McDonald 1998). Our results also bear on a recent suggestion, reflecting the existence of a molecular marker common to all limbic structures, that the limbic system evolved as a coordinated whole (Finlay *et al.* 2001). Our finding that amygdala components evolved with each other independently of other limbic structures indicates that any such globally coordinated limbic evolution must have been limited.

It could perhaps be argued that, whilst strong patterns of correlated evolution such as those reported here certainly indicate evolutionary—and hence functional—unity, this does not necessarily imply that the components are parts of a unified structure. Functional systems, after all, may comprise numerous structures that are spatially, morphologically and molecularly differentiated. We accept this. Our point, however, is that natural selection modifies structure and function in concert. Hence, claims about the structural unity or disunity of brain components make most sense in a functional context. If two or more brain components are parts of a unified functional system, this is necessarily by virtue of their having some structural unity. The components must be linked by important anatomical connections.

Although the evolutionary correlations between amygdala components and non-amygdala structures were generally consistent across taxonomic groups, there were also some differences. These differences may reflect evolutionary shifts in extrinsic connections. For example, the finding that corticobasolateral amygdala and neocortex are correlated in primates but not in insectivores accords with evidence that the cortical connections of the amygdala are particularly profuse in primates (Young *et al.* 1994). Further, because in Old World monkeys and apes the accessory olfactory bulb is either lacking or vestigial (Stephan *et al.* 1987; Meisami & Bhatnagar 1998), the extrinsic connections of the amygdala, especially of the medial nucleus (Swanson & Petrovich 1998), are bound to differ somewhat between these species and those possessing an accessory olfactory bulb. Such taxonomic differences urge caution in extrapolating experimental results between species, and provide strong justification for further comparative studies.

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