A neuroanatomical evaluation of cholinergic, catecholaminergic, serotonergic and orexinergic neural systems in mammals pertaining to the phylogenetic affinities of the Chiroptera

Tanya Calvey

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Supervisor: Prof. P.R. Manger

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Abstract:

One of the few remaining mysteries in mammalian phylogeny is the issue of Chiropteran phylogeny. In order to further investigate the diphyletic hypothesis that states that Megachiroptera evolved from primate-like gliders and that Microchiroptera evolved from insectivores, the cholinergic, catecholaminergic, serotonergic and orexinergic systems were analyzed in, not only five insectivores (*Crocidura cyanea, Crocidura olivieri, Sylvisorex ollula, Paraechinus aethiopicus* and *Atelerix frontalis*) and three prosimian primates (*Galagoides demidoff*, *Perodicticus potto* and *Lemur catta*), but in species from other orders of interest including the Afrotheria (*Potamogale velox*, *Amblysomus hottentotus* and *Petrodromus tetradactylus*), Lagomorpha (*Lepus capensis*) and Scandentia (*Tupaia belangeri*). Brains of the mammals were coronally sectioned and immunohistochemically stained with antibodies against cholineacetyltransferase, tyrosine hydroxylase, serotonin and orexin-A. The presence or absence of 93 nuclei within these neuromodulatory systems was entered into modern cladistics software for analysis of the 13 studied species, as well as an additional 40 previously studied mammals. The majority of nuclei revealed in the current study were similar among the species investigated and to mammals generally, but certain differences in the nuclear complement highlighted potential phylogenetic interrelationships. The Afrotherian, *A. hottentotus*, presented unusual cholinergic interneurons in the cerebral cortex, hippocampus, olfactory bulb and amygdala, and exhibited an unusual foreshortening of the brain, such that a major mesencephalic flexure in the brainstem was evident. The Afrotherian, *P. tetradactylus*, lacked the catecholaminergic A15d nucleus as in a previously studied member of Macroscelididae. The three Insectivoran shrews lacked the cholinergic parabigeminal and Edinger-Westphal nuclei, had a mediodorsal arch of the cholinergic laterodorsal tegmental nucleus, lacked the catecholaminergic A4 and A15d nuclei and presented an incipient ventral division of the substantia nigra which is identical to previously studied Microchiroptera. All three prosimians presented a central compact division of catecholaminergic locus coeruleus (A6c) surrounded by a shell of less densely packed (A6d) tyrosine hydroxylase immunopositive neurons. This combination of compact and diffuse divisions of the locus coeruleus complex is only found in primates and Megachiropterans of all the mammalian species studied to date. *T. belangeri* of the Scandentia contained ChAT+ neurons within the nucleus of the trapezoid body as well as the superior olivary nuclear complex, which has not been described in any mammal studied to date. *L. capensis* of the Lagomorpha presented

the rodent specific rostral dorsal midline medullary nucleus (C3), while *T. belangeri* was lacking both the ventral and dorsal divisions of the anterior hypothalamic group (A15v and A15d), and both species were lacking the primate/Megachiropteran specific compact portion of the locus coeruleus. Our neuroanatomical analysis suggests a phylogenetic relationship between the Soricidae (shrews) and the Microchiropterans, supports the phylogenetic grouping of primates with Megachiropterans, confirms previous molecular evidence of the relationship between lagomorphs and rodents within the super-order Glires, and suggests that primates are phylogenetically closer to Megachiroptera than to any members of the Euarchontoglires. The cladistic analysis confirmed the neuroanatomical analysis with the most parsimonious tree placing Megachiroptera into the Euarchontoglires as a sister group to primates and the Microchiroptera next to Soricidae within the Laurasiatheria.