The Puzzle of Forebrain Evolution

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The classic paper discussed in this essay:

Bruce LL, Neary TJ (1995): The limbic system of tetrapods: a comparative analysis of cortical and amygdalar populations. *Brain Behav Evol* 46:224–234.

I love puzzles, and the puzzle of how the connections between forebrain and hypothalamic areas of reptiles related to those of frogs and mammals was a wonderful combination of fun, obsession, and frustration. My friend and collaborator in this study, Tim Neary, had discovered an approach to the *Gekko* hypothalamus through the roof of the mouth, so we could place injections there without involving other structures. We hoped that by tracing the connections of the ventromedial and lateral hypothalamus with the forebrain we could identify potential homologues of the mammalian amygdalar nuclei. We began our collaboration in 1988, soon after I moved to Creighton University. At that time the reptilian amygdalar nuclei were believed to be somewhere in the caudal telencephalon, but no one was sure which part of the telencephalon or if they were comparable to specific mammalian amygdalar nuclei.

Tim and I came from different but complementary backgrounds. Tim was schooled in comparative vertebrate neurobiology and politics while a graduate student with Ted Voneida at Case Western and then he followed Glenn Northcutt to the University of Michigan as a postdoctoral fellow. His comparative neuroanato-

my course, taught by Glenn, was cut short by the Kent State massacre, at which time students and faculty quit attending classes to protest. Tim learned a lesson about tear gas instead. Even so, Tim was an extraordinary neuroanatomist. He injected tract tracers into every possible part of the frog brain and understood its organization better than anyone.

As a graduate student I worked first with Earl Kicliter at the University of Illinois and then with Ann Butler at Georgetown University. My studies with Earl included a year at the University of Puerto Rico, where I was fortunate to take a seminar in comparative neuroanatomy that was taught by Earl, Boyd Campbell, Sven Ebbesson, and Jose de Olmos. Discussions with them on how to use different characteristics to identify possible homologies provided the foundation to my fascination for comparative neuroscience. Most memorable was the four of them discussing how best to use a combination of fiber tracts, connections, topology, embryology, histochemistry, and electrophysiology to identify potentially homologous structures in different species. Their mantra was that the greater the number of similar characters between two structures in diverse species, the greater the probability

that they were derived from a common ancestral structure [Campbell and Hodos, 1970]. This was the approach that Tim and I applied to our study of the evolution of the forebrain. Jose de Olmos gave me an appreciation for the intricate organization of the different parts of the amygdala, hypothalamus, and thalamus in mammals, as well as an awareness that little was known about the limbic system in nonmammalian vertebrates. At Georgetown my exposure to evolutionary neurobiology continued through my interactions with Ann Butler, Mark Braford, and Cathy McCormick as I analyzed the connections of the reptilian forebrain for my thesis.

Tim and I began our hypothalamic study with the belief that the reptilian dorsal ventricular ridge (DVR) and avian nidopallium were homologous to parts of the mammalian sensory cortex, as proposed by Karten [1969]. Karten's hypothesis was the best explanation of the data at that time and we never considered questioning it. We expected that hypothalamic injections would label amygdalar nuclei somewhere in the caudal telencephalon. After our first injection in the ventromedial hypothalamus, we were shocked to find a significant cluster of labeled cells in the medial core

of the DVR. We believed, of course, that somehow our injection had involved a fiber tract such as the lateral forebrain bundle or internal capsule. After smaller and smaller injections the DVR cluster was still there. Moreover, we could follow labeled axons from the cluster, through a tract believed to be homologous to the stria terminalis, into our injection site in the ventromedial hypothalamus. Our injections in the lateral hypothalamus labeled cells in areas believed to be amygdalar groups, as well as in the caudal DVR. In other words, our data showed that much of the DVR, with the notable exception of the areas receiving tectothalamic visual and auditory information, was connected with the hypothalamus, and thus amygdala-like. At the same time Tim made injections into the frog hypothalamus and traced connections to particular forebrain areas. Together this work produced a trio of papers on hypothalamic connections in lizards and frogs that were published in *Brain, Behavior and Evolution* [Bruce and Neary, 1995a, b; Neary, 1995].

The challenge, then, was to identify a reasonable evolutionary scheme that required few connectional changes. We devoured piles of connectional studies on the hypothalamus, amygdala, and numerous cortical areas. Initially it seemed like the ancestral brains that gave rise to each vertebrate class had been reshuffled like a deck of cards. Eventually we realized that the forebrain areas that connected to the hypothalamus had unique combinations of connections and topological relationships that were similar in frogs, lizards, and mammals, suggesting that they were highly conserved, homologous groups.

The second modification to the traditional evolutionary scheme came about as Tim and I reexamined pallial connections in frogs. We realized that the frog medial pallium was comparable to both the neocortex and the hippocampus of mammals, whereas the dorsal pallium and dorsal part of the lateral pallium of frogs was an olfactory area comparable to the olfactory cortex of mammals. This meant that the hippocampus and neocortex-like regions were born near the cortical hem of the pallium in frogs, reptiles, and mammals, whereas the hypothalamus-projecting areas of the pallium in frogs (LPv) and reptiles (caudal DVR) arose from an area adjacent to the striatum. The olfactory cortex was located between these two territories and physically separated them in both frogs and reptiles.

Comparisons between the pallial areas of frogs, lizards, and birds were then clear because their connections and topology were conserved. However, identifying a mammalian homologue of the anterior part of the DVR of reptiles and rostral LPv of frogs remained a significant challenge. Why and how could regions that were evolutionarily successful and apparently essential in frogs, reptiles, and birds lose and gain multiple significant connections, change migratory patterns, and become an integral part of the mammalian sensory cortex, as suggested by Karten? Likewise, identifying reptilian and amphibian homologues of the mammalian lateral amygdala, which receives massive sensory cortical input, was challenging. Eventually we considered the possibility that the mammalian lateral amygdala was comparable to the anterior DVR/nidopallium, an idea inspired by two papers describing the sensory thalamic input to the lateral amygdala [LeDoux et al., 1990; Turner and Herkenham, 1991]. Further literature searches revealed that other connections of the sensory recipient DVR were very similar to those of the lateral amygdala, suggesting that they arose from a common anlage. And so the idea of a homology between mammalian basolateral amygdalar complex, reptilian DVR, avian nidopallium, and amphibian LPv was born. Eventually, by considering each connection and topological relationship as a separate character we were led to an evolutionary scheme in which each region had highly conserved features that could be recognized in mammals, birds, reptiles, and amphibians.

We were thrilled beyond belief. Nonetheless, we knew this idea would be controversial and, therefore, reviewed the data repeatedly for another year before we presented it as a poster at the Society for Neuroscience in 1993, then in the 1994 Karger workshop. Finally we wrote our paper describing the rationale for our hypothesis of the evolution of the limbic system [Bruce and Neary, 1995c].

Tim was a very skilled writer, and he deserves a great deal of credit for the clarity and readability of our papers. By the time we finished our paper on the evolution of the limbic system, Tim's health was failing noticeably, largely due to what was eventually diagnosed as multiple sclerosis. Sadly, his poor health kept us from further collaborations.

We realized that our hypothesis challenged current ideas and would not be well

received by other researchers, at least initially. In fact it was not accepted for many years. First, it went against nearly 30 years of tradition, during which Karten's hypothesis was accepted *de rigueur*. Second, our arguments relied heavily on a multitude of connections as well as topology to identify homologues. This method had not been used before to such an extent, and I suspect some people, especially those unfamiliar with connectional specificity, were unsure how rigorous it was. Third, the avian nidopallium was and is a research model for studying cortical functions, such as vocal learning and sensory-motor integration. Perhaps some people felt threatened by the suggestion that their many years of work on the cortex was actually on the amygdala.

Initially our work was largely ignored or cited as a controversial alternative to Karten's hypothesis. It was first considered seriously after Fernandez et al. [1998] showed that the frog LPv, reptilian DVR, avian nidopallium, and mammalian pallial amygdala express a different set of genes from those in cortex. Subsequent studies of genetic patterning detailed the development of different forebrain regions [e.g. Medina et al., 2011]. These results are consistent with our findings, although our connectional and topological analyses identified homologous subpopulations within the genetically identified fields that have yet to be distinguished by gene expression studies. Our hypothesis continues to be challenged, although more and more scientists are considering it seriously, and it is rewarding to know that our hypothesis has contributed to the research of others.

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