

Evolution of the Amygdala: New Insights from Studies in Amphibians

Frédéric Laberge^{a, b} Sabine Mühlenbrock-Lenter^b Wolfgang Grunwald^b
Gerhard Roth^{a, b}

^aHanse Institute for Advanced Study, Delmenhorst, and ^bBrain Research Institute, University of Bremen, Bremen, Germany

Key Words

Amphibians · Amniotes · Evolution · Development · Telencephalon · Amygdala · Limbic system · Comparative neuroanatomy

Abstract

The histology of amphibian brains gives an impression of relative simplicity when compared with that of reptiles or mammals. The amphibian telencephalon is small and contains comparatively few and large neurons, which in most parts constitute a dense periventricular cellular layer. However, the view emerging from the last decade is that the brains of all tetrapods, including amphibians, share a general bauplan resulting from common ancestry and the need to perform similar vital functions. To what extent this common organization also applies to higher brain functions is unknown due to a limited knowledge of the neurobiology of early vertebrates. The amygdala is widely recognized as a brain center critical for basic forms of emotional learning (e.g., fear conditioning) and its structure in amphibians could suggest how this capacity evolved. A functional systems approach is used here to synthesize the results of our anatomical investigations of the amphibian amygdala. It is proposed that the connectivity of the amphibian telencephalon portends a capacity for multi-modal association in a lim-

bic system largely similar to that of amniote vertebrates. One remarkable exception is the presence of new sensory-associative regions of the amygdala in amniotes: the posterior dorsal ventricular ridge plus lateral nuclei in reptiles and the basolateral complex in mammals. These presumably homologous regions apparently are capable of modulating the phylogenetically older central amygdala and allow more complex forms of emotional learning.

Copyright © 2006 S. Karger AG, Basel

Introduction

In his review of emotion and the brain, J.E. LeDoux [2000] rejected a concept of the limbic system which attributes emotions to the ‘old’ cortex and the basal ganglia, arguing that it could not explain the specific brain circuits of emotion. However, he observed that the functional idea of a limbic system could be useful for the formulation of a general evolutionary explanation of the relationship between emotion and brain. The amygdala is generally recognized as an important part of the emotional brain. It has long been proposed to consist of phylogenetically old and new divisions [Johnston, 1923]. Swanson and Petrovich [1998] rejected the classical view of the mammalian amygdala as a structural or functional unit

and instead argued that it is composed of four different functional systems: main olfactory, accessory olfactory, autonomic and frontotemporal cortical. Despite the controversial nature of Swanson and Petrovich's definition of the amygdala, as is the case with the limbic system concept in general, it provides an attractive framework to explore the evolution of the entire amygdaloid complex.

The functional systems (components) of the amygdala are essentially defined by their sensory input from early stations of the chemical senses (olfaction and taste), visceral sensory afferents and higher-order thalamic and cortical input from all sensory modalities, in addition to an output to autonomic and visceral centers in the brainstem and connections to the limbic cortex. Our laboratory has extensively used this functional systems approach to investigate the connectivity of the amphibian amygdala [Roth et al., 2004; Laberge and Roth, 2005]. Accordingly, we propose a morphotype of the tetrapod amygdala [see Northcutt, 1995].

Recent neuroanatomical studies led to diverging views on the organization of the amphibian basal ganglia and amygdaloid complex. Interpretations differ primarily in the identity of the dorsal pallidum and central (autonom-

ic) amygdala, along with different claims of homology regarding structures equivalent to the mammalian basolateral amygdaloid complex. In the following, we first present the functional components of the amygdala as they can be deduced from anatomical and connective data. The situation found in reptiles and mammals is also described to enable comparisons with the amphibian outgroup. Secondly, anatomical data are complemented by recent gene expression studies indicative of the extent of pallial and subpallial territories in the telencephalon. Finally, we assess the competing claims of homology for the components of the amphibian amygdaloid complex.

Amygdaloid Complex in Amphibians

(fig. 1A, B)

In anurans and urodeles, the main olfactory component of the amygdala is found in the ventral part of the caudal lateral pallium. It is distinguished from the more rostral lateral pallium by a projection to the ventral hypothalamus. Scalia and collaborators [1991] previously identified this structure in anurans as the cortical amyg-

Abbreviations used in figures 1 and 2

I	olfactory nerve	DLA	dorsolateral amygdala	NS	nucleus sphericus
II	optic nerve	DP	dorsal pallium	ot	optic tract
ac	anterior commissure	DT	dorsal thalamus	PB	parabrachial nucleus
ADVR	anterior dorsal ventricular ridge	DPAL	dorsal pallidum	PDVR	posterior dorsal ventricular ridge
AOB	accessory olfactory bulb	ExA	external amygdala	PIR	piriform cortex
aot	accessory olfactory tract	LA	lateral amygdala	POA	preoptic area
BLA	basolateral amygdala	LC	lateral cortex	S	septum
BMA	basomedial amygdala	LP	lateral pallium	SAT	striato-amygdaloid transition area
c	caudal	MA	medial amygdala (reptiles)	SP	substance P
CEA	central amygdala	MC	medial cortex	STR	striatum
COApl	cortical amygdala pars lateralis	MEA	medial amygdala (mammals)	VAA	ventral anterior amygdala
COApm	cortical amygdala pars medialis	MOB	main olfactory bulb	VCP	ventral cellular prominence
CP	caudate-putamen	MP	medial pallium	VPA	ventral posterior amygdala
DC	dorsal cortex	MS	medial septum	VT	ventral thalamus

Fig. 1. Functional compartments of the amygdala in tetrapods. The main olfactory (brown), accessory olfactory (green), autonomic (blue) and limbic associative (red) components of the amygdaloid complex are pictured on schematic transverse sections and lateral illustrations of the brain in an anuran amphibian (**A**), a urodele amphibian (**B**), a reptile (**C**) and a mammal (**D**). The dorsal striato-pallidal complex is shown in gray to help illustrate regional topol-

ogy. The asterisks in **A** and **B** show the position that we believe represents the ventral pallidum in amphibians [Mühlenbrock-Lenter et al., 2005; Laberge and Roth, 2005]. The levels of transverse sections is shown by a single bar in **A** and **D**, and numbers in **B** and **C**. The olfactory and optic nerves are shown for position reference. Brain outline and structures in **C** and **D** are modified from Lanuza et al. [1998]. For abbreviations see list.

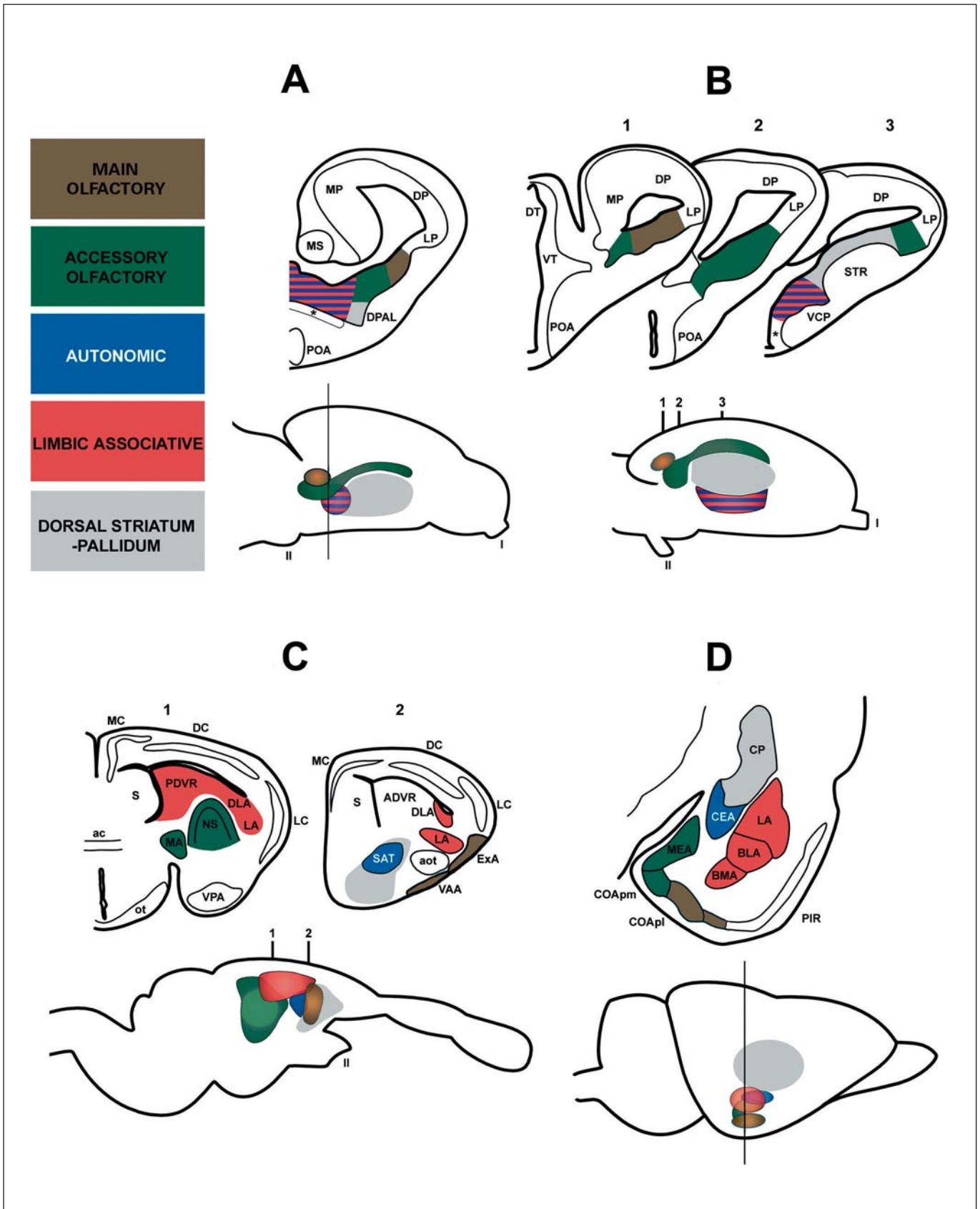


Table 1. Comparison of previous nomenclatures used to describe the components of the amphibian amygdaloid complex with the one used in this article

Present nomenclature	Northcutt and Kicliter [1980]	Scalia et al. [1991]	Bruce and Neary [1995]	Marín et al. [1998] and Moreno and González [2003, 2004, 2005]	Brox et al. [2004]
Main olfactory component (cortical amygdala)	Pars ventralis of the lateral pallium (pv)	Cortical amygdaloid nucleus (am cort)	Caudal ventral part of the lateral pallium (LPv)	Caudal lateral amygdala (LA)	Posterolateral amygdalar nucleus (PLA)
Accessory olfactory component (extended vomeronasal amygdala)	Pars ventralis of the lateral pallium (pv)	–	Rostral ventral part of the lateral pallium (LPv)	Rostral lateral amygdala + anterior amygdala (LA+AA)	Rostral lateral amygdala + anterior amygdala (LA+AA)
	Amygdala pars lateralis (apl)	Medial amygdaloid nucleus (am med)	Lateral amygdala (AL)	Medial amygdala (MEA)	Medial amygdala (MEA)
Mixed autonomic and limbic associative component (extended central amygdala)	Amygdala pars medialis (apm)	–	–	Bed nucleus of the stria terminalis (BST)	Bed nucleus of the stria terminalis + nucleus of the anterior commissure (BST+CA)
Caudal dorsal pallidum	Part of the entopeduncular nucleus (en)	–	Caudal striatum (Stc)	Central amygdala (CeA)	Pallidum (PA)

N.B. Herrick [1948] termed the rostral part of the vomeronasal extended amygdala in salamander, the lateral cellular prominence, and the caudal part receiving strong AOB input, nucleus amygdalae.

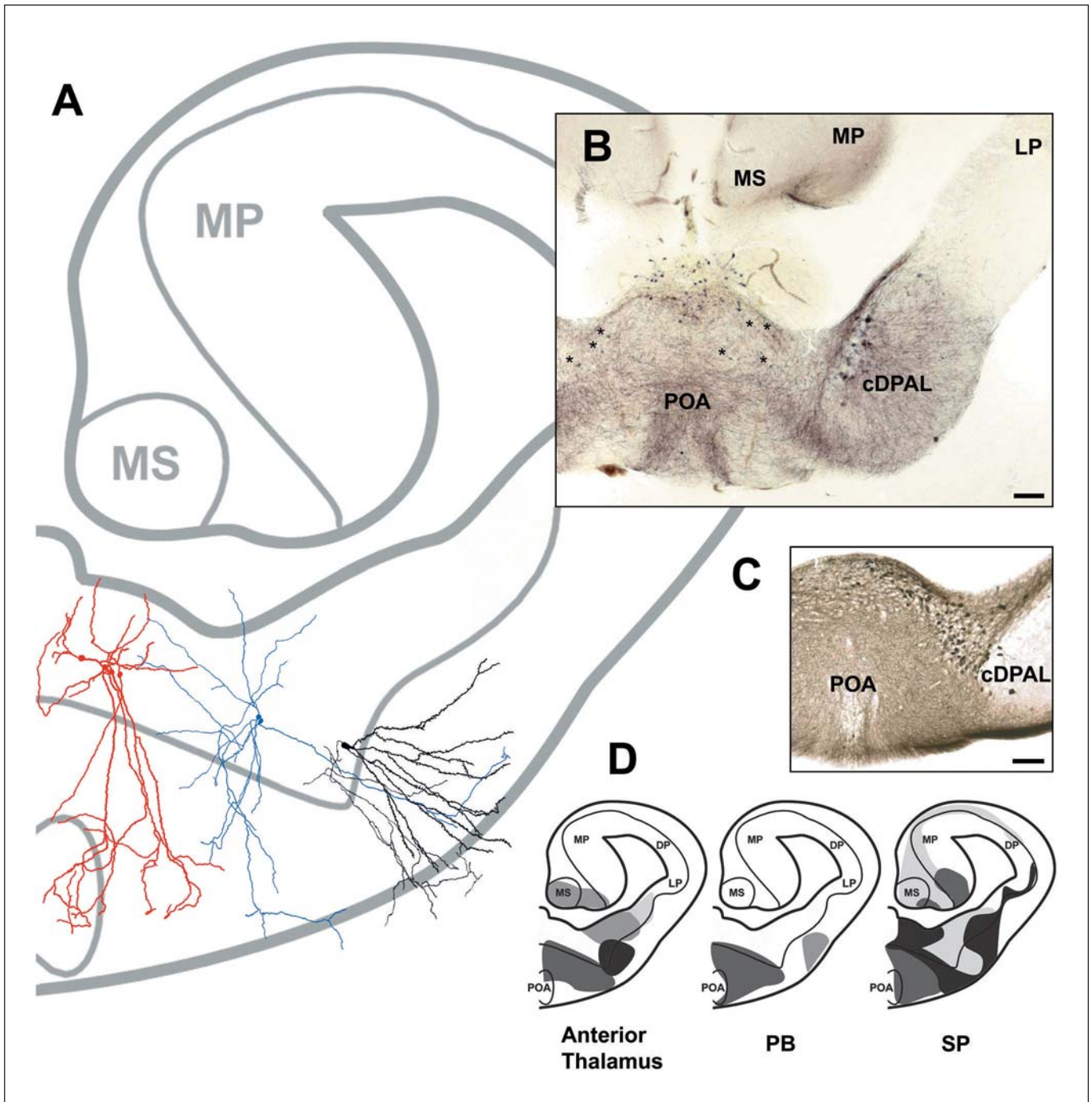
daloid nucleus. Moreno and González [2004] demonstrated that it receives strong input from the main olfactory bulb (MOB) and probably little or no input from the accessory olfactory bulb (AOB) in anurans. In salamanders, the axonal terminal fields of the main and accessory olfactory bulbs are segregated into a lateral and a medial region, respectively, in the caudal pole of the telencephalon paralleling the dendritic fields of lateral and medial neurons situated in this region [Laberge and Roth, 2005]. This suggests that the main olfactory component of the amphibian amygdala receives olfactory input only from the MOB.

The accessory olfactory, or vomeronasal, component is found in the caudal telencephalon just medial to the main olfactory component. Rostrally, it extends dorsal to the striato-pallidum, and caudally it merges with the dorsal preoptic region. Thus, the extended vomeronasal amygdala comprises both the amygdala pars lateralis of Northcutt and Kicliter [1980], also named medial amygdala by Moreno and González [2003], and the striato-pallial transition area [Marín et al., 1997a, b; Roth et al., 2004; Laberge and Roth, 2005], also named anterior amygdala by Marín and collaborators [1998] (see table 1 for a survey of the different nomenclatures describing the amphibian amygdaloid complex). The caudal component of the vomeronasal amygdala receives input from the AOB and projects strongly to the preoptic area and hypothalamus. The

anterior part shares these descending projections with the caudal part, is reciprocally connected with the latter, and might also receive AOB input.

The autonomic component displays reciprocal connections with visceral and autonomic centers. In amphibians, this nucleus also comprises neurons projecting to the limbic pallium (see below) and to the septal region. In anurans, it is found in a position medially adjacent to the accessory olfactory component and the caudal tip of the dorsal pallidum. It extends rostrally below the ventral lateral septum as described by Roth et al. [2004] and Roden et al. [2005]. Neurons of the dorsal pallidum and autonomic amygdala display striking differences in soma size and the morphology of their dendritic trees (fig. 2A).

Fig. 2. The central amygdala in the toad *Bombina orientalis* as seen on transverse brain sections at rostral preoptic level. **A** Intracellular injections of biocytin reveal morphological differences between neurons of the caudal dorsal pallidum and the subpallial amygdala. On the right side, a camera lucida reconstruction of a neuron of the caudal dorsal pallidum (black) shows its large soma and abundant dendritic spines. The dendrites course rostrally inside the dorsal striato-pallidal neuropil. To the left, clusters of two and four neurons illustrate neurons typical of the subpallial amygdala with small somata, smooth dendrites that arborize locally and axonal projec-



tions to autonomic centers (blue) or limbic and visceral regions (red). **B** Micrograph showing a specimen in which biocytin was applied to the cut brain just caudal to the cerebellum. Retrogradely filled neurons project to the medulla and can be seen in the caudal dorsal pallidum and the medial region of the amygdala. Neurons can also be seen more laterally in the amygdala (asterisks). Note that the anterograde labeling of fibers from the medulla does not extend laterally into the main olfactory amygdala and lateral pallium. **C** Same experiment as in **B**, but biocytin was applied to the medial brain in the septum and medial pallium. Retrogradely filled

neurons possess ascending limbic projections and are abundant throughout the subpallial amygdala. Scale bar in **B** and **C** is 100 μm . **D** Schematic representation of the distribution of afferents from the anterior thalamus, parabrachial nucleus (PB) and substance P (SP)-positive fibers to the anuran central amygdala. These projections represent sensory and visceral inputs. The strength of labeling increases from scattered, moderate, high density and highest density according to the intensity of gray. For abbreviations see list.

Figure 2B, D shows that the medial part of the anuran amygdala is connected to visceral and autonomic centers as it is reciprocally connected with the brainstem, and receives parabrachial input and a strong neuropeptidergic innervation. In salamanders, the autonomic component of the amygdala is found in the ventral rostral part of the amygdala and is not continuous with the more caudal olfactory components of the amygdala. It is located in the ventral cellular prominence below the dorsal striatopallidum and caudal to the rostrally-situated nucleus accumbens.

The frontotemporal cortical component is referred to here as the limbic associative component because of the apparent absence of frontal and temporal cortices in amphibians. Limbic regions included in this term are the medial pallium and the septal region, both of which are strongly interconnected. The dorsal pallium could also subservise important limbic functions in amphibians as it is strongly connected with the medial pallium and receives MOB input [Westhoff and Roth, 2002; Laberge and Roth, 2005]. The medial pallium, or at least parts of it, is believed to be homologous to Ammon's horn and subiculum of the hippocampal formation of mammals [Northcutt and Ronan, 1992; Striedter, 1997; González and Lopez, 2002; Westhoff and Roth, 2002; Moreno et al., 2004]. In anurans, the limbic associative component is found in the medial part of the amygdala in combination with the autonomic component. Figure 2C shows neurons of the medial part of the anuran amygdala projecting to the medial pallium, and figure 2D shows thalamic projections of high density in the medial part, but only few scattered thalamic fibers in the lateral part of the amygdala. Reciprocal connections of this region with the medial pallium, septum and thalamus enable the association of sensory thalamic input with the limbic pallium. As mentioned above, the salamander limbic associative component is also mixed with neurons displaying connections characteristic of the autonomic component in a rostral division of the amygdala.

Amygdaloid Complex in Reptiles (fig. 1C)

The brain template chosen in figure 1C is that of a lizard even though our attempt is to give an overview of the general reptilian situation. The main olfactory component is represented by non-cortical structures that receive MOB input. These are the external and the ventral anterior amygdala situated in the superficial rostralateral telencephalon [Lanuza and Halpern, 1998]. The vomero-

nasal component, i.e. nucleus sphericus and medial amygdala, is situated more medially in the caudal telencephalon. Note that the vomeronasal organ is absent in crocodylians and many turtles [Northcutt, 1981]. The striato-amygdaloid transition area (SAT) most likely represents the autonomic component of the reptilian amygdala exhibiting strong reciprocal connections with autonomic and visceral centers [Russchen and Jonker, 1988; area d in Siemen and Künzle, 1994; Bruce and Neary, 1995]. This structure is located in the medioventral part of the telencephalon just caudal to the striatum, which is found quite rostrally in reptiles.

There is more disagreement concerning the identity of the amygdaloid structures involved in associations between the pallium and thalamic sensory input. Lanuza and collaborators [1998] consider that the posterior dorsal ventricular ridge (PDVR), the dorsolateral and the lateral amygdala are functionally equivalent to the mammalian basolateral complex, as illustrated in figure 1C. This view implies that the thalamo-recipient anterior dorsal ventricular ridge (ADVR) of reptiles is not part of the amygdala. An alternative interpretation states that the ADVR is homologous to the mammalian lateral nucleus of the amygdala [Bruce and Neary, 1995; Fernandez et al., 1998]. The thalamic sensory input to the ADVR is segregated across modalities, as opposed to the multimodal input to the PDVR, which is expected for a limbic associative component [Lanuza et al., 1998; see also Striedter, 1997 and Novejarque et al., 2004]. Interestingly, the PDVR of reptiles appears to be involved in emotional behavior, as shown by experimental lesion or stimulation of the reptilian amygdala [Tarr, 1977; Distel, 1978; Sugerman and Demski, 1978]. However, the thalamic sensory input to the lateral nucleus of mammals is also largely segregated across modalities [Linke et al., 2000]. We consider these two competing interpretations on the nature of the ADVR as equally parsimonious in regard to the data presently available.

Amygdaloid Complex in Mammals (fig. 1D)

Mammals are the best-studied group in terms of structure and function of the amygdala. In fact, hypotheses about the functions of the amphibian and reptilian amygdala, derived mostly from anatomical data, depend on an extrapolation of the functional knowledge gathered in rodents, nonhuman primates and human. Figure 1D shows the temporal lobe of a mammal. The main olfactory component is represented by the anterior and posterolateral

cortical amygdala, the piriform cortex-cortical amygdala transition regions and the anterior amygdalar area. The latter structures (except the anterior amygdalar area not pictured) are found ventrolateral to the accessory olfactory component represented by the posteromedial cortical amygdala and the medial amygdala. The visceral-autonomic central amygdala is found dorsomedially, whereas the frontotemporal cortical amygdalar component including the anterior basolateral and lateral amygdala is situated in the cortical subplate [Swanson and Petrovich, 1998; Petrovich et al., 2001]. Note that the latter authors considered the basomedial and posterior basolateral amygdala part of the main olfactory component because of strong connections with regions receiving MOB input. Here we consider the latter two regions to be part of the limbic associative component because of shared limbic-cortical connections with the anterior basolateral and lateral amygdala. The connectivity of the cortical and basolateral complex nuclei suggests that they are responsible for association of multi-modal sensory information [McDonald, 2003]. Moreover, aversive and appetitive conditioning studies suggest that sensory associations between conditioned and unconditioned stimuli do not only take place in the lateral but also in the central amygdala [Kapp et al., 1992; Hatfield et al., 1996; Killcross et al., 1997; Hitchcott and Phillips, 1998; Parkinson et al., 2000; Everitt et al., 2003; Paré et al., 2004].

Developmental Origin of the Amygdaloid Complex

Recent experimental analyses of mutants and gene expression territories in the developing and adult vertebrate telencephalon outline the multiple developmental origins of the nuclei of the amygdaloid complex. The telencephalic vesicle is divided into two major regions: the pallium and the subpallium, the latter found ventral to the former. The following pallial subregions are recognized as field homologues in embryonic and adult vertebrates: medial, dorsal, lateral and ventral pallium [Fernandez et al., 1998; Puelles et al., 2000; Puelles, 2001]. The recent discovery of the ventral pallial region, as well as the location of the pallial-subpallial boundary, greatly helps to solve the problems of homology of amygdalar subdivisions. The identification of developmental origin is sometimes complicated by the fact that in late development cell migration occurs across developmental territories [Puelles et al., 2000; Marín and Rubenstein, 2001; Gorski et al., 2002]. Briefly, the following amygdalar structures

are thought to be subpallial: medial and central nuclei in mammals [Puelles et al., 2000; Stenman et al., 2003], medial nucleus and SAT in reptiles [Medina and Reiner, 1995; Fernandez et al., 1998; Martínez-García et al., 2002], the limbic associative and autonomic components as described by us in amphibians. There is some uncertainty about the pallial or subpallial origin of the vomeronasal component of the amphibian amygdala. Moreno and collaborators [2004] could not conclude a subpallial or ventral pallial origin of the *Xenopus* vomeronasal component, whereas the latest proposal of Brox and colleagues [2002, 2003, 2004] describes a medial subpallial and a lateral ventral pallial division of this component.

There is consensus that the claustramygdaloid complex and the olfactory cortex are formed by nuclei of both ventral and lateral pallial origin [Fernandez et al., 1998; Puelles et al., 2000; Stenman et al., 2003; Medina et al., 2004]. However, these reports disagree on the specifics. The most recent findings of Medina and collaborators [2004] suggest that the lateral and ventral pallial derivatives would give rise to regions of the main olfactory and vomeronasal components, respectively, as well as the basolateral complex. Indeed, they present good evidence that the anterior and posteromedial cortical nuclei as well as the basomedial and lateral nuclei of the mouse derive from the ventral pallium, whereas the posterolateral cortical and basolateral nuclei derive from the lateral pallium.

Corresponding data are minimal in reptiles, but the work of Fernandez and colleagues [1998] suggest that most of the DVR derives from the ventral pallium, whereas the main olfactory component and some part of the DVR derive from the lateral pallium [see also Cordery and Molnár, 1999]. Martínez-García and colleagues [2002] have argued that the PDVR and lateral amygdala are of ventropallial and the dorsolateral amygdala of lateropallial origin. In amphibians, we interpret the results of Brox and collaborators [2004] as indicating that the extended vomeronasal component derives from the ventral pallium, but possibly includes a small subpallial medial portion, whereas the main olfactory component (their posterolateral amygdalar nucleus) derives from the lateral pallium. This interpretation derives from our connectional results in amphibians demonstrating reciprocal connections and a clear continuity between the rostral and caudal parts of the amphibian vomeronasal component [Roth et al., 2004; Laberge and Roth, 2005; G. Roth, unpublished observations]. Therefore, adopting the nomenclature of Brox and collaborators [2004], the anterior part of the lateral amygdalar nucleus and the ventropallial medial amygdalar nucleus appear to represent an ex-

tended vomeronasal amygdala in anurans, as seen in salamanders.

Claims of Homology

As can be seen above, our interpretation of the amygdala and striatopallidum differ substantially from the model recently used in amphibian neuroanatomical literature [Marín et al., 1998; Moreno and González, 2003, 2004, 2005]. Briefly, these authors divide the rostral extension of the amygdala coursing above the striatum into the lateral and anterior amygdala, the latter found more ventral. In the caudal telencephalon, they consider almost the entire medial subpallium as bed nucleus of the stria terminalis (BNST) and the most caudal portion of the classical striatum as central amygdala. They position the medial amygdala and the caudal portion of the lateral amygdala further laterally, which receive vomeronasal and MOB input, respectively. Moreno and González [2003, 2004] proposed that the anuran lateral amygdala is homologous to the amniote basolateral complex and the anuran medial amygdala homologous to the mammalian medial nuclei. In the following, we propose an alternative and more parsimonious view.

(1) Moreno and González [2004] proposed that the ventral part of the caudal lateral pallium (here the main olfactory component) is a multimodal sensory center due to scattered afferent fiber labeling from the dorsal thalamus and parabrachial nucleus. However, these thalamic and visceral inputs are weak at best [Neary, 1990; Roth and Grunwald, 2000; Roth et al., 2003, 2004; Moreno and González, 2004]. Additionally, the lateral amygdala of Moreno and González [2004] receives only a minor projection from neurons situated in the caudal medial pallium and does not project back to any part of the medial pallium, the presumed homologue of the mammalian hippocampal formation. Furthermore, although it projects to the lateral pallium, it is not connected with the dorsal pallium.

The above suggests that the lateral part of the amphibian amygdala is not a limbic associative structure. It should also be noted that, in contrast to the mammalian lateral nucleus and the reptilian PDVR which derive embryologically from the ventral pallium, the anuran lateral amygdala/main olfactory component appears to derive from the lateral pallium [fig. 7 in Brox et al., 2004], which again argues against the homology proposed by Moreno and González [2004]. The amphibian subpallial amygdala, on the other hand, displays strong reciprocal connections with the dorsal thalamus, limbic medial pallium

and visceral centers, enabling an integration of multimodal sensory information under pallial influence [Roth et al., 2004; Laberge and Roth, 2005]. The dorsal thalamic neurons projecting to the pallium in amphibians send axon collaterals to the medial amygdala on their course toward the rostral part of the pallium [Roth and Grunwald, 2000; Roth et al., 2003]. Intracellular recordings demonstrate that neurons of the medial part of the amygdala display excitatory or inhibitory multimodal responses to stimulation of sensory afferents [F. Laberge, unpublished observation] and, thus, appear to receive multimodal sensory input just like the rostral pallium [Karamian et al., 1966]. All this suggests that the amygdala and pallium of amphibians could be simultaneously recruited by sensory input. Interestingly, anatomically similar thalamic cells projecting to both the lateral amygdala and auditory cortex could be involved in mammalian fear conditioning [Doron and LeDoux, 2000].

Due to its massive direct MOB input, the caudal lateral amygdala of Marín et al. [1998] and Moreno and González [2004] corresponds to the main olfactory component and should be homologized with the cortical amygdaloid structures receiving MOB input in amniotes. If we accept this view then the pallial limbic associative component of amniotes is homoplastic, but not homologous, to the limbic associative component of anamniotes – an idea that does not exclude a developmental field homology between the amphibian lateral pallium and parts of the mammalian basolateral complex. It should be noted that Bruce and Neary [1995] proposed that the lateral amygdala of amphibians (their caudal ventral part of the lateral pallium) is homologous to the main olfactory component of the reptilian amygdala and a ventral part of the olfactory cortex as well as to part of the basolateral complex of mammals. For the reasons described above, this appears to us as a field homology and not necessarily a homology of function, which would be expected if the basolateral complex were an amniote innovation.

Interestingly, the main olfactory component of the amphibian amygdala projects to the nucleus accumbens/autonomic extended amygdala continuum [Moreno and González, 2004], as is the case with the reptilian PDVR/lateral nuclei and the mammalian basolateral complex [McDonald, 1991; Novejarque et al., 2004]. The projection of the basolateral amygdala to the ventral striatum is known to be involved in the motivational effects of emotion [Cardinal et al., 2002]. This suggests that some aspects of reinforcement processes independent of stimulus associations could be located in the main olfactory amygdala of amphibians.

Caution is warranted when discussing the vomeronasal/main olfactory divisions of the amygdala of amphibians because divisions in this region are still unsettled, especially regarding the rostral portion. Despite careful investigations, we were unable to identify anterior and lateral divisions of the amygdala in the fire-bellied toad as Marín and collaborators [1998] described in the frog [G. Roth and S. Mühlenbrock-Lenter, unpublished observations]. However, we observed that staining to substance P and NADPH diaphorase is restricted to the caudal part of the olfactory divisions of the amygdala [Mühlenbrock-Lenter et al., 2005], but the significance of these findings is unclear. The observed staining differences could reflect the existence of intrinsic divisions of the amphibian vomeronasal component, as suggested by our connectional results [Roth et al., 2004; Laberge and Roth, 2005; G. Roth, unpublished observations].

(2) The gene expression data suggest that most, or all, of the vomeronasal component [medial amygdala in Moreno and González, 2003] is of ventral pallial origin [Brox et al., 2004], which does not correspond to the mammalian subpallial medial nuclei, but would correspond to the posteromedial cortical amygdala.

(3) The central amygdala of Marín et al. [1998] represents a structure clearly continuous with the dorsal pallidum, as defined by Endepols and collaborators [2004] from connectional and histochemical evidence in anurans. However, figure 2 shows that another structure with autonomic/visceral reciprocal connections exists medial to the caudal tip of the dorsal pallidum [Roth et al., 2004; Mühlenbrock-Lenter et al., 2005], and probably represents the autonomic amygdala. This structure extends rostrally in a position topologically equivalent to the mammalian central extended amygdala [Alheid et al., 1995]. Thus, it does not include the part of the caudal striatum that Marín and colleagues [1998] and Moreno and González [2005] consider to be the autonomic portion of the amygdala in anurans. Interestingly, the above two views could be reconciled if one assumes the existence of medial and lateral divisions of the amphibian autonomic component, as in the case of the mammalian central nuclei [McDonald, 2003]. In this scenario, neurons in the extreme caudal part of the anuran dorsal pallidum exhibiting high NADPH-diaphorase activity [Marín et al., 1998; Mühlenbrock-Lenter et al., 2005] could represent the lateral portion of the autonomic amygdala. Russchen and Jonker [1988] reported that tracer injections involving the medial SAT resulted in additional projections to the nucleus accumbens, septum, preoptic area and hypothalamus compared to the lateral

SAT, which suggest that a medial SAT division with ascending limbic projections could also exist in reptiles. The identity of these nuclei with long descending connections should be studied further by identifying more precisely their sites of axonal terminations.

Conclusions

The analysis of the evolution of the amygdala by a functional systems approach [sensu Swanson and Petrovich, 1998] leads to an interesting observation: the limbic associative component of the amphibian amygdaloid complex is not homologous to the frontotemporal cortical component of mammals because of different developmental origins. Johnston [1923] had already suggested that the mammalian basal and lateral nuclei were new structures formed by an infolding or immigration of cells. Evidently, the anamniote-amniote transition is marked by important changes in dorsal thalamic projections to the telencephalon [Butler, 1994; Striedter, 1997; Puelles, 2001], including the expansion of major ascending thalamic projections to the pallial amygdala and the cortex. This event, as well as the elaboration of cortical afferents, conferred upon the PDVR and basolateral complex the capacity to integrate and further process multi-modal sensory, cognitive and limbic information. Multi-modal sensory associations and plasticity in the central amygdala have recently been confirmed [Everitt et al., 2003; Paré et al., 2004; Samson and Paré, 2005], and it is tempting to suggest that the mammalian central amygdala has conserved its dual autonomic and limbic associative ancestral functions, as the connectivity of the amphibian subpallial amygdala implies.

The ancestral tetrapod morphotype would comprise well-established functional systems of the amygdala, i.e. main olfactory, vomeronasal and mixed autonomic/limbic associative. The absence of a basolateral complex in amphibians would corroborate the view that anamniotes have a limited repertoire of learning responses compared to amniotes. Associative learning is a general phenomenon probably common to all bilateral animals [Bitterman, 1975; Papini, 2002]. Learning research also suggests that brain mechanisms responsible for fear are a conserved feature of vertebrates [Papini, 2003]. For example, goldfish are capable of instrumental avoidance learning – a task thought to depend on the reduction of an internal response of fear induced by a warning [Overmier and Papini, 1986; Portavella et al., 2003, 2004]. On the other hand, learning dependent on frustration resulting

from the unexpected omission of an appetitive reinforcer appears to be present only in mammals [Bitterman, 1975; Papini, 2003]. This led to the suggestion that some emotions are more primitive than others.

Interestingly, physiological responses triggered by aversive emotion such as fever or increased heart rate are absent in fish and amphibians, but present in reptiles and other amniote vertebrates [Cabanac, 1999; Cabanac and Cabanac, 2004]. Another report suggests that conditioned taste aversion is similarly absent in amphibians and present in amniotes [Paradis and Cabanac, 2004]. Because the amygdala participates in emotional learning in mammals, the above observations suggest that the advent of the basolateral amygdaloid complex and its reptilian equivalent brought genuine innovations to vertebrate emotional behavior.

References

- Alheid GF, de Olmos JS, Beltramino CA (1995) Amygdala and extended amygdala. In: *The Rat Nervous System* (Paxinos G, ed), pp 495–578. San Diego: Academic Press.
- Bitterman ME (1975) The comparative analysis of learning. *Science* 188:699–709.
- Brox A, Ferreiro B, Puelles L, Medina L (2002) The telencephalon of the frog *Xenopus* based on calretinin immunostaining and gene expression patterns. *Brain Res Bull* 57:381–384.
- Brox A, Puelles L, Ferreiro B, Medina L (2003) Expression of the genes GAD67 and Distal-less-4 in the forebrain of *Xenopus laevis* confirms a common pattern in tetrapods. *J Comp Neurol* 461:370–393.
- Brox A, Puelles L, Ferreiro B, Medina L (2004) Expression of the genes *Emx1*, *Tbr1*, and *Eomes* (*Tbr2*) in the telencephalon of *Xenopus laevis* confirms the existence of a ventral pallidial division in all tetrapods. *J Comp Neurol* 474:562–577.
- Bruce LL, Neary TJ (1995) The limbic system of tetrapods: a comparative analysis of cortical and amygdalar populations. *Brain Behav Evol* 46:224–234.
- Butler AB (1994) The evolution of the dorsal thalamus of jawed vertebrates, including mammals: cladistic analysis and a new hypothesis. *Brain Res Rev* 19:29–65.
- Cabanac M (1999) Emotion and phylogeny. *Jpn J Physiol* 49:1–10.
- Cabanac AJ, Cabanac M (2004) No emotional fever in toads. *J Therm Biol* 29:669–673.
- Cardinal RN, Parkinson JA, Hall J, Everitt BJ (2002) Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev* 26:321–352.
- Cordery P, Molnár Z (1999) Embryonic development of connections in turtle pallium. *J Comp Neurol* 413:26–54.
- Distel H (1978) Behavior and electrical brain stimulation in the green iguana, *Iguana iguana* L. II. Stimulation effects. *Exp Brain Res* 31:353–367.
- Doron NN, LeDoux JE (2000) Cells in the posterior thalamus project to both amygdala and temporal cortex: a quantitative retrograde double-labeling study in the rat. *J Comp Neurol* 425:257–274.
- Endepols H, Roden K, Luksch H, Dicke U, Walkowiak W (2004) Dorsal striatopallidal system in anurans. *J Comp Neurol* 468:299–310.
- Everitt BJ, Cardinal RN, Parkinson JA, Robbins TW (2003) Appetitive behavior: impact of amygdala-dependent mechanisms of emotional learning. *Ann NY Acad Sci* 985:233–250.
- Fernandez AS, Pieau C, Repérant J, Boncinelli E, Wassef M (1998) Expression of the *Emx-1* and *Dlx-1* homeobox genes define three molecularly distinct domains in the telencephalon of mouse, chick, turtle and frog embryos: implications for the evolution of telencephalic subdivisions in amniotes. *Development* 125:2099–2111.
- González A, Lopez JM (2002) A forerunner of septohippocampal cholinergic system is present in amphibians. *Neurosci Lett* 327:111–114.
- Gorski JA, Talley T, Qiu M, Puelles L, Rubenstein JL, Jones KR (2002) Cortical excitatory neurons and glia, but not GABAergic neurons, are produced in the *Emx1*-expressing lineage. *J Neurosci* 22:6309–6314.
- Hatfield T, Han JS, Conley M, Gallagher M, Holland P (1996) Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. *J Neurosci* 16:5256–5265.
- Herrick CJ (1948) The brain of the tiger salamander. Chicago: University of Chicago Press.
- Hitchcott PK, Phillips GD (1998) Double dissociation of the behavioural effects of R(+) 7-OH-DPAT infusions in the central and basolateral amygdala nuclei upon Pavlovian and instrumental conditioned appetitive behaviours. *Psychopharmacology* 140:458–469.
- Johnston JB (1923) Further contributions to the study of the evolution of the forebrain. *J Comp Neurol* 35:337–481.
- Kapp BS, Whalen PJ, Supple WF, Pascoe JP (1992) Amygdaloid contributions to conditioned arousal and sensory processing. In: *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction* (Aggleton JP, ed), pp 229–254. New York: Wiley-Liss.
- Karamian AI, Vesselkin NP, Belekova MG, Zagorulko TM (1966) Electrophysiological characteristics of tectal and thalamo-cortical divisions of the visual system in lower vertebrates. *J Comp Neurol* 127:559–576.
- Killcross S, Robbins TW, Everitt BJ (1997) Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature* 388:377–380.
- Laberge F, Roth G (2005) Connectivity and cytoarchitecture of the ventral telencephalon in the salamander *Plethodon shermani*. *J Comp Neurol* 482:176–200.
- Lanuza E, Halpern M (1998) Efferents and centrifugal afferents of the main and accessory olfactory bulbs in the snake *Thamnophis sirtalis*. *Brain Behav Evol* 51:1–22.
- Lanuza E, Belekova M, Martínez-Marcos A, Font C, Martínez-García F (1998) Identification of the reptilian basolateral amygdala: an anatomical investigation of the afferents to the posterior dorsal ventricular ridge of the lizard *Podarcis hispanica*. *Eur J Neurosci* 10:3517–3534.

Acknowledgment

The work of F.L. was supported by a fellowship of the Hanse Institute for Advanced Study, Delmenhorst.

- LeDoux JE (2000) Emotion circuits in the brain. *Ann Rev Neurosci* 23:155–184.
- Linke R, Braune G, Schwegler H (2000) Differential projection of the posterior paralaminar thalamic nuclei to the amygdaloid complex in the rat. *Exp Brain Res* 134:520–532.
- Marín O, González A, Smeets WJAJ (1997a) Basal ganglia organization in amphibians: efferent connections to the striatum and the nucleus accumbens. *J Comp Neurol* 378:16–49.
- Marín O, González A, Smeets WJAJ (1997b) Basal ganglia organization in amphibians: efferent connections of the striatum and the nucleus accumbens. *J Comp Neurol* 380:23–50.
- Marín O, Smeets WJAJ, González A (1998) Basal ganglia organization in amphibians: chemoarchitecture. *J Comp Neurol* 392:285–312.
- Marín O, Rubenstein JL (2001) A long, remarkable journey: tangential migration in the telencephalon. *Nat Rev Neurosci* 2:780–790.
- Martínez-García F, Martínez-Marcos A, Lanuza E (2002) The pallial amygdala of amniote vertebrates: evolution of the concept, evolution of the structure. *Brain Res Bull* 57:463–469.
- McDonald AJ (1991) Topographical organization of amygdaloid projections to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. *Neuroscience* 44:15–33.
- McDonald AJ (2003) Is there an amygdala and how far does it extend? An anatomical perspective. *Ann NY Acad Sci* 985:1–21.
- Medina L, Reiner A (1995) Neurotransmitter organization and connectivity of the basal ganglia in vertebrates: implications for the evolution of basal ganglia. *Brain Behav Evol* 46:235–258.
- Medina L, Legaz I, González G, De Castro F, Rubenstein JL, Puelles L (2004) Expression of Dbx1, Neurogenin 2, Semaphorin 5A, Cadherin 8, and Emx1 distinguish ventral and lateral pallial histogenetic divisions in the developing mouse claustroramygdaloid complex. *J Comp Neurol* 474:504–523.
- Moreno N, González A (2003) Hodological characterization of the medial amygdala in anuran amphibians. *J Comp Neurol* 466:389–408.
- Moreno N, González A (2004) Localization and connectivity of the lateral amygdala in anuran amphibians. *J Comp Neurol* 479:130–148.
- Moreno N, González A (2005) Central amygdala in anuran amphibians: neurochemical organization and connectivity. *J Comp Neurol* 489:69–91.
- Moreno N, Bachy I, Rétaux S, González A (2004) LIM-homeodomain genes as developmental and adult genetic markers of *Xenopus* forebrain functional subdivisions. *J Comp Neurol* 472:52–72.
- Mühlenbrock-Lenter S, Endepols H, Roth G, Walkowiak W (2005) Immunohistological characterization of striatal and amygdalar structures in the telencephalon of the fire-bellied toad *Bombina orientalis*. *Neuroscience* 134:705–719.
- Neary TJ (1990) The pallium of anuran amphibians. In: *Cerebral Cortex, Vol. 8A, Comparative Structure and Evolution of Cerebral Cortex, Part 1* (Jones EG, Peters A, eds), pp 107–138. New York: Plenum.
- Northcutt RG (1981) Evolution of the telencephalon in nonmammals. *Ann Rev Neurosci* 4:301–350.
- Northcutt RG (1995) The forebrain of gnathostomes: in search of a morphotype. *Brain Behav Evol* 46:275–318.
- Northcutt RG, Kicliter E (1980) Organization of the amphibian telencephalon. In: *Comparative Neurology of the Telencephalon* (Ebbesson SOE, ed), pp 203–255. New York: Plenum.
- Northcutt RG, Ronan M (1992) Afferent and efferent connections of the bullfrog medial pallium. *Brain Behav Evol* 40:1–16.
- Novejarque A, Lanuza E, Martínez-García F (2004) Amygdalostriatal projections in reptiles: A tract-tracing study in the lizard *Podarcis hispanica*. *J Comp Neurol* 479:287–308.
- Overmier JB, Papini MR (1986) Factors modulating the effects of telencephalon ablation on retention, relearning, and extinction of instrumental avoidance behavior. *Behav Neurosci* 100:190–199.
- Papini MR (2002) Pattern and process in the evolution of learning. *Psychol Rev* 109:186–201.
- Papini MR (2003) Comparative psychology of surprising nonreward. *Brain Behav Evol* 62:83–95.
- Paradis S, Cabanac M (2004) Flavor aversion learning induced by lithium chloride in reptiles but not in amphibians. *Behav Proc* 67:11–18.
- Paré D, Quirk GJ, LeDoux JE (2004) New vistas on amygdala networks in conditioned fear. *J Neurophysiol* 92:1–9.
- Parkinson JA, Robbins TW, Everitt BJ (2000) Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. *Eur J Neurosci* 12:405–413.
- Petrovich GD, Canteras NS, Swanson LW (2001) Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. *Brain Res Rev* 38:247–289.
- Portavella M, Salas C, Vargas JP, Papini MR (2003) Involvement of the telencephalon in spaced-trial avoidance learning in the goldfish (*Carassius auratus*). *Physiol Behav* 80:49–56.
- Portavella M, Torres B, Salas C (2004) Avoidance response in goldfish: emotional and temporal involvement of medial and lateral telencephalic pallium. *J Neurosci* 24:2335–2342.
- Puelles L, Kuwana E, Puelles E, Bulfone A, Shimamura K, Keleher J, Smiga S, Rubenstein JL (2000) Pallial and subpallial derivatives in the embryonic chick and mouse telencephalon, traced by the expression of the genes *Dlx-2*, *Emx-1*, *Nkx-2.1*, *Pax-6*, and *Tbr-1*. *J Comp Neurol* 424:409–438.
- Puelles L (2001) Thoughts on the development, structure and evolution of the mammalian and avian telencephalic pallium. *Phil Trans R Soc Lond B* 356:1583–1598.
- Roden K, Endepols H, Walkowiak W (2005) Hodological characterization of the septum in anuran amphibians: I. Afferent connections. *J Comp Neurol* 483:415–436.
- Roth G, Grunwald W (2000) Morphology, axonal projection pattern, and responses to optic nerve stimulation of thalamic neurons in the salamander *Plethodon jordani*. *J Comp Neurol* 428:543–557.
- Roth G, Grunwald W, Dicke U (2003) Morphology, axonal projection pattern, and responses to optic nerve stimulation of thalamic neurons in the fire-bellied toad *Bombina orientalis*. *J Comp Neurol* 461:91–110.
- Roth G, Mühlenbrock-Lenter S, Grunwald W, Lamberge F (2004) Morphology and axonal projection pattern of neurons in the telencephalon of the fire-bellied toad *Bombina orientalis*: an anterograde, retrograde, and intracellular biocytin labeling study. *J Comp Neurol* 478:35–61.
- Russchen FT, Jonker AJ (1988) Efferent connections of the striatum and the nucleus accumbens in the lizard *Gekko gekko*. *J Comp Neurol* 276:61–80.
- Samson RD, Paré D (2005) Activity-dependent synaptic plasticity in the central nucleus of the amygdala. *J Neurosci* 25:1847–1855.
- Scalia F, Gallousis G, Roca S (1991) Differential projections of the main and accessory olfactory bulb in the frog. *J Comp Neurol* 305:443–461.
- Siemen M, Künzle H (1994) Connections of the basal telencephalic areas c and d in the turtle brain. *Anat Embryol* 189:339–359.
- Stenman J, Yu RT, Evans RM, Campbell K (2003) *Tlx* and *Pax6* co-operate genetically to establish the pallio-subpallial boundary in the embryonic mouse telencephalon. *Development* 130:1113–1122.
- Striedter GF (1997) The telencephalon of tetrapods in evolution. *Brain Behav Evol* 49:179–213.
- Sugerman RA, Demski LS (1978) Agonistic behavior elicited by electrical stimulation of the brain in western collared lizards, *Crotaphytus collaris*. *Brain Behav Evol* 15:446–469.
- Swanson LW, Petrovich GD (1998) What is the amygdala? *Trends Neurosci* 21:323–331.
- Tarr RS (1977) Role of the amygdala in the intraspecies aggressive behavior of the iguanid lizard, *Sceloporus occidentalis*. *Physiol Behav* 18:1153–1158.
- Westhoff G, Roth G (2002) Morphology and projection pattern of medial and dorsal pallial neurons in the frog *Discoglossus pictus* and the salamander *Plethodon jordani*. *J Comp Neurol* 445:97–121.
- Yao M, Westphal NJ, Denver RJ (2004) Distribution and acute stressor-induced activation of corticotrophin-releasing hormone neurones in the central nervous system of *Xenopus laevis*. *J Neuroendocrinology* 16:880–893.