Forebrain Connections in the Goldfish Support Telencephalic Homologies with Land Vertebrates

Abstract. *Horseradish peroxidase injections into dorsomedial and dorsolateral regions of the goldfish* (Carassius auratus) *telencephalon demonstrate, by retrograde cell labeling, that the teleost telencephalon receives a pattern of projections from the thalamus remarkably similar to those of land vertebrates. The evidence provides support for a homology between the dorsomedial region and the corpus striatum of land vertebrates and a homology between two dorsolateral regions and the dorsal and medial pallium of land vertebrates.*

Teleost fish represent the largest vertebrate radiation, with over 25,000 extant species (*1*). Yet, until recently (*2*) almost nothing was known about the detailed forebrain connections of these animals. The teleost telencephalon was considered to be a relatively undifferentiated structure dominated by secondary and tertiary olfactory connections (*3*) until studies based on new neuroanatomical tracing techniques, such as improved axonal degeneration stains and axonal transport of marker enzymes and amino acids, showed olfactory projections to restricted areas of the teleost telencephalon (*4*). Moreover, recent electrophysiological studies of the teleost telencephalon reveal well-defined visual (*5*) and auditory (*6*) areas.

Since the teleost telencephalon is not solely an olfactory center, it is particularly important to examine afferent and efferent projections of different regions of the teleost telencephalon to permit comparisons with the telencephalons of other vertebrates (*7*). Among the methods used in previous studies were removal of an entire telencephalic hemisphere and staining for axonal and terminal degeneration (*8*) and large applications of horseradish peroxidase (HRP) enzyme to the surface of the telencephalon (*9*). Although these studies have provided information about the total telencephalic output or input, they do not allow adequate resolution of the source of efferent fibers from the telencephalon or the region of termination of afferent fibers to the telencephalon.

Our approach has been to examine the connections of specific areas of the goldfish telencephalon by means of discrete injections of HRP into either medial or lateral areas of the dorsal telencephalon. Goldfish (*Carassius auratus*), 5 to 10 cm in length and 5 to 10 g in weight, were anesthetized by immersion in a 0.1 percent solution of tricaine methanesulfonate (MS-222) for several minutes until all signs of respiratory activity ceased. A small flap of frontal bone was excised, exposing either the right or left telencephalon. An insect pin (No. 000), coated with a small amount of HRP paste, was manually inserted into the desired site at the surface of the telencephalon by use of a dissecting microscope and telencephalic surface sulci landmarks (*10*). Two to four days later, the fish were perfused through the bulbus arteriosus with 0.1*M* phosphate buffer, then with a solution of cold buffered fixative containing 2.0 percent glutaraldehyde in 0.1*M* phosphate buffer. The brains were removed and placed in cold buffered fixative, with 30 percent sucrose, for an additional 2 to 4 hours before being embedded in gelatin. The embedded brains were sectioned at 40 μm on a freezing microtome and sections were collected into five bins containing cold 0.1*M* phosphate buffer. Individual sections were processed with hydrogen peroxide and either tetramethyl benzidine or Hanker-Yates substance (*11*) as substrates for the identification of the HRP enzyme. Sections were serially mounted and counterstained with neutral red, and the locations of cells and fibers were charted.

After injection of HRP into the dorsomedial (Dm) region of the telencephalon (Fig. 1, case 80.14), we observed retrogradely filled cells only ipsilateral to the injection site. Such cells were found in (i) a well-defined central region (Dc) of the telencephalon, (ii) the entopeduncular nucleus (preoptic region), (iii) the dorsal posterior nucleus (*12*) (dorsal thalamus), and (iv) the nucleus preglomerulosus of the diencephalon. We also observed orthograde labeling of telencephalic efferent fibers after Dm injections. Efferent fibers leave Dm ipsilaterally by a ventrocaudal route (Fig. 1A), enter the lateral forebrain bundle (LFB), and pass through the entopeduncular nucleus in the preoptic region of the telencephalon. At the level of the habenula (Fig. 1C), in the anterior diencephalon, some fibers leave the LFB to enter the dorsomedial margin of the optic tectum. Another group of axons proceeds in a ventrolateral direction to enter the nucleus preglomerulosus. The LFB continues caudally into the posterior diencephalon where, at the level of the posterior commissure (Fig. 1D), some fibers continue to enter the optic tectum and the nucleus preglomerulosus, and the remainder terminate in the inferior lobe.

The lateral portion (Dl) of the teleost telencephalon can be divided into three cytoarchitectonically distinct regions (*13*). Our HRP injections into Dl were not discrete enough to allow description of the projections to each subregion and frequently were contaminated by diffusion of HRP into the adjacent dorsolateral area (Dd). Therefore, we restrict this report to a description of the connections of either the medial (Dm) or the more lateral (Dd and Dl) regions of the goldfish telencephalon. After injections into the more lateral regions of the telencephalon such as Dd and Dl (Fig. 1, case 80.09), we observed retrogradely filled cells ipsilaterally in the entopeduncular nucleus and in nucleus preglomerulosus and bilaterally in an anterior nucleus of the dorsal thalamus. Heavy orthograde fiber labeling was apparent in the medial forebrain bundle (MFB), especially after HRP injections into posterior telencephalic areas. Efferent MFB fibers leave the Dd–Dl injection site. Some cross to the contralateral telencephalic hemisphere via the anterior commissure and descend bilaterally. During their progress through the diencephalon, the MFB fibers form a compact bundle within the ventral thalamus, except for the most rostral portions of the diencephalon (Fig. 1G), in which MFB fibers could be traced into the anterior nucleus of the dorsal thalamus bilaterally. The MFB fibers continue caudally into the midbrain where they end in the tegmentum bilaterally.

In several cases in which an entire telencephalic hemisphere was filled with HRP, we observed excellent retrograde cell filling in the superior raphe and bilaterally in the locus coeruleus at the level of the isthmus, just rostral to the medulla. However, the specific projection pattern of these nuclei to the goldfish telencephalon remains obscure.

Our descriptions of the descending projections of the medial and lateral forebrain bundles of the goldfish telencephalon correspond well with the description of the forebrain projections of two other teleosts, *Eugerres* sp. (mojarra) and *Holocentrus* sp. (squirrelfish) (*8*). In the latter study, an entire telencephalic hemisphere was removed and stained for degenerating axons and terminals, and therefore no information about the sources of the telencephalic efferents could be given. In our study, we observed the projections of the LFB after injection of HRP into the Dm region of the goldfish telencephalon and projec-

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tions of the MFB after injections into the posterior portions of the Dd, Dl telencephalic regions (*14*). These observations are consistent with a medial-to-lateral eversion of the teleost telencephalon during embryogenesis (*15*).

Our study confirms the existence of ascending projections to the teleost telencephalon from the nucleus preglomerulosus (*9*). We report evidence for a parcellation of projections from the dorsal thalamus to the teleost telencephalon with the anterior nucleus of the dorsal thalamus projecting primarily to regions Dd and Dl and the dorsal posterior nucleus to region Dm. Unlike previous investigators (*8, 9*) who observed no direct connections of the teleost telencephalon with areas below the midbrain, we report projections to the goldfish telencephalon from the locus coeruleus and the superior raphe.

Northcutt and Braford (*13*) proposed that there is a homology between portions of the Dm and Dc regions of the teleost forebrain and the corpus striatum of land vertebrates. Their proposal is based on three lines of evidence: (i) similarities in the regional histochemistry of these areas (both contain higher concentrations of acetylcholinesterase than any other forebrain region), (ii) the presence of heavy catecholaminergic innervation of Dm (*16*), and (iii) topography compatible with a telencephalic eversion in teleosts. We provide connectional evidence in support of this homology. The Dm region of the goldfish telencephalon receives projections from the posterior region of the dorsal thalamus via the LFB. A projection from the posterior region of the dorsal thalamus to the striatum has been reported in amphibians, reptiles, and mammals (*17*).

The pattern of connections of the Dd and Dl regions of the goldfish telencephalon via the MFB is very similar to the MFB projections to the dorsal and medial pallium in amphibians (*17*) and reptiles (*18*), where the cells of origin in the diencephalon are located, as in the goldfish, in an anterior nucleus of the dorsal thalamus. The efferent MFB projections in the goldfish bear little resemblance to MFB projections in either birds or mammals, and Vanegas and Ebbesson (*8*) suggest that the teleost MFB may be more comparable to the fornix. If this comparison is valid, a major source of the teleost MFB should be a region of the forebrain comparable to the hippocampus (medial pallium) of land vertebrates. Recently, the ventral (Dl-v) and posterior (Dl-p) portions of the Dl region in the teleost forebrain have been homologized with the medial pallium of land vertebrates on the basis of similarities in topography and regional histochemistry (*13*).

Few electrophysiological studies of the forebrains of fish, amphibians, and reptiles exist, and these studies are crucial for establishing telencephalic homologies with birds and mammals. However, evidence obtained thus far sugests that, despite differences in embryogenesis and cellular organization, teleost and land vertebrate forebrains appear more similar in terms of their connections and perhaps functional organization than was previously believed.

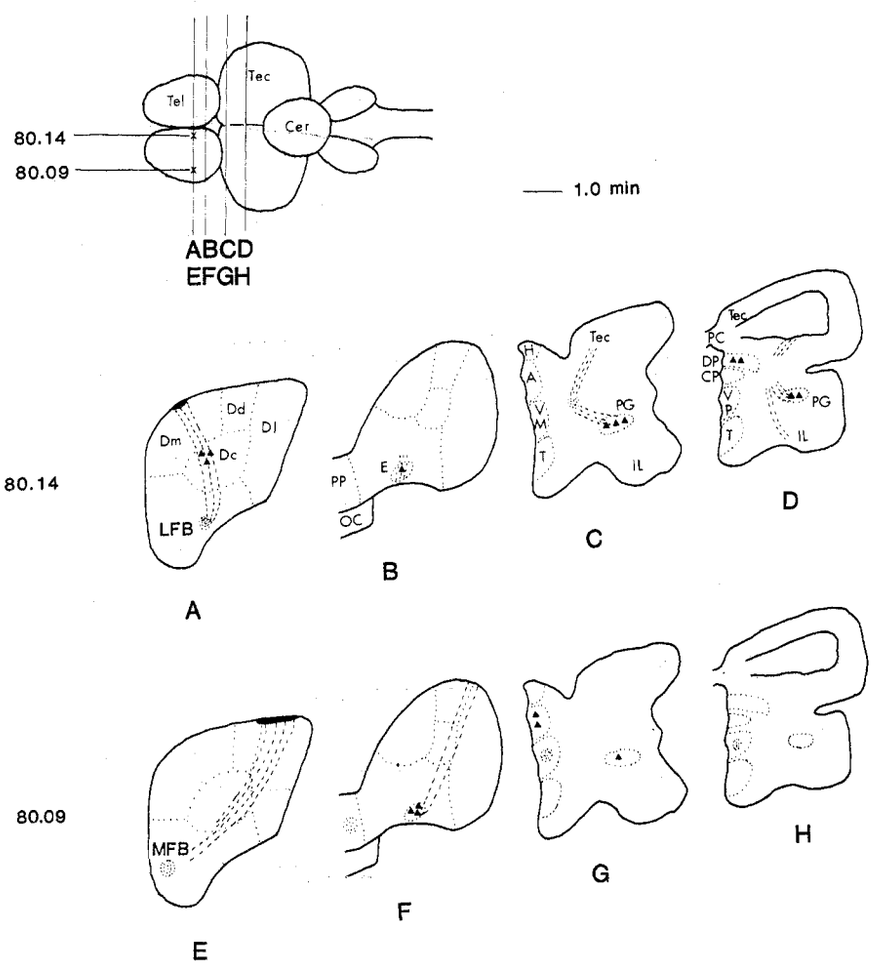

Fig. 1. Connections of regions Dm and Dd–Dl of the goldfish dorsal telencephalon as revealed by HRP histochemistry. The HRP injection sites are labeled X. Projections of case 80.14, a medial pallial injection, and case 80.09, a lateral pallial injection, are compared in cross sections through the same brain regions. For ease of comparison, only the side of the brain ipsilateral to the injection site is shown. Broken lines and stippling indicate axons; filled triangles are HRP cells. Abbreviations: *A*, anterior thalamic nucleus; *Cer*, cerebellum; *CP*, central posterior thalamic nucleus; *Dc*, central zone of dorsal pallium; *Dd*, dorsal zone of dorsal pallium; *Dl*, lateral zone of dorsal pallium; *Dm*, medial zone of dorsal pallium; *DP*, dorsal posterior thalamic nucleus; *E*, entopeduncular nucleus; *H*, habenula; *IL*, inferior lobe of hypothalamus; *LFB*, lateral forebrain bundle; *MFB*, medial forebrain bundle; *OC*, optic chiasm; *PC*, posterior commissure; *PG*, nucleus preglomerulosus; *PP*, nucleus preopticus periventricularis; *T*, tuberal region; *Tec*, optic tectum; *Tel*, telencephalon; *VM*, ventromedial thalamic nucleus; and *VP*, ventroposterior thalamic nucleus.

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Right-Handedness: A Consequence of Infant Supine Head-Orientation Preference?

Abstract. *Most newborn infants (65 percent) preferred to lie with their heads turned to the right, whereas 15 percent showed a distinct preference for the left. Orientation preference is maintained for at least 2 months and predicts preferential hand use in reaching tasks at both 16 and 22 weeks. Right head-orientation preference in early infancy may contribute to the early development of right-handedness.*

Right- and left-handedness are associated with individual differences in a wide variety of psychological phenomena from cognitive styles to recovery of function after brain damage (*1*), but the causes of these associations are unknown. Studying the development of hand preference may disclose some causes of these functional associations. The two characteristics to be explained in human handedness are individual variability, which is common to humans and other mammalian species, and the predominance of right-handedness, which is unique to humans. In nonhuman species, limb preference is randomly distributed among right-, mixed-, and left-limb use (*2*).

Although individual variability could be genetic in origin, the evidence from breeding studies in mice and ontogenetic studies of monkeys favors a nongenetic origin (*3*). Collins reported that mice reared under conditions favoring the use of the right limb develop a right-limb preference, whereas mice reared under conditions favoring the use of the left limb develop a left-limb preference (*4*). If environmental circumstances can bias the distribution of limb preferences of mice, then, since every human culture provides some pressure in favor of the right hand (*5*), culture might constitute the bias responsible for the predominance of right-handedness in humans. If so, however, this would leave unanswered why all cultures should favor right-handedness. Annett has argued that cultural pressures can maintain and increase the bias toward the right hand, but they probably cannot explain its initial occurrence and universal prevalence (*6*). Therefore, she concluded that the source of the right bias must be genetic.

Of the various genetic models of human handedness that have been proposed, only Annett's accounts for all of the genealogical data, particularly the proportion of right-handedness among the offspring of two left-handed parents (*7*). Her model postulates a single allele, which, when present, superimposes a dextral bias on individual variability in handedness, but, when absent—as in the offspring of two left-handed parents—permits individual handedness to assort in the random proportions typical of mammals. Her model also implies that this allele may not affect handedness directly, but instead may produce asymmetries for other functions, which, in turn, produce the dextral bias.

Most newborn infants orient their heads toward their right sides while supine (*8*). This right bias has been thought to contribute to the development of the right bias in handedness by producing lateral asymmetries in visual experience of the hands and differences between the hands in neuromotor activity (*9*). This study was designed to examine the relation between neonatal head-orientation preference and the right bias in handedness during infancy. If head-orientation preference contributes to the development of hand preference, a majority of neonates should prefer to keep their heads turned to the right and a minority toward the left. Furthermore, infants who prefer to orient their heads to the left should exhibit an early left-hand preference. I thus assessed the distribution of neonatal head-orientation preference and examined the association between orientation preference in the neonatal period and hand use during the first half year of infancy.

The direction of supine head orientation was determined for 150 normal, full-term, vaginally delivered neonates (81 males and 69 females) by two separate assessments during the 16 to 48 hours after birth. Each assessment consisted of three 2-minute trials. For each trial the infant's head was first held gently in a midline position for 1 minute and then released. The direction of head orientation (right, chin to the right of the infant's right nipple; midline, chin between the right and left nipple; and left) was recorded immediately thereafter on a check sheet every 6 seconds for 1 minute. The number of 6-second intervals for right, left, and midline orientations were summed independently across three trials.

An infant's head-orientation preference was coded for each assessment period by the formula $(R - L)/(R + L)^{1/2}$, where R equals the number of intervals in which the head was oriented right and L the number oriented left. The protocols were scored for both strength and consistency of head orientation. Infants with scores of ± 1.8 for any assessment were classified as having a strong preference. Infants with two assessment scores of $+1.8$ or greater or -1.8 or less were classified as having a consistent and strong preference to orient their heads toward the right or left side, respectively. Infants with two positive assessment scores, one or both less than 1.8, or two negative scores, one or both greater than -1.8, were classified as biased to the right or left, respectively. Those with one positive and one negative score were classified as having a mixed head-orientation preference.

The distribution of preferences was significantly biased to the right [χ^2 (*4*) = 87.7, $P < .001$] in proportions approximating the right bias in handedness (*10*). The right head bias was present in both males [χ^2 (*4*) = 36.9, $P < .005$] and females [χ^2 (*4*) = 59.7, $P < .001$], and sex differences were not significant [χ^2 (*3*) = 1.7] but corresponded to those sometimes reported for adult handedness (*11*).

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