A knowledge of retained ancient, prehuman structures in human behavior is indispensable for an understanding of all higher mental processes that are built upon them—it is basic in the truest sense of the word. (Konrad Lorenz, 1996, pp. xxvi–xxvii)

Handedness and brain lateralization have long been attributed only to humans; however, more evidence is becoming available about the early ancestry of brain asymmetry and specialization of cerebral hemispheres already in early vertebrates (comprehensively covered in Malashichev & Rogers, 2002; Rogers & Andrew, 2002). This neural feature can be also extended even to invertebrates (for earliest reports, see Bianki & Sheiman, 1985, and Udalova & Karas, 1985; the subject is intensively reviewed in Malashichev, 2006). For example, a common pattern for perceptual lateralization in all vertebrate classes from fish to mammals has been shown previously. The right hemisphere–left eye system is involved in predator escape and interactions with conspecifics, whereas the left hemisphere–right eye system is usually associated with prey capture behavior (Rogers, 2002b). That is, the behavioral lateralization includes asymmetric responses to visual stimuli and results from specialization of the brain hemispheres (Bianki, 1985; Rogers & Andrew, 2002), which appeared early in vertebrate evolution (Vallortigara & Bisazza, 2002). However, the forces that drive the evolution of lateralized behaviors and, particularly, limb preference in vertebrates are still contentious (Corballis, 2003; Crow, 2004; Rogers & Andrew, 2002; Vallortigara & Rogers, 2005, and open peer commentary on that article).

Although responses to visual stimuli usually imply some motor behavior, there are other motor reflexes that do not require any input of visual information (e.g., righting reflexes). A previous review of motor-reflex lateralizations in adult and larval anurans revealed little functional links of pure motor to perceptual asymmetry, suggesting somewhat independent evolution of lateralized functions (for more detail, see the discussion in Malashichev & Wassersug, 2004). In higher vertebrates, only the precise stepping movements (functional walking, e.g., over a barrier or a stumbling stone) are visually guided and involve the control from the motor cortex (via thalamocortical and corticospinal projections), whereas the generation and patterning of automated locomotion have a reflexive nature and spinal control (Grillner, Hegglin, Ménard, Saitoh, & Wikström, 2005; Kandel, Schwartz, & Jessel, 2000). Whether a different neural basis for visually guided and reflexive locomotive behaviors is a prerequisite of independent pattern of lateralization is unknown. Moreover, our current knowledge does not provide a clear answer to the question of whether limb preference is functionally connected with control of locomotion.

Because anuran amphibians have a wide diversity of locomotive habits, they are an interesting and probably the most promising group within which to address the origin of motor lateralization. There are now many examples of such in anurans. For example, anurans show one-sided limb preference when they wipe a foreign object off their snout (snout-wiping test: Bisazza, Cantalupo, Robbins, Rogers, & Vallortigara, 1996, 1997; Goree & Wassersug, 2001; Malashichev, 2002; Malashichev & Nikitina, 2002) and right themselves from an overturned position (righting-response...
test: Malashichev & Nikitina, 2002; Robins, Lippolis, Bisazza, Vallortigara, & Rogers, 1998; Rogers, 2002b). Similarly, turning biases have been reported in anuran larvae (for a review, see Wassersug & Yamashita, 2002).

Our knowledge about motor lateralization in adult anurans is based primarily on studies of toads of the genus *Bufo* (Bufonidae, Neobatrachia). *Bufo bufo* and *Bufo marinus* show significant lateralization of both forelimbs and hind limbs with the bias directed toward the right side (Bisazza et al., 1996, 1997; Robins et al., 1998; Robins & Rogers, 2002), whereas *Bufo viridis* shows a less pronounced but significant left-sided tendency (Bisazza et al., 1997; Malashichev & Nikitina, 2002; Robins et al., 1998). Although capable of jumping, toads of the genus *Bufo* usually walk, using alternating diagonal movements of their forelimbs and hind limbs. The balance between jumping and walking seems to play a key role in establishment of one-sided limb preference in these amphibians. Thus, *B. viridis*, which usually jumps rather than walks (personal observation), is notably less lateralized than *B. bufo* or *B. marinus* (Bisazza et al., 1997; Malashichev & Nikitina, 2002), taxa that always prefer walking to jumping.

In contrast, phylogenetically more basal (see, e.g., Duellman & Trueb, 1986; Ford & Cannatella, 1993) species of the genus *Bombina* (Bombinatoridae, Archaeobatrachia) do not show lateralization of limb use either as subadults shortly after metamorphosis (*Bombina orientalis*, Goree & Wassersug, 2001) or as adults (*Bombina bombina*, Malashichev & Nikitina, 2002). In *B. bombina*, individual preferences vary daily. Some individuals of *B. bombina* also perform the snout-wiping and righting-response tests with both the left and right limbs acting simultaneously (discussed in Malashichev & Nikitina, 2002). It is interesting that species of *Bombina* are semiaquatic anurans, which use simultaneous strokes of their hind limbs for swimming and shallow jumping and never walk by alternating the contralateral limbs.

We hypothesized (Malashichev & Nikitina, 2002) that purely motor (i.e., not guided visually) lateralization of limbs is the function of the mode of locomotion connected with the way in which the frog moves (alternating-limb walking vs. synchronous two-limb pushing when jumping or swimming). However, the disparate phylogenetic positions of Bufonidae and Bombinatoridae (see Figure 1 for phylogenetic position) make unclear whether pronounced handedness in anurans might be derived, whereas ambidexterity is a primitive characteristic (as hypothesized by Goree and Wassersug, 2001). Given the common pattern of brain asymmetry in all vertebrates and improbable independent origin of brain lateralization in amphibians, the latter hypothesis seems unlikely. This article reports results of tests of both hypotheses, however. For this purpose, motor lateralization has been studied in five anuran species using different locomotive habits and belonging to families other than Bufonidae and Bombinatoridae.

Among anurans that actively use alternating-limb locomotion are several families of arboreal frogs (e.g., Hylidae, Neobatrachia), in which most species are well adapted to climbing on tree branches and leaves; they, however, are also good at leaping. One species of this family, *Litoria caerulea*, was used in this study. True frogs (Ranidae, Neobatrachia) are quite different from tree frogs (Hylidae) in that they are accomplished jumpers. They jump much more actively on land, pushing against the substrate with both hind limbs. Alternative-limb walking and climbing widely distributed among bufonids and hylids is not a rule among ranid frogs. To make a broader coverage, I used two species with different ecologies, the more terrestrial *Rana temporaria*, and the more aquatic *Rana lessonae*. The first species is usually found jumping quickly in a certain direction in closed wet biotopes; the second is usually found resting on the bank of a pond, using fast escape jumps to the water when startled. Both species can, of course, move feet alternately but mostly in only a few situations, such as when turning around or when cooled down early on a cold spring morning.

Some frogs are especially interesting for our study of motor lateralization because they use instincts of digging or hiding that are connected with fossorial or semifossorial sit-and-wait life styles. One of them, the spade-toed foot, *Pelobates fuscus* (Pelebotidae, Mesobatrachia), is a subterranean species well known for its digging behavior—alternating shuffling movements of the hind limbs with expanded spadelike tubercles to descend into the substrate, with little role for forelimbs in digging. These toads hide underground during the day and go foraging at night, when they unwillingly hop, spending much of the time sitting motionless. The ornate horned frogs, *Ceratophrys ornata* (Leptodactylidae, Neobatrachia), are sit-and-wait predators that usually watch for prey with their bodies partially buried in the substrate (e.g., leaf litter of the forest floor; see Duellman & Lizana, 1994). The leptodactylid *C. ornata* also alternates hind limbs when hiding in the paper litter in captivity (personal observation). It is an ineffective jumper with relatively short legs. In addition to their burrowing alternating-limb activity, species of this genus also exhibit pedal luring behavior, in which only one hind limb or, in cases of extreme agitation, both hind limbs are used to attract prey (Murphy, 1976; Radcliffe, Chiszar, Estep, Murphy, & Smith, 1986).

The results were analyzed in a broader context of previous reports (Bisazza et al., 1996, 1997; Goree & Wassersug, 2001;...
Malashichev, 2002; Malashichev & Nikitina, 2002; Robins et al., 1998; Rogers, 2002b). Hence, the full analysis involved 11 species of frogs of different phylogenetic position and locomotive habits and allowed high resolution of the study. Experimental paradigms identical to the previous reports were used, which made possible exact comparisons and relative assessment of limb lateralization in all the species involved. Possible links between motor lateralization and locomotive performance (symmetric or simultaneous vs. asymmetric or alternating-limb movements) or the phylogenetic position were tested. I found that there is a strong linkage between the presence of significant motor lateralization and alternation of limbs during locomotion. On the other hand, the available data do not support the hypothesis on increasing motor lateralization within the order Anura. These conclusions are considered in the general context of evolution of brain asymmetry and its behavioral manifestations.

**Method**

**Subjects**

Adult *R. temporaria* (*N* = 35, Ranidae) were collected in Leningrad region of Russia and were tested in the autumn of 2000 in a laboratory in St. Petersburg. These frogs were obtained from a local vendor who supplies animals for research and educational purposes to St. Petersburg State University. They were placed into plastic terraria measuring 40 × 25 × 25 cm (*L* × *W* × *H*: no more than 6–8 frogs per container) with water level of 1–2 cm deep. The frogs were maintained at temperatures lower than 16 °C and were fed live earthworms twice a week. After the study was completed, the frogs went into hibernation and were given to university personnel for educational purposes.

Adult *P. fuscus* (*N* = 15, Pelobatidae) and *R. lessonae* (*N* = 16, Ranidae) were captured in the wild in the Russian regions of Tambov (tested in the summer of 1999) and Pskov (tested in the spring of 2002), respectively. These frogs were on loan to me from the Department of Herpetology of the Zoological Institute of the Russian Academy of Sciences (St. Petersburg) for experiments and were returned to that institution when the study was completed. A maximum of 4 frogs were placed into each plastic terrarium of the same size as described above for *R. temporaria*. A layer of soft, wet soil 3–5 cm thick was provided for *P. fuscus* to allow the frog to dig; *R. lessonae* were provided with a water basin. The food (live cockroaches of different sizes) was available ad libitum every other day. Live prey was given to frogs to ensure their proper nutrition. Because the frogs were caught in the wild, they might have refused to take nonliving food unless they underwent a long period of adaptation to laboratory conditions and training. This was especially applicable to *Rana*, as well as to the tree frogs that usually prey on fast-moving insects.

Adult *L. caerulea* (*N* = 16, Hylidae; tested in the spring of 2004) and *C. ornata* (*N* = 40, Leptodactylidae; tested in the spring of 2001) were captive broods obtained from a local supplier and kept in the St. Petersburg Zoo in accordance with standard recommendations for animal care accepted there. The *Litoria* were kept together in a huge (larger than 1.5 cubic meters) plastic terrarium with a bottom basin and an artificial spring providing water current, tree branches and vegetation positioned on different levels in the terrarium, and top ventilation and light (12 h light:12 h darkness). Horned frogs were placed in plastic boxes (15 × 60 × 40 cm) with side ventilation and wet filter paper as the substrate at densities of 2–5 frogs per box. The frogs were fed live cockroaches and locusts ad libitum every other day. In addition to insects, the *Ceratophrys* also were given dead mice pups once a week as a part of their usual diet in the zoo.

**Testing Procedures**

Subjects were tested in a circular arena with a diameter of 70 cm and walls 25 cm high. The arena was uniformly illuminated with four lamps positioned equidistantly along the wall or with an overhead lamp in umbrella reflector. Testing protocol included two experiments: the snout-wiping test (Bisazza et al., 1996; Malashichev & Nikitina, 2002) and the righting-response test (Malashichev & Nikitina, 2002; Robins et al., 1998) applied to each animal of all five species. In all cases, the snout-wiping test, the less arduous of the two tests for the frogs and toads, preceded the righting-response test. Therefore, the experimental paradigm to assess motor handedness was very similar to those used in the previous reports on lateralization in other anuran species.

For the snout-wiping test (Figure 2A), a 15 mm × 4 mm piece of wet paper was placed across the nostrils on the frog’s or toad’s snout. This size was most appropriate to the size of animals, which varied in the ranges of 5–7 cm for each species. The piece of paper, therefore, could be positioned approximately equally on the snout of all species. Note, however, that in a previous report, the size of the paper strip was different for one species (*B. bombina*) because these toads were substantially smaller (maximum snout-to-vent length, 3 cm) than frogs and toads of other species used in earlier and the current studies. In these experiments, 10 successive trials were given to each animal, with the experimenter alternating the hand holding the animal and the hand operating the strip of paper in each trial. The forelimb that the frog or toad used to remove the paper was recorded.

In the righting-response test (Figure 2B), animals were overturned on the horizontal surface. The experimenter alternated holding the animal with the left and right hands in successive trials, so as not to introduce any lateral bias in position of the animal on its back. Each animal was given 10 successive trials to turn from the overturned position. In the analysis of video recordings of this test, the hind limb used by the animal to push against the substrate was recorded.

**Ethical Note**

If a frog or toad did not respond within 2 min during the trials in either of the aforementioned tests, the trial was stopped and the animal was allowed to rest for up to 1 hr. No animal died, was injured, or was the subject of any disease during the testing period.

**Statistical Analysis**

A standardized set of statistics, which is commonly used with the similar experimental protocols and data (e.g., see Bisazza et al., 1996, 1997), was used to allow correct comparison between the novel and previously published results. Mean percentage of right-limb use in each sample and a proportion of left-handed versus right-handed animals were calculated for both experiments. An animal was scored as right- or left-handed if it wiped or turned on the same side in 7 or more of 10 trials given (70% threshold). Student’s *t* test was used to estimate the probability of distinction of the means (in percentages) from a hypothetical 50% state, whereas a *G* test (log-normalized chi-square test) was used to estimate the probability of distinction of proportional data from 1:1 (see Table 1). The conclusion on the presence of lateralization in forelimb or hind limbs in either experiment in a given species was made only if both *t* and *G* statistics showed significant departures from chance. Heterogeneity chi-square tests were performed for proportional data to learn whether two samples were homogeneous and could be pooled.

**Results**

*L. caerulea*, examined with the snout-wiping test, showed highly significant population preference of the right forelimb (see Table 1 and Figure 3A), suggesting a strong lateralization of the
forelimbs in this species. In the righting-response test, the population bias to use the left hind limb to push against the substrate did not reach significance in t tests but was significant in G tests (see Table 1). Six frogs strongly preferred to use the left hind limb in 70% or more trials, whereas only 1 frog was strongly right-footed (the difference from chance is significant; see Table 1 and Figure 3B).

A similar recent study of *L. caerulea* tested in field conditions (Rogers, 2002a) showed no significant population bias in the direction of pivoting the body in the righting-response test. Nevertheless, a skew toward the right side again existed with 6 frogs showing strong right-side rotation preference versus only 2 with strong left-side preference. Analysis of video recordings from my experiments showed that *L. caerulea* used the left hind limb for pushing against the substrate, which resulted in the frog’s rolling onto its right side. Thus, the proportions of right- and left-footed tree frogs in two samples were similar. Given that the tendency toward left hind limb preference in both samples (mine and Rogers’s) was the same (heterogeneity chi-square test, \( \chi^2(1, N = 36) = 0.0857, p = .82, ns \)), the samples were pooled for a more powerful analysis. In the pooled data, there was a significant population bias toward the use of the left hind limb (see Table 1).

Thus, *L. caerulea* is indeed lateralized in respect to its forelimb and hind limb usage.

Neither *R. temporaria* nor *R. lessonae* revealed any limb lateralization at the population level in either experiment (see Table 1 and Figures 3C–3F). There were equal numbers of individuals preferring to use the right and left forelimb or hind limbs. A few individuals of both species (4, or 11%, of *R. temporaria*; 1, or 4.5%, of *R. lessonae*) occasionally used both hind limbs simultaneously in the course of righting themselves by executing a symmetrical backwards somersault and pivoting on the snout and over the head. This symmetrical turning resembles that reported in *B. bombina* (Malashichev & Nikitina, 2002). Individuals of the latter species, however, rotated their bodies around the hips before landing on their bellies. Such symmetry of fulfillment of the righting reflexes in *Rana* and *Bombina* (along the longitudinal body axis) never was observed in *Bufo* or *Litoria*. These observations along with the results of direct testing suggest that population-level limb lateralization is absent or very low in *R. temporaria* and *R. lessonae*.

The snout-wiping test revealed no preference in forelimb use in *P. fuscus* at population level (see Table 1 and Figures 3G and 3H). Moreover, only 1 animal in the sample population showed a
moderately strong preference to use its forelimbs. This result corresponded well with previous findings in two other populations—*P. fuscus* (Rogers, 2002a) revealed no dominance in forelimb use for snout-wiping at the population level. The tendency to push against the substrate with the left hind limb when righting, was, in contrast, significant (see Table 2). Both *Rana* and *Bombina* preferentially use symmetrical locomotion, such as when contralateral limbs are used synchronously in jumping or swimming (see Table 2).

Contrary to the findings for bufonids and tree frogs, findings for the two other species of neobatrachian ranid frogs (*R. temporaria* and *R. lessonae*) seem to indicate that they are not lateralized in terms of their pure motor righting and wiping reflexes. The results of their testing are similar to previous findings in phylogenetically more basal archaeobatrachians and morphologically more generalized *Bombina* toads (Goree & Wassersug, 2001; Malashichev & Nikitina, 2002). Both *Rana* and *Bombina* preferentially use symmetrical locomotion, such as when contralateral limbs are used synchronously in jumping or swimming (see Table 2).

Finally, *P. fuscus*, which digs in substrate by alternating hind leg movements, was found to possess significant bias in the righting (hind limb operation) but not in the snout-wiping (forelimb operation) reflexes (see Table 2). The leptodactylid *C. ornata*, which uses only short hops, usually hides in leaf substrate by alternating movements of its hind limbs and may unilaterally use its hind limbs in luring the prey, revealed a pattern of lateralization similar to that of *P. fuscus*, although with the opposite direction of bias (see Table 2). Another difference between the two species was that in *Ceratophrys*, the lateralization of the forelimbs approached significance. This was perhaps due to its pronounced habit of wiping foreign objects (litter) from the snout (personal observation).

Collectively, the results of this comparison of 11 anuran species show a strong linkage between one-sided limb preference and alternation of the left and the right limbs in locomotion and some other types of natural motor behavior in anurans (see Table 2).
Snout-wiping test

A. *Litoria caerulea*

B. *Litoria caerulea*

C. *Rana temporaria*

D. *Rana temporaria*

E. *Rana lessonae*

F. *Rana lessonae*

G. *Pelobates fuscus*

H. *Pelobates fuscus*

I. *Ceratophrys ornata*

J. *Ceratophrys ornata*

Righting-response test

Number of animals

Number of right hind limb trials

Number of right forelimb trials

Number of animals

0 1 2 3 4 5 6 7 8 9 10

Number of animals

0 1 2 3 4 5 6 7 8 9 10

Number of animals

0 1 2 3 4 5 6 7 8 9 10
However, the analysis apparently fails to uncover clear evolutionary trends in motor lateralization across species (see Table 2) that are predictive (see the introduction). Indeed, significant prevalence for the animal to use either limb in wiping a foreign object from its snout or righting itself from the overturned position is not a unique characteristic of only archaeobatrachians as hypothesized by Goree and Wassersug (2001) but may occur in some advanced and specialized groups of neobatrachian anurans. The data reported in this and in previous articles correspond more closely to the hypothesis by Malashichev and Nikitina (2002) in that the degree of limb lateralization is functionally connected to the degree of alternation of limbs during locomotion or other aspects of natural behavior. Data from a this study; b Rogers, 2002a; c,d Bisazza et al., 1996, 1997; e Robins et al., 1998; f Malashichev & Nikitina, 2002; g Malashichev, 2002; h Goree & Wassersug, 2001.

Table 2
Limb Preferences in Relation to Phylogenetic Position and Movement Performance

<table>
<thead>
<tr>
<th>Phylogenetic position</th>
<th>Species</th>
<th>Usual locomotion and movement performance</th>
<th>Limb preferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neobatrachia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranidae</td>
<td>Rana temporaria</td>
<td>Synchronous jumping and swimming</td>
<td>Not lateralized</td>
</tr>
<tr>
<td></td>
<td>Rana lessonae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hylidae</td>
<td>Litoria caerulea</td>
<td>Alternating climbing</td>
<td>Lateralized</td>
</tr>
<tr>
<td></td>
<td>Litoria lutropalmata</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptodactyliida</td>
<td>Ceratophrys ornata</td>
<td>Alternating hind limb digging and prey luring</td>
<td>Lateralized (hind limbs only)</td>
</tr>
<tr>
<td>Bufonidae</td>
<td>Bufo bufo</td>
<td>Alternating walking</td>
<td>Lateralized</td>
</tr>
<tr>
<td></td>
<td>Bufo viridis</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bufo marinus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesobatrachia</td>
<td>Pelobates fascus</td>
<td>Alternating hind limb digging</td>
<td>Lateralized (hind limbs only)</td>
</tr>
<tr>
<td>Pelobatidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaeobatrachia</td>
<td>Rana lessonae</td>
<td>Synchronous swimming</td>
<td>Not lateralized</td>
</tr>
<tr>
<td>Bombinatoridae</td>
<td>Bombina bombina</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bombina orientalis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. The list of anuran families and major clades is organized in the order of their presumed divergence to currently available phylogenies (Figure 1). Note the lack of any clear phylogenetic trend in degree of lateralization. On the other hand, lateralized limb preference is qualitatively associated with the alternation of limbs during locomotion or other aspects of natural behavior. Data from a this study; b Rogers, 2002a; c,d Bisazza et al., 1996, 1997; e Robins et al., 1998; f Malashichev & Nikitina, 2002; g Malashichev, 2002; h Goree & Wassersug, 2001.

However, the analysis apparently fails to uncover clear evolutionary trends in motor lateralization across species (see Table 2) that are predictive (see the introduction). Indeed, significant prevalence for the animal to use either limb in wiping a foreign object from its snout or righting itself from the overturned position is not a unique characteristic of only archaeobatrachians as hypothesized by Goree and Wassersug (2001) but may occur in some advanced and specialized groups of neobatrachian anurans. The data reported in this and in previous articles correspond more closely to the hypothesis by Malashichev and Nikitina (2002) in that the degree of limb lateralization is functionally connected to the degree of alternation of limbs in a given species. The more pronounced the habit is to use limbs unilaterally in a certain type of behavior (e.g., alternating-limb locomotion), the greater is the motor lateralization in the species. One trivial explanation for this is that the alternate contraction of the contralateral muscles during quadruped locomotion or any other unilateral action requires differential neural control and, thus, a greater functional asymmetry in the neural system. It is interesting that other authors have found a similar phenomenon in mammals in that the degree of limb lateralization depends on the character of the motor task: the more complex the movement to be fulfilled, the greater the asymmetry in the limb use (Fagot & Vauclair, 1991; Forward, Warren, & Hara, 1962; Mikli- aeva, Kulikov, & Ioffe, 1987).

It is possible that at least some asymmetric actions, which might be partially controlled by vision or have partially autonomic regulation (i.e., the snout-wiping reflexes), might be associated also with the control of posture (see, e.g., MacNeilage, 1991; Tommasi & Vallortigara, 1999). However, in discussing this issue, one should distinguish between largely (primates) or exclusively (birds) bipedal species and those (e.g., amphibians) using quadruped locomotion. In the latter, the control of body position may not be as important in unilateral actions with forelimbs or during alternating-limb locomotion as in the former because of the quadrupeds’ better body stabilization, with two or three other limbs resting on the substrate. However, several interesting points emerge when comparing my data on amphibians with those published in recent reports on crawling and walking human infants and nonhuman primates (Corbetta, 2005; Corbetta & Bojczyk, 2002; Dodson, Stafford, Forsythe, Seltzer, & Ward, 1992; Spinozzi, Castorina, & Truppa, 1998). Particularly, bipedal posture during locomotion has been shown to be more favorable to the success of revealing hand preference in the tasks of varying complexity. To the contrary in the quadrupedal stance and locomotion, the motor lateralization declines. It now seems that quadrupedal locomotion itself is not a unified condition with respect to motor lateralization of limbs but rather that the different gait is associated with quite different degrees of motor asymmetry in a species. Corbetta (2005) argued that “hands-and-knees crawling and quadrupedal locomotion can hinder the expression of preferred lateral biases in manual activities precisely because quadrupedal locomotion involves the alternated and bilateral activity of the fore and hind limbs” (p. 201). The view from the amphibian perspective may alter this conclusion in that synchronous quadruped locomotion is even less.

Figure 3 (opposite). The results of the “snout-wiping” (left plots) and the “righting response” (right plots) tests in five anuran species. The distributions of the numbers of frogs and toads that performed right-sided trials in each test are shown. Note the significant population-level rightward bias that is above the level of chance in the data for Litoria caerulea (A, forelimb use) and Pelobates fascus (H, hind limb use) and the significant leftward bias in the data for Ceratophrys ornata (J, hind limb use). No lateral bias was found in Rana temporaria or Rana lessonae in either test. See also Table 1 for statistics.
favorable to lateralization of limbs than alternating-limb walking and running. Therefore, my observations in anuran amphibians and those of others in primates are in good correspondence, reflecting stepwise acquisition of limb motor lateralization in evolution of terrestrial vertebrates.

Animal locomotion is controlled, in part, by a network of intraspinal neurons—a central pattern generator (CPG) that generates a rhythmic output pattern and that has been suggested to have a symmetrical structure (Golubitsky, Stewart, Buono, & Collins, 1999). Snout-wiping and righting reflexes may be quite distinct from gait-pattern generation. Nevertheless, a correlation between the mode of locomotion and the lateralized limb preference implies that either the neuronal network may include an element of asymmetry or that alternating-limb locomotion is directed by neural asymmetry outside the CPG network (e.g., brain stem, higher neural centers, or autonomic neural system). Although our current knowledge of this issue is limited, some parallels found between spinal CPGs that control locomotion in primary aquatic vertebrates and mammalian neocortices (Yuste, MacLean, Smith, & Lansner, 2005) make this hypothesis viable.

The conclusions reached about anurans in this article seem to possess predictive value. They suggest that in species that jump and stroke-swim, the degree of motor lateralization might be lower than that in species that typically walk and climb. Thus, one might expect that fully aquatic frogs like the African smooth clawed frog, *Xenopus laevis* (Pipidae, Mesobatrachia), or the Titicaca water frog, *Telmatobius culeus* (Leptodactylidae, Neobatrachia), which use simultaneous propulsive strokes with their hind limbs, are less lateralized than leiopelmatids and aschaphids (leiopelmatidae, Figure 1), which are basal anurans that use an alternating-limb swimming technique that is unique among anuran amphibians (Abourachid & Green, 1999). Current experiments in our laboratory support this prediction, at least in *X. laevis*; several different tests have been used and so far have revealed no motor lateralization, at least soon after metamorphosis (Kostylev & Malashichev, 2006). However, the degree of lateralization may change during the frog’s life time. It is noteworthy that because they are dependent on the mode of locomotion, the age-related changes in a locomotion pattern may be concordant with the change in the degree of lateralization. For example, in *Bufo* toads, in which the type of locomotion used depends on the size of the toad, smaller toads, especially subadults, jump more often than walk (personal observation); therefore, less motor lateralization can be expected. Indeed, *B. viridis*, which even in adulthood is smaller than other *Bufo* species and which prefers jumping to walking, is also less lateralized than other species of *Bufo* toads that are larger and more likely to walk (see the discussion in Malashichev & Nikitina, 2002). On the other hand, *Bombina* toads, which use symmetrical locomotion, have no motor lateralization regardless of age (Goree & Wassersug, 2001; Malashichev & Nikitina, 2002).

Alternating-limb locomotion produces baseline limb asymmetry, but that alone explains only a part of the overall limb lateralization in quadrupeds. Unilateral forelimb or hind limb motor activities, such as burrowing, luring, grasping, or manipulation, can additionally increase population biases in some species. Among anurans, *Ceratophrys* is a good example. In these frogs, the alternating-limb locomotion is coupled with alternating-limb burrowing and unilateral pedal luring with the hind limbs. These unilateral or alternating-limb activities result in up to a 12-fold excess of strongly lateralized left-footed frogs over right-footers in this species. The percentage of left-footers in a group of *C. ornata* (at least 75%) is greater than that in species that use alternating-limb locomotion alone. Therefore, those species that act with one of the contralateral limbs in some unique behaviors similar to pedal luring of *Ceratophrys* should be also lateralized. I thus predict that foot-flagging demonstrative behavior in frogs (Hödl & Amézquita, 2001; e.g., waterfall-dwelling *Hyloides*, Leptodactilidae) should result in a greater level of lateralization in the hind limbs. The corresponding levels of motor lateralization in higher vertebrates (e.g., mammals, depending on the mode of locomotion they use) are also predictive. Assessing lateralization in species with different locomotive habits could be a useful reference to study the diverse animal gaits (e.g., jumping in kangaroos and jerboas; ambling in horses, camels, and elephants; or a special kind of asymmetric galloping characteristic for rabbits and hares). A pair of interesting examples comes from the studies of insects. Locusts, which jump effectively with the simultaneous pushes of their long hind legs, show a lack of population-level lateralization in hind-leg use during righting from the overturned position (Faisal & Matheson, 2001). Spiders, however, have shown the preferential use of the left foreleg for touching and handling prey (Ades & Novaes Ramirez, 2002). Because handling prey implies specialization of legs and their asymmetrical use, this finding corresponds well with the findings of the experiments on frogs and may indicate a universal application of the findings reported in this article.

Let me finally consider a model of stepwise acquisition of novel asymmetric features of the neural system in the line from fish to humans. In amphibian larvae and in teleost fish, swimming preferences are largely affected by pronounced lateralization in response to visual stimuli, such as conspecifics, predators, or the organism’s mirror image when inspected unilaterally (Sovrano, Bisazza, & Vallortigara, 2001; Sovrano, Rainoldi, Bisazza, & Vallortigara, 1999). The latter are not, however, present in amniotes. Thus, in primary aquatic vertebrates, the main types of lateralizations are distributed in all vertebrate classes, probably even from early chordates (Vallortigara & Bisazza, 2002) through mammals, and have relevance to social aspects of animal behavior (Vallortigara & Rogers, 2002).

Another common type of asymmetric motor behavior in anuran larvae and in fish is the startle response: asymmetric contraction of axial muscles leading to rapid C-start escape turns, which are lateralized and presumably driven by an asymmetrical system of Mauthner neurons (thoroughly reviewed in Wassersug & Mathews, 2002). The latter are not, however, present in amniotes.

C-start turning lateralization in anuran larvae disappears at metamorphosis (Oseen, Newhook, & Wassersug, 2001; Wassersug & Yamashita, 2002), at which time the role of the axial muscles is
less important to locomotion than that of paired limbs. In early tetrapod evolution (Gans & Parsons, 1966), the role of axial musculature decreases in lateral undulatory locomotion, much as it does during amphibian metamorphosis. Data reported on motor preferences in anuran amphibians in this article show that there is consistent motor lateralization in species with alternating-limb movements. This finding suggests that the evolution of paired limbs in fish–quadrupeds transition boundary and nonundulatory patterns of terrestrial locomotion could require emergence of motor limb asymmetry. The neural features that underlie lateralization of this kind of motor behavior may not be necessarily directly involved in regulation of the other two types of lateralization mentioned (perceptual and C-start turning, see also Malashichev & Wassersug, 2004).

The degree of lateralization probably evolved in different vertebrate lineages as the result of adding of new asymmetric characteristics to pre-existing ancient lateralizations related to processes of perceptual analysis. This concept suggests multiple sources and cumulative effects for a high level of behavioral lateralization in some species (e.g., pronounced right-handedness in humans). Handedness here can be linked partially to asymmetry of visual response, which is a heritage of primary aquatic vertebrates, and partially to alternating-limb locomotion regardless of its particular reason—walking or climbing—a later addition of the early tetrapods. Therefore, the locomotion-based lateralization probably predated the stronger hand preference in evolution and is reflected, for example, in the lateralization of feet in humans. A further increase in lateralized responses in primates included postural asymmetry, bipedal locomotion, and corresponding increased manipulative (and often unilateral) activity of the free forelimbs (Corbetta, 2003, 2005; Dodson et al., 1992; MacNeilage, 1991).

The combination of these factors, rather than any single factor alone, created a characteristic pattern of human lateralization through the course of vertebrate evolution. This multiple ancestry for human hand dominance may thus involve several different neural substrates with independent lateral biases in which the alternating-limb locomotion of the early tetrapods was the initial basis for motor lateralization of both hands and feet.

References


