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Lateralized visual and motor responses in the green tree frog, *Litoria caerulea*

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There is increasing evidence of brain lateralization in frogs and toads, based on studies of their responses to various types of visual stimuli and also from their limb preferences. In a range of toad species, agonistic and predatory responses are preferentially directed by the left and right eyes, respectively. We investigated visual lateralization in the adult Australian green tree frog, a species with marked differences in ecological habitat and evolutionary history compared to the bufonids (toads). We also investigated forelimb preferences in a climbing task, using the same group of frogs. Strong and significant left-eye preferences were found for directing agonistic responses at other frogs, contrasting with a nonsignificant trend towards right-eye preferences for predatory responses. Furthermore, the right forelimb was used preferentially as the leading limb to control climbing to an upright position after the frog had been inverted in the vertical plane. In each instance, the laterality of behaviour corresponded with the lateralization of comparable behaviours in higher vertebrates, supporting the hypothesis that brain lateralization is conserved from a common lateralized ancestor.

Over the past 30 years, the consideration of specialized functions served by the left and right brain hemispheres, termed lateralization, has shifted from the belief that it existed only in humans to the realization that lateralization is a characteristic that may be ubiquitous among vertebrates. Consensus on the extent and conservation of different forms of lateralization in modern vertebrates suggests the existence of a lateralized common ancestor. The presence and direction of lateralization are two general properties thought to enable a species to adapt successfully to novel or changing environments. More specifically, the presence of lateralization enables an individual to process information more efficiently than if certain functions were duplicated in both brain hemispheres (Rogers et al. 2004), whereas the direction of lateralization (i.e. that specific functions are carried out by either left or right hemisphere in the majority of the population) is an important correlate with social cohesion and communication (Vallortigara & Rogers 2005). Anuran amphibians provide important models to test current hypotheses on the evolution and adaptive significance of well-conserved forms of vertebrate lateralization: not only are extant anurans phylogenetically closest to the first tetrapods (the ichthyostegans; Beçak & Kobashi 2004), but they also lack the brain structures comparable to the reptilian or avian pallium or mammalian neocortex (Taylor et al. 1995). Anuran species have none the less successfully colonized aquatic, fossorial, terrestrial and arboreal habitats.

Only terrestrial bufonids (the 'true toads') have been tested for visual lateralization as adult anurans. This is an important point, as although the visual and motor systems are conserved throughout the subclass Anura, adaptations to different habitats have led to changes in the various sizes of the anuran brain (Taylor et al. 1995). Relative to body size, anuran species specialized for arboreal habitats possess larger brains than do anurans living in other habitats (i.e. aquatic, fossorial and terrestrial anuran species: Taylor et al. 1995). The enlarged cerebellum in arboreal anurans is the only brain area found to contribute significantly to the larger brain overall. Enlargements in other brain areas are not significantly different between arboreal anurans and anurans from other habitats. An enlarged cerebellum in arboreal anurans could be an adaptation to life in three-dimensional space,

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although it is surprising that the level of sensory and spatial integration demanded by such a habitat did not also result in an enlarged optic tectum or telencephalon (Taylor et al. 1995). None the less, subtle differences in brain structures relating to habitat adaptations may not only correspond with differences in behavioural patterns, but also influence the presence or degree of lateralization observed. We investigated the semiarboreal Australian green tree frog *Litoria caerulea* (Tyler 1999) for evidence of lateralized behaviour, a species with marked differences in evolution and habitat to that of the terrestrial bufonids.

The toad species *Bufo marinus* and *B. bufo* have left-eye (right-hemisphere) preferences for directing agonistic tongue-strikes at conspecifics during group-feeding episodes (Robins et al. 1998; Vallortigara et al. 1998). In addition, left-eye superiority in responding to predators is found in the toads *B. marinus*, *B. bufo* and *B. viridis* (Lippolis et al. 2002). These left-eye specializations are complemented by a right-eye (left-hemisphere) specialization for directing tongue-striking responses at prey items viewed in the right visual hemifield of *B. marinus*, *B. bufo* and *B. viridis* (Vallortigara et al. 1998; Robins & Rogers 2004). The specializations for visual responses in toads correspond with the general forms of lateralization found in the vertebrate brain, the right hemisphere being dominant for tasks requiring immediate responses to changes and potential threats in the immediate surroundings (Andrew & Rogers 2002; Vallortigara & Rogers 2005). In contrast, the left-brain hemisphere is dominant for tasks requiring deliberation, or at least a basic form of logical processing, such as the visual analysis of complex prey stimuli. In the species studied there appear to be variations in the strength of lateralization for antipredator, agonistic and predatory responses (e.g. Robins et al. 1998; Vallortigara et al. 1998; Lippolis et al. 2002). This suggests that even within terrestrial bufonids, the strength of lateralization for a particular task may indeed possess adaptive significance and vary between species according to habitat demands or other evolutionary pressures.

Lateralization of forelimb use has been assessed in a range of anuran species using two main procedures. The ‘snout-wiping’ test scores the forelimb used to remove a strip of wetted paper, plastic-coated wire or small balloon from the snout, in repeated trials (Bisazza et al. 1996, 1997; Goree & Wassersug 2001; Malashichev 2002, In press; Malashichev & Nikitina 2002). Essentially, the snout-wiping task involves directed movement with a low degree of dexterity, and is presumably a task requiring little physical exertion by the animal. The degree of lateralization demonstrated by the snout-wiping test varies from 39 to 59% right-handedness (%R) between species tested (Malashichev, In press). *Litoria caerulea* had a preference of 58%R for removing a wetted paper strip from the snout in repeated trials (Malashichev, In press). In contrast to the snout-wiping test, the ‘aquatic-righting’ test scores the forelimb used physically to direct or control the rotation of the body to the ‘righted’ position after the animal has been overturned in the horizontal plane. When overturned into a water bath and provided with support for the forelimbs physically to pull and then push against, *B. marinus* toads have significant right-handedness of 66%R, compared to a nonsignificant preference of 48%R when tested in a matched snout-wiping test (given three trials daily for 6 consecutive days: Bisazza et al. 1996, 1997). In 10 consecutive trials of the aquatic-righting test, the preference for the use of the right forelimb was 90%R in *B. marinus* (Robins & Rogers 2002). Thus, tasks requiring physical strength of the forelimbs appear to elicit a comparatively higher degree of right-handedness in toads. The tasks used to test motor preferences in anurans require little or no visual input to coordinate the response. Thus, in contrast to studies in other vertebrates in which visuomotor preferences are commonly measured for visually directed reaching or searching behaviours (reviewed in Andrew et al. 2000), righting experiments measure lateralized motor behaviour. Furthermore, comparative studies in mammals and anurans have shown that the initiation of the righting response occurs in the brainstem without the requirement of the motor cortex (Klemm 1977).

Litoria caerulea is a representative of the hylid-like Pelodryadinae subfamily that, like the terrestrial bufonid family, is contained within the superfamily Bufonoidea (Ruvinsky & Maxson 1996). None the less, *L. caerulea* provides a good comparative species with which to test generalizations drawn from similar studies in terrestrial bufonids. It lacks the definitive tongue-striking abilities of the bufonids and most anuran species, possessing a primitive tongue structure found also in the ‘primitive’ frog families Discoglossidae and Ascaphidae and other members of the Pelodryadinae subfamily and Hylidae family (Deban & Nishikawa 1992; Nishikawa 1997; Peters & Nishikawa 1999). In anurans with the primitive ‘mechanical-pulling’ tongue mechanism, predatory behaviours involve jaw prehension by lunging at the prey with the entire head and body, with minimal protrusion of the tongue (maximally 60% of the jaw length: Deban & Nishikawa 1992). This may also be true for agonistic behaviours. Thus, the agonistic behaviour of *L. caerulea* provides an important contrast to that of the bufonids and most other anuran families equipped with an ‘inertial elongation’ tongue mechanism, which protrude the tongue rapidly and to a maximum length of 180% of the jaw length (Nishikawa & Gans 1992; Peters & Nishikawa 1999). Another adaptation distinguishing the bufonids from *L. caerulea* is the evolution of expanded, adhesive toe pads in the latter species, used for climbing (Tyler 1999). We used this adaptation to test forelimb preferences in a task requiring physical strength, scoring climbing behaviour of the frogs when righting the body inverted in the vertical plane.

METHODS

Frogs and Housing Conditions

We used 15 sexually immature *L. caerulea* (i.e. under 18 months of age, 2.5–3.5 cm snout–vent length), supplied by a commercial breeder (Darryn Nijalke, South Australia). Before testing, the frogs had been held for over 3 months in two home tanks at the University of New England, Australia. The home tanks were tall (50 × 50 cm and 100 cm high, constructed from glass panels 6 mm thick), permitting naturalistic expression of the climbing and prey-foraging behaviours of *L. caerulea* (Tyler 1997). Numerous tall artificial plants were included in the tank, thus mimicking the frogs' natural habitat under comparatively sterile conditions. Once daily, water was automatically showered over the entire arena for 15 min via a sprinkler system in the home tank lid, using biologically filtered water recycled from a storage reservoir. The recycled water was also used in a waterfall system that operated continuously and provided a permanent pool in one-third of the home tank floor permitting the frogs to engage in swimming behaviour. The frogs were separated into two housing groups on the basis of size, with the eight largest individuals being contained in one home tank.

The predominant diet of the frogs consisted of laboratory-bred compost-worms, crickets, wood-cockroaches and mealworms. The frogs were found to respond best when fed relatively small amounts of food on a daily basis, and house flies and other insects (e.g. small grasshoppers) were collected and occasionally used to increase the variety of their diet.

The housing room conditions were maintained between 24 and 30°C and the relative humidity between 90 and 95%. Lighting was provided in a 12:12 h photoperiod, using full-spectrum lighting ('Repti-Glo' fluorescent lighting, model PT2151, Exo Terra, Rolf C. Hagen Inc., Montreal, Canada).

Both experiments investigating lateralized visual and motor behaviour were filmed with a Sony Hi-8 video camera (Sony CD-TR2000E). Frame-by-frame analysis of the videotape was made later with a Sony EV-S9000E PAL video recorder.

The procedures for both housing and testing the frogs were approved by the University of New England's Animal Ethics Committee.

Visual Lateralization

This experiment was conducted within the home tanks, an environment familiar to the frogs, on horizontal platforms suspended approximately 20 cm from the top of the home tanks by wire hooks. A test platform was installed in each home tank 2 days before the experiment, for familiarization purposes. The platforms were plastic yellow discs (16.5 cm diameter), selected to resemble yellowed vegetation encountered under natural conditions, and large enough to support eight frogs comfortably. The frogs were able to approach or leave the platform freely during testing. They were fasted for 24 h before the first day of testing.

We conducted the experiment over 3 consecutive days, with tests scheduled between 1500 and 2200 hours. Mealworm larvae (approximately 15 mm long) were used as the test prey. They were dropped on to the test platform from directly above, and at a rate of approximately one mealworm every 20 s. Each test lasted at least 10 min. If five or more mealworms remained on the platform after 10 min we terminated the test, as the frogs were considered unlikely to continue feeding and competing for prey. Otherwise, the test was continued for a further 10 min (i.e. for a total of 20 min). The group of eight larger frogs ate sufficient mealworms to enable testing for 20 min on each of the 3 days of the experiment. The seven frogs in the other tank could be scored for the full 20 min on the third day of the experiment. The full data set, therefore, contained observations made from a total of 100 min. The test platform within each home tank was filmed from directly overhead, through the tank lid of wire netting. We identified individual frogs from the videotape from body markings and body morphology with respect to the other frogs in the tank.

Scoring visual responses

To score agonistic and predatory responses in *L. caerulea*, we used methods developed previously for *B. marinus* toads (Robins et al. 1998; Robins & Rogers 2004) and *Ctenophorus ornatus* lizards (Robins et al. 2005). Thus, agonistic strikes were directed at another frog and predatory strikes at mealworm prey. 'Sideways' agonistic or predatory strikes were those in which the frog turned to direct the strike, scored accordingly as either 'left' or 'right'. 'Midline' agonistic or predatory strikes were those in which the frog lunged directly along its visual midline to connect with either another frog or prey. We subsequently assigned midline strikes as 'left' or 'right' according to the side to which the body of the target frog or prey was mostly located (i.e. over 50% of the visible length of the target resided to either side of the attacker's visual midline: Robins et al. 1998, 2005; Robins & Rogers 2004). Great care was taken to ensure that midline strikes met this criterion: some midline agonistic and predatory strikes (<5% of strikes observed) could not be determined accurately as either 'left' or 'right' side biased, and were discarded from subsequent analyses. These left and right 'midline' strikes were considered separately from left and right 'sideways' strikes. To avoid any influence of bouts, we counted repeated agonistic and predatory strikes within 2 s from an individual frog as a single strike, and scored this as the first strike in the series.

Agonistic strikes were characterized by the partly opened jaws of the attacking frog making contact with the body of the target frog, most often appearing as a rapid 'nip' rather than a sustained bite (i.e. over 0.3 s in duration, or five consecutive frames of videotape operating at 15 frames/s). Regions of the target frog's body were also scored as the sites receiving the agonistic attack. However, owing to the wide area of the target's body encompassed by a strike, body areas were scored as strikes either to the 'body' (including neck, back and limbs) or 'head' (including mouth, eyes and skull). Examples of agonistic striking are shown in Fig. 1a, redrawn from separate frames of the videotape recording from directly above the test platform. Strikes directed at a target frog engaged in consuming a mealworm were possible bona fide predatory strikes as they were often directed at the movement of the prey in the stomach, throat and mouth of the target frog. Hence, all strikes delivered within 5 s of the target frog having

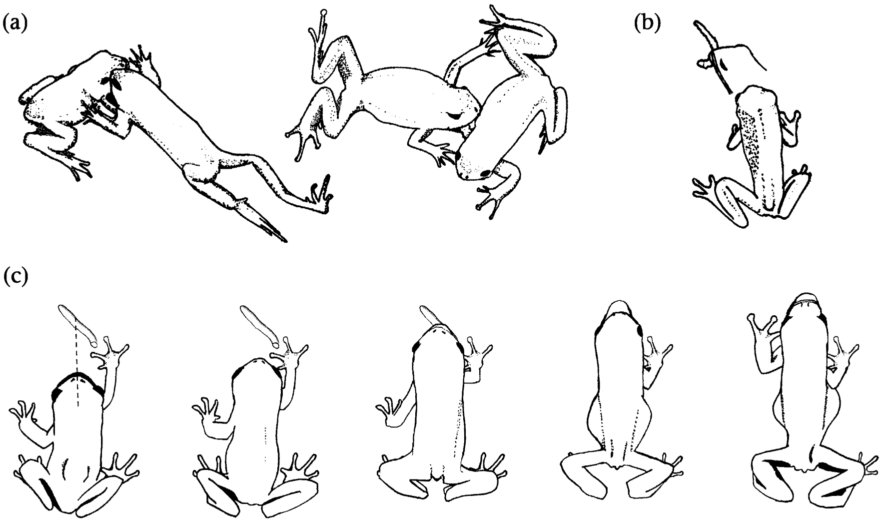

Figure 1. Examples of agonistic and predatory striking behaviour in *L. caerulea*. (a) Two examples of agonistic striking at target frogs in the left visual hemifield of attackers are shown at the moment of impact, traced from overhead videotape. The mouth of the attacking frog is opened on impact with the target frog in both instances, with the lateralization of the respective strikes indicated here by the orientation and position of the actors' bodies. In the first example, a 'sideways, left' strike at the head region of the target frog was scored; the example shows the position of the attacker's right foot from which the strike had been asymmetrically steered. In the second example, a 'midline, left' strike at the right forelimb and mid-torso region of the target frog was scored. (b) Example of a left 'sideways' predatory strike, showing the location of the prey with respect to the frog in the left visual hemifield prior to striking. The trajectory of the frog and point of impact with the prey are indicated from successive frames of videotape. (c) Example of a 'midline' predatory strike biased to the right visual hemifield. This sequence was traced from successive frames of videotape. In the first frame a visual midline has been included to illustrate that nearly 50% more of the mealworm lies in the right than in the left visual hemifield. The frog in this instance does not deviate from the visual midline when directing the body, head and tongue to strike the prey. The wide point of impact between the tongue and prey is occluded by part of the tongue: further examples and explanation of the 'reverse-flip' tongue-striking mechanism of *L. caerulea* can be found in Peters & Nishikawa (1999).

swallowed prey were considered separately from other agonistic behaviour and labelled as 'robbing' strikes. The function of such behaviour might have been to cause the target frog to disgorge its prey, as such strikes were sometimes repeated and invariably in a caudorostral sequence of locations on the body, despite successful attempts to 'rob' the target frog being rarely observed.

Predatory strikes were scored only for clear attempts to catch a mealworm. In addition to scoring such strikes as either 'sideways' or 'midline' with respect to the predator's orientation, we scored predatory strikes as either 'successful' (mealworm captured) or 'unsuccessful'. Figure. 1b, c shows examples of a successful left 'sideways' and a 'right side midline' predatory strike.

Statistical analyses

To analyse the data for agonistic and predatory strikes for the 3 days of testing for lateralization, we used *G* tests, taking into account both the number of scores and the lateral bias of each individual (log-likelihood chi-square test: Zar 1996). Scores as low as a single strike from individual frogs were included in the analysis, but we adjusted for possible overestimation of the group's preference from such inclusions by using the Williams correction on the *G* test scores (Sokal & Rohlf 1995).

To compare the degree of lateralization for agonistic and predatory behaviour for individual frogs, we used Student's *t* tests. For correlations between the incidence and degree of lateralization for agonistic and predatory strikes we used simple linear regression, combining the data from both frog groups. Homogeneity in the number of strikes and degree of lateralization (i.e. mean % left-eye preference) for agonistic and predatory responses of frogs from both housing tanks was checked with unpaired *t* tests. All statistical tests were two tailed, with significance taken at the $P \leq 0.05$ level. For the analyses we used StatView 5.0 (SAS Institute, Cary, NC, U.S.A.) and NCSS Exact Probability Calculator (http://www.ncss.com/download.html; http://www.esf.edu/efb/gibbs/monitor/using-prob-calculator.pdf).

Ethical Note

Agonistic behaviour was defined as those incidences in which open-mouthed frogs collided with the mouth, head or torso of target frogs. We observed no outward signs of irritation or distress as a consequence of the agonistic activity. Although cannibalism is known in the species (e.g. Tyler 1997) we averted the opportunity for such acts by group-housing frogs of similar size. The tests were conducted in a small, introduced area of the home tanks, the frogs being free to enter or leave the filmed area in pursuit of prey or to escape further competitive encounters.

Motor Preferences For Righting

Each frog ($N = 14$) was identified by its individual markings and given 10 consecutive trials on each of 2 days, 1 week apart, to provide a total of 20 trials each. At the start of each trial, we placed the frog on a straight section of nylon tube (34 cm long, 1.5 cm diameter) oriented in the vertical plane. As soon as the frog had positioned itself upright and longitudinally along the tube, we then carefully inverted the tube forwards in the medial plane, completely reversing the frog's caudorostral orientation so that its head was pointed vertically to the floor. The frog was considered to have righted after turning to a position (or perch) where it remained for over 1 s. Frame-by-frame analysis of the videotapes enabled us to see slight positional biases not noted while the tests were carried out; from a possible total of 280 trials, we omitted 14 from the analysis (5%). That is, six frogs had initiated the response from an inverted position not aligned with the vertical climbing tube, with a mean ± SEM of 2.3 ± 0.3 trials discarded. The mean ± SEM number of trials for all 14 frogs was 19.0 ± 0.3.

Scoring righting responses

We used three measures to score lateralized motor preferences in frogs: turning direction (scored as 'left' or 'right'); the leading forelimb first repositioned to initiate the process of turning the body to a righted position; and the numbers of right forelimb repositionings made to lift and turn the body to the righted direction when turning to the right, and the corresponding numbers of left forelimb repositionings when turning to the left. Equivalence between righting responses was made by scoring the first position or perch maintained by the frog for 1 s or more after it moved the body axis away from the vertical plane. From this perch position the frogs may have attempted to leap from the climbing tube, adjust to a second or subsequent perch positions closer to the vertical, or rotate their bodies entirely to continue the vertical climb without further pause. We terminated trials immediately after the frog either leapt from the tube or began the vertical climb. Perch positions in the absolute vertical axis were not observed. We also measured the angle of turning made to reach the righted (perched) position on the frog's left or right side with respect to the climbing tube to investigate whether particular perch positions were favoured.

Statistical analyses

We combined the data from each frog for both series of trials to calculate a '% right-side preference' score (%R); that is, the number of trials in which either the right side was preferred for turning the body or the right forelimb was the leading limb, expressed as a percentage of the total number of trials. We conducted Kolmogorov–Smirnov one-sample goodness-of-fit tests on these data to test the assumption of normality. Side preferences at the group level were then assessed with G tests. To analyse the numbers of forelimb repositionings to the left or right we used a one-way ANOVA. Circular data of the perch angles to either side of the vertical axis were grouped within five sectors of 36°. To compare preferred perch positions within matching sectors on the left and right sides, we used chi-square tests with Bonferroni's correction. We used the Williams–Watson F test on the data comparing mean perch angles to either the left or right side (Sokal & Rohlf 1995). All statistical tests were two tailed.

RESULTS

Lateralized Visual Responses

For agonistic strikes, there was a significant bias for the attacker's left visual hemifield in over 80% of strikes per frog, on average (Table 1). Two frogs were not observed to strike at conspecifics during testing; a mean ± SEM of 3.3 ± 1.0 agonistic strikes were recorded from the remaining 13 frogs. Sideways agonistic strikes were half as common as midline strikes, although the degree of lateralization noted for both types of behaviour was similar (i.e. over 80% of strikes per frog directed within the attacker's left visual hemifield, although found significant for midline strikes only). Table 1 also summarizes the number of recorded 'head' and 'body' strikes: the head of the conspecifics was targeted in 65% of agonistic strikes. The preference for either target area was strong but not significantly lateralized, despite the discrepancy in the numbers of strikes observed.

A total of 38 'robbing' strikes were scored during testing. No side bias for 'robbing' strikes was indicated (12 frogs: 18 leftward strikes, 20 rightward strikes; two-tailed G test: $G_{11,\mathrm{adj}} = 4.84$, $P = 0.94$). With the inclusion of the 'robbing' strikes as agonistic behaviour, left-hemifield bias was reduced in the total of 87 strikes but still biased significantly to the left side (from 14 frogs, mean percentage left ± SEM was 65.0 ± 5.0; two-tailed G test: $G_{13,\mathrm{adj}} = 24.36$, $P = 0.028$).

Table 1. Agonistic striking in *L. caerulea*

		Attacker's hemifield					
	N	Left	Right	*df*	*G*	*P*	% Left ($\bar{X}$ ± SEM)
Total strikes	49	36	13	12	28.42	0.005	82.8±6.3
Sideways strikes	16	12	4	7	13.80	0.054	81.3±13.2
Midline strikes	33	24	9	11	21.25	0.031	80.7±9.0
'Head' strikes	32	22	10	8	14.85	0.062	78.8±7.6
'Body' strikes	17	14	3	8	14.41	0.072	87.0±8.7

Total scores observed from 13 of 15 frogs are presented, with the data for individual scores analysed using Williams-corrected G tests (two-tailed). Sideways and midline strikes have been combined in 'head' and 'body' strikes.

All 15 frogs fed on mealworms during testing (mean number of mealworms eaten ± SEM = 10.1 ± 1.8). The mean number of successful plus unsuccessful predatory strikes (mealworms eaten and missed ± SEM) was 13.2 ± 2.5. For the total scores of predatory strikes there was no significant bias to either the left or right hemifield of the frogs (Table 2). Midline and sideways predatory strikes analysed separately revealed no lateralization, and neither successful nor unsuccessful strikes, in terms of gaining mealworm prey, were lateralized. A consistently weak (and nonlateralized) tendency to favour the right visual hemifield was observed across all subcategories of predatory strike behaviour (Table 2).

There was a significant difference between the degree of lateralization expressed for agonistic strikes and that for predatory strikes (mean difference = 35.0% left-hemifield preference; Student's *t* test: $t_{12} = 3.18$, $P = 0.008$). Although there was a significant correlation between the incidence of agonistic strikes and the incidence of predatory strikes (linear regression: $F_{1,11} = 19.71$, $R^2 = 0.61$, $P = 0.001$), no significant correlation was found between the degree of lateralization for agonistic strikes and that for predatory strikes ($F_{1,11} = -2.04$, $R^2 = 0.21$, $P = 0.066$). Hence, for this study the degree of lateralization expressed for agonistic strikes was apparently independent of the lateralization for predatory strike behaviour.

Unpaired *t* tests confirmed the homogeneity of responsiveness between the frogs of both housing tanks, since no significant differences were found between the two groups in terms of (1) the number of agonistic strikes observed (mean difference = −2.64 agonistic strikes; $t_{13} = -1.36$, $P = 0.20$) and their degree of lateralization (mean difference = −16.50% left-hemifield preference; $t_{11} = -1.32$, $P = 0.21$), and (2) the number of predatory strikes (mean difference = −6.80% predatory strikes; $t_{13} = -1.40$, $P = 0.19$) and their degree of lateralization (mean difference = 14.87% left-hemifield preference; $t_{13} = 1.18$, $P = 0.26$).

Lateralized Motor Responses

Kolmogorov–Smirnov one-sample goodness-of-fit tests revealed that the %R distributions for side turned towards when the frog moved from the vertical position and leading forelimb were not significantly different from expected normal distributions (side turned towards: Z=0.14, $\chi^2_2 = 0.57$, $P > 0.99$; leading forelimb: Z=0.21, $\chi^2_2 = 1.29$, $P > 0.99$). For preferred side when turning, two frogs had a left-side preference and five frogs had a right-side preference to provide a marginal although significant group preference to turn to the right (mean %R ± SEM = 52.3 ± 4.7; *G* test: $G_{13} = 29.52$, $P < 0.01$; Fig. 2a). A moderate and significant preference at the group level was found for the right forelimb to be used as the leading limb, with no frogs showing a preference to use the left forelimb and six frogs showing right forelimb preferences (mean %R ± SEM = 57.1 ± 3.2; *G* test: $G_{13} = 46.40$, $P < 0.001$; Fig. 2b). Significantly, fewer repositionings were made with the right forelimb when leading the turn to the right side ($N = 127$) than were made with the left forelimb when leading to the left side ($N = 139$; one-way ANOVA: $F_{1,264} = 27.45$, $P < 0.0001$; Fig. 3a).

Perch angle orientations within the lower sectors either side of the vertical were rare (<5%) and we combined the data with data for the adjacent sectors (i.e. sector 5 data were combined with sector 4 data; Fig. 3b). No sector was preferred to its matching sector on the opposite side of the climbing tube. The mean overall preferred angle was inclined to the right side of the vertical (mean ± SEM = 353.3 ± 6.1°, $R_{\text{total}} = 0.39$). Mean angles of perch orientation to either side of the climbing tube were not significantly different ($\overline{X} \pm \text{SEM} = 61.3 \pm 3.07°$ left, 63.3 ± 2.85° right; Williams–Watson *F* test: $R_{\text{left}} = 105.6$, $R_{\text{right}} = 116.8$, $F_{1,264} = 0.21$, $P = 0.64$).

DISCUSSION

Visual Lateralization

The preference to direct agonistic strikes at frogs viewed in the left visual hemifield (LVH) in 80% of observations corresponds to the same direction of lateralization found in the toad species *B. bufo* (65% LVH: Vallortigara et al. 1998) and *B. marinus* (60% LVH: Vallortigara et al. 1998) and also in other vertebrate species (lizards Anolis spp.: Deckel 1995; Hews & Worthington 2001; Hews et al. 2004; chicks, *Gallus gallus domesticus*: Rogers & Anson 1979; Rogers 1991; Vallortigara et al. 2001; gelada baboons, *Theropithecus gelada*: Casperd & Dunbar 1996). A consistent pattern to use preferentially the LVH for directing agonistic responses was observed across the

Table 2. Predatory striking in *L. caerulea*

		Predator's hemifield					
	N	Left	Right	*df*	*G*	*P*	% Left ($\overline{X}$ ± SEM)
Total strikes	198	95	103	14	12.52	0.56	45.4±6.4
Sideways strikes	75	37	38	14	6.90	0.94	44.2±8.3
Midline strikes	123	58	65	13	11.34	0.58	49.9±6.3
Successful	151	75	77	14	5.06	0.99	47.6±6.6
Unsuccessful	46	20	26	9	9.14	0.42	44.6±10.2

Total scores observed from 15 frogs are presented, with the data for individual scores analysed with Williams-corrected *G* tests (two-tailed). Sideways and midline strikes have been combined in 'head' and 'body' strikes.

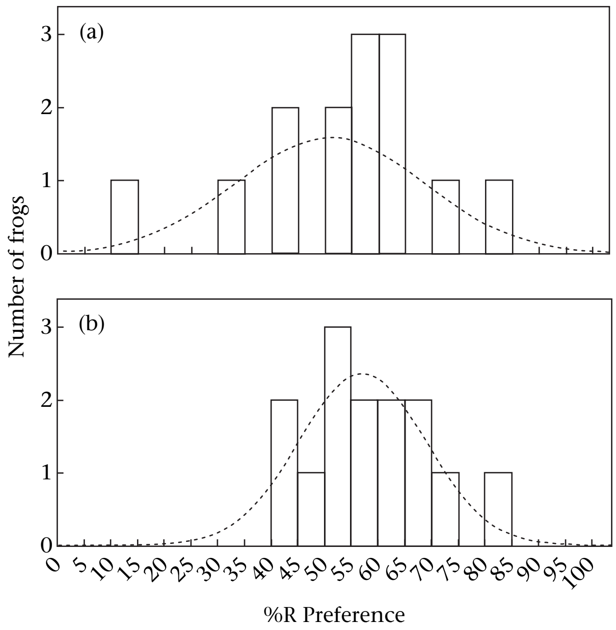

Figure 2. Behavioural preferences in frogs climbing to a righted position after being inverted in the vertical plane. (a) Turning direction preference when climbing to the righted position, expressed as % right-side preference (%R). The normal curve calculated from the data is superimposed on the histogram, with a group mean of 52.3%R. (b) Leading forelimb preference when climbing to the righted position, expressed as % right-forelimb preference (%R) with superimposed normal curve. The group mean is 57.1%R.

subcategories of sideways, midline, head and body strikes. The tendency reached significance for midline agonistic strikes only, indicating that greater sample sizes are required before further conclusions can be drawn.

Although a nonsignificant tendency to direct predatory strikes within the right visual hemifield was found for *L. caerulea*, this tendency was none the less consistent with the right visual hemifield lateralization for predatory responses found in *B. bufo*, *B. viridis* and *B. marinus* when tested individually with automated prey stimuli (Vallortigara et al. 1998; Robins & Rogers 2004). The tendency to prefer the right visual hemifield for predatory responses in the anuran species corresponds with the right-eye system lateralization for feeding responses found in lizards (*C. ornatus* tested in groups: Robins et al. 2005) and in various bird species (reviewed in Andrew & Rogers 2002; Vallortigara & Rogers 2005). Together, the data from the visual experiment support findings of complementary specialization of the vertebrate brain hemispheres for controlling agonistic (i.e. right-hemisphere) and feeding responses (i.e. left-hemisphere).

An appreciably higher degree of lateralization of agonistic behaviour was found in *L. caerulea* than reported previously in the bufonid species. There are two possible reasons why this may be so. First, the frogs used in this study had been captive bred and thus the stresses of captive housing and possibly crowding may have predisposed the individuals to aggressive behaviour, as found in captive crocodiles (Warwick 1990), and possibly this also accentuated the degree of laterality. Second, *L. caerulea* possesses strong site fidelity and possibly territoriality associated with seasonal breeding in natural contexts (Cogger 1992; Tyler 1999). This is in marked contrast to the bufonids, which tend to be opportunistic breeders without well-defined home ranges. Thus, the laterality expressed in agonistic responses during feeding periods here may be linked to other forms of laterality such as spatial awareness, which is unreported in anurans but is a known left-eye/right-brain hemisphere specialization found in other vertebrates (e.g. chicks tested monocularly perform better when using the left eye than the right eye to locate concealed food with the aid of spatial cues; Tommasi & Vallortigara 2001).

The total proportion of midline agonistic strikes in *L. caerulea* (63%) was higher than that found in *B. marinus* (26% Robins et al. 1998). This may undoubtedly be related

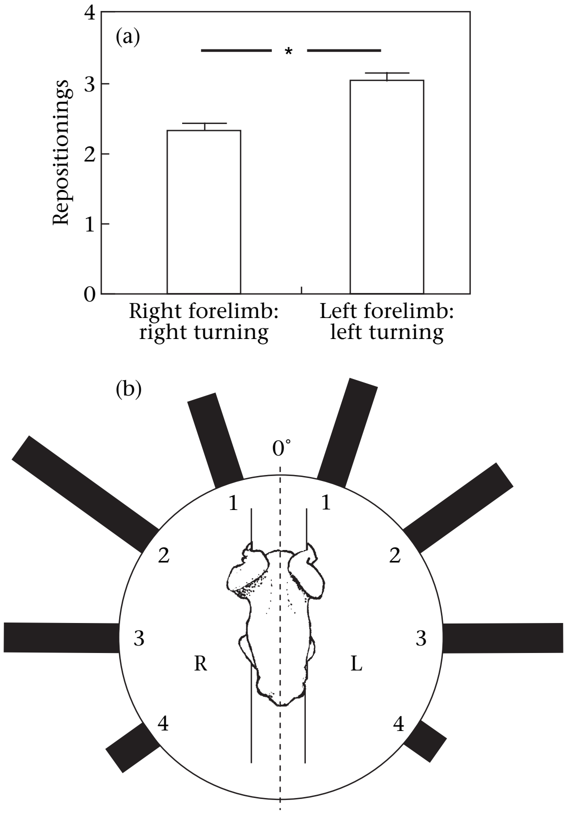

Figure 3. Patterns of turning behaviour in climbing tree frogs. (a) Mean ± SEM number of times forelimbs were repositioned when leading the climb to the righted position (right forelimbs repositioned when turning to the right side, left forelimbs repositioned when turning to the left side). One-way ANOVA, two-tailed: $*P < 0.0001$, Fisher's protected least-significant differences (b) Circular histogram showing the distribution of perch orientations in frogs righting from the vertical axis. The orientation of the frogs on the vertical climbing tube immediately after being inverted is presented in the centre illustration. Perch orientation data are shown grouped in 36° sectors on either side of the vertical axis (0°). There was no significant difference in the number of perch orientations within sectors matched on the right (R) and left (L) sides (chi-square tests with Bonferroni's correction: sector 1: $\chi^2_1 = 0.37$; sector 2: $\chi^2_1 = 2.42$; sector 3: $\chi^2_1 = 0.05$; sector 4: $\chi^2_1 = 0.62$; $P > 0.1$ for all comparisons).

to the relatively inflexible and short tongue of *L. caerulea*, tending to restrict frogs to lunge straight ahead with little sideways deflection to direct an agonistic strike with an opened mouth. In other words, the deflections from the midline of attacking *L. caerulea* were less frequent than those found in *B. marinus*, a species that can direct its tongue at various angles from the midline (Peters & Nishikawa 1999). The tendency for *L. caerulea* to direct agonistic strikes at the body and also head of other frogs viewed within the attacker's LVH was in marked contrast to *B. marinus*, for which the eyes of conspecifics were the selected targets of lateralized agonistic strikes (Robins et al. 1998).

No comparable behaviour to the robbing strikes observed in *L. caerulea* has been seen in the bufonid species tested. The incidence of robbing strikes may indicate that under circumstances of high arousal, the frogs differentiate poorly between visual cues associated with 'prey' and those of 'conspecific'. Despite the behavioural differences in the anurans, *L. caerulea*, none the less, possessed a strong left-hemifield specialization for directing agonistic behaviour, which is a lateralization corresponding with that found in other vertebrate groups.

The lack of evidence of right-eye visual specialization for predatory responses in *L. caerulea* is in contrast with results on bufonids. While there are important differences in methodology (i.e. the bufonids were tested individually with a single, automated prey stimulus: Vallortigara et al. 1998), our finding none the less also differs from the strong right-eye specialization found in group-tested lizards *C. ornatus* for responses to freely moving crickets (Robins et al. 2005). One important difference between *L. caerulea* and the bufonid species tested is that the frogs are sit- and-wait 'ambush' predators, unlike the actively foraging bufonids. Thus, the frogs may be more opportunistic in their sidedness for prey catching, in contrast to the bufonids, which may be using a lateralized search image strategy for feeding. An alternative possibility is presented by the difference in prey used: for example, insect-like stimuli reveal lateralized predatory responses in *B. marinus*, whereas predatory responses are not lateralized for simple 'wormlike' stimuli lacking obvious body segmentation and legs (Robins & Rogers 2004). Thus, it is possible that insect prey may similarly reveal lateralized predatory responses in *L. caerulea*.

Motor Lateralization

Lateralization for the right forelimb was present in *L. caerulea* in a form of righting response that involved climbing. The right-limb lateralization was shown in two ways: (1) as the leading forelimb when initiating the righting response after the body had been inverted and (2) fewer forelimb repositionings made with the right forelimb when turning the body to the right than repositionings made with the left forelimb when turning the body to the left. The right-forelimb lateralization found in *L. caerulea* was in the same direction as that found in *B. marinus*, albeit for a different righting task (Bisazza et al. 1996, 1997; Robins & Rogers 2002). Despite some obvious differences in methodology, necessitated by the small size of the frogs and their adhesive toe pads, right-limb preferences are shown in both species for tasks requiring the limbs to grasp or push forcefully against a surface. The most parsimonious explanation linking the responses found in both species is that when physical strength is required, the right and not left forelimb is used preferentially for the task.

The type of lateralization for the use of the right forelimb in *L. caerulea* for climbing was similar to that found in *B. marinus* for the aquatic-righting test. However, there was weak support for lateralization of forelimb use in *L. caerulea* when righting from the vertical, with right-forelimb preference found for leading the process of rotating the body to a new position of support. This lateralization was found despite a comparatively weaker directional preference for turning to the right side of the body. We infer the relative strength of the right forelimb from the result that, although no preferential perch (righted) orientation or angle was found on either side of the frog's body, the right forelimb was repositioned on significantly fewer occasions to achieve the righted position.

The forelimb preference in *L. caerulea* for righting from the vertical suggests that right-forelimb preferences for tasks requiring strength may be widespread throughout the anuran subclass, as a similar preference was also found in *B. marinus* (Bisazza et al. 1996; Robins & Rogers 2002). This result differs markedly from the left-hindlimb preference determined in anurans when righting from the horizontal (Robins et al. 1998), without the availability of a support for the forelimbs to clasp when assisting the rotation of the body. Hindlimb preferences have been studied in *L. caerulea* in righting experiments in which individual frogs were overturned on to a horizontal surface (Rogers 2002; Malashichev, In press). Although the hindlimbs are used for pushing forcibly against a horizontal surface, the preference for the left and not right hindlimb suggests a different mechanism for the postural reflex and is not yet explained.

General Considerations

Despite the procedural differences for testing visual and motor responses in *L. caerulea* reported here, compared to similar studies in other anuran species, the results support growing evidence that functional specializations are widespread and well conserved throughout the Anura and across the vertebrate kingdom.

The similarity in the degree of lateralized visual and motor responses in *L. caerulea* and the bufonids is remarkable, as they possess different derived morphological specializations reflecting their divergent evolution. That is, bufonids possess the inertial tongue mechanism that *L. caerulea* does not, and *L. caerulea* possess expanded toe pads for climbing which is absent in the terrestrial bufonids. Thus, despite some species differences observed for agonistic responses, the results are consistent within the anuran subclass, and correspond with similar forms of lateralization found across the tetrapod family.

The preference for the right forelimb in *L. caerulea* was associated with climbing, a task requiring significant postural demand. The finding suggests that a preference for the right limb first appeared in early terrestrial anurans for functions requiring strength and was conserved in arboreal and comparatively more ambidextrous anurans (Gray et al. 1997). The possibility is then raised that strength-related lateralization for forelimb use, as hypothesized for arboreal primates by MacNeilage (MacNeilage et al. 1987; MacNeilage 1991), emerged well before the evolution of mammals. Indeed, as righting responses are initiated at the level of the vertebrate brainstem (Klemm 1977), strength-related lateralization for forelimb use may predate the evolution of motor and visuomotor lateralizations found in vertebrates with a cortex or neocortex.

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