

Motor and postural asymmetries in marsupials: Forelimb preferences in the red-necked wallaby (*Macropus rufogriseus*)



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ABSTRACT

In the last decades, several studies on mammal motor lateralization have been carried out. However, data on marsupials are still underrepresented in the literature, despite their importance in tracing the evolution of motor laterality and its functional value. This study aimed at investigating motor lateralization in a sample of captive red-necked wallabies (*Macropus rufogriseus*), considering different daily unimanual activities and forelimb implication in postural support. Data on forelimb preference for food reaching, pulling food out of the mouth, self-scratching and starting locomotion from quadrupedal posture were collected; furthermore, to investigate the role of posture in determining the forelimb laterality of wallabies, data on forelimb use for postural support in tripodal stance were recorded. Our results revealed significant group-level left forelimb preferences for self-scratching and starting locomotion, as well as for providing postural support in tripodal stance. These results are discussed in the light of theories for a right hemisphere dominance for emotion processing and for postural influences on forelimb dominance throughout evolution. The reported left biases in forelimb use for different behaviours are in agreement with previous literature on macropods.

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Motor lateralization has been widely reported in both vertebrate (Vallortigara et al., 1999; Vallortigara et al., 2011; Rogers and Vallortigara, 2015) and invertebrate taxa (Frasnelli et al., 2012; Versace and Vallortigara, 2015). In particular, limb preferences in vertebrates seem to characterize a large amount of species, including amphibians, reptiles, birds and mammals (Rogers et al., 2013), suggesting an early evolution of these asymmetries. To trace the phylogeny of motor lateralization, an increasing number of taxa and species should be studied; in addition, several factors presumably involved in shaping motor asymmetries should be considered. In the last decades several mammalian species have been included in laterality studies, especially non-human primates. Although some research on marsupial lateralization has been carried out (Giljov et al., 2012a,b, 2013, 2015) data on these species are still

underrepresented in the literature, despite their importance in tracing the evolution of motor laterality and its functional value (e.g. Vallortigara, 2015).

Previous studies on marsupials have been focusing on both sensory and motor lateralization. In one study, stripe-faced dunnarts (*Sminthopsis macroura*) have been found to be more reactive to predators perceived in the left than in the right visual field (Lippolis et al., 2005). Regarding forelimb lateralization, a forelimb preference on different unimanual actions (feeding on inanimate food, catching insects, nest material collection and body postural support) have been found at both the group and individual-level in brush-tailed bettongs (*Bettongia penicillata*), with a bias toward the left side (Giljov et al., 2012a). Similar results emerged in the grey short-tailed opossum (*Monodelphis domestica*), in which a group-level left forelimb preference in the skilled reaching of food was reported (Ivanco et al., 1996). On the other hand, in the brush-tailed possum (*Trichosurus vulpecola*) individual but not group-level forepaw preference on reaching for food into a tube was found (Megirian et al., 1977).

According to the Postural Origin Theory, ancestral primate models were characterized by a right hand preference for postural support and a left hand specialization for catching live prey, as an adaptation to their arboreal life-style. Along with the evolution of more terrestrial primate species, such as Old World monkeys and

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great apes, the right hand becomes free from postural duties and specializes in manipulation and precise movements (MacNeilage et al., 1987; MacNeilage, 2007).

One of the main hypotheses trying to explain the emergence of human handedness suggests that this asymmetry could be linked to bipedalism; indeed, in human and non-human primates the upright posture would facilitate object manipulation and manual laterality (Corbetta, 2003). In addition, several studies on non-human primates reported a stronger manual laterality related to upright posture in prosimians (Ward, 1995; Ward et al., 1993), monkeys (Westergaard et al., 1998; Hashimoto et al., 2013) and apes (De Vleeschouwer et al., 1995). More bipedal primates have been found to show a greater manual laterality than more quadrupedal species in both prosimians (Ward et al., 1993; Ward, 1995; Dodson et al., 1992) and great apes (Hopkins, 1993; Hopkins et al., 2003; Hopkins, 2006); therefore, species-specific postural habits and constraints seem to be important in the determination of handedness (Giljov et al., 2012a). Similar results have been reported among marsupials: during feeding, bipedal hopper species which are more strictly reliant on bipedal posture have a higher percentage of lateralized individuals than species depending more on quadrupedal locomotion (Giljov et al., 2012a). In particular, bettongs and wallabies are the most bipedal among marsupials involved in limb laterality research; in these species, a group-level left forelimb preference for unimanual activities was found. On the other hand, in the obligatory quadrupedal opossums (Megirian et al., 1977) and gliders (Giljov et al., 2013), individual but not group-level paw preferences were reported. In addition, at the individual-level, a higher percentage of subjects have been found to be lateralized in forelimb preference in bettongs and wallabies than in gliders and opossums (Giljov et al., 2012a).

In general, in early vertebrates such as anuran amphibians, the lateralization in different activities has been found to be related to the species locomotion habits; in particular, motor lateralization seems to be more pronounced in species with alternating-limb locomotion (Malashichev, 2006); therefore, studying limb lateralization in *taxa* with various locomotion patterns could be important to investigate the evolution of lateralized behaviours.

Studies in marsupials, especially species moving by bipedal hopping, are appealing for several reasons. First, placentals and marsupials are an example of parallel evolution; thus, data on motor laterality in both groups are extremely important to investigate the phylogenetic origin of manual laterality and its adaptive value (Giljov et al., 2013); however, little research has been focusing on marsupial motor lateralization. Second, as well as humans, marsupial bipedal hoppers have various adaptations to a stable bipedal stance (Armati et al., 2006); therefore, investigating patterns of paw preference in these species could be optimal to investigate the role of bipedalism in the evolution of the first bipeds (Giljov et al., 2012a,b; Giljov et al., 2013). Third, marsupial species occupy a multiplicity of ecological niches that have resulted in specific sensory, postural or locomotor habits that may or may not lead themselves to the appearance of population or individual-level manual laterality (Giljov et al., 2012b). Finally, studies on kangaroos and wallabies are particularly suitable in the field of manual laterality due to their ability to adopt both quadrupedal and bipedal postures (Giljov et al., 2012b).

According to the laterality-valence hypothesis, in humans the right hemisphere processes and controls negative emotions and withdrawal, whereas the left hemisphere deals with positive emotions and approach (Davidson, 1995). On the other hand, other authors suggest that the right hemisphere processes both positive and negative emotions. In general, the right hemisphere is supposed to play an important role in emotions (Campbell, 1982; Borod et al., 1998; Rogers, 2010) as evidenced from studies on rats (Denenberg and Yutzy, 1985), chicks (Rogers and Andrew,

2002), toads (Lippolis et al., 2002) and fish (Cantalupo et al., 1995); in these species, right hemisphere asymmetries in emotional responses have been found. Furthermore, stressed animals are hypothesised to rely mainly on the right hemisphere, which should therefore have a dominant role in stress responses (Rogers, 2010). Self-directed behaviours, such as self-scratching and self-touching, seem to be related to stress, anxiety and aggressive contexts, and should therefore imply an emotional involvement in both social and non-social situations (Maestripieri et al., 1992; Leavens et al., 2001). Mediation of these behaviours should therefore be under control of the right hemisphere. Nowadays, studies on laterality in self-directed behaviours revealed a left-hand preference for self-directed behaviours in human (Forrester et al., 2014) and non-human primates, particularly great apes (Wagner et al., 2015); however, only few studies were carried out in other animal species. A study of the forelimb preference in the red-necked wallaby revealed a group-level left forelimb bias in self-scratching of the snout (Giljov et al., 2012b), adding evidence that emotions could be important in the determination of behavioural lateralization. Data on self-directed behaviours, in particular scratching, were collected in our study; according to previous literature on the right-hemisphere involvement in emotional responses and on laterality in self-directed behaviours, we could expect a left bias in the forelimb preference for this behavioural category.

Regarding macropod marsupials, forelimb preferences in a variety of behaviours in wild Eastern grey kangaroos (*Macropus giganteus*), red kangaroos (*Macropus rufus*) and Goodfellow's tree kangaroos (*Dendrolagus goodfellowi*) have been investigated (Giljov et al., 2015). With the exception of *D. goodfellowi*, in which only individual-level paw preferences were found, all other kangaroo species displayed a significant group-level bias toward the left forelimb on feeding from both bipedal and quadrupedal position and also while sustaining the body in tripedal stance and self-grooming (Giljov et al., 2015).

Regarding the red-necked wallaby (*Macropus rufogriseus*), to our knowledge, only one study has taken into consideration the forelimb laterality in this species. In particular, Giljov et al. (2012b) investigated individual and population level forelimb preferences in 27 red-necked wallabies hosted in different zoos. They focused on different daily unimanual activities and considered the effect of posture in the determination of limb use asymmetries. First, significant forepaw preferences for feeding from bipedal but not from quadrupedal position were found at both group and individual-level, with a bias toward the left forepaw; the same result emerged for self-grooming directed to the snout. Furthermore, contrary to findings reported in kangaroo species, a right group-level forepaw preference for sustaining the body in tripedal stance was found. The study seemed to underline an important role of posture in the determination of forelimb lateralization, suggesting noteworthy similarities between the patterns of limb lateralization in marsupials and non-human primates (Giljov et al., 2012b). In particular, the red-necked wallaby manual laterality reported in this study seems to support the Postural Origin Theory (MacNeilage et al., 1987; MacNeilage, 2007).

The main aim of the present study was to investigate group and individual-level forelimb preferences on different spontaneous unimanual activities in a colony of red-necked wallabies. Furthermore, in the light of previous findings on this species and marsupials in general, forelimb preference in posture related activities has been also considered. In addition, basing on previous reports of a right-hemisphere implication in emotional processing, forelimb preference for self-directed behaviours has been investigated. According to previous literature, we expected red-necked wallabies to display both group and individual-level left forelimb preferences for at least some unimanual behavioural categories, particularly feeding and self-directed behaviours, together with

a right forelimb preference in posture related behavioural tasks. Despite the low sample size, this study aims at presenting a new forelimb laterality dataset for a species that has been underrepresented in the literature.

1. Methods

1.1. Study subjects and area

The study was carried out from May to July 2008 with a colony of ten adult red-necked wallabies, seven females and three males. All subjects were housed at Parco Natura Viva, a zoological garden in Verona (Italy). The wallabies were housed in a 1750 sqm naturalistic enclosure made of wide grassy areas, plants and trees, rocks, artificial shelters and a water pool. Wallabies were fed twice a day so that food and water were available to the animals for 24 h. The diet was mainly made of fruits, vegetables, hay, fresh grass, pellet and fresh browse (when accessible); fruits and vegetables could be provided to the wallabies in whole form or in large pieces. During the data collection period, salad, apples and other fruits and vegetables were hung around in the enclosure, at the height of the wallabies' head, to induce the use of the forelimbs in bringing the food to the mouth. All wallabies of the study were captive born. Wallabies were not used to interact with zoo-keepers and humans in general (human intervention was kept to the minimum, the keepers entered the enclosure only for cleaning and feeding procedure).

To minimize disturbance to the wallabies, the observer collected data from the visitor path, after a three weeks habituation period.

This study was carried out using naturalistic observations of the wallaby behaviour and non-invasive or stressful techniques were employed. The study procedure was carried out in accordance with the EU Directive 2010/63/EU for animal research.

1.2. Procedure

Data were collected through the live observation of the interaction with food hung around in the enclosure and of the daily routine activities of the wallabies. A continuous focal animal sampling method was used to collect frequencies of forelimb use (Altmann, 1974; Martin and Bateson, 1986); twenty 15-min sessions per subject were carried out; the data collection period lasted for ten days, and two sessions per day were done: one in the morning, one in the afternoon. Both daily sessions were carried out in the periods of highest wallaby activity (Giljov et al., 2012b), immediately after food provision.

To assess individual-level lateralization in forelimb use, a minimum of ten responses per behavioural category per subject have been collected (Meguerditchian and Vauclair, 2009; Meguerditchian et al., 2010).

To investigate forelimb preference on spontaneous unimanual activities, four behavioural categories were examined: reaching for food, pulling food out of the mouth, self-scratching and starting quadrupedal locomotion. Other rarer behaviours have not been included in the study because in these cases some wallabies failed to reach the minimum of ten responses. Reaching for food (food reaching) was intended as bringing hung fruits and vegetables found in the enclosure to the mouth; given that food was hung at the height of the wallaby's head, this behaviour was observed only in bipedal position. Pulling food out of the mouth (food pulling) was intended as grasping the surplus food that comes out the mouth and remove or cut it with the hands. Self-scratching was intended as scratching of any part of the body with the exception of the forelimbs. Finally, for starting quadrupedal locomotion, the forelimb used to initiate moving from a quadrupedal stance was considered

(Morcillo et al., 2006; Hopkins, 2008). Only unimanual activities were included in the analysis.

To investigate postural implications in the determination of wallaby motor lateralization, data on the forelimb involved in postural support when a subject was in tripedal stance were collected (Giljov et al., 2012b, 2015). During the tripedal stance, the hindlimbs and one forelimb were standing on the ground, whereas the other forelimb was lifted off the ground and was not involved in any activity (Giljov et al., 2012b). Data on which forelimb was standing on the ground have been recorded in this study. Events of right and left forelimbs' use have been collected for all these behavioural categories. Data were collected by the same observer through the live observation of the animals in their social context.

1.3. Data analysis

Forelimb lateralization in spontaneous unimanual activities belonging to wallaby behavioural repertoire has been investigated at both group and individual-level, considering the frequencies of forelimb use. Given that Shapiro-Wilk tests revealed that all data (HI) were normally distributed (food reaching: $P=0.892$; food pulling: $P=0.849$; self-scratching: $P=0.138$; starting locomotion: $P=0.376$; tripedal stance: $P=0.435$), parametric tests have been used for data analyses. Individual-level forelimb preferences were investigated using binomial z-scores. This method allows a statistical classification of the subjects as left-handed ($z < -1.96$), right-handed ($z > 1.96$) and ambi-preferent ($-1.96 < z < 1.96$) (Michel et al., 2002). In addition, an Handedness Index (HI) was calculated; the HI was given by the formula $(R - L)/(R + L)$, in which R stands for the frequencies of the right forelimb use and L stands for the frequencies of the left forelimb use. The HI ranges from -1 to 1 : negative values indicate a bias towards the left side, whereas positive values indicate a bias towards the right side. The strength of the forelimb preference was assessed and compared between different behavioural categories using the absolute values of the HI (ABS-HI) (Hopkins, 1999, 2013).

Group-level forelimb preference for each behavioural category was assessed performing a one-sample *t*-test with the Handedness Index serving as dependent measure, to assess the symmetry of the forelimb use distribution. To verify whether differences between forelimb use in different behavioural categories were present, an ANOVA with Tukey's HSD (Honest Significant Difference) *post hoc* test was run on both the HI and the ABS-HI. Forelimb preference consistency between different behavioural categories was assessed at both the individual and group-level; in the latter case, pairwise Pearson correlations with all behavioural categories were used (Spinozzi and Truppa, 2007; Lilak and Phillips, 2008); a Bonferroni corrected $P < 0.05/6$ or 0.008 for multiple correlations was used as threshold of significance. All tests were two-tailed. The significance level was set at $P < 0.05$.

2. Results

2.1. Group and individual-level forelimb preferences

The mean HI (\pm SE) was -0.19 ± 0.10 for food reaching, -0.16 ± 0.11 for food pulling, -0.15 ± 0.06 for self-scratching and -0.25 ± 0.08 for starting locomotion with the forelimbs. According to one-sample *t*-tests, no biases in the distributions of the HI were found for food reaching ($t(9) = -1.89$, $P = 0.092$) and pulling ($t(9) = -1.49$, $P = 0.169$), whereas a significant group-level preference for the left forelimb was found for self-scratching ($t(9) = -2.61$, $P = 0.028$) and starting locomotion ($t(9) = -3.23$, $P = 0.010$) (Fig. 1).

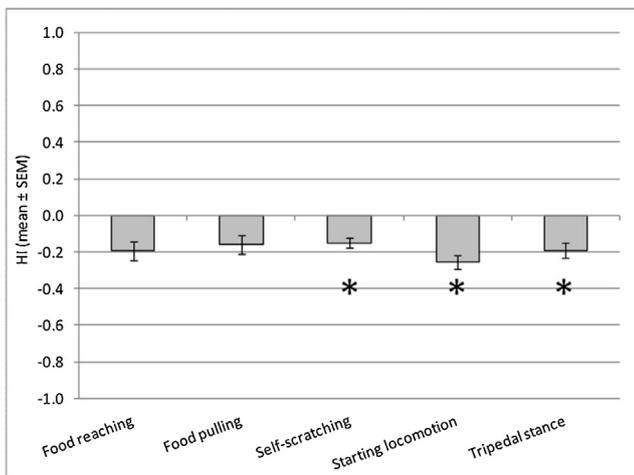


Fig. 1. Forelimb preference (mean HI \pm SEM) in different behavioural categories. Bars stand for standard error of the mean (SEM). Asterisks indicate behavioural categories in which a significant group-level bias was reported (one-sample *t*-test: $P < 0.05$).

Regarding posture, the mean HI (\pm SE) for tripedal stance was -0.19 ± 0.08 . A significant group-level left forelimb preference was apparent ($t(9) = -2.53$, $P = 0.032$) (Fig. 1).

Considering the strength of the forelimb preference, the mean ABS-HI (\pm SE) was 0.29 ± 0.07 for both food reaching and pulling, 0.16 ± 0.05 for self-scratching, 0.31 ± 0.05 for starting locomotion and 0.25 ± 0.05 for tripedal stance.

At the individual-level, the *z*-score analysis revealed that for food reaching, two out of ten wallabies were significantly lateralized and both were left-preferent. For food pulling, five out of ten wallabies showed a significant lateralization; in particular, three of them were left-preferent and two were right-preferent. For self-scratching, only one out of ten subject was significantly lateralized and was left-preferent. For starting locomotion with one forelimb, three out of ten subjects showed a significant lateralization and were all left-preferent.

Regarding posture, for tripedal stance five out of ten subjects were significantly lateralized; four were left-preferent and one was right-preferent (Table 1).

2.2. Hand preference in different behavioural categories

According to a one-way ANOVA, no significant differences between different behavioural categories of the study (food reaching, food pulling, self-scratching and starting locomotion; tripedal stance was not included in this analysis) were found considering neither the HI (ANOVA: $F(3) = 0.29$, $P = 0.833$) (Fig. 1) nor the ABS-HI (ANOVA: $F(3) = 1.296$, $P = 0.291$). Pair-wise comparisons using Tukey HSD *post hoc* test revealed no significant differences between any behavioural category (all $P > 0.8$).

To test consistency of forelimb preference across different behavioural categories (food reaching, food pulling, self-scratching and starting locomotion; tripedal stance was not included in this analysis), Pearson correlations with Bonferroni correction ($P < 0.008$) between all possible pairs of actions were performed. A significant positive correlation was found between food reaching and food pulling (Pearson corr.: $R(10) = 0.86$, $P = 0.002$) and between food reaching and starting locomotion (Pearson corr.: $R(10) = 0.79$, $P = 0.006$) but not in all the other pairs of behavioural categories (see Table 2). Furthermore, to assess the relation between forelimb preference for postural support and other daily activities, the HI for tripedal stance were correlated with the other behavioural categories: food reaching, food pulling, self-scratching and starting

locomotion. No significant correlation between tripedal stance and any other category was found (Table 2).

At the individual-level, four out of ten wallabies showed a consistent forelimb preference across different behavioural categories (food reaching, food pulling, self-scratching and starting locomotion; tripedal stance was not included in this analysis) and all subjects were biased toward the left side (negative HI values) (Table 1). Regarding posture, the HI for tripedal stance were compared with the HI for all other behavioural categories. The higher consistency was found between tripedal stance and self-scratching, in which eight out of ten subjects showed a consistent forelimb preference; in particular, seven were biased toward the left side, one toward the right. Seven out of ten wallabies showed consistency between tripedal stance and food pulling (six were biased toward the left side, one toward the right); six out of ten subjects showed consistency between tripedal stance and starting locomotion (all were biased toward the left side); five out of ten subjects showed consistency between tripedal stance and food reaching (all were biased toward the left side) (Table 1).

3. Discussion & conclusion

First, significant group and individual-level forelimb preferences were found, suggesting a left bias for daily spontaneous activities such as self-directed behaviours and starting quadrupedal locomotion. Second, wallabies displayed a group-level left forelimb preference for postural support during tripedal stance, underlining a role of posture in the determination of limb lateralization. Third, no differences in both direction and strength of the forelimb preferences between different behavioural categories were observed. Finally, consistency of forelimb preference was apparent at both group- and individual-level, at least for some measures.

The finding of a group-level left forelimb preference for self-scratching is in agreement with previous findings on self-directed behaviours in the red-necked wallaby (Giljov et al., 2012b) as well as in primate species, such as great apes and humans (Diamond and Harries, 1984; Rogers and Kaplan, 1996). Self-directed behaviours have been related to contexts implying an emotive involvement, such as anxious and stressful contexts (Maestripieri et al., 1992; Leavens et al., 2001; Rogers, 2010) and, as postulated by the laterality-valence hypothesis, to the right hemisphere (Davidson, 1995). Moreover, other theories link both positive and negative emotions to the right side of the brain (Campbell, 1982; Borod et al., 1998; Rogers, 2010); thus, our results seem to provide further evidence of a right-hemisphere control in emotional situations. Furthermore, this finding seems to highlight similarities between the right-hemisphere implication in the control of emotions in both marsupials and placental mammals. However, future studies with a greater sample of marsupials are needed to further evaluate the relationship between the right hemisphere involvement in the control of emotion and forelimb laterality.

In addition, a significant group-level left forelimb preference for starting locomotion was found in our sample of wallabies. Previous studies on non-human primates reported a group-level right hand preference for starting quadrupedal locomotion (Hopkins and de Waal, 1995; Morcillo et al., 2006; Harrison and Nystrom, 2010) and a bias toward the left forelimb for descending locomotion in great apes. This latter finding highlights that great apes, in particular chimpanzees, lead with the left arm, placing most of the body weight on it (Hopkins, 2008); therefore, the left side of the body has been hypothesized to be involved in postural support, as postulated by the Postural Origin Theory (MacNeilage et al., 1987; MacNeilage, 2007; Hopkins, 2008). The finding of a left forelimb preference for both starting quadrupedal locomotion and tripedal stance in our study does not allow to draw similar conclusions

Table 1

Individual-level forelimb preference in different behavioural categories. For each subject and for each behavioural category the table reports the sex (F = female, M = male), the number of events with the right (R) and left (L) forelimb, the z-score from the Binomial test (z) and the Handedness Index (HI). Asterisks indicate subjects with a significant forelimb preference (left-preferent: $z < -1.96$; right-preferent: $z > 1.96$; $P < 0.05$).

Subject	Sex	Actions												Postural support							
		Food reaching				Food pulling				Self-scratching				Starting locomotion				Tripedal stance			
		R	L	z	HI	R	L	z	HI	R	L	z	HI	R	L	z	HI	R	L	z	HI
Susi	F	40	30	1.08	0.14	113	77	2.54*	0.19	3	8	-1.21	-0.45	18	36	-2.31*	-0.33	21	51	-3.42*	-0.42
Cla	F	15	7	1.49	0.36	102	75	1.95	0.15	16	18	-0.17	-0.06	23	16	0.96	0.18	17	25	-1.08	-0.19
Dane	M	8	8	0.00	0	20	24	-0.45	-0.09	10	23	-2.09*	-0.39	23	18	0.62	0.12	29	41	-1.31	-0.17
Tanta	F	13	20	-1.04	-0.21	45	60	-1.37	-0.14	25	22	0.29	0.06	9	15	-1.02	-0.25	55	32	2.36*	0.26
Fede	M	5	8	-0.55	-0.23	15	29	-1.96*	-0.32	24	25	0.00	-0.02	31	48	-1.80	-0.22	53	115	-4.71*	-0.37
Ire	F	12	31	-2.74*	-0.44	46	138	-6.71*	-0.5	20	28	-1.01	-0.17	12	37	-3.43*	-0.51	30	33	-0.25	-0.05
Chichiño	M	5	9	-0.80	-0.29	15	19	-0.51	-0.12	20	20	0.00	0	11	18	-1.11	-0.24	22	57	-3.83*	-0.44
Lisa	F	9	17	0.80	-0.31	28	48	-2.18*	-0.26	15	16	0.00	-0.03	8	14	-1.07	-0.27	20	25	-0.60	-0.11
Bibi	F	7	9	-0.25	-0.13	42	22	2.38*	0.31	20	24	-0.45	-0.09	7	16	-1.67	-0.39	20	18	0.16	0.05
Vale	F	3	34	-4.93*	-0.84	8	71	-6.98*	-0.8	4	8	-0.87	-0.33	8	34	-3.86*	-0.62	38	104	-5.45*	-0.46

Table 2

Consistency of forelimb preference between different behavioural categories. For each pair of behavioural categories the table reports the R coefficient (R) and the P value (P) from Pearson correlations between the HI scores. Significance level after Bonferroni correction for multiple correlations was set at $P < 0.05/6$ or $P < 0.008$. In the last row, the R and P from Pearson correlations between tripedal stance and all other behavioural categories are reported. Asterisks indicate significant correlation between categories.

MEASURE	Food reaching	Food pulling	Self-scratching	Starting locomotion
Food reaching	-	$R = 0.86$ $P = 0.002^*$	$R = -0.02$ $P = 0.967$	$R = 0.79$ $P = 0.006^*$
Food pulling	-	-	$R = 0.06$ $P = 0.864$	$R = 0.52$ $P = 0.123$
Self-scratching	-	-	-	$R = 0.10$ $P = 0.782$
* Bonferroni correction: $P < 0.008$				
Tripedal stance	$R = 0.14$ $P = 0.704$	$R = 0.25$ $P = 0.486$	$R = 0.44$ $P = 0.207$	$R = 0.09$ $P = 0.811$

for the red-necked wallabies. The reported differences in leading forelimb preference for locomotion between non-human primates and marsupials might be presumably due to morphological features; these could involve both the overall body shape and the limb structure, such as the lower centre of gravity given by the enlarged hindlimbs of macropods (Feldhamer et al., 2015).

The finding of a group-level left forelimb preference for two out of three daily spontaneous activities is in agreement with previous literature on macropod forelimb lateralization; indeed, a greater use of the left than the right forelimb for behaviours such as feeding and scratching has been reported (Giljov et al., 2015); therefore, the patterns of forelimb preference found in bipedal hoppers such as macropods seem to provide support to the hypothesis coming from the non-human primate literature on hand preference, suggesting that the upright posture could be correlated to a more pronounced preference for one hand (Sanford et al., 1984; Ward, 1995; Westergaard et al., 1997; Blois-Heulin et al., 2007; Braccini et al., 2010).

Regarding tripedal stance, our results are not in agreement with previous research on red-necked wallabies, reporting a group-level right bias in the forelimb leaning the body in this position. However, a significant group-level left-forelimb preference for support in tripedal stance was reported in other macropods, particularly red and Eastern grey kangaroos (Giljov et al., 2015), and bettongs (Giljov et al., 2012a). All these studies reported a bias toward the left forelimb in different types of behaviours, specifically food-related and supportive tasks; thus, in contrast to primates (Milliken et al., 2005; Hopkins, 2008), macropods would tend to use the same forelimb for different activities. However, our finding on postural support in tripedal stance should be interpreted with caution; indeed, the sample size is small and only 40% of the wallabies dis-

played a significant left forelimb preference for this behavioural category.

No group-level bias for food reaching was reported in our sample of wallabies; however, findings of a forelimb preference in the same direction for starting locomotion, self-scratching and tripedal stance seem to agree with previous research on bipedal hopper macropods. In these species, the lack of functional specialization of the forelimbs might be due to the fact that 1) manipulative abilities might be less manifested and essential than in primates; 2) bipedal hoppers perform manual activities usually without the need of involving both forelimbs simultaneously (Giljov et al., 2012a).

Although the mean HI score for food reaching was leftward in direction, a lack of group-level forelimb preference was found. This finding is not in accordance with previous literature on red and Eastern grey kangaroos (Giljov et al., 2015), reporting a significant group-level left forelimb preference during feeding (in both quadrupedal and bipedal position); regarding the red-necked wallaby, a group-level left forelimb preference was previously found for feeding from bipedal but not quadrupedal posture (Giljov et al., 2012b). Although only feeding from a bipedal position has been considered in our study, no significant bias emerged in food reaching. Even if these results might be due to the small sample size, this finding is in agreement with past studies on brush-tailed possums (*Trichosurus vulpecula*); in this species, no group-level forelimb preference for food reaching into a tube was found (Megirian et al., 1977).

Finally, to better investigate patterns of limb preference in marsupials, the consistency of forelimb preferences across different activities was considered. Our wallaby sample showed consistent forelimb biases for different activities at both the group and individual-level; however, only a few measures were significantly and positively correlated with each other. In particular, a signifi-

cant positive correlation between food reaching and food pulling was found; this consistency could likely be due to the similarities of motor patterns of the two actions, which are both related to feeding behaviour (Lilak and Phillips, 2008). The consistency between food reaching and starting quadrupedal locomotion is more difficult to explain; this finding seems to be in line with previous results of a left-bias in forelimb use on different behavioural categories. Therefore, our results seem to provide further support to the previously mentioned hypothesis of a lack of functional specialization in macropods (Giljov et al., 2012a).

In conclusion, despite the relatively small number of subjects involved in this study, our findings seem to replicate previous research reporting a left-bias in forelimb use for different types of activities in macropods. Bipedal hoppers seem to display a more pronounced group-level bias in forelimb preference than quadrupedal marsupials; these findings suggest an increasing degree of lateralization in more bipedal species and an important role of bipedalism in the determination of handedness (Giljov et al., 2012a), as reported in humans and non-human primates (De Vleeschouwer et al., 1995; Ward et al., 1993; Ward, 1995; Westergaard et al., 1998; Hashimoto et al., 2013). In addition, results of this study add to the growing body of evidence challenging the hypothesis that handedness is a unique trait of primate evolution (Giljov et al., 2015). In fact, it is becoming clear that motor lateralization in limb usage is observed widely among vertebrates and possibly also among invertebrates (Versace and Vallortigara, 2015).

Further research is needed to increase the sample size and to involve a greater number of marsupial species, with different ecological and morphological adaptations. Furthermore, future studies should better investigate the role of posture in the determination of forelimb preferences; the forelimb use for a same action performed in both bipedal and quadrupedal posture should be considered.

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