Asymmetries in hand usage and perception of communication sounds in early primates (*Microcebus* spp.):
Effects of sex and experience

Thesis

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Declaration

I herewith declare that I autonomously carried out the PhD-thesis entitled “Asymmetries in hand usage and perception of communication sounds in early primates (Microcebus spp.): Effects of sex and experience”.

I did not receive any assistance in return for payment by consulting agencies or any other person. No one received any kind of payment for direct or indirect assistance in correlation to the content of the submitted thesis.

I conducted the project at the Institute of Zoology at the University of Veterinary Medicine Hannover.

The thesis has not been submitted elsewhere for an exam, as thesis or for evaluation in a similar context.

I hereby affirm the above statements to be complete and true to the best of my knowledge.

Lisette Leliveld
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Summary

Leliveld, Lisette. *Asymmetries in hand usage and perception of communication sounds in early primates (Microcebus spp.): Effects of sex and experience*

Cerebral lateralization is proposed to have evolved in two steps (Vallortigara and Rogers 2005), first on the individual level (improving brain efficiency), second on a population level (improving the coordination between group members in social interactions). In this thesis, I aimed to investigate, in accordance with the theory of Vallortigara and Rogers (2005), to which extent lateralization in hand use and the processing of communication calls is present on individual and population level in mouse lemurs, representing the most ancestral primate condition. Furthermore, I aimed to explore how variable cerebral lateralization can be on an *inter*-individual and *intra*-individual level. Therefore, I focused on *inter*-individual differences, such as sex, age and inheritance, and on *intra*-individual variations, caused by different processing mechanisms, such as emotional processing and social recognition. To reach these aims I performed 4 studies, each described in a separate chapter. In chapter 2, I focused on manual lateralization in two mouse lemur species. In chapter 3 and 4, I focused on acoustic correlates to individuality and sex. This provided the basis for chapter 5, in which I focused on the effects of caller characteristics on auditory lateralization in gray mouse lemurs.

In order to gain insight into manual lateralization of early primates, in chapter 2 hand usage was studied in two species of nocturnal lemurs, gray and Goodman’s mouse lemurs. 44 gray mouse lemurs and 19 Goodman’s mouse lemurs were tested in a forced food grasping task. Based on a measure of hand preference (i.e. the hand that is spontaneously chosen for a specific task), both species were found to have manual lateralization at an individual, but not at a population level. Goodman’s mouse lemurs showed stronger individual hand preferences than gray mouse lemurs, probably caused by a species-specific difference of temperament. No sex and age effects were found, but the hand preference of offspring was negatively correlated to that of their mothers, indicating parental influence. Thus, individual differences were found to affect manual lateralization.

In mammals individual signatures in vocalizations provide the basis for individual recognition, and thus play an important role in social behavior. In order to contribute to a better knowledge on acoustic correlates to individuality across the vocal repertoires of nocturnal primates, in chapter 3, a study was conducted to examine to which extent acoustic correlates to individuality can be found in the vocal repertoire of gray mouse lemurs. Four call
types were examined (grunts, tsaks, short whistles and trills) given by subjects under controlled experimental conditions and differing in context and acoustic structure. A discriminant function analysis revealed that all call types were individually distinct, but not to the same degree. These findings provided first evidence of a structurally dependent variation in individual distinctiveness across the vocal repertoire of a nocturnal primate.

In order to study whether the structure of gray mouse lemur vocalizations differs between the sexes, in chapter 4, the acoustic structure of three call types (grunts, tsaks and short whistles) was examined. A discriminant function analysis revealed that tsaks and short whistles, but not grunts, are distinctive by sex. Thus, some mouse lemur vocalizations differ acoustically between sexes, providing a basis for acoustic sex recognition.

Auditory lateralization is suggested to be specialized for the processing of conspecific communication, but can also be affected by emotional processing and social recognition. In order to gain insight into the effects of caller characteristics on auditory lateralization in chapter 5, 17 gray mouse lemurs were tested in a head turn paradigm. The head turn paradigm was established to examine potential functional hemispheric asymmetries on the behavioral level. Subjects were presented with playbacks of two conspecific call types (tsaks and trills) from senders, differing in familiarity (unfamiliar vs. familiar) and sex (same sex vs. other sex). Gray mouse lemurs were able to recognize familiarity and sex of the sender based on some of the vocalizations, as indicated by a different response behavior. Based on the head turn direction towards these calls, I found evidence for a right ear/left hemisphere dominance for the processing of calls of the other sex. Familiarity had no effect on the orientation biases. These results suggest that the sex of the sender affects auditory lateralization in the recipient in this early primate, and therefore possibly also in more recently evolved primate species.

To sum up, mouse lemurs showed individual level lateralization in manual use and population level lateralization in the auditory processing during acoustic sex recognition. These findings match with the theory of Vallortigara and Rogers (2005), because in mouse lemurs coordination of lateralized behavior is more important during inter-sex interactions than during foraging, since mouse lemurs forage solitarily. Furthermore, manual lateralization in mouse lemurs was found to be affected by species-specific temperament and parental influences, causing \textit{inter}-individual variation. In addition, auditory lateralization was found to be affected by processing mechanisms, involved in conspecific communication processing and in sex recognition, causing \textit{intra}-individual variation. These findings illustrate the importance of exploring cerebral lateralization in all its facets, in order to give a full insight into its mechanisms and evolution.
Zusammenfassung

Leliveld, Lisette. Asymmetrien im Handgebrauch und in der Wahrnehmung von Kommunikationslauten bei basalen Primaten (*Microcebus spp.*): Einfluss von Geschlecht und Erfahrung


Zusammenfassung

Bei Säugetieren stellen individuelle Signaturen in Lautäußerungen die Basis für individuelle Erkennung dar und spielen demnach eine bedeutende Rolle im Sozialleben. Um zu einem besseren Verständnis von akustischen Korrelaten von Individualität im vokalen Repertoire eines nachtaktiven Primaten beizutragen, wurde in Kapitel 3 studiert in welchem Maße individuelle Signaturen im vokalen Repertoire des Grauen Mausmakis vorhanden sind. Vier Ruftypen wurden untersucht (Grunzer, Tsäks, Kurzpfiffe und Triller), die von den Versuchstieren unter kontrollierten experimentellen Bedingungen in verschiedenen Kontexten geäußert wurden und sich in ihrer akustischen Struktur unterschieden. Eine Diskriminanzyanalyse zeigte, dass alle Ruftypen individuelle Signaturen aufwiesen, aber nicht im selben Maße. Diese Ergebnisse sind der erste Hinweis darauf, dass sich die akustische Struktur des Ruftyps auf die Diskrimination auswirkt.

Inwiefern sich Lautäußerungen der Grauen Mausmakis in ihrer akustischen Struktur zwischen den Geschlechtern unterscheiden, wird in Kapitel 4 anhand von 3 Ruftypen (Grunzer, Tsäk und Kurzpfiffe) untersucht. Eine Diskriminanzyanalyse zeigte, dass Tsäks und Kurzpfiffe, nicht aber Grunzer, sich zwischen den Geschlechtern unterscheiden. Diese Lautäußerungen besitzen geschlechtsspezifische Signaturen, die die Basis für die akustische Erkennung des Geschlechts darstellen können.

Zusammenfassung

“With growing evidence for manual and cerebral asymmetries in non-human species, there is some doubt about whether there is truly a distinctiveness of the human pattern.”

Corballis (1998)
One of the crucial topics in modern neuroscience is that of cerebral lateralization. A brain is considered to be lateralized (or asymmetrical) if one side (hemisphere or other region) is structurally different from the other and/or performs a different set of functions (Bisazza et al., 1998). The best studied and generally acknowledged forms of lateralization in humans are the left hemisphere dominance for language and hand use (at least in 90% of the human population; Marchant et al., 1995), but there is also extensive evidence for lateralization in other cerebral functions in humans, such as emotion (for a recent review see Demaree et al., 2005), and spatial processing (for a recent review see Jager and Postma, 2003).

Until recently, research on cerebral lateralization focused mainly on humans, while other species have been largely ignored in this respect (Vallortigara and Rogers, 2005). The main reason for this was that cerebral lateralization was (and to some extent still is; Crow, 2004) considered to be a uniquely human trait (e.g., Warren, 1980; Corballis, 1991). In recent years, however, extensive evidence has been gathered of structural, functional, and behavioral lateralization in many non-human species ranging from fishes to non-human primates, indicating ancient evolutionary roots (for reviews see e.g., Bisazza et al., 1998; Vallortigara et al., 1999; Rogers, 2000; Vallortigara and Rogers, 2005; Vallortigara, 2006; see also Table 1.1).

With regard to the evolution of lateralized cerebral functions, Vallortigara and colleagues proposed that lateralization evolved in two steps (Vallortigara and Rogers, 2005; Vallortigara, 2006). First, lateralization evolved on the individual level, improving brain efficiency (e.g., Levy, 1977; Rogers, 2002). Second, lateralization evolved on a population level (with the vast majority showing lateralization in the same direction). Population level lateralization improves the coordination between group members, facilitating social interactions (Rogers, 2000; Vallortigara and Rogers, 2005; Vallortigara, 2006).

On the individual level cerebral lateralization has been suggested to be highly influenced by individual factors, such as ontogeny, sex and age (Warren, 1980). For example, in some species (including humans) the percentage of left handers is found to be higher in males than in females (e.g., Ward et al., 1990; Annett, 2002; Corp and Byrne, 2003), which could be explained by the inhibiting effect of prenatal testosterone on the growth of the left hemisphere in males (Geschwind and Galaburda, 1987).
Table 1.1: Overview of lateralized functions that have been described in various vertebrate species (modified from Vallortigara and Rogers, 2005).

<table>
<thead>
<tr>
<th>Left Hemisphere</th>
<th>Right Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Emotional processing</strong></td>
<td></td>
</tr>
<tr>
<td>- Inhibition of aggression <em>(chicks, humans)</em></td>
<td>- Fear <em>(chicks, rats)</em></td>
</tr>
<tr>
<td>- Inhibition of intense emotions <em>(humans)</em></td>
<td>- Aggression <em>(fishes, toads, lizards, chicks, monkeys)</em></td>
</tr>
<tr>
<td>- Approach of objects <em>(birds, primates)</em></td>
<td>- Courtship and copulatory behavior <em>(fish, newts, birds)</em></td>
</tr>
<tr>
<td></td>
<td>- Expression of intense emotions <em>(monkeys, apes, humans)</em></td>
</tr>
<tr>
<td></td>
<td>- Avoidance/withdrawal <em>(monkeys, apes, humans)</em></td>
</tr>
<tr>
<td><strong>Motor functions</strong></td>
<td></td>
</tr>
<tr>
<td>- Prey catching <em>(fish, toads)</em></td>
<td>- Predator escape <em>(fish, frogs, toads, chicks, dunnarts)</em></td>
</tr>
<tr>
<td>- Manipulation of food items/objects <em>(birds, monkeys, apes)</em></td>
<td></td>
</tr>
<tr>
<td><strong>Visual processing</strong></td>
<td></td>
</tr>
<tr>
<td>- Recognition of categories/attention to large changes <em>(birds, rats)</em></td>
<td>- Contact/monitoring of conspecifics <em>(fish, toads)</em></td>
</tr>
<tr>
<td>- Prey discrimination <em>(fish, toads)</em></td>
<td>- Recognition/analysis of faces <em>(sheep, monkeys, humans)</em></td>
</tr>
<tr>
<td>- Foraging with discrimination of food items <em>(birds)</em></td>
<td>- Recognition of individual conspecifics <em>(birds, sheep, humans)</em></td>
</tr>
<tr>
<td><strong>Auditory processing</strong></td>
<td></td>
</tr>
<tr>
<td>- Recognition of species typical vocalizations <em>(birds, mice, dogs, sea lions, monkeys, humans)</em></td>
<td>- Recognition of individual conspecifics <em>(birds, horses, humans)</em></td>
</tr>
</tbody>
</table>

Nevertheless, still little is known about how the different lateralized functions evolved and how they relate to each other. As far as I know, only one article addresses these questions (Andrew et al., 2000). Andrew and colleagues proposed that the first cerebral lateralization would have appeared in teleost fish, in which the left hemisphere became specialized for visual processing for predation, triggering the specialization of the right hemisphere for recognizing conspecifics.

Currently, cerebral lateralization has been identified in many different cerebral processes, based on anatomical, functional or behavioral evidence (for reviews see Hellige, 1990; Toga and Thompson, 2003). In this thesis I will focus mainly on lateralized cerebral functions, which can be detected on the behavioral level. An advantage of behavioral studies is that they provide direct insight into the extent to which cerebral lateralization affects the interactions of the subjects in relation to their environment, allowing an approach to lateralization from an ecological and evolutionary perspective (Tommasi, 2009). Previous behavioral studies of cerebral lateralization have focused mainly on either the lateralized functions of the motor system (such as hand use and facial expressions) or on the lateralized
Chapter 1. General introduction

sensory perception system (such as vision and hearing). These lateralized processes are interesting to study for themselves, but also often reflect lateralization in other processing mechanisms such as language, spatial processing, emotional processing and social recognition (see Table 1.1). All behavioral studies on cerebral lateralization in vertebrates (from fishes to humans) are based on the knowledge that each hemisphere predominantly controls the processing of perception and motor functions on the contralateral side of the body (Bisazza et al., 1998). As a result, behavioral indications of an enhanced activation on one side of the body can be used as evidence for enhanced activity of the contralateral cerebral hemisphere. Below, I will discuss the major findings on some lateralized cerebral functions that have been studied in both humans and non-human animals.

1.1 Lateralization of emotion

Emotion is well established as a lateralized function (Andrew et al., 2000), but the precise contribution of each hemisphere to emotional processing in humans is still heavily debated (for reviews see Davidson, 1995; Demaree et al., 2005). However, before discussing lateralization of emotion, it is important to note that I will focus here only on the processing that is involved in the experience (=feeling) and on the expression of (one’s own) emotion (the perception of emotional states in others will be discussed in section 1.3). For the lateralization of expression and experience of emotion several hypotheses exist, the two major being the ‘right hemisphere hypothesis’ and the ‘emotional valence hypothesis’ (see Demaree et al., 2005). The ‘right hemisphere hypothesis’ suggests that the right hemisphere is dominant in all emotional processing (Gainotti, 1972). The ‘emotional valence hypothesis’ suggests that the right hemisphere is dominant in processing of negative emotions, while the left hemisphere is dominant in processing of positive emotions (Ahern and Schwartz, 1979). A variation on this latter hypothesis, by Davidson (1995), postulates that approach emotions are processed with a left hemisphere dominance, whereas withdrawal emotions are processed with a right hemisphere dominance (termed the ‘approach-withdrawal hypothesis’). In humans, both hypotheses (‘right hemisphere’ and ‘emotional valence’) are supported by experimental evidence (e.g., Wylie and Goodale, 1988; Lee et al., 2004), but also some studies support the ‘approach-withdrawal’ hypothesis (e.g., Davidson et al., 1990; Sobotka et al., 1992).
In non-human animals a few studies provide evidence for lateralized emotional processing, based on brain imaging investigations (Parr and Hopkins, 2000) and based on facial or vocal expressions of emotion. In chimpanzees (Fernández-Carriba et al., 2002) and rhesus macaques (Hauser and Akre, 2001) a right hemisphere dominance was found for emotional facial expressions, providing support for the ‘right hemisphere hypothesis’. On the other hand, in marmosets support was provided for the ‘emotional valence hypothesis’, since calls of positive emotional valence were accompanied with a larger right hemimouth (= left hemisphere dominance), and calls of negative emotional valence were accompanied with a larger left hemimouth (= right hemisphere dominance; Hook-Costigan and Rogers, 1998).

Although lateralization of emotion is not often studied through perceptual asymmetries in non-human animals, there are nevertheless some indications of emotionally guided visual or auditory asymmetries. For instance, during agonistic interactions, toads (Vallortigara et al., 1998), lizards (Deckel, 1995; Hews et al., 2004) and baboons (Casperd and Dunbar, 1996) showed a preference to observe the opponent with the left eye (increasing the input to the right hemisphere). Also, bushbabies showed a left eye (right hemisphere) preference for observing arousing, negative stimuli (Rogers et al., 1994).

In auditory perception the results are contradictory. On the one hand, male mouse lemurs showed a right ear bias for responding to calls of negative emotional valence (Scheumann and Zimmermann, 2008). On the other hand, in domestic dogs and Campbell’s monkeys, a left ear bias was found for the processing of stimuli of negative emotional valence (Siniscalchi et al., 2008; Basile et al., 2009b). No lateralized perception has so far been reported in non-human animals for stimuli that are of positive emotional valence to the receiver.

In humans and non-human animals, lateralization of emotion is found to be affected by individual characteristics such as sex (e.g., Wager et al., 2003; Scheumann and Zimmermann, 2008), and temperament (e.g., Davidson, 1995; Larose et al., 2006).

To conclude, still little is known about lateralized emotional processing in non-human animals and more research is needed to clarify the role of emotional processing amongst other processing mechanisms (such as language and social recognition) that influence motor and perceptual lateralization, as will be shown in the following sections.
1.2 Motor lateralization

Of all functional asymmetries, motor lateralization is encountered most in the everyday life of humans, since about 90% of the human population is right handed (Marchant et al., 1995) and most football players can shoot better with the right foot than with the left (McLean and Tumilty, 1993). However, asymmetries are also found in other motor functions, such as body turning (Bracha et al., 1987), infant cradling (Harris et al., 2001), and even kissing (Güntürkün, 2003). Generally, these lateralizations may reflect purely cerebral asymmetries in motor control, but sometimes motor lateralizations, such as facial expressions, can also be affected by lateralization in emotional expression (see section 1.1), or by lateralization in language production (Graves et al., 1982).

Although motor lateralization has long been suggested to be restricted to humans (Warren, 1980), in the recent years, non-human vertebrates were also found to be lateralized in e.g., manual functions (for reviews see McGrew and Marchant, 1997; Papademetriou et al., 2005), infant cradling (for a review see Hopkins, 2004), body turning (Dodson et al., 1992) and scratching (Hopkins et al., 2006).

Studies on the primary cortex anatomy of right handers found a deeper anterior central sulcus (which is formed by the posterior precentral gyrus) in the left hemisphere, compared to the right hemisphere (e.g., Amunts et al., 1996; Foundas et al., 1998; Amunts et al., 2000), indicating a larger hand motor area (Amunts et al., 2000). Similar neuro-anatomical asymmetries in the precentral gyrus, have been found in non-human primates (e.g., Hopkins and Cantalupo, 2004; Dadda et al., 2006), suggesting homologies in motor lateralization between humans and non-human primates.

Manual lateralization is proposed to have evolved in ancestral primates that specialized the left hand to grab for food and the right hand to hold on to branches (MacNeilage et al., 1987). However, the lack of evidence of hand preferences on a population level in the majority of the studied non-human primates, provides no support for this so-called ‘Postural Origins Theory’ (McGrew and Marchant, 1997).

Most of the studied species showed only individual level manual lateralization. On the individual level, there is evidence that left or right handedness is influenced by individual characteristics, like sex (e.g., Milliken et al., 1991; Corp and Byrne, 2003), age (e.g., Ward et al., 1990; Hopkins and Leavens, 1998), inheritance (e.g., Hopkins, 1999; Lonsdorf and Hopkins, 2005) and temperament (e.g., Westergaard et al., 2001a; Rogers, 2009). Because of these individual differences and because the evolution of manual lateralization is still not
clearly understood, more studies are necessary to increase our understanding of manual lateralization.

1.3 Perceptual lateralization

Research on perceptual lateralization has primarily focused on the visual- and auditory domain. Hence, I will concentrate on findings in these two domains.

1.3.1 Visual lateralization

Within perceptual lateralization, visual lateralization has been most extensively studied in both humans and non-human vertebrates, and is suggested to be the phylogenically oldest form of lateralization (Andrew et al., 2000). Visual lateralization is to a large extent determined by the object that is observed (see Table 1.1). A right hemisphere dominance has been found for the perception of faces (but not for common objects) in humans (e.g., Kanwisher et al., 1997; Rossion et al., 2003) and rhesus macaques (e.g., Hamilton and Vermeire, 1988; Vermeire and Hamilton, 1998). Apart from that, there is evidence that visual processing is additionally influenced by the visual perception of emotional expressions and by social recognition. The perception of emotional facial expressions has been found to be processed predominantly in the right hemisphere in humans (e.g., Fusar-Poli et al., 2009; Tamietto et al., 2006), and also in chimpanzees (Morris and Hopkins, 1993). Likewise, a left eye/right hemisphere dominance for processing visual recognition of familiairs has been reported in both humans (for reviews see Gainotti, 2007; Brancucci et al., 2009) and non-human animals, such as domestic fowl (e.g., Vallortigara and Andrew, 1991; Deng and Rogers, 2002), quails (Zucca and Sovrano, 2008) and sheep (Peirce et al., 2000).

So, recognition of familiairs is suggested to be a right hemisphere specialty (Brancucci et al., 2009), but little is known about lateralization in the visual recognition of sex. In humans gender judgment in general (e.g., Luh et al., 1991; Butler and Harvey, 2006), and of the female face in particular (Parente and Tommasi, 2008), has been found to be lateralized to the left-half face/right hemisphere, based on studies with chimeric (male-female or female-male) faces. In non-human vertebrates no comparable experiments were performed. Some studies do report lateralized sexual/courtship behavior, but with incongruent findings, i.e. a left eye preference is reported in black-winged silts (Ventolini et al., 2005) and a right eye preference
is reported in five species of poeciliid fish (Bisazza et al., 1997) and zebra finches (George et al., 2006).

1.3.2 Auditory lateralization

Humans have a clear lateralization in auditory perception, with spoken language being processed with a left hemisphere dominance (e.g., Fitch et al., 1997; Friederici and Alter, 2004). This left hemisphere dominance for spoken language processing is suggested to be caused by a left hemisphere specialization for the processing of rapid temporal changes (Belin et al., 1998), whereas the right hemisphere is suggested to be more specialized in the processing of pitch perception (e.g., Zatorre, 1988; Warrier and Zatorre, 2004).

However, like visual lateralization, auditory lateralization is determined by more than one factor; apart from the left hemisphere dominance for spoken language processing, auditory lateralization can also reflect asymmetries in the processing of emotional prosody or in social recognition. Studies on auditory perception in humans indicate a right hemisphere dominance in the processing of emotional prosody (e.g., Blonder et al., 1991; Pell, 2007). Together with the evidence from visual perception, there is, therefore, evidence of a major role of the right hemisphere in the perception of emotion in others in humans.

Only few studies have focused on the lateralized processing of social recognition in the auditory domain. In humans, most studies on voice recognition were done a few decades ago, reporting a right hemisphere dominance in the recognition of familiar voices (Van Lancker and Kreiman, 1987). But, recently also a left hemisphere dominance was reported (Basile et al., 2009b). Sex recognition, based on voices was tested in only one study, which reports that female voices are processed in the right hemisphere, whereas male voices are processed in the left hemisphere (Landis et al., 1982).

In non-human vertebrates, the left hemisphere dominance for processing spoken language and rapid temporal changes is paralleled by evidence of right ear preferences (= left hemisphere dominance) in the processing of acoustic conspecific communication (for a recent review see Taglialatela, 2007). Furthermore, similar structural cerebral asymmetries are found for humans and non-human primates in the planum temporale (which is part of the Wernicke’s area) (humans: e.g., Wittelson and Pallie, 1973; Galaburda et al., 1987; primates: e.g., Gannon et al., 1998; Hopkins et al., 1998) and in the sylvian fissure (humans: e.g., Yenikomshian and Benson, 1976; Good et al., 2001; primates: e.g., Heilbroner and Holloway,
1988; Hopkins et al., 2000). These findings suggest commonalities in the lateralized processing of human speech and non-human conspecific communication.

Still, not many species have yet been studied on auditory laterality and not all of these species showed a right ear preference (=left hemisphere dominance) for the processing of conspecific calls (e.g., Barbary macaques: Teufel et al., 2007; vervet monkeys: Gil-da-Costa and Hauser, 2006). In addition, auditory lateralization is not always uniform across a population, but has been found to differ between age classes (Hauser and Andersson, 1994; Böye et al., 2005), and sexes (Scheumann and Zimmermann, 2008). Therefore, more studies are needed to gain a better understanding of the evolution of lateralization in the auditory perception of conspecific communication.

In non-human vertebrates, the strongest support for a left hemisphere specialization for the acoustic processing of rapid temporal changes (Belin et al., 1998) comes from findings in Japanese macaques, where a right ear advantage for discriminating between conspecific calls was found to be based on temporal processing, rather than pitch perception (Petersen et al., 1978; Beecher et al., 1979; Petersen et al., 1984). However, Petersen and colleagues argued that a communicative significance was essential for establishing this asymmetry, since the control primate species (pigtail macaque, bonnet macaque and vervet monkey) discriminated the Japanese macaque calls based on pitch, rather than on temporal processing, and showed no ear advantage. Furthermore, Ehret (1987) found that mother mice showed a right ear advantage for the recognition of pup calls, whereas virgin females that had been exposed to the pup calls in a non-social context did not. Palleroni and Hauser (2003) found that raptors, that have hunted howler monkeys, showed a right ear preference for the processing of howler monkey calls, whereas raptors that have only heard howler monkey calls (without hunting them), showed a left ear preference for these calls. All these findings suggest that a communicative significance, achieved through exposure to calls in a meaningful context, is essential for establishing a right ear/left hemisphere dominance in the processing of these calls.

In humans paralinguistic information processing, such as voice recognition is suggested to rely on other neural substrates than speech processing, and is more likely to be shared between humans and non-human primates (Belin, 2006). Indeed, a voice recognition region has been identified in the non-human primate brain, located in the middle of the anterior superior-temporal plane (Petkov et al., 2008), suggesting that voice recognition in non-human primates relies on similar neural substrates as in humans. In addition, individual vocal recognition has been reported in many non-human vertebrate species (e.g., Rendall et
al., 1996; McComb et al., 2003; Carter et al., 2008), as indicated by an ability to discriminate between familiar and unfamiliar conspecifics, based on their vocalizations (voices).

Nevertheless, only a few studies have focused on the lateralized acoustic perception of familiar conspecifics. Moreover, the few studies that focused on this found conflicting results. Some found a left ear preference (George et al., 2004), some a right ear preference (Cynx et al., 1992; Basile et al., 2009a), and some found no effect (Böye et al., 2005; Gil-da-Costa and Hauser, 2006). Therefore, more studies are needed on more species to fully understand the effect of familiarity with the sender on auditory lateralization.

Concerning the effect of the sex of the sender on auditory lateralization, to my knowledge, no studies tested this in any non-human vertebrate species. Still, many vertebrate species have shown the ability to perceive the sex of conspecifics, based on their vocalizations (e.g., Hauser, 2007; Smith et al., 2009), providing a basis for a possible effect of the sex of the sender on auditory lateralization.

1.4 **Co-evolution of lateralized processes?**

An interesting finding in the research on cerebral lateralization is that motor- and perceptual lateralization seem to be partially linked in humans. For example, left handers more often have a right hemisphere dominance for language functions than right handers (reviewed by Annett, 2002). Corballis (2003) proposed an evolutionary scenario in which, in human ancestors/non-human primates, manual gestures became incorporated in conspecific communication, not only leading to a population level right handedness, but also to the origin of human language. This scenario received much attention and was discussed controversially (e.g., Hopkins and Cantalupo, 2003; Jürgens, 2003). Alternatively, some have proposed that hand preference and auditory lateralization for conspecific communication evolved independently from each other (Hopkins and Cantalupo, 2004). This suggestion is supported by studies on rhesus macaques (Hauser and Andersson, 1994) and mouse lemurs (Scheumann and Zimmermann, 2008) that found no relation between hand preferences and auditory lateralization for processing conspecific communication calls. Thus, still a lot is not yet understood of how and when the human link between manual and auditory lateralization evolved and more comparisons between manual and auditory lateralization are needed in more species to gain a complete understanding of this possible link.
1.5 Animal model

Primates, as our closest biological relatives have been extensively studied on many different forms of cerebral lateralization, as shown in the previous sections. The research on cerebral lateralization has, however, mostly focused on apes and old world monkeys. Only a few studies have so far focused on prosimians, even fewer on nocturnal prosimians. Nevertheless this group of primates can provide important knowledge for unraveling the evolution of cerebral lateralization across the primate order. Therefore, I will explore in this thesis cerebral lateralization in mouse lemurs, small bodied nocturnal primates, considered to represent the most ancestral primate condition (Martin, 1972). Mouse lemurs are endemic to Madagascar, where they inhabit the fine-branch niche. They have an elaborate vocal repertoire with both low frequency and ultrasonic communication calls (Zimmermann, 1995). Mouse lemurs live in a multi-male, multi-female dispersed social system (Radespiel et al., 1998; Radespiel et al., 2001), meaning that during the night they forage solitarily, while during the day, females form temporary stable sleeping groups.

1.6 Aims

As shown above, the evolution of vertebrate cerebral lateralization is still little understood, since lateralization seems to be very dynamic, causing a high variability in the findings of lateralization from one species or genus. First, individual variation in sex, age, inheritance and even temperament could affect the pattern of cerebral lateralization, resulting in variation in lateralization patterns between different individuals. This will be discussed in this thesis as inter-individual variation in lateralization. Second, there are several lateralized processing mechanisms that underlie perceptual lateralizations, and that could all influence perceptual lateralization. For example, linguistic processing, emotional processing and social recognition can all occur, during the perception of spoken speech, and therefore could all affect the resulting lateralization pattern. Therefore, perceptual lateralization can vary within one individual, depending on the processing mechanisms that are involved. This will be discussed in this thesis as intra-individual variation in lateralization. Thus, the central question here is: To which extent is cerebral lateralization present in early primates and how variable is cerebral lateralization on an inter- and intra-individual level?

To investigate the variability of lateralization, I focused especially on variation of cerebral lateralization on an inter-individual level, i.e. individual characteristics such as sex,
age, or inheritance, and on an intra-individual level, i.e. effects of underlying processing mechanisms. Indications have been found that gray mouse lemurs show manual lateralization only on an individual level, and that male gray mouse lemurs show auditory lateralization on a population level (Scheumann and Zimmermann, 2008). Therefore, I focused mainly on inter-individual variation in manual lateralization (chapter 2) and intra-individual variation in auditory lateralization (chapter 5). In chapter 2 I focused on the differences between individuals (and species) that could influence the pattern of manual lateralization (i.e. inter-individual variation). In chapter 3 and 4 I tested for individual signatures and sex differences in the acoustic structure of gray mouse lemur vocalizations, which provides the basis for an investigation into the effects of caller characteristics on auditory lateralization. In chapter 5 I focused mainly on how auditory lateralization can vary within one individual, depending on which processing mechanisms are involved (such as familiarity and sex recognition), i.e. intra-individual variation. These investigations into inter- and intra-individual variations in cerebral lateralization are aimed to contribute to a better understanding of cerebral lateralization in early primates. This will then ultimately contribute to a better understanding of the evolution of primate cerebral lateralization.

1.6.1 Chapter 2: Manual lateralization in two mouse lemur species

Manual lateralization has been studied in a variety of primate species. Nevertheless, the evolutionary pattern is still unclear. Therefore, the first aim was to explore the variation in lateralization pattern on an inter-individual level that might muddle with the discerning of a general lateralization pattern of a species or genus. Furthermore, the evolution of manual lateralization is still unclear. Knowledge on manual lateralization in nocturnal prosimians (suggested to represent the most ancestral primate condition; Martin, 1972) could help to discern the evolutionary pattern, but this group of primates is still little studied. Therefore, the second aim of this study was to contribute to a better understanding of the evolution of manual lateralization in primates, by focusing on nocturnal prosimians.

In order to achieve the first aim, I tested for differences between sexes and age classes, and looked at parental influences. In order to achieve the second aim, I tested for manual lateralization on a population level in two species of mouse lemurs to understand the general pattern of lateralization of these early primates. In addition, I compared the two species to give insight into the evolution of manual lateralization on a small scale. Finally, I explored the stability of individual manual lateralization by looking at the development of hand biases with increasing experience in the task.
1.6.2 Chapter 3, 4 and 5: Acoustic conveyance and perception of caller characteristics by gray mouse lemurs and its effect on the lateralized auditory processing of communication calls.

As lined out before, auditory lateralization can result from different processing mechanisms, i.e. of conspecific communication processing, emotional processing and social recognition. In male gray mouse lemurs, Scheumann and Zimmermann (2008) found that the left hemisphere dominance for processing conspecific communication was restricted to calls of negative emotional valence. However, nothing is known yet on how social recognition affects auditory lateralization in these (and many other) primates. Therefore, in this study, I aimed to further explore auditory lateralization of conspecific communication in gray mouse lemurs, with regard to effects of emotion and social recognition (familiarity and sex).

As a first step in the study on the lateralized auditory processing of social recognition, I explored whether gray mouse lemur vocalizations encode information about sender identity and sex in their structure, which would enable acoustic discrimination between familiar and unfamiliar senders, and between male and female senders. This predisposition of the communication system of gray mouse lemurs for the transmission of identity and sex is a prerequisite for auditory lateralization in social recognition. Therefore, I tested for individual signatures in gray mouse lemur vocalizations in chapter 3 and for sex differences in the acoustic structure of their vocalizations in chapter 4.

As a second step, in chapter 5, I tested for acoustic recognition of caller characteristics (familiarity and sex) in gray mouse lemurs, and its effect on auditory lateralization. These effects of caller characteristics on auditory lateralization can give insight into the variation of lateralization on an intra-individual level, as they may involve processing mechanisms, such as conspecific communication processing, social recognition and possibly also of emotional processing. In addition, attention was paid to sex differences as an inter-individual level variation.
Manual lateralization in early primates: a comparison of two mouse lemur species

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2.1 Abstract

In humans, 90% of the population is right handed. Although population hand preference has been found in some primate species, the evolution of manual lateralization in primates is not yet clear. In order to gain insight into manual lateralization of ancestral primates, we studied hand usage in unspecialized quadrupedal, nocturnal lemurs, using a large sample size. We compared two closely related mouse lemur species to explore the variation of hand preference within the same genus. We tested 44 gray mouse lemurs and 19 Goodman’s mouse lemurs in a forced food grasping task. The tests were videotaped. Measures of hand preference (i.e. the hand that is spontaneously chosen for a specific task) and successful hand usage (i.e. the hand that is successful in completing a specific task) were taken to explore manual lateralization. Both species showed manual lateralization at an individual, but not at a population level. Goodman’s mouse lemurs showed stronger individual hand preferences than gray mouse lemurs. This suggests that strength in hand preference is variable within the same genus. No sex and age effects were found. The hand preference of offspring was negatively correlated to that of their mothers, but not correlated to that of their fathers. Thus, no clear genetic effect can be derived from these results. In the Goodman’s mouse lemurs, hand preference increased with increasing task experience. However, successful hand usage was not affected by task experience, suggesting that successful hand usage is a more stable measurement for manual lateralization than hand preference.

2.2 Introduction

Right handedness is a prominent indication of cerebral lateralization in humans, which has previously been discussed as being a uniquely human trait (Corballis, 2003). To date, however, various studies have shown that manual lateralization at a population level is also present in a number of different vertebrate lineages, e.g., mammals (e.g., Sanford et al., 1984; Diamond and McGrew, 1994; Hopkins and Bard, 2000; Güven et al., 2003; Lonsdorf and Hopkins, 2005; Meguerditchian and Vauclair, 2006), birds (e.g., Rogers and Workman, 1993) and amphibians (e.g., Bisazza et al., 1996).

To explain the evolution of manual lateralization in primates, MacNeilage et al. (1987) formulated the “postural origins theory”. This theory proposes that in ancestral, bipedal, arboreal primate species, a left hand bias exists for visually guided reaching in conjunction with a right sided asymmetry for postural control. In more recently evolved terrestrial
primates, the right hand became the preferred hand for manipulation, rather than providing postural support. Finally, in the great apes and humans this led to a right hand dominance in all actions. Two meta-analyses, by McGrew and Marchant (1997) and by Papademetriou et al. (2005), found no strong support for the “postural origins theory”, because of the lack of manual lateralization at the population level in many of the studied species and a great variability between species within the same family and genus.

Comparative studies testing different primate species using the same methodology found species differences in manual lateralization between distantly related species of anthropoid primates (e.g., Westergaard and Suomi, 1996; Singer and Schwibbe, 1999; Hopkins et al., 2003b; Phillips and Hopkins, 2007), but also between species of the same genus (Westergaard et al., 2001a). For example, Singer and Schwibbe (1999) found that tamarins showed stronger right hand preferences than marmosets, which they ascribed to a difference in posture and foraging style. Further, Westergaard et al. (2001a) found differences in the direction of hand preference between three species of macaques, which they ascribed to a difference in temperament.

Within a given species manual lateralization is also discussed to be affected by factors such as sex (e.g., Stafford et al., 1990; Milliken et al., 1991; Corp and Byrne, 2003) and age (e.g., Ward et al., 1990; Mason et al., 1995; Hopkins and Leavens, 1998), but also by task experience (e.g., Milner, 1969; Lehman, 1980; Fagot and Vauclair, 1991; Mittra et al., 1997) and inheritance (e.g., Hopkins et al., 1993; Hopkins, 1999; Hopkins et al., 2001; Lonsdorf and Hopkins, 2005; Hopkins et al., 2006). Warren (1980) pointed out that non-human primate manual lateralization patterns are merely a result of human influence and the development of a routine. This statement is supported by studies that showed that manual lateralization tends to increase with experience in a task (Lehman, 1980; Mittra et al., 1997). However, other studies found an opposite pattern, a decrease in manual lateralization with task experience (Milner, 1969; Vauclair and Fagot, 1991). This was explained by the suggestion that novel tasks elicit a stronger manual lateralization than familiar tasks (Fagot and Vauclair, 1991). Although it is widely acknowledged that genetic factors contribute to handedness in humans, the precise nature of these factors remains to be clarified (Laland et al., 1995). In non-human primates, indications of inheritance have been found in chimpanzees (e.g., Hopkins et al., 1993; Hopkins, 1999; Hopkins et al., 2001; Lonsdorf and Hopkins, 2005; Hopkins et al., 2006), capuchins (Westergaard and Suomi, 1997) and macaques (Westergaard et al., 2001b). However, it has been suggested that environmental factors, such as prenatal position, hormones and mother-infant interactions such as cradling, rather than genes, may account for
the transfer of hand preference directions from parents to offspring (Westergaard and Suomi, 1997; Hopkins, 1999; Westergaard et al., 2001b; Hopkins et al., 2006).

Hand preference (i.e. the hand that is spontaneously chosen for a specific task) is the most commonly used measure for manual lateralization, but several authors suggested that successful hand usage (i.e. the hand that is more successful in completing a specific task) gives a better indication of motor lateralization (e.g., Fragaszy and Adams-Curtis, 1993; Preilowski, 1993) and is less affected by repetitive use (Preilowski, 1993). So far, however, only a few studies have focused on successful hand usage in non-human primates (e.g., Fragaszy and Mitchell, 1990; Fragaszy and Adams-Curtis, 1993; Milliken, 1995; Hopkins and Russell, 2004; Spinozzi et al., 2007).

From an evolutionary point of view, it is interesting to find out whether manual lateralization is present in mouse lemurs, which are suggested to represent the most ancestral primate condition (Martin, 1972). Mouse lemurs are small-bodied, arboreal, nocturnal primate species living in the fine branch niche of the Malagasy forests. They show quadrupedal locomotion, but use their hands to catch insects. They live in a dispersed multi-male multi-female system (Radespiel et al., 1998; Radespiel et al., 2001). Infants are raised by the mother, who transports her babies in her mouth (Martin, 1972). Hand preferences and body turn preferences have been found in gray mouse lemurs, based on a small sample size (Dodson et al., 1992; Ward, 1995). Also indications have been found that male gray mouse lemurs are lateralized in the processing of communication sounds of negative emotional valence (Scheumann and Zimmermann, 2008). In this study we investigated manual lateralization in two mouse lemur species, the gray mouse lemur and the Goodman’s mouse lemur, both at an individual and at a population level, using the same paradigm, a forced food grasping task. Furthermore, we tested for effects of sex, age and task experience and for parental influence on manual lateralization. Finally, we compared the measures of hand preference and successful hand usage (e.g., Fragaszy and Mitchell, 1990; Preilowski, 1993).

2.3 Methods

2.3.1 Subjects

We tested 44 gray mouse lemurs (Microcebus murinus), 21 males, 23 females, and 19 Goodman’s mouse lemurs (Microcebus lehilahytsara), seven males and 12 females, of our breeding colony. The mouse lemurs were housed in the animal facility of the Institute of
Zoology, University of Veterinary Medicine Hannover, at a reversed day-night cycle (for details of housing conditions see Wrogemann et al., 2001. Note *Microcebus lehilahytsara* was termed previously *Microcebus rufus*). All subjects were born in captivity. The age of the gray mouse lemurs ranged from 7 months to 9 years, with nine individuals less than 1 year old at the time of testing (=juvenile, Radespiel and Zimmermann, 2003). The age of the Goodman’s mouse lemurs ranged from 6 months to 8 years, with six individuals less than 1 year old at the time of testing (=juvenile, Radespiel and Zimmermann, 2003).

2.3.2 Experimental setup

Each subject was tested alone in a test cage (Ebecco stainless steel cage for marmosets, 80 cm x 87 cm x 50 cm) in a sound-attenuated chamber. The cage was equipped with two wooden bars, a nest box and a transparent box with a small opening (1 x 2 cm). The transparent box was attached to the outside of the cage, so that the animals were forced to grab with one hand inside it, through the small openings between the bars (Fig. 2.1). Since one hand is needed for support, the subject could only use one hand to grab with. The subject’s behavior was videotaped using a digital camcorder (Sony DR-TRV 22E PAL, Nightshoot). The camera was connected to a monitor outside the chamber, where the experimenter sat and observed the subject.

2.3.3 Procedure

Each session was conducted at the beginning of the activity period of each subject. For each session a subject was removed from its home cage, placed in a new nest box and attached to the test cage in the sound-attenuated chamber. Each subject was tested with a forced food grasping task for 15 minutes. In this task a subject had to use one of its hands to grab immobile mealworms through a small opening in a transparent box. For each session 10 immobile mealworms were placed in the transparent box. For some subjects that refused to eat the mealworms, raisins or grape parts were used instead. The session started as soon as the door to the sound attenuated chamber was closed, to rule out any influence of the experimenter.

An experiment consisted of several habituation sessions and a minimum of three test sessions on separate days. Habituation sessions were used to familiarize a subject with the experimental setup and procedure. We defined a subject as habituated when it had positioned itself in front of the box with mealworms, within the first 5 minutes of the session.
Fig. 2.1: Experimental setup. Box with mealworms is attached outside the cage, with an opening towards the inside. This way the mouse lemur can grasp the mealworms through the bars of the cage.

2.3.4 Data and video analysis

We digitized the video tapes using Pinnacle Studio 8 and Intervideo WinDVD creator 2 and analyzed them using Interact 3.1. (Mangold International GmbH). We conducted a frame by frame analysis (25 frames/second). Analyses were done at an individual level. For every mealworm that was grabbed out of the box, we recorded which hand (right or left) was used in the first attempt to grab it (hand preference) and which hand (left or right) was finally successful (successful hand usage). A hand was considered to be successful when it had picked up one or more mealworms out of the box. To test for effects of task experience, we also analyzed the first habituation session in which a mouse lemur retrieved mealworms from the box in exactly the same way as the test sessions.

2.3.5 Statistical analysis

A handedness index was calculated for each subject for the analysis of hand preference (HI of preference) and successful hand usage (HI of success), respectively. Pooling the data of the test sessions, we calculated the handedness index for each subject according to the formula $HI = \frac{\text{number right} - \text{number left}}{\text{number right} + \text{number left}}$ (Lonsdorf and Hopkins, 2005). The outcome of this formula can range from -1 to 1, with positive values reflecting right hand bias and negative values reflecting left hand bias. We additionally used the absolute HI (HI-ABS) value of each subject to compare the strength of the lateralization irrespective of direction. Furthermore, we tested whether a subject used one hand more often than expected by chance, using a Binomial test with 50 % chance level. We defined subjects
as left- or right-handers or ambiguous: right-handers – subjects used the right hand significantly more often than expected by chance (positive handedness index), left-handers – subjects used the left hand significantly more often than expected by chance (negative handedness index), ambiguous – subjects did not use one hand significantly more often than expected by chance.

According to a Kolmogorov-Smirnov test, our data differed significantly from a normal distribution. For this reason, we used non-parametric tests (two tailed). To explore whether a significant majority of the population is lateralized, we used a Chi-square test with the number of left, right, and ambiguous handed individuals to test if this distribution differed significantly from chance (25:25:50, Güven et al., 2003). To test if the population showed a lateralization towards the right or the left, a Binomial test was conducted to test whether significantly more subjects used the right hand more than expected by chance (50:50). To explore whether there are differences in the HI of preference and HI of success between species, sexes and age groups a Mann-Whitney-U test was conducted.

To explore the effect of task experience on manual lateralization, the HI of preference and HI of success, calculated from the first habituation session, were compared to the HI of preference and HI of success, calculated from the last test session. A Wilcoxon test was used for this comparison. Fifteen gray mouse lemurs had already participated in a handedness experiment previously and were considered as experienced in this study. These subjects were therefore excluded from the analyses on task experience effects. Parental influence was determined according to the study of Lonsdorf and Hopkins (2005): The HI of mothers and fathers were correlated with the mean HI of their offspring. All statistical tests were calculated using SPSS 14.

2.4 Results

2.4.1 Hand preference

In both species, almost all subjects (gray mouse lemurs: 80%, Goodman’s mouse lemurs: 100%) showed a significant individual hand preference (see Appendix 2A). No population level hand preference was found. Of the 44 gray mouse lemurs, 14 subjects (32%) showed a significant left hand preference, 21 (48%) a significant right hand preference and 9 (20%) were ambiguous (Fig. 2.2). The numbers of left, right and ambiguous subjects did not fit a 25:25:50 distribution, as expected by chance ($\chi^2 (2) =17.59$: P < 0.001, n=44). But,
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despite the fact that 80% of the gray mouse lemurs were lateralized, there was no population bias (Binomial test: 14L: 21R, P = 0.311).

Of the 19 Goodman’s mouse lemurs all showed a significant individual hand preference, with 9 (47%) having a left hand preference and 10 (53%) having a right hand preference (Fig. 2.3). There were no ambiguous subjects. The left and right handers were equal in numbers, which means that there was no population level hand preference (Binomial test: 9L: 10R, P = 1.000).

2.4.2 Species differences

Although the two species did not have a significant difference in their HI of preference (Mann-Whitney-U test: Z = -0.038, P = 0.970, n₁=19; n₂=44), they did differ in the strength (HI-ABS) of their preference. The Goodman’s mouse lemurs showed a higher HI-ABS than the gray mouse lemurs (Mann-Whitney-U test: Z = -2.740, P = 0.006, n₁=19; n₂=44).

2.4.3 Factors influencing manual lateralization

In both species, no difference was found between males and females in either the direction (Mann-Whitney-U test: gray mouse lemurs: Z = -0.412, P = 0.680, n₁=23; n₂=21; Goodman’s mouse lemurs: Z = -0.343, P = 0.733, n₁=12; n₂=7) or the strength of the HI of preference (Mann-Whitney-U test: gray mouse lemurs: Z = -0.486, P = 0.627, n₁=23; n₂=21; Goodman’s mouse lemurs: Z = -1.029, P = 0.340, n₁=12; n₂=7).

No age effect was found in either species. Juveniles did not significantly differ from adults in direction (Mann-Whitney-U test: gray mouse lemurs: Z = -0.146, P = 0.886, n₁=9; n₂=35; Goodman’s mouse lemurs: Z = -0.045, P = 0.966, n₁=6; n₂=13) and strength of the HI of preference (Mann-Whitney-U test: gray mouse lemurs: Z = -0.983, P = 0.343 n₁=9; n₂=35; Goodman’s mouse lemurs: Z = 0.000, P = 1.000, n₁=6; n₂=13).

The first habituation session of 18 inexperienced gray mouse lemurs and 19 inexperienced Goodman’s mouse lemurs was compared with their last test session. No significant shift in the HI of preference was found in both species (Wilcoxon test: gray mouse lemurs: Z = -0.409, P = 0.683, n=18; Goodman’s mouse lemurs: Z = -0.188, P = 0.851, n=19) (Fig. 2.4a,b). However, the strength (HI-ABS) of the preference did significantly change with task experience for the Goodman’s mouse lemurs (Wilcoxon test: Z = -2.973, P = 0.003, n=19). The mean HI-ABS of preference was higher in the last test session than in the first
habituation session. For the gray mouse lemurs the strength of preference did not change with task experience (Wilcoxon test: \( Z = -0.970, P = 0.332, n=18 \)).

Fig. 2.2: Individual HI of preference values for *Microcebus murinus*. Black bars represent the females, white bars the males. * \( p \leq 0.05 \) using two-tailed Binomial test.

Fig. 2.3: Individual HI of preference values for *Microcebus lehilahytsara*. Black bars represent the females, white bars the males. * \( p \leq 0.05 \) using two tailed Binomial test.
Fig. 2.4: Comparison of HI and absolute HI values between the first habituation trial and the last test trial for the gray mouse lemurs (a) and the Goodman’s mouse lemurs (b). * \( p \leq 0.05 \) using Wilcoxon test.

Fig. 2.5: Correlations between the females HI of preference and the average HI of preference of their offspring (a) and between the males HI of preference and the average HI of preference of their offspring (b). Squares indicate the gray mouse lemurs, triangles the Goodman’s mouse lemurs. Trend line shows trend for the gray mouse lemurs.

The HI of 10 mothers (eight gray mouse lemurs and two Goodman’s mouse lemurs) and of nine fathers (seven gray mouse lemurs and two Goodman’s mouse lemurs) was known in this study (see also Appendix 2B). Parental influence was analyzed by correlating the HI of preference of mothers and fathers with the mean HI of preference of their offspring. There was a significant negative correlation between the mothers and their offspring (Spearman’s rank correlation: \( r_s = -0.905, P = 0.002, n=8 \)) (Fig. 2.5a). In gray mouse lemurs, there was no significant correlation between the fathers and their offspring (Spearman’s rank correlation: \( r_s = 0.643, P = 0.119, n=7 \)) (Fig. 2.5b). The number of Goodman’s mouse lemurs was too low for statistical analyses.
2.4.4 Successful hand usage vs. hand preference

The HI of success was highly correlated with the HI of preference (Spearman’s rank correlation: $r_s = 0.964$, $P < 0.001$, $n=63$). The results based on the HI of success are therefore similar to those found for hand preference. However, there was one difference. Task experience did not affect successful hand usage, while it did affect hand preference. For HI of success we found no difference between the first habituation session and the last test session in the direction (Wilcoxon test: gray mouse lemurs: $Z = -0.804$, $P = 0.421$, $n=18$; Goodman’s mouse lemurs: $Z = -0.652$, $P = 0.514$, $n=19$), and in the strength (Wilcoxon test: gray mouse lemurs: $Z = -0.471$, $P = 0.637$, $n=18$; Goodman’s mouse lemurs: $Z = -1.897$, $P = 0.058$, $n=19$) for both mouse lemur species.

2.5 Discussion

Both mouse lemur species showed a manual lateralization at an individual but not at a population level. The two mouse lemur species differed in the strength of their manual lateralization. The Goodman’s mouse lemurs showed a stronger lateralization than the gray mouse lemurs. Our results showed an effect of task experience and parental influence, but not of sex or age. Even when the data of both species was combined to create a larger sample size in order to test sex and age effects on direction of manual lateralization, no significant effects were found (sex: Mann-Whitney-U test: $Z = -0.674$, $P =0.501$, $n_1=28$; $n_2=35$; Mann-Whitney-U test: $Z = -0.194$, $P =0.846$, $n_1=15$; $n_2=48$). In Goodman’s mouse lemurs, but not in gray mouse lemurs, the HI of preference increased with task experience. As an indication of parental influence, the HI of gray mouse lemur females was negatively correlated to the HI of their offspring. Although the HI of preference and of success gave similar results, we found that the HI of success, in contrast to the HI of preference, was not affected by task experience.

Our findings of manual lateralization at an individual, but not at a population level in mouse lemurs agree with previous findings in gray mouse lemurs, based on a smaller sample size (Dodson et al., 1992). Still, it is interesting to note here that of the gray mouse lemurs that had a significant hand preference, 60% preferred the right hand, which is comparable to findings in chimpanzee populations (Hopkins et al., 2003a). In any case, our findings are not in agreement with the left hand bias at a population level that was found in many other lemur species (see Papademetriou et al., 2005), tested on comparable sample sizes. We have two possible explanations for this. First, Vallortigara and Rogers (2005) suggested that social
constraints are the trigger for the evolution of population level lateralization, so population level lateralization should be more prominent in more social species. Since gray mouse lemurs live in a dispersed social system where only females form temporary stable sleeping groups, (Radespiel et al., 1998), less social pressure would exist for the synchronization of manual lateralization at a population level. Most likely the same is true for the Goodman’s mouse lemurs, for which there is only little information on social ecology (Randrianambinina, 2001). The second explanation comes from Rogers and Workman (1993), who suggested that active use of paws or hands for feeding or searching for food is required for population level manual lateralization. Since mouse lemurs have a strong tendency to retrieve stationary food with the mouth (Ward, 1995), this less active hand use (Rogers and Workman, 1993) might be the best explanation for the lack of population level manual lateralization that is found here.

We found a difference in the strength of manual lateralization, even though the two species are closely related. Overall, the Goodman’s mouse lemurs showed stronger manual lateralization than the gray mouse lemurs. In the first habituation session no significant difference in strength of manual lateralization was found between the species (Mann-Whitney-U test: Z = -0.614, P = 0.539, n₁ = 19; n₂ = 18). Therefore, this species difference can be explained by the fact that only the Goodman’s mouse lemurs showed an increase in the strength of manual lateralization with task experience. This increase in strength contradicts Fagot and Vauclair’s hypothesis (1991) that novel tasks would elicit a stronger manual lateralization than familiar tasks. In our study the opposite result was found. Instead, we found that Goodman’s mouse lemurs, even though they showed individual hand preferences from the beginning of the experiment, showed a development of a routine, which supports Warren’s statement (1980) partially. In contrast to the latter species, the gray mouse lemurs did not increase their manual lateralization with increasing task experience. This is the first time a difference between species was related to a difference in development with task experience.

Differences between closely related species in manual lateralization have been found in other studies as well (Singer and Schwibbe, 1999; Westergaard et al., 2001a). Westergaard et al. (2001a) found differences in direction of hand preference between three macaque species and explained them by differences in temperament. A similar explanation may also hold true for mouse lemurs. Temperament has already been found to influence the direction of hand preference in another prosimian primate, the galago (Watson and Ward, 1996). In our study Goodman’s mouse lemurs needed significantly more habituation sessions to reach the habituation criterion than gray mouse lemurs did (Mann-Whitney-U test: Z = -3.787, P <
0.001, \( n_1=19; n_2=37 \). This may indicate that they are more nervous and stressed in an unknown environment than the gray mouse lemurs. These possible differences in temperament should be explored further.

In our study we found a negative mother-offspring correlation of hand preference for gray mouse lemurs, which coincides with findings in pigtail macaques (Westergaard et al., 2001b), but is not in agreement with the positive mother-offspring correlations in chimpanzees, capuchins and rhesus monkeys (Westergaard and Suomi, 1997; Hopkins et al., 2001; Westergaard et al., 2001b; Lonsdorf and Hopkins, 2005; Hopkins et al., 2006;), and of no mother-offspring correlations in bonnet monkeys (Brooker et al., 1981). These conflicting findings in primates suggest that maternal influences on offspring hand preference vary between primate species (Westergaard et al., 2001b). Furthermore, we did not find an influence of fathers, which is in agreement with findings in chimpanzees and macaques (Westergaard et al., 2001b; Hopkins et al., 2006), but not with the negative father-offspring correlation in capuchins (Westergaard and Suomi, 1997). Between siblings no significant correlations of HI were found. The fact that there was a significant influence of the mother, but not of the father, doesn’t support a clear, straightforward genetic base of manual lateralization in gray mouse lemurs, as would be expected by single factor inheritance theory, which was suggested for humans (Laland, 1995). Further, the fact that the hand preference of the mothers negatively influenced the hand preference of their offspring, suggests that other processes than inheritance may be involved. Hopkins (1999) suggested that apart from genetic effects environmental influences could also play an important role in the transfer of hand preference directions from mothers to their offspring, for example through cradling bias, fetal position and hormones. In chimpanzees (Hopkins and Bard, 1993) maternal cradling biases were found to be correlated to the hand preferences of the young. Mouse lemur mothers do not cradle their young while nursing. Nevertheless, an asymmetric position of the mother during nursing could affect the nursing position of their young, and thereby possibly their later hand preference as well. Therefore, we suggest that environmental factors might also affect the transfer of hand preference directions in mouse lemurs.

In our study the usage of the two measures, HI of preference and success, provided similar results. We found, however, that the success measure was, in contrast to the HI of preference, unaffected by task experience in the Goodman’s mouse lemurs. Although a measure of hand preference is necessary to compare results with other published studies, our results suggest that a measurement of successful hand usage is, as Preilowski (1993) suggested, unaffected by repetitive use and therefore more stable.
In conclusion, gray and Goodman’s mouse lemurs show individual but not population level manual lateralization in a forced food grasping task. The difference in strength between these two closely related species suggests that manual lateralization measures can be variable intragenerically. The lack of inheritance of manual lateralization found in this study argues against explanations based on simple genetic features. Further studies are needed to investigate the role of environmental influences on manual lateralization.

2.6 Acknowledgements

We gratefully acknowledge the financial support given by the EC Sixth Framework Program under contract no. MEST-CT-2005-021014 and by the German Research Foundation (DFG) under contract no. AZ Zi 345/15. We would like to thank Sabine Schmidt and Eckart Altenmüller for critical comments and Frances Sherwood-Brock for polishing the English. We also wish to thank R.W. Brüning for technical support and W. Mehl and A. Sauer for animal care.

2.7 Appendices

2.7.1 Appendix 2A: Handedness Index of preference for each individual.

<table>
<thead>
<tr>
<th>Species name</th>
<th>sex</th>
<th>Age (years)</th>
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<th>HI of preference</th>
<th>P-value</th>
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(continued)
### 2.7.1 Appendix 2A: Handedness Index of preference for each individual. (Continued)

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$^2$ P-value was calculated with a Binomial test.

$^1$ Age is rounded down to years, except for juveniles (<1).
### 2.7.2 Appendix 2B: Pairs of which the hand preference category is known, with their offspring.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Hand preference category: male, female</th>
<th>No. of right handed offspring</th>
<th>No. of left handed offspring</th>
<th>No. of ambiguous handed offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R,L</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>R,R</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>A,L</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>R,A</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>L,A</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>R,L</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>R,A</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>L,R</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Codes: L = left, R = Right, A = Ambiguous
Acoustic correlates of individuality in the vocal repertoire of a nocturnal primate (*Microcebus murinus*)

Submitted to the *Journal of the Acoustical Society of America*
3.1 Abstract

In mammals individual signatures in vocalizations provide the basis for individual recognition, and thus play an important role in social behavior. Differences in individual distinctiveness across call types within the same species were postulated to be linked to variations in context and structure. To explore whether context and structure predict variations in individual distinctiveness across call types in a nocturnal primate, a study was conducted to examine to which extent acoustic correlates to individuality can be found in the vocal repertoire of the gray mouse lemur, a nocturnal primate, living in a dispersed multi-male multi-female social system. Four call types were examined (grunts, tsaks, short whistles and trills) given by subjects under controlled experimental conditions and differing in context and acoustic structure. A discriminant function analysis revealed that all call types are individually distinct, but not to the same degree. These findings provide first evidence for a nocturnal primate that variation in individual distinctiveness across the vocal repertoire is not determined by context, but by structure.

3.2 Introduction

Individual vocal signatures are present in various classes of vertebrates, e.g., frogs (e.g., Bee et al., 2001; Bee, 2004), birds (e.g., Naguib et al., 2001; Searby et al., 2004), and mammals, such as bats (e.g., Bastian and Schmidt, 2008; Knörnschild and Von Helversen, 2008), carnivores (e.g., Yin and McCowan, 2004; Collins et al., 2005), even-toed ungulates (e.g., Blackshaw et al., 1996; Vannoni and McEligott, 2007) and non-human primates (e.g., Macedonia, 1986; Zimmermann and Lerch, 1993; Fischer et al., 1995). However, most of the studies on individual vocal signatures have focused on only one call type of a certain species. A focus on more call types could give a more complete understanding of the correlates to individuality that a species codes acoustically. Indeed, a few studies have shown that individual distinctiveness is not consistent across the entire vocal repertoire within a species, but differs between call types (e.g., Mitani et al., 1996; Rendall et al., 1998; Bastian and Schmidt, 2008; Knörnschild and Von Helversen, 2008). The present study investigates to which extent the individual distinctiveness varies across the vocal repertoire of a nocturnal primate and which factors may explain this variation.

Within our study we addressed the question whether the context, in which a call type is produced, determines individual distinctiveness. Based on the literature, two major
hypotheses emerged. First, a ‘social cohesion hypothesis’ postulates that call types used for social cohesion are better suited for coding individuality than call types that are used in other social circumstances. This is based on the fact that some authors have stressed the importance of individual recognition for facilitating and maintaining social cohesion (e.g., Macedonia, 1986; Janik and Slater, 1998; Maurello et al., 2000). Thus, it is expected that individual vocal signatures are more likely to occur in call types that are used to facilitate social cohesion, independent of the presence or absence of visual contact with the receiver.

Second, a ‘visual contact hypothesis’ suggests that the presence (or absence) of visual contact between the caller and receiver during call production, determines the individual distinctiveness of a call type (e.g., Mitani et al., 1996; Yin and McCowan, 2004). Thus, in wild chimpanzees long-distance pant-hoot calls showed higher individual variability than the close range pant-grunt, in which visual contact seems to make acoustic recognition less necessary (Mitani et al., 1996). This suggests that individual vocal signatures are more likely to occur in call types that are produced in absence of visual contact, independent of the presence or absence of a social cohesive context.

A second question was, whether the acoustic structure of a call type determines individual distinctiveness. While environmental factors will ultimately determine the efficiency of vocal transmissions, certain structural properties optimize the ability of a call to transmit information regarding the sender (direction, distance and identity) (Macedonia, 1986). Calls containing narrow frequency bands (‘pure toned’) are inherently better than calls containing broad frequency bands (noisy) for distance propagation and sound localization (Wiley and Richards, 1978; Brown, 1982). Furthermore, narrow-frequency-band calls permit information transmission through frequency modulations (in addition to amplitude modulations), whereas in broad-frequency-band calls information can only be transmitted through amplitude modulations (Wiley and Richards, 1978). Indeed, in rhesus macaques (Rendall et al., 1998), Indian false vampire bats (Bastian and Schmidt, 2008) and banded mongooses (Müller and Manser, 2008) the individual distinctiveness was found to decrease with an increase of noisiness (broad frequency bands) of the tested call type. This implies that narrow-frequency-band and frequency modulated calls are better suited for the coding of individuality than noisy (broad-frequency-band) calls.

Up to date little is known about individual signatures across the vocal repertoires of nocturnal mammals (Rasoloharijaona et al., 2006; Bastian and Schmidt, 2008). To fill this gap we explored for the first time acoustic correlates to individuality across the vocal repertoire of
Chapter 3. Individual signatures in gray mouse lemur vocalizations

a nocturnal, small-bodied, primate species, living in dense forest vegetation, the gray mouse lemur (*Microcebus murinus*). Mouse lemurs live in a dispersed multi-male multi-female system in which females forage solitary during the night, but form temporarily stable sleeping groups during the day (e.g., Radespiel et al., 1998; Radespiel et al., 2001). Mate choice by females is reportedly found, so that individually distinct calls may help to avoid inbreeding (Zimmermann, 1995; Radespiel et al., 2002; Craul et al., 2004). Furthermore, individual or group distinctiveness in calls may help in the coordination of group reunions (e.g., Braune et al., 2005; 2008). Because of the importance of vocalizations for the regulation of group formation and mate choice in this solitary foraging primate, we expected to find individual vocal signatures in gray mouse lemur vocalizations.

In this study, we analyzed the four most common call types of gray mouse lemurs (Zimmermann, 1995). These four call types (grunts, tsaks, short whistles and trills) varied in social context, in presence or absence of visual contact to receiver, and in acoustic structure. The grunt is a noisy (broad-frequency-band) call produced in defensive threat, usually at the sleeping site (Zimmermann, 1995). The grunt consists usually of two parts, the first is louder and higher pitched, the second softer and lower pitched (see Fig. 3.1a). The tsak is a chevron-shaped, narrow-frequency-band, harmonic call (see Fig. 3.1b), produced during agonistic interactions (Zimmermann, 1995). The short whistle is a narrow-frequency-band, harmonic call with a fairly constant fundamental frequency contour (see Fig. 3.1c), used in a context of general disturbance (Zimmermann, 1995). The trill is a narrow-frequency-band, harmonic call with strong frequency modulations used in social cohesion (Zimmermann and Lerch, 1993) (see Fig. 3.1d). In males individual signatures in the trill have already been found (Zimmermann and Lerch, 1993). Based on the contexts in which the call types are usually produced and based on our own observations, we can derive that grunts and tsaks are produced within visual contact to the receiver, while short whistles and trills are usually directed at receivers that are out of visual contact. With respect to our first question, we expected to find that individual distinctiveness of a call type is determined by the context in which this call type is produced, but the two presented hypotheses predict different outcomes. Based on the ‘social cohesion hypothesis’ we expected that trills, used for social cohesion, are more individually distinct compared to short whistles, tsaks and grunts. Based on the ‘visual contact hypothesis’ we expected trills and short whistles to be more individually distinct than tsaks and grunts, which are produced in the presence of visual contact. With respect to our second question, we expected to find that individual distinctiveness of a call type is determined by acoustic structure. Therefore, we expected a higher individual distinctiveness
in the three, narrow-frequency-band, harmonic calls, compared to the broad-frequency-band, noisy call. Between the narrow-frequency-band calls we expected that due to the degree of modulation in the call structure, trills would be most individually distinct, followed by tsaks, which are still more modulated than short whistles.

3.3 Methods

3.3.1 Subjects and housing

We used calls of 44 gray mouse lemurs (*Microcebus murinus*), which were housed at a reversed day-night cycle in the animal facility of the Institute of Zoology, University of Veterinary Medicine Hannover (for details of housing conditions see Wrogemann et al., 2001). All subjects were born in captivity. Their age at the time of recording ranged from seven months to eight years (see Table 3.1). Body weight was measured once every week by the institute staff.

Fig. 3.1: Representative spectrograms of the four different call types, grunt (a), tsak (b), short whistle (c), and trill (d), produced by four different individuals to show the acoustic variation.
Chapter 3. Individual signatures in gray mouse lemur vocalizations

### Table 3.1: Information on the recorded subjects. Some subjects contributed calls of more than one call type to our study.

<table>
<thead>
<tr>
<th>Call type</th>
<th>Sex</th>
<th>Number of subjects</th>
<th>Age range</th>
<th>Weight range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(years)</td>
<td>(grams)</td>
</tr>
<tr>
<td>Grunt</td>
<td>males</td>
<td>10</td>
<td>2-8</td>
<td>58-141</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>10</td>
<td>1-8</td>
<td>78-147</td>
</tr>
<tr>
<td>Tsak</td>
<td>males</td>
<td>10</td>
<td>0-8</td>
<td>62-150</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>10</td>
<td>0-4</td>
<td>65-112</td>
</tr>
<tr>
<td>Short whistle</td>
<td>males</td>
<td>12</td>
<td>1-7</td>
<td>61-104</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>10</td>
<td>0-5</td>
<td>65-93</td>
</tr>
<tr>
<td>Trill</td>
<td>males</td>
<td>10</td>
<td>3-8</td>
<td>60-79</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

#### 3.3.2 Sound recordings

With exception of the grunts, all calls are produced in the ultrasonic range (Zimmermann, 1995). Different recording equipment was used for recording of low frequency (grunts) and ultrasonic call types (tsaks, short whistles and trills).

Grunts (low frequency) were recorded using a microphone (Sennheiser, K6/ME64, Wennebostel, Germany), linked to a pre-amplifier (M-Audio DMP3, Avid Technology, Tewksbury, USA), digitized at a sampling frequency of 92 kHz (A/D converter: AP192K, M-Audio, Hallbergmoos, Germany), and stored as uncompressed audio files in a computer outside the test room. Recordings were made in Batsound Pro (Version 3.31, Pettersson Elektronik AB, Uppsala, Sweden). Grunts were elicited by a startle paradigm, e.g., by grasping with the hand inside the sleeping box.

Ultrasonic call types were recorded on a laptop, either with the recording software Batsound Pro 3.31 or NiDisk (Version 1.33, Engineering design, Berkeley, USA). When the recordings were made with Batsound Pro 3.31, we connected the high frequency output of a bat detector (U30, Ultra Sound Advice, London, UK) via a control-filter unit (Pettersson F2000, Pettersson Elektronik AB, Uppsala, Sweden) to a high-speed A/D card (PCM DAS 16/330, Measurement Computing Corp., USA; 200 kHz sampling frequency with time expansion 10) in a laptop (Thinkpad, IBM, Armonk, USA). When the recordings were made with NiDisk 1.33, the bat detector was connected to a laptop (Toshiba, Tokyo, Japan) equipped with an A/D converter card (DAQCard-6062E, National Instruments, Austin, USA; 200-500 kHz sampling frequency). In order to record, the subjects were placed in test cages (stainless steel cage for marmosets, 80 cm x 87 cm x 50 cm, Ebeco, Castrop-Rauxel, Germany) in a sound-attenuated room. Calls were recorded in a social encounter paradigm during the mating season. Additionally, short whistles were recorded during habituation of a subject on the sound-attenuated room and during predator playback experiments (see Bunkus,
Chapter 3. Individual signatures in gray mouse lemur vocalizations

2006; Sündermann et al., 2008). Additional calls were obtained from a sound database, established by the Zimmermann group at the Institute of Zoology, University of Veterinary Medicine Hannover, Germany.

3.3.3 Acoustic analyses

All calls were inspected spectrographically using Batsound Pro 3.31 and only calls that showed in the power spectrum a minimum difference of 20 dB from the peak of the fundamental frequency to background noise, and that were not overlapped by other calls, were selected for analyses. For sound analyses we selected a sample of 10 calls per subject, for every call type (see Table 3.1). Sound analyses were performed in Signal (Version 4.0, Engineering design, Berkeley, USA). Short whistles, tsaks and grunts are produced in sequences. Therefore the analyses were made from three to four calls (selected from the middle of a sequence) of three different sequences per subject. The frequency and temporal parameters that were measured differed depending on the structure of the call types (see Appendix 3A, 3B and 3C). The sound analysis of trills was modified from Zimmermann and Lerch (1993).

3.3.4 Statistics

A stepwise Discriminant Function Analysis (‘sDFA’; Bortz, 2005) was performed to explore the probability by which calls could be assigned to the respective caller. For parameter reduction we performed two preparatory analyses prior to the sDFA (Bortz, 2005). A significant difference across the individuals in a One-way ANOVA and no strong correlation to other parameters (Pearson correlation coefficient < 0.90) was the criterion for a parameter to be included in the sDFA. The following parameters were then used for the sDFA, for grunts: four temporal, one amplitude, 12 frequency, and four call consistency parameters, for tsaks: four temporal and 13 frequency parameters, for short whistles: four temporal and six frequency parameters, for trills: seven temporal and 17 frequency parameters (see Appendix 3A, 3B and 3C). In addition to the original classification, we also performed a leave-one-out cross-validation in the sDFA (Bortz, 2005). Furthermore, the sDFA selects the parameters according to their distinctiveness. The distribution of correctly and incorrectly classified calls is then tested against a chance distribution in a chi-square test in order to see whether the classification accuracy is significantly higher than chance (e.g., Fischer et al., 1995; Braune et al., 2005; Volodina et al., 2006). In order to compare individual
distinctiveness between call types, we performed an sDFA on equalized sample sizes, i.e. we reduced the sample sizes of grunts, tsaks and short whistles to the sample size of trills (10 males). Subsequently we calculated the kappa value (measurement of agreement), based on the classification of the calls by the sDFA, in order to categorize the calls to a level of agreement (Landis and Koch, 1977).

To explore to which degree parameters coding for individuality are dependent on age or body weight, we took the age and body weight of each subject (at the point of recording) and correlated these to the average structural call parameters used in the sDFA of every call type using a Pearson correlation. To explore to which degree parameters coding for individuality are dependent on sex, we tested for sex differences in the parameters used in the sDFA. For that we performed an independent samples t-test, based on average values of each individual. We used a sequential Bonferroni correction to control for multiple testing (Bortz, 2005). All statistic analyses were performed in SPSS (Version 16.0, SPSS Inc., Chicago, USA).

3.4 Results

We found evidence for individual signatures in all four call types, but the individual distinctiveness differed between the call types. Means and variations of selected parameters for every call type are shown in Table 3.2.

3.4.1 Grunts

According to the sDFA, calls could be assigned to the respective sender with a correct classification of 64.7% (54.3% cross-validated; N=20; Fig. 3.2a). Correct classification was significantly higher than could be expected by chance (5%; original: $\chi^2=1379.410$, df=1, $p<0.001$; cross-validated: $\chi^2=943.323$, df=1, $p<0.001$, N=20). Eight parameters, consisting of one amplitude and one consistency parameter, four temporal and two frequency parameters, were entered by the sDFA (see Appendix 3D). The first entered parameter by the sDFA was the call duration.

Of the eight parameters that coded for individuality one parameter, ‘relative duration of part 1’, was significantly higher in females than in males, after Bonferroni corrections (t-test: F=7.034, p=0.001, N=20). No significant correlations to age or bodyweight were found after Bonferroni corrections.
Table 3.2: Mean values and standard deviations for the acoustic parameters measured across call types.

<table>
<thead>
<tr>
<th>Call type</th>
<th>call dur (ms)</th>
<th>dur max (ms)</th>
<th>peak start (Hz)</th>
<th>Peak max (Hz)</th>
<th>peak end (Hz)</th>
<th>F₀ start (Hz)</th>
<th>F₀ max (Hz)</th>
<th>F₀ end (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grunt</td>
<td>mean 150</td>
<td>-</td>
<td>442</td>
<td>757</td>
<td>904</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>st.dev. 63</td>
<td>-</td>
<td>151</td>
<td>1166</td>
<td>1587</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tsak</td>
<td>mean 30</td>
<td>15</td>
<td>13384</td>
<td>16697</td>
<td>13950</td>
<td>12560</td>
<td>16137</td>
<td>12637</td>
</tr>
<tr>
<td></td>
<td>st.dev. 6</td>
<td>3</td>
<td>2691</td>
<td>3907</td>
<td>3375</td>
<td>2625</td>
<td>3803</td>
<td>2668</td>
</tr>
<tr>
<td>Short whistle</td>
<td>mean 22</td>
<td>-</td>
<td>15844</td>
<td>-</td>
<td>17379</td>
<td>12915</td>
<td>-</td>
<td>13185</td>
</tr>
<tr>
<td></td>
<td>st.dev. 7</td>
<td>-</td>
<td>4393</td>
<td>-</td>
<td>3564</td>
<td>1573</td>
<td>-</td>
<td>1649</td>
</tr>
<tr>
<td>Trill</td>
<td>mean 628</td>
<td>75</td>
<td>24868</td>
<td>29182</td>
<td>16401</td>
<td>21207</td>
<td>29182</td>
<td>13336</td>
</tr>
<tr>
<td></td>
<td>st.dev. 131</td>
<td>16</td>
<td>6822</td>
<td>4563</td>
<td>3439</td>
<td>3680</td>
<td>4563</td>
<td>2079</td>
</tr>
</tbody>
</table>

3.4.2 Tsaks

The sDFA correctly classified 80.5% of the calls (69.5% cross-validated, N=20; Fig. 3.2b), which is significantly higher than predicted by chance (5%; original: $\chi^2=2400.105$, df=1, p< 0.001; cross-validated: $\chi^2=1751.684$, df=1, p<0.001, N=20). Four temporal parameters and five frequency parameters were entered by the sDFA (see Appendix 3D). The $F_0$ max was the first entered parameter by the sDFA.

After applying Bonferroni corrections, one of the nine parameters that coded for individuality according to the sDFA, i.e. ‘duration till maximum’, was significantly longer in females than in males (t-test: F=0.201, p=0.006, N=20). Two of the nine parameters, i.e. ‘call duration’ and ‘duration till maximum’, significantly increased with age (Pearson: call dur: r=0.623, p=0.003; dur max: r=0.629, p=0.003, N=20) and one of the nine parameters, i.e. ‘end peak frequency’, significantly decreased with body weight (Pearson: r=-0.610, p=0.004, N=20).

3.4.3 Short whistles

The sDFA rendered a correct classification of 89.1% (78.2% cross-validated, N=22; Fig. 3.2c), which is significantly higher than by chance (4.5%; original: $\chi^2=3663.146$, df=1, p<0.001; cross-validated: $\chi^2=2779.249$, df=1, p<0.001, N=22). Three temporal and five frequency parameters were entered by this analysis (see Appendix 3D). The sDFA first entered the parameter $F_0$ start. All eight parameters that coded for individuality according to the sDFA did not correlate significantly with age or body weight or differed significantly between sexes after applying a Bonferroni correction.
3.4.4 Trills

The trill represents the call type with the highest individual distinctiveness, according to the sDFA. This analysis correctly classified 100.0% of the calls (95.7% cross-validated, N=10; Fig. 3.2d). The cross-validated classification accuracy was significantly higher than by chance (10%; cross-validated: $\chi^2=749.932$, df=1, $p<0.001$, N=10). The 12 parameters that were entered by the sDFA consisted of four temporal and eight frequency parameters, with $F_0$ start entered first (see Appendix 3D). Age and body weight were not significantly correlated to any of the parameters, after Bonferroni corrections.

Fig. 3.2: Plot of the two first discriminant functions for the four different call types, grunt (a), tsak (b), short whistle (c), and trill (d), based on the sDFA of 10 males. Every individual is indicated by a different symbol.
3.4.5 Comparison of call types

When a sDFA was run on 10 males for each call type, the percentages of correct classified calls were 79.6% (71.4% cross-validated) in grunts, 88.0% (81.0% cross-validated) in tsaks, 90% (86.0% cross-validated) in short whistles, and 100.0% (95.7% cross-validated) in trills. These percentages of correct classified calls correspond to kappa values of 0.77 for grunts, 0.87 for tsaks, 0.89 for short whistles and 1.00 for trills. According to the categorization by Landis and Koch (1977) the strength of agreement is ‘substantial’ (kappa value range: 0.61-0.80) for grunts, and ‘almost perfect’ (kappa value range: 0.81-1.00) for tsaks, short whistles and trills.

3.5 Discussion

We found evidence for individual signatures in all four tested call types. The sDFA was able to detect more individual distinctiveness in the three harmonic calls (trill, short whistle and tsak) than in the noisy call (grunt). We found only a small influence of sex (grunt and tsak, one parameter), age (tsak, two parameters) and body weight (tsak, one parameter) on the coding of individuality in grunts and tsaks.

Although sex, age and body weight have been reported to influence call structure (e.g., Reby and McComb, 2003; Aubin et al., 2007; Ey et al., 2007b) we found that in gray mouse lemurs, the parameters that coded for individuality usually did not code for sex, age or body weight as well. This does not mean, however, that sex, age and bodyweight have no influence on the call structure of mouse lemur vocalizations, since there might be other parameters coding for this. All in all we found that the coding of individuality in the tested call types of the gray mouse lemur was largely independent of sex, age and body weight.

The correct classification was significantly above chance level in every tested call type of the vocal repertoire of the gray mouse lemur. Other studies in birds, non-primate mammals and primates have reported comparable correct DFA classifications (e.g., Fischer et al., 1995; Frommolt et al., 2003; Rukstalis et al., 2003; Wiley, 2005; Aubin et al., 2007). Moreover, our findings for the trill are in line with previous findings by Zimmermann and Lerch (1993) based on a small sample, who found an 89.9% correct classification, also mainly based on the parameter $F_0$ start.
As expected we found that across the vocal repertoire there was a variation in the individual distinctiveness of the tested call types, ranging from 79.6% in grunts to 100% in trills, when tested on an equal sample size. According to the Landis and Koch (1977) categorization the strength of agreement of the grunts is a level lower than for the three harmonic calls. Although Landis and Koch (1977) note that this categorization is only arbitrary, it does enable us to make a direct comparison between the call types. Thus, we can conclude that trills, short whistles and tsaks contain more acoustic correlates of individuality than grunts.

To address our first question, based on the classification accuracy of the sDFA, we did not find that the individual distinctiveness of a call type is determined by the context in which this call type is produced. Thus, we found no support for the ‘social cohesion’ hypothesis, since, according to the sDFA, trills were not clearly more individual distinctive compared to the non-social cohesion calls. Thus, it seems unlikely that individual signatures have evolved purely for social cohesion purposes in gray mouse lemurs. Also, no support was found for the ‘visual contact’ hypothesis, since the individual distinctiveness of tsaks (within visual contact) falls into the same level (almost perfect) as the individual distinctiveness of trills and whistles (out of visual contact).

In defense of the ‘social cohesion’ and ‘visual contact’ hypothesis one can argue that short whistles, tsaks and grunts could also function for recruiting aid from kin, which means that also in tsaks and grunts receivers could be out of visual contact. Vocal recruitment has been reported in several primate species (e.g., Gouzoules et al., 1984; Slocombe and Zuberbühler, 2007). In gray mouse lemurs vocal recruitment was observed in at least one anecdotal case, where threat vocalizations (either grunt or tsak) from a gray mouse lemur that was captured by a snake, attracted (out of view) conspecifics, which then helped the captured animal to escape (Eberle and Kappeler, 2008). However, since this has not been described in any other studies, this might be an extraordinary case. At least grunts seem an unlikely candidate to be used for recruitment, since noisy calls are less suitable for long distance propagation (Wiley and Richards, 1978). On the other hand, Owren and Rendall (2001) suggested that vocal individuality may serve to influence receiver affect by associating the sender’s calls with specific emotional events, experienced by the receiver. This may explain why acoustic correlates of individuality have also been commonly found in close range calls that are not used for social cohesion, in many species (e.g., Owren et al., 1997; Fischer et al., 2002; Gwilliam et al., 2008).
Although we aimed to measure the calls with acoustic parameters that gave a good representation of the vocal structure of the call type, we cannot be sure that we did not overlook some structural parameters that might enable gray mouse lemurs to individually recognize one another. This applies especially to grunts, in which the noisy structure complicated sound analyses. Furthermore, we must note here that the sDFA results give only an indication of the information regarding the callers’ identity that is encoded in a call. Whether all of this information can be perceived by conspecifics in different social contexts and at different distances, still needs to be determined (e.g., Snowdon and Cleveland, 1980; Falls, 1982). So, even though we found that context does not determine the acoustic cues we found to be relevant for coding individuality, it might still affect non-measured acoustic features that may help receivers to recognize different individuals. Therefore, complimentary evidence of discrimination by the animals in question from playback experiments is necessary to gain a clear understanding of the individual recognition system.

With regard to our second question, we found that individual distinctiveness of a call type is determined by the acoustic structure of that call type, since calls with narrow frequency bands (harmonic calls: trills, short whistles and tsaks) are more individually distinctive compared to broad-frequency-band calls (noisy calls: grunts). The results are furthermore in line with similar findings in rhesus macaques (Rendall et al., 1998), Indian false vampire bats (Bastian and Schmidt, 2008) and banded mongooses (Müller and Manser, 2008), where the individual distinctiveness of a call type decreased with an increase of noisiness (broad-frequency-bands). However, in contradiction to our hypothesis, frequency modulations do not seem to shape the individual distinctiveness, since the short whistles, which have an almost constant fundamental frequency contour, were not less individually distinct than the frequency modulated tsaks and trills. Thus, our data supports our hypothesis in its assumption of narrow frequency bands as a principal determinant of the individual distinctiveness of a call type, but not in its assumption of frequency modulation as an additional determinant.

### 3.6 Conclusion

We found evidence for the presence of individual signatures across the vocal repertoire of a small nocturnal mammal, the gray mouse lemur. Individually distinct acoustic
features provide important information that may be used for memorizing individual identity and governing behavioral decisions in a nocturnal primates’ society, in which individuals are often dispersed in space. Furthermore, we found that across the vocal repertoire of the gray mouse lemur narrow-frequency-band trills, short whistles and tsaks were more individually distinctive than the broad-frequency-band grunt, which indicates the importance of narrow frequency bands for coding individuality.

In order for individual recognition to function, it is first necessary that callers produce calls that are characterized by stereotypy within each individual, but vary noticeably among individuals (Falls, 1982). The second requirement is that the intended recipient must be able to discriminate such calls during the recognition process. In this article we were able to show that in the vocal communication system of the gray mouse lemur, the first requirement is met. The second requirement still remains to be tested.

3.7 Acknowledgements

We would like to thank Christian Schopf, Elisabeth Engelke, Melanie Dietz, Carmen Sautter and Esther Bunkus for recording some of the calls. Also, we would like to thank Simone Schehka for helping with the analysis of the grunts. We thank Sabine Schmidt for critical comments. Furthermore we thank R.W. Brüning for technical support, B. Lohmeier and E. Engelke for conducting the weight measurements, and W. Mehl and H.-J. Sauer for animal care. Financial support was given by the Marie Curie Fellowship for Early Stage Research Training under contract no. MEST-CT-2005-021014.
### 3.8 Appendices

#### 3.8.1 Appendix 3A: Measured parameters for grunts

Parameter codes, description and calculations. For the symbols, used in the calculations refer to the spectrogram.

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp.</td>
<td>*call dur&lt;sup&gt;a&lt;/sup&gt;</td>
<td>call duration (ms)</td>
<td>E&lt;sub&gt;2&lt;/sub&gt;-S&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>*dur 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>duration of part 1 of the call (ms)</td>
<td>E&lt;sub&gt;1&lt;/sub&gt;-S&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>*dur 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>duration of part 2 of the call (ms)</td>
<td>E&lt;sub&gt;2&lt;/sub&gt;-S&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>rel dur 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>relative duration part 1 (ms)</td>
<td>dur 1/call dur</td>
</tr>
<tr>
<td></td>
<td>*int 1 to 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>interval between end of part 1 and start of part 2 (ms)</td>
<td>S&lt;sub&gt;2&lt;/sub&gt;-E&lt;sub&gt;1&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>int to next&lt;sup&gt;a&lt;/sup&gt;</td>
<td>interval between end of the measured call and start of the next call (ms)</td>
<td>S&lt;sub&gt;i&lt;/sub&gt;(next)-E&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>Amp.</td>
<td>*rel amp 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>relative amplitude part 1 (dB)</td>
<td>amplitude part 1/ amplitude part 2</td>
</tr>
<tr>
<td>Freq.</td>
<td>*peak part 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest in part 1 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak low part 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>lowest of the two frequencies at which amplitude is highest in part 1 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak high part 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>highest of the two frequencies at which amplitude is highest in part 1 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak start 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at start (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak low start 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>lowest of the two frequencies at which amplitude is highest at start (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak high start 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>highest of the two frequencies at which amplitude is highest at start (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak middle 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at middle point (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak low middle 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>lowest of the two frequencies at which amplitude is highest at middle point (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
### 3.8.1 Appendix 3A: Measured parameters for grunts (Continued)

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>*peak high middle 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>highest of the two frequencies at which amplitude is highest at middle point (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak end 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at end (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak low end 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>lowest of the two frequencies at which amplitude is highest at end (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak high end 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>highest of the two frequencies at which amplitude is highest at end (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak part 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest in part 2 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak start 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at start (5 ms interval) (part2) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak middle 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at middle point (5 ms interval) (part2) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak end 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at end (5 ms interval) (part2) (Hz)</td>
<td></td>
</tr>
<tr>
<td>Cons.</td>
<td>*mean cons1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>mean taken from correlations of successive 5 ms intervals of part 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*var cons 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>variance taken from correlations of successive 5 ms intervals of part 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*mean cons 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>mean taken from correlations of successive 5 ms intervals of part 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*var cons 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>variance taken from correlations of successive 5 ms intervals of part 2</td>
<td></td>
</tr>
</tbody>
</table>

*: used for the sDFA. <sup>a</sup>: based on measurements in the oscillogram, <sup>b</sup>: based on measurements in the power spectrum.
### 3.8.2 Appendix 3B: Measured parameters for tsaks and short whistles

Parameter codes, description and calculations. For the symbols, used in the calculations refer to the spectrograms.

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp.</td>
<td>* †call dur&lt;sup&gt;a&lt;/sup&gt;</td>
<td>call duration (ms)</td>
<td>E-S</td>
</tr>
<tr>
<td></td>
<td>†dur max&lt;sup&gt;c,d&lt;/sup&gt;</td>
<td>duration from start point till maximum point (ms)</td>
<td>M-S</td>
</tr>
<tr>
<td></td>
<td>* †interval&lt;sup&gt;a&lt;/sup&gt;</td>
<td>interval from end of the measured call till start of the next call (ms)</td>
<td>N-E</td>
</tr>
<tr>
<td></td>
<td>* †rep int&lt;sup&gt;a&lt;/sup&gt;</td>
<td>repetition interval (ms): call dur + interval</td>
<td>N-S</td>
</tr>
<tr>
<td></td>
<td>* †rel call dur&lt;sup&gt;a&lt;/sup&gt;</td>
<td>relative call duration (ms)</td>
<td>call dur/ rep int</td>
</tr>
<tr>
<td>Freq.</td>
<td>* †F&lt;sub&gt;0&lt;/sub&gt; start&lt;sup&gt;b&lt;/sup&gt;</td>
<td>lowest peak frequency at start (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>†F&lt;sub&gt;0&lt;/sub&gt; max&lt;sup&gt;d&lt;/sup&gt;</td>
<td>lowest peak frequency at maximum frequency point (5ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>†F&lt;sub&gt;0&lt;/sub&gt; end&lt;sup&gt;b&lt;/sup&gt;</td>
<td>lowest peak frequency at end (5 ms interval)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* †peak start&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at start (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>†peak max&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at maximum frequency point (5ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* †peak end&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at end (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>* †band start&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bandwidth at F&lt;sub&gt;0&lt;/sub&gt; start (Hz)</td>
<td>maxF&lt;sub&gt;0&lt;/sub&gt;(S) - minF&lt;sub&gt;0&lt;/sub&gt;(S)</td>
</tr>
</tbody>
</table>

(continued)
### 3.8.2 Appendix 3B: Measured parameters for tsaks and short whistles (Continued)

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>†band max(bD)</td>
<td>bandwidth at (F_0) max (Hz)</td>
<td>(\text{max}F_0(M))-(\text{min}F_0(M))</td>
<td></td>
</tr>
<tr>
<td>*†band end(b)</td>
<td>bandwidth at (F_0) end (Hz)</td>
<td>(\text{max}F_0(E))-(\text{min}F_0(E))</td>
<td></td>
</tr>
<tr>
<td>†band 1(b)</td>
<td>bandwidth between (F_0) max and (F_0) start (Hz)</td>
<td>(F_0(M)-F_0(S))</td>
<td></td>
</tr>
<tr>
<td>†band 2(b)</td>
<td>bandwidth between (F_0) end and (F_0) max (Hz)</td>
<td>(F_0(E)-F_0(M))</td>
<td></td>
</tr>
<tr>
<td>slope 1(b)</td>
<td>slope (frequency/duration) from (F_0) start till (F_0) max (Hz/ms)</td>
<td>((\text{max}F_0(M)-\text{min}F_0(S))/\text{dur max})</td>
<td></td>
</tr>
<tr>
<td>†slope 2(b)</td>
<td>slope (frequency/duration) from (F_0) max till (F_0) end (Hz/ms)</td>
<td>((\text{max}F_0(E)-\text{min}F_0(M))/\text{(call dur-dur max)})</td>
<td></td>
</tr>
<tr>
<td>*†slope call(b)</td>
<td>slope (frequency/duration) from (F_0) start till (F_0) end (Hz/ms)</td>
<td>((\text{max}F_0(E)-\text{min}F_0(S))/\text{call dur})</td>
<td></td>
</tr>
</tbody>
</table>

*: used for the sDFA for short whistle, †: used for the sDFA for tsak. \(^a\): based on measurements in the oscillogram, \(^b\): based on measurements in the power spectrum, \(^c\): based on measurements in the spectrogram. \(^D\): Only measured in tsak.

### 3.8.3 Appendix 3C: Measured parameters for trills

Parameter codes, description and calculations. For the symbols, used in the calculations refer to the spectrogram.

![Spectrogram Image](image)

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp.</td>
<td>*call dur(^a)</td>
<td>call duration (ms)</td>
<td>E-S</td>
</tr>
<tr>
<td></td>
<td>*dur whistle(^c)</td>
<td>duration from start till turn point (ms)</td>
<td>T-S</td>
</tr>
<tr>
<td></td>
<td>*dur max(^c)</td>
<td>duration from start till maximum point (ms)</td>
<td>M-S</td>
</tr>
<tr>
<td></td>
<td>*dur ascend(^c)</td>
<td>duration from turn point till maximum point (ms)</td>
<td>M-T</td>
</tr>
<tr>
<td></td>
<td>*rel dur max(^a)</td>
<td>relative duration till maximum point (ms)</td>
<td>dur max/call dur</td>
</tr>
<tr>
<td></td>
<td>*mod rate (1-7)(^c)</td>
<td>modulation rate of first 7 modulations (Hz)</td>
<td>(((\text{max}7-\text{min}1)/7)^{-1} \times 1000)</td>
</tr>
<tr>
<td></td>
<td>*mod rate (-3-0)(^f)</td>
<td>modulation rate of last 4 modulations (Hz)</td>
<td>(((\text{max}e-\text{mine}-3)/4)^{-1} \times 1000)</td>
</tr>
<tr>
<td></td>
<td>mod no(^c)</td>
<td>number of modulations</td>
<td></td>
</tr>
<tr>
<td>Freq.</td>
<td>*(F_0) start(^c)</td>
<td>lowest peak frequency at start (5 ms interval) (Hz)</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
### 3.8.3 Appendix 3C: Measured parameters for trills (Continued)

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>F₀ turn</strong>&lt;sup&gt;b&lt;/sup&gt; lowest peak frequency at turn point (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>F₀ max</strong>&lt;sup&gt;b&lt;/sup&gt; lowest peak frequency at maximum point (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td>*F₀ end&lt;sup&gt;b&lt;/sup&gt;</td>
<td>lowest peak frequency at end (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td>*peak start&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at start (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td>*peak turn&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at maximum point (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td>*peak max&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at turn point (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td>peak end&lt;sup&gt;b&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at end (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td>*band start&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bandwidth at F₀ start (Hz)</td>
<td>maxF₀(S)-minF₀(S)</td>
</tr>
<tr>
<td>*band turn&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bandwidth at F₀ turn (Hz)</td>
<td>maxF₀(T)-minF₀(T)</td>
</tr>
<tr>
<td>*band max&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bandwidth at F₀ max (Hz)</td>
<td>maxF₀(M)-minF₀(M)</td>
</tr>
<tr>
<td>*band end&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bandwidth at F₀ end (Hz)</td>
<td>maxF₀(E)-minF₀(E)</td>
</tr>
<tr>
<td>*F₀ min mod(1-7)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>average of the minimum frequencies of 1st till 7th modulation (Hz)</td>
<td>(min1+…+min7)/7</td>
</tr>
<tr>
<td></td>
<td><strong>F₀ max mod(1-7)</strong>&lt;sup&gt;c&lt;/sup&gt; average of the maximum frequencies of 1st till 7th modulation (Hz)</td>
<td>(max1+…+max7)/7</td>
</tr>
<tr>
<td>*band mod(1-7)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>average bandwidth of 1st till 7th modulation (Hz)</td>
<td>F₀ max mod(1-7) - F₀ min mod(1-7)</td>
</tr>
<tr>
<td>*F₀ min mod(-3-0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>average of the minimum frequencies of 3rd to last till last modulation (Hz)</td>
<td>(minE+…+minE-3)/4</td>
</tr>
<tr>
<td></td>
<td><strong>F₀ max mod(-3-0)</strong>&lt;sup&gt;c&lt;/sup&gt; average of the maximum frequencies of 3rd till last modulation (Hz)</td>
<td>(maxE+…+maxE-3)/4</td>
</tr>
<tr>
<td>*band mod(-3-0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>average bandwidth of 3rdt last till last modulation (Hz)</td>
<td>F₀ max mod(-3-0) - F₀ min mod(-3-0)</td>
</tr>
<tr>
<td>*slope call&lt;sup&gt;b&lt;/sup&gt;</td>
<td>slope from F₀ start till F₀ end (Hz/ms)</td>
<td>(F₀(S)-F₀(E))/call dur</td>
</tr>
<tr>
<td>slope whistle&lt;sup&gt;b&lt;/sup&gt;</td>
<td>slope from F₀ start till F₀ turn (Hz/ms)</td>
<td>(F₀(T)-F₀(S))/dur</td>
</tr>
<tr>
<td>*slope ascend&lt;sup&gt;b&lt;/sup&gt;</td>
<td>slope from F₀ turn till F₀ max</td>
<td>(F₀(M)-F₀(T))/dur</td>
</tr>
<tr>
<td>*band 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bandwidth between F₀ max and F₀ start (Hz)</td>
<td>F₀(M)-F₀(S)</td>
</tr>
<tr>
<td>*band 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>bandwidth between F₀ end and F₀ max (Hz)</td>
<td>F₀(E)-F₀(M)</td>
</tr>
</tbody>
</table>

*: used for the sDFA.  
<sup>a</sup>: based on measurements in the oscillogram,  
<sup>b</sup>: based on measurements in the power spectrum,  
<sup>c</sup>: based on measurements in the spectrogram.
### 3.8.4 Appendix 3D: Variables entered by the sDFA for each call type

<table>
<thead>
<tr>
<th>Step</th>
<th>Grunt</th>
<th>Tsak</th>
<th>Short whistle</th>
<th>Trill</th>
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</thead>
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<td>1</td>
<td>49.383</td>
<td>≥0</td>
<td>57.801</td>
<td>≥0</td>
</tr>
<tr>
<td>2</td>
<td>34.298</td>
<td>≥0</td>
<td>32.436</td>
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<td>3</td>
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<td>26.148</td>
<td>≥0</td>
</tr>
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<td>4</td>
<td>20.540</td>
<td>≥0</td>
<td>22.105</td>
<td>≥0</td>
</tr>
<tr>
<td>5</td>
<td>17.416</td>
<td>≥0</td>
<td>19.248</td>
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<td>6</td>
<td>15.205</td>
<td>≥0</td>
<td>17.212</td>
<td>≥0</td>
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<tr>
<td>7</td>
<td>13.586</td>
<td>≥0</td>
<td>15.647</td>
<td>≥0</td>
</tr>
<tr>
<td>8</td>
<td>12.181</td>
<td>≥0</td>
<td>14.269</td>
<td>≥0</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*a*: significant sex difference, *b*: correlated to age, *c*: correlated to body weight
4

Sex differences in the acoustic structure of gray mouse lemur vocalizations

In preparation as short communication for American Journal of Primatology
4.1 Abstract

Sex differences in the acoustic structure of vocalization have been found in diurnal primates. However, not much is known on acoustic sex differences in nocturnal primates. Gray mouse lemurs are small bodied, nocturnal, primates with an elaborate vocal repertoire. In order to study whether the structure of gray mouse lemur vocalizations differs between the sexes, the acoustic structure of three call types (grunts, tsaks and short whistles) was examined. A permutated discriminant function analysis revealed that tsaks and short whistles, but not grunts, are distinctive by sex. Further analyses showed that the sex differences in the acoustic structure of short whistles were linked to age differences, whereas in tsaks sex differences were independent of age and body weight effects. Thus, some mouse lemur vocalizations differ acoustically between sexes, providing a basis for acoustic sex recognition.

4.2 Introduction

Sex differences in the acoustic structure of communication calls might be important for effective reproduction, especially in species that have a dispersed social system, in which finding a potential mate, at the right location and time, is difficult. Differences in the acoustic structure of male and female vocalizations have been reported in non-human primates (e.g., Rendall et al., 2004; Ey et al., 2007a) and in other mammals (e.g., Pfefferle et al., 2007; Grilliot et al., 2009). In primates, so far, vocal sex differences have been found in diurnal primates, with a cohesive social system (for a review see Ey et al., 2007b). However, not much is known yet about sex differences in vocalizations of nocturnal primates, with a dispersed social system. Therefore we explored whether sex differences are present in the acoustic structure of vocalizations of a nocturnal prosimian primate, living in a multi-male multi-female, dispersed social system, the gray mouse lemur. Based on their nocturnal lifestyle, and socioecology (e.g., Radespiel et al., 1998; Radespiel et al., 2001), we expected to find sex differences in the structure of their vocalizations.

Sex differences in the acoustic structure were analyzed in three vocalizations out of the vocal repertoire of the gray mouse lemur; grunts (noisy calls, produced in defensive threat), tsaks (harmonic calls, produced in agonistic contexts) and short whistles, (harmonic calls, produced in contexts of general disturbance; Zimmermann, 1995). These vocalizations have already been found to be individually distinct (see Chapter 3).
4.3 Methods

4.3.1 Subjects and housing

We used calls of 41 gray mouse lemurs (*Microcebus murinus*), which were housed at a reversed day-night cycle in the animal facility of the Institute of Zoology, University of Veterinary Medicine Hannover (for details of housing conditions see Wrogemann et al. 2001). All subjects were born in captivity. Their age at the time of recording ranged from seven months to eight years (see Table 4.1). Body weight was measured once every week by the institute staff.

4.3.2 Sound recordings

With exception of the grunts, all calls are produced in the ultrasonic range (Zimmermann, 1995). Different recording equipment was used for recording of low frequency (grunts) and ultrasonic call types (tsaks, short whistles).

Grunts (low frequency) were recorded using a microphone (Sennheiser, K6/ME64, Wennebostel, Germany), linked to a pre-amplifier (M-Audio DMP3, Avid Technology, Tewksbury, USA), digitized at a sampling frequency of 92 kHz (A/D converter: AP192K, M-Audio, Hallbergmoos, Germany), and stored as uncompressed audio files in a computer outside the test room. Recordings were made in Batsound Pro (Version 3.31, Pettersson Elektronik AB, Uppsala, Sweden). Grunts were elicited by a startle paradigm, e.g., by grasping with the hand inside the sleeping box.

Ultrasonic call types were recorded on a laptop, either with the recording software Batsound Pro 3.31 or NiDisk (Version 1.33, Engineering design, Berkeley, USA). When the recordings were made with Batsound Pro 3.31, we connected the high frequency output of a bat detector (U30, Ultra Sound Advice, London, UK) via a control-filter unit (Pettersson F2000, Pettersson Elektronik AB, Uppsala, Sweden) to a high-speed A/D card (PCM DAS 16/330, Measurement Computing Corp., USA; 200 kHz sampling frequency with time expansion 10) in a laptop (Thinkpad, IBM, Armonk, USA). When the recordings were made with NiDisk 1.33, the bat detector was connected to a laptop (Toshiba, Tokyo, Japan) equipped with an A/D converter card (DAQCard-6062E, National Instruments, Austin, USA; 200-500 kHz sampling frequency). In order to record, the subjects were placed in test cages (stainless steel cage for marmosets, 80 cm x 87 cm x 50 cm, Ebeco, Castrop-Rauxel,
Germany) in a sound-attenuated room. Calls were recorded in a social encounter paradigm during the mating season. Additionally, short whistles were recorded during habituation of a subject on the sound-attenuated room and during predator playback experiments (see Bunkus, 2006; Sündermann et al., 2008). Further calls were obtained from a sound database, established by the Zimmermann group at the Institute of Zoology, University of Veterinary Medicine Hannover, Germany.

### 4.3.3 Acoustic analyses

We analyzed the acoustic structure of three call types of the gray mouse lemur vocal repertoire, grunts, tsaks and short whistles. In the study of Leliveld et al. (chapter 3), also trills were analyzed on individual signatures. In the present study, however, not enough trills from female senders were available for a reliable study on sex differences in this call type.

All calls were inspected spectrographically using Batsound Pro 3.31 and only calls that showed in the power spectrum a minimum difference of 20 dB from the peak of the fundamental frequency to background noise, and that were not overlapped by other calls, were selected for analyses. For sound analyses we selected a sample of 10 calls per subject, for every call type (see Table 4.1). Sound analyses were performed in Signal (Version 4.0, Engineering design, Berkeley, USA). Short whistles, tsaks and grunts are produced in sequences. Therefore the analyses were made from three to four calls (selected from the middle of a sequence) of three different sequences per subject. The frequency and temporal parameters that were measured differed depending on the structure of the call types (see Appendix 3A and 3B).

<table>
<thead>
<tr>
<th>Call type</th>
<th>Sex</th>
<th>Number of subjects</th>
<th>Age range (years)</th>
<th>Weight range (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grunt</td>
<td>males</td>
<td>10</td>
<td>2-8</td>
<td>58-141</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>10</td>
<td>1-8</td>
<td>78-147</td>
</tr>
<tr>
<td>Tsak</td>
<td>males</td>
<td>10</td>
<td>0-8</td>
<td>62-150</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>10</td>
<td>0-4</td>
<td>65-112</td>
</tr>
<tr>
<td>Short whistle</td>
<td>males</td>
<td>12</td>
<td>1-7</td>
<td>61-104</td>
</tr>
<tr>
<td></td>
<td>females</td>
<td>10</td>
<td>0-5</td>
<td>65-93</td>
</tr>
</tbody>
</table>

Table 4.1: Information on the recorded subjects. Some subjects contributed calls of more than one call type to our study.
4.3.4 Statistics

A nested Permutated Discriminant Function Analysis (PDFA) was performed (Mundry and Sommer, 2007) to explore the probability by which calls could be assigned to the respective caller. Because the two sex groups were based on more than one data point per individual, this had to be controlled for by nesting the calls per subject. This was possible with the PDFA, which is performed in R (R Development Core Team, 2008). For parameter reduction we performed two preparatory analyses prior to the PDFA (Bortz, 2005). A significant difference between the sexes in an independent samples t-test and no strong correlation to other parameters (Pearson correlation coefficient < 0.90) was the criterion for a parameter to be included in the PDFA. The following parameters were then used for the PDFA, for grunts: one temporal, one amplitude, two frequency, and one call consistency parameter, for tsaks: three temporal and two frequency parameters, for short whistles: one frequency parameter (see Appendix 4A and 4B). To test for sex differences in the call structure, we performed first a PDFA. Subsequently, a stepwise DFA was performed to assess the main parameters that contribute to a reliable sex differentiation.

To explore to which degree parameters that code for sex are dependent on age or body weight, we took the age and body weight of each subject (at the point of recording) and correlated these to the mean of the parameters that were used by the stepwise DFA, for each call type, using a Pearson correlation. We used a sequential Bonferroni correction to control for multiple testing (Bortz, 2005). Apart from the PDFA, all analyses were performed in SPSS (Version 16.0, SPSS Inc., Chicago, USA).

4.4 Results

In grunts, sex differences were found in five parameters, but no reliable sex distinction could be found by the PDFA, as the cross-validation classification accuracy was not significantly higher than chance (PDFA: original: 57.5%, cross-validated: 70%; \( \chi^2 \): original: 4.500, \( P=0.034 \), \( N=20 \), cross-validated: 0.800, \( P=0.371 \), \( N=20 \)).

In tsaks, significant sex differences were found in five parameters. The nested PDFA could make a reliable distinction between the calls of the two sexes (PDFA: original: 77.5%, cross-validated: 80.0%, \( N=20 \); \( \chi^2 \): original: 60.500, \( P<0.001 \); cross-validated: 7.200, \( P=0.007 \), \( N=20 \)), based on two (of the five) parameters, ‘relative call duration’ and ‘duration till maximum’. The values of both parameters were higher in males (Fig. 4.1). After applying
Bonferroni corrections, one of the two parameters that coded for sex, according to the stepwise DFA, i.e. ‘duration till maximum’, significantly increased with age (Pearson: r=0.629, p=0.003, N=20). However, in this sample no significant sex differences in age were found (t-test: F=5.798, p=0.238, N=20)

In short whistles only one parameter was included in the nested PDFA, which gave classification accuracies that were significantly above chance (PDFA: original: 78.2%, cross-validated: 68.2%, N=22; χ²: original: 2779.249, P<0.001; cross-validated: 207.605, P<0.001, N=22). This parameter, ‘F₀ start’, was significantly higher in females than in males (Fig. 4.2). ‘F₀ start’ correlated significantly negatively with age (Pearson: r=-0.494, p=0.019, N=22). And in this sample males and females significantly differed in age (t-test: F=1.570, p=0.001, N=22), females being younger than males. No significant correlations to body weight were found.

Fig 4.1: comparison of male and female tsaks in the ‘duration till maximum’ (a), and ‘relative call duration (b)

Fig 4.2: comparison of male and female short whistles in the ‘F₀ start’
4.5 Discussion

Our results suggest that the acoustic structure of short whistles and tsaks differ between sexes in gray mouse lemurs. However, no sex differences were found in the acoustic structure of grunts. Although one of the two parameters that coded for sex in tsaks was found to increase with age, no significant differences were found in age between males and females. Therefore, these sex differences cannot be explained by differences in age. In contrast, in short whistles, age was negatively correlated to the fundamental start frequency and also significantly different between males and females. Thus, it is unclear whether the higher frequency values of females in short whistles (see Fig. 4.2), is an effect of sex or of age. Body weight was not correlated to any of the parameters that coded for sex in both short whistles and tsaks. Therefore, our findings indicate that in gray mouse lemurs, acoustic sexual dimorphism is independent of age and body weight effects in tsaks, but is correlated to age in short whistles.

Our findings of sex differences in the acoustic structure of vocalizations in this small bodied, nocturnal prosimian are in line with previous findings in other prosimians (Rasoloharijaona et al., 2006), anthropoid primates (for review see Ey et al., 2007b) and other mammals (e.g., Sousa-Lima et al., 2002; Pfefferle et al., 2007). In most of these species, however, males and females also differ in body size. In contradiction, in mouse lemurs no great sexual dimorphism in body size/weight exists (Zimmermann et al., 1998). In species that have no sexual dimorphism in visual appearance, sexual dimorphism in acoustic appearance might play an important role in mate choice and territory defense against same sex conspecifics (Morton, 1996). Thus, acoustic sexual dimorphism has been found in this visually monomorphic species. This acoustic sex dimorphism might be a predisposition, still present in anthropoid primates, in which this acoustic sex dimorphism is then strengthened by their body size dimorphism.

In this study grunts were found to be not distinctive by the sex of the sender. In a previous study on individual signatures in gray mouse lemur vocalizations, grunts were also found to be less individually distinct than tsaks and short whistles (chapter 3). This was explained by the noisy structure of the grunt, which makes this call type less suited for transmitting information regarding the sender (Macedonia, 1986). Therefore, the lack of sex distinctiveness in the grunt that we found here provides further support for the assumption that narrow frequency bands play an important role in the transmission of information regarding the sender (Macedonia, 1986).
To conclude, we found evidence of sexual dimorphism in the acoustic structure of some, but not all, call types of the vocal repertoire of the gray mouse lemur. Sexually distinctive vocalizations provide important information that may facilitate the finding of a potential mate for this socially dispersed, nocturnal primate species.

4.6 Acknowledgements

We would like to thank Christian Schopf, Elisabeth Engelke, Melanie Dietz, Carmen Sautter and Esther Bunkus for recording some of the calls. Also, we would like to thank Simone Schehka for helping with the analysis of the grunts. Furthermore we thank R.W. Brüning for technical support, B. Lohmeier and E. Engelke for conducting the weight measurements, and W. Mehl and H.-J. Sauer for animal care. Financial support was given by the Marie Curie Fellowship for Early Stage Research Training under contract no. MEST-CT-2005-021014.
4.7 Appendices

4.7.1 Appendix 4A: Measured parameters for grunts

Parameter codes, description and calculations. For the symbols, used in the calculations refer to the spectrogram.

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp.</td>
<td>call dur$^a$</td>
<td>call duration (ms)</td>
<td>$E_2 - S_1$</td>
</tr>
<tr>
<td></td>
<td>dur 1$^a$</td>
<td>duration of part 1 of the call (ms)</td>
<td>$E_1 - S_1$</td>
</tr>
<tr>
<td></td>
<td>dur 2$^a$</td>
<td>duration of part 2 of the call (ms)</td>
<td>$E_2 - S_2$</td>
</tr>
<tr>
<td></td>
<td>*rel dur 1$^a$</td>
<td>relative duration part 1 (ms)</td>
<td>$dur 1/call dur$</td>
</tr>
<tr>
<td></td>
<td>int 1 to 2$^a$</td>
<td>interval between end of part 1 and start of part 2 (ms)</td>
<td>$S_2 - E_1$</td>
</tr>
<tr>
<td></td>
<td>int to next$^a$</td>
<td>interval between end of the measured call and start of the next call (ms)</td>
<td>$S_1(next) - E_2$</td>
</tr>
<tr>
<td>Amp.</td>
<td>*rel amp 1$^a$</td>
<td>relative amplitude part 1 (dB)</td>
<td>amplitude part 1/ amplitude part 2</td>
</tr>
<tr>
<td>Freq.</td>
<td>peak part 1$^b$</td>
<td>frequency at which amplitude is highest in part 1 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak low part 1$^b$</td>
<td>lowest of the two frequencies at which amplitude is highest in part 1 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak high part 1$^b$</td>
<td>highest of the two frequencies at which amplitude is highest in part 1 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak start 1$^b$</td>
<td>frequency at which amplitude is highest at start (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak low start 1$^b$</td>
<td>lowest of the two frequencies at which amplitude is highest at start (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak high start 1$^b$</td>
<td>highest of the two frequencies at which amplitude is highest at start (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak middle 1$^b$</td>
<td>frequency at which amplitude is highest at middle point (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak low middle 1$^b$</td>
<td>lowest of the two frequencies at which amplitude is highest at middle point (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
### 4.7.1 Appendix 4A: Measured parameters for grunts (Continued)

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<th>Description</th>
<th>Calculation</th>
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<td></td>
<td>peak high middle 1(^b)</td>
<td>highest of the two frequencies at which amplitude is highest at middle point (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak end 1(^b)</td>
<td>frequency at which amplitude is highest at end (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak low end 1(^b)</td>
<td>lowest of the two frequencies at which amplitude is highest at end (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak high end 1(^b)</td>
<td>highest of the two frequencies at which amplitude is highest at end (5 ms interval) (part1) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak part 2(^b)</td>
<td>frequency at which amplitude is highest in part 2 (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak start 2(^b)</td>
<td>frequency at which amplitude is highest at start (5 ms interval) (part2) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak middle 2(^b)</td>
<td>frequency at which amplitude is highest at middle point (5 ms interval) (part2) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*peak end 2(^b)</td>
<td>frequency at which amplitude is highest at end (5 ms interval) (part2) (Hz)</td>
<td></td>
</tr>
<tr>
<td>Cons.*</td>
<td>*mean cons1(^b)</td>
<td>mean taken from correlations of successive 5 ms intervals of part 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>var cons 1(^b)</td>
<td>variance taken from correlations of successive 5 ms intervals of part 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mean cons 2(^b)</td>
<td>mean taken from correlations of successive 5 ms intervals of part 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>var cons 2(^b)</td>
<td>variance taken from correlations of successive 5 ms intervals of part 2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\): used for the PDFA. \(^b\): based on measurements in the oscillogram. \(^b\): based on measurements in the power spectrum.
4.7.2 Appendix 4B: Measured parameters for tsaks and short whistles

Parameter codes, description and calculations. For the symbols, used in the calculations refer to the spectrograms.

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<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
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<tbody>
<tr>
<td>Temp.</td>
<td>† call dur&lt;sup&gt;a&lt;/sup&gt;</td>
<td>call duration (ms)</td>
<td>E-S</td>
</tr>
<tr>
<td></td>
<td>† dur max&lt;sup&gt;‡D&lt;/sup&gt;</td>
<td>duration from start point till maximum point (ms)</td>
<td>M-S</td>
</tr>
<tr>
<td></td>
<td>interval&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>interval from end of the measured call till start of the next call (ms)</td>
<td>N-E</td>
</tr>
<tr>
<td></td>
<td>rep int&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>repetition interval (ms): call dur + interval</td>
<td>N-S</td>
</tr>
<tr>
<td>Freq.</td>
<td>† rel call dur&lt;sup&gt;a&lt;/sup&gt;</td>
<td>relative call duration (ms)</td>
<td>call dur/rep int</td>
</tr>
<tr>
<td></td>
<td>*F&lt;sub&gt;0&lt;/sub&gt; start&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>lowest peak frequency at start (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*F&lt;sub&gt;0&lt;/sub&gt; max&lt;sup&gt;‡D&lt;/sup&gt;</td>
<td>lowest peak frequency at maximum frequency point (5ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F&lt;sub&gt;0&lt;/sub&gt; end&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>lowest peak frequency at end (5 ms interval)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak start&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at start (5 ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak max&lt;sup&gt;‡D&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at maximum frequency point (5ms interval) (Hz)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>peak end&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>frequency at which amplitude is highest at end (5 ms interval) (Hz)</td>
<td></td>
</tr>
</tbody>
</table>

(continued)
### Chapter 4. Sex differences in the acoustic structure

#### 4.7.2 Appendix 4B: Measured parameters for tsaks and short whistles (Continued)

<table>
<thead>
<tr>
<th>Type</th>
<th>Parameter</th>
<th>Description</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>band start(\textsuperscript{a})</td>
<td>bandwidth at (F_0) start (Hz)</td>
<td>[\text{max}F_0(S) - \text{min}F_0(S)]</td>
<td></td>
</tr>
<tr>
<td>band max(\textsuperscript{bd})</td>
<td>bandwidth at (F_0) max (Hz)</td>
<td>[\text{max}F_0(M) - \text{min}F_0(M)]</td>
<td></td>
</tr>
<tr>
<td>†band end(\textsuperscript{b})</td>
<td>bandwidth at (F_0) end (Hz)</td>
<td>[\text{max}F_0(E) - \text{min}F_0(E)]</td>
<td></td>
</tr>
<tr>
<td>band 1(\textsuperscript{nd})</td>
<td>bandwidth between (F_0) max and (F_0) start (Hz)</td>
<td>[F_0(M) - F_0(S)]</td>
<td></td>
</tr>
<tr>
<td>band 2(\textsuperscript{bd})</td>
<td>bandwidth between (F_0) end and (F_0) max (Hz)</td>
<td>[F_0(E) - F_0(M)]</td>
<td></td>
</tr>
<tr>
<td>slope 1(\textsuperscript{bd})</td>
<td>slope (frequency/duration) from (F_0) start till (F_0) max (Hz/ms)</td>
<td>[\frac{(F_0(M) - F_0(S))}{\text{call dur}}]</td>
<td></td>
</tr>
<tr>
<td>slope 2(\textsuperscript{bd})</td>
<td>slope (frequency/duration) from (F_0) max till (F_0) end (Hz/ms)</td>
<td>[\frac{(F_0(E) - F_0(M))}{\text{call dur-dur max}}]</td>
<td></td>
</tr>
<tr>
<td>†slope call(\textsuperscript{b})</td>
<td>slope (frequency/duration) from (F_0) start till (F_0) end (Hz/ms)</td>
<td>[\frac{(F_0(E) - F_0(S))}{\text{call dur}}]</td>
<td></td>
</tr>
</tbody>
</table>

*: used for the PDFA for short whistle, †: used for the PDFA for tsak. \(\textsuperscript{a}\): based on measurements in the oscillogram, \(\textsuperscript{b}\): based on measurements in the power spectrum, \(\textsuperscript{c}\): based on measurements in the spectrogram, \(\textsuperscript{d}\): Only measured in tsak.
5

Effects of caller characteristics on orienting asymmetries to communication calls in an early primate (*Microcebus murinus*)

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5.1 Abstract

Auditory lateralization is suggested to be characterized by a left hemisphere dominance for the processing of conspecific communication. Nevertheless, there are indications that auditory lateralization can also be affected by emotional processing and social recognition. In order to gain insight into the effects of caller characteristics on auditory lateralization, 17 gray mouse lemurs were tested in a head turn paradigm. The head turn paradigm was established to examine potential functional hemispheric asymmetries on the behavioral level. Subjects were presented with playbacks of two conspecific call types (tsak calls and trill calls) from senders, differing in familiarity (unfamiliar vs. familiar) and sex (same sex vs. other sex). Based on the head turn direction towards these calls, evidence was found for a right ear/left hemisphere dominance for the processing of calls of the other sex. Familiarity had no effect on the orientation biases. Nevertheless we found that gray mouse lemurs were able to recognize both familiarity and sex of the sender based on some of the vocalizations, as indicated by a different response behavior. The findings in this study support the growing consensus that auditory lateralization is not only determined by the processing of conspecific communication, but also by other factors like the sex of the sender.

5.2 Introduction

In the last hundred years cerebral lateralization in the processing of language has received much attention and studies have found support for the existence of a left hemisphere dominance in the processing of language in humans (e.g., Ecklund-Flores and Turkewitz, 1996; Belin et al., 1998; Bethmann et al., 2007). This predisposition of the left hemisphere for the processing of language was suggested to arise from a pre-linguistic advantage of the left hemisphere for processing information with a high temporal precision (Belin et al., 1998), whereas the right hemisphere has an advantage for pitch perception (e.g., Zatorre, 1988; Warrier and Zatorre, 2004). However, there are several indications that other factors such as communicative significance, emotional valence, and familiarity with the speaker, affect lateralized auditory processing.

The communicative significance of a sound has been found to be a pre-requisite for left hemisphere dominance in auditory processing in several studies. For instance, a left hemisphere dominance has been shown for the processing of meaningful words, but not for meaningless (unknown) words (Pulvermüller, et al. 2001; Yasin, 2007).
The emotional valence hypothesis suggests that the left hemisphere is specialized in processing positive/approach emotions, while the right hemisphere is specialized in processing negative/withdrawal emotions (e.g., Davidson, 1995; Craig, 2005). For instance, Altenmüller et al. (2002) found a left hemisphere dominance when subjects were listening to a music piece that they had attributed positive emotions to, indicating an effect of emotional valence on auditory processing.

Finally, also familiarity of the speaker has been found to affect the processing of conspecific communication sounds (for a review see Brancucci et al., 2009). In humans, paralinguistic information processing, such as voice recognition is suggested to rely on other neural substrates than speech processing, and it is more likely to be shared between humans and non-human primates (Belin, 2006). Voice recognition was found to be lateralized, with some studies showing a right hemisphere dominance (Van Lancker and Kreiman, 1987) and some studies showing a left hemisphere dominance for familiar voice discrimination (Basile et al., 2009). In addition, some studies found a left hemisphere dominance for unfamiliar voice discrimination (Doehring and Bartholomeus, 1971; Kreiman and Van Lancker, 1988).

The assumption that the lateralized processing of conspecific communication is unique to humans (e.g., Zatorre et al., 2002), is challenged by findings of left hemisphere dominance in the processing of conspecific communication sounds in other animal species, such as rhesus macaques (Hauser and Andersson, 1994; Hauser et al., 1998; Ghazanfar et al., 2001; Poremba et al., 2004), Japanese macaques (Petersen et al., 1978; Beecher et al., 1979; Petersen et al., 1984; Heffner and Heffner, 1986) and sea lions (Böye et al., 2005). This left hemisphere dominance was, like in humans, also explained by its specialization for processing temporal cues (Hauser et al., 1998; Ghazanfar et al., 2001).

However, also in non-human animals there are indications that auditory lateralization of conspecific communication is affected by factors, such as communicative significance, emotional valence, and familiarity with the sender. Communicative significance was first suggested as an important pre-requisite for a left hemisphere dominance by Petersen and colleagues (Petersen et al., 1978; Beecher et al., 1979; Petersen et al., 1984), based on findings that Japanese macaques perceived their conspecific calls with a left hemisphere advantage, whereas other old world monkey species (controls) showed no ear advantage. Also, Ehret (1987) found that mother mice showed a left hemisphere bias for the recognition of pup calls, whereas virgin females that had been exposed to the pup calls in a non-social context did not. Finally, Palleroni and Hauser (2003) found that raptors, that have hunted
howler monkeys, showed a left hemisphere dominance for the processing of howler monkey calls, whereas raptors that never hunted howler monkeys (but have been exposed to their calls) showed a right hemisphere dominance for these calls (Palleroni and Hauser, 2003). All these findings suggest that a communicative significance, achieved through exposure to calls in a meaningful context, is essential for establishing a left hemisphere dominance in the processing of these calls.

Although the lateralized processing of emotion is a well-studied subject in humans (for reviews see Davidson, 1995; Demaree et al., 2005), not many studies have focused on this in non-human vertebrates. Nevertheless, there are a few studies in non-human animals that found an effect of emotional valence on auditory lateralization. For instance, in dogs, leftward turning towards different playback stimuli (conspecific and thunderstorm) was significantly positively correlated to a behavioral index of fear and emotional state, indicating a right hemisphere dominance for the processing of these stimuli (Siniscalchi et al., 2008). On the other hand, Scheumann and Zimmermann (2008) found that male gray mouse lemurs showed a right ear/left hemisphere dominance, which was restricted to conspecific calls of negative emotional valence.

Visual perception of familiar conspecifics is found to be lateralized to the right hemisphere in several vertebrate species, such as domestic fowl (e.g., Vallortigara and Andrew, 1991; Deng and Rogers, 2002), quails (Zucca and Sovrano, 2008), and sheep (Peirce et al., 2000). In contrast, in auditory perception only a few studies tested for lateralized processing of familiar conspecifics (here referred to as ‘familiarity-to-sender effect’). In a study on starlings, George et al., (2004) found that the processing of songs of familiar conspecifics was lateralized to the right hemisphere, which suggests a similar lateralized processing of familiar conspecifics in auditory and visual modalities. However, the findings in auditory lateralization are contradictory, because also a left hemisphere dominance was found in horses (Basile et al., 2009a) and zebra finches (Cynx et al., 1992).

Since both humans and several non-primate species show this ‘familiarity-to-sender’ effect, one would expect to find it also in non-human primates. Indeed, recently a voice recognition region has been identified in the primate brain, located in the middle of the anterior superior-temporal plane (Petkov et al., 2008). This suggests that voice recognition in non-human primates relies on similar neural substrates as in humans. Moreover, many primate species have shown to be able to discriminate between familiar and unfamiliar individuals, based on their vocalizations (e.g., Rukstalis and French, 2005; Sproul et al., 2006). Indeed, recently Basile et al. (2009b) found that Campbell’s monkeys showed a left
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Hemisphere dominance exclusively for the processing of calls of familiar senders. However, the unfamiliar subjects in this study were also heterospecific, and therefore it is unclear whether the familiarity or conspecificity of the sender had an effect on the lateralized processing of these calls. Adversely, based on conspecific calls, Gill-da.Costa and Hauser (2006) did not find a ‘familiarity-to-sender effect’ on auditory lateralization in vervet monkeys. Because of these contradictory findings in humans, non-human primates and other vertebrate species, there is a need for more studies on the ‘familiarity-to-sender effect’ on auditory lateralization. Therefore, the first aim of our study is to explore the ‘familiarity-to-sender effect’ on the lateralized processing of conspecific communication sounds in a prosimian primate, the gray mouse lemur, which is suggested to represent the most ancestral primate condition (Martin, 1972).

The second aim of our study is to explore the effect of another characteristic of the caller on lateralized processing of communication sounds, namely its sex, here referred to as the ‘sex-of-sender effect’. As far as we know, no study has so far compared the lateralized auditory processing of sounds of male and female senders in non-human animals, and only one study tested this in humans. In humans, Landis et al. (1982) reported a right ear advantage for the recognition of female voices and a left ear advantage for the recognition of male voices, indicating that the sex of the sender could effect the lateralized processing of the sound, at least in humans. In non-human animals, a ‘sex-of-sender effect’ on auditory lateralization can be expected, based on the assumption that calls from males and females are of different emotional valence to the receiver, especially in the context of courtship. Indeed, lateralized behavior has been found in courtship approach, but with contradicting findings, since male black winged silts prefer to use the left eye in courtship behavior (Ventolini et al., 2005), whereas males in poeciliid fish approach females with a right eye preference (Bisazza et al., 1997).

In addition, studies have found that males and females respond differently to calls, depending on the sex of the caller in non-human primates (e.g., Masataka, 1987; Hauser, 2007; Smith et al., 2009) and other vertebrates (e.g., Yamaguchi, 1998; Kazial and Masters, 2004). These findings indicate not only that the sex of the caller could be recognized from their calls, but also that male and female calls represent different emotional valence to the receiver, as shown in the different behavioral responses after playback of these calls.
In this study we investigated for the first time the effect of sender characteristics (familiarity and sex) on the lateralized auditory processing of communication calls in an early primate. Our model species is the gray mouse lemur (*Microcebus murinus*), a small bodied nocturnal prosimian species, endemic to Madagascar. Mouse lemurs have an elaborate vocal repertoire with both low frequency and ultrasonic communication calls (Zimmermann, 1995). In a previous study on auditory lateralization in this species Scheumann and Zimmermann (2008) already found that auditory lateralization of male gray mouse lemurs varied between call types of different emotional valence. This species is therefore ideal to further explore the mechanisms that affect the lateralized auditory processing of conspecific communication calls.

Many of the studies that explored auditory lateralization in non-human animals have used a so-called head turn paradigm, developed by Hauser and Andersson (1994). In this paradigm a sound is played back to a subject from a loudspeaker that is placed 180 degrees behind the subject. It is assumed that turning one ear towards the source of the sound causes an increase in the intensity of the signal at that ear and consequently an auditory-input bias to the contralateral hemisphere (Hauser et al., 1998). Hence, turning the right ear towards the sound indicates a left hemisphere dominance for the processing of this sound, whereas turning the left ear indicates a right hemisphere dominance for the processing of this sound.

In order to explore the effect of caller characteristics on auditory lateralization in gray mouse lemurs, we studied the orienting asymmetries in both male and female gray mouse lemurs in response to playbacks of two conspecific call types, the tsak call (used in agonistic contexts) and the trill call (used in social cohesion contexts). Both call types were found to be distinctive by caller (see chapter 3), enabling the receiver to individually recognize the sender. Sex differences in the call structure have not yet been studied in the trill call, but in the tsak call sex differences have been found (see chapter 4). Therefore, we expect to find that mouse lemurs are able to discriminate between familiar and unfamiliar, and between male and female senders based on their vocalizations. In the study by Scheumann and Zimmermann (2008) male mouse lemurs showed a right orientation bias towards tsak calls, but no orientation bias towards trill calls. However, only calls from unfamiliar female senders were used for the playbacks. Therefore, we wanted to test in this study whether caller-specific information (sex and familiarity), coded in the calls, might have affected this orientation bias. Accordingly, this study includes calls from unfamiliar males, unfamiliar females and of
familiar, same sex conspecifics (cage mate). In order to test for a familiarity-to-sender effect, we compared the responses of the subjects to calls (trill calls and tsak calls separately) of familiar, same sex, conspecifics with the response to calls of unfamiliar, same sex conspecifics (see Fig. 5.1). In order to test for sex-of-sender effect, we compared the responses of the subjects to calls (trill calls and tsak calls separately) of unfamiliar, other sex, conspecifics with the response to calls of unfamiliar, same sex, conspecifics (Fig. 5.1). Based on the majority of studies that indicated a right hemisphere dominance for the perception of familiar conspecifics, we expected to find a familiarity-of-sender effect, causing a left ear/right hemisphere dominance for processing calls of the familiar, same sex conspecific in the tsak calls, as well as in the trill calls. Furthermore, based on the previous findings of Scheumann and Zimmermann (2008) we expected that sex-specific information coded in the calls might have affected the sex-specific auditory lateralization that was found previously. Therefore we expect to find a sex-of-sender effect on orientation biases, mainly in tsak calls.

5.3 Material and Methods

5.3.1 Subjects

We tested 17 gray mouse lemurs (12 males, five females) of our breeding colony, housed in the animal facility of the Institute of Zoology, University of Veterinary Medicine Hannover (for details in housing conditions see Wrogemann et al., 2001). All subjects were born in captivity. Their age ranged from nine months to eight years. 15 subjects were housed together with one or two other individuals of the same sex at the time of testing. Two subjects had been separated from their (same sex) cage mate shortly before the start of the experiment.

![Diagram](image.png)

**Fig. 5.1:** Schedule of the different playback stimuli, and how they are compared to test the effects of familiarity-to-sender and sex-of-sender.
5.3.2 Experimental set-up

Each mouse lemur was tested alone in a test cage (Ebecco stainless steel cage for marmosets, 80 cm x 87 cm x 50 cm) in a sound-attenuated chamber. The cage was equipped with two wooden bars, a nest box and a bottle with banana-peach juice. A loudspeaker was placed 180° on the opposite side of the nipple of the juice-bottle (Fig. 5.2). To control for an effect of the nest box, it was placed either on the right (eight subjects) or the left (nine subjects) side of the cage. The playback stimuli were played back using the software NiDisk 1.33 by a Toshiba laptop equipped with a D/A converter card (National Instruments). The laptop was connected via an amplifier (Pioneer a-337) to a high frequency loudspeaker (Panasonic Leaf Tweeter EAS-Th400A, frequency range: 2–70 kHz). Subjects’ behavior was videotaped using a digital camcorder (Sony DR-TRV 22E PAL mini DVD/Sony DCR-SR35E, Nightshoot). When using the Sony DR-TRV 22E PAL, the camera was linked to the tape output of a U-30 bat detector (Ultra Sound Advice) as external microphone. The camera was connected to a monitor outside the chamber where the experimenter sat and observed the subjects.

5.3.3 Playback stimuli

The playback stimuli were created from calls that were recorded from captive gray mouse lemurs of our breeding colony. Playback stimuli differed on three different levels: (1) call type (trill call vs. tsak call), (2) familiarity (familiar vs. unfamiliar sender), and (3) sex (same sex vs. other sex sender), creating six categories of playback stimuli (Fig. 5.1). An unfamiliar sender is defined here as a conspecific that was never housed in the same room at the same time as the subject. A familiar sender is here defined as a conspecific that was housed in the same cage as the subject at the time of testing, or had been separated from the subject no longer than six months before the testing started. Due to these specific requirements concerning the sender of the acoustic stimulus, we created a different playback stimuli set for each subject.

An experimental trial consisted of the presentation of a playback stimulus. All playback stimuli consisted of a sequence of three sounds separated by a constant interval of 3600 ms. The duration of the tsak call sequences was standardized to the duration of the trill call (of each playback category, for each subject) as the longest continuous sound element. All acoustic stimuli were diffused with a sound pressure level of 75 ± 1 dB at a distance of 1 m (RMS measurement, Brüel und Kjær Measuring Amplifier Type 2610).
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5.3.4 Procedure

Each experiment was conducted at the beginning of the activity period of each subject. For the experiment a subject was removed from its home cage, placed in a new nest box and attached to the test cage in a sound-attenuated chamber. During the experiment subjects were able to drink juice from the bottle through licking on the nipple of the bottle. The experiment started as soon as the door to the sound attenuated chamber was closed, to rule out any influence of the experimenter.

We habituated each subject to the experimental set-up and the experimental procedure. We defined a subject as habituated when it licked on the nipple of the bottle within the first five minutes of the experiment. When a subject reached the habituation criterion, we conducted the first test at the next day.

In the test, we started a playback stimulus when the subject was sitting in a defined position, meaning that it was licking on the nipple of the bottle while keeping its head straight and its hands on the wooden bar. Thereby, the loudspeaker was positioned 180° behind the subject. Within one test, three test stimuli were played back to the subject in a random order, with a minimum inter-stimuli interval of five minutes. If the test could not be finished in more than two hours, we tested the remaining acoustic stimuli of this test on a separate day. Tests were separated by two to four days. A minimum of two days (= two sessions) was needed to expose the subject to all six stimuli. Each animal was exposed to each playback stimuli three times (= three sessions). The sessions were separated by a minimum of seven days.
5.3.5 Data and video analysis

When the test were videotaped using the Sony DR-TRV 22E PAL, we digitized all video tapes using InterVideo WinDVD creator 2. When tests were recorded using Sony DCR-SR35E, the already digital files were transferred to an external hard disk. We conducted a frame by frame analysis (25 frames/second) in Interact 3.1. (Mangold International GmbH). We determined the exact time (frame) that the playback was started, using Music Maker Deluxe 2005 Version 10.0 (Music Editor 2.01, Magix AG). This time point was transferred manually to Interact 3.1. We analyzed all experimental trials with regard to the head position at the start of the playback stimulus. Since sometimes subjects did not turn their head in response to the first sound of a playback stimulus, but to the second or third, we determined also the head position at the onset of the second or third sound.

To test for orientation biases (= first head turns towards a playback stimulus), we selected for further analyses all trials, in which the head criterion (= the subject was licking on the nipple of the bottle while keeping its head straight and its hands on the wooden bar) was fulfilled. For the selected trials, we analyzed the head turn direction of the first head turn during the presentation of a sound, head turns during the intercall-intervals were not included. For each trial we scored the following head turn responses: no response – subjects did not turn head more than 90° to either of the two sides within the stimuli playback, right turn - subject turned its head more than 90° to the right side, left turn – subject turned head more than 90° to the left side.

To asses for behavioral indications of acoustic recognition of familiarity or sex of the sender, we selected all trials in which the subject was drinking from the bottle at playback onset. This means that subjects that had shifted their heads whilst drinking (and were therefore not included into analyses of orientation bias) could still be included into these analyses. In these selected trials we measured the latency to stop drinking, latency to turn head (≥45°) and drinking interruption duration after playback. These parameters are an indication of the interest that the subject has for a specific playback stimulus and have been used in other studies on acoustic recognition (e.g., Cheney and Seyfarth, 1980; Rendall et al., 1996).

5.3.6 Statistical analysis

To determine effects of ‘familiarity-to-sender’, we directly compared the responses of each subject to the call of a familiar sender of the same sex (FS) with the responses to the call
of an unfamiliar sender of the same sex (US; Fig. 5.1). To determine effects of sex-of-sender, we directly compared the responses of each subject to the call of an unfamiliar sender of the other sex (UO) with the responses to the call of an unfamiliar sender of the same sex (US). Since we expected different outcomes for the two call types (trill calls and tsak calls), based on the study of Scheumann and Zimmermann (2008), we considered the responses for each call type separately.

To test for orientation biases in the first session, we used the head turn responses of all subjects (population level) for each playback stimulus. We tested whether significantly more subjects turned their head to one side than expected by chance for each of the six acoustic stimuli using the Chi-square test (observed versus expected; Hauser and Andersson, 1994; Böye et al., 2005).

For all sessions together, we used the total number of right and left head turns a subject had made for each stimulus, and defined a subject as right biased or left biased for this stimulus (individual level). We then tested whether significantly more subjects turned their head to one side than expected by chance, using the Chi-square test (observed versus expected).

Based on all trials, in which subjects showed a response towards the playback stimuli, we calculated an Orientation Index (OI) for each stimulus and subject, according to the formula OI = (number right head turns – number left head turns)/(number right head turns + number left head turns). Positive values reflect a right head turn bias – left hemispheric advantage and negative values reflect a left head turn bias – right hemispheric advantage. This index is derived from the Handedness Index (Lonsdorf and Hopkins, 2005), which has also been used in studies on auditory lateralization (e.g., Scheumann and Zimmermann, 2008; Siniscalchi et al., 2008).

To test for behavioral indications of acoustic recognition, we calculated per subject and per playback stimulus the mean of the three sessions for each of the measured behavioral parameters. Since the data was not normally distributed, a Wilcoxon test was used to compare between the different playback categories (FS vs. US, and UO vs. US) per call type. To control for the double comparison of the US category, Bonferroni corrections were applied. To analyze the rate of response to each playback stimulus in the three sessions, we calculated for each individual a Response Index (RI), according to the following formula: RI = (number right head turns + number left head turns)/(number right head turns + number left head turns).
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+ number of no head turns; Siniscalchi et al., 2008). An RI value of one indicates that the subject oriented to all playbacks, an RI value of zero indicates that subject oriented to none of the playbacks.

To test for a relation between orientation biases and behavioral response for each playback stimulus, we correlated the response parameters to the OI, using Spearman’s rank correlation.

Using a Mann-Whitney-U test, we tested whether the Orientation Index is affected by sex of the subject or box position. Furthermore, to test for an age effect, we correlated the OI to the age of each subject (Spearman rank correlation). All statistical tests were calculated using SPSS 16. We found no differences in any behavioral parameters or in the OI, between right or left box position (Mann-Whitney-U: p≥0.481) for all parameters. Also, the overall Response Index (on population level) showed no significant decrease over time (Friedman test: \( \chi^2=4.820, p=0.090, N=17; \) Appendix 5A).

5.4 Results

We found no differences in any behavioral parameters or in the OI between males and females (Mann-Whitney-U: p≥0.279 for all parameters). Since no sex differences were found, we decided to perform all analyses on the entire subject sample (N=17). The RI decreased significantly with the age of the subject (Spearman: R=−0.525, p=0.003, N=17), but none of the other parameters correlated to age (Spearman: p≥0.164 for all parameters).

5.4.1 Orientation biases

In the first session (first playback of every stimulus), a significant right orientation bias was found in the response to a playback of TrUO (trill call of unfamiliar sender of the other sex; \( \chi^2=4.500, p=0.034, N=8; \) Appendix 5B and Appendix 5C). In the response towards TsUO (tsak call of unfamiliar sender of the other sex) a trend to turn right was found (\( \chi^2=2.778, p=0.096, N=9 \)). No other significant orientation biases were found.

Based on the individual biases (based on the total number of right and left turns a subject made during the three sessions) for each playback stimulus, a trend was found to turn right to a playback of TrUO (trill call of unfamiliar sender of the other sex), based on the subjects that showed a bias (\( \chi^2=2.778, p=0.096, N=9; \) Appendix5D and Fig. 5.3), and to turn left to a playback of TrFS (trill call of familiar sender of same sex) (\( \chi^2=3.000, p=0.083, \))
N=12). However, in tsak calls a significant orientation bias was found, as significantly more subjects were right biased in their turns towards TsUO (tsak call of unfamiliar sender of the other sex) than expected by chance ($\chi^2=5.333$, p=0.039, N=12)

### 5.4.2 Discrimination of sender characteristics

The subjects showed a trend for a longer latency to stop drinking and to turn the head in response to trill calls of familiar senders (TrFS), compared to trill calls of unfamiliar senders of the same sex (TrUS; Wilcoxon: latency stop drinking: Z=-1.681, p=0.093, N=17; latency turn head: Z=-1.758, p=0.079, N=17). In the tsak calls, the subjects showed a significantly longer latency to stop drinking when hearing a tsak call of a familiar sender (TsFS), compared to an unfamiliar sender of the same sex (TsUS; Wilcoxon: Z=-2.343, p=0.019, N=17; see Fig. 5.4). In the other behavioral parameters no significant effects were found.

When comparing trill calls of unfamiliar senders of the other sex (TrUO) to trill calls of unfamiliar senders of the same sex (TrUS), the subjects took longer to turn their head towards TrUO’s than towards TrUS’s (Wilcoxon: Z=-2.012, p=0.044, N=17), but this result was no longer significant after Bonferroni corrections. In latency to stop drinking, a trend of the same pattern was found (Wilcoxon: Z=-1.917, p=0.055, N=17). In addition, subjects showed a significantly lower response index towards TrUO’s compared to TrUS’s (Wilcoxon: Z=-2.684, p=0.007, N=16; Figure 5.5). In tsak calls no differences were found between TsUO’s and TsUS’s in the behavioral parameters.

![Fig. 5.3: OI index of the population for each playback stimulus, based on the 3 sessions.](image)

- **Figure 5.3**: OI index of the population for each playback stimulus, based on the 3 sessions.
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**Fig. 5.4:** Latency to stop drinking after playback of tsak calls of familiar sender of the same sex (TsFS), compared to tsak calls of unfamiliar senders of the same sex (TsUS).

**Fig. 5.5:** Response Index after playback of trill calls of unfamiliar senders of the other sex TrUO, compared to trill calls of unfamiliar senders of the same sex (TrUS).
5.4.3 Orientation bias linked to behavioral response

We found no significant correlation between the Orientation Index and the behavioral responses to the playback stimuli, except for TrUS, where a negative correlation was found between latency to stop drinking and OI (Spearman: $R=-0.570$, $p=0.033$, $N=14$). For TsUO there was trend of a negative correlation between latency to turn head and OI (Spearman: $R=-0.465$, $p=0.094$, $N=14$). Both results indicate that positive OI’s (right bias) are accompanied by shorter response latencies and negative OI’s (left bias) by longer response latencies.

5.5 Discussion

We found that orienting asymmetries of gray mouse lemurs towards conspecific communication calls were affected by the sex-of-sender, but not by the familiarity-to-sender. Nevertheless, we found indications of both familiarity and sex recognition on the behavioral level, since gray mouse lemurs were able to discriminate familiar from unfamiliar senders, and other sex from same sex senders on the basis of some of their communication calls.

No familiarity-to-sender effect was found in the orienting asymmetries of gray mouse lemurs towards conspecific communication sounds. These findings contradict the findings in horses (Basile et al., 2009a), starlings (George et al., 2004), and zebra finches (Cynx et al., 1992). Conversely, in vervet monkeys also no effect of familiarity to the sender was found on auditory lateralization (Gil-da-Costa and Hauser, 2006). Therefore, it seems that familiarity does not affect auditory lateralization in prosimians and old world monkeys. The human lateralized auditory processing of familiar voices (Van Lancker and Kreiman, 1987; Basile et al., 2009b) therefore most likely evolved late in primate evolution.

On the other hand, we found an effect of the sex-of-sender on the orientation biases. As far as we know, this is the first study to have focused on such an effect in non-human animals. Because our subjects were tested in the breeding season, our results could be explained by sexual motivation. Therefore, our results might indicate a left hemisphere dominance in sexual behavior in this nocturnal primate, which is in line with the findings of a right eye preference in courtship approach by poeciliid fish (Bisazza et al., 1997), but not in line with findings of a left eye preference in courtship approach by black winged silts (Ventolini et al., 2005). Thus, a sex-of-sender effect on lateralized auditory processing in humans (Landis et al., 1982) might have evolved from the lateralized processing of calls of
other sex senders in early primates. However, the sex of the receiver plays a role in mouse lemurs, but not in humans, suggesting that the sex-of-sender effect on auditory lateralization may be flexible during primate evolution.

Scheumann and Zimmermann (2008) had reported a sex difference in the auditory lateralization of the gray mouse lemur, in that only male mouse lemurs showed a right ear preference to perceive tsak calls and short whistle calls. In the present study, we found no sex differences in perception. The difference between these findings can be explained by the fact that in the previous study both males and females were exposed only to female calls. Since in this study females also showed no orientation bias for the female tsak calls (TsUS), the results of the previous and present study match. So, based on the present findings we can now conclude that this sex difference was not based on perceptual differences between the sexes, but due to the specific lateralization for perceiving the other sex. The fact that in this study we found some evidence for a right orientation bias to trill calls, which was not reported in the previous study, can also be explained by the use of different stimuli: whereas the stimuli in the previous study were recorded in the field and in a female sleeping group context, the present stimuli were recorded from the breeding colony in a laboratory setting and in a female-male context. These latter stimuli might therefore be more relevant to our subjects.

The assumption that auditory lateralization can be explained by the left hemisphere specialization for conspecific communication, based on temporal cues (Belin et al., 1998; Hauser et al., 1998; Ghazanfar et al., 2001), has been brought to question by several studies on humans and non-human animals, that found that conspecific communication is not always processed with a left hemisphere dominance, but can be additionally affected by emotional valence (e.g., Altenmüller et al., 2002; Scheumann and Zimmermann, 2008) and familiarity to the sender (e.g., George et al., 2004; Basile et al., 2009a,b). In addition, our present findings suggest that the sex of the sender can also affect auditory lateralization, at least in gray mouse lemurs.

Acoustic recognition of sex and familiarity has been reported for different species across the vertebrate lineage (familiarity: Miller et al., 2005; Carter et al., 2000; sex: Cure et al., 2009; Smith et al., 2009). However, to our knowledge, this is the first study that reports acoustic recognition of both familiarity and sex in a prosimian primate. And since the gray mouse lemur is suggested to represent the model of the ancestral primate condition (Martin, 1972), these findings indicate that these skills were already present early in primate evolution. Furthermore, in combination with previous findings of individual signatures across the vocal
repertoire (in chapter 3), and with our findings of sex differences in the acoustic structure of
the tsak call (in chapter 4), we can conclude that in the acoustic communication system of the
gray mouse lemur cues to individuality and sex are not only conveyed, but also perceived.

In tsak calls, our findings of a slower response to familiar compared to unfamiliar
conspecifics, indicate a lesser interest to the familiar conspecific. Such a decreased interest to
calls of familiares has also been reported for several other vertebrate species, such as non-
human primates (e.g., Snowdon et al., 1983; Teixidor and Byrne, 1997), non-primate
mammals (Frommolt et al., 2003) and birds (e.g., Price, 1999; Botero et al., 2007). Usually,
the increased behavioral responses, which the subjects in the above mentioned studies showed
towards unfamiliar sender, were of an aggressive nature. This indicates that unfamiliar
conspecifics were perceived as a possible threat. Gray mouse lemurs usually produce tsak
calls in agonistic contexts, which are accompanied by aggressive and threatening behavior of
the sender (Zimmermann, 1995). Thus, our findings suggest that mouse lemurs perceive tsak
calls of unfamiliar conspecifics as of a higher threat than tsak calls of familiar conspecifics.

In contrast, we found no effect of the sex of the sender on the behavioral responses to
tsak calls. These results may be explained by a lack of relevance to distinguish between sexes
in this call type, since unfamiliar conspecifics of both sexes might pose equal threats to the
receiver in an agonistic context.

Our finding of a lesser interest for trill calls of the other sex (produced in a male-
female context), as indicated by a slower and weaker response, coincides with similar
findings of sex recognition by tamarins (Masataka, 1987), macaques (Hauser, 2007),
Australian sea lions (Gwilliam et al., 2008) and two shearwater species (Cure et al., 2009),
tested in similar contexts. These findings were interpreted either as a territorial response to
same sex intruders (Cure et al., 2009; Masataka, 1987), or as an increased interest in the
potential threat of competitors in the breeding season (Gwilliam et al., 2008). Gray mouse
lemurs live in a dispersed multi-male multi-female social system (Radespiel et al., 1998;
Radespiel et al., 2001) and have a seasonal reproduction system (Martin, 1972). Thus, during
the breeding season it might be difficult to find a potential mate, causing a high competition
between same sex conspecifics. Indeed, Perret (1994) reported an increase in male aggressive
behavior during female estrus. In females aggressive competition was not reported, but Perret
(1994) suggested female competition to be based on passive stress. Thus, there are indications
that both male and female mouse lemurs experience increased competition from same sex
conspecifics during the breeding season. Moreover, trill calls were found to function both for
social cohesion as well as for deterring potential competitors (Dietz, 2006). Since in this study
the playbacks were performed in the breeding season, our findings may suggest that mouse lemurs perceive trill calls of same sex conspecifics as of a competitive threat in a breeding context.

In trill calls, no significant effect of the familiarity on the response behavior was found, which could reflect an inability of the subjects to recognize familiaris based on this call type. However, since trill calls were found to be highly acoustically distinct (chapter 3), this seems unlikely. The trill calls that we used for playback were recorded in a male-female context, and therefore do not function as a cohesion call between familiar group members, in contrast to other trill calls that have been described elsewhere (e.g., Zimmermann, 1995). Thus it seems that distinguishing between familiar and unfamiliar same sex conspecifics is irrelevant for mouse lemurs in the perception of trill calls in a male-female context. This suggests that, for the receiver, familiar and unfamiliar senders of the same sex pose similar competitive threat in a breeding context.

We did not find a clear correlation between the Orientation Index and the behavioral responses that were elicited by the playback stimuli. There was only meager evidence of a correlation between OI and behavioral responses: only significant for TrUS and a trend for TsUO. This indicates that positive OI’s (= right orientation bias) are accompanied by shorter response latencies. Thereby, these findings are not in line with the findings of Siniscalchi et al. (2008) who found a strong positive correlation between the OI and strength of reactivity in dogs. Therefore, the orientation biases that we found in mouse lemurs, cannot be explained by the emotional valence of the stimuli that is presented.

In conclusion, we found evidence for a sex-of-sender effect on the lateralized auditory processing of conspecific communication calls in gray mouse lemurs, but we found no evidence for a familiarity-to-sender effect. Nevertheless, acoustic recognition of both familiarity and sex was found in some of the call types.

From an evolutionary perspective, we can extract that our study confirms the previous findings of auditory lateralization in this ancestral primate (Scheumann and Zimmermann, 2008), suggesting that in early primate evolution auditory lateralization is present for some conspecific communication calls (as in some non-primate vertebrates; (Ehret, 1987; Palleroni and Hauser, 2003), but not all (in contrast to some other primates; (Hauser and Andersson, 1994; Ghazanfar et al., 2001). Furthermore, our results, along with the results in vervet monkeys (Gil-da-Costa and Hauser, 2006), suggest that the lateralized processing of familiar voices that is found in humans (Van Lancker and Kreiman, 1987; Basile et al., 2009b), most
likely evolved late in primate evolution. However, sex of the sender does affect the auditory lateralization in this early primate. Therefore, a sex-of-sender effect on auditory lateralization might also be present in more recently evolved primate species. Thus, in addition to effects of communicative significance, emotional valence and familiarity to the sender on auditory lateralization, the present findings suggest that the sex of the sender can also affect auditory lateralization, at least in mouse lemurs.

5.6 Acknowledgements

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5.7 Appendices

5.7.1 Appendix 5A: Mean and standard deviations of the Response Index in each session

![Box plot showing mean and standard deviations of the Response Index in each session.](image)
5.7.2 Appendix 5B: Number of animals that turned their head right, left, or not towards each playback stimulus, in the first session

<table>
<thead>
<tr>
<th>Playback stimulus</th>
<th>Right turns</th>
<th>Left turns</th>
<th>No turns</th>
<th>$\chi^2$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrUO</td>
<td>7</td>
<td>1</td>
<td>7</td>
<td>0.034</td>
</tr>
<tr>
<td>TrUS</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>0.480</td>
</tr>
<tr>
<td>TrFS</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>0.132</td>
</tr>
<tr>
<td>TsUO</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>0.096</td>
</tr>
<tr>
<td>TsUS</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>1.000</td>
</tr>
<tr>
<td>TsFS</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1.000</td>
</tr>
</tbody>
</table>

5.7.3 Appendix 5C: OI index for each playback stimulus, based on the 1st session

5.7.4 Appendix 5D: Number of animals that were right biased (R>L), left biased (L>R) or ambivalent (R=L) in turning towards each playback stimulus, based on the three sessions

<table>
<thead>
<tr>
<th>Playback stimulus</th>
<th>Right biased</th>
<th>Left biased</th>
<th>Ambivalent</th>
<th>$\chi^2$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrUO</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td>0.096</td>
</tr>
<tr>
<td>TrUS</td>
<td>5</td>
<td>7</td>
<td>3</td>
<td>0.564</td>
</tr>
<tr>
<td>TrFS</td>
<td>3</td>
<td>9</td>
<td>4</td>
<td>0.083</td>
</tr>
<tr>
<td>TsUO</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>0.021</td>
</tr>
<tr>
<td>TsUS</td>
<td>8</td>
<td>6</td>
<td>1</td>
<td>0.593</td>
</tr>
<tr>
<td>TsFS</td>
<td>4</td>
<td>9</td>
<td>2</td>
<td>0.166</td>
</tr>
</tbody>
</table>
“Lateralization of the brain appeared early in evolution and many of its features appear to have been retained, possibly even in humans”

Rogers (2000)
Chapter 6. General discussion

The first aim of this thesis was to investigate *inter-* and *intra-*individual level variation in cerebral lateralization, which might muddle with the discerning of the lateralization pattern of a species or genus. The second aim of this thesis was to give insight into cerebral lateralization in an ancestral primate condition, by exploring behavioral lateralization in early primates. In the following section I will summarize the main results of this thesis and discuss them from an evolutionary perspective, in order to contribute to a better understanding of how cerebral lateralization might have evolved in non-human primates, from the ancestral primate brain to the brain of modern humans.

6.1 Manual lateralization

6.1.1 *Inter-individual variation in manual lateralization*

Chapter 2 reports evidence of manual lateralization in the two studied mouse lemur species on an individual, but not on a population level. On this individual level, *inter-*individual variation in manual lateralization was explored. These explorations revealed that manual lateralization in gray and Goodman’s mouse lemurs was not dependent on sex or age, since the Handedness Index (HI) was not significantly correlated to either sex or age. Thus, these results are not in line with studies that found effects of both sex and age on manual lateralization in different non-human primate species (sex: e.g., Milliken et al., 1991; Corp and Byrne, 2003; age: e.g., Ward et al., 1990; Hopkins and Leavens, 1998).

Sex differences in humans are suggested to arise from the inhibiting effect of neonatal testosterone on left hemisphere growth, which is higher in males than in females (Geschwind and Galaburda, 1987), however, to my knowledge, nothing is known on the effect of testosterone on left hemisphere growth in mouse lemurs. Therefore, it is unclear in which aspect (testosterone influence or left hemisphere growth) mouse lemurs differ from humans.

Age effects are usually found in the form of a developmental process, also in prosimians, in which the manual lateralization becomes stronger with maturation (e.g., Mason et al., 1995; Hopkins and Leavens 1998). To my knowledge, nothing is known about the cerebral maturation of mouse lemurs, and about how this maturation might differ from other prosimian species. A possible explanation could be that the mouse lemur reaches cerebral maturity before one year of age. So, possibly, the juveniles in this test were not young enough to test for developmental effects.
As reported in chapter 2, the absolute HI value was higher in Goodman’s mouse lemurs compared to gray mouse lemurs, indicating that Goodman’s mouse lemurs have a stronger manual lateralization, and use their preferred hand more exclusively. Species differences in hand preference direction have also been reported in macaques (Westergaard et al., 2001a), which was explained by a difference of temperament between the tested species. A similar explanation may also hold true for mouse lemurs, since Goodman’s mouse lemurs needed significantly more habituation sessions to reach the habituation criterion than gray mouse lemurs. This may indicate that they are more nervous and stressed in an unknown environment than the gray mouse lemurs.

Temperament has been found to affect manual lateralization in mice (Neveu and Moya, 1997), macaques (Westergaard et al., 2001a), common marmosets (Rogers, 2009), and also another prosimian primate, the galago (Watson and Ward, 1996). Thus, temperament seems to influence manual lateralization in both primate and non-primate mammals, suggesting that this effect evolved already before the advent of primates. However, experimental comparisons of temperament between the two species are needed, in order to determine to which extent the two species differ, and how such a difference might have emerged.

This temperament effect indicates how manual lateralization can vary on an inter-individual level. As shown above, temperament affected manual lateralization in mouse lemurs, which may muddle with the discerning of a lateralization pattern of a genus.

In addition, the Handedness Index of mothers was significantly negatively correlated to the Handedness Indices of their offspring. No significant correlation was found between the Handedness Indices of fathers and their young. The negative mother-offspring correlation in gray mouse lemurs was discussed to argue against a single factor inheritance, as suggested for humans (Laland et al., 1995). Together with similar results in pigtail macaques (Westergaard et al., 2001b) these findings illustrate that still little is understood of how manual lateralization is formed in primates. Indeed, genetic explanations do not seem sufficient here, and environmental factors, like mother-infant interactions (Hopkins and Bard, 1993) are suggested as possible maternal influences on offspring hand preferences (Hopkins, 1999). Thus, the seeming lack of genetic influence that was found in gray mouse lemurs indicates how environmental factors might have caused different lateralization patterns in different individuals, resulting in inter-individual variation, which muddles with efforts to understand the lateralization pattern of a species.
To sum up, although no effects of sex and age were found, the individual level manual lateralization in mouse lemurs was found to be affected by individual differences in temperament and parental influences. In conclusion, manual lateralization in mouse lemurs is found to be variable on an inter-individual level.

6.1.2 Mouse lemur manual lateralization from an evolutionary perspective

The hand preference and performance of the two tested mouse lemur species, as tested in chapter 2, indicate individual manual lateralization in the majority of individuals of both species (only a few subjects had no significant hand bias). According to Levy (1977) and later Rogers (2002), lateralization on the individual level increases brain efficiency, since the two hemispheres complement each other. Indeed, individual level manual lateralization has been found to increase foraging success in chimpanzees (McGrew and Marchant, 1999). Thus, the individual level manual lateralization in gray and Goodman’s mouse lemurs indicates that already in these early primate species, manual function is regulated by well developed and efficient cerebral processing. So, individual level manual lateralization seems to have evolved early in primate evolution (if not before).

The two studied mouse lemur species did not show a population level manual lateralization, as the number of left and right handers were almost equal in both species. Population level cerebral lateralization is proposed to have evolved after individual lateralization and is suggested to be important for regulating social interactions (Bisazza et al., 2000; Rogers, 2000; Vallortigara and Rogers, 2005; Vallortigara, 2006). In the primate order, population level manual lateralization has only been found in humans and some great ape species, while most other species show only individual lateralization, as shown by the meta-analysis of McGrew and Marchant (1997). However, in both humans and chimpanzees population level manual lateralization is most strongly found in manual functions that are likely to involve social coordination, like tool use (e.g., Marchant et al., 1995; Lonsdorf and Hopkins, 2005), whereas in other manual functions, like simple grabbing, no population level manual lateralization was found (e.g., Marchant et al., 1995; Lacqueuse et al., 1999). Therefore, the general findings in primates seem to support the theory of Vallortigara and Rogers (2005) that population level lateralization evolved to regulate social interactions. Also, the manual lateralization pattern of mouse lemurs fits into this theory. Since mouse lemurs are solitary foragers, no social coordination in the hand use for food grabbing would be expected. In conclusion, the findings of manual lateralization in mouse lemurs on an individual, but not on a population level, fit in the evolutionary theory of Vallortigara and Rogers (2005) and
suggests that manual lateralization in food grabbing has not changed much from the ancestral primate to humans.

6.2 Auditory lateralization

6.2.1 Acoustic correlates to caller characteristics as a basis for investigations into auditory lateralization

In chapter 3 individual distinctiveness was found in all four tested calls out of the gray mouse lemur vocal repertoire, but not in the same degree. These findings provided first evidence of a structurally dependent variation in individual distinctiveness across the vocal repertoire of a nocturnal primate. Also, in chapter 5 gray mouse lemurs were found to be able to acoustically discriminate between familiar and unfamiliar conspecifics. Therefore, these findings indicate that the gray mouse lemur communication system fulfills the requirements for social recognition, as stated by Falls (1982). On the one side, callers produce calls that are characterized by stereotypy within each individual, but vary noticeably among individuals. On the other side, receivers are able to extract from the calls information concerning the identity of the sender.

In chapter 4 sex differences were found in the acoustic structure of tsak calls and maybe short whistles, but not in the grunt. These findings provided first evidence of acoustical sex dimorphism in a nocturnal primate. In addition in chapter 5 gray mouse lemurs were found to be able to acoustically discriminate between males and females. Therefore the gray mouse lemur communication system fulfills also the requirements of Falls (1982) with regard to sex recognition. On the one side, the calls are characterized by stereotypy within a sex, but varied noticeably between the sexes. On the other side, receivers are able to extract from the calls information concerning the sex of the sender. These findings that gray mouse lemur vocalizations are distinctive by caller and by sex provided the basis for an investigation into the effects of caller characteristics on auditory lateralization.

6.2.2 Inter-individual variation in auditory lateralization

Considering the analysis of the variability of auditory lateralization on an inter- and intra-individual level, I will first discuss variability of auditory lateralization on an inter-
individual level. In this study, no effects of sex were found on auditory lateralization. Males and females did not show a significant difference in orientation biases for any of the playback stimuli. Sex differences in auditory lateralization in non-human animals have so far been only reported by Scheumann and Zimmermann (2008), which, as discussed in chapter 5, can now be explained by the playback stimuli that were used. Therefore the present finding of no sex difference is in line with the lack of sex differences found in other auditory lateralization studies (reviewed by Taglialatela, 2007).

6.2.3 Intra-individual variation in auditory lateralization

On the level of one organism (intra-individual level) many different lateralized processing mechanisms may be involved in lateralized auditory perception. The lateralization patterns of these processing mechanisms and their interactions would then ultimately determine the lateralization pattern that is shown for the processing of a specific stimulus. In the following part, I would like to assess which processing mechanisms affected gray mouse lemur auditory lateralization.

First of all, conspecific communication, like human language, was suggested to be processed with a left hemisphere dominance. This was suggested to be based on a left hemisphere specialization for the processing of sounds with rapid temporal changes (Belin et al., 1998) Indeed, a left hemisphere dominance is found in most of the tested species (Petersen et al., 1978; Beecher et al., 1979; Petersen et al., 1984; Heffner and Heffner, 1986; Hauser and Andersson, 1994; Hauser et al., 1998; Ghazanfar et al., 2001; Böye et al., 2005). Also, in the previous study by Scheumann and Zimmermann (2008) conspecific communication was found to be a pre-requisite for auditory lateralization in mouse lemurs, as no orienting asymmetries were found towards heterospecific or non-biological sounds. However, in that study, not all conspecific communication sounds elicited orienting asymmetries. In the present study, reported in chapter 5, orienting asymmetries towards heterospecific or non-biological sounds were not tested. Nevertheless, the present study also shows that conspecific communication alone cannot fully account for auditory lateralization. Thereby, this study, as well as the previous study of Scheumann and Zimmermann (2008), adds to an increasing body of research that suggests that auditory lateralization in non-human vertebrates is not determined by a simple dichotomy of conspecific communication vs. other sounds, but might depend also on communicative significance (Ehret, 1987; Palleroni and Hauser, 2003), emotional valence (Siniscalchi et al., 2008) or social recognition (Cynx et al., 1992; George et al., 2004; Basile et al., 2009a). Thus, conspecific communication processing seems to affect
gray mouse lemur auditory lateralization, but it is not the only processing mechanism that determines auditory lateralization.

Second, in chapter 5, no evidence was found for an effect of emotional processing on auditory lateralization. Thereby the present findings seem to contradict the findings of Scheumann and Zimmermann (2008), which suggested an effect of emotional valence on auditory lateralization in gray mouse lemurs. However, in Scheumann and Zimmermann’s study emotional valence was based on the call types that were used (trill calls were defined as of positive emotional valence, and tsak calls as of negative emotional valence), while here emotional processing was assessed, based on the behavioral reaction of the subjects. Thus the findings here cannot be easily compared to the findings of Scheumann and Zimmermann (2008). Still, my findings do not correspond to the findings in dogs, where a strong correlation was found between orienting asymmetries and behavioral response (Siniscalchi et al., 2008), indicating an effect of emotional processing. Hence, these findings suggest that, at least in early primates, lateralization of emotion is not expressed through auditory lateralization.

Finally, I did find indications of a lateralized auditory processing of social recognition in this early primate, although not in the form of familiarity recognition (since no familiarity-to-sender effect was found), in which it is normally described (e.g., Van Lancker and Kreiman, 1987; Basile et al., 2009a,b), but in the relatively unexplored form of sex recognition (sex-of-sender effect) (Landis et al., 1982). The lack of evidence for a familiarity-to-sender effect in chapter 5, in combination with findings of a lack of familiarity-to-sender effect on auditory lateralization in vervet monkeys (Gil-da-Costa and Hauser, 2006), suggests that the human lateralization for the recognition of familiar voices (Van Lancker and Kreiman, 1987), probably evolved late in primate evolution and might not be homologue to acoustic familiarity recognition in non-primate vertebrates (Cynx et al., 1992; George et al., 2004; Basile et al., 2009a). In contrast, the sex-of-sender effect that was found on orienting asymmetries in gray mouse lemurs suggests that the human lateralization for sex recognition (Landis et al., 1982) might have evolved from the lateralized processing of calls of other sex senders in early primates, albeit with some modifications (since in humans, the sex of receiver, plays no role). To prove this hypothesis, however, more studies on other primate species are necessary.

In conclusion, the results indicate that gray mouse lemur auditory lateralization is the result of a combination of a left hemisphere dominance for conspecific communication processing and a left hemisphere dominance for other sex recognition. Thus, auditory lateralization in gray mouse lemurs is found to be variable on an intra-individual level, since
it is dependent on more than one processing mechanism. These findings illustrate the importance of exploring auditory lateralization in all its facets and of controlling for different subjects, different calls and different senders.

6.2.4 Gray mouse lemur auditory lateralization from an evolutionary perspective

As discussed in chapter 5, gray mouse lemurs only show orienting asymmetries to communication calls of an unfamiliar sender of the other sex, but not to same sex senders. This suggests that in gray mouse lemurs, auditory lateralization is determined by a lateralized processing of acoustic sex recognition in conspecific communication calls.

I will now discuss how these findings fit into the theory of Vallortigara and Rogers (2005) on the evolution of cerebral lateralization. This theory proposes that cerebral lateralization first evolved on the individual level, followed by an evolution on the population level. The results of chapter 5 cannot reveal much about individual level lateralization, due to the low sample size per subject (only three playbacks per stimulus). However, a population level lateralization in the processing of acoustic sex recognition was confirmed in chapter 5. According to the theory of Vallortigara and Rogers (2005), population level lateralization is associated with social behavior. More specifically, it suggests that the need to coordinate behavior between lateralized individuals, results in an alignment of the direction of lateralization by the majority of individuals (i.e. population level lateralization). Alignment of lateralized behavioral responses on a population level can be important in coordinating sexual interactions, like courtship behavior (Bisazza et al., 1997; Ventolini et al., 2005). Indeed, Tommasi (2005) suggested that in the courtship context, there would be a strong selective pressure on the population alignment of lateralization for both the expression and assessment of cues related to fitness. In other words, opposite lateralization patterns during courtship might lead to communication disturbances and, ultimately, failure to reproduce. Thus, the population level lateralization in the processing of acoustic sex recognition in gray mouse lemurs suggests that alignment of auditory lateralization in early primates (and possibly also in the rest of the primate order) is caused by the need for social coordination in inter-sex interactions. Thereby, these findings provide support for the theory of Vallortigara and Rogers (2005).

It is interesting to note that both in the previous (Scheumann and Zimmermann, 2008) and in the present study on gray mouse lemur auditory lateralization, not all tested mouse lemurs showed a right turning bias in response to a tsak call of an unfamiliar sender of the other sex (the distribution was 11 to 2 in Scheumann and Zimmermann, 2008; and 10 to 2 in
the present study). Indeed, the alignment of lateralization on the population level is not found to be absolute, but to be restricted to about 60-90% of the population in both humans (Previc, 1991) and non-human vertebrates (Vallortigara and Rogers, 2005). To account for the stability of an minority group that shows the opposite pattern (10-40%), Ghirlanda and Vallortigara (2004) used a game-theoretical analysis to show that this population distribution can arise from two contrasting selection pressures. The first pressure, social coordination, leads to an alignment of lateralization direction (as described before). The second is the pressure on unpredictable behavior, which tends to favor individuals that show an opposite lateralization pattern. For instance, in face of a predator a small minority group may trade-off the group protection for an unpredictable escape pattern (Ghirlanda and Vallortigara, 2004). This unpredictability would not only pay off in predator interactions, but also in intra-specific social interactions, especially in agonistic contexts (Ghirlanda et al., 2009). Thus, possibly the minority group in the tested population of gray mouse lemurs can be explained as a trade-off of an advantage in inter-sex interactions in favor of an advantage in agonistic interactions with same sex competitors. This could be important during the breeding season, when there might be a high competition between same sex individuals for access to potential mates.

6.3 Co-evolution of manual and auditory lateralization?

In humans, manual lateralization and auditory lateralization are suggested to be linked (Annett, 2002). However, in rhesus monkeys (Hauser and Andersson, 1994) and mouse lemurs (Scheumann and Zimmermann, 2008), such a link was not found. Also, in the present population, no correlation was found between the Handedness Index from chapter 2, and the Orientation Index from chapter 5 (Spearman: r=0.385, p=0.194, N=13). Thus, these results confirm the results of Scheumann and Zimmermann (2008) and suggest that at least in early primate evolution manual and auditory lateralization evolved separately. Corballis (2003) proposed that the incorporation of manual gestures in conspecific communication led to a population level right handedness in primates. Since mouse lemurs, as far as I know, do not use manual gestures for communication purposes, my results do not contradict Corballis’ theory. On the other hand, the findings of population level right hand use in gestural communication in e.g., chimpanzees (Hopkins and Leavens 1998) and baboons (Meguerditchian and Vauclair 2006) provide support for Corballis’ theory.
Also, as a pre-requisite for his theory, Corballis (2003) suggested that auditory lateralization should have evolved on a population level before manual lateralization, since the population level manual lateralization is suggested to have evolved from the population level auditory lateralization. Indeed, some primate species that were found to have a population level auditory lateralization (vervets: Gil-da-Costa and Hauser, 2006; rhesus macaques: Hauser and Andersson, 1994; Ghazanfar et al., 2001), did not have a population level manual lateralization (vervets: Harrison and Byrne, 2000; rhesus macaques: Westergaard et al., 2000). The present findings of a population level auditory lateralization and an individual level manual lateralization in gray mouse lemurs are in line with this. Therefore, these findings provide partial support for Corballis’ theory.

6.4 Conclusions

In this thesis I have brought insight into lateralization of hand use, and auditory perception of communication sounds in gray mouse lemurs. Manual lateralization was found to be present on an individual level only, whereas the lateralized auditory processing of conspecific communication calls linked to sex recognition was found on a population level. In mouse lemurs, hand use in foraging takes place in a solitary context, whereas the processing of communication calls and sex recognition takes place in a social context. Therefore these findings fit into the theory of Vallortigara and Rogers (2005), which predicts population level lateralization to occur only under specific social pressure.

Moreover, I found that cerebral lateralized processes in gray mouse lemurs were found to be variable, since they varied on an inter- and an intra-individual level.

First, I found inter-individual variation in manual lateralization. Although sex did not influence auditory lateralization and neither sex nor age affected manual lateralization, temperament and parental influence were found to influence, at least, manual lateralization.

Second, I found intra-individual variation in auditory lateralization. I found no effects of emotional processing or familiarity recognition on auditory lateralization. However, I found that, in gray mouse lemurs, auditory lateralization is affected by the processing mechanisms involved in conspecific communication processing and in sex recognition.

These findings of variability in cerebral lateralization on an inter- and intra-individual level, stress the importance of exploring cerebral lateralization in all its facets and across different social groups and species, in order to fully understand its mechanisms and evolution.
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