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Evidence for semantic communication in titi monkey alarm calls

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Black-fronted titi monkeys, *Callicebus nigrifrons*, produce acoustically distinct vocalizations in response to several predator species. Compared to other primates, the calls are remarkably quiet, high-pitched and structurally simple, suggesting that they may not function uniquely as predator-specific warning calls. To address this, we investigated whether conspecifics were able to respond to these calls in adaptive ways, by playing back call series originally given to a perched raptor (caracara) and terrestrial predatory mammals (oncilla and tayra). Monkeys responded strongly and in predator-specific ways. Specifically, listeners preferentially looked upwards when hearing raptor-related calls, and towards the presumed caller when hearing terrestrial predator-related calls. Locomotor responses were generally uncommon, but if they occurred then they were always in the expected direction. We concluded that black-fronted titi monkeys discriminated between calls given to different predators on the basis of their acoustic features and were able to make inferences about the type or likely location of the predator.

Keywords:
Callicebus
functional reference
New World monkey
playback experiment
predation

Numerous animal studies have shown that vocal signals alone can be sufficient to elicit appropriate responses from listeners in the absence of the natural events that normally elicit them (Seyfarth et al. 1980a, b; Macedonia & Evans 1993; Evans & Marler 1995; Zuberbühler et al. 1997; Zuberbühler 2001; Manser et al. 2001; Templeton et al. 2005). It has become customary to refer to such signals as 'functionally referential', provided they are produced in context-specific ways and elicit specific adaptive responses in listeners. The use of this terminology is further supported by evidence that the 'referent' (e.g. a predator type) may be mentally represented as a natural concept, the 'reference' (Ogden & Richards 1923; Seyfarth & Cheney 1980; Macedonia & Evans 1993; Zuberbühler et al. 1999). The classic example is the vervet monkey, *Chlorocebus aethiops*, alarm call system. In this species, individuals produce several acoustically distinct alarm calls, each tightly associated with the detection of a distinct predator type, such as a python, eagle or leopard (Struhsaker 1967). Playback experiments have demonstrated that these different alarm call types trigger specific locomotor behaviour that is generally appropriate to the hunting technique of the predator, as if the listeners had spotted the predator itself. Upon hearing an alarm call originally given to an eagle, for instance, monkeys respond by running into dense vegetation, whereas in response to calls originally given to a leopard they might climb a nearby tree (Seyfarth et al. 1980a, b). This and similar studies have caught the attention of a wider field because of the apparent parallels with symbolic reference, a key feature of human language (e.g. Seyfarth et al. 1980a). More recent examples have come from Diana monkeys, *Cercopithecus diana* (Zuberbühler 2000a, b), Campbell's monkeys, *Cercopithecus campbelli* (Zuberbühler 2001) and Guereza colobus monkeys, *Colobus guereza* (Schel et al. 2010). However, it is unlikely that functional reference is a unique feature of simian primates. Evidence for comparable phenomena has come from several other taxa, including birds (Rainey et al. 2004; Templeton et al. 2005), prairie dogs, *Cynomys gunnisoni* (Slobodchikoff et al. 1991), suricates, *Suricata suricatta* (Manser et al. 2001) and ring-tailed lemurs, *Lemur catta* (Macedonia 1990). Some of the nonprimate examples have matched or surpassed the primate ones in sophistication and complexity. For example, chickadees, *Poecile atricapilla*, and suricates possess systems by which referential and urgency information is combined in ways not yet described for primates (Manser et al. 2001; Templeton et al. 2005).

Despite this wealth of research, evidence of functional reference is conspicuously sparse in New World monkeys, with few exceptions. Kirchhof & Hammerschmidt (2006) found that two sympatric species of tamarins (*Saguinus fuscicollis* and *Saguinus mystax)* responded with appropriate antipredator reactions after hearing playbacks of calls originally given to aerial and terrestrial

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disturbances. The conclusion was that the aerial and terrestrial alarm calls of *S. mystax* were functionally referential, but that in *S. fuscicollis* functionally referential calls were only present to aerial but not terrestrial predators. Similarly, Wheeler (2010) demonstrated that tufted capuchin monkeys, *Cebus apella*, showed appropriate responses after hearing 'barks' (aerial predator calls) and 'hiccups' (generalized disturbance call), and that these calls were made in the appropriate contexts.

Apart from these studies, we are not aware of any systematic research, which is surprising because New World monkeys are as exposed to predation as other groups of primates. In addition, as mainly arboreal, forest-living species, visual communication is of only limited use, suggesting that vocal signals are likely to play an important role when dealing with predation. New World monkeys are an independent radiation within the primate lineage with essential differences in life history (for example, two of three families have a rapid reproductive rate) and socioecological features (all species are arboreal and male involvement in infant care is common) compared to Cercopithecines and lemurs (Strier 2007). More generally, evidence from New World monkeys is crucial for evolutionary theories of primate vocal behaviour (Snowdon 1989). For these reasons, research on how New World monkeys use specific vocal signals when interacting with predators has considerable theoretical implications, both for theories of communication, including the emergence of complex vocal behaviour, and cognitive evolution. For theories of human language evolution, data on New World species may even reveal something about the phylogenetic history of some of the components of the human language faculty.

In this study, we focused on titi monkeys (*Callicebus* spp.), a highly diverse group of New World monkeys, which consists of 30 different species (van Roosmalen et al. 2002; Wallace et al. 2006; Defler et al. 2010). Their vocal behaviour has been studied before, which has given them the reputation of being particularly complex vocal signallers (Moynihan 1966; Robinson 1979; Cäsar et al. 2012). Although visual displays do occur, especially during intergroup encounters, communication within and between groups appears to be mainly vocal (Moynihan 1966).

In previous work, we have found that black-fronted titi monkeys, *Callicebus nigrifrons*, produce alarm calls to predators in the following way. High-frequency, low amplitude A-calls ('chirps') were given to raptors, regardless of whether they were flying, perched or calling, but also to predatory black capuchin monkeys, *Cebus nigritus*, and other disturbances in the canopy (e.g. models of terrestrial predators). In contrast, high-frequency B-calls ('cheeps') were given to terrestrial predators, such as felids or tayras, *Eira barbara*, and in a number of nonpredatory contexts, such as when descending, when feeding in the lower canopy, during intergroup encounters and in response to nonpredatory animals on the ground (Cäsar 2011; Cäsar et al. 2012). The common theme for this call type is that all call-eliciting contexts involve the possibility of danger on the ground. In this way, the alarm call behaviour of titi monkeys resembles that of Old World primates, such as the putty-nosed monkeys, *Cercopithecus nictitans*, which produce series of 'hacks' to eagles and series of 'pyows' both spontaneously and to terrestrial disturbances (Arnold & Zuberbühler 2006). What is unusual in titi monkey predator calls is the highly inconspicuous acoustic structure, especially compared to the loud and structurally complex alarm calls of many Old World monkeys (e.g. Fischer et al. 2001a, b; Arnold & Zuberbühler 2006; Schel et al. 2010).

To address the question of whether these quiet calls functioned as predator warning calls, we conducted playback experiments with members of several habituated groups for which we already had recordings of alarm calls to natural predators or to predator models (Cäsar 2011; Cäsar et al. 2012). We predicted that if the calls denoted the type or likely location of the predator then monkeys should respond as if they themselves had witnessed the event that elicited the call. Thus, in response to call A we expected monkeys to scan the sky and avoid exposed areas. In response to call B we expected monkeys to scan the forest floor and orient towards or approach the playback speaker (i.e. the presumed caller), to obtain information about the reason for calling and potentially to mob the predator, a behaviour regularly observed during terrestrial predator encounters (Cäsar 2011; Cäsar et al. 2012).

METHODS

Study Site and Subjects

The study was conducted at the 'Reserva Particular do Patrimônio Natural Santuário do Caraça', an 11 000 ha private natural heritage reserve in Minas Gerais, southeastern Brazil (20°05′S, 43°29′W). The reserve contains fragments of Atlantic forest in different stages of ecological succession, surrounded by savannah ('cerrado') and xeric forest with small thorny trees and shrubs ('caatinga'; Coelho et al. 2008). The following primate species can be found in the forested parts: black-fronted titi monkeys, black-tufted-ear marmosets, *Callithrix penicillata*, white-fronted marmosets, *Callithrix geoffroyi*, and black capuchin monkeys.

Black-fronted titi monkeys, our study species, are endemic to the Atlantic forests of southeastern Brazil. As one of the largest *Callicebus* species, there is no sexual dimorphism, with a maximum adult weight of 1650 g (Rowe 1996). Predators of monkeys at the study site include raptors (crowned eagles, *Harpyhaliaetus coronatus*, black-chested buzzard-eagles, *Geranoaetus melanoleucus*, black hawk-eagles, *Spizaetus tyrannus*, and caracara, *Caracara planctus*), black capuchin monkeys and various medium-to-large cats (ocelots, *Leopardus pardalis*, oncillas, *Leopardus tigrinus*, jaguarondis, *Herpailurus yagouaroundi*, pumas, *Puma concolor*) and tayras.

We carried out our research with 11 individuals belonging to four groups of black-fronted titi monkeys between July 2009 and July 2010. Individual recognition was based on a combination of natural features, such as body size, fur characteristics and other body parts. All individuals were fully habituated to human presence, tolerating observation distances of around 3 m. Group composition varied because of births, migration and disappearances as follows: group A consisted of up to three adult (>30 months) males, one adult female, one subadult (18–30 months) male, one juvenile (6–18 months) male and one infant (0–6 months); group D consisted of up to one adult male, one adult female, one subadult male and one infant; group M consisted of up to one adult male, two adult females, one subadult female and one infant; group R consisted of up to three adult males and one adult female.

The research was carried out in compliance with all relevant Brazilian laws and was approved by the University of St Andrews Psychology Ethics Board.

Playback Stimuli and Experimental Procedure

Alarm calls used as playback stimuli were recorded from the five study groups (for details, see Cäsar 2011). Call sequences were edited from complete vocal responses, originally given to presentations of a perched raptor in the canopy (caracara) and two largely terrestrial predators (tayra, oncilla) positioned close to the ground. Because call rate varied in response to the three predators (Table 1) we standardized the call series to 30 s total duration. We only used recordings of satisfactory acoustic quality. In some cases, we had to replace poor-quality calls with high-quality calls of the same type

Table 1
Playback stimuli and natural alarm call behaviour of different groups of wild *C. nigrifrons* in response to natural and model predators (first 30 s of response)

Stimulus	*N* groups	Mean number of calls	Range
A-call series			
Perched live raptor	4	7.75	4–16
Perched model raptor	5	19.2	8–29
Playback stimulus	5	13.0	7–24
B-call series			
Spotted live cat	1	51.0	51
Live oncilla	5	54.4	39–83
Live tayra	5	45.6	15–79
Playback stimulus	3	69.0	55–83

Entries are based on the total number of calls produced by each group during the first 30 s in response to both natural and model predators. Calls were produced mainly by one, but sometimes two or more individuals.

produced later during the same response. To avoid pseudoreplication, each playback stimulus consisted of unique exemplars of calls.

From the available master recordings, we were able to produce eight different playback sequences of sufficient quality (A-call series: $N = 5$; B-call series: $N = 3$). Some call series were used more than once, but never more than four times. Each individual was tested only once for a given stimulus type (i.e. A-call series, B-call series). Representative exemplars of each call type are shown in Fig. 1. To avoid habituation, individuals were not retested for at least 10 days, with one exception (a male in group R was retested after 3 days). Since multiple individuals were tested in each group, we ensured that individuals that were not tested were out of range (more than 40 m away) during an experiment. If this was not possible, we ensured that we adhered to the 10-day trial-free criterion. We videotaped all playback trials, using a camcorder CANON MD205. In two trials, a family member was near the focal individual and could thus be recorded at the same time. In these cases, we scored the first glance of both individuals, but only considered the response of the focal individual for all other variables.

When administering playback trials, we ensured that the experiment simulated a realistic scenario for the subject. If calls of other family members were broadcast, we made sure that the playback speaker was positioned so that the calls came from the actual direction of the caller. If calls from a member of a neighbouring (i.e. nonfamily) group were broadcast, we made sure they were played back from a realistic location, that is, an area visited by the subject and his or her neighbours. This also removed possible side effects of simulating the presence of an intruder in the subject's core area.

Playback stimuli were broadcast with an Apple iPod Nano, connected to a Kenwood KAC-5203 Power amplifier and a PRO-BASS SF 250 speaker. The speaker, attached to an extendable pole, was positioned at an elevation of 3 m off the ground, circa 12–20 m from the subject, outside its visual range. The volume of the iPod was adjusted so that all playback stimuli were broadcast within their natural amplitude range to sound natural to a human observer at a distance of about 20 m.

Behavioural Measures

All videos were coded with ADOBE PREMIERE PRO CS4 software with a time resolution of 25 frames/s (duration of a single frame = 0.04 s). The following measures were taken from the videos: (1) the 'latency to the first reaction' of the focal animal (mostly head turns) by counting the frames, beginning from the first call during the call series; (2) 'direction of the first gaze'; and (3) the 'looking duration', by counting the frames the focal animal devoted to looking in different directions during the 30 s of playback. Direction of gaze was classified as: (1) 'looking up', defined as looking beyond the immediate substrate, with the head oriented at least 45° above the horizontal line; (2) 'looking towards speaker', defined as looking beyond the immediate substrate, with the head oriented within 45° relative to the axis formed with the speaker; (3) 'looking down', defined as looking below the immediate substrate, with the head oriented at least 45° below the horizontal line; (4) 'looking elsewhere', defined as looking in any other direction, including scanning.

Owing to the density of the forest and the fact that the playbacks often elicited movement out of view, looking duration was only coded for the first 15 s of each trial. We then calculated the relative looking duration as the time a subject spent looking in each direction divided by the total time looking to any direction. Thus, we did not consider the time during which they were moving, hidden or not visible when determining the relative proportion of each looking direction. To test further whether a subject's looking direction was really in response to the playback stimulus, we also compared the monkeys' looking behaviour in the 15 s before and 15 s immediately after the end of a playback.

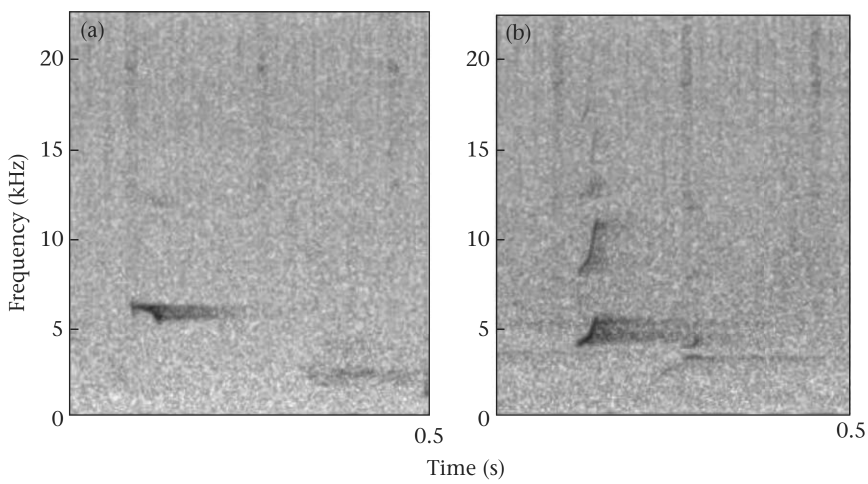

Figure 1. Spectrographic representations of the two call types used as playback: (a) call A, (b) call B. Playbacks consisted of series of A- or B-calls, respectively.

Locomotor responses were scored as 'movement' versus 'no movement'. If movement occurred, we scored whether it was in the horizontal or vertical plane.

To estimate the accuracy of the coding, we carried out an interobserver reliability test between C.C. and a second coder (E.M.), who was naïve to the hypotheses. E.M. recoded the first gaze direction of all trials ($N = 22$) and, unaware of the speaker's location, was instructed to score the direction (left, right, front, behind) and angle (straight line, up, down) of the first head movement immediately after the first call.

Statistical Analysis

To test for differences in the direction of the first gaze (upwards, downwards, towards speaker, elsewhere) we analysed all valid trials ($N = 22$, including playbacks from family and nonfamily members) using chi-square and binomial tests. For differences in latency and looking duration, we only considered individuals tested with stimuli produced by a nonfamily member and if tested in both conditions (to obtain matched samples). We used linear mixed models (LMMs) to investigate differences in the duration of looking in each direction between call types A and B. We included the condition as a fixed factor in the model, that is, before (baseline), during or after the call was presented, and its interaction with call type. We also included a random effect for individual. We used proportional data transformed to normality using ASIN transformation. To check for differences between conditions we used the conditional *t* test, as recommended by Pinheiro & Bates (2000, page 90). Finally, we used nonparametric Mann–Whitney *U* tests to compare response latencies to A-call and B-call series, and to check for presentation order effects on monkeys' responses. If individuals were tested in two matched conditions, we used Wilcoxon signed-ranks tests instead. Analyses were performed with SPSS 18 for Windows (SPSS Inc., Chicago, IL, U.S.A.) and R 2.13.1 (The R Foundation for Statistical Computing, Vienna, Austria). Alpha levels were set at 0.05.

RESULTS

Sample Size

A total of 24 playback experiments were conducted with 11 different individuals. Each individual was tested at least once with both a raptor and a terrestrial predator alarm call series, with calls sourced from another family member or a member of a neighbouring group (Table 2). Two trials had to be excluded from analysis because animals were already looking towards the speaker when the stimulus was broadcast, yielding a final sample of $N = 22$ valid cases (Table 2).

Latency

Alarm call type had no significant influence on response latency (Mann–Whitney *U* test: $U = 90.5$, $N_1 = 10$, $N_2 = 12$, $P = 0.111$; Fig. 2). Likewise, in matched comparisons using only calls by neighbouring individuals, response latency was not significantly affected by alarm call type (Wilcoxon test: $Z = -0.987$, $N_1 = N_2 = 8$, exact $P = 0.391$). Presentation order, that is, whether subjects heard A- or B-calls first, also had no effect (Mann–Whitney *U* test: $U = 58.5$, $N_1 = 10$, $N_2 = 2$, $P = 0.194$).

Looking Duration

For the subsequent analyses, we compared all subjects tested with A- and B-call series given by neighbours ($N = 8$; Table 2).

Table 2
Individuals tested with playbacks of A- and B-call series, originally produced by a family member or a member of a neighbouring group

Subject	Age–sex	Group	Condition (call provider)
Apolo	AM	A	**1 (D)–2 (D)**
Ana	AF	A	**1 (D)–2 (D)**
Aquiles	AM	A	**1 (D)–2 (P)**
Aguirre	AM	A	**1 (D+A)–2 (D)**
André	JM	A	**1 (R)–2 (D)**
Desbotado	AM	D	**1 (R)–2 (A)**
Diego	AM	D	1 (D)–2 (A+D)
Roberto*	AM	R	**1 (A)–2 (D)**
Rosa	AF	R	**1 (A)–2 (D)**
Rafael	AM	R	2 (D)
Marion	AF	M	2 (A)

AM = adult male, AF = adult female, JM = juvenile male; condition 1 = A-call series; condition 2 = B-call series; subjects tested with neighbour calls in both conditions used for LMM analysis are shown in bold.

* During playback of A-call series Roberto and Rosa sat next to each other. Although both reacted identically, only Roberto was included in the duration analysis.

Listeners looked upwards for significantly longer periods in response to A- than B-call series (LMM: $t_{32} = 4.45$, $P < 0.001$; Fig. 3a). Moreover, in response to A-call series they looked upwards significantly longer in comparison to baseline, that is, before the stimulus (LMM: $t_{32} = 3.84$, $P = 0.0005$). The duration of looking upwards after the end of the stimulus was not statistically different from before the stimulus (LMM: $t_{32} = 1.58$, $P = 0.125$). Furthermore, there was no difference in the time spent looking upwards after hearing call B across all conditions ($P > 0.05$).

There was no significant difference in the duration of downwards looking after hearing A- and B-call series (LMM: $t_{32} = 0.90$, $P = 0.374$; Fig. 3b). Listeners looked downwards significantly less during playbacks of B-call series than prior to hearing the stimulus (LMM: $t_{32} = 2.56$, $P = 0.016$). There were no differences in looking duration downwards across other conditions for call B, both before versus after (LMM: $t_{32} = 0.98$, $P = 0.333$) and during versus after trials (LMM: $t_{32} = 1.25$, $P = 0.220$). Although monkeys looked downwards for longer after than during the playback of this call, the difference was not significant (LMM: $t_{32} = 1.801$, $P = 0.081$).

Monkeys looked significantly longer towards the speaker after hearing B- than A-calls (LMM: $t_{32} = 3.53$, $P = 0.001$; Fig. 3c). They

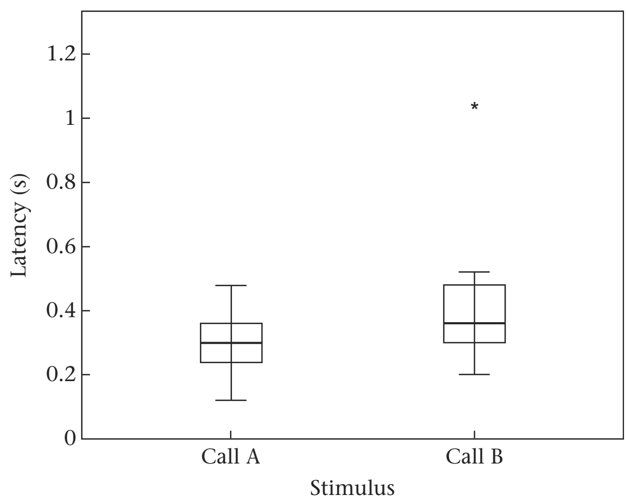

Figure 2. Box plots indicating the latencies in response to the different playback types. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range, and the asterisk indicates an extreme case. Number of trials: 10 for call A and 12 for call B.

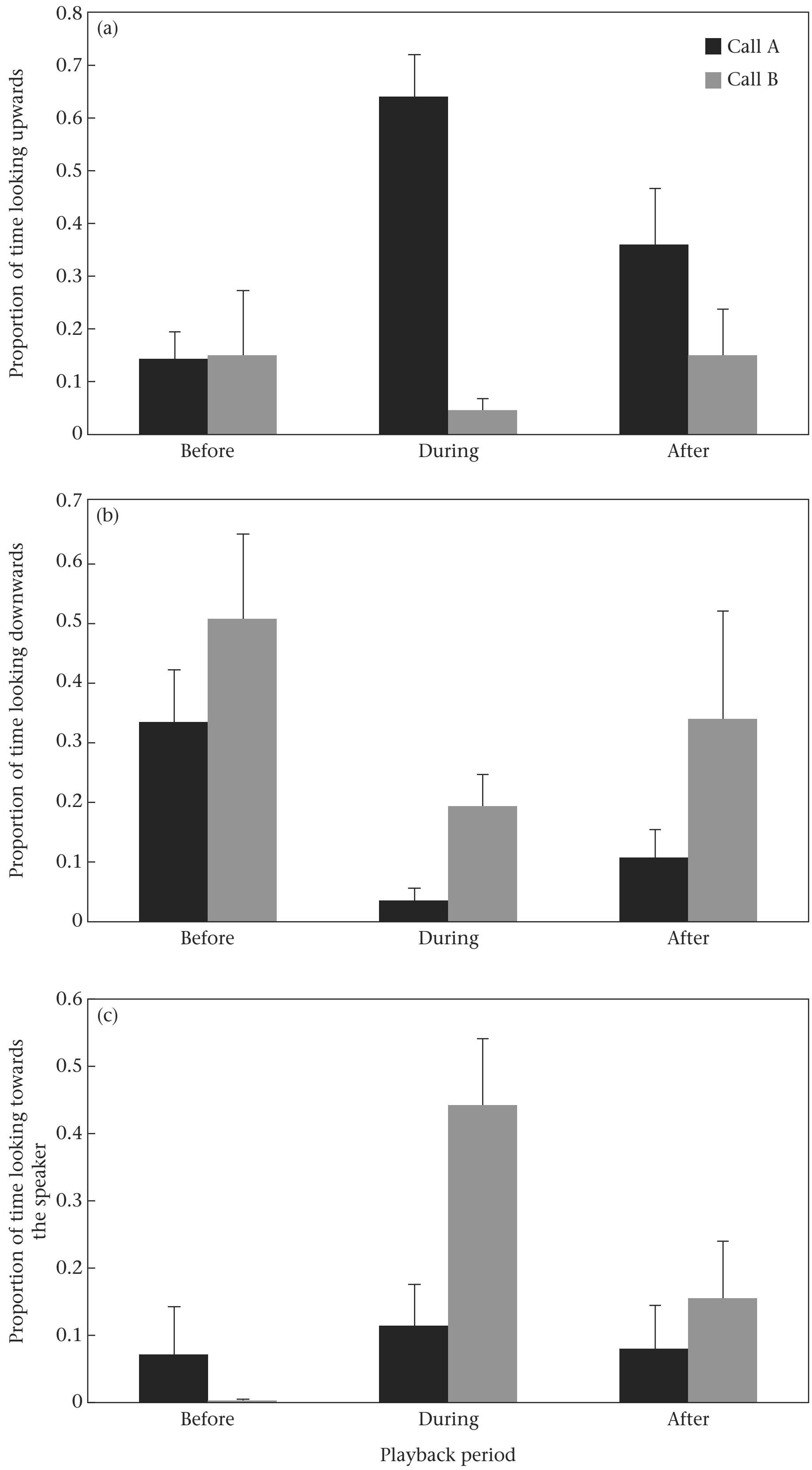

Figure 3. Looking durations: (a) upwards, (b) downwards and (c) towards the speaker (means ± SE).

also looked significantly longer towards the speaker during (LMM: $t_{32} = 4.611$, $P = 0.0001$), but not after (LMM: $t_{32} = 1.245$, $P = 0.222$) playbacks of B-call series, compared to the period before stimulus exposure. Monkeys spent less time looking towards the speaker after the playback of call B than during the playback of this call (LMM: $t_{32} = 2.786$, $P = 0.009$). For call A, there were no differences in the time spent looking towards the speaker in any condition ($P > 0.05$).

There were no differences in time spent looking in other directions (elsewhere) in all comparisons ($P > 0.05$). Presentation order, that is, whether subjects heard A- or B-calls first, also had no effect (Mann–Whitney *U* test: all comparisons $P > 0.05$).

Direction of First Gaze

In 20 of 22 trials individuals immediately changed their looking direction in response to the first call played back. In the remaining two trials, individuals only reacted after a few seconds, possibly because they were distracted or did not hear the stimuli.

First gaze direction depended significantly on call type played back (Table 3). First gazes were never down after hearing playbacks indicating the presence of a raptor, and never up after playbacks indicating the presence of a terrestrial predator. When we compared the direction of first gaze after hearing call A with that after hearing call B the difference was significant (two-tailed chi-square: $\chi^2_3 = 20.444$, $P < 0.0001$). The interobserver reliability between C.C. and the second coder (E.M.) was perfect (100%).

Locomotor Responses

Hearing an alarm call series had no significant effect on locomotor behaviour (A-calls: $N_{Move} = 2$, $N_{Not\ move} = 6$, two-tailed P value $= 0.289$; B-calls: $N_{Move} = 3$, $N_{Not\ move} = 5$, two-tailed P value $= 0.727$; binomial tests). If moving occurred to B-calls ($N = 5$ trials), then it was frequently away from the speaker (two of three cases) or higher up within the canopy (one of three cases). Both individuals that did move after hearing call A went away from the caller to hide somewhere in the vegetation.

DISCUSSION

Titi monkeys' responses to playbacks of their own calls were related to the type and probable location of the predator, suggesting that the two call types designated different external objects or events to hearers. This was evident for A-calls given to raptors, normally detected within the canopy, and B-calls to medium-sized cats or tayras, normally detected on the ground. In addition, A-calls elicited behaviour typically given to aerial predators, while B-calls elicited behaviour typically given to terrestrial predators.

In previous work, we have shown that titi monkeys produce two main alarm call types as part of longer series (Cäsar et al. 2012). Call A is produced to raptors, regardless of their behaviour (flying, perched or calling), but it is also found at the beginning of call sequences to other predators in the canopy (Cäsar et al. 2012). Playbacks of A-calls elicited longer looking skywards, indicating that the monkeys were anticipating a relevant event above, such as a raptor attack. Based on these results, we conclude that call A (chirps) functions to refer to danger within the canopy, especially raptors. Future work will have to determine whether there are subtle acoustic differences within the chirps and whether such variation is related to the event type experienced by the caller. This is unlikely given the very basic morphology of the calls. However, monkeys sometimes produce multiples of these calls in specific contexts, for instance B-call doublets and triplets to terrestrial predators, but never to nonpredatory disturbances (Cäsar 2011). Similar variation in syllable numbers has been reported in other species, usually in relation to specific external events (Schel et al. 2010) or high urgency (e.g. Templeton et al. 2005).

Call B is produced to terrestrial predators, to nonpredatory disturbances on the ground, while descending or foraging near the ground, during some intergroup encounters, and to capuchin monkeys discovered within the canopy (Cäsar et al. 2012). Playback of B-calls elicited longer looking towards the (suspected) caller, suggesting that listeners were searching for additional cues to determine the call-triggering event, for example by assessing the caller's own behaviour or orientation, as previously reported from putty-nosed monkeys (Arnold & Zuberbühler 2006). This is an adaptive response especially in cases where the referent of a call is ambiguous, and can only be identified by additional information, for example what the caller is looking at. These findings are in line with other studies that have shown that, even if predator-associated calls are given to a wide variety of events, including in nonpredatory situations, they can still elicit predator-specific reactions, despite their low levels of context specificity (e.g. Fichtel & Kappeler 2002; Kirchhof & Hammerschmidt 2006; Wheeler 2010).

In natural predator encounters, black-fronted titi monkeys usually give very different vocal and locomotor responses after detecting raptors and terrestrial predators. After hearing the calls of an individual responding to a raptor, monkeys mostly scan the canopy or sky, freeze or flee rapidly, usually by moving lower towards a protected place. In response to terrestrial predators, they instead scan the forest ground or lower canopy, look for and approach the first caller, and usually gather to harass the predator cooperatively (Cäsar et al. 2012). Contrary to such observations, we were unable to elicit similar behaviour when playing series of B-calls (Cäsar et al. 2012). It is possible that in experimental situations, listeners did not have enough time or motivation to approach the caller, as our playbacks were very short (15 s). Natural vocal responses to terrestrial predators, in contrast, can last for up to 1 h (Cäsar 2011). Moreover, the willingness of other monkeys to approach and join in with alarm calling appears to depend on their own distance from the caller (C. Cäsar, unpublished data). Another difference is that subjects could not see the caller in the experiments, which may have further lowered their motivation to approach.

In the closely related *Callicebus cupreus*, individual differences in 'chirrups' (a probable variant of B-calls) have already been demonstrated (Moynihan 1966; Robinson 1979), suggesting a similar effect for the B-calls of *C. nigrifrons*. It is thus possible that, because we used calls of neighbouring individuals, subjects recognized them as neighbour calls and were not prepared to assist them in mobbing a potential predator. For example, in one trial a young male responded faster and approached more to B-calls by his own father than when hearing a neighbour's calls. It is also relevant that listeners did not show any conspicuous display behaviour, such as pilo-erection, tail lashing or body arching, when hearing neighbouring alarm calls, in contrast to regular intergroup encounters (Moynihan 1966; C. Cäsar, personal observation). These explanations are not exclusive and may act in combination. Moreover, they also explain why listeners did not produce their own

Table 3
Direction of first glance following playback stimulus (frequency of looking direction)

Looking direction	Playback condition		Binomial test
	A-call series (raptor in canopy)	B-call series (cat or tayra on ground)	
Up	10*	0	**0.0005**
Down	0	2	0.250
Speaker	0	8	**0.004**
Other	0	2	0.250

Other: first glances of two single adult males were towards paired group males (the presumed fathers). Significant one-tailed *P* values are shown in bold.

* Responses to A-call series included one individual scored simultaneously with the focal individual.

alarm calls after hearing a terrestrial predator alarm call series. For raptors, nonvocal responses are expected. In natural encounters, only the first individual to see a raptor produces calls, while others call only if they discover the predator independently afterwards (Cäsar et al. 2012).

One relevant finding in this study was that two structurally simple and perceptually inconspicuous calls could have strong behavioural effects in recipients. Structurally, the two call types vary mainly in the shape of the main frequency transition, which appears to be sufficient to convey biologically highly relevant information on external events (Cäsar et al. 2012). The fact that predator alarm calls in many Old World monkeys tend to be acoustically complex and perceptually conspicuous suggests that alarm call structure may be a relatively flexible trait in primate evolution. Titi monkeys are considerably smaller than most Old World monkeys, which may make them vulnerable to a much larger range of predators. Natural selection may have favoured the evolution of structurally inconspicuous signals that are more difficult for a predator to locate. This is remarkable in light of the fact that alarm call use and responses to alarm calls are not very different from what has been reported in Old World monkeys (e.g. Zuberbühler 2000a; Arnold & Zuberbühler 2008; Schel et al. 2010).

The fact that *Callicebus* monkeys possess an unusually complex vocal repertoire during normal social interactions (Moynihan 1966; Robinson 1979; Cäsar et al. 2012) further highlights the relative ease by which vocal complexity can evolve in primates, which is especially true for humans. A future challenge will be to understand the function and meaning of complex vocal behaviour in titi monkeys, how listeners integrate the numerous, highly graded signals with pragmatic cues, and what the relationship is between single calls, call sequences and call combinations. Obtaining statistically meaningful sample sizes will remain a major challenge in this primate species, which form small groups and are not easily habituated in the wild.

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