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Estrogen and Progestogen Correlates of the Structure of Female Copulation Calls in Semi-Free-Ranging Barbary Macaques (*Macaca sylvanus*)

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Abstract Females of many Old World primates produce conspicuous vocalizations in combination with copulations. Indirect evidence exists that in Barbary macaques (*Macaca sylvanus*), the structure of these copulation calls is related to changes in reproductive hormone levels. However, the structure of these calls does not vary significantly around the timing of ovulation when estrogen and progestogen levels show marked changes. We here aimed to clarify this paradox by investigating how the steroid hormones estrogen and progesterone are related to changes in the acoustic structure of copulation calls. We collected data on semi-free-ranging Barbary macaques in Gibraltar and at La Forêt des Singes in Rocamadour, France. We determined estrogen and progestogen concentrations from fecal samples and combined them with a fine-grained structural analysis of female copulation calls ($N=775$ calls of 11 females). Our analysis indicates a time lag of 3 d between changes in fecal hormone levels, adjusted for the excretion lag time, and in the

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acoustic structure of copulation calls. Specifically, we found that estrogen increased the duration and frequency of the calls, whereas progestogen had an antagonistic effect. Importantly, however, variation in acoustic variables did not track short-term changes such as the peak in estrogen occurring around the timing of ovulation. Taken together, our results help to explain why female Barbary macaque copulation calls are related to changes in hormone levels but fail to indicate the fertile phase.

Keywords Acoustic structure · Copulation call · Estrogen · Progesterone · Steroid hormone

Introduction

Animal vocalizations provide important information about the vocalizer, including identity (Owren and Rendall 2003; Price *et al.* 2009; Rendall 2003; Rendall *et al.* 1998; Semple 2001) and caller attributes such as rank (Fischer *et al.* 2004; Harris 2006; Vannoni and McElligott 2008) and age, sex, and size (Ey *et al.* 2007). In addition, vocal behavior appears to be strongly influenced by reproductive hormones. For example, in male vertebrates, testosterone levels affect both the usage and structure of vocalizations, e.g., in fish (Fine *et al.* 2004; Remage-Healey and Bass 2005), frogs (Penna *et al.* 1992; Rhodes *et al.* 2007), birds (Arnold 1992; Balthazart and Ball 1995; Meitzen *et al.* 2007; Rybak and Gahr 2004; Smith *et al.* 1997; van Duyse *et al.* 2002), and nonhuman primates (Hollien 1960; Meitzen *et al.* 2007; Newman *et al.* 2000; Rybak and Gahr 2004; Saida *et al.* 1990; van Duyse *et al.* 2002), as well as ontogenetic development and short-term changes in vocal performance (Meitzen *et al.* 2007; Newman *et al.* 2000; Penna *et al.* 1992; Remage-Healey and Bass 2005; Smith *et al.* 1997; van Duyse *et al.* 2002).

Although many researchers report a relationship between acoustic structure and reproductive hormones in males, less is known for females. In women, voice characteristics such as frequency, harmonics, and intensity can change during the menstrual cycle (Abitbol *et al.* 1999; Brodnitz 1979). For example, fundamental frequency increases during high- vs. low-fertility days (Bryant and Haselton 2009), suggesting a role of estrogens in voice modulation. Further, changes in acoustic structure of the human female voice have also been reported after women enter menopause (Abitbol *et al.* 1999; Boulet and Oddens 1996; Caruso *et al.* 2000) and after hormone replacement therapy (Gerritsma *et al.* 1994; Lindholm *et al.* 1997). It is hypothesized that the structural modulation of the voice is the result of changes in the level of reproductive hormones eliciting hormone-dependent morphological changes in organs responsible for voice production, such as the vocal cords (Gerritsma *et al.* 1994) and larynx, tissues known to contain receptors for sex steroids (Newman *et al.* 2000; Saez and Sakai 1976; Voelter *et al.* 2008; *cf.* Schneider *et al.* 2007).

In nonhuman primates, the relationship between acoustic characteristics and reproductive hormones is largely untested. To date, most research has centered on female vocalizations that putatively serve to attract mates (estrus calls) or that are uttered during or immediately after copulation (copulation calls). For instance, in Tonkean macaques (*Macaca tonkeana*), the frequency of occurrence of female estrus calls correlates positively with estrogen levels (Aujard *et al.* 1998). In addition, studies in baboons and macaques have shown changes in temporal (using hormone measures to determine cycle state: Pfefferle *et al.* 2008a; using swelling size as indicator of cycle state: Deputte and Goustard 1980; O'Connell and Cowlishaw 1994; Semple *et al.* 2002) and spectral (using hormone measures to determine cycle state: Pfefferle *et al.* 2008a; using swelling size as indicator of cycle state: Semple and McComb 2000; Semple *et al.* 2002) parameters of female copulation calls during the course of the menstrual cycle, suggesting, at least indirectly, a relationship between reproductive hormones and the structure of these calls. Significantly, however, such variations in copulation call structure within the ovarian cycle do not, at least in Barbary macaques, seem to be temporally related to the time of ovulation, even though it is around this time that the most marked changes in both absolute and relative levels of estrogen and progesterone occur (Pfefferle *et al.* 2008a). Although interpretation of this finding is somewhat difficult, it does not necessarily exclude a relationship between call structural parameters and female reproductive hormones because other hormone-dependent modalities, such as female sexual behavior and anogenital swelling, also do not always change significantly around the time of ovulation (Engelhardt *et al.* 2005; Higham *et al.* 2009). Clearly, the existence, or otherwise, of a direct link between copulation call structure and levels of reproductive steroids in female Barbary macaques requires further investigation.

The investigation of this link is the objective of the present study, in which we extend our previous investigations of the functional significance of copulation calls in Barbary macaques. Previously, we postulated that in Barbary macaques, copulation calls act as a signal to the mating partner, influencing his likelihood of ejaculation (Pfefferle *et al.* 2008a), and also as a signal to other male group members, announcing a successful copulation (Pfefferle *et al.* 2008b) and thereby inciting the interest of other males in the calling female (Semple 1998). However, our inability to demonstrate a temporal relationship between call structure/frequency and ovulation raises the question as to whether hormonal changes during the periovulatory period of the female cycle are too small to induce a significant effect on the structural parameters of the call or whether any direct link between vocal parameters and reproductive hormone levels exists at all.

Accordingly, we examined the relationship between levels of female reproductive hormones and the structure of copulation calls in detail in 2 different situations: 1) within the time course of the normal ovulatory conception cycle and 2) during the postconception cycle, which refers to the temporally distinct period of increased sexual activity during early (usually within 2–6 wk) pregnancy, a phenomenon occurring in several macaque species, e.g., Japanese macaques (*Macaca fuscata*: Nigi *et al.* 1990), pig-tailed macaques (*Macaca nemestrina*: Hadidian and Bernstein 1979), and long-tailed macaques (*Macaca fascicularis*: Engelhardt *et al.* 2007) including Barbary macaques (Kuester and Paul 1984; Möhle *et al.* 2005). Postconception cycles are characterized by an increase in the estrogen-to-progestogen ratio that is qualitatively similar to that seen in conception cycles, but absolute levels of both hormones are markedly elevated in the postconception cycle (Möhle *et al.* 2005).

Here, we take advantage of this quantitative difference in endocrine profiles to examine whether and, if so, how changes in female estrogen and progestogen levels affect the vocal characteristics of female Barbary macaque copulation calls. Given our previous finding that there was no significant variation in call structure within females during the periovulatory period (Pfefferle *et al*. 2008a), we predict that estrogen and progestogen levels do not or only weakly correlate with the acoustic structure of Barbary macaque female copulation calls during the conception cycle. In contrast, because hormonal effects on biological functions are often dose dependent (Cooke *et al*. 2003; Phillippe *et al*. 1991) and because absolute hormone levels are markedly elevated during the postconception cycle (Möhle *et al.* 2005), the link between reproductive hormones and acoustic structure might be more easily detectable during this period of the female reproductive cycle. Thus, if acoustic parameters of female copulation calls are influenced by female reproductive hormone concentrations, we expect to find a clearer relationship between estrogen and progestogen levels and structural parameters of the calls in the postconception cycle vs. the conception cycle. In addition, we tested for differences in the structure of copulation calls in the conception and postconception cycles, to examine the influence of variation in absolute levels of reproductive hormones on vocalizations.

Materials and Methods

Study Site and Subjects

We studied 11 semi-free-ranging adult female Barbary macaques from 2 populations (Table I). The first, Middle Hill group, lived in the Upper Rock Nature Reserve in Gibraltar (Möhle *et al*. 2005). At the time of our study (mating seasons 2003–2004 and 2004–2005), this group consisted of 18–20 adult individuals, including 5–6 adult males and 13–14 adult females. The second population was housed in the La Forêt des Singes monkey park in Rocamadour, France (De Turckheim and Merz 1984). We studied 2 groups in La Forêt des Singes during the mating season 2005–2006. The Petit Bassin group consisted of 18 adult males and 29 adult females, and the Grand Bassin group consisted of 8 adult males and 21 adult females. Both study populations were health monitored and food provisioned. Subjects were habituated to human observers and individually recognizable using natural markings or tattoos. We analyzed 12 conception and 10 postconception cycles from the 11 focal females, which covered all rank and age classes (Table I) and were not on hormonal contraception.

Acoustic Recordings and Analysis

We recorded copulation calls from a distance of 2-4 m. We made recordings *ad libitum* with a sampling frequency of 44.1 kHz using either a SONY TCD-D100 DAT recorder (SONY Corporation, Japan) or a Marantz PMD 670 Professional Portable Solid State Flash Card Recorder (D & M Professional, Longford, UK) and a Sennheiser directional microphone (Sennheiser, Wedemark, Germany; K6 power module with Rycote Modular Windshield System and a Rycote Windjammer, Rycote, Stroud, U.K.). Overall, we collected 958 calls, of which 775 were of sufficient quality, e.g., not disturbed by any background noise, for acoustic analysis.

Table I General information concerning the focal females

ID	Study site	Group	Mating season	Age	Rank	Cycles available	Conception	Most likely day of ovulation
FA	Gibraltar	MH	03/04	7	Middle	cc & pcc	Yes	12/21/2003
JU	Gibraltar	MH	03/04	8	High	cc & pcc	Yes	12/21/2003
JU	Gibraltar	MH	04/05	9	High	cc & pcc	Yes	12/24/2004
SU	Gibraltar	MH	03/04	14	Low	cc & pcc	Yes	12/14/2003
JA	Gibraltar	MH	04/05	6	High	cc & pcc	Yes	01/14/2005
RE	Gibraltar	MH	04/05	10	High	cc & pcc	Yes	12/12/2004
B354	Rocamadour	GB	05/06	7	middle	cc & pcc	Yes	11/27/2005
E380	Rocamadour	GB	05/06	4	Low	cc & pcc	Yes	12/05/2005
L170	Rocamadour	PB	05/06	19	Low	cc	Yes	10/27/2005
T264	Rocamadour	GB	05/06	14	High	cc & pcc	Yes	12/03/2005
T267	Rocamadour	PB	05/06	14	Low	cc	Yes	10/28/2005
M189	Rocamadour	PB	05/06	18	Low	cc & pcc	Yes	10/25/2005

cc = conception cycle; pcc = postconception cycle; rank: high = individual belonged to the upper third of group hierarchy; middle = middle third of group hierarchy; low = lower third of group hierarchy

Female Barbary macaque copulation calls are composed of a series of call units (Fig. 1), characterized by temporal and spectral parameters. Because units are short and reveal little to no frequency modulation, we focused on variables that describe the distribution of the amplitudes in the frequency spectrum (DFA) and the location of the dominant frequency bands (DFB) to describe the call spectrally. We calculated minimum, maximum, and mean values of the first and second amplitude quartiles (DFA1 and DFA2), as well as minimum, maximum, and mean values of the first and second dominant frequency bands (DFB1 and DFB2). In addition, we determined the minimum, maximum, and mean peak frequency (PF), which is the frequency of the highest amplitude in a certain time segment. We chose call duration as a temporal parameter, which describes the overall length of a call. Owing to a high degree of multicollinearity among the DFA, DFB, and PF parameters, we restricted our

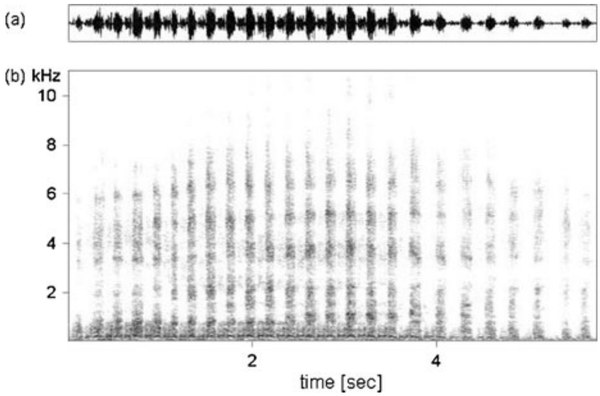

Fig. 1 (**a**) Waveform and (**b**) spectrogram of a female Barbary macaque copulation call. The single units making up the call (panted grunts) are clearly visible.

analysis to means, i.e., DFA2mean for DFA, DFB1mean for DFB, and PFmean for PF. These variables correspond to acoustic features used in previous analyses (Pfefferle *et al.* 2008a; Semple and McComb 2000), and have been shown to describe the call structure (Fischer 1998; Fischer *et al.* 1998; 2001; 2002; Neumann *et al.* 2010). Their salience is supported by playback studies showing the congruence between the macaques' classification of sounds and classification based on these acoustic features (Fischer 1998). Further, this limited set of variables alleviates the problems associated with multiple testing. For more detailed information on how we assigned acoustic parameters see Pfefferle *et al.* (2008a), and for a description of the algorithms see Hammerschmidt (1990) and Schrader and Hammerschmidt (1997).

Fecal Sample Collection and Hormone Analysis

We collected a mean of 47 (range 30–70) fecal samples per focal female (mean: 1 sample every 1.8 d). We collected only fresh samples, which we homogenized with a wooden stick. We then placed 3–5 g of the fecal sample in a polypropylene tube containing 10 ml of absolute ethanol. At the end of the field period, we shipped all samples to the German Primate Center endocrine laboratory for hormone analysis. For this, we homogenized the samples in their solvent and extracted them twice according to the method described by Ziegler *et al.* (2000). The efficiency of this extraction procedure with Barbary macaque feces is 90% (Möhle *et al.* 2005). We measured fecal extracts for levels of immunoactive total estrogens (E_{total}) and 5α-reduced 20-oxo pregnanes (5-P-3OH) using enzyme immunoassays that had been previously validated for monitoring ovarian endocrine function in Barbary macaques (Heistermann *et al.* 2008; Möhle *et al.* 2005). Sensitivity of the assays at 90% binding was 19.6 pg for 5-P-3OH and 3.9 pg for E_{total}. Intra- and interassay coefficients of variation, calculated from replicate determinations of high- and low-value quality controls, were 8.2% (N=16, 5-P-3OH), 7.9% (N=16, E_{total}; high) and 10.4% (N=16, 5-P-3OH), 9.2% (N=16, E_{total}; low) and 18.7% (N=102, 5-P-3OH), 16.6% (N=82, E_{total}; high) and 16.2% (N=102, 5-P-3OH), and 16.7% (N=82, E_{total}; low).

All females conceived during the study as indicated by maintenance of elevated 5-P-3OH levels beyond the length of a normal luteal phase. We determined the day of ovulation (= day of conception) for each female using the significant postovulatory 5-P-3OH rise (Heistermann *et al.* 2008). After determining the day of conception we backdated all hormone values by 2 d to adjust for the time lag between secretion of progesterone and estrogen in the blood and excretion of its metabolites into feces (Shideler *et al.* 1993). We based all subsequent analyses on these adjusted hormone values.

Data and Statistical Analysis

For each female we defined the period from the date of the first observed copulation (average 23.5 d before ovulation, range 14–32 d) to d 15 after conception as the conception cycle. The beginning of the postconception cycle is indicated by a vaginal bleeding occurring *ca.* 16 d after conception (Kuester and Paul 1984; Möhle *et al.* 2005), and is accompanied by a marked decline in progestogen levels and a marked rise in estrogen levels (Möhle *et al.* 2005; Fig. 2). The end of the

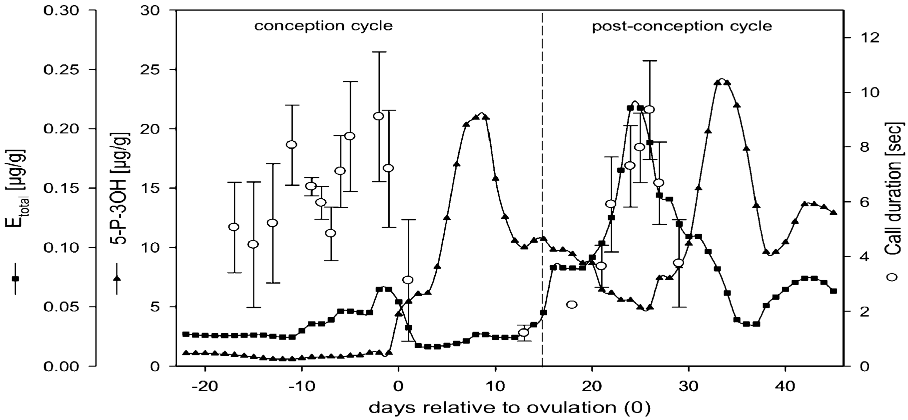

Fig. 2 Representative pattern of estrogen (E_{total}) and progestogen (5-P-3OH) levels, and the time course of call duration (mean ± SD of the females recorded calls at a particular day) during the conception and postconception cycles of female JU (mating season 03/04). The occurrence of a value for call duration also indicates the occurrence of a copulation call on this particular day. The figure includes the 3-d time lag of the hormonal effect on copulation call occurrence and length, i.e., the acoustic data are shifted 3 d backwards.

postconception cycle is more difficult to define; we applied the definition of Kuester and Paul (1984), who used an average length of 28 d. Thus, d 16–44 after conception represents the postconception cycle.

Because of weather conditions and partial unavailability of individuals, we were unable to collect fecal samples every day. Because our time-scale analysis of the effect of hormonal changes on copulation call structure requires daily hormonal values, we interpolated missing hormonal data points using the aspline function of the akima package in R (Akima 1970, 1991a,b). To reduce the error resulting from short-term hormone fluctuations due to, e.g., food intake, stress, we smoothed the resulting curves of the individual hormonal data using the running median procedure in R (function = runmed, window size = 3; Cassidy *et al.* 1995; Gann *et al.* 2001; Mohr *et al.* 1996). All resulting hormone profiles were in accordance with previously published female Barbary macaque hormone profiles (Möhle *et al.* 2005). We used the resulting values in statistical analyses.

Influence of Steroid Hormones on Copulation Call Structure

We applied a linear mixed model to analyze the effect of the continuous variables estrogen and progestogen on the dependent continuous variables call duration, peak frequency (PFmean), distribution of the first dominant frequency band (DFB1mean), and distribution of the second dominant amplitude quartile (DFA2mean). We log10-transformed E_{total} and 5-P-3OH measures to achieve normal distribution and included female ID as a random effect.

Because any modulating effect of hormones can be delayed up to several days (Losel and Wehling 2003), we checked for a possible time lag in the relationship between endocrine and acoustic parameters. We tested a time lag of 0 to ≤7 d, which covers the reported range hormones are known to need for exerting a biological effect (Gillman 1940; Losel and Wehling 2003; Sherwin and Gelfand 1987). We used the Akaike information criterion (AIC) to select from the set of models the one that best approximates the data, i.e., that best explains the effect of the 2 hormones on call structure. A lower AIC indicates that a model has a better fit than a higher value, controlling for the number of parameters in the model. Because the absolute hormone levels in a female's conception and postconception cycles differ markedly and because the magnitude of the hormone change is much greater during the postconception cycle vs. the conception cycle (Möhle *et al.* 2005), we analyzed the types of cycles separately. We implemented the models in the R statistical computing environment (R Development Core Team 2008) using the glmer function (Bates 2005). The α level of statistical tests was 0.05.

Comparison of Copulation Calls Uttered During Conception and Postconception Cycles

To test whether acoustic parameters of copulation calls uttered during conception and postconception cycles differ, we compared acoustic values of the 2 cycle types. As temporal reference points for this comparison we chose the peak in estrogen preceding ovulation in the conception cycle and the clearly defined estrogen peak associated with the postconception cycle (Fig. 2 and Möhle *et al.* 2005). These periods of elevated estrogens represented times when mating activity was most pronounced and copulation calls most often given by the females (Fig. 2). We compared the acoustic structure during a 5-d window (day of estrogen peak ± 2 d) around both estrogen peaks using a linear mixed model (SPSS v. 19) incorporating female ID as random effect.

Results

Figure 2 depicts a representative time course of estrogen (E_{total}) and progestogen (5-P-3OH) levels over a conception and a postconception cycle in relation to call duration in an individual female (JU, mating season 03/04). The 2 reproductive hormones follow the typical pattern described for Barbary macaques during the conception cycle and early pregnancy (*cf.* Möhle *et al.* 2005), with clearly elevated estrogen levels occurring in the preovulatory phase and between d 15 and 35 postconception and progestogen levels rising after ovulation and remaining elevated postconception.

Influence of Steroid Hormones on Copulation Call Structure

Levels of both estrogen (E_{total}) and progestogen (5-P-3OH) were related to the structure of the copulation calls (Table II). The AIC model selection revealed a time lag of the hormonal effect on call structure of 3 d. No other time lag yielded significant results (Table II), although there was a trend for DFB1mean with a time lag of 1 d. The hormonal effects on the acoustic structure of copulation calls were most pronounced during the postconception cycle, when call duration, peak frequency, and the location of the second amplitude quartile were associated with

Table II Results of the linear mixed model testing the influence of the predictor variables estrogen (E_{total}) and progestogen (5-P-3OH) ($\log_{10}$-transformed) on the structural call parameters call duration, peak frequency (PFmean), location of the first dominant frequency band (DFB1meam), and the location of the second amplitude quartile (DFA2mean)

		Conception cycle			Postconception cycle		
		Estimate ± SE	*t*	*p*	Estimate ± SE	*t*	*p*
Call duration	(Intercept)	8.42±1.07		0.0001	9.86±0.88		0.0001
	σ_{residual}	2.75			2.25		
	σ_{ID}	1.22			1.64		
	$\log_{10} E_{\text{total}}$	1.58±0.71	2.23	0.024	2.87±0.88	3.26	0.0040
	$\log_{10}$ 5-P-3OH	−0.76±0.39	−1.96	0.053	−2.94±0.85	−3.44	0.0006
	Time lag	3			3		
PFmean	(Intercept)	528±80		0.0001	676±66		0.0001
	σ_{residual}	170			202		
	σ_{ID}	134			28		
	$\log_{10} E_{\text{total}}$	73±49		0.24	71±66	1.08	0.26
	$\log_{10}$ 5-P-3OH	44±25	1.48	0.10	−168±71	−2.36	0.026
	Time lag	0	1.74		3		
DFB1mean	(Intercept)	477±52		0.0001	433±44		0.0001
	σ_{residual}	122			108		
	σ_{ID}	62			76		
	$\log_{10} E_{\text{total}}$	19±34	0.36	0.49	71±37	1.89	0.054
	$\log_{10}$ 5-P-3OH	−15±18	−0.85	0.43	85±45	1.87	0.071
	Time lag	0			1		
DFA2mean	(Intercept)	1034±223		0.0001	1214±202		0.0001
	σ_{residual}	473			487		
	σ_{ID}	384			351		
	$\log_{10} E_{\text{total}}$	−103±137	−0.75	0.56	401±207	1.94	0.04
	$\log_{10}$ 5-P-3OH	40±74	0.54	0.44	278±208	1.34	0.12
	Time lag	3			3		

The intercept describes the value of the acoustic dependent if the females' log10 (E_{total}) and log10 (5-P-3OH) levels are 0. We included female ID as a random factor. Data are for 12 conception and 10 postconception cycles

the E_{total} or 5-P-3OH level, or both (Table II). If the ratio between E_{total} and 5-P-3OH was high, e.g., during the first half of the postconception cycle, copulation calls were longer (6.99 s) vs. periods when this ratio was low, e.g., during the second half of the postconception cycle (call duration = 4.05 s). Similarly, copulation calls uttered during the first half of the postconception cycle (high E_{total}/5-P-3OH ratio) had a higher peak frequency (605 Hz) vs. those uttered during periods of low E_{total}/5-P-3OH ratio (437 Hz). During the conception cycle, the only significant relationship found between hormones and call structure was the influence of E_{total} on call duration (Table II), but the effect of 5-P-3OH levels on call duration was close to significant and negative.

Comparison of Copulation Calls Uttered During Conception and Postconception Cycles

There was no statistically significant difference in the acoustic structure of copulation calls uttered during the conception and postconception cycles (call duration: $F_{(2,743)}=2.27$, $p=0.104$; DFA2mean: $F_{(2,640)}=0.551$, $p=0.58$; DFB1mean: $F_{(2,640)}=1.182$, $p=0.307$; PFmean: $F_{(2,637)}=0.02$, $p=0.981$).

Discussion

Our results support the hypothesis that variation in levels of estrogens and progestogens affects the acoustic structure of female Barbary macaque copulation calls. The relationship between the 2 reproductive hormones and the structure of vocalizations was most pronounced in the postconception cycle, during which estrogen levels were, on average, 3 times higher than preovulatory levels in the conception cycle. Specifically, in the postconception cycle estrogens were positively related to call duration and frequency of the second amplitude quartile, whereas an increase in progestogen levels was related to a decrease in call duration and peak frequency. In comparison, only call duration was positively related to estrogen levels during the conception cycle, but none of the other changes in call parameters could be explained by variation in the levels of the 2 hormones. For the changes in call characteristics, we found a best fit when a time lag of 3 d between changes in hormone levels and acoustic parameters was taken into consideration. The fact that we found no difference in the structure of copulation calls between the conception and postconception cycles suggests that the observed acoustical changes are triggered by relative, rather than absolute, changes in hormone titers.

Although our findings are correlational and do not necessarily indicate causal relationships, they are generally consistent with results of other studies showing that reproductive hormones influence calling behavior and call structure, e.g., humans (Abitbol *et al.* 1999; Bryant and Haselton 2009; Gerritsma *et al.* 1994), nonhuman primates (Aujard *et al.* 1998), and birds (Arnold 1992; Balthazart and Ball 1995; Rybak and Gahr 2004). Interestingly, the relationship between the endocrine and the call parameters was stronger during the postconception vs. the conception cycle, indicating that the modulating effect of the 2 reproductive hormones on call characteristics differed between reproductive stages. The reason for this is not clear, but at least 2, possibly related, explanations exist. First, hormonal effects on biological functions are usually dose dependent (Cooke *et al.* 2003; Phillippe *et al.* 1991), and thus it is likely that the more pronounced change in the relative levels of reproductive hormones during the postconception cycle lead to more pronounced changes in call structure. Second, if, as in other vertebrate species, the regulation of calling behavior and characteristics is dependent on the number of steroid hormone receptors in vocal organs, pronounced acoustic changes might occur only after a certain threshold level of these receptors is reached. Reproductive hormone levels are consistently low in female Barbary macaques outside the breeding season, and because females usually conceive during their first cycle of the mating season (Kuester and Paul 1984; Möhle *et al.* 2005), they are exposed to elevated hormone levels (particularly estrogens) for only a couple of days during the conception cycle. We hypothesize that this time period may be too short to stimulate a sufficient up-regulation of hormone receptors or to induce the morphological changes in the sound production organs necessary for affecting call parameters. This might be particularly the case for spectral parameters whose production depends mainly on morphological changes in the phonation system, e.g., larynx, vocal cords. However, temporal parameters such as duration are mainly associated with alternations in the respiratory system, i.e., lungs, which should not depend (as much) on the hormonal state of the female. The observed relationship between the endocrine measures and call duration might therefore be indirect in the sense that the hormones affect the female's motivation to call longer. Such an indirect effect on call duration may require lower levels of reproductive hormones than a direct influence on call structure, for which morphological changes in the phonation system are first needed. These potentially different endocrine thresholds may also explain why changes in call duration correlate with the endocrine milieu in both the conception and postconception cycles, whereas spectral variations in call structure are related to hormone levels only in the postconception cycle. However, based on the present results we are unable to disentangle the relative contribution and interactions of these different factors, mainly owing to the lack of sufficient measurements of a female's motivational state.

Because steroid hormones generally act by modulating gene expression, their biological effect is usually delayed by hours or even several days (Gillman 1940; Losel and Wehling 2003; Sherwin and Gelfand 1987). Our data showing that a time lag of 3 d best explains the potential effect of the concentrations of the 2 hormones on the acoustic structure of the calls is consistent with this. Moreover, the observed time lag of hormonal action on calling structure is in the range of previously reported delays between endocrinological changes and downstream effects on other behaviors or morphological traits in vertebrates. For instance, the effect of progesterone on the deturgescence of female anogenital swelling size in baboons has a lag of 48 h (Gillman 1940), the initiation of menstrual bleeding occurs 90–96 h after progesterone administration (Gillman 1940), and the effect of estrogen on mounting behavior in rats is delayed by 7 d (D'Occhio and Brooks 1980). Similar time lag periods occur for testosterone action, e.g., treatment of women with testosterone leads to changes in female sexual interest after 1 wk (Sherwin and Gelfand 1987).

In terms of the function of Barbary macaque copulation calls, previous studies have demonstrated that copulation calls do not advertise the female fertile phase, but do affect mating outcome, i.e., ejaculation (Pfefferle *et al.* 2008a). Further, male Barbary macaques are able to distinguish between copulation calls uttered during ejaculatory and nonejaculatory copulations, with the former eliciting a stronger male response in terms of searching for the calling female (Pfefferle *et al.* 2008b). Thus, by stimulating male–male competition and increasing the number of male mating partners, copulation calls may be a means of promoting sperm competition/paternity confusion in this species. Though paternity confusion has been shown to reduce the risk of infanticide by males in several primate species (van Schaik *et al.* 2004), in Barbary macaques it may also set the stage for promoting extensive infant care from multiple males (Paul *et al.* 1992). This does not discount other hypotheses of copulation call function in other species with a different mating system, e.g., Guinea baboons (*Papio papio*), in which copulation calls occur after copulation, leading to increased male mate guarding (Maestripieri *et al.* 2005).

Because the structure of copulation calls uttered during the conception and postconception cycles does not differ, the acoustic modality cannot be used to discriminate these 2 cycle types. Although frequent copulations do occur during the postconception cycle, it remains to be investigated whether their performance is biased toward lower ranking or newly immigrated males, as in long-tailed macaques (Engelhardt *et al.* 2007). If this is the case, males that are familiar with the females may discriminate between conception and postconception cycles using other modalities, e.g., behavior, anogenital swelling, and odor. However, in Barbary macaques, it is difficult to envisage how such a capability would help males to increase reproductive success because females are not monopolized by specific males during the fertile phase (Brauch *et al.* 2008), and >75% females conceive during their first menstrual cycle (Kuester and Paul 1984; Möhle *et al.* 2005). The occurrence of postconception mating in general and the finding of no structural differences between copulation calls uttered during the conception and postconception cycles, together with a rather weak link between reproductive hormone levels and copulation call structure during the conception cycle and a pronounced time lag in the modulation of the call structure by the female endocrine milieu, support the hypothesis that copulation calls function more in the context of paternity confusion than in paternity assurance. Taken together, our data provide a plausible explanation as to why female Barbary macaque copulation calls are related to changes in hormonal levels but do not indicate the fertile phase.

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