Cross-modal signaling in giant pandas

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For solitary species that aggregate to breed, signals, such as sound or odor, transmitted across the landscape can play an important role in mate choice and coordinating breeding activity. Recent work on vocalizations of the giant panda (*Ailuropoda melanoleuca*), bleats and chirps that are emitted during periods of reproductive activity, has revealed that auditory communication in this species is more complicated and developed than once believed. However, playback experiments using these calls have recorded only a few behaviors over short observation times and the influence of these calls on the signaling behavior of receivers remains unknown. Here, we present results from a pilot study in which we played the bleats of male and female giant pandas in estrus to captive animals and measured vocal and chemical signaling response behavior. We found an increase in scent marking behavior, but not vocalizations, in males and females following the playback of calls made by members of the opposite sex in estrus. To our knowledge, this is the first study to detect a chemical communication response, which was marking, to an auditory signal in giant pandas. Our findings reveal a previously unknown relationship between acoustic and chemical signals in this high profile species and suggest that these two forms of signaling may be interdependent in the social lives of giant pandas.

***Ailuropoda melanoleuca*, auditory communication, chemical communication, multiple modality, giant panda**

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Vocalizations are an important method used by animals to communicate [1]. Research has revealed a range of languages, cultures, functions and social contexts in which vocalizations are fundamental in a wide range of species [2–6]. Research on auditory signals can take an acoustic, behavioral or functional approach, or a combination of these [7,8]. Auditory communication in mammals has been shown to have a range of functions. For example, individuals detect, find and choose mates based on the vocalizations of others in free ranging yellow baboons, *Papio cynocephalus cynocephalus* [9,10]. Males use calls to deter rivals when defending territory and competing for access to females and other resources in ungulates, such as red deer *Cervus elaphus* and fallow bucks *Dama dama* [11,12]. Sound can also be used as an alarm in social animals, such

as ground-dwelling Sciurids and primates [13,14]. Vocal signals are also used by gregarious mammals to maintain social bonds, such as for parent-offspring recognition [15–18].

For solitary species that only aggregate to breed, signals, such as sound (audible and ultrasonic song), body display and odor, transmitted across the landscape can play an important role in mate choice and coordinating breeding activity [12,19,20]. Male and female animals often switch from one communication modality to another in response to courtship calls, body display or scent marking during the mating season. For example, female concave-eared torrent frogs (*Odorrana tormota*) make ultrasonic calls instead of audible calls to communicate with courtship males immediately before ovulation [21]. Female swamp sparrows (*Melospiza georgiana*) display significantly more solicitation responses to high-performance versus low-performance

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songs of the same song type produced by different males [22]. The issue of cross-modalities communication and mate choice is an evolving area of research, and even in high-profile species our understanding of these systems is poor. Recent work on giant pandas (*Ailuropoda melanoleuca*) [23–26] has revealed that auditory communication is more complicated than previously believed. Giant pandas are solitary animals that produce 11 different vocalizations across a range of contexts, some of which change in rate and structure during periods of reproductive activity [27,28]. Male and female giant pandas frequently produce vocalizations (bleats and chirps) and deposit scent markings during the mating season [29]. Bleats and chirps are nonaggressive and affiliative calls predominately used in sexual attraction and the synchronization of estrus and mating in giant pandas [30–33]. Acoustic analysis has shown that bleats are individualized and may signal caller sex, age and body size [23]. In addition, female giant panda chirps can signal their precise estrus state [24]. Experiments involving the playback of male calls to females has shown that female giant pandas can discriminate between the vocalizations of males and show preference to potential mates [23]. Male giant pandas can also perceive differences in female chirps and possibly also the time of female fertile phases [24]. Giant panda calls are often produced alongside increases in marking behavior [34,35] and while marking and vocalization behavior have been studied separately, the relationship between these signaling modes remains unknown. Here we present results from a pilot study using captive giant pandas that focused on the auditory and olfactory responses to the bleats of male and female giant pandas during estrus. This research will add to our growing appreciation of communication in giant pandas and other large mammals, and may have applications for the captive management of this iconic species.

1 Materials and methods

1.1 Animals

We conducted this study at the China Conservation and Research Center for the Giant Panda at Wolong Nature Reserve, Sichuan, China. Our study comprised 10 adult females and 8 adult males aged 5–16 years (Table 1) and took place from March to June 2005. Focal males and females were examined during estrus. At this facility, animals are individually housed in typical indoor/outdoor enclosures [36] that are cleaned daily from 07:30–09:00 hours. Animals were fed twice daily (morning and afternoon) fresh bamboo and a mix of corn, bamboo powder, soybean and egg.

1.2 Recordings and playback stimuli

Male bleats were recorded from Wugang during estrus and female bleats were recorded from Maomao during estrus

Table 1 Subjects used in the pilot study^{a)}

Name	Studbook number	Age in years during experiment	Gender
Zhuangzhuang	357	16	Male
Yingying	369	14	Male
Ximeng	399	12	Male
Lingling	424	10	Male
Linlin	455	8	Male
Yuanyuan	488	6	Male
Wugang	502	6	Male
Lulu	503	6	Male
Leilei	374	16	Female
#20	414	15	Female
Haizi	544	11	Female
Feifei	432	10	Female
Guoguo	439	9	Female
Yoyou	474	7	Female
Gongzhu	477	7	Female
Maomao	504	6	Female
Yeye	495	6	Female
Longxin	516	5	Female

a) As of 2005.

from March to June 2005. Wugang and Maomao are not genetically related to our subjects, did not engage in abnormal behavior and showed normal signs of fertility. Calls were captured using a Sony ICD-SX40 MP3 player (Sony, Tokyo, Japan) and Samson S11 microphone (Samson Technologies, Hauppauge, United States). All sounds were recorded at maximum microphone power. We recorded 10–30 calls from each animal during scent marking behavior and saved them in MP3 format. We chose calls that contained the least background noise for use as playback stimuli. Stimuli consisted of 10 bleats (frequency: 60 Hz–13.5 kHz) each separated by 10 s and were played using a Sony ICD-SX40 MP3 player (Sony) with a 70 mW speaker at maximum volume. The MP3 player was hidden from the focal animal behind a fixture within the enclosure.

1.3 Behavioral responses to playbacks

Playbacks were conducted between 8:00–10:30 and 14:00–16:30 hours. Before each observation, the door connecting the indoor and outdoor sections of the enclosure was opened and the animal was enticed inside by animal keepers. To reduce the possible effects of neighboring individual's chemical, auditory and visual signal stimuli, the subject was kept inside the indoor section of the enclosure by closing the door connecting the indoor and outdoor sections while the tests were being run. After entering the indoor area, animals were given 3 min to adapt to the area before a stimulus was played or the control treatment commenced. We chose 3 min because in our experience this is the average time that a giant panda inspects a chamber after

entering and before engaging in stereotypical behavior. For the playback, we played bleats from Wugang to 10 females in estrus and bleats from Maomao to 8 males in estrus. Focal animals were observed for 10 min. The same playback stimulus was played to each focal animal 3 times, separated by 2 d (range: 2–3 d). A control treatment consisted of observing the animal for 10 min with the speaker power on and at maximum volume, but with no input. Control treatments were run only once for each focal animal. For each subject, the test lasted 40 min, which included one 10-min control test and three 10-min playback tests. Thus, for the 18 animals (10 female, 8 male), 720 min of observation and video recordings were collected. The focal animal sampling method and continuous recording method were used for the behavioral observations.

Our subjects were housed within a breeding and research center and have experienced long-term cohabitation within its confines. Given the physical space between enclosures and degree of separation between animals, we doubt that our focal animals have developed a preference for our particular donor male or female based on vocalizations. We chose donors inhabiting enclosures as far as possible from focal animals; we cannot however, rule out the possibility that our focal animals had developed an acoustic memory of the donor animals (Wugang and Maomao) used in the playbacks.

1.4 Behavioral and statistical analyses

We noted all marking and vocalization behavior during the observations. We defined marking behavior as rubbing the anogenital gland against a surface. For vocalization behavior, we noted the number of bleats emitted. Behaviors were recorded in real-time by a single trained observer positioned 2–3 m from the enclosure with an unobstructed view of the focal animal. Observers were aware of the identity of the donor and familiar with the behavior of giant pandas in captivity. For our analyses, we tabulated the frequency of marking and bleating across all three playback trials and three control treatments and then averaged these values to obtain one value for the playback and one for the control. We inspected the distribution of the data for normality. To investigate evidence for an effect of the playback, we used paired-sample *t* tests in SPSS for Windows 13.0 (SPSS Inc, Chicago, United States). The significance level was 0.05 and all tests were two-tailed.

2 Results

Male giant pandas increased marking behavior following the playback of Maomao's bleat (paired *t* test, $df = 7$, $P = 0.001$) and females increased marking behavior following exposure to Wugang's bleats (paired *t* test, $df = 9$, $P = 0.01$). No change in the number of bleats emitted by focal males

and females following the playbacks was observed (female: $df = 9$, $P = 0.293$; male: $df = 7$, $P = 0.300$; Figure 1).

3 Discussion

Chemical and auditory communication are major modes of signaling in the lives of giant pandas [33]. Giant panda male and female urine and male anogenital gland secretions contain information that allow individuals to discriminate age, gender and social status [34,37–41]. We have a basic understanding of chemical signaling in giant pandas [37,38], and recent work on their vocal communications has raised the profile of this aspect of their sociality [23,25]. It is now clear that a great deal of information is coded in the bleats and chirps of giant pandas and ours is the first study to detect a chemical communication response, which was marking, to these auditory signals. This association between chemical and vocal signaling is further supported by work on captive and wild giant pandas [30,33,42]. For example, adults produce more bleats, chirps and markings when are translocated to an enclosure belonging to the opposite sex [34,35] and in captivity male and female giant panda switch from scent marking to chirping and bleating as the breeding season progresses [43]. This mix and switch of auditory and chemical communication is common in captive giant pandas, but data from the wild is still lacking [33,44]. Our findings have shown a clear relationship between cross-modal signals that will hopefully stimulate further research in both the giant panda and other mammals.

One of the goals of the giant panda captive breeding program is to produce healthy individuals with species-typical behavior, such as mating and foraging. However, more than half of annual breeding output is realized via artificial insemination or a combination of artificial insemination and natural mating (Liu D, unpublished data). Further, some

Figure 1 Marking and bleating frequency (times/10 min) of (a) male giant pandas to the bleats of estrus female (black bars) and (b) female giant pandas to the bleats of estrus males (black bars) and a control treatment (white bars). Data are mean $\pm$ SE. * $P < 0.05$, ** $P < 0.01$.

adult males are unable to mate naturally with females because of asynchrony or female sexual inactivity [29]. These issues may be because of a focus on the chemical environment [34,39,45] and a lack of understanding of the role of vocal communication in giant panda mate selection and reproduction. Recent acoustic analyses in combination with our findings suggest auditory communication may play the same role as chemical communication in the social lives of giant pandas [23–25]. Further work is needed before we can apply our understanding of vocal signaling to giant panda captive breeding programs. Our limited number of sound donors means that we are unable to generalize further about the role of vocal signaling in this species, but the exciting finding of cross-modal signaling in this species should guide future research into wild and captive giant pandas.

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