Influence of dominance rank and affiliation relationships on selfdirected behavior in female Tibetan macaques (*Macaca thibetana*)

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Abstract: Self-directed behavior (SDB) is characterized as an indicator of anxiety, frustration and stress in nonhuman primates. In this study, we collected self-directed behavior data from one group of free-ranging Tibetan macaques (*Macaca thibetana*) at Mt. Huangshan, China (September 2012–May 2013) using a combination of behavioral sampling methods including focal animal sampling, behavioral sampling, continuous sampling and instantaneous sampling. Our results showed that females engaged in significantly higher rates of self-directed behavior when they were in proximity to dominant individuals compared to subordinate ones. Conflict losers significantly increased their SDB rates after agonistic episodes, indicating that SDB might also serve as an index of anxiety in *M. thibetana*. We further found that females significantly increased their SDB rates of losers were higher when they stayed with strongly affiliation opponents; if conflicts were not reconciled, the postconflict SDB rates of losers were higher when they stayed with strongly affiliation opponents; if conflicts were reconciliation, while victims of moderately affiliation relationships opponents did not engaged in more SDB rates before reconciliation than after reconciliation. We conclude that both of dominance rank and affiliation relationships might both influence the SDB rates of female Tibetan macaques significantly, suggesting that SDB is not only an index of anxiety in Tibetan macaques, but also can provide a new insight into evaluation of social relationships between individuals.

Keywords: Tibetan macaques (Macaca thibetana); Female; Self-directed behavior (SDB); Dominance Rank; Affiliation relationship

Animals, including humans, can exhibit some activities unconsciously when facing with stress or anxiety (Lantz, 1979; Maestripieri et al, 1992). For example, passerine birds clean their bills or feathers in some sexual or agonistic contexts; non-human primates show self-scratch behavior after being attacked by conspecific members (Maestripieri et al, 1992; Radford, 2012). Thus, a behavior pattern which is "apparently irrelevant" to an animal's ongoing activities called selfdirected behavior (SDB) (Maestripieri et al, 1992). It exists in a wide range of animals such as arthropods, fish, birds and mammals (Duncan & Wood-Gush, 1972; Hansen & Drake Af Hagelsrum, 1984; Roper, 1984; Rowell, 1961). Self-directed behavior, such as selfscratching, self-grooming, self-touching, yawning and body shaking (Schino et al, 1988), have been widely

used as an indicator of anxiety in non-human primates. Pharmacological and behavioral evidence showed that there was a high correlation between SDB and anxiety (Maestripieri et al, 1992). For example, anxiolytic and anxiogenic drugs could reduce or increase the individual SDB rates in wild gregarious non-human primates (Crawley et al, 1985; Maestripieri et al, 1992; Ninan et al, 1982; Redmond Jr & Huang, 1979; Schino et

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al, 1996; Schino et al, 1991). With purely observational method, researchers attempted to link SDB and anxiety by observing the individual SDB rates in different contexts. In the study of Macaca fascicularis, Pavani et al (1991) found that the individual engaged in higher scratching rates when stayed with a dominant neighbor rather than with a subordinate neighbor. Similar results were received in Olive Baboon (Papio anubis) (Castles et al, 1999). Although Captive vervets (Cercopithecus aethiops) didn't engage in more SDB rates when near to a dominant neighbor, it showed higher SDB rates when in postconflict situation (Daniel et al, 2008). Analogous studies about M. fuscata fuscata, M. Sylvanus, M. fascicularis suggested that individual's SDB rates tended to increase in postconflict situation (Aureli, 1997; Aureli & Schaik, 1991; Kutsukake & Castles, 2001).

Earlier studies about SDB in non-human primates mainly concentrated in two uncertain circumstances: (1) close proximity to dominant individuals. Subjects would engaged in anxiety emotion since the dominant neighbor increased the possibility being attacked (Daniel et al, 2008). (2) postconflict. After a conflict, individuals became stressful as a result of 2 ambivalent motivations : 1) withdrawing for fear of renewed attacks and 2) approach to reconcile (Aureli, 1997; Aureli & Schaik, 1991). Thus, the individual's SDB rates after conflict increased significantly. These two uncertain circumstances are both related to dominance rank, that is to say, subordinates will engage in higher SDB rates under threaten or potential threaten from dominants. However, individual's SDB levels are not only influenced by the dominance rank, but also social relationships (Castles et al, 1999). For example, Castles et al (1999) found that the Olive Baboons' SDB rates were higher when proximate to dominants, but had no correlation with the rank distance. Based on this, if the SDB rates are influenced by the dyadic relationships between the partners? Moreover, if the SDB rates are influenced by the quality of the relationship between former opponents after conflict? All these questions remain to be answered.

Tibetan macaques(*M. thibetana*) are highly gregarious and display female philopatry, male dispersal (Li, 1999; Xia et al, 2013). Male-male relationship are mostly competitive, while female-male relationships are affected by different factors such as mating season, non-mating season, temporary spouse relationship and population sex ratio(Li, 1999; Wang et al, 2009). Female Tibetan macaques are reported to form linear dominance hierarchies, establish strong and well-differentiated relationships with other adult females in their group, and female-female relationships play a very important role in maintenance of the group's stability (Xia et al, 2012). Therefore, female Tibetan macaques are ideal model to study influence of dominance rank and dyadic affiliation on SDB. Proximity to dominant individuals and postconflict contexts were set in this study, to examine: (1) the correlation between SDB and anxiety; (2) the effect of dyadic affiliation relationships on SDB.

MATERIALS AND METHODS

Study site and subjects

We conducted this study at the Valley of Wild Monkeys, Mt. Huangshan, Anhui Province, China (E118°10', N30°29'), a well-known tourist destination and research site, environment about this area refer to Li (1999). There are two groups at the site: Yulingkeng A1 (YA1) and Yulingkeng A2 (YA2), for this project we collected behavioral samples in YA1. The nine female adult individuals were selected for study which can be distinguished by facial/body characteristics, and the abbreviations rules of the subjects' name referred to Li (1999). Observations of YA1 continued for 27 years since 1986, recording dynamic data of immigration, emigration, death, birth and so on. During the study period, the YA1 group consisted of 4 adult males, 9 adult females, 5 subadults and 18 juveniles/infants. Table 1 shows the study individuals and duration for sampling during study.

Behavioral definitions

In this study, self-directed behavior definitions see Table 2.

Data collection

This study was conducted from September 2012 to May 2013, using behavioral sampling, focal animal sampling, continuous recording, instantaneous recording to collect behavioral data (Altmann, 1974).

Behavioral sampling was used to record the displacements, aggressions and avoidances among females, these data were used to construct the win/lose matrix in order to confirm dominance hierarchy, 288 samples were recorded.

Focal sample duration (using a digital voice record)

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Table 1 Subjects' duration for sampling and SDB rates							
Subjects	Focal duration (min)	SS (bouts/min)	SG (bouts/min)	ST (bouts/min)	SHAKE (bouts/min)	YAWN (bouts/min)	Total SDB (bouts/min)
YH	1200	0.243	0.049	0.007	0.003	0.002	0.303
Hhui	1200	0.213	0.073	0.011	0.008	0004	0.309
YM	1197	0.203	0.046	0.018	0.005	0.008	0.279
TH	1200	0.168	0.088	0.013	0.011	0.001	0.282
TXX	1193	0.244	0.049	0.013	0.010	0.010	0.326
HH	1196	0.219	0.078	0.024	0.016	0.006	0.343
TR	1200	0.193	0.094	0.023	0.010	0.003	0.323
TT	1200	0.157	0.051	0.017	0.004	0.003	0.231
YZ	1200	0.198	0.068	0.011	0.003	0.003	0.283

Dominance rank of 9 focal female descended from top to bottom.

Table 2 Behavioral definitions							
Catalog	Definition						
Self-scratching (SS)) (usually repeated) Movement of the hand or foot during which the fingertips are drawn across fur or skin.						
Self-grooming (SG)) Picking through and/or slowly brushing aside fur with one or both hands.						
Self-touching (ST)	Other forms of body touching with the hand including wiping eyes, inspecting feet and placing hand to mouth.						
Shaking	Shaking movement of entire body (similar to that of a wet dog).						
Yawning	Brief gaping movement of the mouth. Not recorded as an SDB if accompanied by aggressive signals such as eye flash or canine whetting.						
Postconflict reconci	Postconflict reconciliation behavior						
Teeth-chatter	Clicking sounds are made with the teeth by rapidly moving the jaw up and down. Eyelids are lowered, the chin is raised, and the tongue may move rapidly across the teeth.						
Embrace	One individual approaches another and lipsmack or both individuals hold each other and may lightly bite one another.						
Touch	One individual lightly touches another usually on the head, shoulders, or back.						
Present	One individual displays her bottom to another.						
Genital inspection	One individual touches, licks, or sniffs the genitals of another.						
Groom	One individual orally or manually manipulates the fur of another						
Bridge	A complex sequence of behavior in which an individual approaches another, alternating glances at the receiver and an infant that is carried by either the approacher or the approached. The pair holds the infant between them and simultaneously licks the infant's genitals or body while teeth-chattering vigorously.						
Hold bottom	One individual approaches another and holds or embraces her bottom for a few seconds.						

Self-directed behavior definitions were modified by Schino et al (1988), aggressive behavior definitions were modified by Berman et al (2006) and Li (1999).

was set at 20 min to score the activity of focal individual. To balance samples, we sampled each focal individual randomly, with no subject observed twice before all others were watched once. Focal sampling and continuous recording were used to score grooming, proximity and all that affiliation behaviors to evaluate the dyadic affiliation relationships. During the focal sampling, we also recorded the SDB and set breaks in SDB lasting >2 s or switches to another class of SDB as separate bouts. Whenever we recorded an SDB bout, we also recorded the identity of the nearest individual within 2 m. Instantaneous recording was proceeded with focal sampling to record the identity of the nearest individual at 1-min intervals. Samples were discarded if the focal individuals

disappeared and samples were less than 15min (Castles et al, 1999; Manson & Perry, 2000).

We initiated focal observations on the victim of aggression once a conflict happen and record: victim and aggressor (Li, 1999). Then we collected 5 min postconflict (PC) focal sample, including SDB and affiliative interactions. We also recorded the time both when a SDB and postconflict affiliative interactions (see table 2) occurred. If conflict reoccurred between former opponents during the PC period, we restarted the PC observation after the new encounters. The next day at the same time, we conducted matched control (MC) focal observations of the victim, the contents and methods recorded in MC according to PC. If the subjects were out of vision or involved in a conflict 5min before MC, we postponed the MC until the all conditions were met. If MC observation could not be conducted within one week of the PC, the PC was discarded.

Data analysis

We assessed individual dominance ranks by calculating David's Score(DS). We also calculated linearity for the obtained dominance hierarchy (De Vries, 1995; Gammell et al, 2003). Rank distance is the number of individuals ranking between the focal animal and a given partner, plus 1 (Castles et al, 1999).

Individual ranks calculated with DS: P_{ij} (The proportion of wins by individual *i* in his interactions with another individual *j*)= α_{ij} / n_{ij} , P_{ji} =1- P_{ij} ; α_{ij} is the number of times that *i* defeats *j*, n_{ij} is the total number of interactions between *i* and *j*. If there was no aggression and avoidances between individual *i* and *j*, then P_{ij} = P_{ji} =0. For each member *i*, DS= $w+w_2$ - $l-l_2$, *w* represents the sum of *i*'s P_{ij} ; w_2 represents the summed *w* values (weighted by the appropriate P_{ij} values) of those individuals with which *i* interacted; *l* represents the sum of *j*'s P_{ji} ; l_2 represents the summed *l* values (weighted by the appropriate P_{ji} values) of those individuals with which *j* interacted. Individual dominance rank is determined by DS, the higher the DS value, the higher the rank, and vice versa. For more details, see Gammel et al (2003).

To determine the number of proximity point samples, we followed Castles et al (1999), wherein *s* is the number of SDB bouts shown by focal individual, when individual *X* was nearest neighbor within 2 m; *P* equals the number of proximity point sample in which individual *X* was nearest neighbor within 2 m. For individual *X*, the focal individual's neighbor SDB rates is s/q. According to the way of calculation above, the unit of SDB rates is bouts/min.

We measured the relationship between females by using the Dyadic Association Index (DAI):

$$DAI_{AB} = \frac{\sum (A+B)}{\sum A + \sum B - \sum (A+B)}$$

wherein A is the time individual A was seen, B is the time individual B was seen and A+B is the time A and B were seen together (Nishida, 1968). Then the dyadic scores were ranked individually. In this study, each one of the 9 focal animals corresponds to 8 dyadic relationships, wherein the top quartile were labeled as strongly affiliation relationship and the lowest quartile were labeled as weakly affiliation relationship, and the mid 50% was labeled as moderately affiliation relationship (Arnold & Whiten, 2001; Cords & Aureli, 2000; Preuschoft et al, 2002).

We analyzed only overall rates (all SDBs summed) because most of the individual SDBs, besides scratching, had very low occurrences. We used mean values (\pm *SE*) as quantitative criteria. A paired *t*-test was used to analyze differences of SDB rates influenced by different dominance rank and different affiliation relationship. Spearman Rank Correlation Test was used to test the correlation between dominance rank and SDB rates, also, the rank distance and SDB rates. All analyses were two tailed and carried out using the SPSS 16.0 software, with the significance level set at 0.05.

RESULTS

Dominance hierarchy

Dominance hierarchy for adult females showed a linear dominance hierarchy (h'=0.991, n=9, P<0.001; see Table 3).

		5										
	YH	HHUI	YM	ТН	TXX	нн	TR	TT	YZ	w	w ₂	DS
YH		8(1.0)	13(1.0)	10(1.0)	12(1.0)	5(1.0)	8(1.0)	6(1.0)	5(1.0)	8	28	36
HHUI			9(1.0)	12(1.0)	11(1.0)	3(1.0)	1(1.0)	7(1.0)	3(1.0)	7	21	27
YM				14(1.0)	12(1.0)	1(1.0)	14(1.0)	7(1.0)	2(1.0)	6	15	18
ТН					6(1.0)	11(0.85)	15(1.0)	9(1.0)	7(1.0)	4.85	9.68	7.65
TXX						1(1.0)	12(1.0)	5(1.0)	4(1.0)	4	6.15	0
нн				2(0.15)			11(1.0)	7(1.0)	5(1.0)	3.15	3.73	-7.65
TR								13(1.0)	11(1.0)	2	1	-18
TT									6(1.0)	1	0	-27
YZ										0	0	-36
1	0	1	2	3.15	4	4.85	6	7	8			
l_2	0	0	1	3.73	6.15	9.68	15	21	28			

Table 3	Dominance	hierarchy	for females
Table 5	Dominance	mici ai cii y	IUI IUIIIaius

The individual on the vertical axis scored as winner.

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Correlation between SDB and anxiety in female Tibetan macaques

In the study group, the mean±*SE* SDB rates among 9 subjects was 0.297 ± 0.011 bouts/min (see Table 1). There is no correlation between dominance rank and any SDB rates (overall: *Rs*=-0.050, *n*=9, *P*=0.898; SS: *Rs*=-0.500, *n*=9, *P*=0.170; SG: *Rs*= 0.283, *n*=9, *P*=0.460; ST: *Rs*= 0.393, *n*=9, *P*=0.295; SHAKE: *Rs*=-0.017, *n*=9, *P*=0.966; YAWN: *Rs*= 0.000, *n*=9, *P*=1.000).

All subjects had ≥ 1 other females within 2 m on 34.9% (±4.92%) of the proximity point samples. Focal subjects engaged in significantly more SDB rates when their nearest neighbor was a dominant than the neighbor was a subordinate (*t*=4.629, *n*=7, *P*=0.004) or there was no neighbor(*t*=2.622, *n*=7, *P*=0.039) within 2 m. In addition, they engaged in more SDB rates when there was no neighbor than their nearest neighbor was a subordinate within 2m (*t*=2.898, *n*=7, *P*=0.027) (Figure 1) (There was no dominants near YH and there was no subordinates near YZ, so we did not analysis the neighbors of YH and YZ in this part).

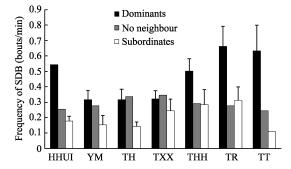


Figure 1 Self-directed behavior rates when a dominant or subordinate conspecific was nearest neighbor within 2 m and when there was no nearest neighbor within 2 m

The SDB rate was not correlated with rank distance (dominant point samples near YH, Hhui, YM and subordinate point samples near YZ, TT, TR were all less than 3, we didn't analysis in this part). Spearman rank correlation coefficients ranged between -0.600 and 0.500 for dominant near neighbors (TH: subordinate partners: Rs=0.500, n=3, P=0.667; TXX: subordinate partners: Rs=0.000, n=4, P=1.000; HH: subordinate partners: Rs=0.100, n=5, P=0.873; TR: subordinate partners: Rs=-0.600, n=6, P=0.208; TT: subordinate partners: Rs=-0.179, n=7, P=0.702; YZ: subordinate partners: Rs=-0.214, n=7, P=0.645. we didn't analysis YZ, TT and TR for the number of subordinates partner of YZ, TT and TR were less than 3); between-0.976 and 0.800 for

subordinate near neighbors (YH: dominant partners: Rs=-0.976, n=8, P=0.000; Hhui: dominant partners: Rs=-0.536, n=7, P=0.215; YM: dominant partners: Rs=0.800, n=4, P=0.200; TH: dominant partners: Rs=-0.300, n=5, P=0.624; TXX: dominant partners: Rs=-0.400, n=4, P=0.600; HH: dominant partners: Rs=-0.500, n=3, P=0.667. we didn't analysis YH, Hhui and YM for the number of subordinates partner of YH, Hhui and YM did not reach 3). Only 1 of 12 coefficients were significant (YH: subordinate partners: Rs=-0.976, n=8, P=0.000).

In this study, we had 60 valid PC-MC observations. Postconflict SDB rates without reconciliation were significantly more than MC (t=6.317, n=8, P=0.000) and after reconciliation (t=5.142, n=7, P=0.002). The SDB rates before reconciliation were significantly more than MC (t=3.675, n=7, P=0.010) and after reconciliation (t=3.654, n=7, P=0.008). It was close to significant that the postconflict SDB rates with no reconciliation were more than before reconciliation (t=-2.550, n=7, P=0.043), while there was no significant differences between after reconciliation and MC (t=1.058, n=7, P=0.331) (Figure 2).

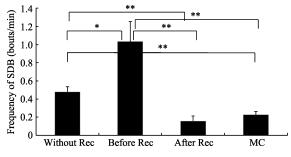


Figure 2 Victims' self-directed behavior rates with reconciliation (Rec) or without reconciliation between opponents and MC

**: P<0.01; *:P<0.05.

There was no correlation between postconflict SDB rates and rank distance (the data of YH, Hhui, YM was not used in this analysis because there were not enough individuals nearby for spearman correlation analysis). Spearman rank correlation coefficients ranged between - 0.522 and 0.718(TH: Rs=-0.866, n=3, P=0.333; TXX: Rs=-0.500, n=3, P=0.667; HH: Rs=-0.112, n=5, P=0.858; TR: Rs=-0.800, n=4, P=0.200; TT: Rs=0.500, n=3, P=0.667; YZ: Rs=-0.316, n=4, P=0.684).

Female affiliation relationship and proximity SDB

Comparisons of individuals' mean SDB rates with

dominant partners of different affiliation relationships, we found that subjects engaged in more SDB rates with a neighbor of strong affiliation relationship than of weakly affiliation relationship(t=-2.818, n=5, P=0.048). There was no significant differences between with a neighbor of strong affiliation relationship and moderately affiliation relationship (t=-0.568, n=6, P=0.595), also, moderately affiliation relationship and weakly affiliation relationship (t=-0.377, n=5, P=0.726).

Comparisons of individual mean SDB rates with subordinate partners of different affiliation relationships, we found no differences among them (strong affiliation relationship and moderately affiliation relationship: t=2.641, n=3, P=0.118; strong affiliation relationship and weakly affiliation relationship: t=0.417, n=4, P=0.705; moderately affiliation relationship and weakly affiliation relationship and weakly affiliation relationship and relationship and weakly affiliation relati

Female affiliation relationship and postconflict SDB

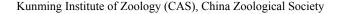
Postconflict with no reconciliation, the subjects engaged in significantly more SDB rates aggressed by individuals with strong affiliation relationship than weakly affiliation relationship (t=0.900, n=4, P=0.003). But there were no differences between attacked by strong affiliation relationship and moderately affiliation relationship (t=0.987, n=5, P=0.379), moderately affiliation relationship (t=1.353, n=5, P=0.247).

Postconflict with reconciliation, subjects engaged in significantly more SDB rates attacked by strongly affiliation relationships before reconciliation than after reconciliation (t=3.008, n=8, P=0.020) and MC (t=3.006, n=8, P=0.020). There were no differences between attacked by moderately affiliation relationships before reconciliation and after reconciliation (t=2.955, n=4, P=0.060) or MC (t=2.039, n=4, P=0.134) (Figure 3).

DISCUSSION

The correlation of SDB and anxiety in female Tibetan macaques

Female Tibetan macaques showed significantly higher SDB rates while proximity to dominants than subordinates, which may be resulted from the strict dominance hierarchy. Aggression among Tibetan macaques is directed predominantly down the hierarchy (Li, 1999), thus a dominant partner will present more danger of receiving aggression than a subordinate partner, and increased anxiety should be expected. The SDB rates is



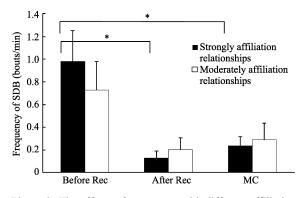


Figure 3 The effects of opponents with different affiliation relationships on self-directed behavior frequency before reconciliation and after reconciliation

*: P<0.05.

significantly higher when there was no neighbor within 2 m than in proximity to a subordinate, suggesting that detachment from near neighbors increased the potential surrounding risk and separation from allies. Moreover, the presence of subordinates reduced the subjects' SDB rates significantly, this is because both the two potential risks presented above were removed, that is, the dominants scarcely received threaten from subordinates, and the potential separation from allies was eliminated, thus the individual was in a state of emotional stability.

Similar with the results reported for long-tailed macaques (M. fascicularis) (Aureli, 1992;1997; Aureli & Schaik, 1991; Aureli et al, 1989; Das et al, 1998), Barbary macaques (M. sylvanus) (Aureli, 1997), Olive baboons (Castles & Whiten, 1998), Chimpanzees (Pan troglodytes) (Fraser et al, 2010), after conflicts, female Tibetan macaques showed higher SDB rates without reconciliation or before reconciliation, while the opponents reconciled, the SDB rates decreased. It can be explained that the victim was fear of renewed attacks without reconciliation or attempt to reconcile before reconciliation (SDB rates was higher before reconciliation than without reconciliation confirmed the second assumption, see Figure 2), thus the presence of anxiety emotion is expected. If this emotion can not be disposed immediately and cumulated, which would increase the likelihood of worse outcome such as growing development, disease resistance and fertility (Henry, 1982; Nederhof & Schmidt, 2012), and even the damage of brain (Uno et al, 1989). When the opponents reconciled, the emotion of anxiety and stress got released, which decreased the bad consequence greatly, suggesting the importance of postconflict reconciliation to individual survival and even the stability of population.

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Both of the two uncertain situation were established on dominance rank difference in social interaction, which showed that there was some correlation between SDB and anxiety in Tibetan macaques.

The influence of affiliation relationship on SDB

In this study, SDB rates of focal subjects had no correlation with rank distance of the proximate partners and the aggressors, suggesting that SDB varies not only according to the dominance rank, Castles et al (1999) supposed that SDB rates also varies according to other aspects of its relationship with the partner in close proximity.

We divided dyadic affiliation relationship into three levels: strongly affiliation relationship, moderately affiliation relationship and weakly affiliation relationship, and found that focal subjects engaged in more SDB rates in proximity of dominants with strongly affiliation relationship than weakly affiliation relationship, which is consistent with the speculation of Castles et al (1999): the comparison of SDB rate across proximity partners could, therefore, provide insight into relationship security. In secure relationship, whether or not the tolerance, low aggressive rates, gaining support would be common remained to be verified in further study.

In the study of genus *Macaca* (*M. fascicularis*), Aureli (1997) found that the victim showed higher SDB rates aggressed by more valuable group members. A similar result was received in female Tibetan macaques that the victim showed more SDB rates attacked by strongly affiliation relationship partners than weakly affiliation relationship partners. Aggressed by strongly affiliation relationship partners with reconciliation, the victim showed higher SDB rates before reconciliation than after reconciliation, while the SDB rates did not show difference before reconciliation and after reconciliation aggressed by moderately affiliation relationship partners. It may be because that the Tibetan macaques are highly gregarious dominated by males, the status of females mainly depended on the supports of male and females alliance particularly (Li, 1999) and female alliance mainly depended on partners with strongly affiliation relationship. If the bond between strongly affiliation relationship partners was break, it would be greatly harmful to females' status and value in the group. Focal subjects were anxious to repair the relationship after conflicts, while, the break of strongly affiliation relationship had worst influence, thus the higher SDB rates performed after conflicts between partners with strongly affiliation relationship is expected.

In conclusion, SDB had a positive correlation with anxiety in Tibetan macaques, and the influence of dyadic affiliation relationship on SDB was reflected in our study.

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