



ELSEVIER

SCIENCE @ DIRECT®

Hormones and Behavior xx (2004) xxx–xxx

Hormones
and Behaviorwww.elsevier.com/locate/yhbeh

Vocal buffering of the stress response: exposure to conspecific vocalizations moderates urinary cortisol excretion in isolated marmosets

Michael Rukstalis^{a,*}, Jeffrey A. French^{a,b}

^aDepartment of Biology, University of Nebraska at Omaha, United States

^bDepartment of Psychology, University of Nebraska at Omaha, United States

Received 15 July 2004; revised 8 September 2004; accepted 13 September 2004

Abstract

For many species, the presence of a significant social partner can lessen the behavioral and physiological responses to stressful stimuli. This study examined whether a single, individually specific signal, signature vocalization (phee call) could attenuate the physiological stress response that is induced in marmosets by housing them in short-term social isolation. Utilizing a repeated-measures design, adult marmosets ($n = 10$) were temporarily isolated from their long-term pair mate and exposed to three conditions: signature vocalizations from the pair mate, phee calls from an unfamiliar opposite sex individual, or no auditory stimuli. Levels of urinary cortisol were monitored as a physiological indicator of the stress response. Urinary cortisol levels were also monitored, while subjects remained undisturbed in their home cages to provide baseline levels. Temporarily isolated marmosets showed significantly higher levels of urinary cortisol than undisturbed marmosets. However, the nature of the acoustic stimulus experienced during isolation led to differences in the excretion of urinary cortisol. Isolated marmosets exposed to a familiar pair mate's vocalization showed significantly lower levels of urinary cortisol than when exposed to unfamiliar marmoset vocalizations ($P < 0.04$) or to no auditory stimuli ($P < 0.03$). Neither the duration of pairing nor the quality of relationship in the pair (indexed by spatial proximity scores) predicted the magnitude of reduction in cortisol in the familiar vocalization condition. The results presented here provide the first evidence that a single, individually specific communication signal can decrease the magnitude of a physiological stress response in a manner analogous to the physical presence of a social partner, a process we term "vocal buffering."

© 2004 Published by Elsevier Inc.

Keywords: Stress; Cortisol; Social buffering; Communication; Signature signals; Vocal buffering

Introduction

For members of a social species, one of the most profound moderators of function in the hypothalamic–pituitary–adrenal (HPA) axis during times of stress may be the presence of significant social partners (DeVries et al., 2003; Hennessy, 1997; Levine, 1993). This model of social support, sometimes known as buffering, postulates that during times of stress, the presence of significant social partners down-regulates activity in the HPA axis and hence serves to buffer

the individual against the stressful stimulus (Cohen and Wills, 1985). Presumably, the beneficial effects associated with the physical presence of a significant social partner derive from the partner's ability to either modify the perceived intensity of the stressor, and/or to down-regulate the magnitude and duration of the HPA response during exposure to the stressful stimulus. This phenomenon has been well studied in the context of mother–offspring interactions. In some species, offspring that are exposed to stressful situations in the presence of the mother display significantly reduced behavioral and glucocorticoid responses to those stressors, relative to offspring exposed to the same stressors in the absence of the mother (see review in Hennessy, 1997). However, this effect of social support is not just limited to mother–infant dyads. Social buffering of behavioral and physiological

* Corresponding author. Department of Biology, AH 418, University of Nebraska at Omaha, 6001 Dodge Street, Omaha, NE 68182. Fax: +1 402 554 3121.

E-mail address: mrukstalis@mail.unomaha.edu (M. Rukstalis).

51 responses to stress has also been reported in studies involving
52 separation in adult nonhuman primates (e.g., heterosexual
53 pairs: [Mendoza and Mason, 1986](#); [Smith et al., 1998](#); same-
54 sex pairs: [Gust et al., 1994](#)).

55 The physical presence of a social partner is defined by an
56 amalgamation of several distinct stimulus attributes (e.g.,
57 visual, olfactory, and auditory signals) as well as by the
58 nature and pattern of social interactions. Presumably, the
59 interactions of these signals function to provide a represen-
60 tation of one individual (the sender) and perhaps the
61 relationship it represents to another individual (the receiver).
62 In the absence of one or more of these signals, as often
63 occurs in visually obscured environments, isolated individ-
64 uals may attempt to maintain the positive effects of social
65 support by using a single individually specific, or signature,
66 signal rather than having to rely on a combination of several
67 signals. Signature signals have been identified across a wide
68 variety of birds and mammals (e.g., [Charrier et al., 2003](#);
69 [Randall, 1989](#); [Sayigh et al., 1998](#); [Searby et al., 2004](#)).
70 Individually distinct communication signals are also prev-
71 alent among many nonhuman primates (e.g., [Cleveland and](#)
72 [Snowdon, 1982](#); [Hammerschmidt and Todt, 1995](#); [Jones](#)
73 [et al., 1993](#); [Jorgensen, 1998](#); [Snowdon and Cleveland,](#)
74 [1980](#); [Snowdon et al., 1983](#); [Symmes et al., 1979](#)).
75 Therefore, if the physical presence of a social partner can
76 moderate responses (i.e., reduce HPA axis activity) to
77 stressful stimuli, it may be that, in addition to identity,
78 these signature signals also communicate the beneficial
79 effects of social support by communicating representations
80 of individuals and the relationship they represent, in turn
81 moderating the consequences of exposure to stressful
82 events.

83 Marmosets, small tropical primates from the New World
84 family Callitrichidae, are characterized by strong socioemo-
85 tional attachments (“pair bonds”) between adult males and
86 females, as well as cooperative infant care and prolonged
87 residence of offspring in extended family groups ([Rylands,](#)
88 [1993](#)). Thus, social interactions are an important attribute of
89 callitrichid life, and the resulting social relationships have
90 profound impacts on marmoset physiology and behavior
91 including reproduction ([Ginther et al., 2001](#); [Smith et al.,](#)
92 [1997](#); [Ziegler and Sousa, 2002](#)), endocrine regulation
93 ([Shepherd and French, 1999](#); [Smith and French, 1997](#);
94 [Smith et al., 1998](#)), and communication ([Elowson and](#)
95 [Snowdon, 1994](#); [Rukstalis et al., 2003](#); [Snowdon and](#)
96 [Elowson, 1999](#); [Snowdon and Hodun, 1981](#); [Vitale et al.,](#)
97 [2003](#)). In addition to these important social characteristics,
98 marmosets also possess rich vocal repertoires that are used
99 in a wide variety of contexts, including intragroup cohesion
100 and maintenance of territories ([Cleveland and Snowdon,](#)
101 [1982](#); [Epple, 1968](#); [Heymann, 1987](#); [Hubrecht, 1985](#);
102 [Stevenson and Poole, 1976](#)). These vocalizations are also
103 known to have “signature” or individually distinct properties
104 which can be used to distinguish individuals among group
105 members ([Jones et al., 1993](#); [Jorgensen and French, 1998](#);
106 [Snowdon and Cleveland, 1980](#)). In addition to strong

107 attachments and signature vocalizations, marmosets have
108 also been shown to experience social buffering of the stress
109 response. [Smith et al. \(1998\)](#) demonstrated that marmosets
110 removed from their home cage and exposed to a novel
111 environment had significantly lower levels of urinary
112 cortisol when their heterosexual pair mate was present
113 versus when they were not. Therefore, the existence of
114 complex social interactions, highly conserved, individually
115 identifiable vocalizations, and evidence of social buffering
116 make callitrichid primates ideally suited for examining the
117 effects of individually specific signals on physiological
118 responses to stressful events.

119 The purpose of the present study was to evaluate whether
120 exposure to a single, individually specific signal (i.e., phee
121 call) could reduce the consequences of social separation and
122 exposure to environmental novelty. Specifically, we pre-
123 dicted that exposure to the phee call of a significant social
124 partner (i.e., pair mate) would reduce urinary cortisol
125 excretion, a common indicator of psychosocial stress, in
126 marmosets isolated and exposed to a novel environment.
127 Additionally, it may be that exposure to vocalizations from
128 individuals other than the focal animal’s pair mate may also
129 influence the physiological consequences of exposure to
130 stressful events. However, given the individually specific
131 nature of marmoset vocalizations and the long-term attach-
132 ments formed between heterosexual pairs, we predicted that
133 exposure to vocalizations from unfamiliar individuals would
134 not reduce urinary cortisol production in socially isolated
135 individuals exposed to novel environments. We also
136 examined the effect of length of pairing and social
137 proximity, if any, on urinary cortisol excretion upon
138 exposure to a pair mate’s vocalization.

139 **Materials and methods**

140 *Subjects and housing*

141 Subjects for this study were 10 Wied’s black tufted-ear
142 marmosets (*Callithrix kuhlii*) housed in five preexisting,
143 long-term heterosexual pairs at the University of Nebraska
144 at Omaha’s Callitrichid Research Center. [Table 1](#) contains
145 demographic information for all marmosets included in the
146 study. The mean age and length of pairing prior to the
147 beginning of the study were 6.8 and 4.4 years, respectively.
148 Wire mesh enclosures for each pair measured approximately
149 1.2 × 0.9 × 2.4 m and were equipped with branches, nest
150 boxes, and various enrichment devices. All colony rooms
151 were maintained at a constant temperature of 20–22°C and
152 were subject to a 12:12 light–dark cycle. Groups residing in
153 the same colony room were denied visual contact but
154 retained olfactory and auditory contact with neighboring
155 enclosures. All animals were fed a mixture of various fresh
156 fruits and vegetables, dairy products, Zupreem marmoset
157 diet ([Hills Brothers](#); [Mission, KS, USA](#)), and Mazuri
158 primate fiber sticks ([Mazuri](#); [St. Louis, MO, USA](#)). All

Table 1				
Demographic information for all marmosets included in the study				
	Subject	Sex	Age (years)	Time paired (years)
t1.4	Ye	M	5	4
t1.5	Iz	F	4	
t1.6	Ca	M	10	4
t1.7	It	F	6	
t1.8	Fl	M	6	3
t1.9	Fo	F	5	
t1.10	Ke	M	12	8
t1.11	Ya	F	10	
t1.12	Bu	M	5	3
t1.13	Ne	F	5	

159 protocols in this study were approved by the University of
 160 Nebraska at Omaha's Institutional Animal Care and Use
 161 Committee (IACUC #03-096-011).

162 *Auditory signals*

163 Phee calls were collected with a Marantz PMD 201
 164 portable analog cassette recorder (Marantz; Itasca, IL, USA)
 165 using a Sennheiser (Sennheiser USA; Old Lyme, CT, USA)
 166 ME 80 directional microphone (frequency range 50–15,000
 167 Hz). Prior to each recording session, animals were allowed
 168 to habituate to the presence of an observer and recording
 169 equipment, located approximately 1.5 m from the enclosure.
 170 Individual callers were identified by speaking the name of
 171 the calling animal directly into the microphone following
 172 the end of the vocalization (Jorgensen and French, 1998).
 173 Unfamiliar vocalizations were collected from marmoset
 174 pairs residing in colony rooms other than the focal animal.
 175 Previous research in our laboratory (Rukstalis et al., 2003)
 176 and others (Elowson and Snowdon, 1994) has indicated that
 177 changes in the demographic makeup of colony rooms can
 178 significantly alter the morphology of marmoset vocaliza-
 179 tions. In light of this information, all calls were obtained
 180 from animals residing in undisturbed colony rooms through-
 181 out the course of this study.

182 *Data collection*

183 Pretrial urine samples were collected the morning of the
 184 experiment, between 0700 and 0800 h, and represented the
 185 first void of the day. At 1200 h, individual marmosets were
 186 removed from their home cages by trained technicians and
 187 placed in small transport cages (0.38 × 0.38 × 0.38 m).
 188 Isolated individuals were then moved to a quiet room
 189 located at least 20 m from the home colony room. Subjects
 190 were isolated from their long-term pair mates for 4 h (1200–
 191 1600 h) and exposed to one of three conditions: isolation
 192 with no auditory stimulation (no call), isolation and
 193 exposure to an unfamiliar opposite sex marmoset's phee
 194 call (unfamiliar call), or isolation and exposure to the
 195 familiar pair mate's phee call (familiar call). Familiar and
 196 unfamiliar vocalizations were played back using a Marantz

model PMD 201 (Marantz) cassette recorder, located 197
 approximately 0.5 m from the enclosure. Playbacks con- 198
 sisted of a single vocal exemplar from either the focal 199
 animal's familiar pair mate or an unfamiliar opposite sex 200
 animal presented at a rate of once every 2 min throughout 201
 the course of the separation. In order to control for possible 202
 order effects and habituation to the protocol, the sequence of 203
 conditions for each animal was assigned in a random 204
 manner. Additionally, for any individual, a period of at least 205
 3 days elapsed between successive trials. Urine samples 206
 were collected every 30 min from clean aluminum pans 207
 placed under the transport cage. Posttrial urine samples were 208
 collected the following morning (0700–0800 h). Urine 209
 samples from these same subjects (control condition) 210
 residing undisturbed in their home cage were collected at 211
 0700 h, during the trial periods (i.e., 1200–1600 h), and on 212
 the following morning to document circadian variation in 213
 urinary cortisol when marmosets that did not undergo social 214
 isolation. All subjects had been previously trained to urinate 215
 in exchange for a preferred food item. Therefore, urine 216
 collection during control trials was collected in hand-held 217
 aluminum pans without the need for separation and/or 218
 isolation. Following collection, all samples were centrifuged 219
 to remove debris, transferred to clean vials, and stored at 220
 –20°C until assay. 221

222 During the control trial, a 15-min behavioral observation
 223 was conducted once an hour (1200–1600 h) by a trained
 224 technician located approximately 1.5 m from the enclosure
 225 using the Observer 4.1 software (Noldus USA; Leesburg,
 226 VA, USA). All animals were allowed 5 min to habituate to
 227 the presence of the observer. An all-occurrences method was
 228 used to record pair proximity and individual approaches and
 229 leaves. Approaches were scored when an animal approached
 230 its pair mate to within one marmoset body length and
 231 remained there for at least 5 s. Leaves were scored when an
 232 animal moved at least one marmoset body length away from
 233 its pair mate and remained there for at least 5 s.

Data analysis

234
 235 Urinary cortisol concentrations were determined using an
 236 enzyme immunoassay (EIA) previously described and
 237 validated for use in *C. kuhlii* (Smith and French, 1997).
 238 Hormone concentrations were measured in 18 assays.
 239 Coefficients of variation (CV) were measured from dupli-
 240 cate evaluations of pooled marmoset urine. Interassay
 241 coefficients of variation (CVs) for high and low pools were
 242 12.05% and 16.95%, respectively. Intraassay CVs for high
 243 and low pools were 4.4% and 3.84%, respectively. To
 244 account for differences in urine sample concentration, all
 245 hormone values were corrected for creatinine concentration.
 246 Creatinine values were determined using a modified Jaffé
 247 end-point assay, previously described and validated for use
 248 in this species (French et al., 1996).

249 Levels of urinary cortisol were examined in samples
 250 collected from individual marmosets exposed to the four

251 conditions. For each subject, cortisol concentrations were
 252 determined for the first-void preisolation sample, the mean
 253 concentration excreted over the first 2 h of a trial, the mean
 254 cortisol concentration during the last 2 h of a trial, and the
 255 first-void sample collected on the morning following a trial.
 256 Previous studies in this laboratory have demonstrated that
 257 changes in urinary cortisol are readily detectable within 2 h
 258 of exposure to a mild stressor (Smith and French, 1997;
 259 Smith et al., 1998). A three-way mixed ANOVA [Sex(2) ×
 260 Condition(4) × Time(4)] was used to assess the impact of
 261 these varying conditions on urinary concentrations of
 262 cortisol. Where appropriate, paired-sample, dependent *t*
 263 tests were used to compare possible differences at individual
 264 time points. Additionally, a two-way ANOVA [Order(3) ×
 265 Time(4)] was used to assess the impact of presentation of
 266 the separation conditions on subsequent levels of urinary
 267 cortisol. An alpha level of 0.05 was adopted for all statistical
 268 tests.

269 Results of the four 15-min behavioral observations were
 270 pooled. The number of individual leaves and approaches to
 271 and from the pair mate was counted. These numbers were
 272 used to calculate an index of the maintenance of social
 273 proximity as an indicator of relationship quality (Hinde and
 274 Atkinson, 1970). A Pearson correlation was used to assess
 275 the relationship between these scores and the reduction in
 276 urinary cortisol excretion between the familiar call and no
 277 call conditions. We also used a Pearson correlation to assess
 278 the relationship between length of pairing and reduction in
 279 urinary cortisol between these same two conditions.

280 Results

281 Social isolation and exposure to environmental novelty
 282 induced a significant physiological stress response in
 283 marmosets. The results of our repeated-measures ANOVA
 284 revealed a significant interaction between time of sample
 285 collection and call condition on levels of urinary cortisol
 286 excretion [$F(9) = 6.39, P < 0.0001$]. After two h, marmosets
 287 separated from their pair mates had significantly higher levels
 288 of urinary cortisol when exposed to any of the three treatment
 289 conditions [unfamiliar calls: $t(9) = 2.76, P = 0.022$; familiar
 290 calls: $t(9) = 2.553, P = 0.011$; or no calls: $t(9) = 4.11,$
 291 $P = 0.003$] than when they were left undisturbed (control) in
 292 their home cages. Although, after 2 h, there was no
 293 significant difference in levels of urinary cortisol when
 294 marmosets were exposed to familiar versus unfamiliar calls
 295 or unfamiliar versus no calls, exposure to familiar vocal-
 296 izations resulted in significantly lower levels of urinary
 297 cortisol compared to when the marmosets were exposed to
 298 no vocalizations [$t(9) = 2.65, P = 0.026$; Fig. 1].

299 Differences in the excretion of urinary cortisol between
 300 conditions continued to be evident after 4 h of separation.
 301 Again, isolation and exposure to any of the three treatment
 302 conditions resulted in significantly higher levels of urinary
 303 cortisol [familiar call: $t(9) = 3.219, P = 0.011$; unfamiliar call:

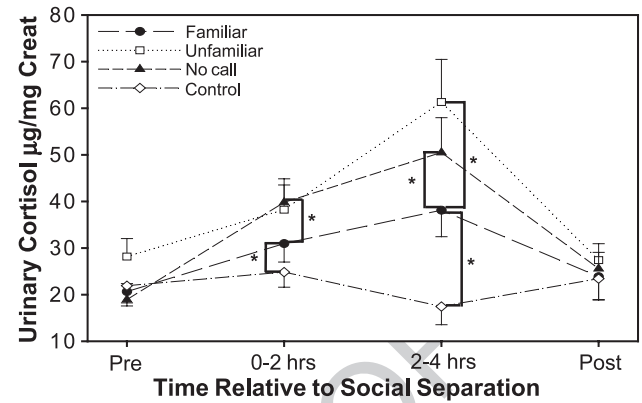


Fig. 1. Mean (\pm SEM) urinary cortisol excretion ($\mu\text{g}/\text{mg}$ creatinine) for all marmosets ($N = 10$), in the familiar condition, versus all other experimental conditions, across the 4-h separation period. * $P < 0.05$. See text for other significant effects.

304 $t(9) = 5.113, P = 0.001$; no call: $t(9) = 4.744, P = 0.001$] than
 305 when marmosets were left undisturbed in their home cages.
 306 At 4 h, there was clear evidence that exposure to familiar calls
 307 reduced the stress response to isolation. Marmosets who had
 308 been exposed to familiar calls displayed significantly lower
 309 levels of urinary cortisol 4 h postisolation than marmosets
 310 exposed to unfamiliar calls [$t(9) = 2.397, P = 0.04$] or no calls
 311 [$t(9) = 2.554, P = 0.031$].

312 The results of the two-way ANOVA indicated that the
 313 order of presentation of the separation conditions had no
 314 effect on subsequent levels of urinary cortisol (mean urinary
 315 cortisol: Presentation 1, $30.52 \pm 3.20 \mu\text{g}/\text{ml}$; Presentation 2,
 316 $36.30 \pm 4.37 \mu\text{g}/\text{ml}$; Presentation 3, $34.06 \pm 4.65 \mu\text{g}/\text{ml}$).
 317 Additionally, neither the duration of pairing nor proximity
 318 maintenance scores appeared to influence the degree of
 319 vocal buffering.

320 Discussion

321 Individual marmosets, isolated from their long-term pair
 322 mate and exposed to a novel environment, showed
 323 significant increases in the production of glucocorticoids
 324 across the 4-h trial period, while urinary cortisol in
 325 marmosets that remained in their undisturbed home cages
 326 was quite stable. Thus, social isolation and exposure to a
 327 novel environment appear to have constituted a significant
 328 source of stress. However, the amount of urinary cortisol
 329 measured in each condition depended on the presence and
 330 identity of vocalizations presented to the isolated individ-
 331 uals. After 2 h of isolation, individuals exposed to phee calls
 332 from their long-term pair mates had significantly lower
 333 levels of urinary cortisol than when no calls were presented.
 334 After 4 h of separation, marmosets isolated and exposed to
 335 their pair mate's phee call had significantly lower levels of
 336 urinary cortisol than when they were exposed to either no
 337 vocalizations or unfamiliar vocalizations. Therefore, expo-

338 sure to a single, individually specific signal modality (i.e.,
339 conspecific vocalization) reduced the physiological con-
340 sequences (i.e., glucocorticoid production) of social iso-
341 lation and exposure to environmental novelty, a process we
342 term vocal buffering.

343 The mechanisms or processes involved in this vocal
344 buffering of the HPA axis are still unknown. However, two
345 distinct possibilities exist as potential explanations for the
346 ability of individually specific vocalizations to reduce a
347 physiological response to stress. In the current study, we
348 isolated individuals from their home cages and long-term
349 pair mates prior to exposing them to various vocal
350 conditions. As has been previously noted, environmental
351 novelty is a potent stimulator of the HPA axis (Hennessy
352 et al., 1997). It may be the case then that exposing
353 individuals isolated in novel environments to familiar
354 vocalizations simply reduced the overall environmental
355 novelty and thereby diminished the perceived intensity of
356 the stressor. In a similar study of marmosets, Smith et al.
357 (1998) reported that removal of an individual's pair mate
358 from the home cage did not cause a subsequent rise in
359 glucocorticoid production, suggesting that environmental
360 novelty rather than social separation induced the stress
361 response. Although the focal animals' pair mates were
362 removed from the home cage, other individuals (i.e.,
363 offspring) were not (Smith et al., 1998). Therefore, it may
364 have been that social interactions with the remaining
365 offspring were able to buffer the individual against the
366 stress of pair mate removal. Alternatively, if social
367 separation rather than exposure to environmental novelty
368 is the true stressor, then the ability of familiar vocalizations
369 to moderate an individual's HPA axis may be due to the
370 individually specific nature of the communication signals.
371 In a study of the individually specific signaling system of
372 the golden hamster (*Mesocricetus auratus*), Johnston and
373 Bullock (2001) and Johnston and Jernigan (1994) provided
374 evidence that identifying scent signals is an integral part of
375 a larger multifactor representation of individuals. The results
376 of these studies suggest that individually specific signals
377 may go beyond mere markers of discrimination and may
378 serve to elicit complex representations of individuality and
379 quite possibly the relationships that identity entails.
380 Callitrichid primates possess highly conserved, individually
381 identifiable vocal signals, characterized by low within-
382 individual and high between-individual variability (Jones
383 et al., 1993; Jorgensen and French, 1998; Snowdon and
384 Cleveland, 1980). In addition to identity, the signature
385 system contained within the vocalizations of these nonhu-
386 man primates may also communicate more complex
387 representations of individuality, thereby reducing the
388 physiological consequences of exposure to stressful stimuli
389 in a manner analogous to the physical presence of the social
390 partner.

391 The results of this study also revealed that marmosets
392 exhibited larger concentrations of urinary cortisol,
393 although not significant, when individuals were exposed

394 to vocalizations from unfamiliar conspecifics versus
395 familiar conspecifics. Again, depending on the source of
396 the stress, this trend might also be explained by either
397 social separation or novelty exposure. Callitrichid primates
398 are generally characterized as highly territorial, group
399 dwelling animals (Heymann, 1987; Hubrecht, 1985;
400 Stevenson and Poole, 1976). Thus, exposure to the calls
401 of an unfamiliar individual when isolated from a social
402 group may be perceived as a threat and therefore could
403 intensify stress rather than alleviating it or having no
404 effect. On the other hand, the vocalizations of callers
405 unfamiliar to the focal animal might be perceived as an
406 additional novel component of the separation environment.
407 Therefore, any rise in physiological indices of stress may
408 simply reflect a reaction to increased novelty rather than a
409 perceived threat from a nongroup member. It should be
410 noted, however, that the effects of novelty exposure and
411 social separation may not be mutually exclusive, and the
412 results presented here may reflect a combination of these
413 two effects.

414 The ability of specific social partners to buffer an
415 individual against stressful stimuli or to stimulate a stress
416 response upon separation can vary greatly depending on the
417 nature and quality of the relationship they share. Filial
418 attachment, the bond that forms from an offspring to its
419 caregiver, can be observed in many species (Mason and
420 Mendoza, 1998). For example, when placed in a novel
421 environment, guinea pig pups show greater elevations of
422 plasma cortisol and ACTH when they are tested alone
423 versus with they are tested with their mothers (Hennessy
424 and Moorman, 1989; Hennessy and Tamborski, 1989;
425 Hennessy et al., 2002). Similarly, squirrel monkey
426 mother–infant dyads experience significant increases in
427 plasma cortisol when separated from each other (Coe et al.,
428 1978; Levine et al., 1993; Mendoza et al., 1978; Wiener
429 et al., 1990). Documentation of attachment between adult
430 heterosexual pairs is less common. However, a notable
431 exception has been reported in a previous study of a socially
432 monogamous primate, the titi monkey. Mendoza and Mason
433 (1986) demonstrated that titi monkey heterosexual pair
434 mates showed a significant increase in plasma cortisol when
435 separated from each other, but not from their offspring. Like
436 titi monkeys, the socially monogamous marmosets are also
437 known for their strong heterosexual pair-bonds. These long-
438 term relationships can take several weeks to form and
439 represent an important social bond for an adult marmoset
440 (Schaffner et al., 1995). In the present study, we used both
441 length of pairing and Hinde and Atkinson index scores
442 (proximity maintenance) to quantify relationship quality.
443 However, in this case, neither was an indicator of the
444 amount of reduction in urinary cortisol (buffering) an
445 individual experienced. However, given the small sample
446 size and restricted range of data (i.e., time paired), a
447 correlation may be difficult to find. Future studies assessing
448 social buffering in callitrichid primates would be benefited
449 by examining the relationship between quantitative meas-

450 ures of relationship quality and amount of social buffering
451 experienced by individuals.

452 The results of the present study may provide a possible
453 framework for disentangling the potential of various
454 relationships and the attachments they represent to influence
455 behavioral and physiological responses to stress. The data
456 presented here showed a significant “vocal buffering” effect
457 of individually specific vocalizations on urinary cortisol
458 excretion in isolated pair mates. Future studies would do
459 well to examine the effect of individually specific stimuli
460 from various social companions (i.e., offspring, same-sex
461 conspecific, siblings, etc.), mating strategies, and social
462 systems in order to shed additional light on the strengths of
463 attachments and existence of vocal buffering in complex
464 social systems.

465 Acknowledgments

466 We wish to thank Heather Jensen and the volunteers and
467 staff of the Callitrichid Research Center at UNOmaha for
468 their excellent care of the marmoset colony. We would also
469 like to thank Jeff Fite for comments on this manuscript. The
470 work was supported, in part, by funds from the National
471 Science Foundation (IBN 00-91030) and the National
472 Institutes of Health (HD- HD 42882).

473 References

474

475 Charrier, I., Mathevon, N., Jouventin, P., 2003. Vocal signature recognition
476 of mothers by fur seal pups. *Anim. Behav.* 65, 543–550.
477 Cleveland, J., Snowdon, C.T., 1982. The complex vocal repertoire of the
478 adult cotton-top tamarin (*Saguinus oedipus oedipus*). *Z. Tierpsychol.*
479 58, 231–270.
480 Coe, C.L., Mendoza, S.P., Smotherman, W.P., Levine, S., 1978. Mother–
481 infant attachment in the squirrel monkey: adrenal responses to
482 separation. *Behav. Biol.* 22, 253–256.
483 Cohen, S., Wills, T.A., 1985. Stress, social support, and the buffering
484 hypothesis. *Psychol. Bull.* 98, 310–357.
485 DeVries, A.C., Glasper, E.R., Detillion, C.E., 2003. Social modulation of
486 stress responses. *Physiol. Behav.* 79, 399–407.
487 Elowson, M.A., Snowdon, C.T., 1994. Pygmy marmosets, *Cebuella*
488 *pygmaea*, modify vocal structure in response to changed social environ-
489 ment. *Anim. Behav.* 47, 1267–1277.
490 Epple, G., 1968. Comparative studies on vocalization in marmoset
491 monkeys (Hapalidae). *Folia Primatol.* 8, 1–40 (Basel).
492 French, J.A., Brewer, K.J., Schaffner, C.M., Schalley, J., Hightower-
493 Merritt, D., Smith, T.E., Bell, S.M., 1996. Urinary steroid and
494 gonadotropin excretion across the reproductive cycle in female Wied’s
495 black tufted-ear marmosets (*Callithrix kuhlii*). *Am. J. Primatol.* 40 (3),
496 231–245.
497 Ginther, A.J., Ziegler, T.E., Snowdon, C.T., 2001. Reproductive biology of
498 captive male cotton-top tamarin monkeys as a function of social
499 environment. *Anim. Behav.* 61, 65–78.
500 Gust, D.A., Gordon, T.P., Brodie, A.R., McClure, H.M., 1994. Effect of a
501 preferred companion in modulating stress in adult female rhesus
502 monkeys. *Physiol. Behav.* 53, 681–684.
503 Hammerschmidt, K., Todt, D., 1995. Individual differences in vocalizations
504 of young Barbary macaques (*Macaca sylvanus*): a multi-parametric
505 analysis to identify critical cues in acoustic signalling. *Behaviour* 132,
506 381–399.

Hennessy, M.B., 1997. Hypothalamic–pituitary–adrenal responses to brief
507 social separation. *Neurosci. Biobehav. Rev.* 21 (1), 11–29. 508
Hennessy, M.B., Moorman, L., 1989. Factors influencing cortisol and
509 behavioral responses to maternal separation in guinea pigs. *Behav.*
510 *Neurosci.* 103 (2), 378–385. 511
Hennessy, M.B., Tamborski, P.S., 1989. The influence of maternal
512 separation on plasma concentrations of ACTH, epinephrine, and
513 norepinephrine in guinea pig pups. *Physiol. Behav.* 45, 11452–11472. 514
Hennessy, M.B., McInturf, S.M., Mazzei, S.J., 1997. Evidence that
515 endogenous corticotropin-releasing factor suppresses behavioral
516 responses of guinea pig pups to brief isolation in novel surroundings.
517 *Dev. Psychobiol.* 31, 39–47. 518
Hennessy, M.B., O’Leary, S.K., Hawke, J.L., Wilson, S.E., 2002. Social
519 influences on cortisol and behavioral responses of preweaning,
520 periadolescent, and adult guinea pigs. *Physiol. Behav.* 76, 305–314. 521
Heymann, E., 1987. Behaviour and communication of mousted
522 tamarins, *Saguinus mystax mystax* (Primates: Callitrichidae), in an
523 outdoor enclosure. *Primate Rep.* 17, 45–52. 524
Hinde, R.A., Atkinson, S., 1970. Assessing the roles of social partners in
525 maintaining mutual proximity, as exemplified by mother–infant
526 relations in rhesus monkeys. *Anim. Behav.* 18, 169–176. 527
Hubrecht, R.C., 1985. Home-range and use and territorial behavior in the
528 common marmoset, *Callithrix jacchus jacchus*, at the Tapacura field
529 station, Recife, Brazil. *Int. J. Primatol.* 6 (5), 533–550. 530
Johnston, R.E., Bullock, T.A., 2001. Individual recognition by use of
531 odours in golden hamsters: the nature of individual representations.
532 *Anim. Behav.* 61, 545–557. 533
Johnston, R.E., Jernigan, P., 1994. Golden hamsters recognize individuals,
534 not just individual scents. *Anim. Behav.* 48, 129–136. 535
Jones, B.S., Harris, D.H.R., Catchpole, C.K., 1993. The stability of the
536 vocal signature in phee calls of the common marmoset, *Callithrix*
537 *jacchus*. *Am. J. Primatol.* 31, 67–75. 538
Jorgensen, D., French, J.A., 1998. Individuality but not stability in
539 marmoset long calls. *Ethology* 104, 729–742. 540
Levine, S., 1993. The influence of social factors on the response to stress.
541 *Psychother. Psychosom.* 60, 33–38. 542
Levine, S., Wiener, S.G., Coe, C.L., 1993. Temporal and social factors
543 influencing behavioral and hormonal responses to separation in
544 mother and infant squirrel monkeys. *Psychoneuroendocrinology* 19
545 (8), 297–306. 546
Mendoza, S.P., Mason, W.A., 1986. Parental division of labour and
547 differentiation of attachments in a monogamous primate (*Callicebus*
548 *moloch*). *Anim. Behav.* 34, 1336–1347. 549
Mendoza, S.P., Mason, W.A., Miner, M.T., Kaplan, J., Levine, S., 1978.
550 Pituitary–adrenal response to separation in mother and infant squirrel
551 monkeys. *Dev. Psychobiol.* 11, 169–175. 552
Randall, J.A., 1989. Individual foot drumming signatures in banner-tailed
553 kangaroo rats *Dipodomys spectabilis*. *Anim. Behav.* 38, 620–630. 554
Rukstalis, M., Fite, J.E., French, J.A., 2003. Social change affects vocal
555 structure in a callitrichid primate (*Callithrix kuhlii*). *Ethology* 109,
556 327–340. 557
Rylands, A.B.e., 1993. Marmosets and Tamarins: Systematics, Behavior
558 and Ecology. Oxford Univ. Press, Oxford. 559
Sayigh, L.S., Tyack, P.L., Wells, R.S., Solow, A.R., Scott, M.D., Irvine,
560 A.B., 1998. Individual recognition in wild bottlenose dolphins: a field
561 test using playback experiments. *Anim. Behav.* 57, 41–50. 562
Schaffner, C.M., Shepherd, R.E., Santons, C.V., French, J.A., 1995.
563 Development of heterosexual relationships in Wied’s black tufted-ear
564 marmosets (*Callithrix kuhlii*). *Am. J. Primatol.* 36, 185–200. 565
Searby, A., Jouventin, P., Aubin, T., 2004. Acoustic recognition in
566 macaroni penguins: an original signature system. *Anim. Behav.* 67,
567 615–625. 568
Shepherd, R.E., French, J.A., 1999. Comparative analysis of sociality in
569 lion tamarins (*Leontopithecus rosalia*) and marmosets (*Callithrix*
570 *kuhlii*): responses to separation from long-term pair mates. *J. Comp.*
571 *Psychol.* 113, 24–32. 572
Smith, T.E., French, J.A., 1997. Psychosocial stress and urinary cortisol
573

- 574 excretion in marmoset monkeys (*Callithrix kuhlii*). *Physiol. Behav.* 62,
575 225–232.
- 576 Smith, T.E., Schaffner, C.M., French, J.A., 1997. Social and developmental
577 influences on reproductive function in female Wied's black tufted-ear
578 marmosets (*Callithrix kuhlii*). *Horm. Behav.* 31 (2), 159–168.
- 579 Smith, T.E., McGreer-Whitworth, B., French, J.A., 1998. Close proximity
580 of the heterosexual partner reduces the physiological and behavioral
581 consequences of novel-cage housing in black tufted-ear marmosets
582 (*Callithrix kuhlii*). *Horm. Behav.* 34, 211–222.
- 583 Snowdon, C.T., Cleveland, J., 1980. Individual recognition of contact calls
584 by pygmy marmosets. *Anim. Behav.* 28, 717–727.
- 585 Snowdon, C.T., Elowson, M.A., 1999. Pygmy marmosets modify call
586 structure when paired. *Ethology* 105, 893–908.
- 587 Snowdon, C.T., Hodun, A., 1981. Acoustic adaptations in pygmy marmoset
588 contact calls: locational cues vary with distances between conspecifics.
589 *Behav. Ecol. Sociobiol.* 9, 295–300.
- 590 Snowdon, C.T., Cleveland, J., French, J.A., 1983. Responses to context-
608 and individual-specific cues in cotton-top tamarin long calls. *Anim.* 591
Behav. 31, 92–101. 592
- Stevenson, M.F., Poole, T.B., 1976. An ethogram of the common marmoset
593 (*Callithrix jacchus*): general behavioral repertoire. *Anim. Behav.* 24,
594 428–451. 595
- Symmes, D., Newman, J.D., Talmage-Riggs, G., Lieblisch, A.K., 1979. 596
Individuality and stability of isolation peeps in squirrel monkeys. *Anim.* 597
Behav. 27, 1142–1152. 598
- Vitale, A., Zanzoni, M., Queyras, A., Chiarotti, F., 2003. Degree of social
599 contact affects the emission of food calls in the common marmoset
600 (*Callithrix jacchus*). *Am. J. Primatol.* 59, 21–28. 601
- Wiener, S.G., Bayart, F., Faull, K.F., Levine, S., 1990. Behavioral and
602 physiological responses to maternal separation in squirrel monkeys
603 (*Saimiri sciureus*). *Behav. Neurosci.* 104 (1), 108–115. 604
- Ziegler, T.E., Sousa, M.B., 2002. Parent–daughter relationships and social
605 controls on fertility in female common marmosets, *Callithrix jacchus*.
606 *Horm. Behav.* 42, 356–367. 607