

Prolactin levels in paternal striped mouse (*Rhabdomys pumilio*) fathers

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Received 24 September 2003; received in revised form 26 November 2003; accepted 18 December 2003

Abstract

Paternal behavior is associated with an increase in prolactin levels in fish, birds and mammals, including rodents. The striped mouse (*Rhabdomys pumilio*) from southern Africa shows highly developed paternal care. We investigated whether striped mouse fathers have higher prolactin levels than nonfathers, and whether there is a relationship between tactile stimulation with pups and prolactin secretion in fathers. We measured serum prolactin in 42 male striped mice assigned to one of four different experimental groups (single males, paired males, fathers housed with mother and pups, and fathers separated from their family by a wire-mesh partition). Our results revealed no increases in prolactin levels in fathers, and fathers with tactile contact with pups did not have higher prolactin levels than the fathers that were prevented from making tactile contact with pups. In contrast, experienced males had higher prolactin levels than inexperienced males. Male striped mice are polygynous in nature, living in groups, with three breeding females, and are permanently associated with pups during the breeding season. In a field study, males had higher prolactin levels during the breeding season than during the nonbreeding season. Thus, prolactin secretion in the polygynous striped mouse might be regulated by environmental stimuli, whereas social stimuli might be important for monogamous species. This is the first study to demonstrate seasonal changes in prolactin levels in a free-living male mammal.

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Keywords: Paternal care; Parental care; Prolactin; *Rhabdomys*; Seasonality

1. Introduction

Whereas biparental care is the rule in birds [1,2], uniparental care by the mother is the norm in mammals, with less than 10% of mammalian species showing paternal care [3]. Both biparental care in birds [4,5] and maternal care in mammals [6,7] are stimulated by the hormone prolactin. Prolactin appears also to be important in the regulation of paternal care in mammals [5,8]. In mammals, paternal care occurs mainly in primates, canids and rodents [3]. All endocrinological studies in mammalian species with naturally occurring paternal care have demonstrated a relationship between prolactin secretion and paternal care. In New World monkeys, fathers have higher prolactin levels than nonfathers [9–11], and fathers of some species experience an increase in prolactin levels after the birth of infants [11–13]. Fathers of the biparental California mouse (*Peromyscus californicus*) have elevated prolactin levels [14], and a

seasonal increase in male prolactin levels occurs during pup rearing in wolves [15,16]. In dwarf hamsters of the genus *Phodopus*, prolactin levels increase in males after the birth of pups in the biparental species *Phodopus campbelli*, but not in the males of *Phodopus sungorus*, which does not display paternal care [17].

While paternally behaving mammal fathers have elevated prolactin levels, the stimuli eliciting increased prolactin secretion are poorly understood [45]. Social stimuli accompanying paternal care, such as cues from infants and a pregnant partner, are likely causes [11,13]. Tactile stimulation from infants can lead to increased prolactin levels in female mammals [18–21], and tactile stimulation through infant carrying leads to increased prolactin levels in common marmoset males (*Callithrix jacchus* [10,22]; however, see Ref. [11] for increased prolactin levels in fathers during the period without infant carrying). Therefore, tactile stimulation might also account for increased prolactin levels in other mammal fathers, such as rodent fathers huddling with their pups.

The striped mouse (*Rhabdomys pumilio*) displays highly developed paternal care [23]. It is a diurnal muroid rodent

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from southern Africa, with a body weight of about 40 g [24,25]. In a previous study [23], we reported that striped mouse fathers show all patterns of parental behavior as seen in mothers, with the obvious exception of nursing. Males lick and huddle with pups to the same extent as females, and males also retrieve pups, although to a lesser extent than females. During the period of pup rearing, there is a threefold increase in the time spent in the nest box by both fathers and mothers.

The social organization of free-living striped mice is flexible, ranging from solitary to group living [26]. In the semiarid succulent karoo of South Africa, the striped mouse is a territorial group living solitary forager, with communal breeding and helpers at the nest [26]. Groups can consist of up to 30 individuals, including 2–4 breeding females, 1 breeding male and their adult offspring of both sexes. Adult offspring remains within their natal group until the next breeding season, without breeding themselves, and helps with nest construction, territorial defense and infant care [26]. The breeding male spends the night together with the group in the same nest, and interacts in the same sociopositive manner with juveniles as mothers do (i.e., grooming and licking juveniles [23]). In addition, free-living breeding males have been observed to groom and lick pups in the nest (unpublished data), and a pup-retrieval experiment demonstrated that free-living males are paternally inclined [23]. The striped mouse is not the only polygynous mammal that shows paternal care, and the historical assumption that paternal care is only associated with monogamy has been refuted [27,28].

The results from both the captivity and field studies indicate that the striped mouse is an ideal model for studying paternal care in mammals [23]. In the present study, we tested whether paternal care is associated with increased prolactin levels in the striped mouse. Additionally, we investigated the hypothesis that increased prolactin levels in mammal fathers might be due to tactile stimulation, by comparing the prolactin levels of fathers living with their family with those of fathers separated from their family by a wire-mesh partition. Furthermore, we measured the prolactin levels in free-living males to ascertain whether there is a relationship between prolactin levels and breeding condition, as is known from several bird species [29,30]. Whereas prolactin has been measured in free-living mammals previously [46,47], to our knowledge, this is the first study which considers seasonal variation in prolactin levels in free-living male mammals.

2. Materials and methods

2.1. Laboratory study

2.1.1. Subjects

Subjects were either wild-born mice from Goegap Nature Reserve in the northwest of South Africa or their

captive-born adult offspring (i.e., first generation, born in captivity). A total of 42 males were used for the study, 11 of which were wild-born and 31 were captive-born. All wild-born males had experienced infant rearing in captivity, whereas none of the captive-born males had such experience before the study. All wild-born males were kept singly for four months prior to this study. Captive-born males were kept either in sibling groups or in family groups without the father, who had been removed at weaning (16 days). All animals were housed under standard laboratory conditions, with a light regime of 14:10-h light–dark cycle, with lights on at 6 a.m., room temperature of 20–24 °C and 30–60% relative humidity. Epol mouse cubes (purchased from Epol, Pretoria West, South Africa) and water were available ad libitum, and seeds (parrot food) were provided daily.

Males were housed in 40 × 12 × 25 cm (l × h × w) Labotec holding cages (purchased from Labotec, Halfway House, South Africa), containing coarse wood shavings as litter and hay as nesting material. Males were assigned to one of four experimental groups (number wild-born/captive-born males): (1) singly housed control males (3/11); (2) paired males (1/12); (3) fathers housed with mother and pups (4/4); and (4) fathers housed in the same cage as mother and pups, but prevented from making tactile contact with the rest of the family by a wire-mesh partition (3/4).

Males of the control group were housed in the same type of cages as of the paired males. Pairs of the Experimental Group 4 (males without tactile contact) were moved into glass tanks (45 cm × 30 cm × 30 cm) 1 week before the expected parturition. The floors of the tanks were covered with wood shavings. Each tank was divided into equal-sized halves using a wire-mesh partition. Two PVC nest boxes (13 × 9 × 10 cm) were present, one on either side of the partition. Each nest box had two openings, one of which was the entrance to the nest box; the other opening was positioned against the wire mesh, directly facing the opening of other the nest box. From their nest boxes, fathers could make visual, auditory and olfactory contact with their pups in the other nest box across the divide.

2.1.2. Collection of blood samples

Blood samples were collected from all 42 males, as well as from the 16 lactating (partners of males that were used in experiments) and 5 nonlactating females (which had produced pups at least 4 weeks before blood sampling) for comparison. Blood samples of the males of the control group were taken 3–4 weeks after they had been housed alone. The blood samples of paired males were taken 2 weeks after pairing. The blood samples of fathers of both Experimental Groups 3 (living with family) and 4 (without tactile contact) were taken on Day 3 after the birth of their litters. Blood sampling was done between 9 and 11 a.m.

It is well known that stress can lead to increased prolactin levels [31,32]. To minimize stress, singly housed males and pairs were transferred in their holding cages from the animal

room to the blood collection room, where the male was immediately anesthetized with 0.2 ml Xylazine and Ketamine (4:1 ratio). Approximately 2–3 min thereafter, 0.5–1.0 ml blood was taken by cardiac puncture, and the male was then immediately euthanized. Mean duration for blood collection (including transport between rooms) was 3.4 min (S.D. \pm 0.8 min), which, in studies with meerkats, was rapid enough to ensure that the levels of prolactin were not influenced by the sampling procedure itself [46]. Collected blood was transferred into a tube and kept at room temperature for 60 min to facilitate clotting. Samples were then centrifuged for 20 min and were finally drawn up and stored for 3 months in a deep freezer, at -20 °C until prolactin analysis.

There was no difference in the time taken to obtain blood samples among the experimental groups ($P=.19$; KW=4.774, Kruskal-Wallis test). There was also no indication that this method leads to increased prolactin levels due to stress because there was no correlation between prolactin levels and the time taken to obtain blood samples ($P>.9$; $r_s=-.02$, Spearman rank correlation; $n=13$; to avoid an effect of treatment, only control males were used for this analyses).

2.1.3. Prolactin assay

A commercial RIA kit for measuring rat prolactin (Biocode Biotechnology, Belgium) was used. Thus, all values are expressed in rat equivalents, and it is possible that the absolute prolactin values for the striped mouse might be different with that of the rat because the cross reaction between the rat antibody and the *Rhabdomys* prolactin is not known. Standards were 6, 27, 65, 130 and 250 ng prolactin/ml. The assay procedure followed the protocols provided by the supplier, except that 25 μ l, instead of 50 μ l in double, was used for the standards and samples because there was insufficient serum for analyzing several individuals. Accordingly, the quantities of tracer, anti-rPRL antiserum and PEG second antibody were also divided by half. As the procedure was altered, a new least detectable dose (LDD) was calculated as being two standard deviations above the mean count for nine samples (in double) at zero dose. The LDD was calculated as 7.4 ng/ml. The sample for one male was below the LDD, and the LDD value was used in this case. Samples from all males were analyzed in one assay, negating the need to obtain an interassay coefficient of variation. Intra-assay variation was determined using two pools of serum from the remaining samples and was calculated as 10.3% ($n=8$) and 17.9% ($n=8$).

To validate the assay for measuring prolactin in *R. pumilio*, several samples were measured using two different quantities, and two pools were measured at several different concentrations. The dilution response of samples and pools demonstrated that the assay measured *Rhabdomys* prolactin (Table 1). Biological validation was indicated by the fact that lactating females had higher prolactin

levels than both males and nonlactating females (see Results).

2.1.4. Behavioral measurements

Fathers in several species have higher prolactin levels than nonfathers [5]. However, a direct relationship between prolactin levels and the degree of paternal care has never been demonstrated, implying that fathers that show more paternal care do not have higher prolactin levels than fathers that show less paternal care. Since striped mouse fathers always have highly developed paternal care under captive conditions [23], we did not perform detailed measurements of paternal behavior. Instead, pairs in Experimental Group 3 were observed for 15 min the day before blood sampling to establish whether paternal care occurred.

2.2. Field study

2.2.1. Field site

The field study was conducted in Goegap Nature Reserve near Springbok in northwest South Africa. The area is arid, and rain (average of 160 mm/year) falls mainly in winter. The vegetation type is succulent karoo [33]. Blood samples were collected outside the breeding season in March 2002 and during the breeding season in September and October 2002.

2.2.2. Subjects

In the nonbreeding season, samples were collected from 24 adult males, none of which was scrotal (i.e., no male had descended testes). During the breeding season, samples were collected from five breeding males, whose social groups were previously established in another study [26]. All five males nested with their group members during the nights and interacted amicably with juveniles in front of the group nests [26]. Nest occupancy was also confirmed by radio-tracking the males during the night (unpublished data). Furthermore, videotaping inside natural nests was possible for two males, and both demonstrated paternal behavior such as licking and huddling the pups (unpublished data). To increase the number of free-living males during the breeding season, blood was sampled from an additional four males that were trapped 5 km away from the field site. These four males were paired and housed in holding cages, which were placed on the veranda of the research station, where the mice experienced natural weather conditions and photoperiod.

2.2.3. Blood collection

For ethical reasons, a different method of blood sampling was chosen for the field than for the laboratory study. Blood was collected from the retro-orbital sinus of the free-living striped mice. For the field (but not for the captive) study, it was essential that wild-living mice were kept alive. This was of major importance, as the death of

Table 1
Dilution response of *R. pumilio* serum samples measured with a kit for rat-prolactin

Sample	ul in double	ng prolactin/ ml measured	expected	% measured of expected
1	50	78.7	65.4	120.3
	25	65.4		reference
2	50	63.7	57.2	111.4
	25	57.2		reference
3	50	116.3	116.3	reference
	100	113.4		97.5
4	75	18.8	20.8	90.4
	25	20.8		reference
5	25	35.9	35.9	reference
	12	31.8		88.6
Pool 1	150	23.5	20.4	115.2
	100	20.6		101.0
	75	23.9		117.2
	50	18.0		88.2
	25	20.4		reference
Pool 2	150	31.2	23.4	133.3
	100	29.0		123.9
	75	29.6		126.5
	50	27.6		117.9
	25	23.4		reference
	12	28.3		120.9

For each sample, prolactin was measured with different quantities of serum (μl in double). Measurements of the same sample with different quantities are shown with the same background color. The last column gives the measured prolactin concentration as a percentage of expected levels. The expected values were based on the quantities measured in the aliquots that had the same quantity as the standards (either 25 or 50 μl , referred to as “reference”).

the breeding male in the free-ranging mice would have, without any doubt, influenced the entire social group. Thus, the stress of taking blood from the retro-orbital sinus of one individual was regarded as being less destructive than stressing an entire population by permanently removing and euthanizing breeding males from several social groups.

In a pilot study, we sampled blood from the retro-orbital sinus of a striped mouse in captivity, under veterinary supervision. Blood was drawn twice, 5 days apart. This mouse was then euthanized and sent for autopsy. The sampled eye showed no inflammation or pathology compared with the control eye that had not been sampled.

To collect blood samples in the field, live traps were placed at the nests of known striped mouse groups. Trapping was done in the morning, i.e., within the first hour of activity of the mice. Traps were watched from a distance of 5 m. When a mouse entered the trap, it was removed from the trap, and a blood sample of 0.2 to 0.5 ml was taken from the retro-orbital sinus. Only one eye was sampled per individual, and only one sample taken per individual. It took 2.06 ± 0.62 min to take samples. After blood sampling, the mice were put into a holding cage and were provided with pieces of apple and sunflower seeds for 30 min. The mice started eating immediately and thus

had the opportunity to regain liquid and energy lost due to the blood sampling before they were released at their group nest. All sampled mice were retrapped in the field over the following few days. In males maintained under semicaptive conditions, blood samples were taken on Day 3 after the birth of their pups. Blood samples were processed in the same way as in the laboratory study.

2.2.4. Prolactin assay

The prolactin assay was performed in the same way as described in the previous section, except that 50 μl , instead of 25 μl , was used in the samples and standards. In 10 cases, not enough serum was available, and we used 2×25 μl serum, increased by the zero standard, to a volume of 2×50 μl . Later, we corrected the measured prolactin levels by multiplying it by two. The intra-assay variation of six samples (a pool of the remaining samples) was 5.7%. As all samples were measured in the same assay, no interassay variation was determined.

2.2.5. Behavioral measurements

All breeding males of the natural groups were observed in the field [26]. The four males in seminatural conditions were tested for retrieving behavior after blood sampling (same day). The female was taken out of the cage, and five pups were placed in a corner away from the nest. The male

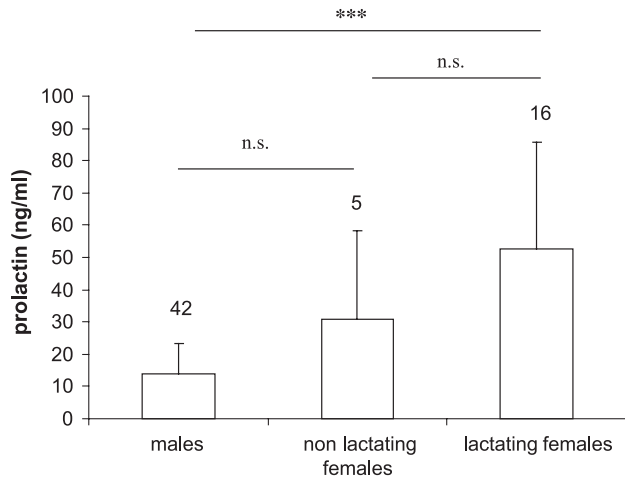


Fig. 1. Prolactin levels in males (all experimental groups combined), nonlactating females and lactating females. Sample sizes, means and S.E.M. are shown. *** $P < .001$.

was then observed for 10 min, and the latency until the retrieval of pups was recorded.

2.3. Statistics

Because of small sample sizes and the nonnormal distribution of the data set, nonparametric statistics were used [34], all being two-tailed. To test for an overall significant difference in prolactin levels, a Kruskal-Wallis test was performed. Analyses were performed using the software Instat and Statview.

3. Results

3.1. Comparison of prolactin levels between females and males

There was an overall significant difference in prolactin levels among males, nonlactating females and lactating

females ($P = .0003$; $KW = 16.048$, Kruskal-Wallis test; Fig. 1). Dunn's multiple comparison post hoc test yielded a significant difference between lactating females and males ($P < .001$, $R = 21.5$; Fig. 1).

3.2. Paternal behavior

In Experimental Group 3, seven of eight males were observed with their pups in the nest ($P < .05$; $T = 1$, Sign test), indicating that they participated in infant care [23]. The male that was not observed in the nest was excluded from further analysis.

3.3. Comparison of prolactin levels between different male groups

The mean serum prolactin levels of single males (19.7 ng prolactin/ml), paired males (16.8 ng prolactin/ml), fathers with (20.2 ng prolactin/ml) and fathers without tactile contact with their family (14.1 ng prolactin/ml) were similar, and there was no overall significant difference ($P > .7$; $KW = 1.365$; Fig. 2). There was no difference between fathers with and without tactile contact ($P > .99$, Mann-Whitney U Test).

It is possible that the absence of significant differences was due to the experienced males (which had offspring several months before the study) having a different prolactin secretion pattern with that of inexperienced males, as is known for New World monkey fathers [9,11]. There was a significant difference in the prolactin levels of experienced males [$n = 11$, 22.5 ± 3.3 ng prolactin/ml (mean \pm S.E.M.)] compared with 11 nonexperienced, singly kept control males (16.1 ± 2.2 ng prolactin/ml; $P = .04$; $U = 29$, Mann-Whitney U Test).

To control for an effect of experience, we performed the same analysis as before, without experienced males ($n = 30$). However, there still was no overall significant difference ($P = .41$; $KW = 2.886$). We also compared the prolactin levels of males that experienced fatherhood (fathers with tactile

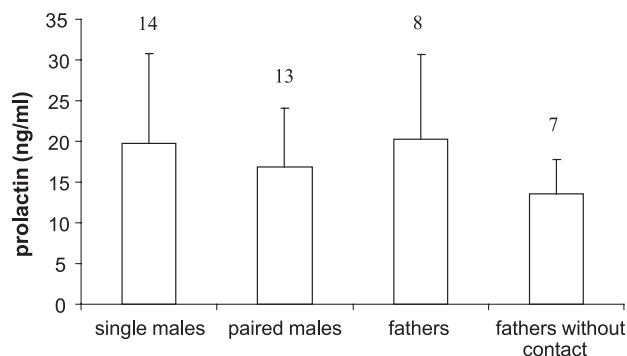


Fig. 2. Prolactin levels of the four experimental male groups (males that were kept singly, paired males, fathers in a family group, and fathers without tactile contact with their mate and pups). Sample sizes, means and S.E.M. are shown. None of the comparisons are significant.



Fig. 3. Prolactin levels of wild males outside and during the breeding season. Sample sizes, means and S.E.M. are shown. * $P < .05$.

contact with their family and experienced males, $n=14$) with males that never experienced fatherhood (23 singly kept control males and males paired with females), but did not find a significant difference ($P>.1$; $U=204$, Mann–Whitney U Test). Neither did the seven fathers of our study have higher prolactin levels than the 23 nonfathers ($P>.4$; $U=64$, Mann–Whitney U Test). Wild-caught males had higher prolactin levels than captive-born younger males ($P=.005$; $m=11$; $n=31$; $U=72$, U test).

3.4. Field data

There was no indication that prolactin levels during the breeding season differed between free-living males and wild-trapped males kept in captivity ($P>.7$; $U=8$, U test). Thus, the data for free-living and captive males were pooled to generate a data set of prolactin levels for the breeding season. Males had significantly higher prolactin levels during the breeding season than during the nonbreeding season ($P<.05$, $m=9$, $n=24$, $U=157$, U Test; Fig. 3). All free-living males showed amicable behavior towards the juveniles of their group, such as sniffing at juveniles, grooming them or sitting in body contact with them, indicating paternal motivation (data presented in Ref. [26]). Three of the four wild-trapped males retrieved pups.

To test for a possible influence of age, we compared data for males that were expected to be more than 1 year old during the nonbreeding season with males that were born during the previous breeding season and were approximately 5 months old. We used body weight as an indicator for age, as we knew that old breeding males are significantly heavier than their adult sons [26]. Ten males with a body weight above 50 g (mean: 55.1 ± 1.2 g; 26.3 ± 4.3 ng prolactin/ml) did not have higher prolactin levels than males with a body weight below 44 g (mean: 37.5 ± 1.9 g; 23.6 ± 3.6 ng prolactin/ml; $n=8$; $P>.7$; $U=32.0$, U test). Five males with a body weight between 44 and 50 g were excluded from this analysis, as it was not possible to assign them to one of the age classes.

4. Discussion

We were not able to demonstrate increased prolactin levels in striped mouse fathers compared with nonfathers, a phenomenon described regularly in so many taxa, that it is regarded as a vertebrate generality [5]. This is also in contrast to the results reported for two other rodent species with highly developed paternal care, the Djungarian dwarf hamster [17] and the California mouse [14]. This is surprising, as in all three species males show the same patterns of parental care (licking, huddling and retrieving) and to the same extent as females (dwarf hamster: Ref. [35]; California mouse: Ref. [36]; striped mouse: Ref. [23]). One reason why we did not find a difference could be that we used both paternally experienced and paternally inexperienced males

in all the experimental groups. In New World monkey fathers, it is known that experienced fathers can have higher prolactin levels than nonfathers [11]. Similarly, experienced striped mouse males had higher prolactin levels compared with inexperienced males. However, we did not find a significant difference in prolactin levels when the data of experienced males were excluded from the analysis. Whereas our sample size was reduced from 41 to 30 males by this procedure, this sample size was still higher than the sample sizes used in the cited studies in New World monkeys. Furthermore, the seven fathers in our study did not have higher prolactin levels than the 23 inexperienced nonfathers, and even a much larger sample size might not have made this comparison statistically significant ($P>.4$).

Captive males of the Djungarian dwarf hamster, California mouse and striped mouse exhibit highly developed paternal care and there is evidence that paternal care occurs also under natural conditions in all three species (dwarf hamster: [35]; California mouse: [37,38]; striped mouse: [23,26]). However, there is an important difference in the social organization among the three. The Djungarian dwarf hamster and California mouse live in socially monogamous pairs [36,37,39], and both parents rear their offspring [38,40–43]. In contrast, the striped mouse lives in groups of up to 30 adult individuals, consisting of up to four breeding females, one breeding male and their adult non-reproducing offspring [23,26]. Striped mouse pups are reared cooperatively, and all group members participate in infant care [26]. Therefore, the regulation of paternal response in the two monogamous species might be different from the polygynous striped mouse.

Striped mouse females have postpartum oestrus [25,44], and females at our field sites have two to three successive litters (unpublished data). This means that the breeding male of a group with three breeding females will encounter six to nine litters within the breeding season of 3 months, and there will be always pups and juveniles of different age classes present in the group nest. Thus, if prolactin is important in paternal care, it is sensible that males have elevated prolactin levels throughout the breeding season. If prolactin secretion is regulated by external environmental stimuli associated with the breeding season, the lack of differences in the prolactin levels in our captive study may be explained by the fact that all males were housed under the same light schedule and temperature, which simulated the conditions during the natural breeding season.

Free-living striped mouse males had higher prolactin levels during the breeding season than during the nonbreeding season. Apart from seasonal environmental differences (e.g., photoperiod), it is possible that the age of males might influence prolactin secretion during the breeding season, as males sampled during the breeding season were a few months older than the males sampled during the nonbreeding season. In captivity, wild-trapped males, which were older, had higher prolactin levels than younger captive-born males. However, we do not know whether this difference

was due to age, experience or any other factor. We doubt that age alone could explain the significant difference we found. In the captive study, wild-trapped males that were about 16 months old, had prolactin levels of 26 ng/ml, but free-living males, which were younger and about 12 months old during the breeding season, had higher prolactin levels (i.e., 35 ng/ml). Furthermore, during the nonbreeding season, heavy males that were regarded as being about 15 months old had similar prolactin levels with lighter males, which were assumed to be only 5 months old. Instead, seasonal changes in prolactin secretion more likely explain our findings than differences due to age. It is known, both from several bird species [29,30] and captive-kept wolves [16], that prolactin secretion increases during the breeding season. This is the first study to measure prolactin levels in free-ranging male mammals across seasons.

The fact that we did not find increased prolactin levels in captive fathers but did in free-living males during the breeding season could be due to two reasons: (1) It is possible that paternal care is independent of hormonal regulation. A contributing factor could be the experience gained by nonreproducing adult males as helpers at the nest [26]. (2) Alternatively, it could be that we failed to observe an increase in prolactin in captivity. When prolactin secretion is regulated by environmental stimuli, we might not have been able to find a difference between the different male groups, as all of them were kept under identical standard laboratory conditions, i.e., same temperature and light regime schedule.

This is the first study to measure prolactin levels in the striped mouse, a polygynous species. Further studies are needed to test the effect of a number of factors on prolactin levels. It would be important to know the exact influence of age and experience on prolactin secretion. In addition, more detailed behavioral studies, especially with regard to the time spent in body contact with pups immediately before blood sampling, might yield interesting results. Furthermore, the reasons for seasonal changes should be investigated. Potential seasonal stimuli that might influence prolactin levels are photoperiodic changes, ambient temperature, food availability, and even social experiences, factors which are likely to differ between the breeding and nonbreeding seasons. Finally, whether changes in prolactin levels influence social behavior remain unknown.

Acknowledgements

We are very grateful to C. Pryce for allowing C. Schradin to perform the hormone analyses in his laboratory in Zurich, Switzerland. Comments by C. Touma and G. Anzenberger significantly improved the manuscript. This study had been approved by the University of the Witwatersrand animal ethics committee (AESC 2002/9/13 and 2002/13/3), and was supported by grants from the Swiss National Science

Foundation, the German Science Foundation (DFG), the National Research Foundation (South Africa), the University of the Witwatersrand, the Schweizerische Gesellschaft für Naturwissenschaften, and the Fonds zur Förderung des akademischen Nachwuchses (FAN of the Zürcher Universitätsverein). C. Schradin thanks G. Anzenberger for his ongoing support and scientific interest, despite Schradin changing his research interest from paternal New World monkeys to paternal murids.

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