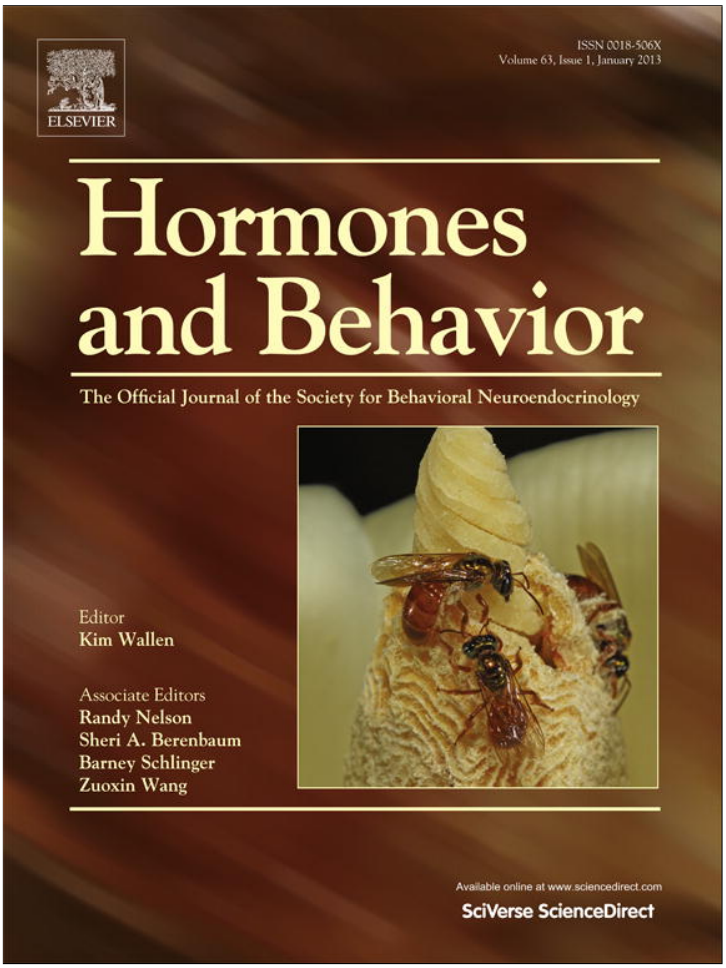

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Staying put or leaving home: endocrine, neuroendocrine and behavioral consequences in male African striped mice

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ABSTRACT

Social flexibility occurs when individuals of both sexes can change their social and reproductive tactics, which in turn can influence the social system of an entire population. However, little is known regarding the extent to which individuals of socially flexible species vary in their social behavior and in the underlying physiological mechanisms that support different social tactics. The present study in African striped mice modeled in captivity three male tactics described from the field: (a) philopatric males remaining in the family; (b) solitary roamers; or (c) group-living breeding males. Sixteen pairs and their offspring were kept in captivity, while one male offspring from the family remained as singly housed after he reached 21 days of age. Differences in behavior, morphology, hormone and neuropeptide levels were tested, and physiological measurements were correlated with behavioral measurements. In standardized arena experiments group-living males (philopatrics and breeders) were significantly more aggressive than singly housed males, in agreement with previous data suggesting that group-living, but not roaming males, are territorial. Philopatric males showed signs of reproductive suppression, small testes, lower testosterone and higher corticosterone levels than their singly housed brothers. Higher levels of arginine vasopressin (AVP) were measured in the PVN and BNST of singly housed males compared to group-living males. Based on these findings we hypothesize that roamers are physiologically primed, and capable, if the opportunity to mate arises, to release AVP, form social bonds and become territorial, thus quickly adopting the tactic as breeding male which would yield a higher reproductive success.

Introduction

Significant within species variations exist in social systems, which in some cases might be due to local adaptation and genetic differences between individuals and populations (Lott, 1991) or due to social flexibility. The term "social flexibility" describes the phenomenon that the social system of a population can be modified if males and females change their social and reproductive tactics (Schradin et al., 2012b). "Tactic" refers here to the behavior shown by an individual which results from the individual decisions rules, which has been called a "strategy" (Gross, 1996). In the case of social flexibility, individuals of a given population may have relatively consistent decisions rules, representing a single strategy with alternative individual tactics (Schradin and Lindholm, 2011). On the population level, such individual strategies can lead to alternative social systems (Schradin et al., 2012b). An example would be living in communal groups, in monogamous pairs, or living solitarily as observed in prairie voles (*Microtus ochrogaster*) (Getz and McGuire, 1993, 1997; Solomon et al., 2009). Such plastic tactics can optimize individual fitness under changing environmental conditions (Schradin et al., 2012b).

While social flexibility seems to be regulated by the environment and is likely to lead to fitness benefits, its proximate mechanisms are poorly understood (Lott, 1991; Schradin et al., 2012b). Often the switches in social behavior are dramatic, for example from pair-living to solitary-living as described for invertebrate and vertebrate species (Getz and McGuire, 1993; Müller et al., 2006; Schradin et al., 2010a). Similarly, offspring that reach adulthood may disperse and become solitary or alternatively remain philopatric, eventually living in extended family groups (Emlen, 1995).

The most parsimonious explanation for individual tactic switches would be that the observed change in behavior results from environmental changes constraining individual behavior. For example if population density declines sharply, due to predation or food shortage, previously group-living individuals might be forced to become solitary, as the other group members disappeared. In such cases, changes in social organization might not represent an actual switch in the behavioral tactic of an individual. Thus, to demonstrate true behavioral flexibility it is necessary to demonstrate that individuals actually do

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change their tactics, i.e. their social behaviors, in the face of a change in environment (Schradin et al., 2012b).

The present experiment examines the hypothesis that environmentally triggered changes in individual social tactics reflect concomitant or antecedent changes in specific physiological mechanisms. For flexible alternative tactics, the relative plasticity hypothesis predicts that changes in steroid hormones, such as testosterone and corticosterone, are components of the mechanisms that allow individuals to change from one tactic to another (Moore et al., 1998). Androgens, and especially testosterone, are powerful mediators of sexual and aggressive behaviors (Wingfield, 2005). In the mammalian brain, testosterone can be converted into estrogen via aromatase, and the estrogen receptor α plays a significant role in the regulation of male social behavior (Cushing and Wynne-Edwards, 2006). Similarly, glucocorticoids such as corticosterone, influence metabolism as well as social behaviors, including pair-bonding and biparental care (Bales et al., 2006; DeVries et al., 1996). Thus, significant changes in steroid hormones are expected to be associated with social flexibility (Schradin et al., 2012b).

In addition to steroid hormones, the neuropeptides oxytocin (OT) and arginine vasopressin (AVP), which are produced and secreted in the brain, have been causally implicated in social behaviors (Bales et al., 2006; Carter, 1998). While OT may be of particular importance to female reproduction and some features of behavior (Lee et al., 2009), the evolutionary closely related AVP plays an central role in male social behavior, especially social bonding, paternal care, and defensive aggression (Carter, 2007). Functional connections among brain regions of the social behavior network (SBN) are assumed to regulate phenotypic variation in behavior. AVP and OT are produced and secreted by neurons in several areas of the SBN including the medial bed nucleus of the stria terminalis (BNST), the supra-optic nucleus (SON) and the paraventricular nucleus (PVN), with well-documented effects on social behaviors (Caldwell et al., 2008; DeVries et al., 1981; Goodson and Kabelik, 2009). AVP contains processes from BNST project into the lateral septum, a brain region which is rich in AVP receptors. AVP has been specifically implicated in male–male aggression (Ferris et al., 1986), social recognition (Dantzer et al., 1988) and social bonding (Winslow et al., 1993). The SON is involved in the regulation of emotional reactivity and mobilization to a variety of stressors (Landgraf et al., 1998). The PVN is of particular importance in the synthesis and release into the brain of neuropeptides implicated in behavior and stress reactivity. For example, neurons containing peptides project from the PVN into both the basal forebrain and brainstem (Sawchenko and Swanson, 1982). AVP from the PVN has been associated with increased anxiety, and in response to stressors, can act as a secretagogue for ACTH release. These neuropeptides and associated changes in their receptors also have been associated with species, population and individual differences in social behavior (Cushing et al., 2001; Heckel and Fink, 2008; Solomon et al., 2009). If OT and AVP play a mechanistic role in social flexibility, we hypothesized that these peptides would differ as a function of different social tactics, possibly regulating the differential expression of social behaviors that form a specific tactic.

One mammalian species that shows high social flexibility is the African striped mouse (*Rhabdomys pumilio*; Schradin et al., 2012b). In this species, both sexes can express alternative reproductive tactics: (a) remaining as a non-breeding philopatric helper at the nest, (b) leaving the natal group and initiating solitary breeding, or (c) group-living as a communally breeding female or as the dominant breeding male within a social group. The present study models in a laboratory setting these conditions for males. In the field under high population density, males typically remain philopatric in their natal group long after reaching sexual maturity (Schradin and Pillay, 2004). Under low population density, males may leave their natal group when they are 3–4 weeks old, living as solitary roaming males (Schoepf and Schradin, 2012), or if a group of communally breeding females becomes available they switch to be territorial breeders (Schradin et al., 2009a). Solitary males have higher testosterone but lower corticosterone levels than philopatric males, which seem to be reproductively suppressed by the breeding male of their group (Schradin et al., 2009a,b). In sum, striped mouse males may either grow up in extended family groups of up to 30 adult mice or leave their natal group and become solitary when they are only a few weeks old (Schradin, 2005).

In the present study we examined patterns of change in behavior, as well as steroid and neuropeptide hormones in male striped mice, originating from the same population, but subsequently randomly assigned in the laboratory to live either alone or remain as a philopatric member of the natal family. The breeder male from each family was also studied. We hypothesized that different living conditions would be associated with changes in social behavior, indicating that the tactic is expressed as an individual trait, rather than a simple consequence of environmental conditions experienced in nature. Specifically, we predicted that solitary males would mature more quickly, with higher testosterone, but lower corticosterone levels. Using immunhistochemistry we also indexed tissue levels of AVP and OT in brain areas of the SBN, testing the prediction that AVP, which has often been implicated in male social behavior and territoriality would be particularly likely to differ as a function of living conditions (Caldwell et al., 2008; Carter, 2007). Finally, we examined correlations among the observed behavioral and physiological changes to test the hypothesis that individual differences in social behavior would be related to patterns of change in the neuropeptides and steroid hormones measured here.

Methods

Housing conditions

The captive colony used in the experiments consisted of descendants from animals originally trapped in 2002 in the Succulent Karoo in South Africa. Animals were kept at the University of Zurich under a 12:12-h light regime. Wild rodents kept in captivity are prone to develop stereotypic behavior (for striped mice see Jones et al., 2010) which is known to affect both social behavior as well as physiology and brain structure (Würbel, 2001). Thus, all animals were kept under environmentally enriched conditions, which were successful in avoiding the development of stereotypic behaviors (Schradin, unpublished data) and may help to reduce weight gain. Families were kept in two glass tanks 50×30×30 cm, connected to one another by a flexible plastic tube ($n=16$ families). Additionally, one plastic cage 20×13×15 cm was connected by another tube, and a water bottle was provided in this cage. Single individuals were kept in a single glass tank connected to two plastic cages. All tanks and cages had 5-cm deep wood shavings for bedding. The tanks contained natural branches and each family and singly kept mouse had one running wheel. Mice were supplied with water ad libitum. Striped mice in the Succulent Karoo display significant weight fluctuations, gaining weight during spring and losing more than 10% during the following dry season (Schradin and Pillay, 2005a), which may explain why this species is prone to extreme obesity in captivity. To further avoid obesity and as a means of behavioral enrichment, striped mice were not fed ad libitum, but rather three times a day: in the morning they received a seed mix of 4.0 g/individual (guinea pig and hamster food, Haefliger AG, Herzogenbuchsee, Switzerland), at noon one piece (approx. 1.0 g) of fruit or vegetable per individual and in the afternoon two mealworms per individual.

Animal ethical clearance was provided by the Kantonale Veterinärmt of the Kanton Zürich in Switzerland (ethical clearance number 91/2006).

Experimental procedure

Three males per family were used for the study: the father (approximately 3 months older than his sons) and two of his sons from the same litter, which were fully developed, scrotal adults (9–10 weeks old) at the end of the experiments. Families were kept together until offspring were three weeks old (weaning is on D16; Brooks,

1982). On D21, one male offspring was randomly assigned to be singly housed. One of his brothers was kept with the family as a philopatric male. Additionally, two siblings from the same litter remained in the family. In 11 families, one male and one female sibling remained. In the other five families two female siblings remained, as no male sibling was available. All surplus offspring were euthanized.

Philopatric males were present for the rearing of the next litter, which was either born when the male was 4 weeks old (10 families) or 7 weeks old (6 families). To avoid crowding in family cages, all juveniles except one male and one female of the second litter were removed when they reached 3 weeks of age. No pair had a third litter within the study period of 10 weeks. For each family, the philopatric male and its singly housed brother were weighed once a week and its reproductive state was determined as either being non-scrotal (testes inside the body) or being scrotal (testes fully descended), until the week both siblings were recorded as being scrotal (on average after 5.3 ± 1.2 weeks). When the male offspring were 9–10 weeks old the experiment was ended. At this age, all males were fully scrotal. This represents the age at which males would have dispersed and become solitary roamers under field conditions of very low population density in the field (Schoepf and Schradin, 2012; Schradin, 2005; Schradin and Pillay, 2005b; Schradin et al., 2010a).

Behavioral testing

Behavioral testing was done at the end of each experiment. All tests were performed in a neutral wooden presentation arena ($80 \times 65 \times 94$ cm) that consisted of two equal sized compartments with a removable partition. The arena was cleaned between tests.

Parental/Allo-parental care

This test was performed once when the male offspring were 8 weeks old. The test animal (father, philopatric male or singly housed male) was put inside the presentation arena. After a habituation period of 3 min, a pup between 0 and 6 days old from another breeding pair was presented for 5 min. The time the test animal huddled over the pup was recorded. In cases in which the pup was attacked (6 cases of 45 experiments), the experiment was immediately terminated and the pup was euthanized. Each stimulus pup was used for one experiment only.

Personality traits

When male offspring were 9 weeks old, all three males within a family (the father, the philopatric male and the singly housed male) were tested for individual differences in behavioral styles, which have been described as "personality traits" (Sih et al., 2004). The method for behavioral classification was adopted from Yuen and Schradin (unpublished data), using the same three testing procedures:

1. Open field test (similar measures have been used to index "anxiety" in a variety of species; in personality research it is used to measure "activity" and "boldness"; Reale et al., 2007): subjects were observed for 5 min and the amount of time they spent with at least half a mouse length away from the arena's walls was recorded. Activity of the subject was recorded using +/0 recording; i.e. we recorded every 15 s whether the mouse was active or not.
2. Exploration (novel objects test): with the subject inside the arena, two novel objects (a fixed object: rubber toy tiger $L \times H \times W =$ 11.0 cm × 4.5 cm × 2.0 cm; and a movable wooden ball, diameter 5 cm) were placed at the opposite end of the arena. Latency to approach (in seconds) and interaction with the novel objects (frequency) were recorded during a 5-min observation period.
3. Social behavior (same sex encounter test): this test has been used previously to measure aggression in wild animals (Schradin et al., 2010b). The partition was inserted and an adult same sex novel conspecific from a sibling group from our captive colony was placed into the other compartment of the arena. In striped mice, dominance is positively correlated with body mass (Schradin, 2004). To maximize the probability that the test animal would be dominant and initiate interactions, a stimulus animal that was lighter than the test animal was used for these tests. After a habituation period of 3 min the partition was removed, followed by 5 min of observation. In 1 of the 48 tests aggression escalated (by the tested breeding male) and the test was terminated after 3 min to avoid damaging fights that otherwise might have led to injury. The frequency of aggressive behaviors (fight, bite, chasing) was recorded as well as the frequency of grooming the stimulus animal by the test animal. The total time spent in body contact also was measured.

Brain and blood sampling

Two to 4 days after behavioral testing, when the males were 9–10 weeks old, experiments were terminated. The body mass of each male was determined (to the closest 0.1 g). It was anaesthetized and a blood sample of 300 μl was taken using sublingual blood sampling (Heimann et al., 2009). The blood was allowed to clot for 1.5 h at ambient temperature and was then centrifuged for 10 min at 10,000*g*. The resulting serum was pipetted and frozen in aliquots. Males were euthanized immediately after blood sampling. Brain tissue was removed and kept at room temperature in 4% paraformaldehyde with slow stirring for passive perfusion. Paraformaldehyde was changed after 10 min, 2 h and 3 h. Brains then were stored at 4 °C in 4% paraformaldehyde for 36 h and then transferred into 20% sucrose solution, which was changed after 36 h.

Hormone assays

All samples were analyzed at the University of Zurich. Commercial kits (EIA) from IBL Hamburg were used for both testosterone and corticosterone. Procedures followed kit manuals. However, due to very high corticosterone levels, samples were diluted 1:50. In some cases, samples for testosterone measurements were too small to be easily managed, and had to be diluted 1:1 with the zero standard (with subsequent conversion of values). All measurements were well within the standard curve of the assay. The kits were previously validated for measurements of hormones in striped mouse serum by demonstrating that serial dilution of striped mouse sample pools (two for each hormone) paralleled the standard curve (Schradin, 2008b). Intra- and inter-assay variability was determined with pools from striped mice serum. For corticosterone, intra-assay variability was 6.4% and inter-assay variability was 11.1%. For testosterone, intra-assay variability was 3.3% and inter-assay variability was 11.4%.

Immunohistochemistry

Brains were sliced at 30 μm. Free-floating sections were rinsed six times for 10 min each with potassium phosphate-buffered saline (KPBS). Sections then were incubated in 1% sodium borohydride for 20 min. After 6 washes of 6 min each in KPBS, sections were incubated in 0.014% phenylhydrazine for 15 min. Tissues were then rinsed six times for 10 min each in KPBS. Sections then were incubated in primary antibody (anti AVP from MP Biomedicals; anti OT generously donated and validated by Mariana Morris (Morris et al., 1980) and used many times previously (Grippo et al., 2007; Kenkel et al., 2012) at a concentration of 1:100,000 (diluted in KPBS + 0.4% Triton X-100) for 1 h at room temperature, and then incubated for 42 h at 4 °C. Sections were then rinsed 10 times for 6 min each with KPBS and afterwards incubated for 1 h at room temperature in anti-rabbit IgG (Vector Laboratories; 1:600). Sections then were rinsed five times during a period of 50 min with KPBS, and then incubated in A/B solution (Vector Laboratories; 45 ml A, 45 ml B per 10 ml KPBS + 0.4% Triton X-100) for 1 h. Sections then were rinsed three times in KPBS for 5 min each and afterwards three times in Tris-buffered saline for 5 min each.

After adding of 41.5 μl of H_2O_2, both AVP and OT were visualized by immediate incubation in diaminobenzadine (DAB) dissolved in Tris-buffered saline, for 15 min at room temperature, and then sections were rinsed three times with Tris-buffered saline and three times with KPBS (5 min each). Stained sections were mounted on gelatin-coated slides, air-dried, dehydrated in a series of ethanol dilutions, cleared with Histoclear (National Diagnostics), and then protected with coverslips using Histomount mounting medium (National Diagnostics). Images were captured using a microscope with camera. The density of AVP- and OT-immunoreactive (ir) cell bodies was determined from pictures taken at 20× using Image J software (NIH, USA). Measurements were taken from selected brain areas of the "social behavior network" that have previously been implicated in social behavior (Caldwell et al., 2008; Goodson and Kabelik, 2009): PVN, BNST, and SON (Fig. 1). OT was measured in an area of 112,500 pixels in the PVN, in the entire area of the SON, while the number of OT-ir containing cells was counted in the BNST. AVP was measured in an area of 112,500 pixels in the PVN, and in the entire slide in the BNST and the SON. Many, but not all animals had more than one slice through a given brain nuclei. In the present study a single representative slice for each individual, which showed the highest binding, was used for analysis. We used sections that were anatomically matched and were as close as possible to the middle of each brain nuclei. All analyses were conducted by an experimentally blinded observer.

Statistics

Morphological data were compared within families using repeated-measures ANOVA followed by the Tukey–Kramer multiple comparison test (q) using the software Instat (GraphPad). Data were tested for normal distribution. Percentage data, though bounded by 0.0 and 100.0%, were treated like normally distributed data if their distribution did not differ significantly from a normal distribution. Of the 16 families, 5 took part in another study, of which the data on hormone levels, body mass, testis and epididymis masses have been presented in a previous paper (Schradin et al., 2012a); these also are presented here along with the data from the additional 11 pairs that did not take part in the previous study. There is no overlap with other data (neuropeptides or behavior) between the two studies. Behavioral scores, histochemical data, and hormone levels were compared among reproductive tactics (breeder, philopatric, solitary male) using a type 3 Generalized Linear Model (equivalent to repeated-measures ANOVA), using the family effect as a blocking factor and reproductive tactic as a fixed effect. Pairwise comparisons were made on the estimated parameters using Tukey–Kramer multiple comparison tests (controlling for experiment-wise error). The effects of hormone levels and histochemical parameters on the behavioral scores were tested in mixed models, with family identity as a random effect. Hence, there were three models to test for the effects of reproductive tactic, hormone levels and histochemical measures upon behavioral scores, while blocking the between-family effect. In addition, to evaluate the extent to which the physiological parameters could interact with the effect of reproductive tactic, or have independent effects on behavioral scores, a full mixed model incorporating all physiological variables as well as reproductive tactic was used. All mixed models used Satterthwaite's method for denominator degrees of freedom estimation. All behavioral scores were log-transformed, as well as OT-ir in the BNST and testosterone levels, if entered as dependent variables, in order to achieve normality (Shapiro–Wilk test, $p > 0.07$ in all ANOVA models). Furthermore, histochemical data were standardized when entered as independent variables, as two batches of chemical kits were used that gave different average measures (differed in staining). Hence, the mean of each batch was subtracted from the individual values, and the result divided by the standard error in each batch. All Generalized Linear Models were derived using SAS (version 9, SAS Institute Inc., SAS/STAT 9.1 User's Guide. 2006, Cary, NC: SAS Institute Inc.). All data are presented as mean ± standard error. As an estimation of effect site, we calculated pairwise Cohen's d between breeders and philopatrics (dBP), breeders and singles (dBS), and between philopatrics and singles (dPS). A Cohen's d of <0.20 indicates a small effect, $d = 0.5$ a medium effect and $d > 0.80$ a large effect.

Results

Body mass and sexual maturity

Breeders were significantly heavier than both philopatric and singly housed males ($p < 0.001$) (Table 1). These body weight differences also were mirrored in significant differences in total brain weight (Table 1) However, when brain mass was corrected for body mass, breeders had relatively smaller brains than both philopatrics and singles (Table 1).

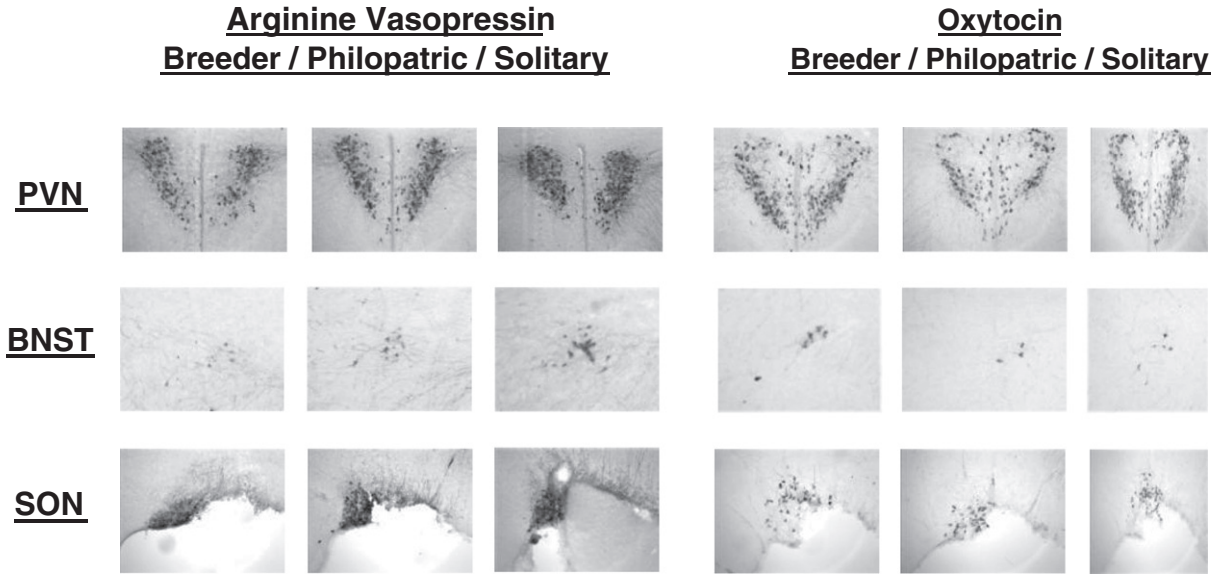

Fig. 1. Images of the distribution of irAVP (left) and irOT (right) in different brain areas of male striped mice.

Table 1
Morphological and physiological parameters (mean values ± SE) measured in male African striped mice from 16 families: breeding males, their adult sons staying in the family (philopatric) and their adult sons (from the same litter as philopatrics) that were singly housed since the age of 3 weeks, representing roamers in the field (singles).

		n		Breeder	Philopatrics	Singles	*F*	*p*	*d*BP	*d*BS	*d*PS
Body mass		16	[g]	64.5 ± 2.9[a]	53.7 ± 1.4	50.0 ± 1.5	**$F_{2,30}$ = 14.569**	**<0.0001**	**0.84**	**1.09**	0.64
Brain	Mass	16	[g]	0.77 ± 0.02[a]	0.73 ± 0.01	0.73 ± 0.01	**$F_{2,30}$ = 4.485**	**<0.05**	0.58	0.56	0.05
	Mass/body mass	16	[%]	1.22 ± 0.04[a]	1.37 ± 0.05	1.47 ± 0.05	**$F_{2,30}$ = 12.421**	**<0.0001**	−0.71	**−1.07**	−0.64
Testes	Testes mass	15	[g]	1.31 ± 0.05[a]	0.90 ± 0.06[b]	1.16 ± 0.08	**$F_{2,28}$ = 21.765**	**<0.0001**	**1.63**	0.72	**−0.99**
	Testis mass/body mass	15	[%]	2.05 ± 010	1.62 ± 0.10[a]	2.32 ± 0.12	**$F_{2,28}$ = 14.455**	**<0.0001**	0.78	−0.70	**−1.31**
	Epididymis mass	15	[g]	0.22 ± 0.02[a]	0.11 ± 0.01[b]	0.15 ± 0.01	**$F_{2,28}$ = 29.545**	**<0.0001**	**2.04**	**1.00**	**−0.97**
	Epididymis mass/body mass	15	[%]	3.2 ± 0.4	1.9 ± 0.3[a]	3.0 ± 0.2	**$F_{2,28}$ = 13.097**	**<0.0001**	**1.58**	0.42	**−1.22**
Hormones	Testosterone	16	[ng/mg]	4.21 ± 0.92	0.76 ± 0.14[a]	2.41 ± 0.50	**$F_{2,30}$ = 16.73**	**0.0001**	**1.27**	0.45	**−1.74**
	Corticosterone	16	[ng/mg]	295 ± 27	364 ± 53	159 ± 28[a]	**$F_{2,30}$ = 8.64**	**0.0011**	−0.31	**0.96**	**0.91**

n: number of families studied. *F* and overall *p* values are provided. "a" and "b" superscripts indicate significant pairwise differences (post-tests) to categories with none or a different subscript. Cohen's *d* for paired comparisons between breeders and philopatrics (*d*BP), breeders and singles (*d*BS), and between philopatrics and singles (*d*PS) are also shown, with ds indicating a large effect (>0.80) being marked in bold.

Breeders had larger testes than philopatrics, both absolutely as well as relatively to body mass (Table 1). Breeders also had larger testes than singles, but not when related to body mass. Singles had larger testes than their family living philopatric brothers, both absolutely and relatively to body mass. The results for epididymis mass followed the same pattern (Table 1).

Singly housed males became scrotal at a younger age (4.1 ± 0.1 weeks) than their family-living brothers (philopatrics; 5.5 ± 0.4 weeks; data not normally distributed, Wilcoxon test, $T = 0$, $p < 0.01$). Singly housed males had a lower body mass than their family-living brothers when they first became scrotal (29.5 ± 1.4 g vs. 36.4 ± 2.3 g; paired $t_{16} = 2.817$ $p < 0.05$).

Behavioral differences of adult scrotal males of the three tactics

Singly housed males remained closer to the wall in the open field and exhibited less exploratory sniffing toward a novel object, possibly indicating higher levels of anxiety, compared to philopatric males, and were less active than males of either of the group-living tactics (Table 2). Breeders and philopatrics did not differ in anxiety, activity, and exploration (Table 2).

Measures of social behaviors indicated that singly housed mice showed more socio-positive (grooming, sitting in body contact) and fewer aggressive behaviors toward same sex strangers when compared to either breeders or philopatrics (Table 2). Breeders spent more time huddling and licking pups than males of any of the two other categories. More breeders (10 of 15) showed care-giving behavior toward pups than either philopatrics (2 of 16, Fisher exact test, $p < 0.01$) or singly housed males (3 of 16, Fisher exact Test, $p < 0.01$).

Physiological differences

Philopatrics had significantly lower testosterone levels than either breeders or singly housed males, while breeders did not differ from singly housed males (Table 2). Singly housed males had significantly lower corticosterone levels than either breeders or philopatric males, which did not differ from each other (Table 2).

Significant differences were measured for AVP-ir in the PVN ($F_{2,26} = 5.46$, $p = 0.01$; dBP = 0.08. dBS = −0.71, dPS = −0.93; Fig. 2A), with a trend toward a difference in the BNST ($F_{2,30} = 3.17$, $p = 0.057$; dBP = 0.13, dBS = −0.62, dPS = −0.52; Fig. 2B). Singly housed males showed higher amounts of AVP-ir (Fig. 2). In contrast, singly housed males had significantly lower amounts of AVP-ir in the SON ($F_{2,23} = 4.73$, $p = 0.02$; dBP = 0.08. dBS = 0.79, dPS = −0.67; Fig. 2B). For OT, we did not find any differences among the male tactics (all $p > 0.20$; Fig. 1).

Physiology and behavior

Only a few regressions of physiological parameters on behavioral scores were found to be significant: corticosterone level were negatively related to both grooming ($F_{1,44} = 5.73$, $p = 0.021$) and body contact ($F_{1,40.7} = 4.13$, $p = 0.049$). Body contact was negatively related to AVP-ir in the SON ($F_{1,28} = 5.89$, $p = 0.022$). OT-ir in the SON was positively related to activity scores ($F_{1,28} = 5.56$, $p = 0.026$). However, none of these regressions remained statistically significant in the full mixed model that included the effect of reproductive tactic.

Discussion

The results of this study demonstrate for the first time behavioral and neuro-endocrine differences between male striped mice, tested under laboratory conditions that modeled three alternative reproductive tactics (ARTs) observed under field conditions. Philopatric males had higher corticosterone but lower testosterone levels, presumably related to previous observation that philopatric males, remaining with their natal family, reach sexual maturity later and have smaller testes than males that leave home (Schradin et al., 2012a). We also found evidence that singly housed males have higher concentrations of AVP-ir cells in comparison to group-living males. This was especially apparent in the PVN with a similar trend in the BNST. We did not detect differences in OT. However, in the present study physiological parameters did not appear to strongly mediate the observed behavioral differences between reproductive tactics, suggesting that larger samples and additional dependent variables might be needed to observe such relationship, if they exist. In spite of these limitations, ours is one of the most comprehensive studies demonstrating morphological, behavioral, hormonal and neuroendocrine differences between males following ARTs, and is a first step toward identifying the physiological mechanisms of social flexibility.

Breeding males were about 3 months older than their sons, and they were the heaviest males, which mimics the situation in the field, where breeders are larger and older than philopatrics and roamers (Schradin et al., 2009b). Accordingly, breeders had larger brains, but when controlled for body mass their brains were significantly smaller than those of philopatrics and singly housed males. Striped mice gain body mass and may become obese when food is abundant, during the breeding season in the field (Schradin and Pillay, 2005a), as well as in captivity (Schradin unpubl. data). Breeders may simply have been able to accumulate fat reserves and increase body mass for a longer period than younger males from the two other tactics, resulting in a lower brain mass to body mass ratio.

Data from this study support the hypothesis that males from different tactics differ in behaviors typically used to index individual "personality traits," (Reale et al., 2007; Sih et al., 2004). A behavioral

Table 2
Behavioral parameters (mean, (SE)) measured in male African striped mice from 16 families: breeding males, their adult sons staying in the family (philopatric) and their adult sons (from the same litter as philopatrics) that were kept single since the age of 3 weeks, modeling roamers in the field (singles).

		n		Breeder	Philopatrics	Singles	*F*	*p*	*d*BP	*d*BS	*d*PS
Behavior	Time spent in open field (anxiety)	15, 16, 16	[s]	$13.93 \pm 6.52^{a,b}$	15.25 ± 2.54^{a}	3.56 ± 1.76^{b}	$\mathbf{F_{2,29} = 6.00}$	**0.0066**	−0.31	0.45	**1.08**
	Activity	15, 16, 16	[frequency]	5.20 ± 1.03	5.94 ± 0.86	2.31 ± 0.71^{a}	$\mathbf{F_{2,29} = 8.88}$	**0.0010**	−0.19	0.78	**0.95**
	Approach latency	15, 16, 16	[s]	144.53 ± 32.60	125.63 ± 26.34	201.00 ± 27.04	$F_{2,29} = 2.31$	0.1171	0.03	−0.48	−0.58
	Novel object sniffing	15, 16, 16	[frequency]	$8.73 \pm 2.52^{a,b}$	11.56 ± 1.88^{a}	3.38 ± 1.06^{b}	$\mathbf{F_{2,29} = 4.92}$	**0.0145**	−0.32	0.38	**0.90**
	Aggression	15, 16, 16	[frequency]	$2.13 \pm 0.80^{a,b}$	2.69 ± 0.88^{a}	0.25 ± 0.17^{b}	$\mathbf{F_{2,29} = 4.95}$	**0.0142**	−0.13	0.74	0.79
	Body contact	15, 16, 16	[s]	41.00 ± 12.77	21.19 ± 6.96	75.75 ± 18.79^{a}	$\mathbf{F_{2,29} = 5.97}$	**0.0068**	0.20	−0.67	**−0.83**
	Grooming	15, 16, 16	[s]	0.60 ± 0.25	1.44 ± 0.41	5.31 ± 0.83^{a}	$\mathbf{F_{2,29} = 21.72}$	**0.0001**	−0.41	**−1.55**	**−1.23**
	Caring for pups	14, 15, 15	[s]	27.07 ± 10.12^{a}	4.73 ± 2.77	3.73 ± 1.90	$\mathbf{F_{2,27} = 8.51}$	**0.0014**	**1.17**	0.75	−0.01

One breeding male was not tested behaviorally, and one family was not tested with pups. *n*: number of families studied. *F* and overall *p* values are provided. "a" and "b" superscripts indicate significant pairwise differences (post-tests) to categories with none or a different subscript. Cohen's *d* for paired comparisons between breeders and philopatrics (*d*BP), breeders and singles (*d*BS), and between philopatrics and singles (*d*PS) are also shown, with *d*s indicating a large effect (>0.80) being marked in bold.

trait may be described as a personality trait if individuals are consistent in these behaviors over time and over situations, and if individuals differ consistently; for example, one individual may be consistently more bold and more explorative than another (Biro and Stamps, 2008). In the current study we did not test for consistency in behavior across time, but this has been demonstrated in free-ranging striped mice (Yuen and Schradin, unpublished data). We found that singly housed males were less exploratory and more anxious than philopatric males and less active than males of either of the group-living tactics. Solitary males may follow a risk-aversive tactic, which could be adaptive since solitary males are in danger of encountering territorial aggression from much larger breeder males (Schradin et al., 2009a), and also from smaller philopatric males (Schradin, 2004). In contrast, breeders and philopatrics did not differ in measures of personality traits.

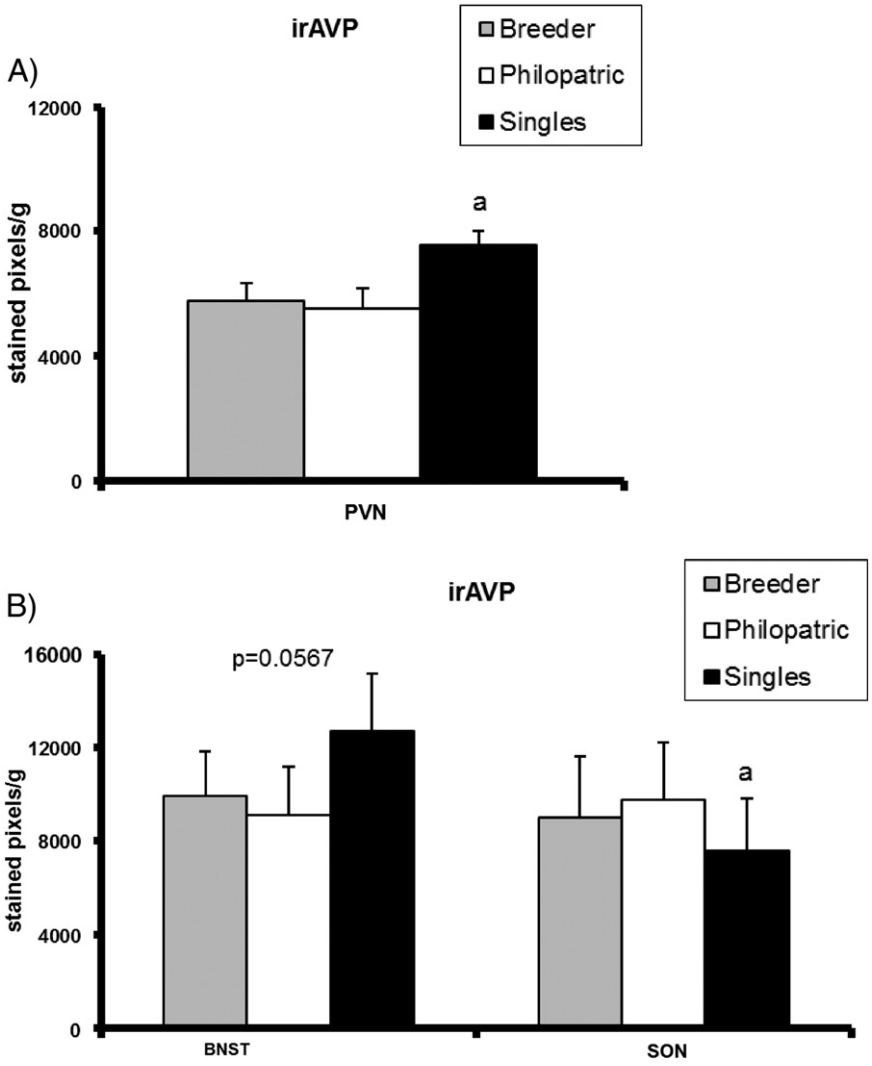

Fig. 2. Differences between the three male tactics in irAVP measured in different brain areas. (A) PVN. B) BNST and SON. Original data (mean ± SE) are shown, while statistic refer to differences of least square means of the model (see text). "a" indicates significant pairwise differences (post-tests) to categories with no subscript. Twelve to 16 males of each tactic were studied (for exact sample sizes and statistics see text).

In the current study, we demonstrated for the first time that, compared to either philopatrics or breeder males, solitary males were less aggressive toward strange males, and showed more socio-positive behaviors such as grooming and sitting in body contact. This is in agreement with the field observation that roamers are non-territorial, while breeders and philopatrics are territorial (Schradin, 2004; Schradin et al., 2009a). Breeders were more responsive toward pups, showing high levels of paternal care, while philopatrics and singly housed males typically ignored pups. This is in agreement with early work showing high levels of paternal care in male breeders (Schradin and Pillay, 2003), and consistent with our finding that breeder males in comparison to males from the two other tactics have higher levels of prolactin (Schradin, 2008a; Schradin and Yuen, 2011), a hormone known to be important for the regulation of paternal care (Schradin and Anzenberger, 1999). This study supports the hypothesis that solitary living males differ in personality traits from group-living males, suggesting that personality traits may not be fixed in striped mouse males, but rather can change when males change their tactic.

Our behavioral results also are consistent with the hypothesis that the tactics observed in nature represent real differences between the males and do not simply reflect different environmental constraints. To demonstrate social flexibility, i.e. the ability of individuals to change their behavior, it is necessary to show that changes in tactics are associated with changes in both social behavior and social motivation (Schradin et al., 2012b). Alternatively, differences observed in the field may not be due to changes within an individual, but rather a transient response to different environmental conditions. For example, low population density may render group-living impossible, while high population density could make dispersal and solitary living impossible. In socially flexible individuals the behavioral changes are the result of an individual choice, which itself is a response to the current environmental conditions (Schradin and Lindholm, 2011). In sum, we found significant differences between males from the different tactics in social behavior, indicating that alternative reproductive tactics also represent alternative social tactics.

As in a previous study (Schradin et al., 2009b) we found here that singly kept males had lower corticosterone levels, higher testosterone levels and reached puberty earlier than their family living brothers. In the previous study mice were kept in standard laboratory cages, in which striped mice are prone to show stereotypic behavior, while in our current study mice had much more space and lived under highly enriched conditions, thus allowing philopatric males more opportunity to avoid the breeding male. Nonetheless, philopatric males still showed indications of reproductive suppression, becoming scrotal at a later age. Even after philopatric males became scrotal, their relative testes and epididymis mass were significantly smaller than that of breeders and roamers (for the same result see Schradin et al., 2012a). Philopatric males were older and at the same time heavier when becoming scrotal, indicating that the observed difference was not due to differences in growth rates between singly and family-housed males, but due to reproductive suppression in the presence of the breeding male or other family members.

We found no indication that males of the three tactics differ in OT concentration in any of the brain nuclei studied. This might be due to the insufficiency in our measures of OT. Alternatively, this finding may support the hypothesis that OT is of less functional importance in the regulation of some aspects of male (versus female) social behaviors (Carter, 2007; de Vries, 2008; but see Kenkel et al., 2012). However, singly housed males had more AVP in both the PVN and the BNST when compared to the two group-living tactics, which did not differ from each other. AVP in the PVN and BNST plays a critical role in the regulation of social bonding, parental care and territorial aggression in male mammals (Caldwell et al., 2008; Goodson and Kabelik, 2009). It might therefore seem surprising that the group-living males, which have social bonds, had lower concentrations of AVP than the singly kept males that had not had an opportunity to form social bonds or exhibit paternal behaviors. However, it is important to note that based on data from prairie voles, defensive aggression and the induction, but not the maintenance, of social bonds are AVP-dependent and arise as a consequence of mating and other behavioral interactions with a female partner (Winslow et al., 1993). It is possible that the measures used here, are indexing stored AVP, which may be released by interactions with a potential mate. Singly housed males might have higher concentrations of stored, immunoreactive AVP because they secrete very little AVP within the nervous system. Furthermore, by maintaining AVP and testosterone synthesis males living alone could retain the physiological capacity to quickly switch their social tactic if environmental conditions changed. For example, roamer males could switch to the breeder tactic, and release AVP if they encounter a group of communally breeding females into which they can immigrate and form social bonds. In this case, roamers could immediately increase AVP release, forming social bonds with the group's females, change their social behavior and switch to the optimal breeding tactic (territorial breeders often have higher fitness than roamers; Schradin and Lindholm, 2011). This would require that the sensitivity of the brain to AVP is the same in roamers as in breeders, and in fact they do not differ in the expression in brain tissue of the AVP V1 receptor a (Schradin, Simon & Bales, unpublished data). The SON is believed to be especially important as a source of peripherally released AVP (Landgraf and Neumann, 2004). Peripheral AVP has several functions including permitting mobilization by increasing blood pressure and regulating fluid retention (Caldwell et al., 2008). It is possible that the somewhat lower levels of AVP we observed in the SON of solitary males were related to the reduced immediate need for mobilization, associated with reduced activity, exploration, and increased anxiety seen in the behavioral profiles of solitarily kept males. Future experiments in which both peripheral and central AVP are monitored or manipulated will be needed to explore these hypotheses.

Flexible species such as striped mice offer new insights into the processes that underlie the expression of social behavior in general, but also social flexibility and suggest mechanisms through which animals may be capable of quickly adapting to changing environmental conditions to maximize their individual fitness. Collectively this flexibility can permit a shift in the social system of an entire population, for example from group to solitary living as we have seen in this species (Schradin et al., 2012b).

The present study supports the hypothesis that changes in environmental including social conditions can influence patterns of behaviors. It may be adaptive in the short run for males living alone to show reductions in activity or exploration, with concurrently low levels of corticosterone, typically necessary for mobilization. However, at the same time animals may maintain flexibility in processes that change more slowly, such as the synthesis of hormones. By maintaining the synthesis of testosterone and central levels of AVP in regions such as the PVN, males might retain the capacity to respond quickly in the face of opportunities to form new bonds or mate. These findings confirm the hypothesis that different reproductive tactics can lead to neuroendocrine changes with accompanying significance for motivational processes and also broad implications for the emergence of social systems.

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