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RESEARCH ARTICLE

Long-day photoperiod interacts with vasopressin and food restriction to modulate reproductive status and vasopressin receptor expression of male golden spiny mice

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SUMMARY

We tested the effects of photoperiod, water and food availability on body mass, reproductive status and arginine vasopressin receptor 1A (Avpr1a) mRNA expression in males of desert-adapted golden spiny mice, *Acomys russatus*. In Experiment 1, males were acclimated to short-day (SD; 8 h:16 h light:dark) or long-day (LD; 16 h:8 h light:dark) photoperiods with either saline (control) or vasopressin treatment for 3 weeks. The results of this experiment revealed that under control conditions, SD mice increased body mass by ~5% while LD mice decreased it by ~4%. SD photoperiod had no effect on reproductive status and leptin levels, whereas LD males increased testes mass and serum testosterone, but the photoperiod had no effect on leptin levels. Vasopressin administration decreased LD-induced reproductive enhancement. Because no consistent effect of SD treatment was found on reproductive status, Experiment 2 was carried out only on LD-acclimated males kept under 75% food restriction (decrease from *ad libitum*) with saline or leptin treatment. Body mass, testes mass, serum testosterone, leptin concentrations and Avpr1a mRNA expression were measured. Food restriction remarkably decreased body mass, with a more potent effect in leptin-treated males, showing enhanced reproductive status and a significant increase in serum leptin compared with controls. Avpr1a expression was significantly upregulated in LD, vasopressin-treated and food-restricted males, with higher levels in the hypothalamus compared with the testes. We conclude that in *A. russatus*, LD photoperiod interacts with water and food availability to advance reproductive responses. Avpr1a is suggested to integrate nutritional and osmotic signals to optimize reproduction by modulating reproductive and energetic neuroendocrine axes at the central level. The interaction between photoperiod and other environmental cues is of an adaptive value to desert-adapted small rodents for timing reproduction in unpredictable ecosystems such as extreme deserts.

Key words: desert-adapted, leptin, testosterone, water and food availability, vasopressin receptor mRNA, photoperiod.

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INTRODUCTION

Reproductive responses in desert-adapted rodent species are primed to coincide with favorable environmental resources and moderate climatic conditions. Accordingly, changes in photoperiod (day length) and water and food availability are utilized by these species as predictive cues for reproductive timing (Bronson, 1989; Bronson, 2009). Among all environmental variables out of the tropics, photoperiod is the most reliable and effective cue for timing reproduction in mammals (Goldman, 2001; Hastings et al., 1985; Malpaux et al., 1999). However, in desert ecosystems, relying solely on photoperiod to anticipate habitat and ecological changes can certainly endanger reproduction and offspring success, as these harsh environments are generally characterized by unpredictable climatic conditions and food and water availability (Noy-Meir, 1973; Bronson, 1985; Bronson, 2009). Several environmental cues have been suggested to interact with photoperiod to form a more reliable network to prime the timing of reproduction in unpredictable environments, including precipitation, food availability and temperature (Bronson and Heideman, 1994; El-Bakry et al., 1998; Karels et al., 2000; Shanas and Haim, 2004).

The golden spiny mouse, *Acomys russatus* (Wagner 1840), is a small desert rodent inhabiting arid regions of Egypt, Israel, Jordan and the Arabian Peninsula (Mendelssohn and Yom-Tov, 1999). This species is highly adapted to survive with a limited supply of water from its food, mainly invertebrates (Kronfeld-Schor and Dayan, 1999). Although *A. russatus* is not a seasonal breeder and reproduction is expected to be continual throughout the year under favorable conditions, reproduction has been reported to occur during long days, such as in the spring and summer (Mendelssohn and Yom-Tov, 1999). Photoperiod manipulations have been shown to modulate reproductive responses in males of *A. russatus* (Wube et al., 2008a) and the common spiny mouse, *A. cahirinus*, from a desert population (Bukovetzky et al., 2012), but had no effect on the counterpart females of the former (Wube et al., 2008a). Additionally, laboratory studies showed that in female (Shanas and Haim, 2004) and male *A. russatus* (Wube et al., 2008b), an increase in salinity stress of the water source can reduce body mass (M_b) and halt reproduction responses by decreasing gonadal mass of both sexes, increasing vaginal closure and suppressing spermatogenesis.

The salinity-induced modulation of reproductive responses in *A. russatus* is suggested to be mediated by the peptide hormone vasopressin. This is an anti-diuretic hormone that is synthesized in the hypothalamus, stored in vesicles at the neurohypophysis and released to the blood in response to hyperosmotic stress. Vasopressin regulates water balance by acting on the distal tubules and collecting ducts in the kidney to increase water retention (Antunes-Rodrigues et al., 2004). Nevertheless, vasopressin is also directly released into the brain acting as a neurotransmitter for social behavior or stress

responses (Landgraf et al., 1999). Although there is no direct evidence of a distinct reproductive role of vasopressin, the hormone has been broadly reported to be involved in regulating supportive reproductive processes including pair-bond formation in male prairie voles, *Microytocinus ochrogaster* (Gobrogge et al., 2009), parental care regulation in prairie and meadow voles, *Microtus pennsylvanicus* (Parker and Lee, 2001; Wang et al., 1994), gonadotropin release stimulation in female mice (Miller et al., 2006), and control of epididymis and uterine motility in humans and other animals (Wathes, 1984). Recently, notable testosterone decrease (Bukovetzky et al., 2012) and spermatogenesis suppression (Wube et al., 2008a) following vasopressin treatment were also specifically observed in *A. russatus* males and in desert-adapted populations of *A. cahirinus*, respectively. Nevertheless, studies on the involvement of vasopressin in modulating reproductive activity are lacking and further research is required to elucidate its cellular mechanisms of action.

Food availability is another proximate environmental factor that can modulate seasonal reproduction in mammals (Bronson, 1989). Reproduction is energetically costly and limiting it during challenging energetic conditions such as food shortage is a life history trait (Speakman, 2008). Results of several studies revealed that food restriction can limit several reproductive processes, including gonadotropin and gonadal hormone secretions, spermatogenesis and reproductive organ development (Blank and Desjardins, 1985; Edmonds et al., 2003; Nelson et al., 1992; Steinman et al., 2012; Young et al., 2000; Zysling et al., 2009). The energy-induced reproductive cessation is expected to be mediated by complex interactions between several hormones that regulate metabolism and development throughout the body and modulate reproduction, by interacting with the hypothalamic-pituitary-gonadal axis (Martin et al., 2008). Among all energy regulating hormones, leptin is the most prominent mediator of food intake and energy expenditure (Tucholski and Otto-Buczkowska, 2011). Leptin is primarily synthesized by the adipocytes within white adipose tissue (WAT) and levels of circulating hormone largely correlate with total WAT mass (Maffei et al., 1995). High levels of leptin stimulate catabolic responses that reduce energy intake and increase energy expenditure, resulting in a loss in body mass (M_b) (Baskin et al., 1999). Conversely, low levels of leptin stimulate anabolic responses that increase energy intake and reduce energy expenditure, resulting in M_b gain (Anubhuti and Arora, 2008). Several lines of direct and indirect evidence support the notion that leptin is a potent modulator of reproductive responses in mammals (Clarke and Henry, 1999; Messinis and Milingos, 1999; Zieba et al., 2005; French et al., 2009). Leptin has been shown to rectify adverse food deprivation impacts on both estrous cycling and lactation infertility in rats (Schneider et al., 1998; Woodside et al., 1998). Furthermore, in both human and animal models, deficiency in leptin levels is likely to be associated with abated reproduction organs and diminished reproductive activities in both genders (Israel and Chua, 2010). Finally, in *A. cahirinus*, a strong direct correlation was demonstrated between leptin levels and both relative testis size and testosterone concentrations in short-day (SD)-acclimated mice (Bukovetzky et al., 2012). The metabolic effects of leptin are mediated by receptors in the hypothalamus and testis; therefore, changes in the leptin signal during food deprivation could interact directly with these receptors to modulate reproductive activity (Clarke and Henry, 1999; Moschos et al., 2002).

Although the roles of photoperiod, water and food availability in modulating reproductive responses are well acknowledged, little is known about the possible interaction between these environmental cues that contribute to reproductive success in small rodent species in unpredictable environments such as deserts. The objective of this study is to explore the relationships between photoperiod, vasopressin, leptin and food restriction in regulating M_b and reproductive status of *A. russatus* males. Testis proportions (expressed as percentage of M_b) and serum testosterone levels were assessed as biological markers for reproductive status. First, we acclimated *A. russatus* to either SD or long-day (LD) photoperiod regimens combined with vasopressin treatment and monitored changes in body mass, reproductive status and arginine vasopressin receptor 1A (Avpr1a) mRNA expression in brain and testes tissues (Experiment 1). Afterwards, we evaluated the effects of LD photoperiod combined with food restriction and leptin treatments on M_b, reproductive status and Avpr1a mRNA expression in brain and testes tissues of *A. russatus* males (Experiment 2). We chose to only evaluate the effect of food restriction and leptin treatments under LD photoperiod, as results of Experiment 1 showed that LD photoperiod is required to initiate reproductive activity in *A. russatus* males whereas the influence of SD photoperiod was not consistent.

We hypothesized that if vasopressin, leptin and food restriction act as proximate cues for uncomplementary water and food availability, then decreases in M_b and reproductive status would be expected in mice treated with these cues.

MATERIALS AND METHODS

All animal procedures were performed according to protocols approved by the Ethics and Animal Care Committee of the University of Haifa. Experiments were performed on 48 adult male golden spiny mice (*A. russatus*) obtained from our established wild-type breeding colony at Oranim campus, University of Haifa, Israel. The founder pairs were trapped in arid parts of the Dead Sea shores, characterized by high temperatures, low humidity and unpredictable annual precipitation (Jaffe, 1988). Before experiments, animals were maintained under a photoperiod regimen of 12 h:12 h light:dark (lights on between 08:00 and 20:00 h) and fed *ad libitum*. Experiments were conducted in a climatic cabinet (1300 liters; MEDITEST, Meyzieu, France) with an ambient temperature of 31±1°C. Mice were housed separately in transparent plastic cages (13×13.5×40 cm) using saw dust as bedding, and rat pellets (Koffolk Ltd, Tel Aviv, Israel; 21% crude protein, 4% crude fat, 4% cellulose, 13% moisture and 7% ash equivalent to 18.7 kJ g^{-1} gross energy) and 0.9% NaCl in 2% agar-gel blocks (20 g of dry agar gel per liter distilled water) as a source of water were provided *ad libitum*, unless otherwise stated. Mice were randomly assigned to one of two photoperiod schedules: short day (SD; 8 h:16 h light:dark, lights on between 07:00 and 15:00 h) and long day (LD; 16 h:8 h light:dark, lights on between 07:00 and 23:00 h).

In Experiment 1, mice were acclimated under the two photoperiod schedules for 3 weeks and thereafter were further arbitrarily allocated into four subgroups of seven to eight mice each: for SD groups, SD-acclimated mice were intraperitoneally (i.p.) injected once every other day for 3 weeks with either (1) 0.5 ml 0.9% saline (control group; SD+SL) or (2) 0.5 ml vasopressin (VP; Sigma-Aldrich, St Louis, MO, USA) at a dose of 50 μg kg^{-1} body mass (experimental group; SD+VP); for LD groups, LD-acclimated mice were i.p. injected with either (3) saline (LD+SL) or (4) vasopressin (LD+VP) at the same dose and frequency as administered to SD groups.

In Experiment 2, mice were acclimated under the LD photoperiod with food restriction (FR groups). LD-acclimated mice were offered 75% of their original *ad libitum* food intake – estimated by the equation $y=0.002M_b+0.382$, where y represents *ad libitum* food intake (Wube et al., 2008a) – and were i.p. injected with 0.5 ml 0.9% saline (FR+SL) or 0.5 ml leptin (LEP; Sigma-Aldrich) at a

dose of 5 mg kg^{-1} body mass (FR+LEP). Injections were administered once every other day for 3 weeks.

Experimental procedures

Changes in M_b

Mice in the SD, LD and FR groups were weighed at day 0 to establish their baseline M_b, and then their M_b values were monitored at days 8 and 20 in SD and LD groups and at days 4, 8, 12, 16 and 20 in the FR groups. Percent change in M_b was calculated individually as the difference between measurements on a given day and the baseline M_b value, measured using a semi-analytical scale (±0.01 g; 1907 MP-8, Sartorius, Goettingen, Germany).

Blood sampling for hormonal assay

Mice were anaesthetized with a cocktail of Ketamin 100 mg kg^{-1} and Rampon 10 mg kg^{-1}. A blood sample (0.3±0.05 ml) was drawn from each mouse by cardiac puncture into a 1 cc heparinized syringe with a 22 gauge needle at days 0, 8 and 20. Blood samples were analyzed for serum testosterone and leptin levels. Serum was separated from all blood samples after centrifugation at 16,099 ***g*** for 10 min and frozen at –70°C for later hormonal analysis. No deaths because of the use of this procedure were recorded.

Hormonal analysis

Serum hormonal analyses were performed using commercial ELISA kits for mouse leptin (Quantikine, no. MOB00, R&D Systems, Minneapolis, MN, USA) and mouse testosterone (no. RE52151, IBL, Hamburg, Germany) according to the manufacturers' provided protocols. The intra- and inter-assay coefficients were 182–1803 pg ml^{-1} (4.3–3.8%) and 187–1736 pg ml^{-1} (7.6–5.0%) for leptin and 0.73–11.26 ng ml^{-1} (4.16–3.34%) and 0.82–11.38 ng ml^{-1} (9.94–4.73%) for testosterone, respectively.

Testes and brain sampling

At the end of 3 weeks of treatment, all mice were killed 3 h after light onset (10:00–12:00 h) using anesthesia and thereafter decapitation. The left testis and the brain were separated from all mice. Testes were washed, cleaned from external fat excesses, weighed, and their length and width were measured. The hypothalamus was removed from all brains, and the testes and the hypothalamus were frozen at –70°C for Avpr1a mRNA expression analysis.

Relative testis mass and estimated testis volume

Relative testis mass (M_t) was calculated as a percentage of M_b and was used as an indicator of reproductive status (Møller, 1989). Furthermore, the reported linear correlation between estimated testis volume (ETV) and M_t in hamster species (Watson-Whitmyre and Stetson, 1985; Gorman and Zucker, 1995) was also validated here for *A. russatus*. ETV was calculated as described earlier (Heideman and Pittman, 2009) using a two-dimensional equation, $ETV=W^2\times L\times 0.523$, where W and L are the width and length of the testis, respectively.

Hypothalamic and testicular Avpr1a expression

RNA was extracted from the hypothalamus and left testis of all mice using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA samples (0.4 µg) were subsequently reverse transcribed using the EZ-First Strand cDNA Synthesis Kit for RT-PCR according to the manufacturer's instructions (Biological Industries, Kibbuts Beit-Haemek, Israel). cDNA was amplified in the Verso cDNA kit (Thermo Scientific, Waltham, MA, USA) with the following primer pairs: Avpr1a – forward 5′-CTG GGA CAT CAC CTA CCG C-3′, reverse 5′-ATC CAC GGG TTG CAG CAG CTG-3′ (291 bp), using the Thermal Cycler DNA Engine (Bio-Rad Laboratories, Hercules, CA, USA). As a reference gene, the housekeeping gene ribosomal protein large, P2 (RPLP2) was used as endogenous control by the following primer pairs: RPLP2 – forward 5′-CGT CGC CTC CTA CTT GCT-3′, reverse 5′-CCA TTC AGC TCA CTG ATG ACC TTG-3′ (135 bp). Avpr1a and RPLP2 primers and probes were designed with Primer Express v.1.5 (Applied Biosystems, Carlsbad, CA, USA). The experimental samples and the reference controls were amplified in duplicate in the same run. The relative expression of Avpr1a was calculated in relation to the reference gene RPLP2 using the single-run delta-delta threshold cycle ($\Delta\Delta C_t$) method (Dussault and Pouliot, 2006; Nordgård et al., 2006). This method measures the number of amplification cycles (C_t) needed to reach an arbitrary threshold cycle set point. The threshold cycle was set at 10 times the fluorescence level above the mean standard deviation of background levels in all reaction wells. The C_t values of Avpr1a were normalized against the endogenous expression of the RPLP2 housekeeping gene. The $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001) was used to estimate the fold change in Avpr1a expression relative to SD+SL controls using the formula: fold change=$2^{-\Delta\Delta Ct}$.

Statistical analysis

Data are presented as means ± 1 s.e.m. In Experiment 1, the statistical effects of photoperiod, vasopressin or leptin on M_b and reproductive status were evaluated by three-way repeated-measures ANOVA (3R-ANOVA) with photoperiod (SD *versus* LD) and treatment (VP *versus* SL) as between-subject factors and days of exposure (days 0, 8 and 20) as the within-subject factor. In Experiment 2, the effects of leptin were analyzed using two-way repeated-measures ANOVA (2R-ANOVA) with treatment (LEP *versus* SL) as the between-subject factor and days of exposure as the within-subject factor. The effects of photoperiod and treatment on percentage M_t and Avpr1a mRNA expression were analyzed using three-way ANOVA with photoperiod (SD *versus* LD), drug administration (SL, VP, LEP) and food availability (*ad libitum versus* FR) as the within-subject factors. Two-way and one-way repeated-measures ANOVA models (2R-ANOVA and 1R-ANOVA, respectively) were also completed independently for photoperiod or treatment if mean differences and relevant first-order interaction effects were significant. The effect of food restriction alone (*ad libitum* LD+SL-mice *versus* 75% FR LD+SL mice) on the dependent factors was evaluated by one-way ANOVA. The ANOVA models were followed by a Bonferroni test for repeated-measures or Tukey's *post hoc* comparison for mean effect factors where appropriate. Paired mean differences between treatments and the control at a given exposure day were computed using Student's *t*-test. Finally, Pearson's correlation coefficient (r) was utilized to assess the statistical relationship between serum leptin, serum testosterone, M_b, M_t and ETV. All statistical analyses were conducted using SPSS software 13.0 for Windows (SPSS, Chicago, IL, USA). The P-value for rejecting the null hypothesis that mean effects are equal was set at $P\leq 0.05$.

RESULTS

Experiment 1: Effect of photoperiod and vasopressin on M_b, serum leptin, reproductive status and Avpr1a mRNA expression

M_b

There were significant effects of photoperiod ($F_{1,26}$=6.83, P=0.015) or vasopressin administration ($F_{1,26}$=12.67, P=0.001) on percentage

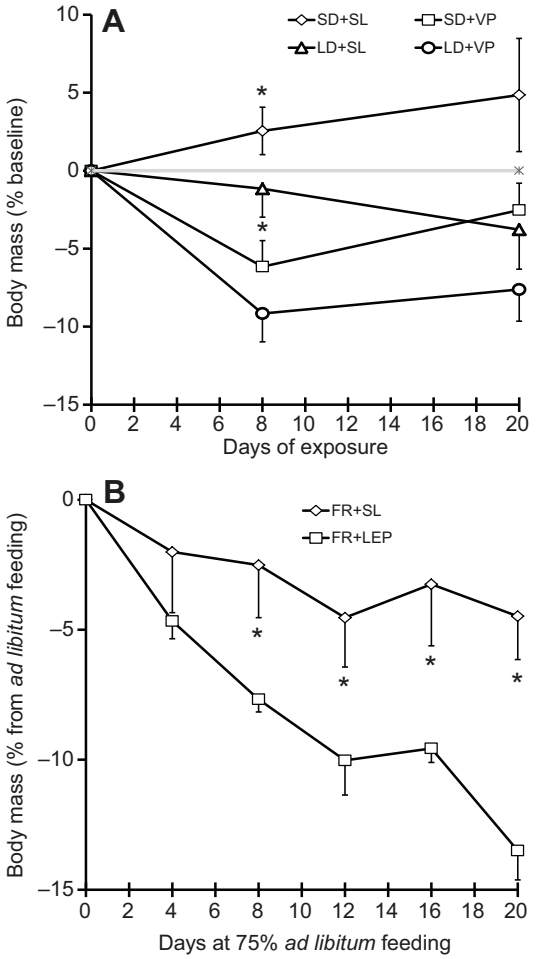

Fig. 1. Percentage change in body mass from baseline in *Acomys russatus* males. (A) Effect of either short-day (SD, 8 h:16 h light:dark, lights off at 15:00 h; *N*=7) or long-day (LD, 16 h:8 h, lights off at 23:00 h; *N*=8) photoperiods with a single saline (SL, 50 µg kg^{-1}) or vasopressin (VP, 50 µg kg^{-1}) intraperitoneal (i.p.) injection at 2 day intervals for 3 weeks. *Significant difference from VP experiment at the same photoperiod (P<0.05, unpaired *t*-test). (B) Effect of a single saline (5 mg kg^{-1}) or leptin (LEP, 5 mg kg^{-1}) i.p. injection at 2 day intervals for 3 weeks in mice kept under both LD photoperiod and 75% food restriction (FR) from *ad libitum* feeding (*N*=7). Values are means ± s.e.m. *Significant difference *versus* LEP (unpaired *t*-test, P<0.05).

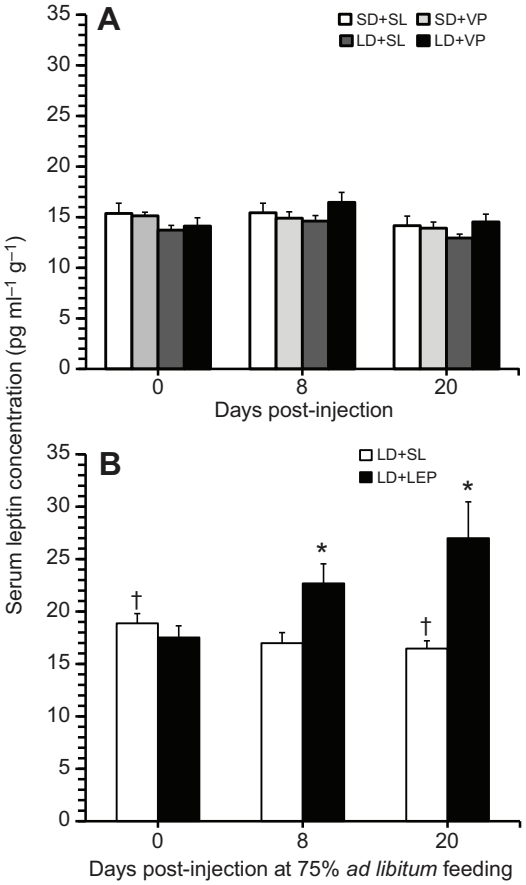

Fig. 2. Serum leptin concentrations of *A. russatus* males. (A) Effect of either short-day (SD, 8 h:16 h light:dark, lights off at 15:00 h; *N*=7) or long-day (LD, 16L:8D, lights off at 23:00 h; *N*=8) photoperiods with a single saline (SL, 50 µg kg^{-1}) or vasopressin (VP, 50 µg kg^{-1}) intraperitoneal (i.p.) injection at 2 day intervals for 3 weeks. (B) Effect of a single saline (5 mg kg^{-1}) or leptin (LEP, 5 mg kg^{-1}) i.p. injection at 2 day intervals for 3 weeks in mice kept under both LD photoperiod and 75% food restriction (FR) from *ad libitum* feeding (*N*=7). Values are means ± s.e.m. *Significant difference *versus* SL; †significant differences *versus* LD+SL (A) at the same day (unpaired *t*-test, P<0.05).

change of M_b (Fig. 1A). No significant photoperiod × vasopressin interaction effects were detected by the 3R-ANOVA model. Photoperiod conditions combined with saline alone have significant effects on M_b ($F_{1,13}$=6.15, P=0.03). At day 20, mean M_b values of LD-treated mice were higher by ~9% compared with SD-treated mice (54.48±3.23 g; Fig. 1A). M_b decreased significantly as a result of vasopressin administration under both SD and LD conditions (SD: $F_{2,12}$=6.11, P=0.015; LD: $F_{2,14}$=15.53, P=0.0001). The percentage change in M_b of LD+VP mice at day 20 was –7.6% and this decrease was approximately threefold higher than that of SD+VP mice (–2.5%).

Serum LEP concentrations

There were no significant effects of photoperiod ($F_{1,26}$=0.37, P=0.55), vasopressin ($F_{1,264}$=0.46, P=0.51) or their interaction ($F_{1,26}$=1.37, P=0.25) on serum leptin levels (Fig. 2A).

Reproductive status

Relative M_t

A significant strong positive correlation between ETV and M_t was revealed (r=0.87, N=42, P=0.0001) for *A. russatus* within all experimental subgroups. A three-way ANOVA yielded a significant main effect of photoperiod, vasopressin administration and food availability on mean relative M_t ($F_{7,43}$=16.4, P=0.0001). A significant interaction with vasopressin was present only for photoperiod ($F_{1,43}$=16.4, P=0.003; Fig. 3).

Serum testosterone concentrations

A 3R-ANOVA established significant effects for photoperiod ($F_{1,26}$=58.96, P=0.0001), vasopressin treatment ($F_{1,26}$=40.89, P=0.0001) and their interaction ($F_{1,26}$=11.64, P=0.002) on serum testosterone levels (Fig. 4A). No significant effects were detected for days of exposure ($F_{2,52}$=1.63, P=0.21). A 2R-ANOVA detected significant effects for days of exposure ($F_{2,28}$=23.49, P=0.0001), vasopressin ($F_{1,14}$=62.56, P=0.0001) and their interaction ($F_{2,28}$=9.26, P=0.001) in LD mice. Vasopressin administration

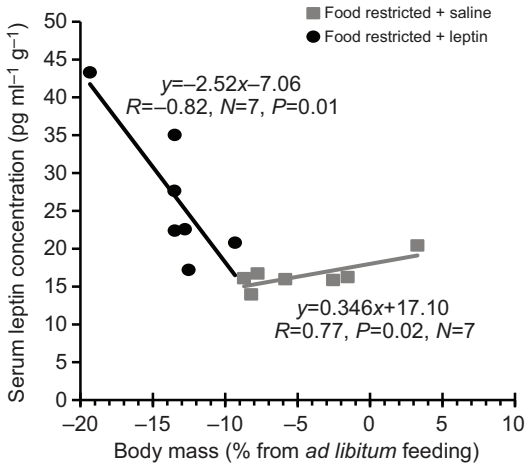

Fig. 3. Correlation between relative body mass and serum leptin concentrations in LD-acclimated males of *A. russatus* treated (i.p.) with either saline (5 mg kg^{-1}) or leptin (5 mg kg^{-1}). Values are means ± s.e.m. of *N*=7.

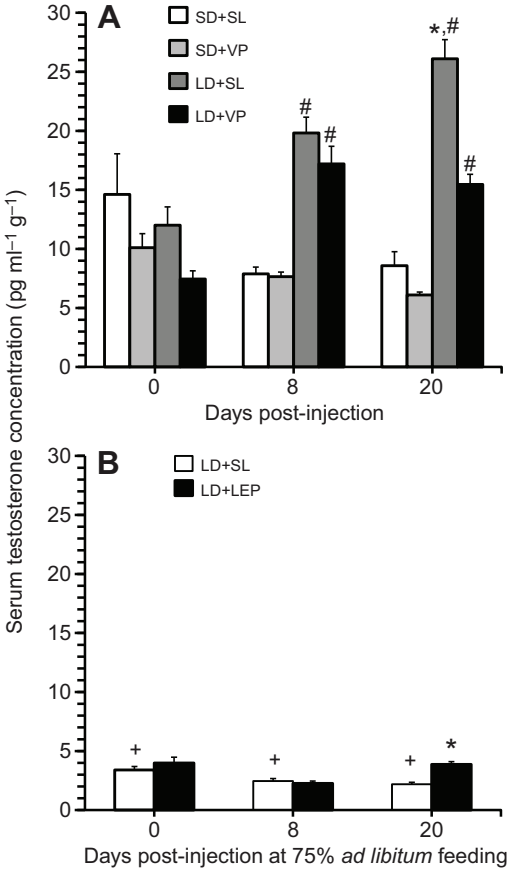

Fig. 4. Serum testosterone concentrations in *A. russatus* males. (A) Effect of either short-day (SD, 8 h:16 h light:dark, lights off at 15:00 h; *N*=7) or long-day (LD, 6 h:8 h, lights off at 23:00 h; *N*=8) photoperiods with a single saline (SL, 50 µg kg^{-1}) or vasopressin (VP, 50 µg kg^{-1}) intraperitoneally (i.p.) injection at 2 day intervals for 3 weeks. *Significant difference from LD+VP mice; #significant difference from counterpart SD mice (*P*<0.05 unpaired *t*-test). (B) Effect of a single saline (5 mg kg^{-1}) or leptin (LEP, 5 mg kg^{-1}) i.p. injection at 2 day intervals for 3 weeks in mice kept under both LD photoperiod and 75% food restriction (FR) from *ad libitum* feeding (*N*=7). All values are means ± s.e.m. *Significant difference from SD; +significant difference from LD+SL (A) at the same day (*P*<0.05, unpaired *t*-test).

significantly decreased mean serum testosterone levels to 11.40±0.74 pg ml^{-1} g^{-1} compared with 19.31±0.52 pg ml^{-1} g^{-1} in SL-treated mice (Bonferroni, *P*=0.0001). Under SD conditions, significant effects were detected by the general 2R-ANOVA model for days of exposure on serum testosterone levels ($F_{2,24}$=6.09, *P*=0.01), but not for vasopressin or the days of exposure × vasopressin interaction.

Avpr1a mRNA expression

A three-way ANOVA detected significant main effects of vasopressin administration and food availability but not photoperiod on Avpr1a mRNA expression in the hypothalamus ($F_{5,18}$=0.34, *P*=0.0001). No significant interaction effects were detected for any of the factors tested (Fig. 5). Vasopressin administration resulted in an approximately threefold increase in hypothalamic Avpr1a mRNA expression under both SD and LD photoperiods compared with controls. In testes, the main significant effect was detected for vasopressin administration, but not for photoperiod or food availability (three-way ANOVA, $F_{5,13}$=7.05, *P*=0.002).

Experiment 2: Effect of leptin on M_b, serum leptin, reproductive status and Avpr1a mRNA expression

M_b

The 2R-ANOVA detected significant effects of leptin ($F_{1,12}$=15.69, *P*=0.002), days of exposure ($F_{5,60}$=12.04, *P*=0.0001) and their interaction ($F_{5,60}$=2.89, *P*=0.02) on M_b of the 75% FR LD mice (Fig. 1B). M_b of FR+LEP mice significantly decreased throughout the 3 week acclimation period and at day 20, a 15% decrease was recorded from baseline levels ($F_{5,30}$=39.16, *P*=0.0001), whereas the 75% FR from the *ad libitum* baseline alone did not cause a significant changes in M_b during the acclimation period.

Serum LEP concentration

2R-ANOVA analysis showed significant effects of days of exposure ($F_{2,24}$=5.61, *P*=0.04), leptin administration ($F_{1,12}$=7.17, *P*=0.02) and their interaction ($F_{1,24}$=8.00, *P*=0.002) on serum leptin levels of 75% *ad libitum* restricted mice (Fig. 2B). Food restriction unaccompanied by other treatments significantly (2R-ANOVA: $F_{1,12}$=16.13, *P*=0.002) increased serum leptin concentrations in FR+SL mice (17.44±1.32 pg ml^{-1} g^{-1}) compared with 100% *ad libitum* fed LD mice (14.03±0.59 pg ml^{-1} g^{-1}). Additionally, Pearson's correlation coefficient revealed a strong correlation between serum leptin levels and percentage change in M_b in the FR+SL (*r*=0.77, *N*=7, *P*=0.02) and FR+LEP (*r*=–0.82, *N*=7, *P*=0.01) experimental groups (Fig. 6). Furthermore, a significant correlation between serum leptin and relative M_t values was only established for FR-SL-treated mice (–0.78, *N*=7, *P*=0.01).

Reproductive status

Relative M_t

FR+LEP treatment of LD mice resulted in a significantly (*post hoc* Tukey's test, *P*<0.05) higher mean relative M_t values than those of FR+SL-treated mice (0.24 and 0.16%, respectively) after 3 weeks at 75% *ad libitum* feeding (Fig. 3). Furthermore, 75% *ad libitum* feeding significantly (*post hoc* Tukey's test, *P*<0.05) decreased mean relative M_t values of FR+SL LD mice (0.16%) compared with those calculated for 100% *ad libitum* fed LD mice (0.47%; Fig. 3).

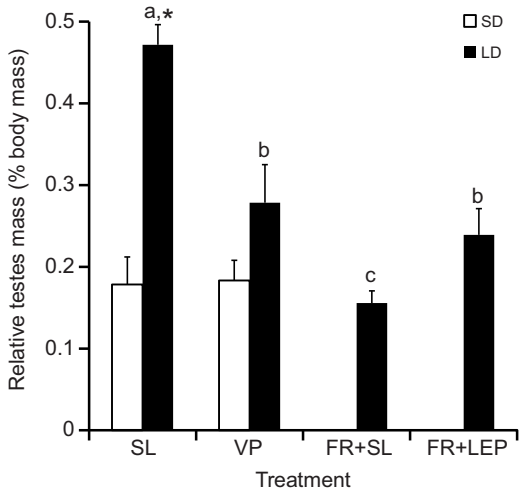

Fig. 5. Relative testes mass (% body mass) in *A. russatus* males. In Experiment 1, mice were kept under either short-day (SD, 8 h:16 h light:dark, lights off at 15:00 h; *N*=7) or long-day (LD, 6 h:8 h, lights off at 23:00 h; *N*=8) photoperiods with a single saline (SL, 50 µg kg^{-1}) or vasopressin (VP, 50 µg kg^{-1}) injection. In Experiment 2, mice were kept under both LD photoperiod and 75% food restriction (FR) from *ad libitum* feeding and received a single saline (5 mg kg^{-1}) or leptin (LEP, 5 mg kg^{-1}) injection (*N*=7). Drugs were injected intraperitoneally (i.p.) at 2 day intervals for 3 weeks. All values are means ± s.e.m. Bars with different letters are significantly different (one-way ANOVA with *post hoc* Tukey's test, *P*<0.05). *Significant difference *versus* SD+SL mice (*t*-test: *t*=–6.85, d.f.=12, *P*=0.0001).

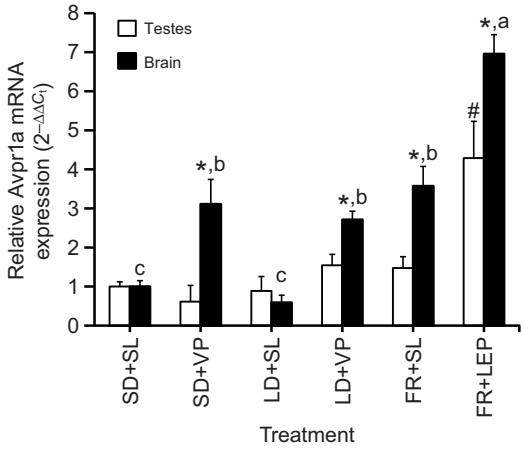

Fig. 6. Differential expression of Avpr1a mRNA in the testes and the brain of *A. russatus* males. In Experiment 1, mice were kept under either SD or LD photoperiods with a single saline (SL, 50 µg kg^{-1}) or vasopressin (VP, 50 µg kg^{-1}) injection. In Experiment 2, mice were kept under both LD photoperiod and 75% food restriction (FR) from *ad libitum* feeding and received a single saline (5 mg kg^{-1}) or leptin (LEP, 5 mg kg^{-1}) injection. All values are means ± s.e.m. of *N*=3–4. Drugs were injected intraperitoneally (i.p.) at 2 day intervals for 3 weeks. $\Delta\Delta C_t$ values were expressed in respect to the housekeeping gene RPLP2 as internal reference and SD+SL samples as external controls. The horizontal gray line represents the control expression level. Bars with different letters are significantly different (one-way ANOVA with *post hoc* Tukey's test, *P*<0.05). *Significant difference *versus* testes (unpaired *t*-test, *P*<0.05); #significant difference *versus* values in testes in the other subgroups (*post hoc* Tukey's test, *P*<0.05).

Serum testosterone concentrations

A 2R-ANOVA showed significant effects of days of exposure ($F_{2,24}$=17.55, *P*=0.0001), leptin administration ($F_{1,12}$=4.76, *P*=0.05) and their interaction ($F_{2,24}$=8.92, *P*=0.001) on serum testosterone levels under 3 weeks of 75% FR feeding (Fig. 4B). Leptin administration to LD mice resulted in a significant (Bonferroni, *P*=0.05) decrease of serum testosterone levels (2.68±0.27 pg ml^{-1} g^{-1}) compared with SL mice (3.37±0.34 pg ml^{-1} g^{-1}). Significant correlations were detected between serum testosterone and leptin levels for FR+SL control mice (*r*=0.62, *N*=7, *P*=0.04). Serum testosterone concentrations of 75% FR+SL LD mice (2.68±0.27 pg ml^{-1} g^{-1}) were significantly (2R-ANOVA: $F_{1,13}$=635.54, *P*=0.0001) lower, with a value of 7.4-fold, compared with control mice under LD conditions that were 100% *ad libitum* fed (Fig. 4A,B).

Avpr1a mRNA expression

Leptin administration in FR LD mice led to 6.96-fold and 4.29-fold increases in hypothalamic and testes Avpr1a mRNA expression, respectively, which are the highest expressions among all experimental groups (Fig. 5).

DISCUSSION

We discovered significant photoperiodic effects on the reproductive status of male desert-adapted golden spiny mice, *A. russatus*, where LD mice had higher M_t and testosterone levels compared with those of SD mice. The increased relative testes mass and the higher serum sex hormone levels detected for LD mice suggest that long photoperiod is an effective environmental signal regulating breeding in *A. russatus* males. Photoperiodic regulation of reproduction has been documented for *A. russatus* and other *Acomys* species at least as an initial cue (Shanas and Haim, 2004; Wube et al., 2008b; Bukovetzky et al., 2012). For LD-acclimated *A. russatus*, testicular histology showed more progress toward spermatogenesis compared with the counterpart SD-acclimated males, while females of *A. russatus* were irresponsive to photoperiod manipulations. However, both sexes of the nocturnal sympatric congener *A. cahirinus* from a Mediterranean population demonstrated similar gonadal activity in responses to SD and LD acclimation, suggesting unresponsiveness to photoperiod (Wube et al., 2008b). Conversely, serum testosterone concentrations in males of *A. cahirinus* were prominently increased in SD-acclimated mice from a desert-adapted population compared with LD mice (Bukovetzky et al., 2012). In another study, SD photoperiod provoked testicular quiescence in four desert-adapted species including *A. cahirinus*, but photoperiodic changes alone failed to induce significant responses in the reproductive status of these species (El-Bakry et al., 1998).

In unpredictable ecosystems such as deserts, photoperiod in combination with a variety of environmental cues may regulate reproduction in small rodent species (El-Bakry et al., 1998; El-Bakry et al., 1999; Medger et al., 2012). For example, water availability is an important regulator of small rodents' reproduction in desert ecosystems (Bronson, 1985; Prakash and Ghosh, 1975). In our study, LD-acclimated male *A. russatus* failed to increase M_t and serum testosterone levels when treated with vasopressin, suggesting that osmotic signals, mediated by vasopressin levels, may block reproductive responsiveness to photoperiod. Our results are consistent with previous findings from our laboratory showing that in female *A. russatus*, increased diet salinity (to induce an osmotic stress) reduced the size of reproductive organs and prompted vaginal closure, suggesting a reproductive hiatus (Shanas and Haim, 2004). Furthermore, previous studies revealed decreased serum testosterone concentrations (Bukovetzky et al., 2012) and impaired spermatogenesis (Wube et al., 2008a) in male *A. russatus*

as a response to vasopressin administration under SD conditions. Several laboratory studies have repeatedly shown that water restriction inhibits reproduction activity in small rodent species (Nelson and Desjardins, 1987; Nelson et al., 1983; Yahr and Kessler, 1975). In the present study, reproductive responsiveness to vasopressin stimulus was most prominent under LD but not SD acclimation. The mechanism by which vasopressin modifies reproductive responsiveness to photoperiod is unknown. However, vasopressin can directly modulate the hypothalamic-pituitary-gonadal axis at the central level as it is a conspicuous neurotransmitter and neuromodulator at the central nervous system, including the hypothalamic vasopressin cell bodies (Kosekova et al., 1993). Thus, vasopressin can modify reproductive activity by directly regulating gonadotropin release at the hypothalamic level. Receptors for vasopressin were identified also on the gonads and therefore vasopressin may have a direct effect on testosterone production.

In our study we used both vasopressin and leptin under different photoperiods to explore the effect of osmotic and nutritional signals on hypothalamic and testes Avpr1a mRNA expression. Our results show that food restriction increased hypothalamic and testes Avpr1a mRNA expression in both saline- and leptin-treated mice compared with control LD mice, with remarkably higher levels in the brain compared with in the testes. Additionally, moderate expression of Avpr1a mRNA was also detected in response to vasopressin administration under LD conditions. Photoperiod alone did not influence Avpr1a mRNA expression either in the brain or in the testes. In rats intracerebroventricular administration of leptin significantly increased vasopressin mRNA expression in the supraoptic nucleus of the hypothalamus (Yamamoto et al., 1999). In another study central administration of leptin to rats, has been shown to activate Avpr1a in the paraventricular nucleus of the hypothalamus to regulate neuroendocrine axes such as the hypothalamus-pituitary-adrenal axis (Morimoto et al., 2000). Accordingly, the significant increase in Avpr1a mRNA expression in the present study, particularly in the hypothalamus of FR mice is likely to be induced by the repeated leptin injections throughout the acclimation period. We suggest that in males of *A. russatus* leptin is involved in the regulation of energy balance during chronic food restriction by central modulation of vasopressin and vasopressin receptor expression. Vasopressin, as a potent hypothalamic neurotransmitter (Kalsbeek et al., 2010), acts *via* variant neuroendocrine axes regulating stress, metabolic and reproductive responses to meet environmental challenges such as food and water deprivations particularly in unpredicted ecosystems.

In the present study, 75% food restriction under LD acclimation significantly decreased M_t and serum testosterone levels, but did not affect serum leptin concentrations compared with mice fed *ad libitum*. Nevertheless, leptin administration to FR *A. russatus* significantly increased testis mass and testosterone levels compared with FR mice under the same photoperiod conditions. Our results are in agreement with those of previous studies that showed remarkable gonadal regression in response to food restriction treatment. In white-footed mice (*Peromyscus leucopus*) and Siberian hamster (*Phodopus sungorus*) kept under LD conditions, 70% food restriction from *ad libitum* feeding resulted in a significant decrease in reproductive organ masses (Young et al., 2000; Zysling et al., 2009). Consistently, the inhibitory effect of food restriction on reproductive activity was also demonstrated in other rodent species under both LD and SD conditions (Edmonds et al., 2003; Nelson et al., 1992; Steinman et al., 2012), including the closely related *A. cahirinus* (Bukovetzky et al., 2012). Reproductive hiatus during unfavorable environmental conditions such as food restriction and water deprivation is expected to play an important role in survival, where energy is allocated to cover maintenance of important homeostatic processes for survival (Hart and Turturro, 1998; Kirkwood, 1992; Shanley and Kirkwood, 2000).

The effect of food restriction on reproductive activity may be mediated by leptin, which is produced by WAT, and the amount of the released hormone to circulation is directly related to the amount of body WAT (Tucholski and Otto-Buczkowska, 2011). Generally, animals use WAT reserves to overcome periods of low food availability and subsequently leptin levels would be decreased as a direct response to the increased consumption of WAT. Thus, the decreased levels of leptin could provide a central regulatory signaling mechanism for modulating neuroendocrine responses, including the hypothalamic-pituitary-gonadal axis (Bates and Myers, 2004; Donato et al., 2011). The results of our study revealed increased testicular mass and serum testosterone concentrations of FR mice following leptin treatment in comparison with untreated FR mice. This result suggests that leptin, at least in males of *A. russatus*, may be directly involved in regulating reproductive responses and thus could serve as an ultimate environmental cue for timing reproduction in unpredictable ecosystems. The molecular mechanism of leptin action remains unclear and further research is required.

In the present study, M_b of *A. russatus* males kept under SD and LD conditions changed significantly over time and with opposite phase. SD mice notably increased their M_b during the course of the experiment while LD mice moderately decreased it. Moreover, FR mice and leptin-treated FR mice considerably decreased their M_b

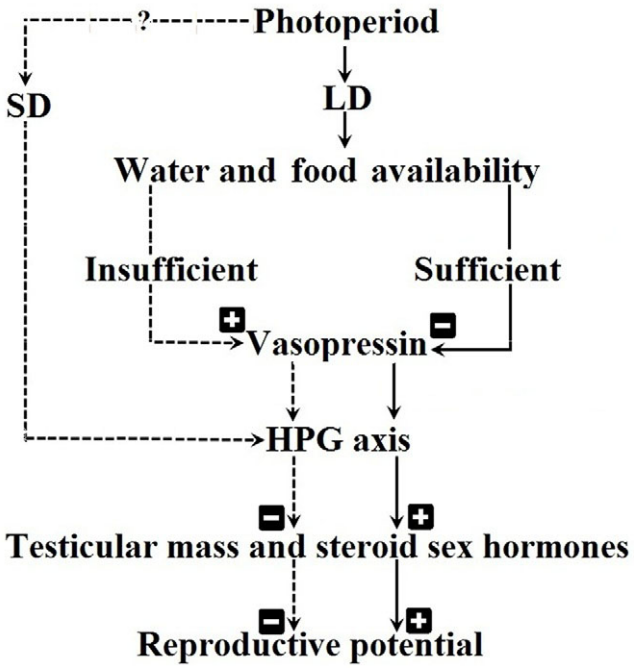

Fig. 7. Schematic model for the modulation of reproductive activity of *A. russatus* males by different environmental variables, expected to be mediated by the hypothalamic-pituitary-gonadal (HPG) axis. General stimulatory and inhibitory pathways are represented by solid and dashed black lines, respectively. When environmental resources are adequate, reproduction presumably is regulated by photoperiod (SD: short day; LD: long day). Conversely, when resources are limited, vasopressin interacts with photoperiod to maximize survival by priming the energetic trade-off between reproduction and basic maintenance costs.

compared with *ad libitum*-fed LD mice, with the most potent effect in leptin-treated mice. The moderate M_b decrease of FR mice is likely related to the low metabolic rate of this species (Haim and Borut, 1981; Rubal et al., 1992) and the remarkable ability of this species to regulate M_b even at 50% FR from baseline feeding. The direct effect of leptin on M_b was also demonstrated in the Djungarian hamster (*Phodopus sungorus*), where exogenous injection of leptin for 10 days decreased the M_b of LD-acclimated hamsters (Klingenspor et al., 2000). The leptin-induced decrease in M_b is suggested to be mediated by inhibition of food intake and stimulation of energy expenditure (Baskin et al., 1999; Bowles and Kopelman, 2001).

In conclusion, the results of our study provide clear support for the effect of photoperiod as an initial environmental cue on male *A. russatus* reproductive status, where the LD photoperiod is more suitable for reproductive timing in this desert-adapted species. Furthermore, we also provide evidence that nutritional and osmotic signals may interact with photoperiod to regulate reproduction in *A. russatus* as ultimate cues for activating the reproductive system (Fig. 7). This finding is of particular ecological interest because it confirms that in unpredictable ecosystems such as the desert, small rodent species utilize other environmental cues that interact with photoperiod to regulate water and energy status with reproduction (Louw and Seely, 1982). This interaction is suggested to be mediated by the hormone leptin, which in turn interacts with central vasopressin pathways to modulate an energetic trade-off between reproduction and the maintenance of homeostasis. Furthermore, leptin and vasopressin signaling could also act separately *via* central or peripheral receptors. The physiological and molecular involvement of leptin and vasopressin in regulating collaborated neuroendocrine responses during periods of deprived resources is not completely understood and further research is needed.

LIST OF SYMBOLS AND ABBREVIATIONS

Avpr1a	arginine vasopressin receptor 1a
C_t	threshold cycle
ETV	estimated testis volume
FR	food restriction
LD	long day
LEP	leptin
SD	short day
VP	vasopressin
WAT	white adipose tissue
M_b	body mass
M_t	relative testis mass

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AUTHOR CONTRIBUTIONS

A.H. developed the study concept and all authors contributed to the study design. Testing and data collection were performed by I.B.-Z. I.B.-Z. and A.E.Z. performed the data analysis and interpretation in consultation with A.H. I.B.-Z. and A.E.Z. drafted the manuscript, and all authors provided critical revisions. All authors approved the final version of the manuscript.

COMPETING INTERESTS

No competing interests declared.

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