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

Israel Ben-Zaken\textsuperscript{a}, Abraham Haim\textsuperscript{a,\textb} and Abed E. Zubidat\textsuperscript{a}

\textsuperscript{a}Department of Evolution and Environmental Biology, University of Haifa, Mount Carmel, Haifa 31905, Israel, \textsuperscript{b}The Israeli Center for Interdisciplinary Research in Chronobiology, University of Haifa, Mount Carmel, Haifa 31905, Israel

Abstract

We tested the effects of photoperiod, water and food availability on body-mass, reproductive status, and vasopressin mRNA receptor 1a (Avpr1a) expression in males of desert-adapted golden spiny mice, \textit{Acomys russatus}. In experiment 1, Males were acclimated to short-day (SD; 16D:8L) or long-day (LD; 8D:16L) with either saline (control) or vasopressin treatment for three weeks. The results of this experiment revealed that under control conditions, SD-mice increased body-mass by \textasciitilde5\% while LD-mice decreased it by \textasciitilde4\%. SD had no effect on reproductive status and leptin levels, whereas LD-males increased testes mass and serum testosterone, but had no effect on leptin levels. Vasopressin administration decreased LD-induced reproductive enhancement. Since 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 from \textit{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 more potent effect in leptin-treated males showing enhanced reproductive status and significant increase in serum leptin compared with controls. Avpr1a expression was significantly up-regulated in LD, vasopressin, and food restricted males, with higher hypothalamic levels compared with testes. We conclude that in \textit{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 unpredicted ecosystems as extreme deserts.
Key words
Desert-adapted; Leptin; Testosterone; Water and food availability; Vasopressin receptor mRNA, photoperiod

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), water and food availability are utilized by these species as predictive cues to reproductive timing (Bronson, 1989; 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 ecological changes can certainly endanger reproduction and offspring prosperity, as these harsh environments are characterized generally by unpredicted climate conditions, food and water availability (Noy-Meir, 1973; Bronson, 1985; 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, is a small desert rodent inhibiting arid regions of Egypt, Israel, Jordan, and Arabian Peninsula (Mendelsson and Yom-Tov, 1999). This species is highly adapted to survive with limited supply of water from its food; mainly invertebrates (Kronfeld 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 as in the spring and summer seasons (Mendelsson and Yom-Tov, 1999). Photoperiod manipulations have been shown to modulate reproductive responses in males of A. russatus (Wube et al., 2008a) and 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 females (Shanas and Haim, 2004) and males (Wube et al., 2008b) A. russatus, an increase in salinity stress of the water source can reduce body mass (Wb) 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 (VP). 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. VP 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, VP is also directly released into the brain acting as a neurotransmitter for social behavior or, stress responses (Landgraf et al., 1998). Although there is no direct evidence of distinct reproductive role of VP, the hormone has been broadly reported to be involved in regulating supportive reproductive processes including pair bond formation in male prairie vole, *Microtus 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 human and non-human animals (Wathes, 1984). Recently, notable TSTO decrease (Bukovezky et al., 2012) and spermatogenesis suppression (Wube et al., 2008a) following VP treatment were also specifically observed in *A. russatus* males and in desert adapted populations of *A. cahirinus* respectively. Nevertheless, studies on the involvement of VP 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 (FR) can confine several reproductive processes including gonadotropins and gonadal hormone secretions, spermatogenesis, and reproductive organs 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 energetic-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 (LEP) is the most prominent mediator of food intake and energy expenditure (Tucholski and Otto-Buczkowska, 2011). LEP is primarily synthesized by the adipocytes within white adipose tissues (WAT) and levels of circulating hormone largely correlate with total WAT mass (Maffei et al., 1995). High levels of LEP stimulate catabolic responses that reduce energy intake and increase energy expenditure resulting in body mass (Wb) loss (Baskin et
al., 1999). Conversely, low levels of LEP stimulate anabolic responses that increase energy intake and reduce energy expenditure resulting in $W_b$ gain (Anubhuti, 2008). Several lines of direct and indirect evidence support the notion that LEP is a potent modulator of reproductive responses in mammals (Clark and Henry, 1999; Messinis and Milingos, 1999; Zieba et al., 2005; French et al., 2009). LEP 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 LEP 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 LEP levels and both relative testis size and TSTO concentrations in SD-acclimated mice (Bukovetzky et al., 2012). The metabolic effects of LEP are mediated by receptors in the hypothalamus and testis; therefore changes in the LEP signal during food deprivation could interact directly with these receptors to modulate reproductive activity (Clarke and Henry, 1999; Moschos et al., 2002).

Although the role of photoperiod, water and food availability in modulating reproductive responses is 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 as deserts. The objective of this study is to explore the relations between photoperiod, VP, LEP, and FR in regulating $W_b$ and reproductive status of $A. russatus$ males. Testis proportions (expressed as percentage of $W_b$) and serum TSTO 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 VP treatment and monitored changes in $W_b$, reproductive status, and VP mRNA receptor 1a (Avpr1a) expression in brain and testes tissues (Experiment 1). Afterwards, we evaluated the effects of LD photoperiod combined with FR and LEP treatments on $W_b$, reproductive status, and Avpr1a expression in brain and testes tissues of $A. russatus$ males (Experiment 2). We chose to only evaluate the effect of FR and LEP treatments under LD photoperiod, as results of experiment 1 showed that LD photoperiod is required to initiate reproductive activity in $A. russatus$ males as was demonstrated for the counterpart LD photoperiod.

We hypothesized that “if VP, LEP, and FR act as proximate cues for uncomplimentary water and food availability, then decreases in $W_b$ 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 forty eight adult male golden spiny mice (A. russatus) obtained from our wild-type established 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 capricious annual precipitations (Jaffe, 1988). Before experiments, animals were maintained in a photoperiod regimen of 12L:12D (Light:Dark cycle, lights on between 08:00 – 20:00 h) and fed ad libitum. Experiments were conducted in climatic cabinet (MEDITEST 1300 liters; Meyzieu, France) with ambient temperature of 31±1 °C. Mice were separately housed in transparent plastic cages (13 X 13.5 X 40 cm) using saw dust as bedding and rat pellets (Koffolk Ltd., 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 source of water were provided ad libitum, unless otherwise stated. Mice were randomly assigned to one of two photoperiod schedules: short-day (SD; 8L:16D, lights on between 07:00 – 15:00 h) and long-day (LD; 16L:8D, lights on between 07:00 - 2300 h). In experiment 1, mice were acclimated under the two photoperiod schedules for three weeks and thereafter, were further arbitrarily allocated into four subgroups of 7 - 8 mice each:

1) SD-groups: SD-acclimated mice were intraperitoneally (i.p.) injected once every other day for three weeks with either 0.5 ml 0.9% saline (control group; SD+SL) or 0.5 ml vasopressin (VP; Sigma) at a dose of 50 μg·kg⁻¹ per Wb (experimental group; SD+VP).

2) LD-groups: LD–acclimated mice were i.p. injected with either saline (LD+SL) or VP (LD+VP) at the same does and frequency as administered to SD groups.

In experiment 2, mice were acclimated under LD photoperiod with food restriction (FR-groups). LD-acclimated mice were offered 75% of their original ad libitum food intake estimated by the equation \( Y = 0.002W_b + 0.382 \) (1), whereas \( Y \) represents ad libitum food intake (Wube et al., 2008a) and i.p. injected with 0.5 ml 0.9% saline (FR+SL) or 0.5 ml leptin (LEP; Sigma) at a dose of 5 mg·kg⁻¹ per Wb (FR+LEP). Injections were administered once every other day for three weeks.
Experimental procedures

Changes in \( W_b \). Mice under SD-, LD-, and FR-groups were weighed at day 0 to establish their baseline \( W_b \) and then their \( W_b \)s were monitored at days 8 and 20 in SD- and LD-groups and at days 4, 8, 12, 16, and 20 in FR-groups. Percent change in \( W_b \) was calculated individually as the difference between measurements at a given day and baseline \( W_b \) value, measured using semi-analytical scale (±0.01g; Sartorius: 1907 MP-8; 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 1 cc heparinized syringe with 22 gauge needle at days 0, 8, and 20. Blood samples were analyzed for serum TSTO and LEP levels. Serum was separated from all blood samples after centrifugation at 12,000 rpm for 10 min and frozen at -70 °C for later hormonal analysis. No deaths were recorded due to the use of this procedure.

Hormonal analysis: Serum hormonal analyses were performed using commercial ELISA kits for Mouse LEP (Quantikine, #MOB00, R&D Systems Inc.; Minneapolis, USA) and Mouse TSTO (#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 LEP 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 TSTO, respectively.

Mice sacrificing: At the end of three treatment weeks all mice were sacrificed, three hours after light onset (10:00 h – 12:00 h) using anesthesia and thereafter decapitation. Left testis and brain were separated from all mice. Testes were washed, cleaned from external fat excesses, weighed, and their length and width were measured. Testes and hypothalamus removed from all brains were frozen at -70 °C for VP mRNA receptor 1a (Avpr1a) expression analysis.

Relative testis mass (\( W_t \)) and estimated testis volume (ETV): Relative \( W_t \) as calculated as a percentage of \( W_b \) and was used as an indicator for reproductive status (Møller, 1989). Furthermore, the reported linear correlation between ETV and \( W_t \) 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 diminution equation, \( ETV = W^2 \times L \times 0.523 \) (2), where \( W \) and \( L \) are the wide and length of the testis, respectively.

*Hypothalamic and testicular Avpr1a expression*: RNA was extracted from hypothalamus and left testis of all mice using the RNeasy Mini Kit (Qiagen; Hilden, Germany) according to the manufacturer’s instructions. 0.4 \( \mu \)g RNA samples were subsequently reverse transcribed using EZ-First Strand cDNA Synthesis Kit for RT-PCR according to the manufacturer’s instructions (Biological Industries; Kibbut Be’er Haemek, Israel). cDNA was amplified in Verso cDNA kit (Thermo scientific; Waltham, 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; Hercules, USA). As a reference gene the housekeeping gene ribosomal protein large, P2 (RPLP2) was used as endogenous control by the primers pairs: RPLP2 – Forward 5’- CGT CGC CTC CTA CTG-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, USA). The experimental samples and the reference controls were amplified in duplicates 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 ten times fluorescence level above the mean standard deviation of background levels in all reaction well. The \( C_t \) values of Avpr1a were normalized against the endogenous expression of 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: \( \text{Fold change} = 2^{-\Delta \Delta C_t} \) (3).

**Statistical analysis**

Data are presented as means and one standard error (s.e.m.) of the means. In experiment 1, the statistical effects of photoperiod, VP, or LEP on \( W_b \) and reproductive status were evaluated by three-way repeated-measure ANOVA (3R-ANOVA) with photoperiod (SD vs LD) and treatment (VP vs saline,) as between-subject factor and days of exposure (days: 0, 8, and 20) as the within-subject factor. In experiment 2, the effects of LEP were analyzed using two-way repeated-measure ANOVA (2R-ANOVA) with treatment (LEP vs saline) as
between-subject factor and days of exposure as the within-subject factor. The effects of photoperiod and treatment on percentage \( W_t \) and \( \text{Avpr1a} \) mRNA expression were analyzed using three-way ANOVA (3-ANOVA) with photoperiod (SD vs LD), drug administrations' (SL, VP, LEP), and food availability (\textit{ad libitum} vs FR) as the within-subject factors. Two and one-way repeated-measure 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 FR alone (\textit{ad libitum} LD+SL-mice vs 75% FR LD+SL-mice) on the dependent factors was evaluated by one-way ANOVA (1-ANOVA). The ANOVA models were followed by Bonferroni for repeated-measure or Tukey post-hoc comparison for mean effect factor where appropriate. Paired mean differences between treatments and control at a given exposure day were computed using student’s \( t \)-test. Finally, Person correlation coefficient (\( r \)) was utilized to assess statistical relationship between serum LEP, serum TSTO, \( W_b \), \( M_t \), and ETV. All statistical analyses were conducted using SPSS software 13.0 for windows (SPSS Inc., Chicago, Illinois, 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 VP on \( W_b \), serum LEP, reproductive status, and \( \text{Avpr1a} \) mRNA expression**

\( W_b \): There were significant effects of photoperiod (\( F_{1,26}=6.83, \ P=0.015 \)), VP administration (\( F_{1,26}=12.67, \ P=0.001 \)) on percentage change of \( W_b \) (Fig. 1A). No significant photoperiod X VP interaction effects were detected by the 3R-ANOVA model. Photoperiod conditions combined with saline alone have significant effects on \( W_b \) (\( F_{1,13}=6.15, \ P=0.03 \)). At day 20, mean \( W_b \) values of LD-treated mice were higher by \( \sim 9\% \) compared with SD-treated mice (54.48±3.23 g; Fig. 1A). \( W_b \) decreased significantly as a result of VP 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 \( W_b \) of LD+VP mice at day 20 was -7.6% and this decrease was about 3-fold higher than that of SD+VP mice (-2.5%).

**Serum LEP concentrations:** There were no significant effects of photoperiod (\( F_{1,26}=0.37, \ P=0.55 \)), VP (\( F_{1,26}=0.46, \ P=0.51 \)), and photoperiod X VP interaction effects (\( F_{1,26}=1.37, \ P=0.25 \)) on serum LEP levels (Fig. 2A).
Reproductive status

Relative Wt: A significant strong positive correlation between ETV and Wt was revealed (r = 0.87, N = 42, P = 0.0001) for A. russatus within all experimental subgroups. A 3-ANOVA yield significant main effect of photoperiod, VP administrations, and food availability for mean relative Wt (F7,43=16.4, P=0.0001). A significant interaction with VP was present only for photoperiod (F1,43=16.4, P=0.003; Fig. 3).

Serum TSTO concentrations: A 3R-ANOVA established significant effects for photoperiod (F1,26=58.96, P=0.0001), VP treatment (F1,26=40.89, P=0.0001), and photoperiod X VP interaction effects (F1,26=11.64, P=0.002) on serum TSTO levels (Fig. 4A). No significant effects were detected for days at treatment (F2,52=1.63, P=0.21). A 2R-ANOVA detected significant effects for days at treatment (F2,28=23.49, P=0.0001), VP (F1,14=62.56, P=0.0001), and days X VP interactions (F2,28=9.26, P=0.001) in LD-mice. VP administration significantly decreased mean serum TSTO 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 for days at treatment (F2,24=6.09, P=0.01), but neither VP nor days X VP interactions on serum TSTO levels were detected by the general 2R-ANOVA model.

Avpr1a mRNA expression: A 3-ANOVA detected significant main effects of VP administrations and food availability but not photoperiod on Avpr1a mRNA expression in the hypothalamus (F5,18=0.34, P=0.0001). No significant interaction effects were detected for all factors tested (Fig. 5). VP administration resulted in a about 3-fold increase in hypothalamic Avpr1a mRNA expression under both SD and LD photoperiods compared with controls. In testes, main significant effect was detected for VP administrations, but not for photoperiod and food availability (3-ANOVA, F5,13=7.05, P=0.002).

Experiment 2: Effect of LEP on Wb, serum LEP, reproductive status, and Avpr1a mRNA expression

Wb: The 2R-ANOVA detected significant effects of LEP (F1,12=15.69, P=0.002), days at treatment (F5,60=12.04, P=0.0001), and significant LEP X days interaction effects (F5,60=2.89, P=0.02) on Wb of the 75% FR LD-mice (Fig. 1B). Wb of FR+LEP mice significantly decreased throughout the three weeks acclimation period and at day 20, a 15% decrease was recorded from baseline levels (F5,30=39.16, P=0.0001), whereas the 75% FR from ad libitum baseline alone did not cause a significant changes in Wb during the acclimation period.
Serum LEP concentration: 2R-ANOVA analysis showed significant effects of days at treatment (F_{2,24}=5.61, P=0.04), LEP administration (F_{1,12}=7.17, P=0.02), and days X LEP interactions (F_{1,24}=8.00, P=0.002) on serum LEP levels of 75% ad libitum restricted-mice (Fig. 2B). FR unaccompanied with other treatment, significantly (2R-ANOVA: F_{1,12}=16.13, P=0.002) increased serum LEP 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 coefficient revealed strong correlation between serum LEP levels and percentage change in W\(_b\) in FR+SL (r = 0.77, N = 7, P = 0.02) and in FR+LEP (r = -0.82, N = 7, P = 0.01) experimental groups (Fig. 6). Furthermore, a significant correlation between serum LEP and relative W\(_t\) values was only established for FR-SL treated mice (-0.78, N = 7, P = 0.01).

Reproductive status
Relative W\(_t\): FR+LEP treatment to LD-mice resulted in a significantly (Tukey post-hoc, P<0.05) higher mean relative W\(_t\) values than those of FR+SL-treated mice (0.24% and 0.16%, respectively) after three weeks at 75% ad libitum feeding (Fig. 3). Furthermore, 75% ad libitum feeding significantly (Tukey post-hoc, P<0.05) decreased mean relative W\(_t\) values of FR+SL LD-mice (0.16%) compared with those calculated for 100% ad libitum fed LD-mice (0.47%; Fig. 3).

Serum TSTO concentrations: A 2R-ANOVA showed significant effects of days at treatment (F_{2,24}=17.55, P=0.0001), LEP administration (F_{1,12}=4.76, P=0.05), and days X LEP interaction effects (F_{2,24}=8.92, P=0.001) on serum TSTO levels under three weeks of 75% FR feeding (Fig. 4B). LEP administration to LD-mice resulted in a significant (Bonferroni, P = 0.05) decrease of serum TSTO 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 TSTO and LEP levels for FR+SL control mice (r = 0.62, N = 7, P = 0.04). Serum TSTO 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 folds, compared with control mice under LD-conditions that were 100% ad libitum fed (Fig. 4A&B).

Avpr1a mRNA expression: LEP administration in FR LD-mice led to 6.96-fold and 4.29-fold increase 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 reproductive status of desert adapted golden spiny male mice *A. russatus* where, LD-mice had higher $W_t$ and TSTO 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 spermatogenesis compared with counterpart SD-acclimated males while female of *A. russatus* were irresponsive to photoperiod manipulations. However, in 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 TSTO 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 males of *A. russatus* failed to increase $W_t$ and serum TSTO levels when treated with VP, suggesting that osmotic signals, mediated by VP levels, may block reproductive responsiveness to photoperiod. Our results are in consistence with previous findings from our laboratory showing that in females of *A. russatus* increased diet salinity (to induce an osmotic stress) reduced reproductive organs and prompted vaginal closure, suggesting reproductive hiatus (Shanas and Haim, 2004). Furthermore, previous studies revealed decreased serum TSTO concentrations (Bukovetzky et al., 2012) and impaired spermatogenesis (Wube et al., 2008a) in males of *A. russatus* as a response to VP administration under SD conditions, respectively. 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 VP stimulus was most prominent under LD, but not SD-acclimation. The mechanism by which VP modifies reproductive responsiveness to photoperiod is unknown. However, VP can directly modulate the hypothalamic-pituitary-gonadal axis at the central level as it is a conspicuous neurotransmitter and neuromodulator at the central nerves system, including the hypothalamic VP cell bodies (Kosekova et al., 1993). Thus, VP can modify reproductive activity by directly regulating gonadotropin release at the hypothalamic level. Receptors for VP were identified also on the gonads and therefore it may have a direct effect on TESTO production.

In our study we used both VP and LEP under different photoperiods to explore the effect of osmotic and nutritional signals on hypothalamic and testes Avpr1a mRNA expression. Our results show that FR increased hypothalamic and testes Avpr1a mRNA expression in both saline and LEP-treated mice compared with controlled LD-mice, with remarkable higher levels in the brain compared with those of the testes. Additionally, moderate expression of Avpr1a mRNA was also detected in response to VP administration under LD conditions. Photoperiod alone did not influence Avpr1a mRNA expression neither in the brain nor in the testes. In rats intracerebroventricular administration of LEP significantly increased VP mRNA expression in the supraoptic nucleus of the hypothalamus (Yamamoto et al., 1999). In another study central administration of LEP 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 LEP injections throughout the acclimation period. We suggest that in males of *A. russatus* LEP is involved in the regulation of energy balance during chronic FR by central modulation of VP and VP receptors expression. VP, 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% FR under LD-acclimation, significantly decreased \( W_t \) and serum TSTO levels, but did not affect serum LEP concentrations compared with *ad libitum*-fed mice. Nevertheless, LEP administration to FR *A. russatus* significantly increased testis mass and TSTO 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 FR treatment. In white-footed mice (*Peromyscus leucopus*) and Siberian hamster
(Phodopus sungorus) kept under LD, 70% FR 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 FR on reproductive activity was also demonstrated in other rodent species both under 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 FR 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 FR on reproductive activity may be mediated by LEP that is produced by white adipose tissues (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 LEP levels would be decreased as a direct response to the increased consumption of WAT. Thus, the decreased levels of LEP 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 TSTO concentrations of FR-mice following LEP treatment in comparison with untreated FR-mice. This result suggest that LEP, at least in males of A. russatus, may be directly involved in regulating reproductive responses and thus could serve as ultimate environmental cue for timing reproduction in unpredicted ecosystems. The molecular mechanism of LEP action remains unclear and further research is required.

In the present study, Wb of A. russatus males kept under SD and LD-acclimations, changed significantly over time and with opposite phase. SD-mice notable increased their Wb during the course of the experiment while LD-mice moderately decreased it. Moreover, FR-mice and LEP-treated FR-mice considerably decreased their Wb compared with ad libitum-fed LD-mice, with most potent effect in LEP-treated mice. The moderate Wb decrease of FR-mice is likely related to the low metabolic rates of this species (Haim and Borut 1981; Rubal et al., 1992) and the remarkable ability of this species to regulate Wb even at 50% FR from baseline feeding. The direct effect of LEP on Wb was also demonstrated in the Djunarian hamster (Phodopus sungorus), where exogenous injection of LEP for 10 days decreased Wb of LD-acclimated hamsters (Klingenspor et al., 2000). The LEP-induced decrease in Wb 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 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 unpredicted ecosystems such as desert, small rodent species utilize other environmental cues that interact with photoperiod to regulate status of water and energy with reproduction (Louw and Seely, 1982). This interaction is suggested to be mediated by the hormone LEP, which in turn interacts with central VP pathways to modulate energetic trade-off between reproduction and homeostasis maintenance. Furthermore, LEP and VP signaling could also act separately *via* central or peripheral receptors. The physiological and molecular involvement of LEP and VP 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**: Vasopressin mRNA receptor 1a
- **ETV**: Estimated testis volume
- **FR**: Food restriction
- **LD**: Long-day
- **LEP**: Leptin
- **SD**: Short-day
- **TSTO**: Testosterone
- **VP**: Vasopressin
- **WAT**: Adipose tissues
- **W_b**: Body mass
- **W_t**: Relative testis mass

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References


Figure legends

Figure 1.
Percentage of changes in body mass from baseline of *A. russatus* males A) Effect of either short-day (SD, Light:Dark 8L:16D, lights off at 1500 h; N = 7) or long-day (LD, 16L:8D, lights off at 2300 h; N = 8) photoperiods with a single saline (SL, 50 μg/kg) or vasopressin (VP, 50 μg/kg) intraperitoneally (*i.p.*) injection at two days intervals for three weeks. *- significant difference from VP experiment at the same photoperiod (P < 0.05, unpaired *t*-test). B) Effect of a single saline (SL, 5 mg/kg) or leptin (LEP, 5 mg/kg) *i.p.* injection at two days intervals for three 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 vs. LEP (P < 0.05, unpaired *t*-test).

Figure 2.
Serum leptin concentrations of *A. russatus*, males A) Effect of either short-day (SD, Light:Dark 8L:16D, lights off at 1500 h; N = 7) or long-day (LD, 16L:8D, lights off at 2300 h; N = 8) photoperiods with a single saline (SL, 50 μg/kg) or vasopressin (VP, 50 μg/kg) intraperitoneally (*i.p.*) injection at two days intervals for three weeks. B) Effect of a single saline (SL, 5 mg/kg) or leptin (LEP, 5 mg/kg) *i.p.* injection at two days intervals for three 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 vs. SL and + significant differences vs LD+SL (A) at the same day (P < 0.05, unpaired *t*-test).
Figure 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) or leptin (5 mg/kg). Values are means ± s.e.m. of N = 7. P, the probability value of the Pearson's correlation coefficient (R).

Figure 4.
Relative testes mass from body mass in *A. russatus* males. In experiment 1, mice were kept under either short-day (SD, Light:Dark 8L:16D, lights off at 1500 h; N = 7) or long-day (LD, 16L:8D, lights off at 2300 h; N = 8) photoperiods with a single saline (SL, 50 μg/kg) or vasopressin (VP, 50 μg/kg). In experiment 2, mice were kept under both LD photoperiod and 75% food restriction (FR) from *ad libitum* feeding and received a single saline (SL, 5 mg/kg) or leptin (LEP, 5 mg/kg) injection (N = 7). Drugs were injected intraperitoneally (i.p.) at two days intervals for three weeks. All values are means ± s.e.m. Bars with different letters are significantly (p<0.05) different as revealed by 1-ANOVA with Tukey post hoc test. *- significant difference vs SD+SL mice (t-test: t=-6.85, d.f.=12, P=0.0001).

Figure 5.
Serum testosterone concentrations in *A. russatus* males. A) Effect of either short-day (SD, Light:Dark 8L:16D, lights off at 1500 h; N = 7) or long-day (LD, 16L:8D, lights off at 2300 h; N = 8) photoperiods with a single saline (SL, 50 μg/kg) or vasopressin (VP, 50 μg/kg) intraperitoneally (i.p.) injection at two days intervals for three weeks. *- significant difference from LD+VP mice and #- significant difference from counterpart SD-mice (P < 0.05 unpaired t-test). B) Effect of a single saline (SL, 5 mg/kg) or leptin (LEP, 5 mg/kg) i.p. injection at two days intervals for three 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 and +- significant differences from LD+SL (A) at the same day (P < 0.05, unpaired t-test).

Figure 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) or vasopressin (VP, 50 μg/kg). In experiment 2, mice were kept under both LD photoperiod and 75% food restriction (FR) from *ad libitum* feeding and received a single saline (SL, 5 mg/kg) or leptin (LEP, 5 mg/kg) injection. All values are Means ± s.e.m. of N =
3–4. Drugs were injected intraperitoneally (i.p.) at two days intervals for three weeks. ΔΔCt were expressed in respect to the house-keeping gene RPLP2 as internal reference and SD+SL samples as external controls. The dashed-gray line represents the control expression level. Bars with different letters are significantly (p<0.05) different as revealed by 1-ANOVA with Tukey post hoc test.*- significant difference vs. testes (P < 0.05, unpaired t-test) and #-significant difference vs. values detected in testes (Tukey post-hoc, P < 0.05.).

Figure 7.
Schematic model for the modulation of reproduction 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 at premium, reproduction presumably is regulated by photoperiod (SD: short-days; LD: long-days). Conversely, when resources are limited vasopressin interacts with photoperiod to maximize survival by priming energetic trade-off between reproduction and basically maintenance costs.
A

Serum leptin levels (pg·ml⁻¹·g⁻¹)

Days post injection

0  8  20

B

Serum leptin levels (pg·ml⁻¹·g⁻¹)

Days post injection at 75% ad libitum feeding

0  8  20
The graph shows the relationship between serum leptin levels (pg·mL⁻¹·g⁻¹) and body mass (% from ad libitum feeding) for two groups: Food restricted + Saline and Food restricted + Leptin.

For Food restricted + Saline:
- Equation: \( Y = -2.52X - 7.06 \)
- Correlation coefficient: \( R = -0.82 \)
- Sample size: \( N = 7 \)
- P-value: \( P = 0.01 \)

For Food restricted + Leptin:
- Equation: \( Y = 0.346X + 17.10 \)
- Correlation coefficient: \( R = 0.77 \)
- P-value: \( P = 0.02 \)
- Sample size: \( N = 7 \)