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Predator threat, copulation effort and immunity in male rats (*Rattus norvegicus*)

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Abstract

Parental investment theory predicts that organisms should increase their current reproductive investments when they perceive a threat to future reproduction. In such a situation, animals are expected to pay the cost of reproduction by committing a terminal reproductive effort (TRE). We aimed to induce a TRE in male Wistar rats by exposing experimental animals to predator (cat) odour, while the control group was exposed to the odour of rabbit. As response variables, we measured the parameters of male copulatory activity (copulation effort) and resistance to the experimental infection with a neurotropic nematode, *Toxocara canis*. Contrary to our expectations, control animals received a greater average number of ejaculations in *ad libitum* copulation trials than predator-exposed animals. Other parameters of copulatory activity (intromission and ejaculation latencies, numbers of mounts and intromissions, ejaculation efficiency, duration of post-ejaculatory interval) did not differ between treatment groups. Exposure to predator odour resulted in growth retardation and immune suppression as revealed by the elevated urinary corticosterone levels and higher parasite counts in brain and muscle tissues as compared with control animals. We failed to detect any immunological cost of increased copulatory activity as no significant correlations between measures of copulatory activity and immune parameters could be found. Individual mount number correlated positively with urine creatinine concentration (an index of muscular activity), suggesting that this aspect of male copulatory behaviour is energetically most demanding. Our experiment thus failed to support the hypotheses that exposure to predator odour increases copulatory activity in male rats and that intense copulatory activity leads to immune suppression. The hypothesis that predator odour exposure leads to immune suppression and reduced infection resistance was supported.

Introduction

The basic premise of life-history theory is that for iteroparous organisms, future reproduction is traded off against the current one (Williams, 1966; Roff, 1992; Stearns, 1992). It follows that individuals are anticipated to pay reproductive costs when current reproduction is likely to make a greater contribution to fitness than expected future reproduction. (Gadgil & Bossert, 1970; Trivers, 1972; Pianka & Parker, 1975; Clutton-Brock, 1984; Pärt, Gustafsson & Moreno, 1992). Assuming that the same resources can be allocated for maintenance and reproduction, commitment of such terminal reproductive effort (TRE) should be evidenced by increased shunting of maintenance resources to reproduction in situations when individuals perceive a threat to their future reproduction.

Although the TRE is an elementary concept for the life-history theory, the empirical evidence for this phenomenon is still fragmentary. Most of the experimental evidence for increased reproductive investment in response to reduction in residual reproductive value originates from studies of invertebrates (e.g. Minchella & Loverde, 1981; Geller, 1990; Roitberg *et al*., 1993; Polak & Starmer, 1998; Adamo, 1999; Agnew, Koella & Michalakis, 2000; McCurdy, Forbes, & Boates, 2000; Javoiš & Tammaru, 2004). Correlative studies in vertebrates have documented increased maternal investment in aged red deer *Cervus elephus* (Clutton-Brock, 1984), Californian gulls *Larus californicus* (Pugesek, 1981) and collared flycatchers *Ficedula albicollis* (Pärt *et al*., 1992). An experimental study in sticklebacks *Gasterosteus aculeatus* has suggested that males in poor condition invest more in current reproduction (by sexual signalling) than males in good condition (Candolin, 1999), while female lizards *Uta stansburiana* produced heavier offspring following autotomal tail loss associated with reduced survival probability (Fox & McCoy, 2000).

Currently, it is widely assumed that individual's reproductive investments can be traded off against its ability to

defend itself against pathogens and parasites (reviewed in Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000; Zuk & Stoehr, 2002). Hence, it can be hypothesized that in animals committing TRE, increased reproductive effort is achieved at the cost of increased susceptibility to infections. The aim of our study is to test this idea in the model of copulatory behaviour in male laboratory rats. The male rat's sexual behaviour constitutes a highly ordered sequence of motor acts involving both striate and smooth muscles (Ågmo, 1997). When exposed to a receptive female, a male rat vigorously mounts, intromits and ejaculates (usually five to eight times) for up to 4 h. Thereafter, it usually reaches a state of sexual exhaustion and loses an interest in the female. However, a male rat that has reached sexual satiety can be induced to copulate again if the initial female is replaced with a novel receptive female (the so-called Coolidge effect; reviewed in Ågmo, 1999; Rodríguez-Manzo, 1999). We hypothesized that continuous copulation effort would lead to depletion of maintenance resources and immunosuppression in the male rat (1) because of the energetic costs of copulatory activities and (2) the continuous exposure to increased levels of potentially immunosuppressive testosterone, required for sperm production.

In order to trigger a TRE (manipulate male motivation to invest in copulation), we applied the predator odour exposure paradigm, widely used for application and assessment of psychological stress in laboratory rats (reviewed by Blanchard *et al*., 1998; Williams, 1999; Dielenberg & McGregor, 2001). Despite their long history in captivity, laboratory rats have not lost the fear of predator odours. Exposing them to the collar worn by domestic cats results in behaviours and reactions typical to situations of fear, anxiety and stress. We assumed that exposure of rats to cat odour would mimic a natural situation of predator threat, which should be perceived as a cue for reduced residual reproductive value. Thus, male rats exposed to predator odour were expected to increase their investment in copulatory behaviour as compared with control males exposed to neutral odour.

In order to assess the possible immunosuppressive effects of increased copulatory investment, we experimentally infected animals after copulation trial with a neurotropic nematode *Toxocara canis*. *Toxocara canis* is an ascarid parasite of carnivores, using rodents as paratenic hosts. Subsequent to oral infection with embryonated eggs, infective larvae (L_3; Brunanska, Dubinsky & Reiterova, 1995) of *T. canis* migrate through host tissues and contribute to pathology. Neurotropic larvae accumulate in the rodent brain 14 days after infection and presumably make the paratenic host vulnerable to predation for the final carnivorous host. Hence, this parasite should be suitable for detection of the fitness consequences of immune suppression in rodent hosts because the fitness of the parasite depends on (1) its ability to survive the attacks of host's immune system and (2) its ability to debilitate host behaviour and turn it into vulnerable prey (e.g. Cox & Holland, 1998; Cuéllar *et al*., 2001).

Furthermore, the effect of psychological stress upon infection success of *T. canis* deserves attention in the context of psychoneuroimmunology, where the model pathogens have predominantly involved viral and bacterial (see Moynihan & Ader, 1996) or protozoan (Barnard, Behnke & Sewell, 1996) parasites. Additionally, such information is relevant in the context of research in immunoecology where the scarcity of knowledge about relationships between host immunocompetence and actual parasite resistance has been recognized for some time (e.g. Braude, Tang-Martinez & Taylor, 1999; Owens & Wilson, 1999; Buchanan, 2000; Read & Allen, 2000; Ryder, 2003). We therefore also measured indirect indices of metabolic activity, stress and immune system activation, such as the urine creatinine and corticosterone concentration, leucocyte differentials and growth patterns, and examined their variation in relation to predator stress treatment and parameters of male copulatory activity. Examination of such correlations has a potential to contribute to the understanding of the nature of the possible costs associated with different aspects of copulatory activity. So far, the research on costs of copulation for males has been almost exclusively limited to invertebrates (see Telford & Webb, 1998 for a review), and only two studies (Kress *et al*., 1989; Ostrowski *et al*., 1989) have addressed these issues in mammals.

Our study thus aimed to explain whether exposure to predatory cues induces the following: (1) increased copulatory activity and (2) immune suppression in male rats; (3) whether intense copulatory activity is associated with immune suppression and (4) which aspects of copulatory activity are most costly in terms of energy expenditure and/or immune suppression.

Methods

Rat maintenance and screening tests

Thirty male and 12 ovariectomized female outbred Wistar (Brl:Wist@Mol) rats were obtained from Taconic M & B, Ejby, Denmark, at the age of 8 weeks on 25 September 2002. All rats were housed together in a separate room under standard vivarium conditions with 12-h light/12-h dark periods (lights off at 08:00 h) at a temperature of $21 \pm 2\,^{\circ}C$. Rats were housed in polypropylene cages (type 80-134G006) with 1820 cm^2 floor area and external dimensions of $59.5 \times 38 \times 20$ (height) cm, covered with wire lid, and bedded with aspen shavings. Animals were housed in groups of five (males) or six (females). Tap water and food pellets (R 70, Lactamin AB, Kimstad, Sweden) were available *ad libitum*. Subsequent to the period of acclimatization and quarantine of 14 days, rats were habituated with the researchers by daily handling (15 min per cage) for 1 week. Body masses of rats were recorded before the formation of experimental groups, before the sexual exhaustion experiment and afterwards weekly (Fig. 2) using a Mettler Toledo (Greifensee, Switzerland) PL1501-S balance with a precision of 0.1 g.

At the age of 11 weeks, screening tests of male copulatory abilities were started. Screening tests were aimed at separating 'sluggish copulators' from males with a normal pattern

of sexual activity (see Ågmo, 1997 for details). Males were considered sexually inactive when they did not intromit within 30 min. Each male participated in four screening tests during which sexually active individuals (20 out of 30) obtained at least one ejaculation. The mating arena consisted of a glass tank of $40 \times 60 \times 40$ (high) cm with a bottom covered with cardboard, which was changed each time before a new pair of rats entered the trial. A male was placed in the arena and left for habituation for 10 min; after that, a receptive female was introduced and the subsequent behaviour of rats was videotaped and protocolled. Mating trials lasted between 12:00 and 16:30 h and were performed under dim light conditions. Ovariectomized females were turned sexually receptive by subcutaneous injection 0.2 ml of oestradiol 3-benzoate [E-8515, Sigma-Aldrich Co, St Louis, MO, USA; 100 μg/ml, dissolved in paraffin oil for PCR (Acros Organics, NJ, USA)], and injection of 0.2 ml progesterone (P-0130, Sigma-Aldrich Co, St Louis, MO, USA; 5 μg/ml, dissolved in paraffin oil). Oestrogen was injected 52 h before and progesterone 4 h before mating trial.

Formation of experimental groups and odour treatment

After separation of sexually active males, they were rehoused to form experimental units in separate cages (four cages of five rats each). These 20 rats were divided into two experimental groups, so that both groups had similar average body masses (see Fig. 2) and similar parameters of pre-test copulatory activity (intromission and ejaculation latencies). Rats were transferred to another experimental facility with similar housing conditions at 16 weeks of age and allowed to habituate with their new cage mates for 14 days. Subsequently, rats were subjected to predator or control odour treatments that lasted 2 h day^{-1} during 6 successive days.

Predator odour originated from two soft nylon collars (TRIXIE, Jarplund-Weding, Germany) worn by two cats (male and female, domestic pets) for 3 weeks. Collars were cut into three pieces of equal length and stored in airtight plastic bags at –18 °C until use. For odour treatment, a cage with five rats was moved into a separate room and a piece of collar was attached to the lid on the end of the cage that was opposite to the compartment where food pellets were located. Odour was applied at different times (between 08:00 and 20:00 h) each day, and collars of different cats were presented on successive days. Rats responded to the odour exposure by strong fear reactions: after sniffing the collar, they hid under the food-containing compartment of the cage, where they remained immobile until the piece of collar was removed after 2 h. After removal of the cat odour source, the behaviour of rats quickly normalized, and their cage was moved back to the room where all the rats were maintained. Moving the cage with odour-exposed rats back to the main room did not apparently affect the behaviour of other rats, consistent with the notion that cat odour is not particularly volatile (Dielenberg & McGregor, 2001). Behaviour of rats in response to predator odour exposure was similar on the first and sixth day of exposure, suggesting that no habituation to cat odour occurred during the 6-day period. As a control (non-predator) odour, we used a piece of cloth that had been kept in the cage of a rabbit for 3 weeks and exposed to the rats under the same protocol as the cat odour. Rats displayed no fear reactions in response to the rabbit odour but rather elicited a curious behaviour towards it.

Measurements of copulation effort

On the next day after the last odour treatment, rats were subjected to the sexual exhaustion experiment that lasted 6–7 h, starting at 12:15 h. Five male rats from the same cage were placed in separate mating arenas and exposed to receptive females prepared as in the screening tests. All males were allowed to copulate with a single female for 4 h, which is considered sufficient for reaching sexual exhaustion (see Ågmo, 1999 and Rodríguez-Manzo, 1999). Then, a novel female was introduced. If the male was able to ejaculate with a new female, he was assigned yet another female after a period of 30 min without sexual activity subsequent to last ejaculation. Otherwise, the experiment was terminated if the male had not reached an intromission with a novel female during 30 min. The behaviour of rats was videotaped and the following standard parameters of sexual behaviour were registered: intromission latency – time from introduction of the female until the first intromission (vaginal penetration); ejaculation latency – time from the first intromission until ejaculation; post-ejaculatory interval – time from ejaculation until the next intromission; number of mounts, intromissions and ejaculations during first 4 h of the experiment, and the total number of ejaculations obtained during the whole experiment. Additionally we recorded the total time spent with the female (mostly contributed by the time spent chasing the female) and ejaculation efficiency as the number of ejaculations obtained during the first 4 h of experiment divided by the time spent with the female. The frequency of the Coolidge effect was recorded as a percentage of animals obtaining ejaculations with more than one female. All rats (10 predator odour-exposed and 10 rabbit odour-exposed) were processed in batches of five animals from the same cage. Predator and rabbit-exposed rats were tested in alternate trials.

Urine collection, corticosterone and creatinine analysis

One day before the beginning of odour treatment, the first urine samples for corticosterone analysis were collected at 19:00 h. The second urine samples were collected immediately after termination of the copulation experiment at about 19:00 h. For collection of urine, the animal was placed in a plastic box of $20 \times 30 \times 20$ (height) cm size, and urine was collected with a micropipette. If the animal had not urinated in the box during 5 min after the copulation experiment, the urine was collected with a pipette on its ventral side during intraperitoneal injection of anaestheticum for

the parasite inoculation experiment (most animals urinated during the injection). We were able to collect the samples of *c*. 0.1–0.5 ml urine. Samples were frozen and stored at −18° C until analysis. Urine corticosterone was measured using a ^{125}I radioimmunoassay kit (RS 490 11; IBL, Hamburg, Germany). Urinary creatinine was measured using a Jaffe method on a Cobas Integra 400 analyser (Roche Diagnostics Ltd, Basel, Switzerland). Urinary corticosterone concentrations were expressed as ng corticosterone per ml creatinine to correct for differences in urine production rates between rats and across days (e.g. Brennan *et al*., 2000).

Blood sampling and analyses

Subsequent to urine collection after termination of the copulation experiment, animals were anaesthetized with an intraperitoneal injection of Diazepam (Nycomed Austria GmbH, Linz, Austria; 5 mg ml^{-1}) 1 ml per 1 kg. About 10 min after the injection, the tails of animals were warmed in about a 37 °C water bath to ensure adequate blood flow. Blood (*c*. 1 ml) was collected from the lateral caudal vein. Blood for analysis of the total leucocyte concentration (WBC) was stored in a Cellpack Diluent (Sysmex Reagents) buffer (44.7 µl blood per 179 µl buffer) until analysis within 1 h after collection with automated blood analyser (Sysmex XE2100; Sysmex Europe GMBH, Hamburg, Germany). Blood smears for differential leucocyte counts were stained with Leishmann–Giemsa method and examined by an experienced observer in order to determine the proportions of lymphocytes and neutrophils per 100 leukocytes. The concentrations of lymphocytes and neutrophils were obtained by multiplication of their proportions with WBC.

Experimental infection and estimation of parasite burden

Immediately after blood collection (when animals were still under anaesthesia), rats were orally inoculated with *c*. 10 000 *T. canis* embryonated eggs via a stomach tube. This dose was used because it was shown to yield a detectable larval recovery from the brains in a pilot experiment (however, no symptoms of aberrant behaviour or distress due to infection were recorded). Eggs were obtained from uteri of female intestinal adult worms, collected from 1-month-old German Shepherd dog puppies (domestic pets) 2 days after deworming with fenbendazole (5 mg kg^{-1} body weight). The upper part of the uterus was removed, crushed on a Petri dish and the eggs were collected into a test tube with tap water. Collected eggs were suspended in 0.1% formalin and poured into tissue culture flasks to a maximal depth of 5 mm and incubated at 25 °C for *c*. 1 month to embryonate. After embryonation, eggs were stored at 4–8 °C until use. Before experimental infections, the egg suspension was washed with tap water three times. Seventeen days after infection, animals were killed by chloroform fumes in a 3 l excsiccator in a separate well-ventilated room, preventing contact of personnel with chloroform fumes. This method of euthanasia was used because of its rapid action and minimal handling stress caused to the animals, e.g. compared with intraperitoneal injections. Brains and whole muscle tissue were collected. Larvae from brains, and muscle tissue were obtained by the HCl–pepsin digestion technique, modified after Kapel & Gamble (2000). Samples were digested in an artificial gastric fluid consisting of 10 ml 38% HCl and 10 g of pepsin powder (1:10 000) dissolved in 1 l tap water. Whole brains were gently crushed with tweezers, while muscle tissues were minced with a cleaver; muscle samples were divided into four portions for digestion. Thereafter, the samples were processed in thermostat at 46 °C with a digestion fluid on a magnetic stirrer for 2–3 h until the tissue had digested. Digested samples were left to settle for about 30 min, and then the supernatant was removed and fresh water was added. This procedure was repeated three to six times to remove tissue remnants. Cleaned sediment was transferred to a 50 ml tube, allowed to settle and the larvae were counted from the remaining 5 ml of the sediment on Petri dishes under a binocular microscope at 14 × 7 magnification. All animal procedures were approved by the Tartu University Animal Care Committee in accordance with the European Community Directive of 24 November 1986 (86/609/EEC).

Statistics

Changes in corticosterone levels and body masses during the experiment were analysed with repeated-measures ANOVA. Immune parameters and indices of copulatory activity were compared between treatment groups by *t*-tests or Mann–Whitney *U*-tests (*z*-adjusted for ties) when parameter distributions deviated from normality. Spearman rank correlations were used to test for associations between copulatory parameters and health variables. Sample sizes varied between different tests due to our inability to obtain sufficient amounts of blood and urine from all rats. All tests are two-tailed. The significance level was set at 0.05.

Results

Rats in control and predator odour treatment groups did not differ in terms of the parameters of copulatory activity, with the exception of the total number of ejaculations, which was higher in the control group (Table 1). Urine creatinine concentrations increased with increasing number of mounts and mounts + intromissions (Table 2). Although no significant correlations between leukocyte concentrations and parameters of copulatory activity emerged, there was a tendency for WBC and lymphocyte haemoconcentrations to decrease with increasing number of mounts and intromissions ($P = 0.06$–0.08; Table 2). No significant correlations emerged between the resistance to *T. canis* infection (i.e. the number of larvae detected from the brain) and copulatory activity parameters (Table 2).

Rats exposed to the predator odour treatment revealed increases in urinary corticosterone secretion (Fig. 1) and growth retardation (Fig. 2) subsequent to the odour exposure.

Table 1 Comparison of copulatory parameters of male rats *Rattus norvegicus* exposed either to predator or control (rabbit) odour during 6 days (2 h day^{-1}) before copulation

Parameter	Predator (mean ± SD)	Control (mean ± SD)	Statistic	*P*
Mounts 4 h^{-1}	44.1 ± 17.6	62.5 ± 50.4	$z=0.61$	0.54
Intromissions 4 h^{-1}	47.0 ± 18.3	50.4 ± 17.3	$t=0.42$	0.68
Ejaculations 4 h^{-1}	5.4 ± 1.4	7.0 ± 2.2	$z=1.66$	0.097
Total number of ejaculations	**5.7 ± 1.7**	**7.8 ± 2.5**	**z = 1.96**	**0.050**
Intromission latency (s)	27.5 ± 34.9	99.4 ± 257.1	$z=0.38$	0.70
Ejaculation latency (min)	22.7 ± 15.8	23.5 ± 13.3	$z=0.08$	0.94
Time spent with female (s)	187.5 ± 178.7	117.0 ± 66.4	$z=0.53$	0.63
Ejaculation efficiency[a] (times s^{-1})	0.056 ± 0.043	0.081 ± 0.054	$t=1.13$	0.27
Average post-ejaculation interval (s)	503 ± 84	531 ± 135	$t=0.53$	0.60
Frequency of the Coolidge effect	30%	30%	$\chi^2=0$	1

[a]Number of ejaculations during 4 h per time spent in contact with female. Sample sizes are 10 individuals per group with the exception of intromission latency where $n=6$ for predator odour treatment.
Bold indicates a statistically significant result.

Table 2 Spearman rank correlations between parameters of copulatory activity and measures of stress or immunocompetence

Parameter R_S *P*	Mounts 4 h^{-1}	Intromission 4 h^{-1}	Mounts + intromission 4 h^{-1}	No. of ejacuations 4 h^{-1}	Total no. of ejaculations	Time with female	Ejaculation efficiency
Creatinine	**0.64**	0.60	**0.63**	−0.46	−0.25	0.29	−0.42
($n=13$)	**0.018**	0.085	**0.023**	0.108	NS	NS	NS
Corticosterone/creatinine	−0.39	−0.15	−0.43	−0.12	−0.30	0.05	−0.08
($n=11$)	NS	NS	NS	NS	NS	NS	NS
WBC	−0.46	−0.41	−0.50	0.33	0.34	−0.02	0.11
($n=15$)	0.082	NS	0.060	NS	NS	NS	NS
Lymphocyte count	−0.49	−0.32	−0.49	0.39	0.30	0.09	0.028
($n=15$)	0.064	NS	0.064	NS	NS	NS	NS
Neutrophil count	−0.19	−0.22	−0.17	0.28	0.34	−0.20	0.27
($n=15$)	NS	NS	NS	NS	NS	NS	NS
No. of larvae in	−0.16	−0.02	−0.15	−0.28	−0.36	0.20	−0.26
brain ($n=20$)	NS	NS	NS	NS	NS	NS	NS

All *P*-values greater than 0.1 are labelled as not significant (NS).
Bold indicates a statistically significant result.

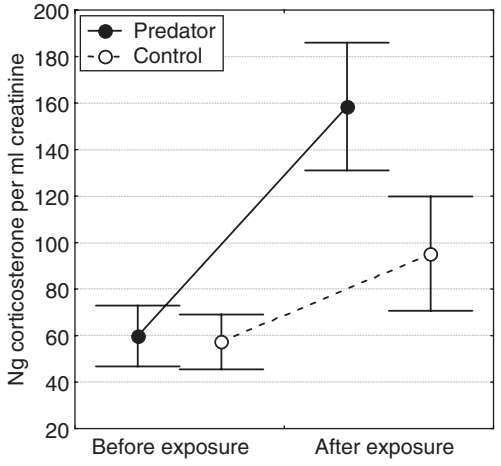

Figure 1 Changes in the mean (± SE) urinary corticosterone levels in rats *Rattus norvegicus* exposed to the predator and control odours. $n=4$ for the predator group and $n=5$ for the control group. $F_{1,7}=29.0$, $P=0.001$ for the effect of time, $F_{1,7}=1.6$, $P=0.245$ for the effect of treatment and $F_{1,7}=5.7$, $P=0.048$ for the time × treatment interaction in repeated measures ANOVA.

Seventeen days following infection, predator-treated rats had significantly more nematode larvae in their brains and muscle tissue (Fig. 3).

Discussion

This study aimed at explaining whether exposure to predatory cues induces (1) increased copulatory activity and (2) immune suppression in male rats; (3) whether intense copulatory activity is associated with immune suppression and (4) which aspects of copulatory activity are most expensive in terms of energy expenditure and/or immune suppression.

The answer to the first question was clearly negative as no evidence of increased copulatory activity could be detected in the predator-exposed group (Table 1). One possible explanation for such a result might be that some neural and/or hormonal pathways activated due to predator-induced stress response may have suppressed sexual behaviour, although such a reasoning would perhaps contradict previous findings on enhancement of sexual behaviour in male rats by mild stress (reviewed by Smith, Stewart &

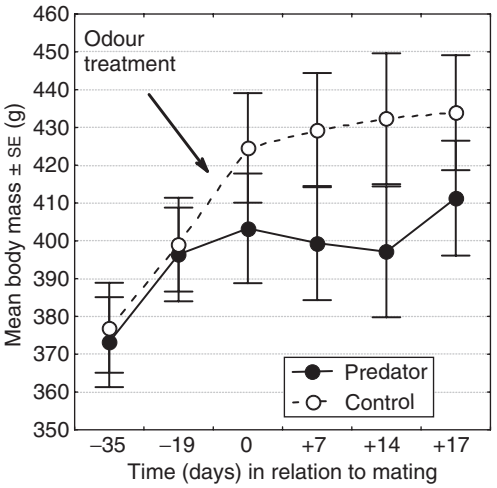

Figure 2 Changes in the body mass of rats *Rattus norvegicus* during the course of experiment. $n = 10$ for both groups. $F_{5,90} = 42.6$, $P < 0.001$ for the effect of time, $F_{5,90} = 0.93$, $P = 0.347$ for the effect of treatment, and $F_{5,90} = 6.3$, $p = 0.004$ for the time × treatment interaction in repeated measures ANOVA (Greenhouse–Geisser adjusted *P*-values).

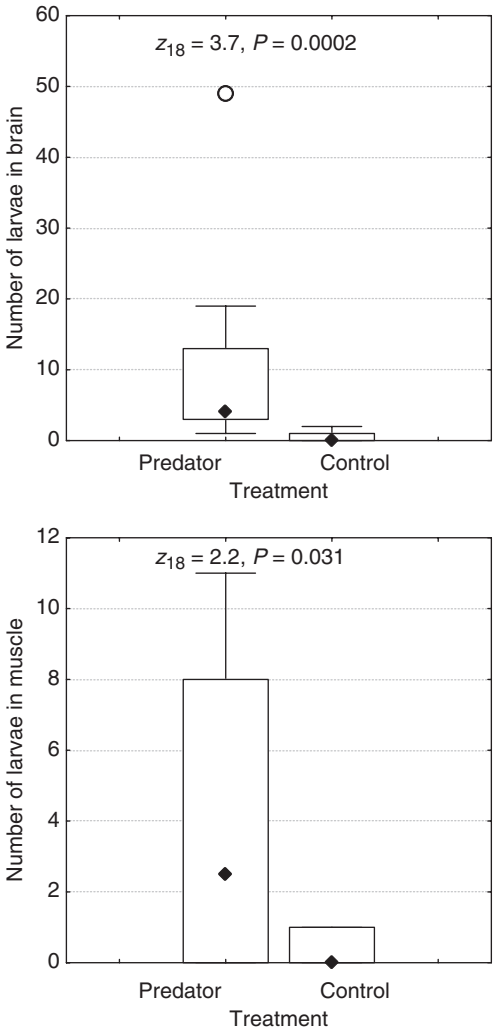

Figure 3 Number of *Toxocara canis* larvae in brains (upper) and muscle tissue (lower) of predator odour-exposed and control rats *Rattus norvegicus* 18 days after odour exposure and 17 days p.i. Diamonds indicate medians, boxes cover a range between the 25th and the 75th percentile and whiskers indicate the non-outlier range (1.5 × box length). Single dot represents an observation outside this range.

Pfaus, 1997). An alternative explanation for the failure of our experiment could be that our predator odour exposure protocol did not successfully mimic the natural situation triggering TRE. Aiming to simulate the presence of predators, we exposed rats to cat odour in their home cages 2 h day^{-1} during six consecutive days. It is possible that the stress generated by the odour exposure may have appeared unnaturally severe (e.g. because the rats had no means to escape from the odour) and its physiological consequences could have obscured the normal anxiogenic pathways, leading to increased sexual activity. Finally, at least theoretically, we also cannot exclude the possibility that male rats have evolved their reproductive behaviour under selection pressures that preclude triggering of TRE in response to predatory cues.

Evidence about costs of copulation in our experiment remains equivocal as well. Only two parameters of copulatory activity (frequency of mounts and mounts + intromissions) correlated significantly with one of the six physiological parameters measured, namely the urine creatinine concentration. Urine creatinine concentrations can be considered as indicative of muscle catabolism because creatinine excretion increases markedly during physical exercise (e.g. Calles-Escandon *et al.*, 1984). Hence, our results indicate that for male rats, mounting and intromitting appear to be the most costly components of copulatory activity energetically, e.g. more costly than chasing the female or achieving a high number of ejaculations. However, we cannot currently assess whether these costs are of sufficient magnitude as to impinge on any component of fitness. It should be noted that quantitative evidence on costs of copulation in male mammals (besides those associated with sexually transmitted diseases) is surprisingly scarce. We are aware of only two studies (in golden hamsters *Mesocricetus auratus*) that have detected an immunosuppressive effect of intense copulatory activity in males (Kress *et al.*, 1989; Ostrowski *et al.*, 1989).

Predator-induced stress and immune suppression

Our experiment established a strong immunosuppressive effect of predator odour treatment in male rats. Exposure to cat odour resulted in increased corticosterone levels, present at least 23 h subsequent to the last exposure (Fig. 1). Additionally, for predator-exposed rats, growth was arrested for at least 2 weeks after exposure as they remained about 30 g lighter than controls (Fig. 2).

Importantly, we demonstrated that this immune suppression indeed reduced the resistance of rats to neurotropic nematode infection (Fig. 3). To our knowledge, this is the first evidence for the effect of stress-induced immune suppression on susceptibility to helminth infection in the rodent host. One implication of this finding is that exposure of rats to cat odour at $2\,h\,day^{-1}$ during 6 days can be considered as an extremely severe chronic stress treatment, at least under conditions where the rats cannot escape. This is probably worth taking into account while developing predator stress models for testing of anxiolytic drugs. On the other hand, our results also indicate that the infection susceptibility of paratenic hosts of *T. canis* is sensitive to psychological stress. This finding may explain the large variation in disease severity in hosts infected with a standard dose of embryonated eggs (e.g. Cox & Holland, 1998). It also implies that experiments with the *T. canis* model in rodents should very carefully control for standardizing the housing and handling conditions.

The prevailing view in the ecological immunology is that stress-induced immunosuppression is a result of reallocation of energy or other resources from immune function to costly behaviours like reproductive effort (e.g. Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000; Zuk & Stoehr, 2002). In our experiment, however, immune suppression was not caused by an increase in reproductive effort, which raises the question regarding why the exposure to predator odour increases infection susceptibility. At present, we have no answer to this question as the evolutionary reasons for immune suppression induced by chronic stress have remained obscure (e.g. Maier & Watkins, 1998; Lekander, 2002) despite the extensive studies that date back to the pioneering work of Hans Seyle in 1925.

Conclusions

Although our experiment failed to induce a terminal copulatory effort in male rats, it succeeded in exploring some causes and consequences of variation in copulatory behaviour and effects of predatory stress-induced immunosuppression upon parasite resistance in a novel experimental system. Particularly, our results suggest that *T. canis* infection in a paratenic host is a promising model for immunoecological research. On the other hand, this study also raises a number of questions about psychoneuroendocrine mechanisms linking responses to predatory cues and sexual behaviour, e.g. whether milder or acute treatments with predatory odour would induce different behavioural and immunological responses compared with the effects of chronic predator exposure. These aspects are worth further examining, especially in the context of paucity of experimental evidence for Terminal Reproductive Effort in vertebrates.

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