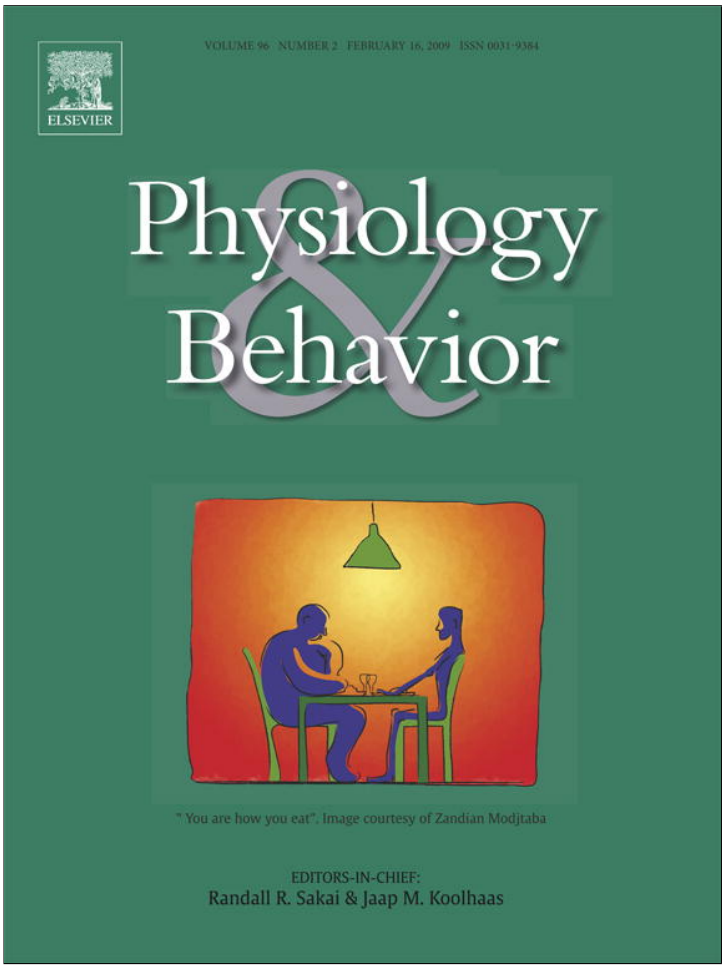

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Immunodepression reduces learning performance in male laboratory mice (*Mus musculus*)

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ABSTRACT

Several theoretical and empirical studies have suggested that immunocompetence may act as a constraint on learning, due to a trade-off in investment in the two processes. Here we tested whether experimentally depressing immune responsiveness of male BKW mice using antithymocyte serum (ATS) would lead to reduced learning performance in a radial maze task. Correct choices in the maze were indicated by the presence of familiar odours, incorrect choices by unfamiliar odours. We showed that temporarily depressing cellular immunity led to a reduction in performance in terms of a reduced proportion of correct choices. We also found a positive relationship between the proportion of correct entries over the period of testing and haemagglutination titre, indicating that mice showing greater immune responsiveness performed better in the maze. We conclude that depressing the immune system reduces learning performance in a combined odour/spatial learning task, and that some individuals are better able to compensate for the experimental immunodepression. In contrast to previous studies, there was no evidence that the effect of ATS was mediated by associated changes in corticosterone or testosterone secretion. There were no significant effects of the presence of female odour on learning performance, and therefore no evidence that the down regulation of learning in relation to immune depression was influenced by apparent reproductive opportunity.

1. Introduction

Interrelationships between the immune system, resistance to disease and behaviour have been the subject of much interest in recent years [1–4] Of particular interest has been the potential mediating role of steroid hormones, such as androgens and gluccocorticoids in trade-offs between behavioural and/or other phenotypic traits and immune function [5,6]. Our own studies of wild and laboratory rodents have revealed several associations between measures of circulating testosterone and corticosterone, and both behaviour and immune function (see [6]). We have also shown that hormonal and behavioural modulation in relation to immune function is influenced by both social and physical environmental factors e.g. [7–12]. Recently we have investigated the adaptive modulation of higher order aspects of behaviour, particularly learning, in relation to immune function [6,13].

There is good evidence that learning ability is influenced by immunity status, in terms of being both reduced when the immune system is under stress during infection (e.g. [14–16]; but see e.g. [17]), and enhanced when cytokines are experimentally boosted (e.g. [18–20]). In two recent studies of laboratory mice, we have shown that learning itself has an impact on immune function and the ability of mice to resist a subsequent experimental infection. The degree of difficulty of learning the correct response was varied in a spatial [13], and an odour learning task [21]. Mice faced with a harder learning task showed less resistance to a subsequent infection of a blood protozoan (*Babesia microti*), and resistance declined in a linear relationship with the degree of learning shown by individual subjects (i.e. better learning led to decreased resistance). In both studies there was a tendency for mice with higher initial concentrations of corticosterone to have a reduced resistance to infection. However, the relationship between corticosterone and learning was *positive* in the case of the maze task (fewer errors with higher levels of corticosterone) but *negative* for odour, or social, learning (more errors with higher levels of corticosterone). Differences in the relationship between corticosterone and learning across different contexts have been found in several other studies (e.g. [22–25]).

The above work suggests that there is a trade-off between immune function and learning. If investment is required for learning there will be fewer resources available to fight infection [13,21], and vice versa, investment in immune function reduces resources available for learning [14,26]. These studies looked at concurrent relationships between learning, immune function and resistance, and were thus essentially correlational studies. We assume that the increased resources expended on learning the more difficult task were no longer available for an immune response leading to reduced resistance i.e. a trade-off in the resources

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available for the two processes. However, to infer cause and effect more reliably, and determine whether there is indeed a trade-off, it is necessary to manipulate the immune system experimentally, i.e. if you decrease the resources available for the immune system, via immunodepression, do subjects, adaptively, decrease their investment in learning?

In the present paper, therefore, we report an experiment in which we experimentally depressed immune responsiveness in male mice with the prediction that immunodepressed subjects would show reduced learning performance. We used the same radial maze procedure as previously [13], so that we could control for any confounding depressing effects of treatment on overall activity (since mice have actively to move around to solve spatial tasks). However, in the current study the correct arm was indicated by the presence of a familiar odour, so the task included both a spatial (remembering the layout of the maze) and a social (recognizing a familiar odour) learning component. The aim was to use an ecologically relevant task, i.e. finding food using conspecific odour as a cue. All mice were exposed to the same number of male odours, so differences in the apparent level of competition are not a confounding factor with experimental treatment.

To manipulate immune responsiveness, we treated mice with heterologous antithymocyte serum (ATS) (see [9,10]) because the technique is relatively innocuous and without the general side effects of other forms of immunodepressive therapy, such as cytotoxic drugs, whole-body irradiation or ablation of primary lymphoid organs (e.g. [9,10,27,28]), and acts primarily on T-lymphocytes, the helper cells that are essential for efficient antibody responses and cellular immunity [26,29]. ATS treatment was preferred to treatment with monoclonal antibodies that would have required the administration of two reagents (antiCD4 and antiCD8) and suitable controls to achieve the same end.

Finally, if immune function is traded off against other components of life history, we might expect a reduced tendency to decrease investment in learning in response to immunosupression, if there appears to be a potential reproductive gain [2,30]. In a previous study, we showed that the addition of female odour (suggesting reproductive opportunity) to the home cage of a group of males was sufficient to override the down-regulation of testosterone and aggressive behaviours, and the up-regulation of sleep in ATS-treated males [10]. We therefore repeated this procedure here by exposing half the subject males in the maze task to female odour in their home cages, with the prediction that any reduction in learning ability seen in ATS-treated males would be less marked, or absent, in those exposed to female odour.

2. Materials and methods

2.1. Animals

Subjects were 90 adult male BKW mice (B&K Ltd, Hull, UK) that were 75 days of age at the start of the experiment. Since we have already shown that dominance relationships between BKW males housed together affect learning performance in the mazes used here [31], mice were housed singly from purchase and throughout the experiment in standard opaque polypropylene laboratory cages (48×15×13 cm; model M3, North Kent Plastics, UK) to standardize social experience prior to the experiment. Animals were maintained throughout on a 12 h:12 h reversed light:dark cycle with white lights on at 20.00 h, and all tests were carried out under dim (40 W) red illumination during the dark phase.

2.2. Experimental groups

After a two week settling period, mice were assigned to one of six groups (15 mice per group) for the experimental procedure. See Fig. 1 for a schematic of the schedule.

(i) ATS-treated, no female odour
(ii) Naïve serum control, no female odour
(iii) Sham-inoculated control, no female odour
(iv)–(vi) As (i)–(iii) but with female odour.

On Day 1 (after settling) mice were injected with 0.5 ml ATS (Groups i and iv) or 0.5 ml naïve rabbit serum (Groups ii and v), or sham-inoculated (Groups iii and vi). On Days 2 and 4 the mice were again injected. A blood sample was taken from a caudal vein on Days 2 and 5 for later serum assays. On day 6, mice were weighed and each injected with 0.2 ml of a sheep erythrocyte suspension (SRBC) containing 25×10^7 erythrocytes ml (each mouse receiving 5×10^7 cells) for haemagglutination assay at the end of the experiment.

2.3. Preparation of anti-thymocyte serum

Anti-thymocyte serum (ATS) was obtained by dissecting out the thymuses of 55 3-week old C57BL/10 mice. Adult female New Zealand white rabbits (5) were injected with a thymocyte single-cell suspension (ca. $2–5\times10^8$ thymus cells/rabbit), using aseptic techniques as described for lymphocytes [32], differing only in that cell suspensions were prepared in RPMI medium without foetal calf serum. Rabbits were injected intravenously into the lateral ear vein with the required number of cells in a volume not exceeding 0.9 ml. This procedure was repeated 2–3 weeks later and the rabbits were exsanguinated after a further 7 days. The serum was separated after clotting at 4 °C, heat inactivated at 56 °C for 45 min, aliquoted, and stored at −80 °C until required. Naïve serum was obtained from female New Zealand white rabbits (5) of approximately the same age.

2.4. Odour treatment

Groups (iv)–(vi) were subjected to female odour in the maze home cage, from Day 6 and throughout testing. Odours were obtained by housing adult female mice singly on a sawdust substrate 48 h prior to testing. Approximately 100 cm^3 of soiled sawdust from the cages of two of the females (c. 50 cm^3 from each) was scattered evenly over the floor of the home cages of those males in the 'female odour' treatments, with the same quantity of clean sawdust scattered in the cages of mice in the 'no female odour' treatments. A different pair of females contributed the sawdust to each of the 'female odour' treated mice.

2.5. Maze training

We used three 7-arm enclosed radial mazes made of acrylic and Perspex [31]. Each maze consisted of a central octagonal area 24 cm in diameter, with seven identical arms radiating at equal angles from it. The arms were 28 cm long and 8 cm wide with 7.5 cm high walls along their entire length. Each arm contained an opaque Perspex suspended swing door 16 cm in from the central area, which could be locked manually with a pin. During training and tests, food in the form of crushed pellets was placed beyond the doors as procedures required (see below). Food availability was regulated to meet the hourly food requirement of each subject as calculated from their food intake during settling. A 25 cm clear Perspex tunnel ran from the remaining aperture in the central area to a standard laboratory cage identical to the home cages above. A manually removable opaque guillotine door in the aperture of the central area opened or closed the entrance from the cage to the maze as required.

Mice were introduced individually to the 'home' cage of one of the mazes from Day 7. Following [31], introduction took place at the end of the dark phase to allow the animal 12 h to settle before being allowed into the maze for the first time. Food (standard pellets) and water were available *ad libitum* in the cage during the settling period. During the subsequent two (training) dark phases, however, food was available only in the end compartments of the arms of the maze. Mice were allowed free access to the maze through the 12 h dark period during which food

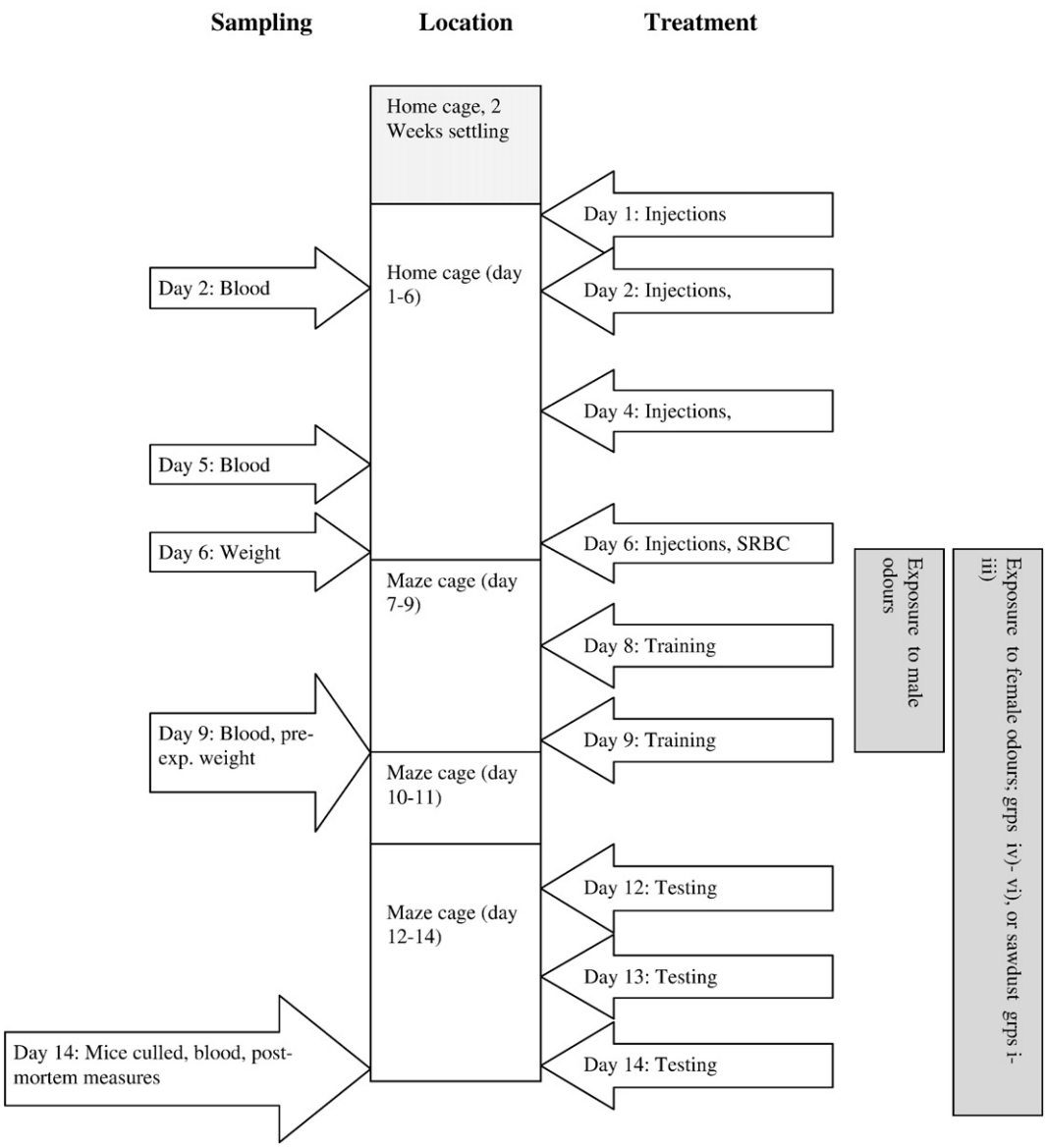

Fig. 1. Schematic of experiment time line, showing the time when mice were injected with treatments (ATS, Naïve Rabbit serum, Sham); when blood samples were taken; and when odour treatments were given in relation to maze training and testing.

(crushed pellets) was available (doors unlocked) in all arms of the maze. At the end of each 12 h period of access, mice were once again confined to the attached 'home' cage with ad libitum food for the light phase. Following the second training period of access, each mouse was weighed (pre-experimental weight) and an 88 μl blood sample (pre-experimental sample) taken from a caudal vein following e.g. [9,10]. Mice were then allowed 36 h to re-settle in the 'home' cage of the maze before experimental testing (see Fig. 1 for schematic of procedure).

2.6. Maze testing

During training, mice were exposed to the urinary odours of two unfamiliar adult males for four 30 minute periods, one of these odours later being used to indicate the available arm of the maze (see below). All males were exposed to the same number of odours, and the learning task was the same for all mice. To obtain the urine, previously unfamiliar males (N=43) were housed singly in a bare polypropylene cage for up to 60 min. All urine produced during this time was drawn up into a syringe and then frozen at −40 °C until required for familiarization, or testing. Mice were familiarized with odours by introducing a strip (2 cm×2 cm) of Benchkote marked with a 3 μl drop of urine into the maze 'home' cage. During the maze testing procedure, strips (2×2 cm) of Benchkote, marked with a 2 μl drop of urine from one unfamiliar (to the subject) male were placed at the entrance of each locked (food unavailable) arm. The unlocked (food available) arm was marked by the urine of one of the males to which the test mouse had already been exposed in its home cage. In all treatments, food was placed in the end compartment of all arms to control for effects of food odour, but the doors of the unavailable arms remained locked and the food therefore inaccessible [31].

Testing took place during the dark phase as three 10 min trials within a one 1 h session which was repeated four times a day, over three days i.e. 12 trials per day for three days, (see [13] for details of the schedule i.e.). Sessions within a day were separated by a 1 h interval during which the mouse was confined to the maze 'home' cage. Each mouse was deprived of food for 3 h before entering its first trial, and no food was available in the 'home' cage during inter-session intervals. As in the training phase, however, food was available *ad libitum* in the cage overnight (light phase).

After each 10 min trial, the maze was cleaned with detergent (Tego; Goldschmidt, Germany) and thoroughly rinsed with water, and

the positions of the (detachable) arms rearranged to control for any effects of the subject's odour cues from previous visits [31]. In addition, the position of all odour cues was rearranged in a random manner. The odours were then refreshed with different unfamiliar stimulus odours used in each trial; the familiar male odour (indicating the presence of food) being alternated between the two odours to which the test male had been exposed to in its home cage. Behaviour in the mazes was recorded via an overhead camera (Panasonic WV-BP 330/8) and relayed to a remote video recorder in an observation room for transcription using the Observer 3.0 (Noldus Ltd, Wageningen, The Netherlands) event recorder package. As well as recording successful entries to correct end compartments, we also recorded the total number of entries to all arms as a measure of overall locomotory activity, so that any covariation between locomotory activity and treatment could be taken into account in subsequent analyses of correct responses (see below).

At the end of testing, mice were weighed for the final time (terminal weight), killed by inhalation of CO_2 (UK Home Office approved Schedule 1 method), and exsanguinated by cardiac puncture (terminal sample). They were then autopsied and the following organs carefully dissected out and weighed: thymus, spleen, heart, kidneys, adrenal glands, testes, seminal vesicles, preputial glands and coagulating glands. Left and right paired organs were dissected out and weighed separately. All blood samples were spun down and the serum frozen and stored at −40 °C until assayed later, by standard ELISA, for testosterone, corticosterone, haemagglutination titre against SRBCs, and total IgG (mg/l) concentrations (see [33,34]).

In a small number of cases, limited blood samples meant it was not possible to undertake all three physiological assays or all infection measures; sample sizes therefore varied slightly in subsequent analyses according to the variables included. Measures taken at each sample point are summarized in Table 1.

2.7. Ethical considerations

The experiment involved mild food deprivation, taking intercurrent blood samples and challenging animals with ATS to induce transient immunodepression. All procedures were carried out under licence (to CJB, JND and JMB) from the UK Home Office, were approved by the University of Nottingham's Ethical Review Committee and were well within the limits imposed by the 'Mild' severity band of the licences.

3. Results

3.1. Pre-experimental measures

Two-way analysis of variance (ANOVA) showed no significant bias in pre-experimental body weight, testosterone or corticosterone concentrations with respect to later ATS or female odour treatments (Table 1). However, there was a significant difference in pre-experimental IgG concentration with respect to ATS treatment ($F_{2,68}=5.81$, $p=0.005$), with later ATS-treated mice turning out to have a lower IgG concentration than naïve serum controls (Table 1). There was no difference in pre-experimental IgG concentration with respect to female odour treatment.

3.2. Effects of ATS treatment on immune capacity

Two-way ANOVA of the post-experimental haemagglutination titre against SRBCs, with ATS treatment and female odour as factors, and terminal body weight, the mean number of arms visited per day during tests (as a measure of general activity in the mazes [13]) and pre-experimental IgG concentration as covariates, showed a significant difference between ATS treatments in the expected direction ($F_{2,63}=76.77$, $p<0.001$) i.e. ATS treated mice showed a reduced immune response. Haemagglutination titre was lowest for ATS-treated mice (mean±SE 1/dilution: ATS=22.67±12.07, NRS control=78.08+23.16, sham-inoculated=2284.46±220.38). Note that the very high values in the sham-inoculated group were primarily driven by the disproportional allocation of high responders to this group despite randomization. There was no effect of female odour treatment and no significant relationship with any of the covariates. The difference between ATS and NRS controls remained significant when compared in the absence of the sham-inoculated treatment ($F_{1,42}=6.34$, $p<0.016$). ATS treatment thus had the expected effect of reducing immune responsiveness to SRBC challenge (see also [9,10]).

ANOVA of terminal IgG concentration, again controlling for terminal body weight, the number of arms visited and pre-experimental IgG, also showed a significant difference across ATS treatments ($F_{2,63}=15.47$, $p<0.001$). However, the significant effect was due to an inexplicably lower terminal concentration in the sham-inoculated controls (see Table 1) rather than the difference between ATS-treated mice and NRS controls.

Table 1
Mean+SE values for measures of testosterone (ng/ml), corticosterone (ng/ml), and total IgG concentrations (mg/l) and body weights (g) for mice in relation to ATS and female odour treatments (see text)

	No female odour			Female odour		
	ATS	NRS	Sham	ATS	NRS	Sham
Testosterone						
Pre training	4.80±1.33	4.19±0.63	4.33±0.79	3.58±0.77	5.10±0.86	6.02±1.01
Pre experimental	5.38±1.29	4.95±0386	5.18±1.38	2.61±0.53	5.04±0.62	6.41±1.05
Terminal	5.04±1.97	6.33±1.23	6.98±2.41	3.19±0.89	6.94±0.79	6.67±1.29
Corticosterone						
Pre training	98.25±24.78	135.25±15.72	90.49±17.07	124.30±28.33	95.48±20.76	84.14±17.27
Pre experimental	142.0±20.94	157.8±23.58	123.0±20.96	155.4±28.33	116.8±20.99	101.9±17.56
Terminal	64.27±18.47	75.95±19.02	35.20±12.34	33.18±11.44	42.08±10.15	47.58±14.74
IgG						
Pre training	0.55±0.019	0.62±0.016	0.58±0.016	0.57±0.017	0.62±0.015	0.61±0.020
Pre experimental	0.69±0.018	0.73±0.016	0.63±0.014	0.72±0.040	0.79±0.029	0.63±0.022
Terminal	0.76±0.018	0.81±0.027	0.71±0.020	0.74±0.041	0.86±0.019	0.69±0.022
Weight						
Pre training	32.75±0.58	33.40±0.57	33.51±0.69	33.03±0.68	33.07±0.68	33.26±0.47
Pre experimental	32.78±0.63	33.66±0.59	33.71±0.69	33.03±.67	33.46±0.63	33.53±0.44
Terminal	33.37±0.65	34.47±0.62	34.46±0.68	33.50±0.57	33.89±0.61	33.92±0.43

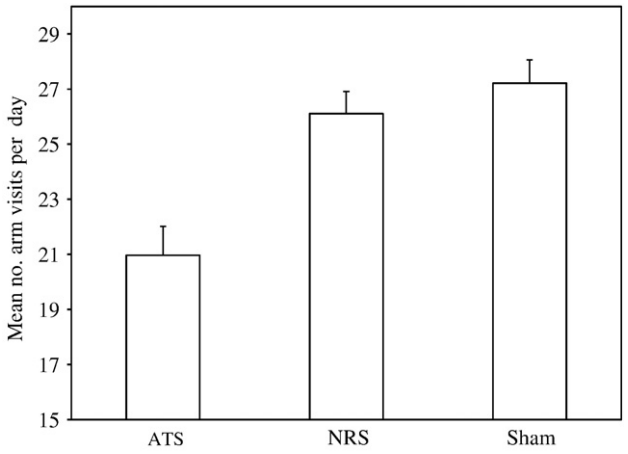

Fig. 2. The mean (±SE) number of arm entries per day of testing in relation to ATS treatment.

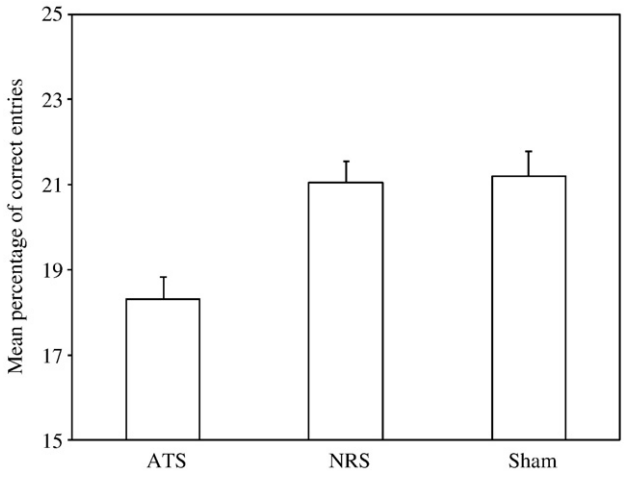

Fig. 4. The mean (±SE) percentage of correct arm entries in relation to ATS treatment.

3.3. Effects of treatment on learning

As our measures of learning performance, we followed [13] in using the proportion of correct entries to arms in the maze (i.e. pushing through the unlocked door at the end of the appropriate arm), and latency to the first correct choice. Repeated measures ANOVA showed there was no significant change in the number of arm entries per trial, i.e. activity, across the three successive days of testing ($F_{2,134}=0.13$, NS). However, a two-way ANOVA with ATS and female odour treatment as factors, showed there was a significant difference in arm entries across ATS treatments ($F_{2,67}=12.47$, $p<0.001$), with the number of entries increasing from ATS-treated mice to sham-inoculated controls (Fig. 2). ATS-treated mice were thus less active in the mazes than the two control groups (Bonferroni $p<0.05$). There was no significant effect of female odour treatment ($F_{1,67}=1.62$, NS) and no ATS x female odour treatment interaction ($F_{2,67}=0.05$, NS).

Repeated measures ANOVA, with ATS and female odour treatments as between-subjects factors, and the mean number of arms visited per day (averaged over the three days) as a covariate to control for the difference in overall activity across ATS treatments, showed a significant increase in the proportion of correct entries across days ($F_{2,130}=6.68$, $p=0.002$; Fig. 3a). The proportion of correct entries overall was similar in range (15–29%) to that found by [13] and, on all three days of testing, the mean proportion was considerably, and significantly, above chance expectation (14.3%) (*t*-test for a known standard for Day 1: $t_{71}=42.48$, $p<0.001$; for Day 2: $t_{73}=52.20$, $p<0.001$; for Day 3: $t_{73}=49.49$, $p<0.001$).

A two-way ANOVA with ATS and female odour treatment as factor, showed there was no significant relationship between proportion of correct entries (averaged across three days) and the mean number of arm entries across days ($F_{1,65}=1.11$, NS) and no significant effect of female odour treatment ($F_{1,65}=0.44$, NS), or interaction between female odour and ATS treatments ($F_{2,65}=0.02$, NS). However, there was a significant effect of ATS treatment on the mean proportion of correct entries ($F_{2,65}=3.88$, $p=0.026$; Fig. 4), with ATS-treated mice showing the poorest performance (Bonferroni, $p<0.05$) compared to the other two treatments.

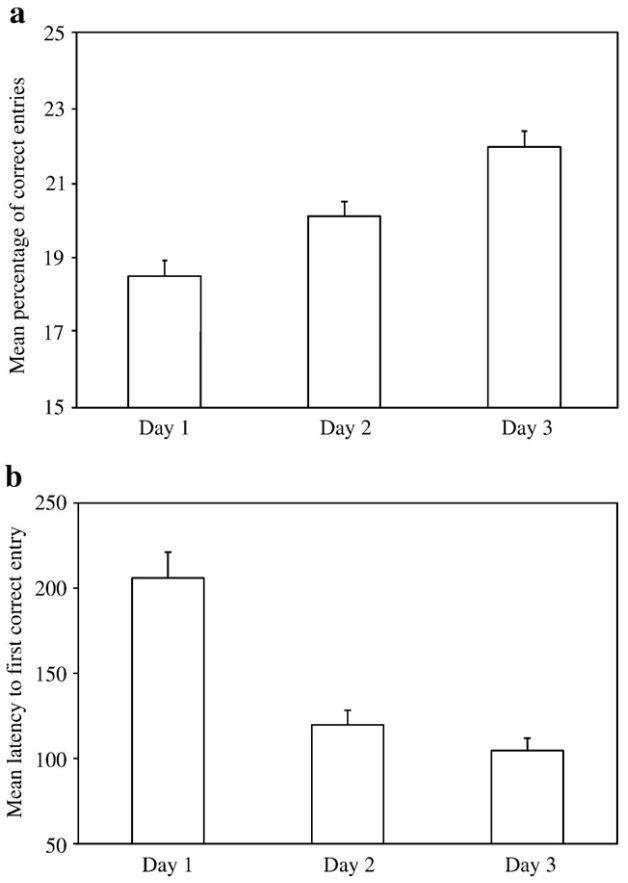

Fig. 3. (a) The mean (±SE) percentage of correct arm entries, and (b) the mean (±SE) latency (s) to first correct arm entry over successive days of maze testing.

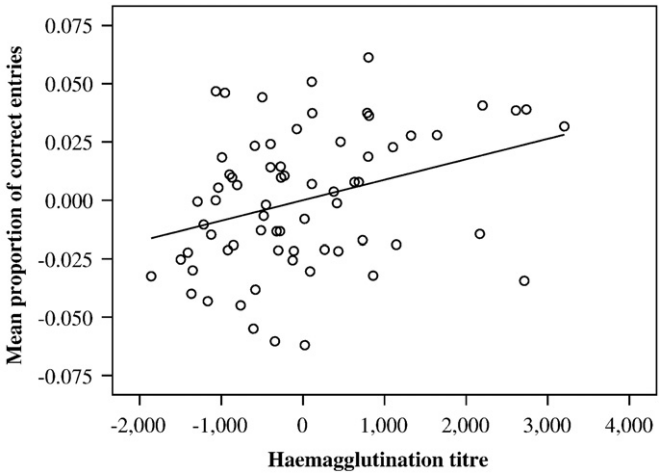

Fig. 5. Component effect plot from partial regression analysis for the relationship between haemagglutination titre (1/dilution) and the mean proportion of correct arm entries over the period of testing.

Table 2
Mean+SE values for measures of organ weights for mice in relation to ATS and female odour treatments (see text)

	No female odour			Female odour		
	ATS	NRS	Sham	ATS	NRS	Sham
Spleen (g)	0.23±0.026	0.14±0.006	0.12±0.007	0.22±0.034	0.14±0.007	0.12±0.006
Heart (g)	0.17±0.003	0.16±0.003	0.16±0.005	0.17±0.004	0.17±0.003	0.17±0.004
Mean Kidney (g)	0.34±0.009	0.34±0.006	0.33±0.008	0.35±0.009	0.34±0.008	0.34±0.008
Mean testes (g)	0.11±0.005	0.12±0.002	0.11±0.003	0.12±0.003	0.12±0.003	0.12±0.003
Seminal Vesicles (g)	0.21±0.017	0.22±0.002	0.23±0.017	0.21±0.16	0.21±0.011	0.20±0.009
Thymus (mg)	55.39±3.96	70.76±4.53	73.90±3.21	53.64±2.32	62.18±2.66	64.82±2.69
Mean adrenal (mg)	3.59±0.21	3.27±0.13	3.19±0.18	3.27±0.18	4.30±1.08	3.35±0.18
Mean preputial (mg)	46.49±1.95	52.65±3.80	53.93±3.55	46.00±3.08	49.66±2.96	52.10±2.50
Mean coagulating (mg)	11.28±0.44	12.13±0.38	12.61±0.61	13.59±0.74	13.01±0.77	11.85±0.53

Means refer to mean of left and right organ.

A repeated measures ANOVA of latency to first correct choice showed that latency declined significantly across days ($F_{2,134}=34.36$, $p<0.001$; Fig. 3 b) with, as might be expected, a strongly significant negative relationship between latency and the number of arms visited per day ($F_{2,67}=149.62$, $p<0.001$). However, there were no significant effects of ATS or female odour treatments on mean latency (Two-way ANOVA: $F_{2,67}=0.33$, NS and $F_{1,67}=0.49$, NS, respectively).

3.4. Changes in immunity and hormone measures during testing

Although there was a slight increase in testosterone concentration over the period of testing (Table 1), repeated measures ANOVA showed the change was not significant ($F_{1,65}=0.036$, NS), and there was no significant difference in the magnitude of change with ATS ($F_{2,65}=1.33$, NS) (c.f. [9]) or female odour ($F_{1,65}=094$, NS) treatments. Neither was there any interaction between ATS and female odour treatments ($F_{2,65}=1.66$, NS). The same was the case for corticosterone (ATS treatment: $F_{2,67}=1.86$, NS; female odour treatment $F_{1,67}=0.86$, NS). A similar ANOVA for the change in total IgG across the period of testing showed that IgG concentration increased significantly ($F_{1,67}=5.14$, $p=0.027$). There was also a significant effect of ATS treatment ($F_{2,67}=12.71$, $p<0.001$), with the increase in IgG being lower in ATS-treated mice than NRS controls, but once again, with a paradoxically lower increase still in sham-inoculated controls (Bonferroni $p<0.05$). Partial regression analysis revealed no significant association between the magnitude of change in either testosterone or corticosterone over the period of testing and change in IgG concentration (c.f. [7]), haemagglutination titre, or thymus weight (c.f. [9,10]).

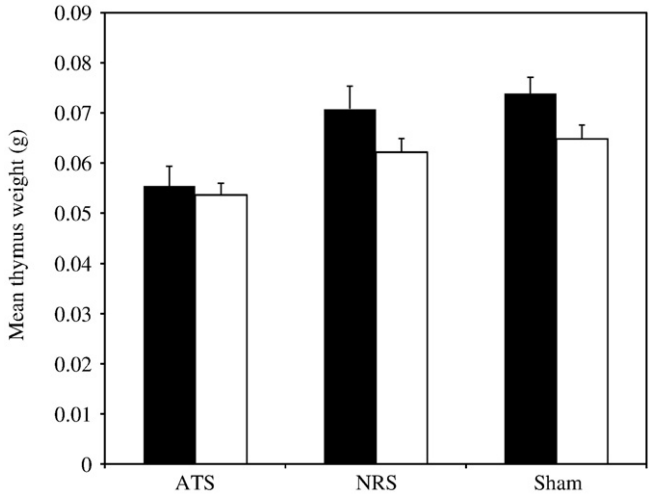

Fig. 6. Mean (±SE) thymus weight (g) in relation to ATS Treatment and female odour treatment (black bars = no odour; white bars = female odour).

3.5. Relationships between learning performance, immune responsiveness and hormone concentrations

The results above suggest that learning performance, in terms of the proportion of correct responses, was affected by ATS treatment independent of the apparently depressing effect on overall activity of ATS treatment itself. An important question, therefore, is whether learning performance showed any direct relationship with measures of immune responsiveness, and with the potentially immune-modulating hormones testosterone and corticosterone.

We carried out a stepwise partial regression analysis with the proportion of correct entries as the dependent variable and haemagglutination titre, the change in IgG, testosterone and corticosterone concentrations across the period of maze testing, terminal body weight, the number of arms visited per day and pre-experimental IgG concentration as independent variables. The only variable to enter the equation was haemagglutination titre, which showed a significant positive relationship with the proportion of correct entries ($t_{67}=2.31$, $p=0.024$; Fig. 5). A second stepwise regression, with latency to first correct choice as the dependent variable showed no significant relationship with any of the independent variables. The same was true of regression analysis of hormone measures only, including pre-experimental testosterone and corticosterone concentrations as additional independent variables.

3.6. Organ weights

Two-way ANOVA of organ weights with ATS and female odour treatments as factors (see Table 2) and terminal body weight as a covariate showed a significant effect of both factors on thymus weight (ATS treatment: $F_{2,65}=9.48$, $p<0.001$; female odour: $F_{1,65}=5.18$, $p=0.026$), with thymus weight being lower in ATS-treated mice (Bonferroni $p<0.05$), and lower in mice exposed to female odours (Fig. 6). No significant effects of either factor emerged for adrenal, testes, seminal vesicles, preputial gland, coagulating gland, kidney or heart weights (see Table 2). However, spleen weight differed significantly with ATS treatment ($F_{2,65}=21.83$, $p<0.001$), with ATS-treated mice having the largest spleens compared to the other two treatments (Bonferroni $p<0.05$).

Partial regression analysis showed that the proportion of correct maze entries decreased as seminal vesicle weight increased ($t_{68}=-2.30$, $p=0.025$) and the weight of the heart increased ($t_{68}=2.30$, $p=0.025$), i.e. mice with larger hearts and smaller seminal vesicles showed better learning. A similar analysis of latency to first correct entry resulted in a significant positive relationship with seminal vesicle weight ($t_{68}=2.07$, $p=0.042$), but a negative relationship with testes weight ($t_{68}=-2.90$,

$p=0.005$); i.e. mice with smaller seminal vesicles and larger testes found the correct arm more quickly.

4. Discussion

The results suggest that temporarily depressing cellular immunity in mice affected maze learning performance in the direction expected from previous studies of the relationship between learning and immune responsiveness in the BKW strain [13,21]. Mice with an ATS depressed immune system showed a reduced learning performance, in terms of proportion of correct entries. Interestingly across all groups those mice that showed the greatest immune responsiveness (haemaggluttination titre) showed the best learning performance. However, there was little evidence that this was related to any underlying hormonal changes associated with learning [13,21], as we found no relationship between hormone concentration and learning performance.

That ATS treatment depressed immune responsiveness was confirmed by reduced haemagglutination and total IgG titres, and lower thymus weights in ATS-treated animals compared with NRS controls, though sham-inoculated controls showed paradoxically low outcomes in the case of IgG. An increase in spleen weight in the ATS animals was as found previously [9,10] and was expected as a consequence of increased haemopoiesis following partial reduction in erythrocytes through cross-reactivity with ATS [26]. While ATS-treated animals emerged as having by chance had lower concentrations of total IgG prior to testing, the chance difference was controlled for in subsequent analyses. Interestingly, however, and in contrast with previous results, ATS-treated mice did not show a reduction in testosterone concentration and there was no association between the magnitude of change in testosterone concentration over the period of testing and any measure of immune responsiveness (change in total IgG, haemagglutination titre, thymus weight) (c.f. [9,10]). Thus there was no evidence here of testosterone being modulated in response to current immune status, perhaps due to the fact that mice have been exposed to male odours indicating the possibility of competition , consistent with the results from our previous study involving exposure to male odours during learning [21].

Learning over the period of exposure to the maze was shown by the increase in the proportion of correct entries and reduced latency to first correct choice over time. Males treated with ATS, however, made fewer correct choices over the period of testing than control mice, though latency to first correct choice was unaffected. The effect of ATS on the proportion of correct entries was independent of any changes in overall levels of locomotory activity, measured here as the total number of arm entries (see also [13]), so was not due to general physical malaise. Thus reduced learning performance appeared to be attributable to induced immune depression. The positive relationship between the proportion of correct entries and haemagglutination titre also suggests that learning effort was influenced by current immune responsiveness, with mice showing greater responsiveness performing better in the maze. This is consistent with the hypothesis that there is a trade-off between learning and immune function, i.e. mice with greater immune 'resources' can invest more in learning.

In contrast to previous studies [13,21], which showed task-dependent relationships between learning and corticosterone (see also [23,24,35]), there was no evidence that learning is related to circulating corticosterone concentration. Neither did any relationships emerge with testosterone. There was thus no evidence that the impact of ATS treatment on learning was mediated by associated changes in steroid hormone secretion. Given that in our previous studies we found a positive relationship between corticosterone and spatial learning [13], and a negative relationship between corticosterone and odour learning [21], it is possible that in this task the combination of spatial and odour components resulted in no relationship. Previous studies have also found contrasting results (e.g. [23]), which indicates that the effect corticosterone has upon learning the effects are context dependent, and probably weak.

Whether or not female odours were present in the subjects' home cage had no effect on learning performance, although mice exposed to the odours of females had reduced thymus glands. Thus, in contrast to the effects of female odours on other behaviours showing sensitivity to current immune status, such as aggression and sleep [9,10], learning, at least as measured here, appeared not to be influenced by them. One possible reason is that female odours were not present in the maze itself, which instead contained the odours of a range of males.

Previous studies have shown that increasing the demands of a learning task leads to a reduction in immune performance [13,21]. Here, we show for the first time that experimentally reducing immune performance reduces learning ability. In addition, individuals with the least responsive immune system show the greatest learning deficit. In our previous studies mice that learned the task well had reduced resistance to *Babesia* [13,21], but the learning task took place before the challenge to the immune system, i.e. mice invested in learning without any information on the need for a future immune response. In the current study, although immunodepressed mice show reduced learning, mice that had the resources to be able to respond most to the immune challenge were also able to afford more investment in learning, although what mediates this effect is unclear. In conclusion, this study shows that the functioning of the immune system has a direct response on learning ability, unrelated to the direct effects such as malaise, or activity levels. A discussion of the mechanism whereby immune function and performance in cognitive tasks interact is beyond the scope of this paper. However, recent work suggests that the trade-off between learning and immune function is mediated by cytokines (e.g. [19,20,36]), and/or T cells [37,38], see reviews [39,40]. T cells have been shown to be important in neurogenesis [41], and mice with severe immune deficiency have been shown to have impaired memory acquisition (although not retrieval) which can be restored by transfer of T cells [37]. Given that ATS treatment reduces the T cell population, it is possible that our results are explained by a lack of memory acquisition related to the T cell deficit.

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