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Enhanced habituation and decreased anxiety by environmental enrichment and possible attenuation of these effects by chronic α-tocopherol (vitamin E) in aging male and female rats

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ABSTRACT

Middle-aged 330-day-old male and female hooded rats were group-housed for nearly 5 months in either standard cages, or in cages containing objects. Each cage also provided either pure water, or a solution of vitamin E (DL-α-tocopherol acetate) for drinking. Records were kept of averages for each cage of the rats' body weights and the volume of fluid/100 g average body weight drunk. The average daily dose of tocopherol was approximately 162 and 173 mg/kg for males and females respectively. Males (but not females) kept in enriched cages weighed less than those from standard cages. They also drank less fluid than females who also drank more tocopherol solution than males. When 490+ days old, for rats provided with water, enrichment led to decreased open-field ambulation and increased within-session decrements in the response (habituation). Enrichment also led to decreased occupancy of the center of the apparatus for males only and, for all rats combined, increased grooming behavior. It was concluded that the effects of enrichment on aged rats were due to increased within-session habituation to novelty and decreased anxiety similar to what has been suggested for younger animals. Tocopherol appeared to interfere with effects of enrichment possibly because of pro-oxidant-related increased anxiety.

1. Introduction

There is increasing acceptance of the view that environmentally enriching experiences can slow down aging of the brain in both humans (Shimamura et al., 1995) and animals (Frick and Fernandez, 2003) and possibly provide some protection against neurodegenerative changes that accompany disorders such as Alzheimer's Disease (Arendash et al., 2004; Jankowsky et al., 2005). Current interest in environmental enrichment is largely derived from over 40 years of research with rats and mice that has demonstrated the benefits for brain and behavioral development of early exposure to enriched social, physical activity-related and perceptual stimulation (Renner and Rosenzweig, 1987). Enrichment has typically been provided by the provision of a number of objects (or toys) for group-housed animals that are designed to encourage exploration, manipulation and physical activity. In the intact brain, some of the reasons that have been proposed for enrichment-related behavioral outcomes, such as improved learning and memory (Fordyce and Farrar, 1991; Renner and Rosenzweig, 1987), have included increased hippocampal neurogenesis, dendrite branching and synapse formation, enhanced long-term potentiation, and increased central monoaminergic and cholinergic activity (van Praag et al., 2000). In addition to producing cognitive changes, environmental enrichment has also been reported to decrease anxiety and associated stress-related responses in both mice (Benaroya-Milshtein et al., 2004; Fernández-Teruel et al., 1997) and rats (Roy et al., 2001).

There are a number of studies on record of the neurobehavioral status of older rats and mice exposed to environmental enrichment from a much earlier age (Kobayashi et al., 2002; Leal-Galicia et al., 2008; Pham et al., 1999; Segovia et al., 2008). For example, open-field locomotor activity has been shown to either increase (Gardner et al., 1975) or more commonly decrease (Elliot and Grunberg, 2005) in animals exposed to enrichment when they were younger. Such variability in past outcomes might have arisen from the procedure's propensity for reflecting either exploration of novel stimuli, or fear-motivated attempts to escape from the apparatus (Renner and Rosenzweig, 1987). It is therefore conceivable that either effect could result from low fear namely, increased ambulation due to more curiosity-related exploration, or decreased ambulation due to lower levels of escape motivation. Alternatively, it has been suggested that the lower activity shown by enriched animals may have been due to faster rates of habituation to the novelty of the open field (Schrijver et al., 2002; Zimmerman et al., 2001). Although there have been fewer investigations of the effects of enrichment that is not experienced until older ages are reached, there is some evidence of behavioral

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benefits for rats (Doty, 1972) and mice (Frick and Fernandez, 2003) and changes in the brain (such as neurogenesis in the mouse hippocampus, Kempermann et al., 1998, 2002) in older-exposed animals. Therefore, a major aim of the present study was to determine if environmental enrichment that was not experienced until rats were middle-aged to elderly could have similar effects on open-field behavior to those observed with much younger-exposed subjects. The main focus was on possible changes in habituation of ambulation along with the extent to which fear or anxiety might have played a part. Since all rats were housed in groups of three, social enrichment was not manipulated. Rather, environmental enrichment was confined to the presence of regularly changed objects in standard laboratory cages.

There is also evidence that chronic treatment with antioxidants can benefit cognition in elderly humans and animals (Martin et al., 2002; Socci et al., 1995) presumably because of the reduction in oxidative stress associated with greater aging-related sensitivity to reactive oxygen species that lead to cell death and cognitive declines (Cantuti-Castelvetri et al., 2000; Golden et al., 2002). In this respect, a particular antioxidant that has received a considerable amount of attention is vitamin E which has been shown to attenuate impairments during the normal aging process in both humans and animals (Cantuti-Castelvetri et al., 2000). But in addition to its importance for cognition, vitamin E has also been associated with anxiety. For example, vitamin E deficiency and higher anxiety has been described for mice that were deficient in phospholipid transfer protein (Desrumaux et al., 2004) or α-tocopherol transfer protein (Gohil et al., 2003). Although 6 days of treating normal rats with vitamin E was shown to attenuate stress-induced changes in brain biochemical indices of stress (Shaheen et al., 1993), chronic treatment of rats characterized by increased sensitivity to oxidative stress did not reduce their high anxiety as measured by ambulation, defecation and occupancy of center squares in an open field, and open-arm entries in a plus maze (Kolosova et al., 2006). However, in the same study, normal rats showed evidence of increased anxiety with vitamin E. In view of these conflicting outcomes, it was decided to include chronic vitamin E treatment with environmental enrichment in order to assess the vitamin's specific effect on anxiety-related behavior as well as within-session habituation of open-field ambulation, and also the extent to which it might interact with enrichment. The possible role of anxiety in any observed effects of either treatment (or their combination) was investigated by recording open-field defecation and center square occupancy, and preferences for the light versus dark half of a light–dark box. This apparatus measures rodents' fear of entering and spending time in the more aversive illuminated environment (Hascöet et al., 2001; Sánchez, 1996).

As with many areas of biobehavioral research involving laboratory rodents (Hughes, 2007), the vast majority of studies of later effects of enrichment and vitamin E have been conducted with male subjects only. In view of the relative lack of information about females and some evidence of sex differences in the behavioral and/or physiological effects of early environmental enrichment (Elliot and Grunberg, 2005; Joseph, 1979; Juraska et al., 1985; Peña et al., 2006) as well as tocopherol deficiency (Amelink et al., 1991) or supplementation (Salonen et al., 2003), rats of both sexes were investigated in the present study.

2. Methods

2.1. Subjects

The subjects were 36 male and 33 female PVG/C middle-aged hooded rats approximately 330 days old that had been bred in the Animal Facility of the Department of Psychology, University of Canterbury. They were housed in groups of three same-sexed individuals in 550×360×220 mm-high plastic cages with a 500 ml communal drinking bottle, and free access to standard laboratory food. The cages were kept in a room with an ambient temperature of 22 °C±2 °C, humidity of 48%±10% and a 12 h light/dark cycle (lights on at 08.00 h). All treatment and testing procedures complied with the requirements of Parts 5 (Codes of Welfare) and 6 (Use of Animals in Research, Teaching and Testing) of the New Zealand Animal Welfare Act (1999) and had been approved by the Animal Ethics Committee of the University of Canterbury.

2.2. Environmental enrichment

Enrichment was provided in half of the cages in the form of four different objects or "toys" randomly selected from a pool including large glass marbles, various sized glass jars and metal lids, PVC and plastic tunnels, plastic cups, wooden see-saws, pottery ornaments and small metal household utensils. These objects were chosen to encourage manipulation and physical activity as well as general exploration. Once a week the objects were replaced with new objects in such a way that exactly the same set of four was not experienced by each cage of rats more than once. The remaining half of the cages comprised the standard condition since they were devoid of any objects (other than each rat's other cage-mates) for the duration of the study.

2.3. Tocopherol treatment

For 10 days prior to imposition of the cage environment conditions, the average body weights and volume of water drunk by each cage of male and female rats were recorded each day. The average weight for males was 380 g and 210 g for females. The average volume of water drunk/day by males was 22 ml (or 5.79 ml/100 g) and 32 ml (or 13.97 ml/100 g) for females. These data were used to calculate the amount of vitamin E (in the form of 500 mg/ml DL-α-tocopherol acetate water immiscible liquid, Merck Chemicals Ltd) needed to be added to the communal water bottle in each cage to provide approximate daily doses of 150–200 mg/kg/rat which were in the range of those previously shown to be behaviorally effective in aged mice and rats (Mcdonald et al., 2005; Socci et al., 1995). The calculations resulted in tocopherol drinking solutions of 3.5 mg/ml for males, and 1.5 mg/ml for females. Half the rats in each enrichment condition were provided with tocopherol in the communal drinking bottles, and the other half were provided with pure water. The water bottles were replenished with the appropriate drinking fluid once or twice a week at which times the volume drunk was measured. From the beginning until the end of the 4+ months of treatment, all rats were weighed approximately once a week and, for each cage, the average body weights and volumes of fluid drunk (and thus also the average doses of tocopherol/average weight) were calculated. These averages for 9 occasions (approximately 2 weeks apart) when the recording of weights and fluid/doses consumed coincided comprised the data used in subsequent statistical analyses.

While the practice of providing tocopherol in communal drinking water does not enable accurate assessments of dose intake for individual rats, it avoids the stress of socially isolating animals or subjecting them to aversive long-term daily injection or oral gavage administration procedures. And since it was intended that social density, and thus social enrichment, should be kept constant for all groups, individual housing was not an option. Besides, the provision of tocopherol in communal drinking water has been successfully employed in a number of earlier studies (Ishihara et al., 2000; Kelso et al., 2002; Zhang et al., 2004).

2.4. Behavioral testing apparatus and methods

All rats were given a single trial in an open field, followed several minutes later by one in a light–dark box.

2.4.1. Open field

The open field comprised a 600×600-mm square Perspex arena with transparent 300 mm-high walls, and a black floor which was divided into 16 numbered squares by means of a grid of white intersecting lines. It sat on a 700-mm-high table and was illuminated by dim fluorescent room lighting (47 lx).

Each rat was placed in the center of the apparatus for a 5-min trial. The observer stood approximately 1.5 m away from the open field in a position that enabled the rat to be clearly seen through the transparent walls. Every 3 s (signaled by a tone from a battery-operated timer and delivered through an ear-piece) the particular numbered square the rat was occupying, and if it was rearing on its hind legs or grooming were manually recorded on a prepared data sheet. A total of 100 such records were made during the session. At the end of the trial, fecal boluses left in the apparatus (defecation) were counted and removed. From the square occupancy data, the total distance traveled in the apparatus (ambulation) was later estimated by counting the number of times the rat occupied a square that was different from that occupied on the occurrence of an immediately preceding 3-s signal (Hughes and Beveridge, 1987). It was also possible to determine the number of signals when it had been observed occupying one of the four numbered center squares of the apparatus. Consequently rearing, grooming and center occupancy data comprised frequency counts of each response. Not withstanding concerns about whether the open-field procedure actually measures exploration or fear (Renner and Rosenzweig, 1987), lower frequencies of open-field ambulation and rearing, and higher frequencies of grooming and defecation are often regarded as indices of higher anxiety (Archer, 1973; Belzung, 1999; Brain and Marrow, 1999). However, within-session changes in these responses (especially ambulation) may also reflect rates of habituation to the novelty of the apparatus irrespective of whether the responses are motivated by curiosity or fear. Therefore, because of the suggestion that enrichment-related reductions in ambulation were due to faster habituation by enriched animals (Schrijver et al., 2002; Zimmerman et al., 2001), the numbers of times individual rats occupied an open-field square that was different from that occupied during a preceding observation were calculated for the two halves of each 5-min observation period. The differences in ambulation scores between the first and second halves of the observation period were then calculated as a percentage of the first half scores to provide a measure of per cent decrement.

2.4.2. Light–dark box

The light–dark box consisted of two 300-mm long×200-mm wide×300-mm-high compartments separated by a wooden partition. Movement between the compartments was possible by a 100-mm×100-mm opening in the center of the partition that could be closed with a removable horizontal slide. A hinged wooden lid covered the dark side of the apparatus lid while the light side was covered by a clear Perspex lid. The box sat on a 700-mm-high table and was illuminated by 47 lx fluorescent room lighting.

Each rat was left in the dark side for about 30 s with the slide separating the two compartments in place which was then withdrawn thereby allowing the rat free access to both sides. The observer stood approximately 1 m away from the apparatus in a position that enabled the rat's emergence to be clearly seen. For 5 min, the total time spent in the light side was recorded with a manually operated stop-clock during which time the number of entries of this side was also counted. At the end of the trial, the total number of fecal boluses left in the apparatus (defecation) was noted.

2.5. General procedure

Assignment of the rats to the cage environment and drinking solution conditions began when they were approximately 340 days old and thus were middle-aged (Conrad and Roy, 1995; Wolden-Hanson et al., 2000). This treatment lasted for nearly 5 months. From about 490 days after birth, by which time they would have been entering old age (Chakraborti et al., 2008; Lee et al., 2004; Le Grevès et al., 2002), the rats experienced their open-field and light–dark box tests. Each piece of apparatus was thoroughly cleaned and washed with disinfectant between rats.

2.6. Statistical analyses

Average body weights, fluid consumption and tocopherol dose during the treatment period were subjected to separate measurements (9)×cage environment (standard, enriched)×drinking solution (water, tocopherol)×sex (male, female) repeated measures ANOVAs. When significant effects occurred, individual post hoc comparisons were made by means of Scheffé tests ($P<0.05$). The data points used for these analyses were averages for each cage in the eight possible combinations of the three experimental conditions namely, cage environment, drinking solution and sex of the rats.

Except for open-field ambulation, all behavioral data (including per cent decrement in open-field ambulation between the first and second halves of the observation periods) were subjected to separate sex×cage environment×drinking solution ANOVAs. Following any significant interactions, post hoc comparisons between groups were made with Scheffé tests ($P<0.05$). In the ANOVA for open-field ambulation, the repeated within-session factor (scores during each

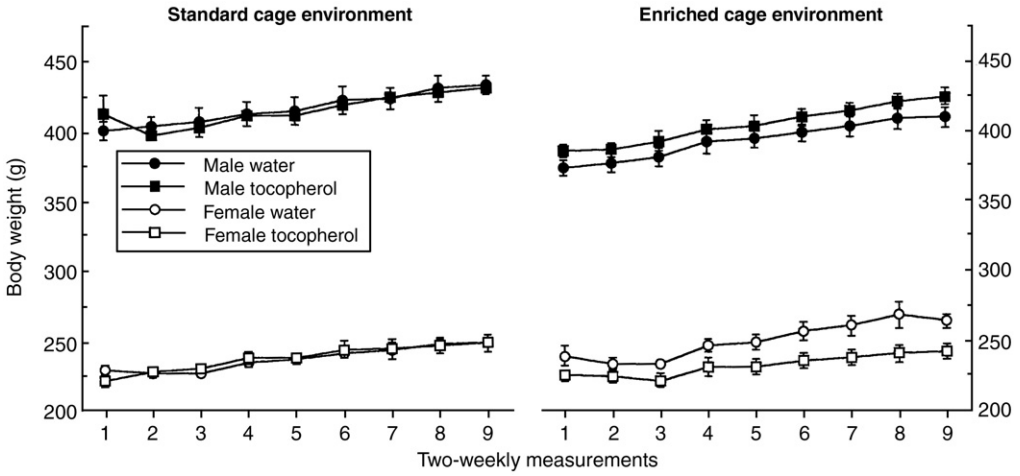

Fig. 1. Mean ± S.E.M. average body weights for male and female rats kept in standard and enriched cages, and that were supplied with water or a tocopherol drinking solution.

half of the observation periods) was included with the other three factors.

3. Results

Near the end of enrichment and tocopherol treatment and before the beginning of behavioral testing, one male and two females died for unknown reasons other than aging. The male rat was in the enriched/water condition, and both females were in the standard/tocopherol condition.

3.1. Average body weights, fluid consumption and tocopherol dose

3.1.1. Average body weights

The average body weights of cages of male and female rats kept in standard and enriched conditions and supplied with water or tocopherol solution for drinking are outlined in Fig. 1.

The average weights of cages of all female rats combined (mean ± S.E.M. = 238.99 ± 3.55 g) were obviously significantly lighter than those for males (407.03 ± 4.32) right throughout the measurement period [$F(1,15) = 901.18$, $P<0.0001$]. For each sex, the average weights increased during the treatment period. These increases were significant for both sexes combined [$F(8,128) = 111.9$, $P<0.0001$]. Although neither the cage environment nor drinking solution main effect was significant [$F(1,15) = 3.00$, $P>0.1$; $F(1,15) = 0.26$, $P>0.6$, respectively], there was a significant sex × cage environment interaction [$F(1,15) = 7.75$, $P<0.015$]. From inspection of Fig. 1, it is clear that this arose from males kept in the enriched cages weighing significantly lighter than those kept in standard cages [$P<0.05$]. There was no significant effect of cage environment on female average weight.

3.1.2. Average fluid consumption

Average fluid consumption at each of the 9 measurements was calculated as ml drunk per 100 g average body weight. For each measurement, results of these calculations for male and female rats kept in standard and enriched cages and supplied with water or tocopherol solution are shown in Fig. 2.

For all measurements combined, average fluid consumption was significantly higher for female rats [mean ± S.E.M = 12.63 ± 0.39] than for males [4.77 ± 0.11, $F(1,15) = 111.64$, $P<0.0001$], and for both sexes combined, the main effect of two-weekly measurements was also significant [$F(1,15) = 8.74$, $P<0.0001$]. However, a significant interaction between sex and measurements [$F(8,128) = 10.64$, $P<0.0001$] revealed that, as is apparent in Fig. 2, the change over time was confined to females. They tended to drink slightly more of either fluid near the end of the measurements than at the beginning. There was also a significant interaction between sex and drinking solution [$F(1,15) = 16.74$, $P<0.001$] which, as shown in Fig. 2, can be accounted for by females (but not males) in each cage environment that were provided with tocopherol solution consuming significantly more fluid than those provided with water [$P<0.05$].

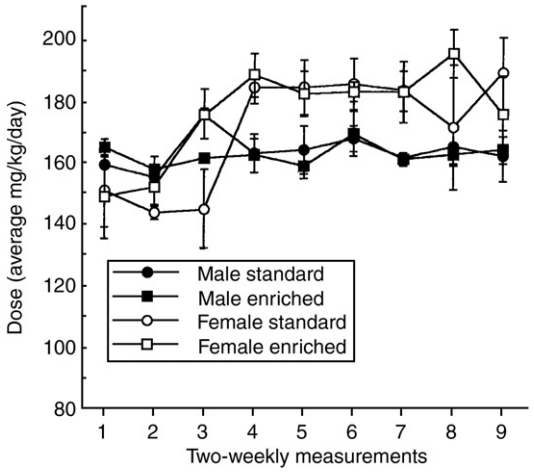

Fig. 3. Mean ± S.E.M. average daily dose of tocopherol/kg average body weight recorded on 9 occasions during the 4+ months of environmental enrichment and tocopherol treatment for male and female rats kept in standard and enriched cages, and that were supplied with water or a tocopherol drinking solution.

3.1.3. Average tocopherol dose

For the cages of tocopherol-treated rats, average doses consumed at each of the measurements were calculated separately for males and females kept in standard and enriched cages from the average volume of fluid drunk in relation to the amount of tocopherol (mg/ml) it contained. The results are outlined in Fig. 3.

The overall sex difference in average dose consumed for both cage environments combined was significant [males = 162.44 ± 3.43 mg/kg/day, females = 172.65 ± 6.43 mg/kg/day, $F(1,8) = 6.78$, $P<0.035$], as were the changes during treatment for both sexes and cage environments combined [$F(1,8) = 5.69$, $P<0.0001$]. However, as shown by a significant sex × measurements interaction [$F(8,64) = 4.39$, $P<0.001$], for the first three measurements, there were no significant sex differences for both cage environments combined, but then females consumed significantly higher doses than males for the remainder [$P<0.05$]. This was due to a significant increase from the

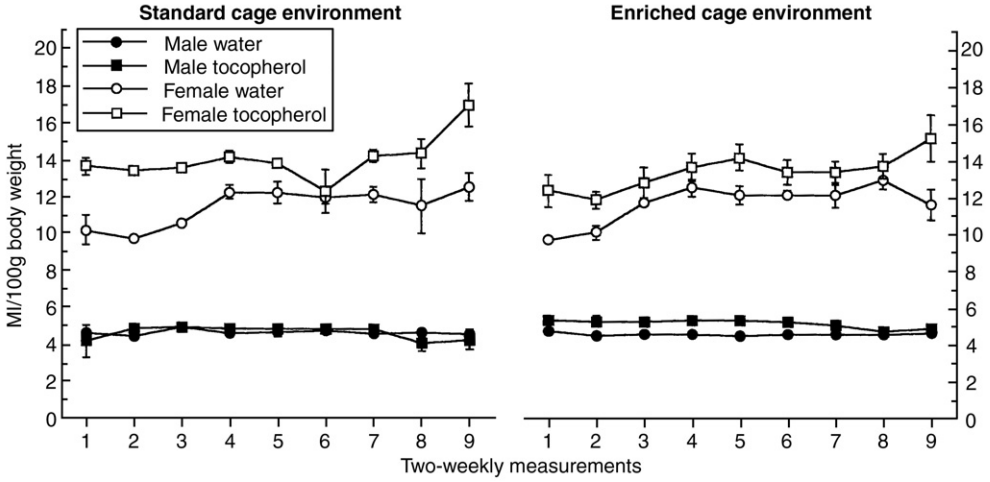

Fig. 2. Mean ± S.E.M. average fluid consumption/100 g average body weight recorded on 9 occasions during the 4+ months of environmental enrichment and tocopherol treatment for male and female rats kept in standard and enriched cages, and that were supplied with water or a tocopherol drinking solution.

Table 1
Mean (±S.E.M.) 3-s observations of open-field ambulation and their per cent (%) decrement from the first to the second half of each observation period for male and female rats kept in standard and enriched cages that were supplied with water or tocopherol drinking solutions (see text for ANOVA results for main effects and interactions).

	Standard cage environment				Enriched cage environment			
	Water drinking solution		Tocopherol drinking solution		Water drinking solution		Tocopherol drinking solution	
	Males	Females	Males	Females	Males	Females	Males	Females
	(n=9)	(n=9)	(n=9)	(n=7)	(n=8)	(n=9)	(n=9)	(n=9)
Ambulation[a,c]	53.91 (±2.90)	58.62 (±3.87)	54.80 (±2.81)	41.33 (±4.43)	42.56 (±2.15)	45.12 (±5.37)	47.45 (±2.93)	46.89 (±2.73)
% decrement[b,c]	22.55 (±11.18)	34.49 (±6.98)	26.84 (±6.26)	56.64 (±6.80)	42.89 (±6.70)	49.92 (±7.48)	24.15 (±8.47)	30.72 (±6.34)

[a] Cage environment main effect significant.
[b] Sex main effect significant.
[c] Cage environment × drinking solution interaction significant.

first three to the last six measurements for all females [$F(8,40)=5.69$, $P<0.0001$], but not for males [$F(8,40)=1.48$, $P>0.1$].

3.2. Behavioral changes

3.2.1. Open-field responses

Effects of sex, enrichment and tocopherol treatment for males and females on ambulation and per cent decrement from the first to the second half of the observations are outlined in Table 1.

The ANOVA for ambulation also included (as a repeated factor) differences between frequencies of the response recorded in each half of the observation periods. Mean ± S.E.M. frequencies for the first and second halves for all rats combined were 29.60 (±0.73) and 18.98 (±0.91) respectively. Although the difference between them was significant [$F(1,57)=14.88$, $P<0.0001$], there were no significant interactions between this repeated factor and any of the other three non-repeated factors. For all rats combined, ambulation was significantly affected by cage environment [$F(1,57)=7.18$, $P<0.01$], but not by either drinking solution [$F(1,57)=1.17$, $P>0.2$] or sex [$F(1,57)=0.36$, $P>0.3$]. Percent decrement in the response was significantly affected by sex, with females displaying greater decrements than males [$F(1,57)=6.15$, $P<0.05$], but not by either cage environment [$F(1,57)=0.10$, $P>0.7$] or drinking solution [$F(1,57)=0.27$, $P>0.6$]. However, for both measures, the results are more appropriately considered in the light of significant interactions between the two manipulations [$F(1,57)=4.93$, $P<0.05$; $F(1,57)=8.26$, $P<0.01$, respectively] that are outlined in Figs. 4 and 5 for both sexes combined.

Enrichment significantly decreased ambulation for rats provided with water for drinking, but not for those provided with the tocopherol solution. Similarly, the level of decrement in the response was significantly greater for enriched animals provided with water (Fig. 5), thereby suggesting faster habituation, but not for those provided with tocopherol. Tocopherol also significantly reduced the decrement for rats kept in enriched but not for those kept in standard cages.

Effects of sex, enrichment and tocopherol treatment for male and female rats on the four remaining responses recorded in the open field can be seen in Table 2.

For all rats combined, cage environment had no significant main effect on center occupancy [$F(1,57)=1.13$, $P>0.2$], rearing [$F(1,57)=2.79$, $P>0.1$] or defecation [$F(1,57)=0.35$, $P>0.5$], but there was a significant interaction between cage environment and the sex of the rats for center occupancy [$F(1,57)=8.58$, $P<0.005$] described below. However, all rats kept in enriched cages groomed significantly more often than those kept in standard cages [enriched mean ± S.E.M = 1.59 ± 0.40, standard = 3.67 ± 0.65, $F(1,57)=5.97$, $P<0.05$]. There was no significant main effect of drinking solution on any measure [center occupancy, $F(1,57)=0.09$, $P>0.7$; rearing, $F(1,57)=2.86$, $P>0.1$; grooming, $F(1,57)=0.03$, $P>0.8$; defecation, $F(1,57)=0.62$, $P>0.4$]. Although all male rats occupied the center squares of the apparatus more often than all females [male mean ± S.E.M = 19.12 ± 1.45, females = 14.74 ± 5.66, $F(1,57)=5.66$, $P<0.05$], this result will be evaluated below in the light of the significant interaction between sex and cage environment for this measure. The two sexes did not differ significantly in either rearing [$F(1,57)=0.08$, $P>0.8$] or grooming [$F(1,57)=3.53$, $P>0.06$]. However, all males [mean ± S.E.M = 1.94 ± 0.33] defecated significantly more often than all females [mean ±

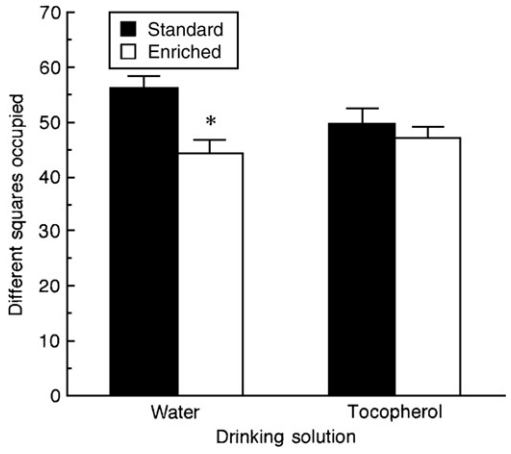

Fig. 4. Mean ± S.E.M. open-field ambulation (different squares occupied) for male and female rats combined kept in standard and enriched cages that were provided with water or tocopherol drinking solutions. *Difference between the standard and enriched cage conditions significant ($P<0.05$) for that particular drinking solution.

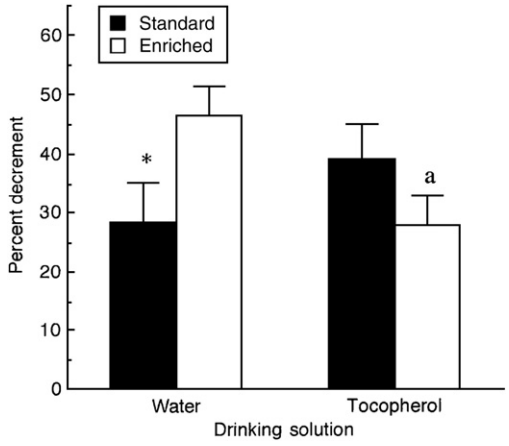

Fig. 5. Mean ± S.E.M. per cent decrement in ambulation from the first to the second half of the observation periods for male and female rats combined kept in standard and enriched cages that were provided with water or tocopherol drinking solutions. *Difference between the standard and enriched cage conditions significant ($P<0.05$) for that particular drinking solution. [a]Difference between the water and tocopherol conditions significant ($P<0.05$) for that particular caging condition.

Table 2
Mean (±S.E.M.) 3-s observations of open-field center occupancy, rearing, grooming and defecation for male and female rats that were kept in standard and enriched cages, and that were supplied with water or tocopherol drinking solutions (see text for ANOVA results for main effects and interactions).

	Standard cage environment				Enriched cage environment			
	Water drinking solution		Tocopherol drinking solution		Water drinking solution		Tocopherol drinking solution	
	Males	Females	Males	Females	Males	Females	Males	Females
	($n=9$)	($n=9$)	($n=9$)	($n=7$)	($n=8$)	($n=9$)	($n=9$)	($n=9$)
Center occupancy[c]	21.76 (±3.18)	15.25 (±2.86)	23.87 (±2.36)	9.83 (±1.40)	14.53 (±2.26)	14.12 (±3.36)	16.31 (±2.75)	18.11 (±2.26)
Rearing	22.46 (±3.51)	26.88 (±2.72)	20.24 (±2.42)	18.33 (±3.14)	18.13 (±2.46)	20.38 (±2.28)	20.13 (±2.42)	17.22 (±2.20)
Grooming[a]	0.54 (±0.34)	1.75 (±0.86)	1.43 (±0.80)	3.50 (±0.96)	3.66 (±1.66)	4.38 (±1.25)	1.99 (±1.11)	4.11 (±1.26)
Defecation[b]	1.50 (±0.60)	0.25 (±0.16)	1.39 (±0.50)	1.33 (±0.67)	2.07 (±0.69)	0.50 (±0.38)	2.68 (±0.84)	0.11 (±0.10)

[a] Cage environment main effect significant.
[b] Sex main effect significant.
[c] Sex × cage environment interaction significant.

S.E.M = 0.48 ± 0.18, $F(1,57) = 13.67$, $P<0.001$]. As outlined in Fig. 6, the significant interaction between the sex of the rats and their cage environment for center occupancy revealed that males (but not females) from enriched cages occupied the center squares of the apparatus significantly less often than those from standard cages.

Although the main sex effect was shown to be significant for this response, the afore-mentioned interaction showed that it was only significant for all rats kept in standard cages. Females from these cages occupied significantly fewer center squares than their male counterparts. As it seemed possible that center square occupancy was merely a product of overall ambulation, the two responses were correlated for each sex separately. In neither case was the correlation significant [males, $r(33) = 0.30$, *ns*; females, $r(30) = 0.19$, *ns*].

3.2.2. Light–dark box responses

Effects of sex, enrichment and tocopherol treatment for males and females on the three responses recorded in the light–dark box are shown in Table 3.

For all rats combined, cage environment had no significant main effect on either entries of the light side [$F(1,57) = 0.06$, $P>0.7$] or time spent in this side [$F(1,57) = 0.02$, $P>0.8$]. However, all rats kept in enriched cages [mean ± S.E.M = 0.52 ± 0.16] defecated less often than those kept in standard cages [1.12 ± 0.21, $F(1,57) = 4.77$, $P<0.05$] There were no significant main effects of either drinking solution or sex respectively on any measure [entries of the light side, $F(1,57) = 0.86$, $P>0.3$, $F(1,57) = 0.86$, $P>0.3$; time spent in the light side, $F(1,57) = 0.07$, $P>0.7$, $F(1,57) = 0.04$, $P>0.8$; defecation, $F(1,57) = 0.02$, $P>0.8$, $F(1,57) = 0.08$, $P>0.6$].

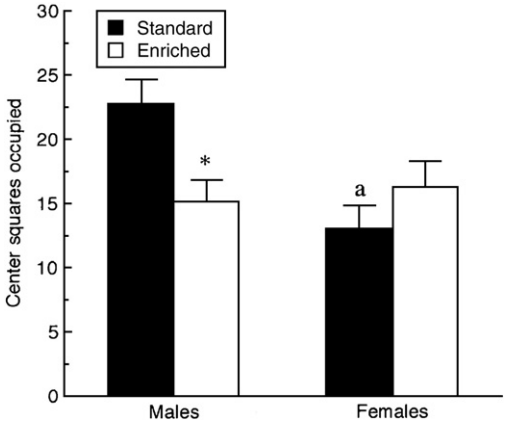

Fig. 6. Mean ± S.E.M. occupancy of open-field center squares for male and female rats (water and tocopherol conditions combined) kept in standard and enriched cages. *Difference between the standard and enriched cage conditions significant ($P<0.05$) for that particular sex. [a]Difference between the sexes significant ($P<0.05$) for that particular caging condition.

4. Discussion

4.1. Average body weights, fluid consumption and tocopherol dose

While the average body weights of all rats combined significantly increased during the nearly 5 months of enrichment and tocopherol treatment, the males kept in enriched cages were significantly lighter throughout than those in the standard condition. Similar findings for male rats exposed to environmental enrichment at earlier ages than the rats in the present investigation have been reported by several other authors (Diamond et al., 1965; Fiala et al., 1977; Hoffmann et al., 2009; Hughes, 1971; Moncek et al., 2004; Peña et al., 2006) even though the opposite effect has been reported to be the general outcome (Chapillon et al., 2002). The lack of a similar effect for female rats differed from that shown in an earlier study with younger-exposed rats in which females kept in enriched conditions were also lighter than those kept in standard cages (Peña et al., 2006). This suggests that, for the lighter females, either enrichment effects on body weight might be age-related or else the present result was due to a floor effect. While it is not possible to conclusively state why the male rats kept in enriched cages were lighter than those kept in standard conditions, it is possible that they spent less time eating (Fiala et al., 1977) because of curiosity-invoking distractions provided by the various objects. Alternatively, the weight difference might have been due to the observation that enrichment appears to make rats more active in their cages (Moncek et al., 2004) thereby contributing to their lower weights because of higher energy expenditure. Unfortunately, neither the amount of food eaten nor activity in the rats' cages was recorded in the present study. Interestingly, research with mice suggests the opposite effect of enrichment to that described for rats namely heavier body weights for males (Chapillon et al., 1999; Roy et al., 2001) but lighter weights for females (Chapillon et al., 1999) thereby demonstrating that, in addition to mouse strain (Chapillon et al., 1999), species might also be important in determining enrichment effects on body weight.

The higher fluid intake relative to body weight shown by female rats is consistent with other findings and has been accounted for by more diuretic activity than males because of their lower levels of circulating hormones (such as arginine vasopressin) that are related to antidiuresis (McGivern et al., 1996; Wang et al., 1980). There was also a sex difference in the pattern of drinking over time and the effects of the addition of tocopherol to the drinking solution — females (but not males) drank more of the solution near the end of the treatment period than at the beginning thereby suggesting a possible age-related increase in diuresis during the 4+ months. Females alone also drank more tocopherol solution than water possibly because of the vitamin's diuretic properties that can accompany high doses in humans (Vogelsang and Shute, 1946). Alternatively, since the male rats were provided with a more concentrated (and possibly more aversive) solution of tocopherol than females (3.5 mg/ml versus 1.5 mg/ml), this

Table 3
Mean (±S.E.M.) entries of and time (s) spent in the light side of the light–dark box, and fecal boluses (defecation) left in the apparatus for male and female rats kept in standard and enriched cages, and that were supplied with water or tocopherol drinking solutions (see text for ANOVA results for main effects and interactions).

	Standard cage environment				Enriched cage environment			
	Water drinking solution		Tocopherol drinking solution		Water drinking solution		Tocopherol drinking solution	
	Males	Females	Males	Females	Males	Females	Males	Females
	($n=9$)	($n=9$)	($n=9$)	($n=7$)	($n=8$)	($n=9$)	($n=9$)	($n=9$)
Entries of light	3.40 (±0.41)	3.38 (±0.32)	3.41 (±0.38)	3.33 (±0.21)	3.07 (±0.46)	4.38 (±1.02)	2.96 (±0.30)	3.00 (±0.29)
Time in light	81.72 (±14.89)	83.38 (±14.80)	113.93 (±11.39)	87.83 (±17.86)	99.93 (±27.18)	95.38 (±15.88)	77.38 (±26.99)	94.89 (±17.42)
Defecation[a]	1.27 (±0.43)	0.75 (±0.31)	1.38 (±0.44)	1.17 (±0.60)	0.61 (±0.38)	0.75 (±0.41)	0.22 (±0.20)	0.44 (±0.24)

[a] Cage environment main effect significant.

may have decreased their consumption of the solution relative to water. Although all female rats combined consumed slightly higher doses of tocopherol than all males, this sex difference occurred only for the last six measurement dates. For the first three of these, the sex difference was negligible. This situation clearly arose from the higher doses consumed by females (but not males) as the treatment period progressed because of their increasing fluid intake during this time span (discussed above). However, it seems unlikely that the small difference between females and males in overall doses consumed (<6%) was an important factor in determining subsequent outcomes.

4.2. Behavioral changes

The lower ambulation in the open field confined to rats from enriched cages that had been provided with water, is consistent with what has been reported for animals exposed to enrichment when much younger (Elliot and Grunberg, 2005). Because low levels of locomotor activity are often believed to reflect heightened anxiety (Archer, 1973; Belzung, 1999), it might appear that enrichment had increased, rather than produced the expected decrease in this state reported by others (Benaroya-Milshtein et al., 2004; Fernández-Teruel et al., 1997; Roy et al., 2001). However, because of the same pattern of results for the measure of within-session habituation, namely percent decrement in ambulation from the first to the second half of the observation periods, it seems more likely that enrichment had led to faster habituation to novelty, as suggested for animals kept in enriched cages when younger (Kempermann et al., 2002; Schrijver et al., 2002; Zimmerman et al., 2001). Contrary to what might have been expected from the beneficial effects of tocopherol reported earlier (Mcdonald et al., 2005; Socci et al., 1995), the vitamin interfered with rather than enhanced this effect of enrichment. (This was evident in the significant reduction in habituation shown by tocopherol-treated rats kept in enriched cages.) It is conceivable that this outcome could have arisen from tocopherol-induced anxiety similar to that noted previously (Kolosova et al., 2006). To ascertain this, it would be useful in future research to employ a range of doses and varying periods of administration along with more precise tests of anxiety.

However, the situation regarding possible enrichment effects on habituation is complicated by evidence of lower occupancy of the center squares of the open field by males (but not females) from enriched cages. Since this measure was not significantly correlated with the ambulation scores, it was probably not just a reflection of lower enrichment-related locomotor activity. This result along with evidence of more grooming behavior (often considered in recent times to indicate higher anxiety, Kalueff and Tuohimaa, 2005) amongst all rats from enriched cages might therefore suggest an increase in their anxiety. Increased grooming by rats exposed to enrichment when younger has also been reported elsewhere but not accounted for (Pham et al., 1999). But, there is by no means complete agreement that grooming is always a reflection of anxiety or responsiveness to stress. For example, Bolles (1960) preferred to regard it as a response that occurs on completion of a sequence of other acts. This was later supported by a demonstration of a within-session decrease in exploration accompanied by an increase in grooming over a 15-min observation period in a novel exploration box during which anxiety (and thus grooming) might have been expected to decrease (Hughes, 1968). A similar within-session increase in grooming has been reported more recently along with the conclusion that, any restorative function the response might have following exposure to stress is delayed and not directly related to the strength of the stressor (van Erp et al., 1994). Also, if center squares occupancies were indeed a measure of anxiety, then the sex difference favoring males for rats kept in standard cages is inconsistent with this sex being more anxious than females (Gray, 1971). However, the males' higher level of open-field defecation in the present study suggests that they were indeed more anxious than females. And finally, the lower defecation rate shown by all enriched subjects in the light–dark box is indicative of lower enrichment-related anxiety reported previously (Benaroya-Milshtein et al., 2004; Fernández-Teruel et al., 1997; Roy et al., 2001). Therefore, on balance, it seems unlikely that the enrichment effects on open-field ambulation were due to higher anxiety. Rather, they probably arose from enhanced within-session habituation, as has been suggested for rats exposed to enrichment at younger ages (Kempermann et al., 2002; Schrijver et al., 2002; Zimmerman et al., 2001). The sex difference in the within-session decline in ambulation supports earlier reports of female rats habituating more rapidly to novelty than males (Green et al., 1975; Hughes, 1990; Walsh and Cummins, 1976).

In conclusion, the results of this study demonstrated for the first time that enrichment led to lower body weights in aging male rats, as shown for those exposed to environmental enrichment when much younger (Diamond et al., 1965; Fiala et al., 1977; Hoffmann et al., 2009; Hughes, 1971; Moncek et al., 2004; Peña et al., 2006). However, this did not characterize aging females. More importantly, the results also showed that, as reflected in within-session decrements in open-field ambulation, the aging male rats exposed to environmental enrichment show reduced ambulatory activity that was most likely due to enhanced habituation to novelty in a similar fashion to what has been described for younger-exposed animals (Kempermann et al., 2002; Schrijver et al., 2002; Zimmerman et al., 2001). In the absence of results from specific tests for learning and memory it was not possible to definitely conclude that there had been enrichment-related benefits for cognitive functioning as shown for younger rats (Van Praag et al., 2000). Nevertheless, it is likely that the enriched rats were indeed superior in this respect to those kept in standard cages since habituation to novelty can be viewed as an elementary form of learning and memory dependant on hippocampal cholinergic activity (Barnes et al., 1991; Thiel et al., 1998). It is therefore possible that short-term working memory had been improved by enrichment. In future research, it would be useful to test for a similar effect on longer-term reference memory by observing between-session habituation in open-field habituation for which there is clearly a strong memory component (Leussis and Bolivar, 2006). The presence of sex differences in effects of enrichment on average body weights and occupancy of the center squares of the open field emphasizes the

value of investigating both male and female rats in biobehavioral research (Hughes, 2007).

While it is likely that the lower level of defecation in the light–dark box shown by rats kept in enriched cages was a reflection of their lower anxiety, one should be mindful of how few fecal boluses were left by rats from any of the groups. (This caution also applies to the significant sex difference in open-field defecation.) Unexpectedly, chronic tocopherol treatment did not mimic or potentiate the effects of enrichment. On the contrary, the vitamin seemed to interfere with its effects possibly because of heightened anxiety that can accompany its action (Kolosova et al., 2006). It is possible that the doses used might have been too high and thus had pro-oxidant properties (Rietjens et al., 2002), even though they were in the range previously shown to have beneficial effects in aged rats (Socci et al., 1995) and mice (Mcdonald et al., 2005). If pro-oxidant effects were indeed a factor in the present study, this possibility could be assessed in future research by additional treatment with other co-antioxidants, such as ascorbic acid, that prevent tocopherol's pro-oxidant activity (Brigelius-Flohé and Traber, 1999).

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References

Amelink CJ, van der Wal WAA, Wokke JHJ, van Asbeck BS, Bär PR. Exercise-induced muscle damage in the rat: the effect of vitamin E deficiency. Pflüg Eur J Physiol 1991;419:304–9.
Archer J. Tests for emotionality in rats and mice: a review. Anim Behav 1973;21: 205–35.
Arendash GW, Garcia MF, Costa DA, Cracchiolo JR, Wefes IM, Potter H. Environmental enrichment improves cognition in aged Alzheimer's transgenic mice despite stable [beta]-amyloid deposition. NeuroReport 2004;15:1751–4.
Barnes NM, Costall B, Kelly ME, Murphy DA, Naylor RJ. Cognitive enhancing actions of PD123177 detected in a mouse habituation paradigm. Neuroreport 1991;2:351–3.
Belzung C. Measuring rodent exploratory behaviour. In: Crusio WE, Gerlai RT, editors. Handbook of molecular-genetic techniques for brain and behavior research. Amsterdam: Elsevier; 1999. p. 738–49.
Benaroya-Milshtein N, Hollander N, Apter A, Kukulansky T, Raz N, Wilf A, et al. Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. Eur J Neurosci 2004;20:1341–7.
Bolles RC. Grooming behavior in the rat. J Comp Physiol Psychol 1960;53:306–10.
Brain PF, Marrow L. Rodent models of human neuroses and psychoses. In: Haug M, Whalen RE, editors. Animal models of human emotion and cognition. Washington: American Psychological Association; 1999. p. 59–75.
Brigelius-Flohé R, Traber MG. Vitamin E: function and metabolism. FASEB J 1999;13: 1146–55.
Cantuti-Castelvetri I, Shukitt-Hale B, Joseph JA. Neurobehavioral aspects of antioxidants in aging. Int J Dev Neurosci 2000;18:367–81.
Chakraborti A, Gulati K, Ray A. Age related differences in stress-induced neurobehavioral responses in rats: modulation by antioxidants and nitrergic agents. Behav Brain Res 2008;194:86–91.
Chapillon P, Manneché C, Belzung C, Caston J. Rearing environmental enrichment in two inbred strains of mice: 1. Effects on emotional reactivity. Behav Genet 1999;29: 41–6.
Chapillon P, Patin V, Roy V, Vincent A, Caston J. Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. Dev Psychobiol 2002;41:373–87.
Conrad CD, Roy EJ. Dentate gyrus destruction and spatial learning impairment after corticosteroid removal in young and middle-aged rats. Hippocampus 1995;5:1-15.
Desrumaux C, Risold P-Y, Schroeder H, Deckert V, Masson D, Athias A, et al. Phospholid transfer protein (PLTP) reduces brain vitamin E content and increases anxiety in mice. FASEB J 2004. doi:10.1096/fj.04-2400fje.
Diamond M, Rosenzweig MR, Krech D. Relationships between body weight and skull development in rats raised in enriched and impoverished conditions. J Exp Zool 1965;160:29–35.
Doty BA. The effects of cage environment upon avoidance responding of aged rats. J Gerontol 1972;27:358–60.
Elliot BM, Grunberg NE. Effects of social and physical enrichment on open field activity differ in male and female Sprague–Dawley rats. Behav Brain Res 2005;165:187–96.
Fernández-Teruel A, Escorihuela RM, Castellano B, Gonzalez B, Tobeña A. Neonatal handling and environmental enrichment effects on emotionality, novelty/reward seeking, and age-related cognitive and hippocampal impairments: focus on the roman rat lines. Behav Genet 1997;27:513–26.
Fiala B, Snow FM, Greenough WT. "Impoverished" rats weigh more than "enriched" rats because they eat more. Dev Psychobiol 1977;10:537–41.
Fordyce DE, Farrar RP. Enhancement of spatial learning in F344 rats by physical activity and related learning-associated alterations in hippocampal and cortical cholinergic functioning. Behav Brain Res 1991;46:123–33.
Frick KM, Fernandez SM. Enrichment enhances spatial memory and increases synaptophysin levels in aged mice. Neurobiol Aging 2003;24:615–26.
Gardner EB, Boitano JJ, Mancino NS, D'Amico DP, Gardner EL. Environmental enrichment and deprivation: effects on learning, memory and exploration. Physiol Behav 1975;14:321–7.
Gohil K, Schock BC, Chakraborty AA, Terasawa Y, Raber J, Farese Jr RV, et al. Gene expression profile of oxidant stress and neurodegeneration in transgenic mice deficient in α-tocopherol transfer protein. Free Radic Biol Med 2003;35:1343–54.
Golden TR, Hinerfeld DA, Melov S. Oxidative stress and aging: beyond correlation. Aging Cell 2002;1:117–23.
Gray JA. Sex differences in emotional behaviour in mammals including man: endocrine bases. Acta Psychol 1971;35:29–46.
Green SE, Joyce D, Summerfield A. Effects of scopolamine on habituation of exploratory activity in rats. Physiol Psychol 1975;3:400–4.
Hascöet M, Bourin M, Dhonnchadha BA. The mouse light–dark box paradigm: a review. Prog Neuropsychopharmacol Biol Psychiatry 2001;25:141–66.
Hoffmann LC, Schütte SRM, Koch M, Schwabe K. Effect of "enriched environment" during development on adult rat behavior and response to the dopamine receptor agonist apomorphine. Neuroscience 2009;158:1589–98.
Hughes RN. Behaviour of male and female rats with free choice of two environments differing in novelty. Anim Behav 1968;16:92–6.
Hughes RN. Reactions to novelty following differential postweaning rearing and handling. Percept Mot Skills 1971;32:883–6.
Hughes RN. Sex-dependent habituation to novelty in rats. Curr Psychol Res Rev 1990;9: 277–84.
Hughes RN. Sex does matter: comments on the prevalence of male-only investigations of drug effects on rodent behaviour. Behav Pharmacol 2007;18:583–9.
Hughes RN, Beveridge IJ. Effects of prenatal exposure to chronic caffeine on locomotor and emotional behavior. Psychobiology 1987;15:179–85.
Ishihara M, Itoh M, Miyamoto K, Suna S, Takeuchi Y, Takenaka I, et al. Spermatogenic disturbance induced by d-(2-ethylhexyl) phthalate is significantly prevented by treatment with antioxidant vitamins in the rat. Int J Androl 2000;23:85–94.
Jankowsky JL, Melnikova T, Fadale DJ, Xu GM, Slunt HH, Gonzales V, et al. Environmental enrichment mitigates cognitive deficits in a mouse model of Alzheimer's Disease. Neurobiol Dis 2005;25:5217–24.
Joseph R. Effects of rearing and sex on maze learning and competitive exploration in rats. J Psychol 1979;101:37–43.
Juraska JM, Fitch JM, Henderson C, Rivers N. Sex differences in the dendritic branching of dentate granule cells following differential experience. Brain Res 1985;333:73–80.
Kalueff AV, Tuohimaa P. The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioural stress research. J Neurosci Methods 2005;143:169–77.
Kelso GF, Porteous CM, Hughes G, Ledgerwood EC, Gane AM, Smith RAJ, et al. Prevention of mitochondrial oxidative damage using targeted antioxidants. Ann NY Acad Sci 2002;959:263–74.
Kempermann G, Kuhn HG, Gage FH. Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 1998;18:3206–12.
Kempermann G, Gast D, Gage FH. Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann Neurol 2002;52:135–43.
Kobayashi S, Ohashi Y, Ando S. Effects of enriched environments with different durations and starting times on learning capacity during aging in rats assessed by a refined procedure of the Hebb–Williams maze task. J Neurosci Res 2002;70:340–6.
Kolosova NG, Trofimova NA, Fursova AZh. Opposite effects of antioxidants on anxiety in Wistar and OXYS rats. Bull Exp Biol Med 2006;141:734–7.
Leal-Galicia P, Casteñeda-Bueno M, Quiroz-Baez R, Arias C. Long-term exposure to environmental enrichment since youth prevents recognition memory decline and increases synaptic plasticity markers in aging. Neurobiol Learn Mem 2008;90: 511–8.
Lee Y-B, Lee HJ, Won MH, Hwang IK, Kang T-C, Lee J-Y, et al. Soy isoflavones improve spatial delayed matching-to-place performance and reduce cholinergic neuron loss in elderly male rats. J Nutr 2004;134:1827–31.
Le Grevès M, Steensland P, Le Grevès P, Nyberg F. Growth hormone induces age-dependent alteration in the expression of hippocampal growth hormone receptor and *N*-methyl-D-aspartate receptor subunits gene transcripts in male rats. Proc Natl Acad Sci U S A 2002;99:7119–23.
Leussis MP, Bolivar VJ. Habituation in rodents: a review of behavior, neurobiology, and genetics. Neurosci Biobehav Rev 2006;30:1045–64.
Martin A, Youdim K, Szprengiel A, Shukitt-Hale B, Joseph J. Roles of vitamins E and C on neurodegenerative diseases and cognitive performance. Nutr Rev 2002;60:308–34.
Mcdonald SR, Sohal RS, Forster MJ. Concurrent administration of coenzyme Q_{10} and α-tocopherol improves learning in aged mice. Free Radic Biol Med 2005;38:729–36.
McGivern RF, Henschel D, Hutcheson M, Pangburn T. Sex difference in daily water consumption of rats: effect of housing and hormones. Physiol Behav 1996;59: 653–8.
Moncek F, Duncko R, Johansson BB, Jezova D. Effects of environmental enrichment on stress related systems in rats. J Neuroendocrinol 2004;16:423–31.
Peña Y, Prunell M, Dimitsantos V, Nadal R, Escorihuela RM. Environmental enrichment effects in social investigation are gender dependent. Behav Brain Res 2006;174:181–7.

Pham TM, Ickes B, Albeck D, Söderström S, Granholm A-Ch, Mohammed AH. Changes in brain nerve growth factor levels and nerve growth factor receptors in rats exposed to environmental enrichment for one year. Neuroscience 1999;94:279–86.
Renner MJ, Rosenzweig MR. Enriched and impoverished environments: effects on brain and behavior. New York: Springer-Verlag; 1987.
Rietjens IMCM, Boersma MG, de Haan L, Spenkelink B, Awad HM, Cnubben NHP, et al. The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ Toxicol Pharmacol 2002;11:321–33.
Roy V, Belzung C, Delarue C, Chapillon P. Environmental enrichment in BALB/c mice: effects in classical tests of anxiety and exposure to a predatory odor. Physiol Behav 2001;74:313–20.
Salonen RM, Nyyssönen K, Kaikkonen J, Porkkala-Sarataho E, Voutilainen S, Rissanen TH, et al. Six-year effects of vitamin C and E supplementation on atherosclerotic progression: the antioxidant supplementation in Atherosclerosis Prevention (ASAP) Study. Circulation 2003;107:947–53.
Sánchez C. 5(HT1A) receptors play an important role in the modulation of behavior of rats in a two-compartment black and white box. Behav Pharmacol 1996;7:788–97.
Schrijver NCA, Bahr NI, Weiss IC, Würbel H. Dissociable effects of isolation rearing and environmental enrichment on exploration, spatial learning and HPA activity in adult rats. Pharmacol Biochem Behav 2002;73:209–24.
Segovia G, Del Arco A, Garrido P, de Blas M, Mora F. Environmental enrichment reduces the response to stress of the cholinergic system in the prefrontal system during aging. Neurochem Int 2008;52:1198–203.
Shaheen AA, Hamdy MA, Kheir-Eldin AA, Lindström P, Abd El-Fattah AA. Effect of pretreatment with vitamin E or diazepam on brain metabolism of stressed rats. Biochem Pharmacol 1993;46:194–7.
Shimamura AP, Berry JM, Mangels JA, Rusting CL, Jurica PJ. Memory and cognitive abilities in university professors: evidence for successful aging. Psychol Sci 1995;6:271–7.
Socci DJ, Crandall BM, Arendash GW. Chronic antioxidant treatment improves the cognitive performance of aged rats. Brain Res 1995;693:88–94.
Thiel CM, Huston JP, Schwarting RKW. Hippocampal acetylcholine and habituation learning. Neuroscience 1998;85:1253–62.
van Erp AMM, Kruk MR, Meelis W, Willekens-Bramer DC. Effect of environmental stressors on time course, variability and form of self-grooming in the rat: handling, social contact, defeat, novelty, restraint and fur moistening. Behav Brain Res 1994;65:47–55.
van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci 2000;1:191–8.
Vogelsang A, Shute EV. Effect of vitamin E in coronary heart disease. Nature 1946;157:772.
Walsh RN, Cummins RA. The open-field test: a critical review. Psychol Bull 1976;83:482–504.
Wang Y-X, Edwards RM, Nambi P, Stack EJ, Pullen M, Crofton JT, et al. Sex difference in the antidiuretic activity of vasopressin in the rat. Am J Physiol 1980;265:R1284–90.
Wolden-Hanson T, Mitton DR, McCants RL, Yellon SM, Wilkinson CW, Matsumoto AM, et al. Daily melatonin administration to middle-aged rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. Endocrinology 2000;141:487–97.
Zhang B, Tanaka J, Yang L, Yang L, Sakanaka M, Hata R, et al. Protective effect of vitamin E against focal brain ischemia and neuronal death through induction of target genes of hypoxia-inducible factor-1. Neuroscience 2004;126:433–40.
Zimmerman A, Stauffacher M, Langhans W, Würbel H. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behav Brain Res 2001;121:11–20.