Dietary fats influence 'open-field' behaviour of male and female laboratory mice

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Summary

Swiss mice of differing ages (juvenile and adult) and sexes were fed four specially formulated, pelleted diets containing respectively 8% saturated vegetable fat, 8% soya oil, 8% olive oil and 2% soya oil (with identities hidden from the experimenter) or a local commercial chow (3% crude fat) for 3 or 6 weeks. Subjects were individually housed and were assessed under red lighting for behaviour in a modified 'open field' (a 30×20 cm box with a black floor). Videotaped records were analysed using 'The Observer' system, quantifying transitions between inner and outer zones, rearing, freezing, grooming and defaecation as well as location in the two equal-sized zones. Clearly, these non-isocaloric diets differed in palatability, producing complex effects on growth as well as physiological and behavioural measures. Many indices were influenced by age, sex, and the duration of dietary exposure. Interactions between factors were common. Defaecation does not seem to provide a useful index of 'emotionality' in this type of study and investigations lacking a wide range of indices seem unlikely to provide unequivocal support for postulated links between dietary lipids and behaviour. The study broadly supports the contention that dietary fats subtly influence mood in mice.

Keywords Swiss mice; dietary fats; male and female; open field; eliminative activity

Most (95%) fat supplied in the diet of human or animals is in the form of triglycerides. The different classes of fatty acids may have variable effects on lipid metabolism and the fatty acid portion of the lipid molecule appears to be responsible for its characteristics (Grundy & Denke 1990). Fatty acids occurring in food stuffs contain even numbers of carbon atoms in unbranched chains, e.g. lauric or dodecanoic acid. Dietary fatty acids can be classified into four types namely saturated, monounsaturated, (n-3) polyunsaturated and (n-6) polyunsaturated fatty acids. A fatty acid with one double bond is referred to as a monounsaturated fatty acid (e.g. olive oil), whereas a fatty acid containing two or more double bonds is considered a polyunsaturated fatty acid (e.g. soybean oil). N-3 and n-6 fatty acids such as linoleic, linolenic, and arachidonic acids are widespread in food sources. They are considered essential fatty acids (EFAs) because they must be supplied in the diet (the body cannot synthesize them in significant amounts) to humans (Bjerve 1989) as well as to rodents (Burr & Burr 1929). EFAs are important constituents of neural membranes, making up about 20% of the dry matter of the brain. EFAs and their eicosanoid derivatives have profound effects on membrane functions, leading to changes in nerve conduction, neurotransmitter re-uptake and post-synaptic transmitter effects (Horrobin *et al.* 1989).

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The relationship is complex. Feeding pregnant mice an imbalanced diet with inadequate EFAs (low (n-6):(n-3) and high levels of docosahexaenoic acid) results in growth retardation and associated behavioural retardation in their pups similar to that seen in malnourishment (Wainwright *et al.* 1999).

The above strongly suggests that lipids have profound effects on the central nervous system (CNS) and its activities. It has been argued that behaviour is the most sensitive indicator of subtle CNS changes, and many researchers (e.g. Wurtman 1982) have implicated dietary factors in behavioural changes. Malnutrition over the period of rapid CNS growth results in morphological, neurochemical and behavioural changes that persist after nutritional rehabilitation (Morgane *et al.* 1978). Dietary deficiency of 18:2ω-6 and 16:3ω-3 in developing animals results in impaired CNS development (e.g. Bourre *et al.* 1989). For example, the offspring of female rats fed soybean oil have enhanced learning ability compared with counterparts fed a safflower oil diet (Lamptey & Walker 1976). 20:4ω-6 and/or 22:6ω-3 have been implicated in such studies in altered learning behaviour and visual function. Rats fed a diet containing 20% polyunsaturated fat (soybean oil) for 3 weeks performed better on a water maze task than counterparts fed either a diet with 20% saturated fat (lard) or a Purina Lab Chow diet (4.5% mixed fats) (Coscina *et al.* 1986). Yehuda and Carasso (1987) compared rats fed diets containing soybean oil, sunflower oil, or lard with control animals. They found that the soybean oil diet improved learning ability and increased the pain threshold (as assessed using the hot-plate test). Yehuda (1987) suggested that dietary manipulations change behaviour by altering the neural membrane lipid composition. Activity level changes in rodents, however, influence the opportunity to learn a maze and it has been reported that rats with a n-3 deficient diet are more likely to explore such situations (Enslen *et al.* 1991).

Most of the literature (reviewed in Benton 1997) relates to the impact of dietary fat on cognitive behaviour. Building on studies comparing the effects of feeding diets of differing fatty acid composition to animals, this present study has focused on easily measured spontaneous behavioural changes. An extremely mild variant of the open-field test was selected for this purpose. More than 20 different behaviours have been quantified in the open-field test, only a few of which are reliable in rats (Walsh & Cummins 1976) and other rodents. These include the most commonly used ambulation, rearing and defaecation (Ivinskis 1968). Interpretation of behavioural measures obtained in open-field testing is problematic. Measures of motor behaviour have been mostly viewed as indices of arousal or exploration, whereas the defaecation measure has generally been suggested to reflect 'emotionality' or autonomic system reactivity (Russell *et al.* 1987).

It was felt that using a substantial array of measures (rather than simply using ambulation and defaecation) would enhance the interpretation of the effects (if any) of dietary fat on expressed behaviour. The present study's concentration on spontaneous behaviour may reflect subtly altered CNS activity more effectively than may tests concerned with learning or pain.

Method

Animal husbandry

This study was carried out on the Swiss strain albino mice, bred and housed in the Animal Facility of the King Fahad Research Medical Centre (Jeddah, Kingdom of Saudi Arabia). The subjects were kept under lighting conditions with white lights on from 08:00 to 20:00 h (local time). Temperature was maintained between 20–25°C and relative humidity ranged from 45 to 55%. Animals were bred in pairs and the litters were not culled at birth, i.e. there was a certain variation in rates of development in litters of different size. Post-weaning (at 21 days of age), the mice were housed in groups of five in transparent cages (type M II m.DO II. F30, E. Becker & Co, Germany) measuring $26 \times 20 \times 14$ cm with stainless steel wire tops. Laboratory rodent pellets (Grain Silos & Flourmills Organization, Western Region, PO Box 5529, Jeddah 21432, Saudi Arabia, see formulation in Table 1) and water were provided

Table 1 The commercial diet provided by Grain Silos & Flourmills Organization contained a mixture of wheat, soybean, corn, powdered alfalfa, and palmetic oil as well as a vitamin/mineral mixture

Crude protein	200 g/kg
Crude carbohydrate	630 g/kg
Crude fat	30 g/kg
Crude fibre	55 g/kg
Vitamin/mineral mixture	85 g/kg
Energy	2.850 kcal/g

ad libitum to the mice. The sawdust substrate was supplied by a commercial carpenter and changed every 2 days.

The mice were transferred shortly before being used in experiments to a separate room in the Medical Centre (their age of transfer depended on whether they were in the juvenile or adult category). In this new location, they were maintained under a reversed lighting schedule [white fluorescent lights on from 21:30 to 09:30 h (local time)], which meant that behavioural observations were performed under dim red lighting. Ambient temperature was maintained between 20 and 23°C. Each mouse was housed in a transparent plastic cage (type THF/2152/Aa, EHRET, Germany) measuring $26 \times 20 \times 14$ cm with a stainless steel wire top.

The experimental protocol was performed under the Joint Supervision Programme between the King Abdulaziz University and the University of Wales Swansea, and the study complied with the UK Animals Scientific Procedures Act 1986.

Food intake

Forty-eight mice of each sex were assigned for each diet. Half the animals of each sex were young (around 3 weeks at the commencement of the experiment) and half were adult (5–6 weeks). The mice were fed one of four experimental pelleted diets (see Table 2), or the control commercial diet with which they were familiar. The experiments with the diets were run in three replications of four mice for each group. The duration of these experiments was 3 or 6 weeks. The daily amounts of food (between 10.10 and 10.99 g) were weighed on a digital balance (Model PB302, Mettler, Switzerland), and were provided to each mouse in their food hoppers. Unconsumed food (that remaining in the hoppers or collected as spillage from the sawdust substrate) was weighed after 24 h using the same balance to calculate the quantities eaten.

'Open-field' behavioural recording

To minimize stress and maximize the mouse's familiarity with its conditions, a highly modified 'open-field' behavioural test was carried out under the identical conditions of illumination (dim red light), temperature, and background noise level to those prevailing in the animal room. At the end of the feeding period (3 or 6 weeks), individually-housed mice from each diet category were transferred to a black arena measuring $40 \times 30 \times 20$ cm. Here, activities were recorded using a video camera (Model SSC-M 370CE CCD Black, Sony, Japan) positioned 69 cm above the arena. The arena had to be black to provide sufficient contrast with the albino mouse under the red lighting conditions. Each mouse was placed in the arena at approximately the same location in the bottom right-hand corner of the outer area, and a 10 min recording was carried out without any disturbance from the experimenter. At the end of recording, the numbers of faecal boli were counted and the mouse was returned to its cage. All tests were conducted between 10:00 and 13:00 h (local time) to minimize any effects of a circadian activity rhythm.

Table 2 SDS (PO Box 705, Witham, Essex, CM8 3AB, UK) provided the experimental diets[*]

Diet A contained 8% saturated vegetable fat (vegetarian lard)
Diet B contained 8% soya oil
Diet C contained 8% olive oil
Diet D contained 2% soya oil
Control: local commercial diet

All contained 270 g/kg casein, 402 g/kg corn starch, 50 g/kg Alphacel (fibre), an added vitamin/mineral mix of 76 g/kg and 4.486 kcal/g. They differed with respect to the source and percentage of fat that they contained. Initially, the diets were coded such that the experiments were conducted 'blind', removing the possibility of inadvertent bias in measurements

[*]These diets not only differed in energy content but the fat influenced the integrity of the pellets

Behavioural analysis of the videotapes

This was carried using the Video Timecode Generator, IEC, ProGAMMA (from Noldus Information Technology BV, Costerweg 5, PO Box 268, 6700 AG Wageningen, The Netherlands) which was connected to a computer (Phaser 486DX/50, Noldus Information Technology BV). A special program (The Observer) was used in this study to assess the durations of a number of behavioural elements. These included:

- *Supported rearing*: when the mouse stands on its hind legs, with its forelegs supported by the walls of the arena.
- *Internal rearing*: when the mouse stands on its hind legs, away from the wall, with its forelegs unsupported, and appears to scan the area.
- *Self-grooming*: when the mouse scratches, nuzzles its fur, or cleans its whiskers.
- *Freezing*: which is a period of total cessation of locomotor activity for more than 5 s.
- *Defaecation*: (deposition of faecal boli on the floor of the arena) was assessed at the termination of the test.

The arena was divided into an external zone adjacent to the walls and an internal zone in the centre (both of 600 cm^2). The total time spent in each area was assessed in a new analysis of each tape.

Statistical analyses

Means and standard errors were calculated for all measures using the SPSS package for Windows version 6.0 (Copyright SPSS Inc., 1989–1993). General factorial ANOVA was completed by SPSS. The results were looked at starting with the interaction terms, as they may explain spurious main effects. *Post hoc* Duncan's tests were carried out using the SAS program (Proprietary Software 6.02 Copyright 1985, SAS Institute Inc., Cary, NC 27511, USA). Although the *post hoc* tests produced modest results and the relatively large number of such tests is viewed as a problem by some authorities, this pattern of ANOVA followed by Duncan's tests is strongly advocated by statistical texts and is routine in this area of science.

Results

Although factorial ANOVAs were performed on all the measures obtained in this study, only some representative measures are reported. These are generally indices likely to be of most interest to experimental workers.

Defaecation

Table 3 shows the mean numbers of faecal pellets produced in the arena by mice of different ages and sexes given five different diets for 3 or 6 weeks.

General factorial ANOVA demonstrated significant effects of diet (df = 4 and 440, $F = 4.21$, $P < 0.002$), age (df = 1 and 440, $F = 5.12$, $P < 0.02$) and sex (df = 1 and 440, $F = 17.4$, $P < 0.0001$). There were also diet × age (df = 4 and 440, $F = 3.04$, $P < 0.02$), and diet × duration (df = 4 and 440, $F = 3.05$, $P < 0.02$) interactions. Further analysis with Duncan's test revealed that mice given diet D produced significantly ($P < 0.05$) more faecal pellets in the arena than subjects on any other diet. Paired comparisons on the young and adult mice given different diets only revealed that younger subjects given the control diet had significantly ($P < 0.05$) lower values than adult mice. Similar comparisons for mice given the diets for 3 or 6 weeks revealed that mice fed on diet B (8% soya oil) for 6 weeks produced significantly ($P < 0.05$) more faecal pellets than counterparts exposed for 3 weeks.

Defaecation in the 'open field' or any other novel arena by rats and mice has generally been regarded as a positive indicator of emotionality (see Archer 1973). It has been suggested, however, that in male mice it reflects territorial marking (see Brain & Nowell 1970, Brain *et al.* 1971). The current data suggest that mice given the 2% soya oil diet produce more faecal pellets than do any other subjects. Rather than reflecting increased emotionality, the most obvious explanation is that the first-mentioned diet is eaten in greater amounts than are the others (as was confirmed in direct measurements). This conclusion is supported by the factor analysis that shows that defaecation is not inversely related to ambulation in this test

Table 3 Mean (±SE) number of faecal pellets produced in the arena by male and female mice of different ages given different diets for variable periods of time (n = 12)

			Diet				
Sex	Age	Duration (weeks)	A	B	C	D	Control
Male	Young	3	6 ± 1	5 ± 1	6 ± 1	7 ± 1	5 ± 1
		6	5 ± 1	6 ± 1	8 ± 1	7 ± 1	4 ± 1
	Adult	3	7 ± 1	5 ± 0	6 ± 1	6 ± 1	6 ± 1
		6	6 ± 1	6 ± 1	6 ± 1	8 ± 1	7 ± 1
Female	Young	3	5 ± 1	4 ± 1	6 ± 1	6 ± 1	3 ± 1
		6	4 ± 1	6 ± 1	5 ± 1	7 ± 1	4 ± 1
	Adult	3	6 ± 1	4 ± 1	4 ± 1	6 ± 1	7 ± 1
		6	5 ± 1	5 ± 1	5 ± 1	7 ± 1	4 ± 1

A: 8% saturated vegetable fat diet; B: 8% soya oil diet; C: 8% olive oil diet; D: 2% soya oil diet; Control: local commercial diet

and is weighted on a separate factor. Unremarkably, adults produced more faecal pellets than younger animals but, somewhat atypically (female rodents are generally regarded as more emotional than males), males produced more faecal pellets than females (territorial marking?). In a majority of the diets used, adult animals produced more faecal pellets than younger animals (see the sex difference) and subjects given the diets for 6 weeks produced more pellets than those on the diets for 3 weeks.

Transitions between external and internal areas

Table 4 shows the mean total transitions between the external and internal areas of the arena by mice of different ages and sexes given one of five different diets for 3 or 6 weeks. General factorial ANOVA showed significant effects of diet (df = 4 and 440, $F = 3.35$, $P < 0.01$), age (df = 1 and 440, $F = 8.63$, $P < 0.003$), sex (df = 1 and 440, $F = 3.81$, $P < 0.051$) and duration (df = 1 and 440, $F = 8.09$, $P < 0.005$). There was also an age × duration × diet interaction (df = 4 and 440, $F = 2.92$, $P < 0.021$). Further analysis with Duncan's test revealed that mice given diet B (8% soya oil) showed significantly ($P < 0.05$) fewer transitions than mice fed on other diets. Duncan's test revealed that adult mice fed on diet D (2% soya oil) for 6 weeks showed significantly ($P < 0.05$) more total transitions between the external and internal areas of the arena than younger subjects exposed for 6 weeks or adult subjects exposed for 3 weeks. Younger mice given control diet

Table 4 Mean (±SE) total transitions between external and internal areas of the arena by male and female mice of different ages given different diets for variable periods of time (n = 12)

			Diet				
Sex	Age	Duration (weeks)	A	B	C	D	Control
Male	Young	3	105.5 ± 8.29	106.6 ± 8.40	103.7 ± 6.68	124.5 ± 7.85	119.0 ± 7.95
		6	125.1 ± 6.43	115.3 ± 6.94	125.8 ± 4.91	116.8 ± 8.68	128.9 ± 7.59
	Adult	3	136.3 ± 6.53	114.2 ± 9.06	125.8 ± 8.84	118.9 ± 6.58	120.5 ± 8.73
		6	130.4 ± 8.82	129.9 ± 9.12	130.8 ± 8.84	144.3 ± 7.56	119.3 ± 6.99
Female	Young	3	118.3 ± 8.59	102.4 ± 5.73	128.4 ± 6.81	123.8 ± 6.24	116.1 ± 9.99
		6	125.8 ± 8.82	116.5 ± 6.71	132.3 ± 7.49	133.3 ± 5.97	148.8 ± 10.2
	Adult	3	125.0 ± 5.77	126.1 ± 7.04	131.0 ± 9.82	130.8 ± 7.62	142.3 ± 12.2
		6	130.3 ± 5.17	118.8 ± 5.29	120.2 ± 7.25	148.9 ± 14.1	121.7 ± 7.93

A: 8% saturated vegetable fat diet; B: 8% soya oil diet; C: 8% olive oil diet; D: 2% soya oil diet; Control: local commercial diet for 6 weeks had significantly ($P < 0.05$) higher values than young mice fed on the same diet for 3 weeks.

The data showed that subjects fed on the diet containing 8% soya oil made fewer transitions than counterparts on the other diets. Such a change would generally be interpreted that these animals showed lowered emotionality. Adults, females and subjects exposed to the diets for longer had higher scores on this measure than their counterparts, which may be interpreted as suggesting that they are more exploratory and less fearful. The 3-way interaction suggests that adults treated with 2% soya oil diet for 6 weeks show an impressive rise in this measure compared with younger counterparts or adults fed on the same diet for 3 weeks. Younger mice fed on control diet for 6 weeks ($P < 0.05$) showed more activity than counterparts given the diet for 3 weeks.

Duration spent rearing in the external zone

Table 5 shows the mean total time spent rearing in the external zone of the arena by mice of different ages and sexes given one of five different diets for 3 or 6 weeks.

General factorial ANOVA showed significant effects of diet (df = 4 and 440, $F = 2.64$, $P < 0.033$), age (df = 1 and 440, $F = 11.71$, $P < 0.001$), and sex (df = 1 and 440, $F = 44.88$, $P < 0.0001$). There were also age × duration (df = 1 and 440, $F = 10.45$, $P < 0.001$), sex × duration (df = 1 and 440, $F = 4.83$, $P < 0.029$), and sex × duration × age (df = 1 and 440, $F = 7.87$, $P < 0.005$) interactions. Duncan's test revealed that mice given diets B (8% soya oil) and A (8% saturated vegetable fat) spent significantly (both $P < 0.05$) more time rearing in the external zone of the arena than counterparts fed on diet D (2% soya oil). Mice fed on control diet spent significantly ($P < 0.05$) longer durations than counterparts receiving diet A (8% saturated vegetable fat). Duncan's test showed that adult mice exposed to the different diets for 3 weeks differed significantly ($P < 0.05$) on this measure with respect to counterparts exposed for 6 weeks, whereas male subjects fed for 3 or 6 weeks differed ($P < 0.05$) from females fed for the same periods. Further analysis with Duncan's test revealed that young male mice fed for 6 weeks differ from young females and adult males fed for 6 weeks (both $P < 0.05$). Adult male mice given diets for 3 weeks devoted significantly ($P < 0.05$) more time to rearing in this zone than adult male mice exposed for 6 weeks and adult female mice fed for 3 weeks. Young male mice given diets for 3 weeks spent longer ($P < 0.05$) rearing here than young female subjects fed for the same period.

Mice given the 2% soya or the control diets appear to devote more time to exploratory rearing in the external area than subjects eating diets containing 8% soya oil

Table 5 Mean (±SE) total duration (seconds) spent rearing in the external zone of the arena by male and female mice of different ages given different diets for variable periods of time (n = 12)

Sex	Age	Duration (weeks)	Diet A	B	C	D	Control
Male	Young	3	122.5 ± 11.7	106.3 ± 7.6	120.8 ± 10.0	147.0 ± 9.6	142.9 ± 10.4
		6	127.8 ± 12.4	129.6 ± 10.4	153.3 ± 13.7	137.4 ± 11.2	146.6 ± 10.5
	Adult	3	145.3 ± 7.6	138.5 ± 9.5	125.5 ± 12.1	141.8 ± 13.4	134.1 ± 14.1
		6	93.8 ± 9.5	104.3 ± 12.3	85.9 ± 7.9	119.2 ± 9.8	127.3 ± 13.0
Female	Young	3	94.8 ± 12.6	95.4 ± 12.2	104.8 ± 6.8	131.0 ± 8.7	107.7 ± 15.1
		6	117.9 ± 9.4	118.2 ± 11.8	109.3 ± 10.6	109.3 ± 7.9	114.1 ± 6.7
	Adult	3	71.0 ± 10.6	102.8 ± 7.7	108.8 ± 9.5	95.3 ± 12.0	103.6 ± 16.2
		6	103.3 ± 14.7	94.3 ± 9.1	99.8 ± 8.8	107.9 ± 11.2	95.5 ± 11.7

A: 8% saturated vegetable fat diet; B: 8% soya oil diet; C: 8% olive oil diet; D: 2% soya oil diet; Control: local commercial diet or 8% saturated vegetable fat. Consequently, high-fat diets may reduce exploratory behaviour. Young animals and males also showed higher incidences of this behaviour than their counterparts. This seems to be best interpreted as an enhanced exploratory activity in these animals compared with their counterparts. The higher incidences in young animals were only evident in mice exposed to the diets for 6 weeks, but the differences between males and females were most evident in mice exposed for 3 weeks. The 3-way interaction between duration $\times$ age and sex appears to be due to the fact that all categories showed a modest increase with exposure duration, except for the adult males who showed a dramatic decline. The high rearing incidence and long duration spent in the external area by young, and also by animals fed on low fatty acid diets (2% soya oil), may reflect high emotionality and fearfulness.

Discussion

For many years, people have believed that the food they eat can have powerful effects on their behaviour. Many studies have confirmed that diet can influence behaviour and specifically that manipulating dietary fat can cause behavioural changes (e.g. Coscina *et al.* 1986, Yehuda *et al.* 1986). In humans, reducing plasma cholesterol values is said to actually increase the incidence of death, and the association between low cholesterol levels and depression has been commented upon on several occasions (Benton 1997, LaRosa 1997). Specifically, it has been found (Wells & Read 1996) that human volunteers fed high fat/low carbohydrate diets reported feelings of lassitude and of being less vigorous and imaginative. Fat was said to depress alertness and mood. It appears (Wells *et al.* 1997) that these changes are due to the release of the hormone cholecystokinin. The effects of consuming food are relatively quickly seen as changed physiology. In rats, for example, plasma triglyceride levels increase in monitored blood vessels (Surina-Baumgartner *et al.* 1996). This paper continues the tradition of looking for evidence of the impact of dietary factors on physiology and behaviour by using an animal model. It attempts to be more wide-ranging than earlier studies.

Unremarkably, food consumption clearly generally stimulates body weight gain. The only exception in the present study was that the body weights of the mice fed on the 2% soya oil diet showed the lowest increase despite these animals consuming the highest quantities of food. The modest body weight gain in these subjects may be due to the low-fat content of that diet (which would reduce its calorific content). Other studies on the same mice (Brain *et al.* 2000) have shown that these non-isocalorific diets differed in palatability, producing complex effects on growth as well as metabolic measures. Many of these physiological indices were influenced by age, sex and duration of dietary exposure. Interactions between factors were common. It has also been shown (Cook *et al.* 1997) that outbred colonies of rats have varied preferences for fat and carbohydrate (i.e. they have innate macronutrient preferences) and this influences food intake and weight gain. Studies have shown (Kinney & Antill 1996) that male albino mice have a significant preference for high-fat food in a choice test and that this choice is determined by olfactory cues. Rats also prefer long-chain fatty acids in their diet and select these using olfactory and gustatory cues (Tsuruta *et al.* 1999). Consequently, the mice in the present study could 'self dose' themselves with different quantities of the varied diets on the basis of their preferences. These preferences might be influenced by age, sex and duration of exposure (Brain *et al.* 2000).

As noted above, the plethora of physiological consequences of exposure to different diets might be expected to subtly influence an animal's behaviour or its mood. Given the test employed, it is appropriate to say something about the measures used and their limitations before attempting to interpret the changes.

As noted earlier, 'novel environment' tests (e.g. the open field) use simple measures such as defaecation, ambulation, rearing, grooming, and freezing which are said to be reliable measures of 'emotionality' (Archer 1973).

Defaecation and ambulation in the open field are the most frequently used measures in this situation (Ivinskis 1968). Defaecation, rearing, and grooming are all increased in rats by exposure to intense noise, whereas no significant effects on ambulation were seen (Weyers *et al.* 1994). This suggests that the first-mentioned measures are influenced by fear-provoking stimuli.

In the open-field and other novel environment tests, female rats generally show more rearing, ambulation, and sniffing, but less grooming, eating and drinking than males (e.g. Archer 1974). The literature suggests that female rodents are less emotional than male counterparts. Gray (1971) argued that the sex difference in fearfulness depends, in part, on the level of circulating oestrogen (which decreases fearful behaviour in female rodents). Sex differences comparable with the literature cited above were evident with the females appearing less emotional and more prone to explore than males on the basis of a number of measures employed in these studies.

In actuality, factor analysis of the whole activity of animals in these present tests revealed that the elements reflected several tendencies including exploration, general locomotion and eliminative behaviour (Maimanee 1999).

Defaecation

Defaecation in the present study is clearly influenced by food intake, sex and age. Although this activity might simply be dismissed as an eliminative behaviour, it has a well-documented use in providing an index of fearful or 'emotional' behaviour. The validity of defaecation as a measure of emotionality has been postulated by many early research workers (e.g. Broadhurst 1957, Hall 1934) who felt that rats low in emotionality would show low incidences of faecal pellet production (an autonomic response). Livesey and Egger (1970) have, in contrast, claimed that a low defaecation score in young rats is indicative of fearfulness. Brain and Nowell (1969) and Bruell (1969) have even suggested that defaecation may be positively correlated with open-field ambulation in male mice and may have a role in territorial marking. In contrast, it has been claimed that a negative correlation exists between these measures in female mice (Bruell 1969). Generally, the female rodent is said to be less fearful than the male (Gray 1971), but several studies in mice indicate that the sexes have similar defaecation scores (see Archer 1975). Archer (*op cit*) has reviewed the relation between food intake and defaecation in tests of 'emotionality' in male rats and has claimed that strains of rats with a greater food intake showed more defaecation. This also seems true of both sexes of mouse in the present study where the greater the food consumption, the greater the number of faecal boli they produced. These issues have to be considered when attempting to use defaecation as an index of 'mood', and cast considerable doubt on its validity under the present circumstances.

The data in this paper show that males of this strain in this mild situation produce more faecal pellets than females (but this is unlikely to prove that the former are more fearful or even more territorial). Of great relevance here is the observation (Nagy & Holm 1970) that different diets change both defaecation and ambulation in mice in the open field. Some of the complications between food intake and defaecation have already been dealt with but it seems likely that this measure cannot be used reliably in this type of study in mice where food intakes vary widely.

Transitions between external and internal zones

Female mice are said to ambulate more than males in an open field (e.g. Priestnall 1973) but there are also many cases where no significant sex differences have been found (e.g. Candland & Nagy 1969). A few reports indicate that males ambulate more in an open field than females (see Archer 1975), leading to the suggestion that animals with lower locomotion scores ('freezers') are behaving in a highly emotional fashion. This is in direct contrast to rats where general exploratory behaviour is usually suppressed by fear (e.g. Montgomery & Monkman 1955).

Livesey and Egger (1970) claimed, however, that high ambulation scores in young rats are indicative of an escape reaction. The present study suggests that the number of transitions made between external and internal areas of the arena (one form of ambulation) is higher in adult mice and females than in counterparts. Using such criteria, one could interpret the behaviour of male and young mice as their being more fearful than their counterparts. The evident sex difference is in broad agreement with the above literature.

Current data also show that mice fed on 2% soya oil diet evidence a higher incidence of transitions between the external and internal areas of the arena. These mice also had low blood cholesterol levels, whereas counterparts fed on 8% soya oil diet showed few transitions and had high cholesterol values (Maimanee 1999). Subjects on the 2% soya oil diet may consequently be less fearful than counterparts on the 8% soya oil diet. Although it is difficult to base such conclusions on one measure only, it is tempting (given the literature) to postulate a relationship between anxiety and concentrations of polyunsaturated fatty acids.

Rearing

The total duration of rearing has been used as an indication of exploration (Weyers *et al.* 1994). In an open field, rearing is often positively correlated with ambulation (e.g. Satinder 1968). In the present study, internal (unsupported) and external (supported) forms of rearing were measured separately. However, sex, age, dietary fat composition and duration of diet identically influenced both measures. Younger and male mice showed more rearing activity than their counterparts. The 2% soya oil diet increased the duration of rearing activity which, taken with data for previous parameters (it increased the number of transitions between the two areas), suggests it lowers emotionality and increases exploration.

General comments

Because of the complications revealed in this study, one cannot deduce that mice fed the 2% soya diet (high defaecation) are more emotional than subjects fed the 8% soya oil diet (low defaecation). One could, of course, use the defaecation measure when animals are on the same diet and eat roughly similar quantities of food. Although the precise interpretation of the changes is difficult, it is clear that dietary fats do have subtle effects on the mood of mice as expressed in this type of test.

As well as being interesting in its own right, it was also thought to be of considerable scientific interest to assess whether diet influenced some of the traditional measures of 'open-field' behaviour. Such a finding could have a major impact on our interpretation of tests designed to assess the impacts of psychoactive drugs (especially anxiolytics). If diet influences such behaviour, it means that traditional measures of emotionality may not be reliable in all cases and their interpretation may have to be changed. It could also partially account for variability between studies carried out in different parts of the world, as differences in diet (as well as general husbandry conditions) are rarely emphasized. The complexity of the data obtained from the open field also suggest that one should even be careful when using such tests to assess the impact of environmental enrichment.

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