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Exposure to a maternal cafeteria diet changes open-field behaviour in the developing offspring

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Highlights

- Feeding a lactational cafeteria diet increases energy intake in the dam
- Cafeteria feeding increases fat and sugar intake, but reduces protein intake
- Cafeteria diet impacts on maternal behaviour
- Cafeteria feeding reduces anxiety in adolescent offspring in the open-field

Abstract

The early postnatal period is a sensitive period in rodents as behavioural systems are developing and maturing during this time. However, little is currently known about the behavioural effects of feeding a hyper-energetic cafeteria diet (CD) during the lactational period when offspring behaviour is tested during early adolescence. To this end, 23 days old offspring from dams (Wistar) fed on CD during lactation were tested in either the open-field or the elevated plus-maze for exploration and anxiety-related behaviour. On postnatal day 9, maternal behaviour and non-maternal behaviour of the dam was assessed. It was hypothesized that lactational CD feeding would reduce anxiety in the offspring. CD-fed dams had a higher energy intake, due to an overconsumption of sugars and fats. When offspring from these dams were exposed to the open field after weaning, their locomotor activity was increased. They entered the more aversive inner zone of the open-field after a shorter latency, made more entries into and spent more time in the inner zone. Anxiety-related behaviour was not affected upon exposure to the elevated plus maze, suggesting anxiolysis in the open-field only. Increased maternal licking/grooming behaviour could possibly contribute to the anxiolytic phenotype as observed in the offspring from the CD group. In conclusion, we demonstrate that lactational overfeeding impacts on the development of behaviour in the early adolescent rat.

Cafeteria diet; plus maze; open-field; lactation; anxiety; maternal behaviour, rat
INTRODUCTION

The association between obesity and various health hazards is well established. Considering the ever increasing number of obese people in the population, obesity puts an immense burden on society and the health system in particular (Allender and Rayner, 2007; Lette et al., 2016; Wang et al., 2011).

Despite the existing links with diabetes and cardio-vascular diseases, more recently, obesity has also been suggested to be a risk factor for developing dementia (Gustafson, 2008; Xu et al., 2011). However, hyperenergetic and obesogenic diets may also affect other behaviours as for example emotional behaviours (Maniam and Morris, 2010; Murphy and Mercer, 2013; Warneke et al., 2014; Wright et al., 2011a). Among various experimental hyperenergetic diets, the cafeteria diet has been well established as an obesogenic diet in rodents and as a model of a Western-style diet. In short, a cafeteria diet consists of various hyperenergetic and highly palatable human food items (Rothwell and Stock, 1979; Sampey et al., 2011; Sclafani and Springer, 1976).

In rodents, cafeteria diets have been shown to change a variety of behaviours, often in a sex-specific manner (Murphy and Mercer, 2013; Warneke et al., 2014; Wright et al., 2011a; Wright et al., 2014). In a series of experiments, we previously demonstrated that feeding a cafeteria diet during lactation leads to reduced anxiety, but also to changes in satiety and, gender-dependent, effects on memory in the offspring when tested at adult age (Wright et al., 2011a; Wright et al., 2011b; Wright et al., 2014). Although these results suggest nutritional programming of behaviour, less is known about the more immediate behavioural consequences of early postnatal exposure. This is important since information on how dietary manipulations impact on the early development of behaviour is not available. Psychiatric diseases are increasingly as associated with developmental disorders with origins during early sensitive periods (Andersen, 2015; Eiland and Romeo, 2013; Leonardo and Hen, 2008; Maccari et al., 2016; Trotman et al., 2013; van Elst et al., 2014), and dietary factors could contribute to behavioural changes in children (Rofey et al., 2009; Waring and Lapane, 2008) although the clinical relevance requires further investigation (Nigg et al., 2016). In humans, maternal high fat diet-induced obesity can programme detrimental effects on anxiety and aggression and maternal obesity due to high fat diet consumption has been linked to deteriorated mental health (Sullivan et al., 2014). In rats, early postnatal exposure to overfeeding can also programme offspring behaviour, even when maternal obesity is not involved (Spencer and Tilbrook, 2009; Wright et al., 2011b; Wright et al., 2014). Thus, the nature and composition of the postnatal diet itself could become an important factor when it comes to dietary effects on behaviour. For example, a direct comparison of high fat and cafeteria diets revealed that the cafeteria diet induces more of the symptoms in rats that also occur in human obesity (Sampey et al., 2011) and hence could represent a more appropriate model to study behavioural effects of hyperenergetic diets (Alfaradhi and Ozanne, 2011). Here, we exposed lactating dams to a cafeteria diet (Akyol et al., 2009; Wright et al., 2011a) and tested the offspring, shortly after weaning, for explorative and anxiety-related behaviour in the open-field and the elevated plus maze.

A behavioural effect of lactational diet could be due to direct nutritional effects of the diet itself, but could also be mediated by a change in maternal behaviour. Increased maternal licking and grooming of the pups have been shown to reduce anxiety-related behaviours in the offspring (Caldji et al., 1998;
Liu et al., 1997) and diet can impact on maternal behaviour (Levay et al., 2008; Purcell et al., 2011). Thus, we also explored maternal behaviour following exposure to the diet for several days.

**METHODS**

*Animals*

Twelve pregnant female Wistar rats (Harlan, UK) were housed individually through the whole gestation period. They were provided with ad libitum access to a standard laboratory chow (Harlan Teklad Global 18%, UK) and water. Animals were maintained under a 12-hour light-dark cycle (lights on 08:00 hours), between 20-22°C. At birth, litters were reduced to 4 pups of each sex, and randomly allocated to the chow fed group (control), or the experimental group which was fed on a cafeteria diet (CD). Litters of both groups were kept under the same ambient conditions. Cage sizes for both groups were identical (55cm x 33cm x 23cm). Pups were weaned on postnatal day (PND) 21. After weaning, offspring were kept in groups of four/cage with males and females kept in separate cages.

*Lactational feeding*

Whereas the control group was fed on chow throughout lactation, the experimental group was fed the same chow in conjunction with the experimental CD allowing the dams to choose between the two diets. CD consisted of a range of highly palatable human foods (pork pie, pate, cocktail sausages, cheese, crisps, jam, fruit and nut chocolate, golden syrup cake, shortbread and peanuts (Akyol et al., 2009). Four of these food items were provided in a bowl on the cage floor daily in excess quantities and two of those was changed daily to maintain variety. At PND 21 the offspring were weaned, group housed with littermates of the same sex and maintained on the chow control diet for the remainder of the study. Food consumption was monitored every other day. Energy intake (kJ) and macronutrient consumption (carbohydrates including sugar, fat, and protein) were calculated from the manufacturers’ data (Table 1). Weight loss due to evaporation was measured in triplicate samples of each individual food item placed in empty cages. The average daily percentage change in the weight of foods ranged from 0 to 6.2 % and corresponded to an average overestimation of energy intake by 2.51 % (7.5 kJ/d), which can be considered within an acceptable error of measurement (Akyol et al., 2009).

*Behavioural assessments and testing*

All behavioural tests with the exception of activity monitoring, where conducted between 0900 and 1200 h.

*Maternal behaviour and dam behaviour in the home cage*

On PND 9, maternal and non-maternal behaviours of the dam were observed for 45 minutes. This took place in a separate observation room. The home cage was placed in this observation room 20 minutes prior to the beginning of the observation period to allow the dam to habituate. Behaviours where recorded manually by the same person each time, as well being digitally recorded in case there was need to revisit the material at a later date. In total, 8 dams per housing condition were observed.

The duration of the following four nursing behaviours was recorded: 1. Hovering: Dam is positioned over all or most of the pups. 2. Low crouch: Dam’s back is slightly arched. 3. High crouch: Dam’s back
is highly arched with significant limb extensions. 4. Supine: Dam is lying on her side. A total nursing time was calculated from the individual nursing positions. In addition licking and grooming of the pup’s body or anogenital region was monitored. Nesting behaviour, i.e. moving nesting material around the cage and/or manipulating the original nest was also recorded (Rees, 2005).

Furthermore, the duration of the non-maternal behaviours feeding, drinking, self-grooming and the frequency of rearing (vertical exploration with both front feet off the ground) were recorded.

**Home cage locomotor activity**

24-hour home cage locomotor activity was measured shortly before weaning (PND 20 to PND 21). At this time, the pups were already roaming the cage. To register locomotor activity, a data logger (Mouse-E-Motion, INFRA-E-MOTION GmbH, Hamburg, and Germany) was mounted above each cage. The logger was connected to an infrared sensor (resolution: 1 motion detection/second; recording interval 30 minutes) to cover the complete space of the cage underneath. The data measured by each Mouse-E-Motion device was downloaded onto a personal computer and processed with Microsoft Excel. Data is expressed as counts/24 hours and represents the total number of detections over the whole observational period for each cage. Activity was analysed in eight litters per feeding condition.

**Offspring behaviour**

To exclude any direct effects of the experimental diet on behaviour, all pups were weaned onto standard chow and behavioural testing was performed on PND 23, i.e. on the second day after weaning. A total of 96 offspring were bred, 88 of which (44 each sex) were tested. The remaining 8 offspring were not used for this study. All animals were tested once to avoid interactions between tests. To avoid any litter effect, they were randomly allocated to behavioural testing. The pups were moved from the holding room to the adjacent behavioural suite and left to habituate for 20 minutes before commencing the tests. Behaviours were recorded using Ethovision XT (version 7.0, Noldus, Netherlands). Testing arenas were cleaned with 70% ethanol between each animal to remove olfactory cues.

**Open-field test**

Offspring were exposed for 5 min to an open-field. The open-field was made out of dark grey plastic and its dimensions were 100cm x 100cm x 50cm. An inner zone was defined as a square, 20 cm in from the walls of the arena. Light intensity in the centre of the open-field was 70lx.

Each rat spent 5 minutes in the open-field and the total distance travelled and latency to first entry into the inner zone, as well as number of entries and time spent in the inner zone, were automatically recorded by the software. The frequency of rearing was recorded manually using Ethovision.
**Elevated plus maze (EPM)**

The elevated plus maze (Montgomery, 1955; Voigt et al., 2005) consisted of two opposite open arms (50cm x 15cm) and two opposite closed arms (50cm x 15cm) arranged in a cross position elevated approximately 60cm from the ground. All arms extended from a central zone (15cm x 15cm). Two standing lights providing 50 lx (in closed arms) to 70 lx (open arms) were used to illuminate the maze. All animals were exposed to the EPM for 5 minutes. Rats were initially placed on the centre platform facing a corner, allowing equal choice of entering an open or a closed arm. The time spent on the open and closed arms, the entries into the arms and the total distance travelled were monitored. The ratio of open arm entries to total arm entries was calculated. The frequency of rearing was recorded manually using Ethovision.

**Data analysis**

The statistical unit for maternal behaviour was the dam. This part of the study was powered to detect a difference of 30% in total time (s) nursing, based upon σ = 500 (determined from previous studies) and an α value of 0.5 at 80% power. For analysis, all nursing behaviours were aggregated into one single parameter and reported as nursing. Dam behaviours and home cage locomotor activity were compared by Student’s t-test.

Body weight data (dam, offspring) were analysed by Two-way RM ANOVA (time, diet).

An initial within treatment analysis (ANOVA) of behavioural data confirmed that there were no significant differences between litters (P>0.05). Hence offspring behavioural data were analysed by Two-way ANOVA (sex, diet) with the offspring as statistical units.

All data was analysed and all graphs were created using GraphPad Prism Version 6.00 for Windows (GraphPad Software, USA). Significance was accepted at a P-value < 0.05. All graphs are shown as mean + SEM.

All experimental procedures were approved by the institutional Ethical Committee.

**RESULTS**

**Energy, macronutrient intake and body weight**

Lactating CD-fed females had a higher daily energy intake (t=2.48; P<0.05) due to overconsumption of fat (t=9.35; P<0.001) and sucrose (t=8.53; P<0.001), although the overall carbohydrate intake was similar to chow fed controls. Protein intake was reduced in CD fed dams by 23% (t=2.19; P<0.05) (Table 2).
Litters of both the control group (6.0 ± 0.2 g) and the CD group (6.2 ± 0.2 g) and the respective dams (C = 281 ± 12.2 g; CD = 268.0 ± 8.3 g) had a similar body weight at the start of the study. Both litter (C = 55.6 ± 1.9 g; CD 57.7 ± 2.0 g; P < 0.0001) and dam (C = 292.9 ± 12.9 g; CD = 288.6 ± 10.5 g; P < 0.0001) body weight increased during the lactational period, whereas CD had no significant effect upon body weight neither in pups nor in dams.

**Maternal behaviour and dam behaviour in the home cage**

Following 8 days of exposure of dam and litter to the cafeteria diet, changes in maternal behaviour were observed. Whereas the total time nursing was not affected by the diet, CD fed dams spent more time licking/grooming the pups (t=2.5; P<0.05). There was no impact of the diet on the duration of nesting behaviour (Fig. 1) and none of the non-maternal behaviours (feeding, drinking, self-grooming and rearing) were affected (data not shown).

**Home cage activity**

The cafeteria diet had no effect on home cage locomotor activity when measured during the last 24 hrs before weaning (control: 28838 ± 2013 counts/24 hrs vs. CD: 26181 ± 1601 counts/24 hrs, n=8/group).

**Offspring behaviour**

**Open-field**

No effects of sex or interactions have been observed for any of the analysed parameters. Thus, data from male and female offspring are presented together. When sex specific effects of diet were observed, data for males and females were also presented within figure 2.

The cafeteria fed offspring travelled a significantly higher distance than the chow fed pups (F(1,36) =6.161, **P<0.01), along with an increased frequency of rearing (F(1,36) = 4.270, *P<0.05). Cafeteria fed pups entered the more aversive inner zone of the open-field after a significantly shorter latency than the chow fed pups (F(1,36) = 4.259, *P<0.05). In addition, they made more entries into (F(1,36) = 6.331, *P<0.05) and spent more time in the inner zone (F(1,36) = 7.462, **P<0.01) (Fig. 2).

**Elevated Plus maze**

As in the open-field, no sex differences or interactions were observed. When sex specific effects of diet were observed, data for males and females were presented within figure 3.

Offspring from both groups travelled the same distance on the plus maze. Both groups spent a similar amount of time on the aversive open arms and the number of entries into the open arms in relation
to total entries was not different either. By contrast, and similar to the open-field, rearing was increased in the offspring fed on a lactational cafeteria diet \(F(1, 44) = 7.628, P < 0.01\) (Fig. 3).

**DISCUSSION**

Cafeteria feeding led to a significant increase of energy intake in lactating dams that was due to overconsumption of sugar and fat, whereas protein intake was reduced. These changes in energy and macronutrient intake are in line with previous data from our laboratories, indicating robustness of the feeding model (Akyol et al., 2012; Wright et al., 2014). This imbalance in lactational diet caused changes in offspring open-field behaviour and, to a lesser extent, in the elevated plus maze. The changes in the open-field reflect an overall increased locomotor activity in offspring fed on the experimental diet, whereas only rearing was increased in both the plus maze and open field. Remarkably, the open-field data clearly indicates more approaches to and more time spent in the more aversive central field of the arena. Such a structural change of open-field exploration has been interpreted as anxiolysis (Prut and Belzung, 2003; Ramos et al., 1997). By contrast, rats with signs of increased anxiety-related behaviours in a variety of tests, would spend less time in the aversive centre of an open-field (Voigt et al., 2005).

Increased locomotor activity in this test can occur for a variety of reasons, mainly motivational and emotional, all of which could possibly interact with other variables such as age (Boguszewski and Zagrodzka, 2002; Lynn and Brown, 2010). However, we have previously demonstrated that reducing the aversiveness of the open-field, by dimming the ambient light, increases locomotor activity in otherwise anxious rats (Voigt et al., 2005). This suggests a modulatory effect of anxiety on this parameter. The structural changes in in open-field locomotor activity, as observed in the current study, indicate a qualitative change rather than just a simple increase. In addition, no diet related changes in home cage activity have been observed. The latter rules out alternative interpretations such as an unspecified diet-induced increase in general activity.

However, when the changes in open-field behaviour are interpreted as being anxiolytic, the lack of such anxiolytic changes in the elevated plus maze is puzzling. The development of the anxiety system in the rat begins pre-weaning, but is probably not fully developed at the current time of testing (Ganella and Kim, 2014). In the present study, offspring from chow fed control dams showed a distinct highly anxious profile on the elevated plus maze with less than 15% time spent on the aversive open arms and approximately 25% of total entries into these arms. These values are almost identical when data from male and females rats from a previous study in our laboratory were averaged (Li et al., 2016). Although comparisons across laboratories can be obscured by environmental differences down to breeding conditions (Rex et al., 1996), previous studies (Bert et al., 2001; Rex et al., 1999; Thongsaard et al., 1996) demonstrated between 31 and 36% open arm entries. Rats with approximately 49% open arm entries have been described as “low anxiety” rats (Ho et al., 2002). Of note, although slightly older than the ones tested here, early adolescent rats spent a similarly low percentage of time on the aversive open arms of the plus maze, as reported in the current study (Lynn and Brown, 2009). Therefore it is likely that a “mild” effect of the diet would not be sufficient to induce anxiolysis in highly anxious rats. The behaviour of chow fed offspring is in keeping with previous
results demonstrating a decrease in anxiety related behaviour between adolescence and adulthood (Lynn and Brown 2010), but also within adolescence itself where early adolescent rats (PND 24-32) show the highest level of anxiety. Around weaning, anxiety increases from the early pre-weaning period to post weaning (PND 22-24) (Albani et al., 2015). We hypothesize that, due to the high innate anxiety during the time of testing, rats are less susceptible to anxiolytic dietary manipulations when tested in the elevated plus maze. This raises questions as to why there was a behavioural effect of the diet in the open-field. In a previous study, the percentage of time on the open arms of the EPM was positively correlated with the open-field measures ‘locomotor activity’ and ‘entries into the centre of the arena’ (Lynn and Brown 2009). In addition, factor analysis has shown that the approach to the centre of the open-field and to the open arms of the elevated plus maze load both on the same factor that is sensitive both to anxiolytic and anxiogenic drugs (Ramos et al., 1997). However, after analysing a total of 19 variables in models and another anxiety test, the black and white box, Ramos et al. (1997) failed to produce one main single factor that reflects a unique phenotypical dimension of anxiety and accounts for all anxiety-related behavioural changes. Hence the authors suggested a rather multidimensional construct that is expressed differently across tests. This would be in line with pharmacological evidence that shows that clinically active anxiolytic drugs can produce a variety of behavioural effects in the open-field (Prut and Belzung, 2003). Anxiety is not only a multidimensional construct (Ramos et al., 1997), but also matures post weaning (Ganella and Kim, 2014). Hence, the observed differences between open-field and plus maze behaviour could be due, at least to some extent, to the ongoing development and maturation of the anxiety system.

The increased rearing does not seem, at least in the plus maze, to be directly anxiety related and may reflect increased vertical exploration as stimulated by novelty. Nevertheless any explorative behaviour is susceptible to modulation by anxiety (Cruz et al., 1994; Lever et al., 2006). Interestingly, rats selected for a high rearing trait, showed reduced anxiety in the open-field, although this was not consistent with other models of anxiety (Borta and Schwarting, 2005; Thiel et al., 1999) and some ambiguity of this parameter has been discussed (Ennaceur, 2014).

The lactational period in rats is particularly sensitive to environmental, including nutritional manipulations (Alfaradhi and Ozanne, 2011; Daniel et al., 2014; Kaffman and Meaney, 2007; Li et al., 2016; Plagemann et al., 1992; Wright et al., 2011b; Wright et al., 2014). Behavioural effects due to early dietary manipulations can be long lasting and therefore a programming effect can be assumed. Thus, early exposure to a cafeteria diet, but also nonspecific overfeeding, had an anxiolytic effect in adult age, both in the open-field and the plus maze (Spencer and Tilbrook, 2009; Wright et al., 2011a). However, a direct comparison to our previous study (Wright et al., 2011a) is hampered by the fact that changes in anxiety-related behaviours emerge with puberty when sex differences start to occur (Johnston and File, 1991; Zitman and Richter-Levin, 2013). In line with the present results, such sex differences have not been observed following exposure of adolescent rats to the open-field and the plus maze or during object exploration (Beatty and Fessler, 1976; Heyser and Ferris, 2013; Imhof et al., 1993). Sex differences in the open-field and elevated plus maze emerge and become prominent in late adolescence (Beatty and Fessler, 1976). Although one study (Lynn and Brown, 2009) found some sex differences already in early adolescent rats, the entries into the inner zone of the open-field were in line with our results and not different between males and female in that study. By contrast, (Lynn and Brown, 2009) observed sex differences in the elevated plus maze, but their rats were tested at 30-32 days of age whereas we tested 23 day old rats.
The behavioural effects observed in the offspring fed on cafeteria diet during lactation could be due to a direct effect of the diet as milk composition reflects the composition of a cafeteria diet (Rolls et al., 1986) and cafeteria feeding has been also shown to have anxiolytic effects in adult rats. However, diet may also induce its effects indirectly on maternal behaviour, as both undernutrition and overnutrition have been shown to change maternal behaviour (Levay et al., 2008; Purcell et al., 2011).

Maternal care provided by the dam determines the early postnatal environment of the pups. The quantity of pup licking/grooming is a reliable measure to assess maternal care (Champagne et al., 2003; Hellstrom et al., 2012); increased licking/grooming of pups is associated with a reduction in neuroendocrine responses to stress in rat offspring (Liu et al., 1997), and this altered response may persist throughout life (Caldji et al., 1998). Maternal licking and grooming can also alter the cytosine methylation of the glucocorticoid receptor promotor thus programming the offspring epigenome (Weaver et al., 2004). In the current study, licking/grooming was increased in dams fed on cafeteria diet.

It could be argued that the present analysis of maternal behaviour is limited, because, and in contrast to other studies (Champagne et al., 2003), it is based on a single observation at a single time point during lactation. However, pups are heavily dependent on the mother around that time (Pachon et al., 1995) and dams show a particular high intensity of maternal behaviour during the light phase (Ader and Grota, 1970; Grota and Ader, 1969; Leon et al., 1984). Moreover, a single observation period has also been used successfully in the past (Levay et al., 2008). Admittedly, the maternal behaviour data is preliminary and cannot provide causative evidence. However, studies have also shown that dam-pup interactions are initiated and regulated by the pups. It is both olfactory and ultrasound emissions that have an effect on the levels of maternal behaviour, especially anogenital licking, expressed by the dam (Brouette-Lahlou et al., 1992). Thus, the current results suggest that the nutritional environment during lactation impacts on offspring behaviour not only via the nutritional route, but possibly also via behavioural interactions between dam and pups during this developmental period.

The present study extends previous findings where an exposure to a cafeteria diet during lactation programmed behavioural changes during adulthood (Wright et al., 2011a; Wright et al., 2014). Here, we demonstrate that lactational diet impacts on the development of behaviour in the early adolescent rat. Considering that psychiatric diseases are increasingly seen as developmental disorders, an early obesogenic diet could be an under-investigated contributing factor.

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REFERENCES


Legends

**FIGURE 1** Duration of nursing, licking/grooming and nesting over a 45 min observation period. Dams from the cafeteria group (black columns) show increased grooming/licking of the pups. Mean + SEM. n = 12/group *P < 0.05. Student’s t-test.

**FIGURE 2** Offspring open-field behaviour. Offspring exposed to a cafeteria diet during lactation (black columns) show behavioural signs of decreased anxiety when compared to offspring from control group (white columns). m = males, f = females (see text). Mean + SEM. n = 20/group *P < 0.05; **P < 0.01. Significant effect of diet. Two-way-ANOVA.

**FIGURE 3** Offspring plus maze behaviour. Offspring exposed to a cafeteria diet during lactation (black columns) show only an increased rearing but no other behavioural change. m = males, f = females (see text). Mean + SEM. n = 24/group, **P < 0.01. Significant effect of diet. Two-way-ANOVA.
Maternal Behaviour

Total nursing

Licking/grooming

Nesting
Open Field

Distance travelled

Distance (cm)

<table>
<thead>
<tr>
<th>Diet</th>
<th>C</th>
<th>CD</th>
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<tbody>
<tr>
<td>m</td>
<td>718 ± 95</td>
<td>914 ± 11</td>
</tr>
<tr>
<td>f</td>
<td>691 ± 100</td>
<td>967 ± 70</td>
</tr>
</tbody>
</table>

Frequency of rearing

<table>
<thead>
<tr>
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<th>C</th>
<th>CD</th>
</tr>
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<tbody>
<tr>
<td>m</td>
<td>7.4 ± 1.0</td>
<td>13.0 ± 3.1</td>
</tr>
<tr>
<td>f</td>
<td>6.9 ± 1.9</td>
<td>12.3 ± 2.2</td>
</tr>
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</table>

Latency to first entry into inner zone

<table>
<thead>
<tr>
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<th>C</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>270 ± 16.9</td>
<td>228 ± 28.6</td>
</tr>
<tr>
<td>f</td>
<td>291 ± 9.1</td>
<td>227 ± 37.9</td>
</tr>
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Frequency of entries into inner zone

<table>
<thead>
<tr>
<th>Diet</th>
<th>C</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>m</td>
<td>0.3 ± 0.2</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>f</td>
<td>0.3 ± 0.2</td>
<td>1.3 ± 0.6</td>
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Duration in inner zone

<table>
<thead>
<tr>
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<th>CD</th>
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<tbody>
<tr>
<td>m</td>
<td>0.1 ± 0.1</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>f</td>
<td>0.3 ± 0.2</td>
<td>1.3 ± 0.6</td>
</tr>
</tbody>
</table>
Elevated Plus Maze

**Distance travelled**

- **Diet**
  - C
  - CD

**Open arm entries**

- **Diet**
  - C
  - CD

**Time on open arms**

- **Diet**
  - C
  - CD

**Frequency of rearing**

- **Diet**
  - C
  - CD

\[ m = 7.9 \pm 1.1 \quad m = 10.4 \pm 1.3 \]
\[ f = 6.8 \pm 0.9 \quad f = 10.2 \pm 0.9 \]
Table 1

Macronutrient and sodium content of diet (per 100g)

<table>
<thead>
<tr>
<th></th>
<th>Energy (kJ)</th>
<th>Protein (g)</th>
<th>CHO (g)</th>
<th>Sugars (g)</th>
<th>Fat (g)</th>
<th>Saturated Fat (g)</th>
<th>Na %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cake</td>
<td>1510</td>
<td>3.8</td>
<td>61.5</td>
<td>38.3</td>
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<td>2472</td>
<td>29.6</td>
<td>11.6</td>
<td>5.9</td>
<td>46</td>
<td>8.7</td>
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<td>2230</td>
<td>5</td>
<td>52.8</td>
<td>0.7</td>
<td>32.7</td>
<td>3.3</td>
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<tr>
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<td>2263</td>
<td>5.8</td>
<td>62.9</td>
<td>57.1</td>
<td>29</td>
<td>18.8</td>
<td>0.2</td>
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<tr>
<td>Sausages</td>
<td>1075</td>
<td>12.9</td>
<td>12.8</td>
<td>1.5</td>
<td>16.7</td>
<td>6.1</td>
<td>1.5</td>
</tr>
<tr>
<td>Pork pie</td>
<td>1556</td>
<td>9</td>
<td>25.7</td>
<td>3.1</td>
<td>25.3</td>
<td>10.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Cheese</td>
<td>1740</td>
<td>25.4</td>
<td>0.1</td>
<td>0.1</td>
<td>34.9</td>
<td>21.7</td>
<td>1.8</td>
</tr>
<tr>
<td>Jam</td>
<td>1033</td>
<td>0.3</td>
<td>61.2</td>
<td>48.6</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<tr>
<td>Pate</td>
<td>1472</td>
<td>14</td>
<td>3.3</td>
<td>2.8</td>
<td>31.2</td>
<td>12.7</td>
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<tr>
<td>Biscuits</td>
<td>2121</td>
<td>5.7</td>
<td>60</td>
<td>16.6</td>
<td>26.4</td>
<td>16.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

| Chow diet | 1297 | 18.6 | 44.2 | 3.5 | 6.2 | 0.9 | 0.5 |

CD items contained an average of 0.9±0.2 % Na⁺ as compared to 0.5% in chow. On average, total fat contained 38.3 % saturated fat in CD and 14.5% saturated fat in chow.
Table 2
Average daily energy and macronutrient intake in lactating dams

<table>
<thead>
<tr>
<th>Diet</th>
<th>Energy intake (kJ/d)</th>
<th>Carbohydrate Sucrose (g/d)</th>
<th>Fat (g/d)</th>
<th>Protein (g/d)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Chow</td>
<td>694.70</td>
<td>63.14</td>
<td>23.67</td>
<td>2.15</td>
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<td></td>
<td>1.97</td>
<td>0.18</td>
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<td></td>
</tr>
<tr>
<td>Cafeteria</td>
<td>908.00*</td>
<td>58.52</td>
<td>19.98</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>5.95****</td>
<td>0.43</td>
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</tbody>
</table>

Data represent mean values from 12 dams/group as collected over 21 days of lactation.

Student’s t-test. *P<0.05, ****P<0.0001 vs. chow fed controls.