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The impact of cafeteria feeding during lactation in the rat on novel object discrimination in the offspring

Thomas M. Wright[1], Madeleine V. King[2], William G. Davey[1], Simon C. Langley-Evans[3,] Jörg-Peter W. Voigt[1*]

[1] School of Veterinary Medicine and Science, [3]School of Biosciences, University of Nottingham, Sutton Bonington, Loughborough LE12 5RD, UK

[2]School of Life Sciences, University of Nottingham Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, UK.

* corresponding author:

School of Veterinary Medicine and Science

University of Nottingham

Sutton Bonington Campus

Loughborough

LE12 5RD

United Kingdom

Tel: +44 (0)115 9516408

Fax: +44 (0)115 9516440

peter.voigt@nottingham.ac.uk

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Abstract

There is increasing evidence that hyperenergetic diets impact on memory in rodents. However, it is largely unknown how diets, such as a cafeteria diet (CD), that mimic a Western diet act on learning and memory, in particular when fed during early stages of development. Here, we fed lactating dams a cafeteria diet and exposed both male and female offspring to a novel object discrimination (NOD) task, a two-trial test of recognition memory in which rats exposed to two identical objects during a training/familiarisation trial can discriminate a novel from a familiar object during the subsequent choice trial. The choice trial was performed following inter-trial interval (ITI) delays of up to 4 h. Maternal diet did not impact on exploration of the objects by either sex during the familiarisation trial. Control males discriminated the novel from the familiar object indicating intact memory with an ITI of 1h, but not 2 or 4h. CD delayed this natural forgetting in male rats such that discrimination was also evident after a 2h ITI. In contrast, control females exhibited discrimination following both 1 and 2h ITIs, but CD impaired performance. In summary, the present study shows that maternal exposure to CD programmes NOD in the adult. In better performing females dietary programming interferes with NOD whereas NOD was improved in males after lactational CD feeding.

1. Introduction

Chronic exposure of rodents to hyperenergetic diets can impair learning and memory [1; 2]. Such diet-induced memory impairments have largely been shown for hippocampal-dependent spatial tasks and less so for perirhinal-dependent object discrimination [3; 4; 5; 6; 7; 8; 9; 10]. There is some evidence that obesity induced by chronic sucrose or high fat feeding to rats impairs performance in object recognition memory tests which measure the extent to which animals can discriminate between novel and familiar objects [11; 12]. It is therefore well-established that obesogenic diets influence a range of behaviours in rats. There is now great interest in whether exposure to similar diets during early life can have similar effects. A number of studies have focused upon exposures during fetal life or the early neonatal stages. In rats, maternal obesity, due to overfeeding, can impair reversal learning [13]. Interestingly, and in contrast to the detrimental effects of adult high fat diet feeding [2] , maternal obesity had a positive impact on spatial water maze learning in the offspring when tested in adulthood [14]. In contrast, maternal obesity due to high fat feeding seems to interfere with operant learning in adulthood [15] and spatial learning is also impaired in offspring from obese mice [16].

Whilst the effects of early life exposure to high fat or high sugar diets are documented, less is known about behavioural effects of Western-type diets like the cafeteria diet (CD) [17; 18; 19; 20]. CD, when compared to a high fat diet, is particularly effective in modelling obesity related metabolic abnormalities [21]. A direct comparison of CD and a high fat diet also revealed differences in their effects on memory, suggesting differences between these diets beyond the induction of obesity [22]. Early developmental stages are a sensitive period for inducing long lasting effects of cafeteria feeding on metabolism [23; 24; 25]. However, little is currently known about the behavioural effects of early cafeteria feeding. A study by White et al. [22] demonstrated that exposure to CD or a high fat diet had different sensitising effects on water maze retention following a re-exposure to the same diet in adulthood. We recently demonstrated that early, in particular lactational, cafeteria feeding does not only programme a pre-obese state in adult offspring, but also programmes feeding behaviour and anxiolysis when tested between 10 and 15 weeks of age [26; 27]. However, beyond programming of satiety regulation and anxiolysis, it remains unknown if lactational exposure to CD impacts on non-spatial memory. The present study therefore explored the consequences of lactational CD feeding on recognition memory in adult offspring. Memory was tested in a novel object discrimination (NOD) paradigm. Originally devised by [12], the NOD procedure has been widely utilized to investigate the impact of genetic, physiological and

pharmacological manipulations on recognition memory in rodents [for review see [28]], and also proved sensitive to nutritional manipulations [4; 11]. In contrast to the water-maze, the NOD test does not involve high levels of stress or anxiety. In high-arousal memory tests, anxiolytic effects of hyper-energetic diets [29] can contribute to the diet-induced memory impairment [30]. Our previous finding that lactational CD feeding programmes anxiolytic effects in the offspring [27], would therefore preclude the aversive water-maze as a test of choice. As direct exposure of rats to hyperenergetic diets has been reported to induce memory deficits, it was hypothesized that maternal exposure to CD might induce a deficit in recognition memory in adult offspring.

2. Experimental Procedures

Pregnant female Wistar rats (Harlan, UK) were housed individually with *ad libitum* access to a standard laboratory chow (Teklad Global 18%, Harlan, 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 either a standard laboratory chow diet (control), or fed the same chow in conjunction with the experimental CD. The latter 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. [31] Four of these food items were provided daily and one of those was changed daily. At postnatal day 21 the offspring were weaned, group housed with littermates of the same sex and maintained on the chow control diet for remainder of the study.

For the behavioural testing, a total of 16 dams/feeding conditions was used and 8 pups from each litter were randomly allocated to a testing condition (n=10/condition). Food consumption of the dams during lactation was closely monitored in an additional 8 dams, four from each feeding condition. This was done in independent litters to avoid any possible handling-induced interference with behavioural testing. Energy intake (kJ) and macronutrient consumption (carbohydrates including sugar, fat, and protein) were calculated from the manufacturers' data. 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[31]. Body weight of both dams and pups were measured at the beginning and the end of the study.

NOD testing was undertaken between 11 and 13 weeks of age, which is in the range of previous studies related to the subject [26]. Ten pups of each sex have been used for behavioural testing. The methodology used in the present study was modified from King *et al.* [32]. Briefly, rats were habituated to the test arena (54cm × 38cm × 40cm) in the absence of any objects for one hour the day before testing. On the day of testing animals received an additional 3-minute habituation session and were returned to the home-cage for 1-minute, before being placed into the observation arena for the training (familiarisation) trial with two identical objects for 3-minutes. In three independent experiments, each animal was then returned to the observation arena for 3-minutes for the test (choice) trial with one of the two objects replaced by a similar but novel object, either after a 1, 2 or 4-hour inter-trial-interval (ITI). The remaining object from the familiarisation trail was left untouched (familiar object).The objects were 150ml water-filled plastic bottles with three horizontal stripes of either white (W) or black (B) 1.2 cm wide masking tape being randomly assigned for each animal during the training schedule. The objects were positioned 13 cm from the length side and 11 cm from the width side of the arena in opposite corners. Arena and objects were cleaned with 70% ethanol between experiments to eliminate olfactory cues. During the two trials exploration of each object (sniffing, licking, chewing, or approaching the object otherwise at a distance < 1cm) was recorded on video and later analysed manually using Ethovision 3.1 (Noldus, Netherlands). Testing was undertaken in constant light (80 lux) between the times of 08:30-hours and 15:00-hours.

The statistical unit for macronutrient and energy intake was the dam. Nutritional data and body weight of dams and pups were analysed using Student's *t*-test. Statistical unit for behavioural testing was the pup. The study was powered to detect a difference of 40% for time spent in exploration, based upon sigma=4.8 (determined from published studies) and an alpha value of 0.5 at 80% power. Object preferences during each NOD trial were assessed using three-way repeated measures ANOVA (with object as the within-subject factor and diet and ITI as between-subject factors) applied separately to each gender and followed by Bonferroni's multiple comparison post-hoc test. Statistical analysis was conducted with SPSS 21 (IBM, UK) and GraphPad Prism 6 (GraphPad, USA). Values are expressed as mean + SEM. $P < 0.05$ was regarded as statistically significant for all tests.

All procedures were performed under licence from the Home Office, in accordance with The Animals (Scientific Procedures) Act 1986 and after approval from the University of Nottingham Ethical Review Committee.

3. Results

Lactating CD-fed females had a higher energy intake due to overconsumption of fat and sucrose, although the overall carbohydrate intake was similar to chow fed controls. Protein intake was reduced in CD fed dams (Table 1). Body weight as measured following parturition was similar in both groups (data not shown), CD fed dams gained more weight during lactation (29.8 ± 1.3 g) than chow fed controls (17.8 ± 2.2 g) ($P<0.01$). By contrast, CD feeding did not impact on body weight in pups in this (data not shown) and in a previous study[26].

Neither male nor female offspring demonstrated any spatial preference for either identical object during the familiarisation trial and there was no impact of diet on total levels of object exploration by either gender (data not shown).

After a 1-hour ITI male offspring were able to distinguish the novel from the familiar object, regardless of whether dams received chow ($P<0.001$) or CD ($P<0.0001$) (Fig. 1a). After a 2-hour ITI male offspring from CD-exposed dams distinguished the novel from the familiar object ($P<0.01$), but controls showed no signs of memory, and neither group exhibited preferential exploration of the novel object after a 4-hour ITI (Fig. 1a).

Female offspring from control dams successfully discriminated the novel object after ITIs of both 1 ($P<0.05$) and 2 h ($P<0.001$), but in each case discrimination was absent in female offspring from CD fed dams (Fig. 1b). However, there was a tendency in these CD-fed females to discriminate the novel object after a 1-hour IT ($P<0.10$). Taken together these findings suggest that maternal exposure to CD during lactation exerts a differential effect on cognitive performance in male and female offspring with lactational CD exposure delaying memory decay in males and accelerating memory decay in females. Irrespective of maternal diet, neither gender showed any behavioural signs of memory after a 4-hour interval.

4. Discussion

This study tested the hypothesis that exposure to cafeteria feeding during the suckling period would impact upon recognition memory in adult life. This was of interest given previous observations that feeding and anxiety-related behaviours are targets for nutritional programming at this stage of life. Our findings confirmed that lactational CD influenced the learning behaviour of Wistar rats.

The present study demonstrated that offering dams a cafeteria diet during lactation led to an increased energy intake, largely due to overconsumption of fat and sucrose. We noted reduced protein intake, which has been reported in previous [31; 33], but not in all cafeteria studies [34; 35]. Although protein intake was significantly lowered by CD feeding, the 23% reduction was not sufficient to impact upon pup growth, suggesting that the protein deficit was modest compared to the over-consumption of energy, fat and sugars. We would rather

198 suggest that programming and behavioural effects of diets mimicking a Western diet are
199 complex and cannot be attributed to a nutritional imbalance of a single macronutrient.
200
201 Feeding of a hyperenergetic cafeteria diet to rat dams during lactation had a significant
202 impact on object recognition memory of the offspring in adult age. This finding provides
203 further evidence that the lactational period is not only important for metabolic programming
204 [25; 36], but also for programming of behaviour, as we found both reduced anxiety and
205 reduced behavioural satiety in parallel studies under identical conditions [26; 27].
206 The observed gender differences in chow fed controls appear consistent with previous non-
207 spatial NOD studies, where females proved superior to males, although the opposite is true
208 for spatial versions of the test [37; 38]. Although not controlled for in the present study,
209 estrogen (E2) is associated with better NOD performance [39] and could potentially modulate
210 NOD through interactions with the brain serotonergic system [40 for review]. Serotonin (5-HT)
211 plays a role in NOD [28 for review; 32] and seems to be affected by early cafeteria feeding as we
212 found in the hypothalamus in offspring of cafeteria fed dams [26]. Hence 5-HT-estrogen
213 interactions may therefore account for the observed gender differences in the effect of early
214 cafeteria programming on NOD, although an additional contribution of glucose levels is also
215 possible.
216 In obese rats, fasting glucose levels are negatively correlated with NOD [11]. Although
217 lactational CD per se only predisposes the offspring to obesity and has little impact on
218 fasting glucose level [25; 27; 31], male rats exposed to CD in the lactation period show a more
219 rapid glucose clearance in blood following a glucose challenge, whereas in females
220 lactational chow lead to faster glucose clearance [25]. As exogenous glucose can enhance
221 memory [41] and brain glucose fluctuates depending on local demand [42], one could
222 speculate that diet-programmed and gender-dependent differences in glucose
223 metabolism/clearance could contribute to differential effects of lactational cafeteria feeding
224 on NOD learning in male and female offspring.
225 Maternal obesity, either due to high fat feeding or a sucrose enriched diet, impaired reversal
226 learning in the offspring, regardless of the type of hyper-energetic diet [13]. This and other
227 studies [22] provide evidence that in rodents an obesogenic environment in early life impacts
228 on cognitive functions in adult age. However, the precise outcome, either being positive or
229 negative, depends on diet, memory model and is possibly gender-dependent. In general,
230 these rodent studies are relevant to the situation in humans where cognitive deficits have
231 been attributed also to maternal obesity [43; 44].
232

In conclusion, the present study shows that maternal exposure to CD can programme NOD in the adult. In better performing females dietary programming interferes with NOD whereas NOD was improved in males after lactational CD feeding.

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Conflict of Interest

None

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Legend

Fig. 1 The effect of maternal lactational diet on novel object discrimination in adult offspring following ITIs of 1-4h. Duration (sec, mean± SEM) spent by A males and B females exploring familiar (open bars) and novel (filled bars) objects during the choice trial (n=8-10 per group). * $P<0.05$; ** $P<0.01$; *** $P<0.001$; **** $P<0.0001$ versus the familiar object in the same gender following the same maternal diet and ITI (three-way repeated measures ANOVA with Bonferroni's multiple comparison post-hoc test).