

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

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24 Short Running Title: Nutritional programming of memory

25 Key Words: Cafeteria diet; rat; nutritional programming; memory; novel object
26 discrimination; sex differences

27 **Abstract**

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29 There is increasing evidence that hyperenergetic diets impact on memory in rodents.
30 However, it is largely unknown how diets, such as a cafeteria diet (CD), that mimic a
31 Western diet act on learning and memory, in particular when fed during early stages of
32 development. Here, we fed lactating dams a cafeteria diet and exposed both male and
33 female offspring to a novel object discrimination (NOD) task, a two-trial test of recognition
34 memory in which rats exposed to two identical objects during a training/familiarisation trial
35 can discriminate a novel from a familiar object during the subsequent choice trial. The choice
36 trial was performed following inter-trial interval (ITI) delays of up to 4 h. Maternal diet did not
37 impact on exploration of the objects by either sex during the familiarisation trial. Control
38 males discriminated the novel from the familiar object indicating intact memory with an ITI of
39 1h, but not 2 or 4h. CD delayed this natural forgetting in male rats such that discrimination
40 was also evident after a 2h ITI. In contrast, control females exhibited discrimination following
41 both 1 and 2h ITIs, but CD impaired performance. In summary, the present study shows that
42 maternal exposure to CD programmes NOD in the adult. In better performing females dietary
43 programming interferes with NOD whereas NOD was improved in males after lactational CD
44 feeding.

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56 1. Introduction

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58 Chronic exposure of rodents to hyperenergetic diets can impair learning and memory ^(1; 2).
59 Such diet-induced memory impairments have largely been shown for hippocampal-
60 dependent spatial tasks and less so for perirhinal-dependent object discrimination ^{(3; 4; 5; 6; 7; 8;}
61 ^{9; 10)}. There is some evidence that obesity induced by chronic sucrose or high fat feeding to
62 rats impairs performance in object recognition memory tests which measure the extent to
63 which animals can discriminate between novel and familiar objects ^(11; 12). It is therefore well-
64 established that obesogenic diets influence a range of behaviours in rats. There is now great
65 interest in whether exposure to similar diets during early life can have similar effects. A
66 number of studies have focused upon exposures during fetal life or the early neonatal
67 stages. In rats, maternal obesity, due to overfeeding, can impair reversal learning ⁽¹³⁾.
68 Interestingly, and in contrast to the detrimental effects of adult high fat diet feeding ⁽²⁾,
69 maternal obesity had a positive impact on spatial water maze learning in the offspring when
70 tested in adulthood ⁽¹⁴⁾. In contrast, maternal obesity due to high fat feeding seems to
71 interfere with operant learning in adulthood ⁽¹⁵⁾ and spatial learning is also impaired in
72 offspring from obese mice ⁽¹⁶⁾.

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

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

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101 **2. Experimental Procedures**

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103 Pregnant female Wistar rats (Harlan, UK) were housed individually with *ad libitum* access to
104 a standard laboratory chow (Teklad Global 18%, Harlan, UK) and water. Animals were
105 maintained under a 12-hour light-dark cycle (lights on 08:00-hours), between 20-22°C. At
106 birth, litters were reduced to 4 pups of each sex, and randomly allocated to either a standard
107 laboratory chow diet (control), or fed the same chow in conjunction with the experimental
108 CD. The latter consisted of a range of highly palatable human foods (pork pie, pate, cocktail
109 sausages, cheese, crisps, jam, fruit and nut chocolate, golden syrup cake, shortbread and
110 peanuts. ⁽³¹⁾ Four of these food items were provided daily and one of those was changed
111 daily. At postnatal day 21 the offspring were weaned, group housed with littermates of the
112 same sex and maintained on the chow control diet for remainder of the study.

113 For the behavioural testing, a total of 16 dams/feeding conditions was used and 8 pups from
114 each litter were randomly allocated to a testing condition (n=10/condition). Food
115 consumption of the dams during lactation was closely monitored in an additional 8 dams,
116 four from each feeding condition. This was done in independent litters to avoid any possible
117 handling-induced interference with behavioural testing. Energy intake (kJ) and
118 macronutrient consumption (carbohydrates including sugar, fat, and protein) were calculated
119 from the manufacturers' data. Weight loss due to evaporation was measured in triplicate
120 samples of each individual food item placed in empty cages. The average daily percentage
121 change in the weight of foods ranged from 0 to 6.2 % and corresponded to an average
122 overestimation of energy intake by 2.51 % (7.5 kJ/d), which can be considered within an
123 acceptable error of measurement⁽³¹⁾. Body weight of both dams and pups were measured at
124 the beginning and the end of the study.

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

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

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

158

159 **3. Results**

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

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

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

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

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185 **4. Discussion**

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

192 The present study demonstrated that offering dams a cafeteria diet during lactation led to an
193 increased energy intake, largely due to overconsumption of fat and sucrose. We noted
194 reduced protein intake, which has been reported in previous ^(31; 33), but not in all cafeteria
195 studies ^(34; 35). Although protein intake was significantly lowered by CD feeding, the 23%
196 reduction was not sufficient to impact upon pup growth, suggesting that the protein deficit
197 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

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

236

237 **Acknowledgements**

238 The expert technical support of Asli Akyol, Carol Armett, Sarah Kirkland, Richard Plant and
239 Karen Swift is gratefully acknowledged.

240

241 **Financial Support**

242

243 This study was supported by grant RSF5103 (JPV and SLE), University of Nottingham. Th.
244 W. was supported by an IDTC studentship from the School of Veterinary Medicine and
245 Science and the School of Biosciences, University of Nottingham.

246

247 **Conflict of Interest**

248

249 None

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254

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408 **Legend**

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

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