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Reinforcement of anticipatory eating by short as well as long fasts

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This paper is based on research presented in a dissertation for the degree of PhD at McGill University by Soghra Jarvandi, MD. The raw data re-analysed here were collected by her (Jarvandi, Booth & Thibault, 2007a; Jarvandi, Thibault & Booth, 2007b, 2009) and two other graduate students, Yehmin Yiin (Thibault & Booth, 2006) and Jennifer White (White et al., 2001). The collaborative work in Montreal was supported by a grant to Prof. Thibault by the Natural Sciences and Engineering Council of Canada, with travel funded from infrastructure support in grants for research led by Prof. Booth from Agri-Food at the Biotechnology and Biological Sciences Research Council, U.K.
Abstract

Rats can learn to anticipate the omission of subsequent meals by increasing food intake. Our previous reports have analysed group means at each trial but that does not allow for rats learning at different speeds. This paper presents instead a rat-by-rat analysis of all the raw data from previous experiments. The re-analysis supports the published evidence that the capacity for reinforcement generated by withholding of food is greater after a longer fast than after a shorter fast, but that the learning is quicker after the shorter fast. The individualised analyses also extend the evidence that the pattern of learning, extinction and re-learning with shorter fasts is similar to that with longer fasts. These findings indicate that, contrary to our previous interpretation, a single learning mechanism can explain the effects of both durations of food deprivation.

Keywords

anticipatory eating; food deprivation; length of fast; metabolic repletion; reinforcement; individual analyses; rats
**Introduction**

When rats are repeatedly deprived of food for 8-12 hours, they can learn to eat more of the food presented before the fast (Jarvandi, Booth & Thibault, 2007; Jarvandi, Thibault & Booth, 2009; Le Magnen, 1957, 1999; Thibault & Booth, 2006). They can also develop greater intake of a food that is provided before a fast of 2-3 hours (Thibault & Booth, 2006). However the increase in intake of the food before the shorter fast is less than that before the longer fast. Indeed, anticipatory eating has usually been measured as relatively greater intake acquired before the longer fast (Le Magnen, 1957; Thibault & Booth, 2006; White, Mok, Thibault & Booth, 2001).

Nevertheless the learnt increases in food intake have varied over trials in a remarkably similar pattern with both lengths of fast. Figure 1 combines the data from two experiments that were plotted separately in our first major paper on anticipatory eating (Thibault & Booth, 2006). Intake increases from trial to trial with both lengths of fast. However, intake of the distinctive food before each fast length reaches a peak and declines. This partial extinction of the learnt response is limited; the learning of increased intake resumes, creating a trough in the progression over trials (Figure 1). With both longer and short fasts, the trial group means after the trough increase to another peak, followed by a second trough. There is a subsequent rise in intake in parallel between the groups, but more trials would be needed to see if a third peak is reached (Figure 1).

The most recently published experiment in this series addressed the question whether learning of anticipatory eating depends on a contrast between the longer and shorter fasts that hitherto were double-alternated between successive trials (Jarvandi et al., 2009). Instead of having both lengths of fast in the series of training days, this time the rats were trained only on long fasts or only on short fasts. The series of long fasts induced greater anticipatory intake, both absolutely and relative to intake before the fast in another group of rats trained on a series of short fasts. This finding was replicated in the same two groups when the lengths of fast were switched. Hence a contrast in length of fast between successive training days is not necessary to the learning of anticipatory eating. The learning mechanism with long fasts operates in isolation from what happens on days with short fasts.
That experiment was not designed to test for such learning from the shorter duration of food deprivation. Indeed, the paper maintained the assumption from the start of our series of papers on this phenomenon, that any increase in intake before a short fast arose from a different mechanism from that of the increase before long fasts (White et al., 2001). Nonetheless, there was a hint in the data from the series of short fasts that this assumption could be wrong. The relative and absolute increase in intake before the longer fast replicated the clear pattern of peaks of learning and troughs of extinction seen in previous experiments (e.g., Figure 1 here). Intake before the shorter fast showed a remarkably similar pattern, although at much lower amplitude (Figure 2 in Jarvandi et al., 2009; cp. Figure 1 here). Furthermore, despite the low amplitude of the oscillation between peaks and troughs, that complex pattern was statistically supported for the shorter fast by analysis of orthogonal contrasts (rows 1 and 2 of Table 2 in Jarvandi et al., 2009). This distinctive pattern in separate series of each fast length opens the possibility that intake is increased by the same mechanism for short and long periods of food deprivation. This brief paper presents a new analysis of the published data which explores that hypothesis.

All previous reports of anticipatory learning of intake (including Jarvandi et al., 2009) used analyses of group means at each trial. If individual rats learn at different speeds, that approach to the raw data could blur the incidences and sizes of peaks and troughs in intake. This paper presents analysis that starts by identifying the first peak in intake of each rat, i.e. the start of the cycles of learning, extinction and re-learning as far as observable within a limited number of trials. The same analysis has also been applied to all the raw data from the decade of experiments; the outcomes are summarised in a table in an Appendix to this brief paper.

Method

Design

In the original experiment on anticipatory eating, intake of a distinctive food during a fixed period of access was used both to train the rat and also to test for learnt intake (Le Magnen, 1957, 1999). Successive training and testing trials differed in the consequences from which the rats might learn: restored access to maintenance food was delayed for either 12 hours or 3 hours. All our experiments on anticipatory eating followed this design with only minor variants (Figure 2). Each experiment had a succession of trials of 1.5 or 1 hour’s access to 20-30 g of food having a distinctive odour or texture, followed by a delay either of 2 or 3 hours or of 8 or 12.5 hours
before the maintenance food was returned (Appendix Table, column 2, sub-column 2).

Maintenance food was withdrawn 3 hours before each trial in order to motivate immediate eating when the food was presented. Between trials there were always at least 31.5 hours of continuous access to maintenance food, in order to prevent carry-over effects of the different lengths of fast.

Figure 2 about here

Our experiments were effectively identical in the housing of the animals and their adaptation to the conditions of maintenance and the experimental period, the contrast in deprivation periods, the randomisation and balancing of pairings of cues placed in the trial food, the scheduling of tests of intake and the collection of food intake data. Full details of each experiment were given in the original publications.

All meaningful variations among experiments are listed in an Appendix to this paper. The rats were always maintained on a standard laboratory diet (Appendix, column 2, sub-column 1), but fat was added to it in one study (Appendix, Experiment 3). The experiments varied in energy nutrient composition of the food presented ad libitum at trials (Appendix, column 2, sub-column 3). Two experiments used as trial food a complete diet containing a mixture of protein and carbohydrate (Appendix, column 2, Experiments 3 and 6). Other experiments tested protein alone or carbohydrate alone in a complete trial food, used solely maltodextrin, or compared low-fat with high fat trial foods (Appendix, column 2, Experiments 4-5, 7, and 1-2 respectively).

Analysis

The published reports presented evidence for learning from data grouped across rats at each training-testing trial through the sequence. The present paper presents the results of calculating for the first time each individual rat’s learnt responses. Intake (in grams) before a longer fast is called ‘L’ and intake before a shorter fast is ‘S.’ In our earlier reports, the relative effect of the two periods of deprivation at each stage of training was measured as L g minus S g (L - S). The individualised approach avoids that comparison between conditions. Anticipatory eating is measured within each condition by exploiting the peak in intake that is produced each time that extinction sets in after some learning (Thibault & Booth, 2006; Jarvandi et al., 2009). The height and trial number of the first peak in the learning curve are measures of, respectively, the amount and speed of learning of anticipatory eating.

Please set a minus sign (not a dash) in the formula “L - S” (L minus S) with a space before and after the minus.
However, to compare the outcomes of individualised analysis among experiments, the difference in heights between the first L and S peaks was used. Since p value depends on N, which varied across studies and sometimes was relatively small, experiments were compared by sizes of effect, estimated as the mean difference between L and S divided by the standard deviation (the d score of J. Cohen, 1988; Lomax, 2007), to give a standardised L - S value for the troughs and the peaks (Appendix, columns 9 and 14). These effect sizes of the individually acquired responses were compared among the experiments, using ANCOVA to adjust for each rat’s L - S score at the first pair of trials, followed by Dunnett’s test for multiple comparisons. When an effect was estimated across all experiments, the mean for each experiment was weighted into the single grand mean by taking the value for each rat in all the experiments and dividing by the total number of rats.

**Results**

For the most recently reported experiment, using maltodextrin as the trial food (Jarvandi et al., 2009; Appendix Experiment 7), the learning by eight individual rats is plotted in Figure 3 in each of the two series of ten trials followed by either shorter fasts (S; 2 hours) or longer fasts (L; 8 hours). In each rat, food intake increased over the initial trials with both durations of fast. Trial-to-trial variability produces small peaks and troughs. Nevertheless, if a mean or median for each trial is estimated by eye within each panel (fast length) in Figure 3, a fairly steady increase across trials can be seen (with opposite anomalies in two rats at Trial 3 on the longer fast). That central tendency peaks at Trials 3-4 with the shorter fast (S, upper panel of Figure 3) and Trials 6-7 with the longer fast (L, lower panel). There is a notable decline in the scatter at Trials 6 and 7 with the shorter fasts (upper panel).

Figure 3 about here

**Relative rates of learning by each rat**

In the series of S trials, all the rats’ first peak in learnt intake was in or before Trial 4, whereas all but one of the first peaks for the series of L trials were in Trial 5 onwards (Figure 3). That distribution approached non-random with p < 0.07 by two-tailed t test.

This outcome from reanalysis was consistent across all the experiments. First the group counts of individuals’ trough and peak of L - S need to be tested against random incidences across trials in each of the seven experiments. The probability of difference from a random
distribution across cycles of the number of rats at each cycle that showed a peak or a trough was estimated by using Fisher’s exact test (Appendix Table columns 6-7 and 11-12). Combining all experiments, these individuals’ peak intakes were non-randomly distributed over trials, \( p < 0.0001 \) for both L and S.

Furthermore, also as visible in some of the previously reported trial-grouped means, individual rats’ peaks in intake before the short fast were usually earlier (often before the 4\(^{th}\) trial) than peaks before the long fast (which were seen most often from the 6\(^{th}\) trial onward). That difference in timing of the maximum acquired intake of individual rats was reliable when the energy nutrients in the trial food were protein only, a mixture of carbohydrate and protein (all \( p < 0.0001 \)) or high in fat (\( p < 0.02 \)).

**Relative amounts of learning**

In contrast to faster learning with the shorter delay in refeeding, greater intake was reinforced eventually by effects of the longer delay (Figure 3). Mean intake at each rat’s peak for the L trials was 16.9 g (95% confidence limits: 14.6, 19.2), whereas it was 13.6 g (12.2, 15.0) for the S trials, \( p < 0.01 \). The effect sizes of these individualised peaks were 5.08 for L trials and 6.91 for S trials; however, this difference in the direction opposite to that expected was not reliable, \( p > 0.3 \).

Across all seven experiments run so far, individual animals’ peak intake of the food discriminative of the subsequent duration of deprivation was greater preceding the longer period of 8-12 hours (L) than it was before the shorter period of 2-3 hours (S) at the same number of trials. Weighted by the number of rats in each experiment, the grand mean ± SD of peak intake before the long fast was \( L = 9.27 ± 3.70 \) g, with \( S = 6.23 ± 2.49 \) g at the same number of trial before the short fast -- a ratio for L/S of 1.49.

**Cycles of learning and extinction**

Presumably the learning of greater intake before a long fast reduces the reinforcement resulting from that deprivation and so intake declines; that restores reinforcement and so intake rises again. When only short fasts were imposed, their trial-mean intakes also showed signs of such oscillation from learning to extinction and re-learning, albeit very slight; nevertheless, as pointed out in the Introduction, there was statistical support for peaks and troughs (Jarvandi et al., 2009). The plots of individual rats indicate why (Figure 3, upper panel): with this shorter fast, the rats strongly converged on a trial in the second peak of learning followed by extinction.
Discussion

The individualised analyses confirmed and extended the evidence from the previously published trial-grouped analyses. The capacity for reinforcement generated by withholding maintenance food is greater after longer deprivation than after a shorter fast. In addition, learning is quicker with the shorter delay between response and reinforcement than with the longer delay. Thirdly, the self-extinguishing effect of increased intake before long fasts is readily seen also before short fasts.

This evidence for anticipatory eating with short as well as long fasts does not distinguish among possible mechanisms of such learning. Either duration without food could reinforce intake negatively, avoiding later depletion, as our papers to date have proposed. Alternatively, each length of fast could reinforce positively, i.e. reward intake as behaviour that leads eventually to a state of repletion.

It should be noted that Le Magnen (1957) made the quite different suggestion that a short fast induces an anticipatory satiety that the long fast cannot. Since absolute intake increases before the shorter fast as well as before the longer fast, we suggest that it is more natural to interpret the phenomenon as learnt hunger rather than learnt satiety, or as anticipatory eating before both lengths of fast.


Captions to Figures

Figure 1. Intake of training and testing food (g, mean ± SEM) before shorter fast (S: 3 hr) and longer fast (L: 10 hr) with either protein or carbohydrate food (Experiments 4 and 5; Thibault & Booth, 2006).

Figure 2. Sequence of procedures before, during and after a trial of training and testing for anticipatory eating in laboratory rats. Compare the Figure 1 in each of Le Magnen (1957, 1999), White et al. (2001), Thibault and Booth (2006) and Jarvandi et al. (2007). T/T Fd: the distinctive food (Fd) presented at the training and testing trial (T/T) before one of the designed delays in restoring access to maintenance food.

Figure 3. Individual rats’ intakes of training and testing food over ten successive trials with a fixed deprivation period following that intake. Upper graph: shorter fasts (S: 2 hr). Lower graph: longer fasts (L: 8 hr). The first series of trials had the shorter fast in half the group of eight rats and the longer fast in the other four rats, with lengths of fast reversed in the second series (Jarvandi et al., 2009).
Figure 1
Figure 2
Setter: to save space, please do not enlarge this graphic vertically at all.

TRAINING / TESTING TRIAL FOR ANTICIPATORY EATING

<table>
<thead>
<tr>
<th>0</th>
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<th>36</th>
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<tr>
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Maintenance food *ad libitum*

<table>
<thead>
<tr>
<th>&lt; 3 h</th>
<th>T/T</th>
<th>&lt;varied fast length&gt;</th>
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<tr>
<td>Fd</td>
<td>2-3 h</td>
<td>or 8-12.5 h</td>
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Maintenance food withheld

Maintenance returned *ad lib.*
Figure 3.
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### Appendix. Maximum observed differences in individual rats between intakes before longer or shorter periods of withholding of food.

<table>
<thead>
<tr>
<th>Exp. no.</th>
<th>Design</th>
<th>Greater intake before shorter deprivation (L &lt; S)</th>
<th>Greater intake before longer deprivation (L &gt; S)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>N of rats at cycle</td>
<td>Mean L - S intakes (g) (95% CLs)</td>
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<tr>
<td>1</td>
<td>Chow 2 High Fat</td>
<td>10</td>
<td>8, 0, 2</td>
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<tr>
<td>2</td>
<td>Chow 2 Low Fat</td>
<td>10</td>
<td>7, 1, 2</td>
</tr>
<tr>
<td>3</td>
<td>Chow 2 Chow + fat [CHO+Pro]</td>
<td>8</td>
<td>7, 1, 0</td>
</tr>
<tr>
<td>4</td>
<td>Chow 2 Pro</td>
<td>16</td>
<td>14, 2, 0</td>
</tr>
<tr>
<td>5</td>
<td>Chow 2 CHO</td>
<td>16</td>
<td>15, 1, 0</td>
</tr>
<tr>
<td>6</td>
<td>Chow 2 CHO + Pro</td>
<td>16</td>
<td>11, 1, 4</td>
</tr>
<tr>
<td>7</td>
<td>Chow 1 MD</td>
<td>8</td>
<td>4, 4, 0</td>
</tr>
</tbody>
</table>

[Notes on next page of MS]
Abbreviations: L, trial intake before longer period of food deprivation; S, trial intake before shorter deprivation; CLs, 95% confidence limits; SD, standard deviation; CHO, carbohydrate; Pro, protein; MD, maltodextrin.

a The lowest score of L-S for each rat after the first cycle of training.
b The highest score of L-S for each rat after the first cycle of training.
d Probability that the counts were random by Fisher's Exact Test.
e Paired comparison with the first cycle of training.

Setter: please include the whole Table and its footnotes within ONE printed page at the very END of the paper (or as Table 1 in a break of References or Discussion if necessary to keep Table and Notes all on the same page).