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Individual differences in saccharin acceptance predict rats' food intake

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HIGHLIGHTS

- Saccharin acceptance predicts subsequent chow intake.
- Elevated plus maze measure related to weight gain.
- Running did not predict any outcome.

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ABSTRACT

Following previous results indicating that low acceptance of saccharin-sweetened yoghurt was associated with slower weight gain, the aim of this experiment was to determine which of three measures of individual differences would predict subsequent chow consumption, body weight gain, and fat mass. Pre-test measures consisted of amount of running in an activity wheel, amount of 0.1% saccharin solution consumed over 24 h, and performance on an elevated plus maze (EPM). Rats were then maintained for three weeks on a diet of standard chow and water. Subsequent post-testing repeated the procedures used in pre-testing. The rats were then culled and fat pads excised and weighed. Pre-testing revealed a negative correlation between saccharin acceptance and activity, while neither measure correlated with anxiety in the EPM. Pre-test saccharin acceptance was positively correlated with subsequent chow consumption, percent weight gain, and g/kg fat mass. Multiple regression analyses including all three pre-test measures confirmed saccharin acceptance as a predictor of chow consumption and, marginally, of fat pad mass, while high anxiety predicted low percent body weight gain.

1. Introduction

There is currently disagreement as to whether the use of artificial (or non-nutritive) sweeteners (NNS) in commercial foods and beverages conveys any benefit with regard to body weight changes in comparison to the use of sugars such as sucrose or High Fructose Corn Syrup (HFCS) as sweeteners. Two recent reviews have concluded that the use of NNS can be beneficial; for example, meta-analysis of sustained human intervention studies suggests that use of NNS reduces bodyweight to a small extent in comparison to sugar-sweetened equivalents [1,2]. The opposing view, namely that the use of NNS is counter-productive and promotes more rapid weight gain than the use of sugars, has been advanced partly on the basis of rat experiments that have compared the effects of NNS – usually saccharin – with those of glucose [3–6].

In a recent experiment undertaken to follow up on a comparison between glucose- and saccharin-sweeteners [7] we exposed rats in an initial stage to both glucose- and saccharin-sweetened yoghurt, as well as plain yoghurt, and then their acceptance of the two kinds of sweet yoghurt was measured. An incidental finding from this experiment was that acceptance of saccharin-sweetened yoghurt, i.e. amount consumed over a 2-h period, predicted body weight gain over the subsequent 12 weeks of the experiment, whereas acceptance of glucose-sweetened yoghurt did not [8].

The main aim of the present study was to test the generality of this finding by first measuring acceptance of a saccharin solution, rather than saccharin-sweetened yoghurt, and then measuring weight gain over a period when rats were given a diet of standard chow and water, rather than the more complex diet we had previously used [8]. As first noted over five decades ago, albino rats tend to show lower and more variable preferences for saccharin solutions than hooded rats [9,10]. Furthermore, male rats tend to show lower and more variable saccharin preferences than female rats [e.g.11]. Such variability

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has also been found in albino rats' response to the artificial sweetener, sucralose, where again males show lower preferences than females [12]. In recent experiments in our laboratory that have involved giving male albino rats saccharin solutions we have also found considerable individual differences in acceptance of such solutions [13].

Rats bred for low acceptance of saccharin have been found to be more anxious, as indicated by an emergence test and amount of defecation in an open field, than those with high saccharin acceptance [11]. In the present experiment anxiety was a second individual difference measure but, instead of an open field test, this was assessed in terms of performance on an elevated plus-maze [14,15].

Another way in which individual rats can differ greatly is in how much they run when placed in an activity wheel [16]. As we had also noted large individual differences in amount of running in our laboratory [17], we were interested to test whether amount of voluntary running might also predict weight gain.

The present study started with a pre-test stage in which all rats were measured on the three variables: Saccharin acceptance, running, and EPM performance. They were then maintained for three weeks on a chow and water diet, before a post-test similar to the pre-test was carried out. Finally, the rats were culled and their fat pads measured.

2. Method

2.1. Subjects

Thirty male Sprague Dawley rats were purchased from ARC Perth, WA. They were 6–7 weeks old on arrival, when they were initially housed 4–5 in a large plastic cage. A week after arrival they were handled daily for 4 days before pre-testing began, as described below. After the first three days of pre-testing they were transferred to individual cages, measuring 44 × 28 × 29 cm, and remained housed in these cages until the end of the experiment in a colony room maintained under a normal 12:12 lighting cycle, with lights on at 0700 h. The rats' mean weight at the start of the experiment was 334 g (SEM 4.8 g). Throughout the experiment they had unrestricted access to the standard rodent chow used widely in Australia (Specialty Feeds, 14.2 kJ/g; 20% protein, 4% fat, 60% carbohydrate; http://www.specialtyfeeds.com) and to water, except during the initial training described below.

2.2. Apparatus

Drinking chambers consisted of fifteen steel cages with steel rod flooring and sides, 20 × 20 × 30 cm high. Plastic drinking bottles of 100-ml capacity with stainless steel ball-bearing spouts could be attached to the drinking chambers using a metal bracket, entering at a 45° angle through the steel wire door.

Activity wheels consisted of a set of twelve commercial wheels, 10 cm wide and 1.1 m in diameter in circumference (MED Product #: ENV-042A; www.med-associates.com) and a set of four custom-built wheels, also 10 cm wide and 1.1 m in circumference. For both types of wheel, revolutions were stored in 30-s intervals using Labview© software.

The Elevated Plus-Maze (EPM) consisted of two runways, 101 × 11 cm, intersecting at a central open square, 11 × 11 cm, at a height of 70 cm above the ground. One runway was enclosed by 41-cm high walls ('closed arms'), while the other runway had no walls ('open arms'). Behavior on the EPM was automatically scored by video tracking software.

2.3. Procedure

Rats were first given three 30-min training sessions to ensure that they would readily drink water in the drinking chambers. Over these three sessions water restriction was reduced from an initial 17 h to 1 h prior to a session.

On each of the first three days of the pre-test stage all rats were given 1-h access to 0.1% saccharin solution in the drinking chambers and 1-h access to an activity wheel. Rats were placed in a different wheel on each of the three days, in order to partially compensate for possible differences between wheels. Half the rats were given 1 h of saccharin followed by 1 h of running, and the other half was given the reverse sequence. This factor was partially counterbalanced across days so that the order of wheel and saccharin sessions alternated across days for each rat. Thus, for 16 rats (4 home cages) the order was wheel-saccharin on Days 1 and 3, and saccharin-wheel on Day 2, whereas the other 14 rats (3 home cages) received saccharin-wheel on Days 1 and 3 and wheel-saccharin on Day 2. Rats were given only a single EPM test, with ten rats tested each day after they had spent at least 90 min in their home cages since their morning saccharin and wheel tests. Since rats drank very little saccharin solution in their three 1-h initial tests, after four days of acclimatization to their single cages, saccharin intake was measured in all rats over a 24-h period. For this 24-h test saccharin solution was presented in a larger 300 ml bottle.

During the subsequent 3-week Diet stage the rats were given unrestricted access to their normal chow and water. They were weighed twice each week, together with their chow consumption.

The subsequent Post-test stage was similar to the initial test stage, in that the rats were again given 1-h access to the activity wheels on three successive days and ten rats were tested each of these days on the EPM. On the other hand, unlike in the initial test, no access to saccharin was given on these three days. Instead, four days later they were given 24-h access to saccharin, as in the initial test.

Finally, the rats were culled by intraperitoneal injection of sodium pentobarbital, and retroperitoneal, visceral and epididymal fat pads were excised and weighed.

2.4. Data analysis

Running was measured as the mean number of wheel turns per session over the three 1-h sessions in both the pre-test and post-test. Saccharin acceptance was measured as amount consumed in the 24-h pre- and post-tests. Anxiety was measured as the proportion of time spent in the closed arms of the EPM over the total time spent in both arms. In preliminary analyses, correlations between pairs of these three measures were calculated at both pre- and post-tests. The stability over time of the three individual difference measures was assessed by correlating pre- and post-test scores on each measure. Finally, correlations were calculated between each of these measures and chow intake (g/kg/day) over the three weeks, percentage body weight gain and total fat pad mass (g/kg, summed over the three sites).

The main results were obtained from sequential multiple regression analyses that examined predictors of chow consumption per kg of body weight, percentage weight gain over the 3-week diet stage and fat pad mass per kg of body weight. The three predictor variables were entered in the following order: Saccharin acceptance at pre-test; running at pre-test; anxiety at pre-test.

3. Results

During the 24-h saccharin consumption test the mean intake was 61.96 ml (SEM: 6.58). In the EPM test the proportion of time spent in the open arms of the maze was generally high (mean 39.5 ± 2.7%) and this may relate to the fact that rats were handled regularly prior to the pre-test phase. While EPM behavior can be modulated by handling, we found no differences between the subsets of rats tested on Days 1, 2, or 3 of the pre-test phase ($F < 1$), suggesting this was unlikely to have affected interpretation of our results.

More importantly, the degree to which the three pre-test measures were correlated is shown in Fig. 1. There was a strong negative correlation between saccharin acceptance and running, but neither of these measures was correlated with anxiety as measured on the EPM.