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Dehydration-anorexia derives from a reduction in meal size, but not meal number

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ABSTRACT

The anorexia that results from extended periods of cellular dehydration is an important physiological adaptation that limits the intake of osmolytes from food and helps maintain the integrity of fluid compartments. The ability to experimentally control both the development and reversal of anorexia, together with the understanding of underlying hormonal and neuropeptidergic signals, makes dehydration (DE)-anorexia a powerful model for exploring the interactions of neural networks that stimulate and inhibit food intake. However, it is not known which meal parameters are affected by cellular dehydration to generate anorexia. Here we use continuous and high temporal resolution recording of food and fluid intake, together with a drinking-explicit method of meal pattern analysis to explore which meal parameters are modified during DE-anorexia. We find that the most important factor responsible for DE-anorexia is the failure to maintain feeding behavior once a meal has started, rather than the ability to initiate a meal, which remains virtually intact. This outcome is consistent with increased sensitivity to satiation signals and post-prandial satiety mechanisms. We also find that DE-anorexia significantly disrupts the temporal distribution of meals across the day so that the number of nocturnal meals gradually decreases while diurnal meal number increases. Surprisingly, once DE-anorexia is reversed this temporal redistribution is maintained for at least 4 days after normal food intake has resumed, which may allow increased daily food intake even after normal satiety mechanisms are reinstated. Therefore, DE-anorexia apparently develops from a selective targeting of those neural networks that control meal termination, whereas meal initiation mechanisms remain viable.

1. Introduction

Anorexia is the inhibition of feeding behavior despite an ongoing state of negative energy balance. We have extensively investigated the mechanisms and neural circuitry underlying anorexia in a rat model where anorexia develops as an adaptive response to cellular dehydration (DE). To induce DE, rats are given hypertonic saline (HS; 2.5% NaCl) to drink instead of water for up to 5 days. Although chow is freely available during this time, rats voluntarily and robustly limit their food intake (DE-anorexia) [1]. Nocturnal food intake progressively declines to approximately 20% of baseline intake and body weight is typically reduced by 15–20% [2,3]. When water is returned rats rapidly exhibit a very reproducible sequence of behaviors that corrects the accrued energy and fluid deficits [2,4].

Our knowledge of spontaneous eating and drinking patterns during DE-anorexia has been limited to two measures: total nocturnal and diurnal consumption, and the measurement of compensatory eating and drinking that follow the return of water [1,2,4]. However, these gross intake measures provide little insight into what aspect of feeding is compromised during DE-anorexia. Since the meal is considered the biological unit of feeding behavior [5,6], any change in the amount of food consumed is the direct result of a change in meal size, meal number, or both [7,8]. To determine which specific components of ingestive behavior are altered during the development and recovery from DE-anorexia we use the BioDAQ Intake Monitoring System to perform a detailed analysis of spontaneous meal patterns before, during, and after the onset of DE-anorexia.

A meal is traditionally defined as a cluster of smaller feeding bouts that are separated from other feeding clusters by an inter-meal interval (IMI) where feeding is absent. Furthermore, the relationship between eating and drinking has been extensively studied, and several reports have demonstrated that approximately 70–85% of water intake is temporally associated with meals [8–10]. Kissileff has emphasized the distinction between food-associated drinking, in which eating and drinking occur discretely but are temporally close, and prandial drinking, which occurs in rapid, alternating succession with feeding bouts, thus occurring within a meal [9]. More recently, Zorrilla and colleagues have extended this definition by proposing that drinking is an explicit component of the meal [11], and thus consider both feeding and drinking data in their analyses. This "drinking-explicit" analysis provides a method for validating an IMI that is used to define meals

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when both food and liquid intake are continuously monitored [11]. Given that DE-anorexia is provoked by drinking hypertonic saline, we now incorporate the notion of within-meal drinking into the framework for studying relationships between eating and drinking behavior before, during, and after the expression of DE-anorexia. We now use detailed meal pattern analyses to determine which aspects of feeding and drinking are modified as DE-anorexia develops and is reversed. In turn, changes in one or more meal components will provide powerful insights about the underlying mechanisms and neural networks that control ingestive behaviors during DE-anorexia.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (Harlan Laboratories; 250–275 g) were individually housed in polysulfone home cages with sanitized wood chips. Cages were equipped with the BioDAQ® Food and Liquid Intake Monitors, a product of Research Diets Inc. (New Brunswick, NJ). Rats were maintained on a 12/12-h light–dark cycle (lights on at 06.30 h) in a temperature-controlled environment (22–23 °C), with ad libitum access to food (Teklad rodent chow 8604) and water, except where noted. Body weights were measured daily throughout the experiment (between 09.00 h and 10.00 h), and food and liquid intake were monitored as described below. All procedures have been approved by the Institutional Animal Care and Use Committee of the University of Southern California.

2.2. BioDAQ food and liquid intake monitoring system

The BioDAQ Food and Liquid Intake Monitoring System provides accurate and continuous collection of food and fluid intake data with minimal experimenter intervention. The system consists of multiple hoppers each coupled to a precision strain gauge-based load cell, or peripheral sensor controller (PSC), that is wired into a central controller. Each PSC outputs raw data to a laptop, as has been previously described in detail [12]. For our experiments, each cage was equipped with two PSCs; one coupled to the food hopper, and the second to an inverted fluid bottle with a ball-bearing spout. Each of a cage's two PSCs was independently wired to the central controller to allow independent monitoring of food and fluid intake. The BioDAQ food hoppers have horizontal slots that allow rats to gnaw and paw at the chow but not remove entire pellets. The design of the hopper retains the crumbs that are not eaten due to gnawing, chewing, etc. Also, the design limits hoarding. The spillage beyond this retention is minimal, typically less than 0.5%. Cages were examined daily for food in the bedding, which was also minimal or not present. Recordings were halted for approximately 1 h per day (between 09.00 h and 10.00 h) for animal maintenance, during which animals did not have access to food or water. This means that results reported for any 24 h period consists of 23 h of data collection and 1 h of down time. Data were recorded using BioDAQ Monitoring Software 2.1.00, and analyzed using DataViewer 2.2.02 and Microsoft Excel 2004 and 2008 for Mac.

2.3. Euhydration, dehydration, and recovery

Rats were given at least 5 days of acclimation to the BioDAQ Monitoring system before any data were collected. After acclimation, ingestive behaviors were monitored for 5 days when chow and water were freely available. At 10.00 h on experimental day 6, drinking water was replaced with hypertonic saline (HS; 2.5% NaCl (w/v) solution), which was then the only fluid available for the next 5 days (experimental days 6–10). At 12.00 h on experimental day 11, water was returned and remained available for the remaining 5 days (experimental days 11–15). These three periods were designated as euhydration (EU), cellular dehydration invoked by drinking HS (DE), and recovery after the return of water (RE). A repeated measure design was implemented, by which each animal acted as its own control for the duration of the study to account for possible variations in between-animal body weights. Prior to replacing water with HS on day 6, rats were of similar body weight (297 ± 5 g). Over the course of the DE period, rats lost on average 25 ± 0.7% of body weight, and on the last day of RE had reached 102 ± 0.9% of their EU body weight.

2.4. Drinking-explicit analysis of meal patterning during DE-anorexia

2.4.1. Meal definition

Experiments used both the food and liquid intake monitors to determine interactions between eating and drinking behavior during the development and recovery from DE-anorexia. To capture this interaction we employed a drinking-explicit analytical method validated by Zorrilla and colleagues [11]. Here a meal is defined as any intake episode that contains at least 0.225 g of food (minimum meal size), and is separated from other burst clusters by an IMI of 300 s. In this study, the minimum meal size was defined as 0.23 g, as the BioDAQ software limits this value to two decimal places.

We also implemented a meal elimination criterion to separate and remove any large feeding cluster that resulted from mechanical errors, operator error, etc. Thus, any feeding cluster with an ingestion rate greater than 0.5 g/min was eliminated if two or more of the following rules were met: the feeding cluster consisted of less than 2 bouts; the feeding cluster contained a single bout that is greater than 1.0 g; the feeding cluster was independent of a drinking cluster.

2.4.2. Combining ingestive clusters into composite meals

The feeding and drinking data collected from each rat were manually combined into composite meals by first segmenting independent feeding and drinking files for each animal for each day into clusters using a 300 s IMI and a 0.23 g minimum meal size criterion (Microsoft Excel Mac2004/2008, Redmond, WA). Independent feeding and drinking clusters were then listed chronologically by cluster start time and scanned for feeding clusters that were separated from drinking clusters (and vice versa) by 300 s or less (Microsoft Excel). Clusters within this limit were assigned to a composite meal, the duration of which was taken as being between the start time of the first cluster and the end of the last response of the final cluster. Note that transition times between eating and drinking (inter-cluster interval) were included in the total meal duration. Drinking clusters not combined to a feeding cluster that did not fulfill the 0.23 g minimum food criteria were omitted from most meal-focused analysis. However, these drinking clusters were included in calculations of overall fluid consumption.

Fig. 1 shows an example of two composite meals derived from 75 min of data collected from a EU rat. The figure also provides an illustration of various terms used in this paper. The terms and layout of this schema are derived from the microstructural analysis of licking established by Davis and Smith [13], together with the methods of Zorrilla and colleagues [11].

2.5. Expt 1) Assessment of feeding patterns before the onset of dehydration-anorexia

Five days of EU feeding and drinking data were collected for each animal ($n = 10$). Each 24 h day (from lights on to lights on) was used to calculate descriptive statistics of average daily meal structure. Parameters measured (units) were number of composite meals; average composite meal duration (min); average IMI (min); percentage of total meals initiated during the nocturnal phase, average within-meal feeding and drinking intake (g or mL); average within-meal feeding and drinking duration (min); average food-to-liquid ratio for both intake (g/mL) and duration (min/min). Total daily intake of food (g), liquid (mL), and the ratio between the two (g/mL) were calculated from the difference in hopper weights over the 23 h-recording period; these data were not subjected to the criteria used to define a meal. Composite