Neuroscience 281 (2014) 44–53

PROGRESSIVE ANTICIPATION IN BEHAVIOR AND BRAIN ACTIVATION OF RATS EXPOSED TO SCHEDULED DAILY PALATABLE FOOD

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Abstract—Scheduled and restricted access to a palatable snack, i.e. chocolate, elicits a brief and strong anticipatory activation and entrains brain areas related with reward and motivation. This behavioral and neuronal activation persists for more than 7 days when this protocol is interrupted, suggesting the participation of a time-keeping system. The process that initiates this anticipation may provide a further understanding of the time-keeping system underlying palatable food entrainment. The aim of this study was to analyze how this entraining protocol starts and to dissect neuronal structures that initiate a chocolate-entrained activation. We assessed the development of anticipation of 5 g of chocolate during the first 8 days of the entrainment protocol. General activity of control and chocolate-entrained rats was continuously monitored with movement sensors. Moreover, motivation to obtain the chocolate was assessed by measuring approaches and interaction responses toward a wire-mesh box containing chocolate. Neuronal activation was determined with c-Fos in reward-related brain areas. We report a progressive increase in the interaction with a box to obtain chocolate parallel to a progressive neuronal activation. A significant anticipatory activation was observed in the prefrontal cortex on day 3 of entrainment and in the nucleus accumbens on day 5, while the arcuate nucleus and pyriform cortex reached significant activation on day 8. The gradual response observed with this protocol indicates that anticipation of a rewarding food requires repetitive and predictable experiences in order to acquire a temporal estimation. We also confirm that anticipation of palatable food involves diverse brain regions.

Key words: food entrainment, circadian rhythms, accumbens, prefrontal cortex, food anticipatory activity.

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Abbreviations: ACC, accumbens; ANOVA, analysis of variance; ARC, arcuate nucleus; BL, baseline; CH, chocolate-entrainment; FAA, food elicits anticipatory activity; PBS, phosphate-buffered saline; PBSGT, phosphate-buffered saline, 0.25% nutritive gelatin and 0.5% triton; PFC, prefrontal cortex; PiCX, pyriform cortex; SCN, suprachiasmatic nucleus.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.036
0306-4522/

INTRODUCTION

Food-related signals are powerful time cues for physiological and behavioral systems. In rodents, scheduled daily access to food elicits anticipatory activity (FAA) to the daily meal. This FAA starts 2–3 h prior to food access and is characterized by behavioral arousal and activation, foraging, increased approach to a feeder and the search for food (Mistlberger, 1994). Associated with FAA plasma corticosterone and core temperature increase, indicating that feeding schedules also modify hormonal and metabolic functions (Krieger and Hauser, 1978; Honma et al., 1983). Likewise daily access to a palatable snack (5 g of chocolate) induces rodents to develop anticipatory activity and has shown to be a powerful stimulus to entrain behavior and neuronal activity in brain areas mediating motivation and reward (Mistlberger and Rusak, 1987; Mendoza et al., 2005; Hsu et al., 2010). Rats exposed to palatable food entrainment show a brief and precise anticipatory activation, and when the protocol is interrupted, rats continue anticipating precisely at the scheduled time for up to 5 days indicating the involvement of a clock mechanism (Angeles-Castellanos et al., 2008). Daily scheduled palatable food entrains c-Fos activation and PER1 and PER2 oscillations in corticolimbic areas, particularly in the nucleus accumbens (ACC), the prefrontal cortex (PFC), amygdala and arcuate nucleus (ARC), which are involved in producing motivational and reward responses (Angeles-Castellanos et al., 2008; Verwey et al., 2007). Such entrained temporal patterns of activation may underlie the time-keeping system that drives the search and craving for palatable food (Webb et al., 2009; Escobar et al., 2011).

Visual inspection of actograms suggests that anticipation of a daily palatable snack (chocolate) requires several days before a clear anticipatory response is observed, which suggests that animals require several cycles to compute the time signal and possibly to induce daily oscillations in brain areas that will be involved in estimating time for anticipation. This evidence suggests that individuals monitor the time and phase when food is available in order to elaborate the anticipatory strategy (Mistlberger, 1994). The detection of brain areas involved in the progressive development of anticipation of a palatable snack may serve as a strategy to uncover structures that function as coordinators or pacemakers for palatable food anticipation.

Based on this possibility, the present study aimed to explore the development of anticipatory activity and neuronal activation to palatable food because, our automated recording system for general activity has indicated that anticipatory activity for chocolate is very brief (Angeles-Castellanos et al., 2008; Mendoza et al., 2005). We developed an additional strategy to evidence the motivation to obtain the daily snack. Based on a report by Valdés et al. (2010) rats were challenged with a wire-mesh box containing a piece of chocolate and the effort to obtain the chocolate was evaluated. Moreover core temperature was monitored with intraperitoneal sensors in order to further evidence a physiological response associated with FAA. Data here provided indicate that the development of anticipatory activity to chocolate is gradual, that behavioral markers of effort and general activity increased progressively associated with evolving neuronal activity in the PFC, ACC, ARC, and the pyriform cortex (PiCX). The prelimbic region of the PFC and the ACC, both involved in processing reward responses, exhibited early anticipatory activation, which indicates their relevant role for emergence of anticipation of a palatable snack.

EXPERIMENTAL PROCEDURES

Subjects and general conditions

Male Wistar rats weighting 250–300 g were obtained from the general bioterium in the Medical Faculty at the Universidad Nacional Autónoma de México. Rats were housed in individual transparent acrylic cages with food (Rodent Laboratory Chow 5001) and water *ad libitum* throughout the experiments. Cages were placed in lockers (eight subjects/locker) with a controlled 12:12-h light/dark cycle (light onset at 0700), constant temperature (22 ± 1 °C) and continuous air flow. Rats were acclimated to environmental conditions for 1 week before starting the experimental procedures. Animal handling procedures were conducted according to the national guide for care and use of animal experimentation (Decreto ley de protección a los animales del Distrito Federal, Gaceta oficial del Distrito Federal 26/02/02), which is in agreement with international requirements for animal handling.

Experimental design

Rats were randomly assigned to one of two groups: (1) *Control rats:* remained in their cage with food and water *ad libitum*; (2) Chocolate-entrainment (CH) group: rats received daily in their home cage 5 g of chocolate at 13:00 h for 8 days (kinder maxi™; 5 g = 28.6 kcal; contains 10.3% proteins, 54.2% carbohydrates and 35.5% fat). A piece of chocolate was given to the rats and left in the cage until they finished, without time limitation. For assessment of neuronal activity, all rats were sacrificed at 14:00 h. Rats from the CH group were divided in two groups, one group was sacrificed 1 h *after chocolate ingestion* and the other group sacrificed anticipating *chocolate*. The time of sacrifice was projected based on the time of expression of c-Fos protein which shows a peak after 60 min and lasting around 90 min. Rats of the control and chocolate-entrained groups shared lockers, thus at the moment of chocolate delivery, control rats were exposed to the opening of the doors and the brief presence of the experimenter.

Anticipatory activity to chocolate in general locomotion and core temperature

One set of 12 rats was used to monitor general activity and eight of the same rats received an intraperitoneal temperature sensor. During the adaptation week, rats underwent surgery in order to place intra-abdominal temperature sensors (iButton Sensor-Temperature Logger; Maxim Integrated Products, Dallas, Semiconductor, USA). Rats were anaesthetized with an intramuscular administration of Xylazine (Procin 0.01 mL/100 g bodyweight) and Ketamine (Inoketam 0.02 mL/100 g body weight). Under deep anesthesia a small incision was cut in the abdominal cavity and a sterilized temperature sensor was introduced in the peritoneum. Anterior abdominal muscles and skin were sutured by layers with absorbable catgut 000 and rats were left to recover for 1 week before starting the baseline (BL). The iButtons were programed to collect temperature data every 30 min for the total handling interval and are shown as daily mean activity patterns for BL, days 1, 2, 3, 5 and 8 of chocolate entrainment. Also, the mean temperature exhibited 6, 4, and 2 h prior and 2 h after chocolate access was calculated.

General activity was recorded with an automatic monitoring system using tilt switches placed under the individual cages as previously reported (Escobar et al., 2007). These sensors detected continuously the animal's movements during the 24 h every day. Behavioral events were collected with a digitized system and were automatically stored every minute in a PC for further analysis The system for monitoring and collection of data was developed by the Mexican biomedical company Omnialva SA de CV. Analysis was performed with the program for PC SPAD9 (Sistema de Procesamiento y Adquisición de Datos, version 1.1.1) designed for this system and based on Matlab (Omnialva SA de CV, Mexico City, Mexico). General activity counts were organized in 15-min bins, for which each time point represents the sum of the previous activity counts for the interval. Data are shown as mean activity temporal patterns for BL, days 1, 2, 3, 5 and 8 of chocolate entrainment.

Evaluation of motivation for chocolate with the wire-mesh box

A different set of control (C; $n = 8$) and chocolate-entrained rats (CH; $n = 8$) were used to test the motivation for the palatable snack, C and CH rats were exposed during 5 min to a sealed wire-mesh box containing a piece of chocolate (based on the strategy reported by Valdés et al. (2010) and the interaction with the box was monitored. First, during BL, all rats were exposed to an empty box. On days 1, 2, 3, 5 and 8 of chocolate entrainment C and CH rats were evaluated while exposed to a box containing 5 g of chocolate. An additional control group ($n = 8$) was tested with an empty box during the same days as a control for daily exposure to the box.