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Operant behavior to obtain palatable food modifies neuronal plasticity in the brain reward circuit

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Abstract
Palatability enhances food intake by hedonic mechanisms that prevail over caloric necessities. Different studies have demonstrated the role of endogenous cannabinoids in the mesocorticolimbic system in controlling food hedonic value and consumption. We hypothesize that the endogenous cannabinoid system could also be involved in the development of food-induced behavioral alterations, such as food-seeking and binge-eating, by a mechanism that requires neuroplastic changes in the brain reward pathway. For this purpose, we evaluated the role of the CB_1 cannabinoid receptor (CB_1-R) in the behavioral and neuroplastic changes induced by operant training for standard, highly caloric or highly palatable isocaloric food using different genetics, viral and pharmacological approaches. Neuroplasticity was evaluated by measuring changes in dendritic spine density in neurons previously labeled with the dye DiI. Only operant training to obtain highly palatable isocaloric food induced neuroplastic changes in neurons of the nucleus accumbens shell and prefrontal cortex that were associated to changes in food-seeking behavior. These behavioral and neuroplastic modifications induced by highly palatable isocaloric food were dependent on the activity of the CB_1-R. Neuroplastic changes induced by highly palatable isocaloric food are similar to those produced by some drugs of abuse and may be crucial in the alteration of food-seeking behavior leading to overweight and obesity.

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1. Introduction

The availability of highly palatable food is a crucial factor that promotes overeating in developed countries (Saper et al., 2002) and finally can lead to overweight, obesity and associated illnesses. The prevalence of obesity has increased substantially since the mid-twentieth century in virtually every country (Caballero, 2007), and obesity prevalence in adults has doubled in the US since 1980, where 34% of US adults are obese (Khan et al., 2009).

Overeating behavior shares similarities with the loss of control and compulsive taking behavior observed in drug-addicts. Thus, deficiencies in inhibitory capacity have been reported in obese humans with excessive food intake (Nederkoorn et al., 2006; Rosval et al., 2006) that are similar to the elevated impulsivity leading to relapse in drug addicts (Moeller et al., 2001). In addition, over-consumption of highly caloric food produces neuroadaptive changes in the brain reward system that can drive the development of compulsive eating (Johnson and Kenny, 2010). Food, like drugs of abuse, activates the mesocorticolimbic system, which mediates the hedonic and motivational aspects of different rewarding stimuli. Repeated activation of this brain pathway may lead to neuroadaptive changes and structural reorganization that could participate in the behavioral alterations promoted by drugs of abuse (Russo et al., 2010). Early studies showed a correlation between changes in neuroplasticity and locomotor sensitization induced by cocaine (Li et al., 2004), although more recent studies have reported conflicting results (for review see Russo et al., 2010). It is currently unclear whether similar neuroplastic alterations occur with repeated exposure to natural rewards. Indeed, most studies have failed to observe changes in structural plasticity in the mesocorticolimbic system induced by food (Robinson et al., 2001; Crombag et al., 2005), although another natural reward, sex, has been recently reported to modify dendritic morphology in the reward circuit (Pitchers et al., 2010).

The endogenous cannabinoid system is a key modulator of the hedonic value of natural rewards (Di Marzo and Matias, 2005) and drugs of abuse (Maldonado et al., 2006). These rewarding responses seem to be mediated, at least in part, by the activation of CB_1-R in the mesocorticolimbic system (Maldonado et al., 2006). CB_1-R are also involved in mediating neuroplastic changes induced by drugs of abuse (Ballesteros-Yáñez et al., 2007). Considering the key role of CB_1-R in food rewarding effects, these receptors could also be involved in any possible neuroplasticity change induced by the exposure to a natural reward.

In this study, we have investigated the ability of palatable food-induced seeking behavior to promote neuroplasticity and the possible involvement of CB_1-R in these changes. For this purpose, CB_1 knockout mice ($CB_1-/-$) and wild-type littermates ($CB_1+/+$) were trained to lever-press to obtain standard, highly caloric or highly palatable isocaloric pellets and the changes in morphological plasticity were evaluated. We found that operant behavior to obtain highly palatable food produced changes in structural plasticity in specific areas of the mesocorticolimbic system in $CB_1+/+$, but not in $CB_1-/-$.

2. Experimental procedures

2.1. Animals

The experiments were carried out in male $CB_1-/-$ and $CB_1+/+$ littermates from 8 to 12 weeks old at the beginning of the experiments. The generation of $CB_1-/-$ and $CB_1+/+$ was described previously (Zimmer et al., 1999). Briefly, homozygous $CB_1+/+$ and $CB_1-/-$ were bred by back-crossing of chimeric and heterozygous animals to C57BL6/J and interbreeding of heterozygous animals for at least 10 generations in order to obtain a pure C57BL6/J background. The animals were individually housed and maintained in a controlled temperature ($21\pm1\ ^{\circ}C$) and humidity ($55\pm10\%$) room with a 12:12-h reversed light/dark cycle (on at 8 p.m and off at 8 a.m.). All the experiments were performed during the dark phase of the dark/light cycle. Animals were habituated to the experimental room and handled for one week before starting the experiments with ad libitum access to standard chow and water. All animal procedures were conducted in accordance with the standard ethical guidelines (European Communities Directive 86/60-EEC, Animal Welfare Assurance #A5388-01, IACUC Approval Date 06/08/2009) and approved by the local ethical committee (Comitè Ètic d'Experimentació Animal—Institut Municipal d'Assistència Sanitària—Universitat Pompeu Fabra).

2.2. Acquisition of operant responding maintained by food

Operant responding maintained by food was evaluated in mouse operant chambers (Model ENV-307A-CT, Med. Associates, Georgia, VT, USA). The chambers were made of aluminum and acrylic, had grid floors (EVV-414, Med. Associates Inc., St. Albans, USA), and were housed in sound and light-attenuated boxes equipped with fans to provide ventilation and white noise. The chambers were equipped with two retractable levers, one randomly selected as the active and the other as the inactive. Pressing on the active lever resulted in a pellet delivery (standard, highly caloric or highly palatable isocaloric pellet) together with a stimulus-light during 2 s (associated-cue), while pressing on the inactive lever had no consequences. A food dispenser equidistant between the two levers permitted delivery of food pellets when required. The beginning of the each operant responding session was signaled by turning on a house light placed on the ceiling of the box for 3 s that was then turned off during the remaining duration of the session. The side of the active and inactive lever was counterbalanced between animals. Each session started with a priming delivery of one pellet. A time-out period of 10 s was established after each pellet delivery. During this period, the cue-light was off and no reinforcer was provided after responding on the active lever. Responses on the active lever and all the responses performed during the time-out period were also recorded. The session was terminated after 100 reinforcers were delivered or after 1 h, whichever occurred first. One hour daily sessions were conducted seven days per week during a period of 41 days. The animals were food deprived five days before starting sessions to maintain their weight at 90% of their ad libitum initial weight adjusted for growth, and this food restriction regime was maintained during the first 10 sessions of the operant behavior training to permit the appropriate acquisition of the task. Additional standard and highly palatable isocaloric-yoked groups were included. These groups were subjected to the same experimental conditions except that no reinforcer and no cue-light were presented after pressing in any of the two levers exposed in the operant chamber. However, standard and highly palatable isocaloric-yoked groups received passively the same amount of pellets and with the same frequency as the $CB_1+/+$ that were trained to obtain them.