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Title: Long-lasting deficits in hedonic and nucleus accumbens reactivity to sweet rewards by sugar overconsumption during adolescence

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ABSTRACT

Adolescence represents a critical period characterized by major neurobiological changes. Chronic stimulation of the reward system might constitute an important factor of vulnerability to pathological development. In spite of the dramatic increase in the consumption of sweet palatable foods during adolescence in our modern societies, the long-term consequences of such exposure on brain reward processing remain poorly understood. Here, we investigated in rats the long-lasting effects of sugar overconsumption during their adolescence on their adult reactivity to the hedonic properties of sweet rewards. Adolescent rats with continuous access to 5% sucrose solution (from post-natal day 30 to 46) showed escalating intake. At adulthood (post-natal day 70), using two-bottle free choice tests, Sucrose-exposed rats showed lower intake than non-exposed rats suggesting decreased sensitivity to the rewarding properties of sucrose. In Experiment 1, we tested their hedonic-related orofacial reactions to intraoral infusion of tasty solutions. We showed that Sucrose-exposed rats presented less hedonic reactions in response to sweet tastes leaving the reactivity to water or quinine unaltered. Hence, in Experiment 2, we observed that this hedonic deficit is associated with lower c-Fos expression levels in the nucleus accumbens, a brain region known to play a central role in hedonic processing. These findings demonstrate that a history of high sucrose intake during the critical period of adolescence induces long-lasting deficits in hedonic treatment that may contribute to reward-related disorders.

INTRODUCTION

Adolescence is a key developmental period of major cognitive and neurobiological changes characterized by an increase in specific behaviors such as impulsivity, novelty seeking or risk-taking (Spear, 2000) and those might provide a window of vulnerability to pathological development (Andersen, 2003; Adriani & Laviola, 2004; Paus *et al.*, 2008). Adolescents are more sensitive to rewards such as drugs or palatable foods (Crews *et al.*, 2007). The consumption of sweet foods dramatically increased during the last few decades especially in adolescents (Wang *et al.*, 2008; Lustig *et al.*, 2012). However, the long-term consequences of sugar overconsumption during adolescence on reward-related processes remain poorly understood.

Like drugs of abuse, the repeated stimulation of the reward system by palatable foods may lead to a loss of control over consumption (Volkow & Wise, 2005; Avena *et al.*, 2008; Kenny, 2011; Kendig, 2014). Several studies have demonstrated that the sensitivity of the reward system to palatable foods is at the highest during adolescence (Spear, 2000; Wilmouth & Spear, 2009; Friemel *et al.*, 2010). Recently, we and others showed that continuous access to a sucrose solution either during the specific period of adolescence or between weaning at adulthood (encompassing adolescence) alters the motivation to obtain natural rewards at adulthood, suggesting a decrease in the rewarding properties of sweet foods (Frazier *et al.*, 2008; Vendruscolo *et al.*, 2010a; Vendruscolo *et al.*, 2010b).

Reward processing can be dissociated in "wanting" and "liking" processes whose underlying neuronal circuits can be partially dissociated (Berridge & Robinson, 1998; Barbano & Cador, 2007; Castro & Berridge, 2014a). "Wanting" refers to the attribution of motivational or incentive value to relevant actions or stimuli thereby reinforcing their associations with the reward and inducing approach or instrumental behavior. This process is thought to be mediated by a broad set of regions including the amygdala (Wassum *et al.*, 2009; Mahler & Berridge, 2012; Robinson *et al.*, 2014), the hypothalamus (Stanley *et al.*, 1993; Castro *et al.*, 2015) and the dopamine system (Baldo *et al.*, 2002; Montague *et al.*, 2004; Richard & Berridge, 2011). In contrast, "liking" processes refer to the pleasure experienced by reward sensory stimulation and are mostly mediated by interactions between the nucleus accumbens, the ventral pallidum and **endogenous opioid and cannabinoid systems (Pecina & Berridge, 2005; Smith & Berridge, 2005; Mahler *et al.*, 2007; Smith & Berridge, 2007)**.

The aim of the present study was to investigate the long-term consequences of sucrose overconsumption during adolescence on the hedonic perception and neurobiological processing of sweet rewards at adulthood. In Experiment 1, we first used a taste reactivity test to measure affective reactions elicited by intraoral infusion of taste solutions. Orofacial stereotypic reactions induced by specific tastes are well described and represent a direct measure of the hedonic properties of these tastes independently of motivational factors (Steiner, 1973; Grill and Norgren, 1978; Berridge, 2000). In Experiment 2, we quantified c-Fos immunostaining induced by a sweet taste in a different set of animals in order to reveal changes in the aforementioned brain circuits following adolescence sucrose overconsumption.

MATERIAL AND METHODS

Experiment 1: Taste reactivity after sucrose overconsumption during adolescence

Subjects

Male Wistar Han rats (Charles River Laboratories, France) were received at the average of postnatal day (P) 26 and were individually housed in plastic cages and maintained under an inverted 12 h light/dark cycle (light on at 8:00 P.M.) in a temperature (22±1°C) and humidity controlled room. The experiments took place during the dark phase of the cycle.

Food (A04, Scientific Animal Food & Engineering, France) and water were provided *ad libitum*. All experiments were conducted in agreement **with the French (council directive 2013-118, February 1, 2013) and international (directive 2010-63, September 22, 2010, European Community) legislations**. The experiments received the approval # 5012088-A from the Bordeaux Ethics Committee (CNREEA #50).

Exposure to sucrose during adolescence

After 4 days of acclimatization, adolescent rats (P30) were given continuous access in their home cage to an additional bottle containing water (Control group, n = 7) or a Sucrose solution (5% w/v, Sucrose group, n = 7) for 16 days. Liquid and food consumption as well as the weight of rats were measured every 2 days. At P46, the sucrose bottle was removed and all rats were given access to two bottles of water. Rats were kept undisturbed in their home cages until adulthood (P70) before behavioral testing (**Figure 1**).

Surgery

For taste reactivity testing, a unilateral oral cannula was implanted to allow oral infusions of different taste solutions. Adult rats were anesthetized with a mixture of ketamine (75 mg/kg, i.p.) and xylazine (7.5 mg/kg, i.p). Polyethylene tubing (PE-10 Tygon, int. Ø 0.1 cm / ext Ø 0.2 cm) was introduced in the mouth by the upper cheek lateral to the first maxillary molar, ascended under the skin and exited at the dorsal head cap (Grill & Norgren, 1978). The tubing was connected to a guide cannula (Plastic One) and fixed to the skull using dental cement. After surgery, rats were treated for 3 days with carprofen (5 mg/kg s.c.) and ampicillin (7 mg /kg, s.c.) to prevent pain and infection. Rats were allowed to recover at least one week before behavioral testing. Cannulae were flushed daily with water.

Behavioral procedures

Two-bottle free choice test

Adult rats (> P70) underwent two free choice tests. Rats were given access in their home cage to 2 bottles filled with either water or saccharin solution (0.13% w/v). Forty-eight hours after this first test, a second test was conducted in the same manner except that rats had a choice between sucrose solution (5% w/v) and water. Total consumption (ml) over the 24 h period of each test was measured for each solution.

Taste reactivity testing

Before taste reactivity testing, rats were first habituated to the experimental chamber during 3 days. Each day, rats were individually placed in a clear plastic cylinder (25x25 cm) with a transparent floor. The days of taste reactivity tests, the oral cannula was connected to a PE-10 tubing attached to a syringe placed on an infusion pump (KD Scientific). After a 5 min habituation period, 1 ml of a specific taste solution was infused in the mouth of the rat at the rate of 1 ml/min. A mirror placed under the transparent floor reflected the rat's face allowing us to videotape orofacial reactions. All rats were tested for their reactions to 4 solutions, each with a specific taste: neutral (water), sweet (5% sucrose and 0.13% saccharin) or bitter (0.1% quinine).

Orofacial reactions were scored off-line in slow motion (1/4 actual speed) under blind conditions (Grill & Norgren, 1978; Berridge, 2000). Hedonic reactions were classified as tongue protrusions and paw licks. Negative reactions were classified as forelimb flails, gapes, head shakes and face wipes. Neutral reactions were classified as small mouth movements and passive drips. Gapes, forelimb flails and head shakes were counted as discrete events. Repetitive actions were counted in time bins: tongue protrusions (2 s), paw licks (5 s), face wipe (5 s), mouth movements (5 s) and passive drip (5 s). This analysis allows the harmonization of each type of reactions which could be summed into total hedonic, aversive or neutral scores. Data are expressed in number of events *per* min.

Experiment 2: c-Fos expression induced by sucrose taste after sucrose overconsumption during adolescence

Subjects, surgery and behavioral procedure

For Experiment 2, new groups of experimentally naive male Wistar Han rats were used (Control group, n = 7 / Sucrose group n = 6). The sucrose exposure during adolescence, the surgical procedure, behavioral apparatus and taste reactivity testing were the same as was described in Experiment 1. In Experiment 2, only the reactivity to the sucrose taste was tested at adulthood and rats were killed 90 min after sucrose injection to measure the expression of the immediate early gene c-Fos induced by sucrose intraoral stimulation.

Immunohistochemistry and c-Fos counting

Ninety minutes after sucrose intraoral infusion, rats were deeply anesthetized with an overdose of pentobarbital sodium (Ceva Santé Animale) and perfused transcardially with 0.9% NaCl solution, followed by 4% PFA solution in 0.1 M PB. The brains were then post-fixed overnight in 4% PFA. Serial coronal sections (40 µm thick) were cut on a vibratome (Leica). Free floating sections were first rinsed in PBS 0.1M (3 x 5 min) and PBS-Triton 0.3% (1 x 5 min). They were then incubated in PBS-Triton 0.3%/H2O2 2% for 30 min and rinsed with PBS-Triton (3 x 10 min). Sections were incubated 1 h in a blocking solution (4% donkey serum and 0.2% Triton X-100 in PBS 0.1 M), then with primary rabbit antibody (anti-c-Fos 1/15,000 in PBS-Triton 0.3% and bovine serum albumin 1%, Calbiochem) for 24 h at room temperature. After rinses in PBS 0.1M (3 x 10 min), sections were then incubated with biotinylated goat anti-rabbit (1/2,000 in PBST 0.3%, Jackson ImmunoResearch) for 2 h at room temperature. They were then incubated with avidin-biotin-peroxydase complex (1/2000 in PBS, Vector Laboratories) for 30 min at room temperature. After rinses in PBS (2 x 10 min) and TBS 0.1M (2 x 10 min), the staining was revealed in a solution of diaminobenzidine (DAB 0.02%, Sigma-Aldrich) / nickel 0.004% in TBS) and hydrogen peroxide (0.07%). The reaction was stopped by adding cold TBS. Sections were mounted on gelatin-coated slides, dehydrated and coverslipped with Eukitt solution.

Labeled sections were scanned using a NanoZoomer (Hamamatsu Photonics, Bordeaux Imaging Center) with 20x lens. Digital microphotographs of the region of interest in each hemisphere were examined with 5x virtual lens. Quantification was performed using an automated method developed in the laboratory with ImageJ software with the same threshold applied for each section. c-Fos positive nuclei were quantified in the nucleus accumbens (NAc, core and shell, from bregma + 2.5 to + 1.0 mm), the ventral pallidum (VP, from bregma + 0.4 to - 0.2 mm), the basolateral and central amygdaloid nuclei (BLA and CEA respectively, from to bregma – 2.3 to – 3.0 mm), the lateral hypothalamus (LH, from bregma – 2.3 to – 3.0 mm) and the ventral tegmental area (VTA, from bregma – 5.3 to – 6.0 mm) according to the Paxinos and Watson atlas (2007). Moreover, we also quantified c-Fos positive cells in the gustatory part of the insular cortex (GCx, from bregma +2.2 to + 0.4 mm), and the gustatory thalamus (parvicellular part of the posteromedial ventral thalamic nucleus or VPPC, from bregma - 3.6 to – 4.2 mm) to control for gustatory sensitivity and taste processing. For each brain region, between two and four sections were examined bilaterally by an experimenter blind of group conditions and the number of positive nuclei/mm² was averaged.

Data analysis

All values were expressed as mean ± standard error of mean (SEM). Behavioral data were analyzed using two-way ANOVAs with Group as between-factor and Age (Sucrose exposure), Bottle (Consumption tests) or Reactions (Taste reactivity tests) as within-factors. The c-Fos data were analyzed using two-way ANOVAs with Group as between-factor and Region as within-factor. Bonferroni *post hoc* tests were performed when required. Linear regression and Pearson's correlation tests were used to investigate relationships between positive orofacial reactions and c-Fos levels. All the analyses were performed using GraphPad Prism (v 6.01). The alpha risk for the rejection of the null hypothesis was 0.05.

RESULTS

Control and Sucrose-exposed rats from the two experiments were pooled for the sucrose exposure and 2-bottle test data (Control, n = 14; Sucrose, n = 13), as no significant interaction between Groups (Control / Sucrose groups) and Experiments (Experiments 1 and 2) were found (all $\boldsymbol{F < 1.9, P > 0.2}$).

Sucrose exposure during adolescence

We first investigated the liquid and food consumption of rats with (Sucrose group) and without (Control group) access to a bottle of sucrose during adolescence (P30-P46). Total liquid consumption escalated across days for both groups (**Figure 2A**, Age $\boldsymbol{F_{7,175} = 14.7, P < 0.001}$). Moreover, the Sucrose group consumed more than the Control group throughout exposure days (Group $\boldsymbol{F_{1,25} = 36.7, P < 0.001}$). Sucrose-exposed rats drank, on average, 5±1 ml of water and 81±9 ml of sucrose solution for two days compared to Control rats which drank 31±2 ml of water. This overconsumption of sucrose was associated with a decrease in food consumption (**Figure 2B**, Group $\boldsymbol{F_{1,25} = 13.7, P = 0.001}$; Age $\boldsymbol{F_{7,175} = 54.7, P < 0.001}$). However, the differences in fluid intake did not affect the weight of rats (**Figure 2C**, Group

***$F_{1,25}$* = 0.2, *P* = 0.7**) nor the rate of weight gain (Group x Age interaction ***$F_{8,200}$* = 1.4, *P* = 0.2**).

Consumption of sweet rewards at adulthood

When rats reached adulthood (> P70), we investigated their consumption of sweet non caloric or caloric rewards using two consecutive 2-bottle consumption tests of 24 h each (water *vs* saccharin 0.13%, **Figure 3A** / water *vs* sucrose 5%, **Figure 3B**). During both tests, all rats consumed more of the sweet solution than water (Bottle all ***F* > 61.9, *P* < 0.001**). Despite all rats consuming a similar amount of water during the tests, Sucrose-exposed rats consumed less of the sweet solutions compared to Control rats. ANOVAs revealed a significant effect of Group during the two tests (**Saccharin test: *$F_{1,25}$* = 4.3, *P* = 0.04; Sucrose test: *$F_{1,25}$* = 8.8, *P* = 0.006**) as well as a Group x Bottle interaction (Saccharin test: ***$F_{1,25}$* = 5.8, *P* = 0.02; Sucrose test: *$F_{1,25}$* = 8.3, *P* = 0.008**). *Post hoc* Bonferroni comparisons revealed a significant difference between Control and Sucrose groups for the consumption of sweet rewards (**all *P* < 0.01**) but not for the consumption of water (**all *P* > 0.9**).

These results demonstrate that the overconsumption of sucrose during adolescence induces a protracted decrease in the consumption of sweet rewards at adulthood. Interestingly, this deficit in intake was not only observed for the previously consumed reward (sucrose) but also for another sweet but non caloric solution (saccharin) suggesting a long-lasting global deficit in the processing of sweet tastes.

Experiment 1: Hedonic taste reactivity at adulthood after adolescent sucrose overconsumption

Next, we investigated the impact of sucrose overconsumption during adolescence on affective reactions to different tastes at adulthood (Control, n = 7; Sucrose, n = 7). To do this, rats were implanted with intraoral cannula allowing direct infusions of specific taste solutions into the mouth and spontaneous orofacial expressions induced by each solution were measured.

Infusion of water elicited very few positive reactions and a mix of negative and neutral reactions (**Figure 4A**). Both groups presented the same level of each type of reactions. An ANOVA with Group and Reactions as factors confirmed this description, revealing no effect of Group ($\boldsymbol{F_{1,12} = 0.04, P = 0.8}$) or Group x Reactions interaction ($\boldsymbol{F_{2,24} = 0.1, P = 0.9}$) but a significant effect of Reactions ($\boldsymbol{F_{2,24} = 17.0, P < 0.001}$). Bonferroni *post hoc* tests confirmed that negative reactions were higher than positive and neutral reactions (**all** $\boldsymbol{P < 0.01}$) which did not differ from each other ($\boldsymbol{P > 0.1}$).

Infusion of 0.1% quinine, a strong bitter taste induced a high level of negative reactions associated with no or very few positive and neutral reactions (**Figure 4B**). As for the water infusion, the pattern of orofacial reactions was similar between Control and Sucrose-exposed rats. An ANOVA confirmed a significant effect of Reactions factor ($\boldsymbol{F_{2,24} = 172.1, P < 0.001}$) but no significant effect of Group ($\boldsymbol{F_{1,12} = 2.6, P = 0.1}$) or Group x Reaction interaction ($\boldsymbol{F_{2,24} = 0.1, P = 0.1}$). Bonferroni *post hoc* tests confirmed that negative reactions were much higher than positive or neutral reactions (**all** $\boldsymbol{P < 0.001}$) which did not differ from each other ($\boldsymbol{P > 0.9}$).

The infusion of 5% sucrose solution induced a different pattern of reactions between the two groups. As expected, Control rats showed a high level of positive reactions induced by a sucrose sweet taste (**Figure 4C**). Sucrose delivery was also associated with few neutral reactions and a very low level of negative reactions, demonstrating the hedonic properties of this sweet reward. In contrast, Sucrose-exposed rats presented a marked decrease of their positive reactions in response to sucrose infusion. This decrease was associated in some rats with an increase in negative reactions. This was confirmed by the statistical analysis which revealed no significant effect of Group (**$F_{1,12}$ = 1.2, *P* = 0.3**) or Reactions (**$F_{2,24}$ = 2.1; *P* = 0.1**) but a Group x Reaction interaction (**$F_{2,24}$ = 6.2, *P* = 0.007**). Separate analysis on each type of reactions confirmed that the Sucrose group expressed less positive and more negative reactions than Control group (all ***P* < 0.05**, Bonferroni *post hoc* tests) but a similar level of neutral reactions (***P* > 0.9**).

Interestingly, a similar pattern of results was observed after intraoral infusion of 0.13% saccharin, a sweet but non caloric reward (**Figure 4D**), on the same rats (4 rats were excluded from the saccharin infusion due to blocked catheters: Control n= 5, Sucrose n = 5). While Control rats mainly expressed positive reactions, Sucrose-exposed rats presented a lower level of positive orofacial mimics associated with a small increase in the number of negative and neutral reactions. An ANOVA revealed a significant Group x Reactions interaction (**$F_{2,16}$ = 5.1, *P* < 0.02**) in spite of the absence of simple Group (**$F_{1,8}$ = 0.5, *P* = 0.5**) or Reactions effects (**$F_{2,16}$ = 1.2, *P* = 0.3**). Furthermore, *post hoc* tests revealed a significant difference between groups for positive reactions (***P* < 0.05**) but not for negative or neutral reactions (**all *P* > 0.4**).

While the processing of neutral and bitter tastes is not affected by an adolescent sweet diet, Sucrose-exposed rats showed a clear deficit in their hedonic reactivity to sweet tastes demonstrating that the overconsumption of sucrose during adolescence alters hedonic processing of rewarding solutions at adulthood.

Experiment 2: c-Fos expression induced by sucrose taste at adulthood after adolescent sucrose overconsumption

Processing of palatable rewards is mediated by several brain circuits centered on the NAc. Hedonic processes are primarily mediated by complex NAc-VP interactions. In contrast, incentive processes are mediated by the dopamine mesolimbic pathway in interaction with amygdalar and hypothalamic regions (Berridge & Robinson, 1998; Kelley *et al.*, 2003; Barbano & Cador, 2007). Given the differences observed in the taste reactivity test between Control and Sucrose-exposed rats, we investigated whether these behavioral alterations could be related to differences in the recruitment of these brain circuits using c-Fos immunostaining (Control, n = 7; Sucrose, n = 6).

As in the previous experiment, Sucrose-exposed rats showed a decrease of positive reactions to the sucrose sweet taste (**Figure 5A**). An ANOVA revealed a main effect of Group (**$F_{1,11} = 7.7$, $P = 0.02$**), and Reaction (**$F_{2,22} = 15.8$, $P < 0.001$**) but also a significant Group x Reaction interaction (**$F_{2,22} = 15.9$, $P < 0.001$**). *Post hoc* analysis conducted on each reaction type confirmed that the Control group presented more positive reactions than the Sucrose group (**$P < 0.001$**). However, no differences between groups were observed for negative or neutral reactions (**all $P > 0.4$**).

After the completion of the taste reactivity test, the level of c-Fos-related cellular activation was first quantified in brain regions underlying hedonic processing of rewards (**Figure 5B**). As depicted in the photomicrographs (**Figure 5C**), we observed less c-Fos positive cells in the Sucrose group compared to the Control group in both the core and shell part of the nucleus accumbens. In the VP, the number of c-Fos immunoreactive cells was low in both groups. Statistical analysis revealed no Group effect (**$F_{1,11} = 3.1$, $P = 0.10$**) but a main effect of Region (**$F_{2,22} = 88.1$, $P < 0.001$**) and a significant Group x Region interaction (**$F_{2,22} = 7.3$, $P = 0.004$**). The density of c-Fos cells appeared to differ between groups in the NAc core (**$P < 0.05$**) and shell (**$P < 0.01$**) but not in the VP (**$P > 0.3$**). Moreover, there was a positive correlation between the number of positive reactions to sucrose and c-Fos levels observed in the shell (**$r^2 = 0.42$, $P = 0.02$**; **Figure 5D**) but not in the core (**$r^2 = 0.15$, $P = 0.19$**) or the VP (**$r^2 = 0.26$, $P = 0.09$**).

In contrast to the NAc-VP circuit, no major differences in c-Fos levels were observed between Control and Sucrose groups in amygdalar nuclei, LH or VTA (**Figure 5E**). A repeated measures ANOVA showed a significant difference of c-Fos levels between regions (**$F_{3,33}$ = 3.1, P = 0.04**) but neither an effect of Group (**$F_{1,11}$ = 0.6, P = 0.4**) nor an interaction between these two factors (**$F_{3,33}$ = 1.6, P = 0.2**).

Importantly, the analysis of c-Fos levels in brain regions involved in taste processing (thalamic relay of gustatory information, VPPC, and the gustatory part of the insular cortex, GCx) revealed no significant differences between groups (**Group: $F_{1,11}$ = 4.7, P = 0.06; Group x Region: $F_{1,11}$ = 1.3, P = 0.2**) in spite of different levels of c-Fos between the two regions (**$F_{1,11}$ = 54.6, P < 0.001**). Thus, sucrose overconsumption during adolescence does not seem to alter gustatory sensitivity and neural processing of sweet tastes at the first processing relays.

DISCUSSION

In the present study, we investigated the effects of an overconsumption of sucrose during adolescence on the consumption of sweet rewards and on the sensitivity to their hedonic properties later at adulthood. We demonstrate here that a previous history of sucrose consumption during adolescence induces a decrease in the consumption of sweet rewards associated with a decrease in positive orofacial reactions to sweet tastes (Experiment 1). Furthermore, the investigation of patterns of cellular activation following taste reactivity to sucrose revealed a decrease in c-Fos immunoreactive cells in Sucrose-exposed rats in the nucleus accumbens, a region involved in hedonic processing (Experiment 2). Taken together, this indicates that sucrose exposure during adolescence results in major changes in the processing of the hedonic properties of sweet foods.

Adolescent sucrose exposure induces a protracted decrease in sweet reward consumption

The rewarding properties of food can be dissociated into two separate but interacting components: motivational and hedonic reward-related processes (Berridge & Robinson, 1998; Castro & Berridge, 2014a). We and other have previously reported that sucrose overconsumption during adolescence decreased the motivation of rats (Vendruscolo *et al.*, 2010a; Vendruscolo *et al.*, 2010b) or mice (Frazier *et al.*, 2008) to obtain palatable foods at adulthood, using operant progressive ratio schedule. **Interestingly, such effects not observed with drugs of abuse such as cocaine or alcohol (Vendruscolo *et al.*, 2010a; Vendruscolo *et al.*, 2010b) indicating some differences in the neurobiological substrates underlying the intake of food or drug of abuse (DiLeone *et al.*, 2012).** Here, in accordance with the previous studies, we report that sucrose overconsumption during adolescence induced a protracted decrease in the consumption of both sucrose and saccharin using a 2-bottle free choice test.

Preference tests are one of the most extensively used measures of hedonic perception and anhedonia states in depressive-like behaviors (Willner *et al.*, 1987). However, several studies have reported that an increase in food intake or in food-seeking responses can occur without an increase in hedonic perception (Berridge & Robinson, 1998; Kelley, 2004; Barbano & Cador, 2007). As the preference tests used in the current study require that the animal voluntarily consumes the solutions, we cannot exclude that the observed decrease in consumption of palatable rewards is related to the motivational aspect of food intake more than a pure deficit in hedonic processing.

Adolescent sucrose exposure induces a dramatic decrease in hedonic processes at adulthood

In order to measure hedonic processing of palatable foods without the interference of motivational processes, we used a taste reactivity test involving intraoral infusion of solutions with specific tastes to elicit spontaneous orofacial responses (Steiner, 1973; Grill and Norgren, 1978; Berridge, 2000). This behavior is highly conserved between species and can be used in rodents, monkeys or humans. Specific tastes induce distinct orofacial reactions that can be classified as positive or "liking" reactions (paw licks, tongue protrusions) or as negative or "disgust" reactions (gapes, head shakes, forelimb flails, face washes). Interestingly, very different tastes can induce similar orofacial reactions (e.g. sucrose and diluted sodium chloride; Grill & Norgren, 1978) demonstrating that they indeed reflect the hedonic properties of the taste.

As expected, Control rats expressed a specific pattern of reactions depending on the taste: positive reactions to sweet tastes (sucrose / saccharine), negative reactions to a bitter taste (quinine) and a mix of positive, negative and neutral reactions to water. In contrast, Sucrose-exposed rats showed a different pattern since they presented a clear decrease of positive "liking" orofacial reactions to sucrose compared to control non-exposed rats. Interestingly, this effect was not limited to the previously consumed reward but it was also observed for the sweet non caloric reward saccharin suggesting a specific alteration of sweetness hedonic properties.

One first explanation could be a primary deficit in sweetness perception within the primary sensory regions. Specific tastes are detected through receptors located on the tongue and the sensory signal is sent to the gustatory cortex via hindbrain nucleus and gustatory thalamic nucleus. From the hindbrain, the taste signal reaches the limbic system for reward processing. Deletion of the taste signaling machinery at tongue level alters sweet taste detection and preference development (Zhang *et al.*, 2003; de Araujo *et al.*, 2008; Beeler *et al.*, 2012). Furthermore, high caloric diets decrease taste cells responses (Maliphol *et al.*,

2013) and the sensitivity to sweet tastes (Robinson *et al.*, 2015). It does not appear to be the case in our experiment since we did not observe any alterations of orofacial reactions to neutral or aversive tastes suggesting that peripheral processing is preserved in Sucrose-exposed rats, at least for water and bitter tastes. Moreover, we did not report any difference in sucrose-induced cellular activation in the gustatory thalamic nucleus or insular cortex by sucrose suggesting that peripheral detection and primary sensory treatment of sweet tastes is preserved in sucrose-exposed rats. As a consequence, the decrease in positive reactions to sweet taste reported in the present study appears to be related to a deficit in the hedonic treatment of sweet rewards rather than in sensory processing.

Adolescence sucrose exposure induces changes in reward-related neuronal circuits

We report here that the deficits in hedonic reactivity to a sweet taste in Sucrose-exposed rats are associated with a decrease in the activation of nucleus accumbens (NAc) cells. The NAc plays a central role in the processing of food rewarding properties, integrating affective and cognitive information from cortical and subcortical regions. The NAc is a heterogeneous structure that can be dissociated in core and shell regions both involved in different aspects of reward processing. Numerous studies pointed the involvement of the NAc shell in the control of food intake and consummatory behaviors (Kelley, 2004; Barbano & Cador, 2007; Castro & Berridge, 2014a; Berridge & Kringelbach, 2015). Pharmacological manipulations of the NAc shell, especially its rostral part, induce changes in food consumption (Stratford & Kelley, 1997; Stratford *et al.*, 1998; Faure *et al.*, 2008; Richard & Berridge, 2011) and in hedonic reactivity to sweet tastes (Pecina & Berridge, 2005; Mahler *et al.*, 2007; Faure *et al.*, 2010; Castro & Berridge, 2014b). These studies demonstrate that changes in c-Fos levels in the NAc shell are directly related to hedonic processing which is consistent with our positive correlation between shell c-Fos level and positive orofacial reactions to sucrose.

The NAc core has rather been involved in instrumental actions performance and in general motivational effects of incentive stimuli (Parkinson *et al.*, 2000; Corbit *et al.*, 2001; Corbit & Balleine, 2011) more than in hedonic processing. Only few studies have reported changes in the NAc core activity in response to tasty stimuli that correlate with reward consumption and orofacial reactivity (Roitman *et al.*, 2005; Taha & Fields, 2005; Wheeler *et al.*, 2008). However, in contrast to the NAc shell, we did not observe a significant correlation between positive orofacial responses to sucrose and c-Fos levels in the NAc core suggesting an additional parallel processing of sweet taste, different from the treatment its hedonic properties. Therefore, we cannot rule out the possibility that sucrose itself could be processed for its incentive properties involving the NAc core, despite the limitation of motivational components in our experimental task. In this case, the deficit in the representation of reward incentive values could lead to a decrease in sucrose intake and in instrumental performance as we previously observed (Vendruscolo *et al.*, 2010a; Vendruscolo *et al.*, 2010b).

The NAc reciprocally interacts with the ventral pallidum to generate “liking” reactions (Smith & Berridge, 2007). Pharmacological manipulations of NAc or VP activity drive c-Fos activity in the other region and amplify taste “liking” reactions. Surprisingly, we did not report any difference in c-Fos levels in the VP between Control and Sucrose-exposed rats. One hypothesis might be that the deficit in NAc activity is sufficient to lead to “liking” deficits despite preserved activity in the VP. For instance, local infusion of naloxone, an opioid receptor antagonist, in the NAc is sufficient to block the increase in hedonic reactions induced by VP stimulation. Furthermore, the VP is involved in the expression of disgust reactions since excitotoxic lesions or temporary inactivation of the VP, but not NAc, produce intense aversive (or “disgust”) reactions to a sweet taste (Ho & Berridge, 2014). Interestingly, we did not observe a significant increase in aversive reactions to the sweet taste in Experiment 2 which could explain the absence of a difference between groups in VP c-Fos levels.

Furthermore, we did not observe any difference in c-Fos levels in amygdalar and hypothalamic regions. In the amygdala, both the BLA and CEA receive sensory information from the hindbrain but play differential roles in reward processes (Balleine & Killcross, 2006). Several studies highlight the central role of CEA in reinforcement processes. Lesions of CEA abolish the general incentive effect of Pavlovian stimuli on action performance (Holland & Gallagher, 2003; Corbit & Balleine, 2005). In contrast, stimulation of the CEA increases the incentive value of relevant actions and stimuli (Mahler & Berridge, 2012; Robinson *et al.*, 2014). The CEA has also been shown to be involved in the development of habits which require the formation of stimulus-response associations (Lingawi & Balleine, 2012). In contrast, much evidence shows that the BLA encodes the incentive value of outcomes by combining the sensory-specific properties of the reward with associated stimuli or actions to correctly guide behavior (Corbit & Balleine, 2005; Coutureau *et al.*, 2009; Parkes & Balleine, 2013). Interestingly, pharmacological manipulations of BLA activity affect action adaptation to changes in outcome value without affecting the detection of changes in reward palatability (Wassum *et al.*, 2009; Wassum *et al.*, 2011) demonstrating the selective role of BLA in incentive processes. In the present study, we used a taste reactivity task in order to restrict our analysis to hedonic processes without incentive influences which could explain the absence of a difference in c-Fos levels in the amygdala.

The lateral hypothalamus (LH) represents the unique output region from the NAc to the hypothalamic region and plays a crucial role in the regulation of food-seeking behavior but also in addictive- and depressive-like states (Kelley *et al.*, 2003; Kelley, 2004; Aston-Jones *et al.*, 2010). Previous studies have shown that local stimulation of LH neurons or NAc inhibition increase c-Fos levels in LH and stimulate feeding behavior (Stanley *et al.*, 1993;

Zheng *et al.*, 2003; Baldo *et al.*, 2004). However, its involvement in “liking” processes is not clear. Berridge and Valentstein (1991) found that electrical stimulation of LH failed to increase orofacial positive reactions to palatable food. Conversely, orexin infusions into the VP, that mimic the activation of the LH-VP pathway, enhance “liking” reactions, supporting a role of the LH in hedonic processes (Ho & Berridge, 2013). Thus, the absence of difference in LH c-Fos levels in the present study in Sucrose-exposed rats might be more in favor of the involvement of this region in the “wanting” circuit rather than in “liking”. An alternative explanation is that adolescent sucrose exposure does not alter “liking” processes through LH – VP pathway. Taken together, our results indicate that protracted deficits in “liking” processing of palatable food following adolescence sucrose consumption can be mainly attributed to alterations in NAc functioning.

Finally, while we observed significant c-Fos levels in the VTA, we did not detect any difference between Control and Sucrose-exposed rats. The dopamine system plays a central role in reward processes and is one of the main afferent regions projecting to the NAc (Berridge & Robinson, 1998; Schultz, 2000; Montague *et al.*, 2004). Several studies using microdialysis or fast-scan voltammetry report an increase in dopamine release in the NAc during consumption or intra-oral injection of sweet solutions (Hajnal & Norgren, 2001; Bassareo *et al.*, 2002; Hajnal *et al.*, 2004; Roitman *et al.*, 2008). Interestingly, changes in dopamine levels are higher for sucrose than for non-caloric sweet rewards in food-deprived animals (de Araujo *et al.*, 2008; Beeler *et al.*, 2012; McCutcheon, 2015) and can be transferred to predictive cues (Day *et al.*, 2007) suggesting an involvement of the NAc dopamine signaling in associative and motivational processes. Conversely, the dopamine system does not seem to be involved in the hedonic treatment of food rewards. Indeed, neither lesions of dopamine neurons nor increases in dopamine signaling appear to alter hedonic reactivity to sweet tastes (Berridge & Robinson, 1998; Pecina *et al.*, 2003), despite modulating motivation for rewards (Faure *et al.*, 2010; Richard & Berridge, 2011; Smith *et al.*, 2011).

The mechanism by which adolescent sucrose overconsumption, leads to long-lasting deficits of the hedonic treatment of sweet rewards at adulthood is not completely understood. Adolescence is an important period of cognitive and brain maturation (Spear, 2000). Notably, the reward system exhibits a delayed development. Indeed, the anatomical organization and functioning of the dopamine system reach maturity between adolescence and adulthood especially in cortical and striatal areas (Andersen, 2003; Huppe-Gourgues & O'Donnell, 2012; McCutcheon *et al.*, 2012; Naneix *et al.*, 2012; Naneix *et al.*, 2013). Furthermore, the opioid system, which plays a central role in hedonic and incentive processes (Pecina & Berridge, 2005; Smith & Berridge, 2007; Wassum *et al.*, 2009; Castro & Berridge, 2014a), also shows a delayed development during postnatal life (Talbot *et al.*, 2005). Interestingly, the chronic consumption of palatable foods or drugs of abuse is known to alter the functioning of both dopamine and opioid systems (Kelley *et al.*, 2003; Volkow & Wise, 2005; Avena *et al.*, 2008; Kenny, 2011; Robinson *et al.*, 2015). Thus, the prolonged stimulation of the reward system by the overconsumption of sweet foods during adolescence could lead to developmental alterations of these systems which may underlie hedonic and motivational deficits (Frazier *et al.*, 2008; Vendruscolo *et al.*, 2010a; Vendruscolo *et al.*, 2010b). This issue warrants further investigation.

Conclusion

In summary, this study provides evidence that overconsumption of sugar during adolescence induces long lasting effects on the hedonic perception of these rewards at adulthood associated with deficits in “liking” neurobiological circuits. Anhedonia and motivational deficits are the hallmarks of several psychiatric disorders including depression, schizophrenia, substance abuse or eating disorders, all emerging during adolescence (Paus *et al.*, 2008). Given the current increasing consumption of sweet palatable foods and drinks in adolescents (Guthrie & Morton, 2000; Wang *et al.*, 2008; Lustig *et al.*, 2012), our results might bring some insights in the understanding of reward-related disorders.

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The authors declare no conflict of interest.

ABBREVIATIONS

BLA, basolateral amygdaloid nucleus; CEA, central amygdaloid nucleus; GCx, gustatory part of the insular cortex; LH, lateral hypothalamus; NAc, nucleus accumbens; P, postnatal day; VP, ventral pallidum; VPPC, parvicellular part of the posteromedial ventral thalamic nucleus; VTA, ventral tegmental area.

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