EJN European Journal of Neuroscience

FENS Federation of European Neuroscience Societies

European Journal of Neuroscience, pp. 1–10, 2016 doi:10.1111/ejn.13149

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

Fabien Naneix,[1,2] Florence Darlot,[1,2] Etienne Coutureau[1,2] and Martine Cador[1,2]
[1]CNRS, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, UMR 5287, Bordeaux, France
[2]University of Bordeaux, Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, UMR 5287, F-33076 Bordeaux, France

Keywords: c-Fos, liking, limbic system, palatability, rat

Edited by Jeffrey Dalley
Received 9 October 2015, revised 12 November 2015, accepted 17 November 2015

Abstract

Adolescence is a critical period characterized by major neurobiological changes. Chronic stimulation of the reward system might constitute an important factor in 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 postnatal day 30–46) 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 behaviours 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 has increased dramatically 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 and adulthood (encompassing adolescence) alters the motivation to obtain natural rewards in adulthood, suggesting a decrease in the rewarding properties of sweet foods (Frazier *et al.*, 2008; Vendruscolo *et al.*, 2010a,b).

Reward processing can be dissociated into '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 behaviour. 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

Correspondence: Martine Cador, [2]Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, as above.
E-mail: martine.cador@u-bordeaux.fr

interactions between the nucleus accumbens NAc), the ventral pallidum (VP) and endogenous opioid and cannabinoid systems (Pecina & Berridge, 2005; Smith & Berridge, 2005, 2007; Mahler *et al.*, 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 in 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 & 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.

Materials 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 age 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 20.00 h) 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 French (council directive 2013-118, February 1, 2013) and International (directive 2010-63, September 22, 2010, European Community) legislation. The experiments received the approval no. 5012088-A from the Bordeaux Ethics Committee (CNREEA no. 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 behavioural testing (Fig. 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; internal diameter 0.1 cm, external diameter 0.2 cm) was introduced into the mouth via 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 for at least 1 week before behavioural testing. Cannulae were flushed daily with water.

Behavioural procedures

Two-bottle free-choice test. Adult rats (> P70) underwent two free-choice tests. Rats were given access in their home cage to two 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 for 3 days. Each day, rats were individually placed in a clear plastic cylinder (25 × 25 cm) with a transparent floor. On the days of taste reactivity tests, the oral cannula was connected to 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 into the mouth of the rat at a 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 four solutions, each with a specific taste: neutral (water), sweet (5% sucrose or 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;

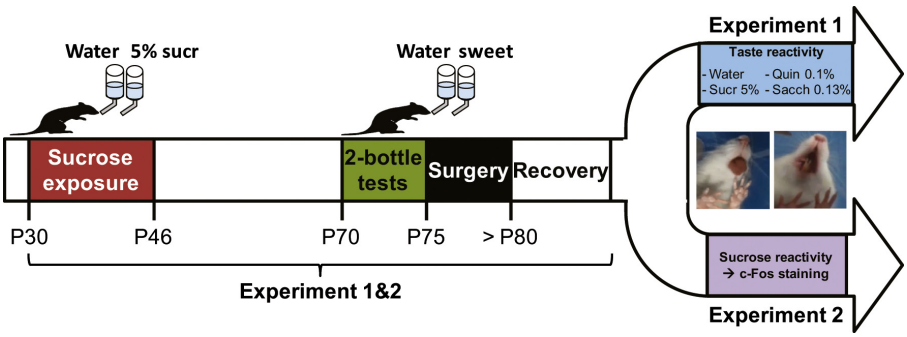

FIG. 1. Schematic representation of the experimental design. In both experiments, rats had access to 5% sucrose solution during adolescence (P30–P46). At adulthood, rats were first tested for their consumption of sweet rewards in two-bottle free-choice tests. They were then implanted with an intra-oral cannula and tested for their hedonic reactivity to different tastes (water, quinine 0.1%, sucrose 5% and saccharine 0.13%; Experiment 1). In Experiment 2, rats were killed 90 min after sucrose intra-oral infusion to investigate c-Fos brain expression.

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 different types of reaction which could be summed into total hedonic, aversive or neutral scores. Data are expressed as number of events per minute.

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

Subjects, surgery and behavioural 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, behavioural apparatus and taste reactivity testing were the same as described in Experiment 1. In Experiment 2, only the reactivity to the sucrose taste was tested in 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 0.1 M (3 × 5 min) and PBS-Triton 0.3% (1 × 5 min). They were then incubated in PBS–Triton, 0.3%, with 2% H_2O_2 for 30 min and rinsed with PBS-Triton (3 × 10 min). Sections were incubated for 1 h in a blocking solution (4% donkey serum and 0.2% Triton X-100 in 0.1 M PBS), 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 0.1 M PBS (3 × 10 min), sections were then incubated with biotinylated goat anti-rabbit (1/2000 in 0.3% PBS-Triton; Jackson ImmunoResearch) for 2 h at room temperature. They were then incubated with avidin–biotin–peroxidase complex (1 : 2000 in PBS; Vector Laboratories) for 30 min at room temperature. After rinses in PBS (2 × 10 min) and 0.1 M TBS (2 × 10 min), the staining was revealed in a solution of diaminobenzidine (0.02%; Sigma–Aldrich) and 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.

Labelled sections were scanned using a NanoZoomer (Hamamatsu Photonics, Bordeaux Imaging Center) with a 20× lens. Digital microphotographs of the region of interest in each hemisphere were examined with 5× 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 NAc (core and shell, from bregma +2.5 to +1.0 mm), the 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 atlas of Paxinos & Watson (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; 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 to group conditions, and the number of positive nuclei/mm^2 was averaged.

Data analysis

All values are expressed as mean ± SEM. Behavioural data were analyzed using two-way ANOVAS with Group as between-subjects factor and Age (sucrose exposure), Bottle (Consumption tests) and Reactions (Taste reactivity tests) as within-subjects factors. The c-Fos data were analyzed using two-way ANOVAS with Group as between-subjects factor and Region as within-subjects 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 two-bottle test data (Control, $n = 14$; Sucrose, $n = 13$), as no significant interaction between Groups (Control and Sucrose groups) and Experiments (Experiments 1 and 2) were found (all $F < 1.9$, $P > 0.2$).

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 (Fig. 2A; Age, $F_{7,175} = 14.7$, $P < 0.001$). Moreover, the Sucrose group consumed more than the Control group throughout exposure days (Group $F_{1,25} = 36.7$, $P < 0.001$). Sucrose-exposed rats drank, on average, 5 ± 1 mL of water and 81 ± 9 mL of sucrose solution in 2 days compared to control rats which drank 31 ± 2 mL of water. This overconsumption of sucrose was associated with a decrease in food consumption (Fig. 2B; Group, $F_{1,25} = 13.7$, $P = 0.001$; Age, $F_{7,175} = 54.7$, $P < 0.001$). However, the differences in fluid intake did not affect the weight of rats (Fig. 2C; Group, $F_{1,25} = 0.2$, $P = 0.7$) nor the rate of weight gain (Group × Age interaction, $F_{8,200} = 1.4$, $P = 0.2$).

Consumption of sweet rewards in adulthood

When rats reached adulthood (> P70), we investigated their consumption of sweet non-caloric or caloric rewards using two consecutive two-bottle consumption tests of 24 h each (water vs. saccharin 0.13%, Fig. 3A; water vs. sucrose 5%, Fig. 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 than did control rats. ANOVAS revealed a significant

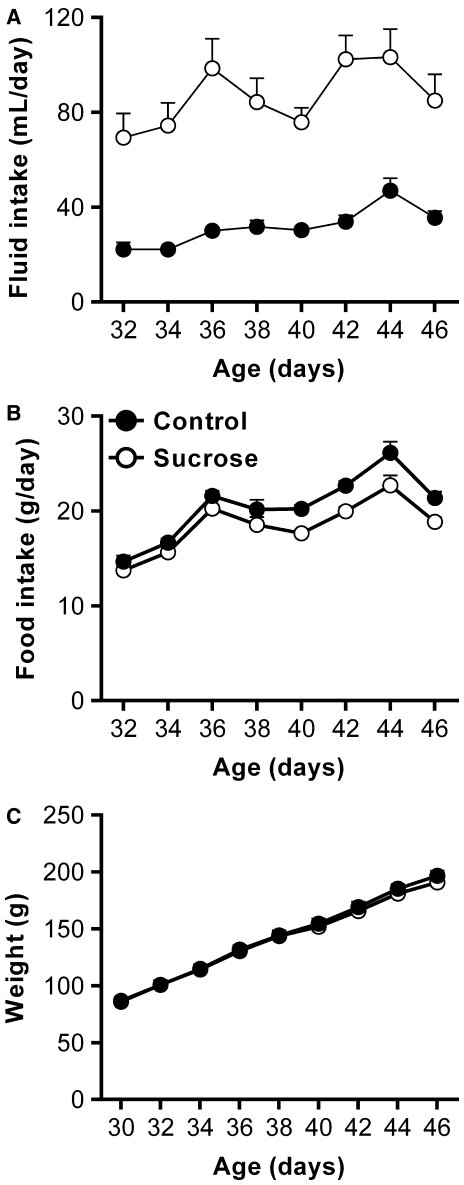

FIG. 2. Sucrose overconsumption during adolescence (P30–P46). Daily (A) fluid and (B) food consumption (water or water + 5% sucrose) during adolescence for control non-exposed (black circles, $n = 14$) and sucrose-exposed (white circles, $n = 13$) rats. (C) Evolution of the weight of the two groups during the sucrose exposure phase at adolescence. Data are expressed as mean + SEM.

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 × 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 in adulthood. Interestingly, this deficit in intake was observed not only 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.

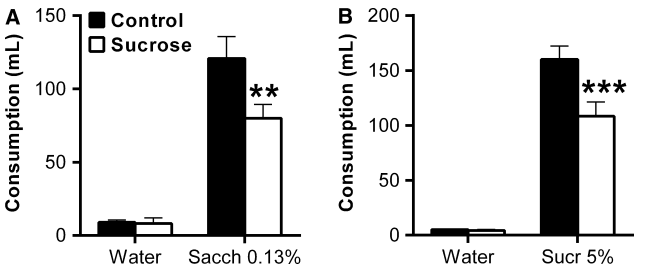

FIG. 3. Intake of sweet rewards in adulthood in two-bottle free choice consumption tests for Control (black bars, $n = 14$) and Sucrose (white bars, $n = 13$) groups. (A) Consumption of 0.13% saccharin vs. water during the 24-h consumption choice test. (B) Consumption of 5% sucrose vs. water during the 24-h consumption choice test. Data are expressed as mean + SEM. $^{**}P < 0.01$ and $^{***}P < 0.001$ (Group effect, two-way ANOVA followed by Bonferroni *post hoc* test).

Experiment 1: hedonic taste reactivity in adulthood after adolescent sucrose overconsumption

Next, we investigated the impact of sucrose overconsumption during adolescence on affective reactions to different tastes in adulthood (Control, $n = 7$; Sucrose, $n = 7$). To do this, rats were implanted with an 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 (Fig. 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 ($F_{1,12} = 0.04$, $P = 0.8$) or Group × Reactions interaction ($F_{2,24} = 0.1$, $P = 0.9$) but a significant effect of Reactions ($F_{2,24} = 17.0$, $P < 0.001$). Bonferroni *post hoc* tests confirmed that negative reactions were higher than positive and neutral reactions (all $P < 0.01$), which did not differ from each other ($P > 0.1$).

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 (Fig. 4B). As for the water infusion, the pattern of orofacial reactions was similar in control and sucrose-exposed rats. An ANOVA confirmed a significant effect of Reactions factor ($F_{2,24} = 172.1$, $P < 0.001$) but no significant effect of Group ($F_{1,12} = 2.6$, $P = 0.1$) or Group × Reaction interaction ($F_{2,24} = 0.1$, $P = 0.1$). Bonferroni *post hoc* tests confirmed that negative reactions were much higher than positive or neutral reactions (all $P < 0.001$), which did not differ from each other ($P > 0.9$).

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 sweet sucrose taste (Fig. 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 in 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 × Reaction interaction ($F_{2,24} = 6.2$, $P = 0.007$). Separate analysis on each type of reaction confirmed that the Sucrose group expressed less positive and more negative reactions than the 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

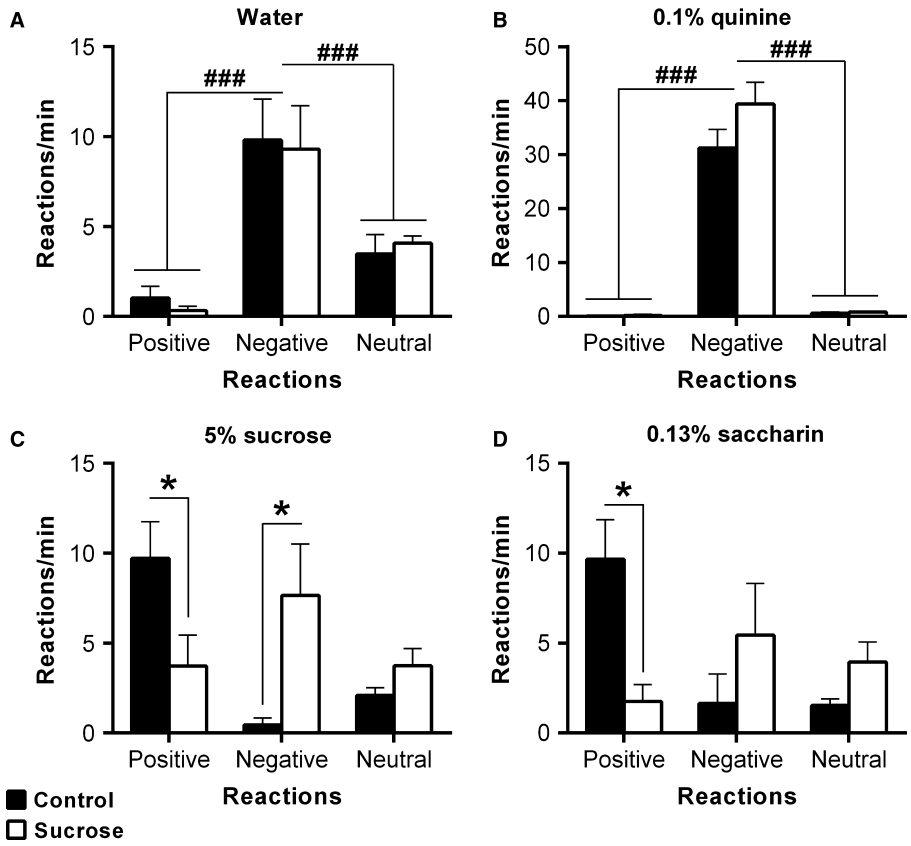

FIG. 4. Experiment 1: Positive, negative and neutral orofacial reactions in response to intraoral infusion of (A) neutral (water), (B) bitter (0.1% quinine) or (C and D) sweet (C, 5% sucrose; D, 0.13% saccharin) tastes for control (black bars, $n = 7$) and sucrose-exposed (white bars, $n = 7$) rats. Data are expressed as mean + SEM. $^{###}P < 0.001$ Reaction effect; $^{*}P < 0.05$ Group effect (two-way ANOVA followed by Bonferroni *post hoc* tests).

reward (Fig. 4D), on the same rats (four 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 × 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 ($F_{2,16} = 1.2$, $P = 0.3$) effects. 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 in adulthood.

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

Processing of palatable rewards is mediated by several brain circuits centred 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 behavioural 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 in positive reactions to the sweet sucrose taste (Fig. 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 × 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 (Fig. 5B). As depicted in the photomicrographs (Fig. 5C), we observed fewer c-Fos-positive cells in the Sucrose group than in the Control group, in both the core and shell part of the NAc. 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 × 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

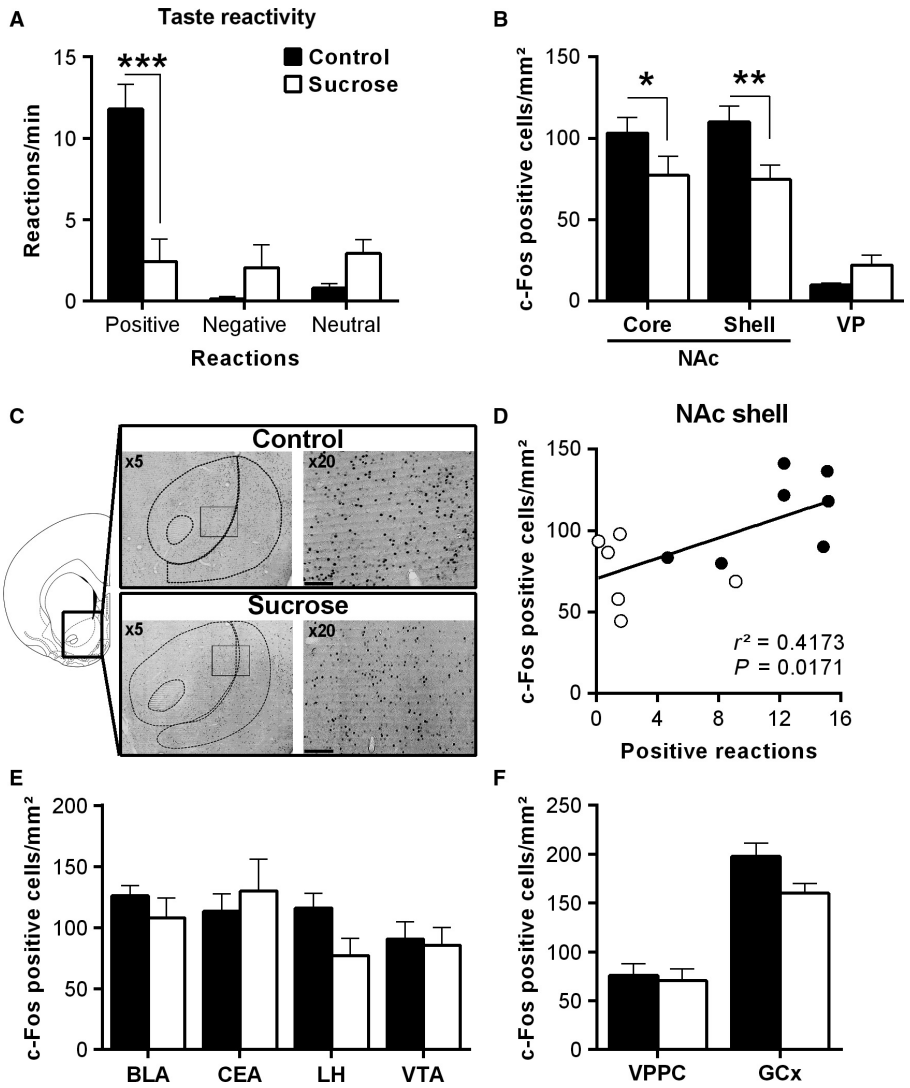

FIG. 5. Experiment 2: Intraoral infusion of 5% sucrose induced different patterns of cellular activity within hedonic-, gustatory- and incentive-related regions in control (black bars, $n = 7$) and sucrose-exposed (white bars, $n = 6$) rats. (A) Positive, negative and neutral orofacial reactions in response to sucrose. (B) Quantification of c-Fos positive cells density (cells/mm^2) in the NAc core and shell and the VP. (C) Representative microphotographs of c-Fos immunostaining in the NAc (bregma +2.0 mm according to the atlas of Paxinos & Watson, 2007) for control (upper) and sucrose (lower) rats at lower (×5, left) and higher (×20, right) magnification. (D) Positive correlation between the number of positive orofacial reactions to sucrose and c-Fos positive cells levels in the NAc shell. (E) Quantification of c-Fos-positive cells in amygdalar nuclei (BLA and CEA), LH and VTA. (F) Quantification of c-Fos-positive cells in gustatory sensory regions including gustatory thalamic nucleus (parvicellular part of the posteromedial ventral thalamic nucleus; VPMpc) and the GCx. Data are expressed as mean + SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ Group effect (two-way ANOVA followed by Bonferroni *post hoc* tests). Scale bar in C, 100 μm.

($r^2 = 0.42$, $P = 0.02$; Fig. 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 (Fig. 5E). A repeated-measures ANOVA showed a significant difference in c-Fos levels between regions ($F_{3,33} = 3.1$, $P = 0.04$) but no effect of Group ($F_{1,11} = 0.6$, $P = 0.4$) and no 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 × 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 in adulthood. We demonstrate here that a previous history of sucrose overconsumption during adolescence induces a decrease in the consumption of sweet rewards, associated with a decrease in positive orofacial reactions to sweet tastes (Experiment 1) at adulthood. 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 NAc, a region involved in hedonic pro-

cessing (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,b) and mice (Frazier *et al.*, 2008) to obtain palatable foods in adulthood, using operant progressive ratio schedules. Interestingly, such effects have not been observed with drugs of abuse such as cocaine or alcohol (Vendruscolo *et al.*, 2010a,b), indicating some differences in the neurobiological substrates underlying the intake of food and of drugs 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 two-bottle free choice test.

Preference tests are one of the most extensively used measures of hedonic perception and anhedonia states in depressive-like behaviours (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 the possibility 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 in 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 & Norgren, 1978; Berridge, 2000). This behaviour is highly conserved between species and can be used in rodents, monkeys and 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 and 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 as they presented a clear decrease in 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 nuclei and gustatory thalamic nuclei. From the hindbrain, the taste signal reaches the limbic system for reward processing. Deletion of the taste signalling machinery at the 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 cell responses (Maliphol *et al.*, 2013) and the sensitivity to sweet tastes (Robinson *et al.*, 2015). This does not appear to be the case in our experiment as we did not observe any alterations in 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 as a result of 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 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 into core and shell regions, which are involved in different aspects of reward processing. Numerous studies point to the involvement of the NAc shell in the control of food intake and consummatory behaviours (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, in contrast, been involved in instrumental action 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 a 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 of 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,b).

The NAc reciprocally interacts with the VP 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 as 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 behaviour (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, 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 LH is the unique output region from the NAc to the hypothalamic region and plays a crucial role in the regulation of food-seeking behaviour 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 behaviour (Stanley *et al.*, 1993; Zheng *et al.*, 2003; Baldo *et al.*, 2004). However, its involvement in 'liking' processes is not clear. Berridge & Valenstein (1991) found that electrical stimulation of LH failed to increase orofacial positive reactions to palatable food. Conversely, orexin infusions into the VP, which 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 favour 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 the 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 signalling 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 signalling 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 in the hedonic treatment of sweet rewards in 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, 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 in these systems which may underlie hedonic and motivational deficits (Frazier *et al.*, 2008; Vendruscolo *et al.*, 2010a,b). 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 in adulthood associated with deficits in 'liking' neurobiological circuits. Anhedonia and motivational deficits are the hallmarks of several psychiatric disorders including depression, schizophrenia, substance abuse and 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.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work is supported by the CNRS, the Conseil Régional d'Aquitaine, the LABEX BRAIN ANR-10-LABX-43 and the Fond Français Alimentation

Santé (grant no. C11022). F.N. is supported by the Fond Français Alimentation Santé. We thank Mariangela Martini and Sophie Pilon for their assistance in the implementation of the taste reactivity paradigm, Gilles Courtand, Angélique Faugère and Yoan Salafranque for technical help and Dr Shauna L. Parkes for her helpful discussions and comments on the manuscript. The microscopy was done in the Bordeaux Imaging Centre, a service unit of the CNRS-INSERM and Bordeaux University, member of the France BioImaging national infrastructure, with help from Christelle Poujol and Sébastien Marais.

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; VPPC, parvicellular part of the posteromedial ventral thalamic nucleus; VP, ventral pallidum; VTA, ventral tegmental area.

References

Adriani, W. & Laviola, G. (2004) Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behav. Pharmacol.*, **15**, 341–352.

Andersen, S.L. (2003) Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci. Biobehav. R.*, **27**, 3–18.

de Araujo, I.E., Oliveira-Maia, A.J., Sotnikova, T.D., Gainetdinov, R.R., Caron, M.G., Nicolelis, M.A. & Simon, S.A. (2008) Food reward in the absence of taste receptor signaling. *Neuron*, **57**, 930–941.

Aston-Jones, G., Smith, R.J., Sartor, G.C., Moorman, D.E., Massi, L., Tahsili-Fahadan, P. & Richardson, K.A. (2010) Lateral hypothalamic orexin/hypocretin neurons: a role in reward-seeking and addiction. *Brain Res.*, **1314**, 74–90.

Avena, N.M., Rada, P. & Hoebel, B.G. (2008) Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci. Biobehav. R.*, **32**, 20–39.

Baldo, B.A., Sadeghian, K., Basso, A.M. & Kelley, A.E. (2002) Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behav. Brain Res.*, **137**, 165–177.

Baldo, B.A., Gual-Bonilla, L., Sijapati, K., Daniel, R.A., Landry, C.F. & Kelley, A.E. (2004) Activation of a subpopulation of orexin/hypocretin-containing hypothalamic neurons by GABAA receptor-mediated inhibition of the nucleus accumbens shell, but not by exposure to a novel environment. *Eur. J. Neurosci.*, **19**, 376–386.

Balleine, B.W. & Killcross, S. (2006) Parallel incentive processing: an integrated view of amygdala function. *Trends Neurosci.*, **29**, 272–279.

Barbano, M.F. & Cador, M. (2007) Opioids for hedonic experience and dopamine to get ready for it. *Psychopharmacology*, **191**, 497–506.

Bassareo, V., De Luca, M.A. & Di Chiara, G. (2002) Differential expression of motivational stimulus properties by dopamine in nucleus accumbens shell versus core and prefrontal cortex. *J. Neurosci.*, **22**, 4709–4719.

Beeler, J.A., McCutcheon, J.E., Cao, Z.F., Murakami, M., Alexander, E., Roitman, M.F. & Zhuang, X. (2012) Taste uncoupled from nutrition fails to sustain the reinforcing properties of food. *Eur. J. Neurosci.*, **36**, 2533–2546.

Berridge, K.C. (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neurosci. Biobehav. R.*, **24**, 173–198.

Berridge, K.C. & Kringelbach, M.L. (2015) Pleasure systems in the brain. *Neuron*, **86**, 646–664.

Berridge, K.C. & Robinson, T.E. (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Brain Res. Rev.*, **28**, 309–369.

Berridge, K.C. & Valenstein, E.S. (1991) What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? *Behav. Neurosci.*, **105**, 3–14.

Castro, D.C. & Berridge, K.C. (2014a) Advances in the neurobiological bases for food 'liking' versus 'wanting'. *Physiol. Behav.*, **136**, 22–30.

Castro, D.C. & Berridge, K.C. (2014b) Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness "liking" and "wanting". *J. Neurosci.*, **34**, 4239–4250.

Castro, D.C., Cole, S.L. & Berridge, K.C. (2015) Lateral hypothalamus, nucleus accumbens, and ventral pallidum roles in eating and hunger: interactions between homeostatic and reward circuitry. *Front. Syst. Neurosci.*, **9**, 90.

Corbit, L.H. & Balleine, B.W. (2005) Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. *J. Neurosci.*, **25**, 962–970.

Corbit, L.H. & Balleine, B.W. (2011) The general and outcome-specific forms of Pavlovian-instrumental transfer are differentially mediated by the nucleus accumbens core and shell. *J. Neurosci.*, **31**, 11786–11794.

Corbit, L.H., Muir, J.L. & Balleine, B.W. (2001) The role of the nucleus accumbens in instrumental conditioning: evidence of a functional dissociation between accumbens core and shell. *J. Neurosci.*, **21**, 3251–3260.

Coutureau, E., Marchand, A.R. & Di Scala, G. (2009) Goal-directed responding is sensitive to lesions to the prelimbic cortex or basolateral nucleus of the amygdala but not to their disconnection. *Behav. Neurosci.*, **123**, 443–448.

Crews, F., He, J. & Hodge, C. (2007) Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol. Biochem. Be.*, **86**, 189–199.

Day, J.J., Roitman, M.F., Wightman, R.M. & Carelli, R.M. (2007) Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci.*, **10**, 1020–1028.

DiLeone, R.J., Taylor, J.R. & Picciotto, M.R. (2012) The drive to eat: comparisons and distinctions between mechanisms of food reward and drug addiction. *Nat. Neurosci.*, **15**, 1330–1335.

Faure, A., Reynolds, S.M., Richard, J.M. & Berridge, K.C. (2008) Mesolimbic dopamine in desire and dread: enabling motivation to be generated by localized glutamate disruptions in nucleus accumbens. *J. Neurosci.*, **28**, 7184–7192.

Faure, A., Richard, J.M. & Berridge, K.C. (2010) Desire and dread from the nucleus accumbens: cortical glutamate and subcortical GABA differentially generate motivation and hedonic impact in the rat. *PLoS One*, **5**, e11223.

Frazier, C.R., Mason, P., Zhuang, X. & Beeler, J.A. (2008) Sucrose exposure in early life alters adult motivation and weight gain. *PLoS One*, **3**, e3221.

Friemel, C.M., Spanagel, R. & Schneider, M. (2010) Reward sensitivity for a palatable food reward peaks during pubertal developmental in rats. *Front. Behav. Neurosci.*, **4**, 39, 1–10.

Grill, H.J. & Norgren, R. (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Res.*, **143**, 263–279.

Guthrie, J.F. & Morton, J.F. (2000) Food sources of added sweeteners in the diets of Americans. *J. Am. Diet. Assoc.*, **100**, 43–51, quiz 49-50.

Hajnal, A. & Norgren, R. (2001) Accumbens dopamine mechanisms in sucrose intake. *Brain Res.*, **904**, 76–84.

Hajnal, A., Smith, G.P. & Norgren, R. (2004) Oral sucrose stimulation increases accumbens dopamine in the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **286**, R31–R37.

Ho, C.Y. & Berridge, K.C. (2013) An orexin hotspot in ventral pallidum amplifies hedonic 'liking' for sweetness. *Neuropsychopharmacology*, **38**, 1655–1664.

Ho, C.Y. & Berridge, K.C. (2014) Excessive disgust caused by brain lesions or temporary inactivations: mapping hotspots of the nucleus accumbens and ventral pallidum. *Eur. J. Neurosci.*, **40**, 3556–3572.

Holland, P.C. & Gallagher, M. (2003) Double dissociation of the effects of lesions of basolateral and central amygdala on conditioned stimulus-potentiated feeding and Pavlovian-instrumental transfer. *Eur. J. Neurosci.*, **17**, 1680–1694.

Huppe-Gourgues, F. & O'Donnell, P. (2012) D(1)-NMDA receptor interactions in the rat nucleus accumbens change during adolescence. *Synapse*, **66**, 584–591.

Kelley, A.E. (2004) Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neurosci. Biobehav. R.*, **27**, 765–776.

Kelley, A.E., Will, M.J., Steininger, T.L., Zhang, M. & Haber, S.N. (2003) Restricted daily consumption of a highly palatable food (chocolate Ensure (R)) alters striatal enkephalin gene expression. *Eur. J. Neurosci.*, **18**, 2592–2598.

Kendig, M.D. (2014) Cognitive and behavioural effects of sugar consumption in rodents. A review. *Appetite*, **80**, 41–54.

Kenny, P.J. (2011) Common cellular and molecular mechanisms in obesity and drug addiction. *Nat. Rev. Neurosci.*, **12**, 638–651.

Lingawi, N.W. & Balleine, B.W. (2012) Amygdala central nucleus interacts with dorsolateral striatum to regulate the acquisition of habits. *J. Neurosci.*, **32**, 1073–1081.

Lustig, R.H., Schmidt, L.A. & Brindis, C.D. (2012) Public health: the toxic truth about sugar. *Nature*, **482**, 27–29.

Mahler, S.V. & Berridge, K.C. (2012) What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. *Psychopharmacology*, **221**, 407–426.

Mahler, S.V., Smith, K.S. & Berridge, K.C. (2007) Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. *Neuropsychopharmacology*, **32**, 2267–2278.

Maliphol, A.B., Garth, D.J. & Medler, K.F. (2013) Diet-induced obesity reduces the responsiveness of the peripheral taste receptor cells. *PLoS One*, **8**, e79403.

McCutcheon, J.E. (2015) The role of dopamine in the pursuit of nutritional value. *Physiol. Behav.*, **152**, 408–415.

McCutcheon, J.E., Conrad, K.L., Carr, S.B., Ford, K.A., McGehee, D.S. & Marinelli, M. (2012) Dopamine neurons in the ventral tegmental area fire faster in adolescent rats than in adults. *J. Neurophysiol.*, **108**, 1620–1630.

Montague, P.R., Hyman, S.E. & Cohen, J.D. (2004) Computational roles for dopamine in behavioural control. *Nature*, **431**, 760–767.

Naneix, F., Marchand, A.R., Di Scala, G., Pape, J.R. & Coutureau, E. (2012) Parallel maturation of goal-directed behavior and dopaminergic systems during adolescence. *J. Neurosci.*, **32**, 16223–16232.

Naneix, F., Marchand, A.R., Pichon, A., Pape, J.R. & Coutureau, E. (2013) Adolescent stimulation of D2 receptors alters the maturation of dopamine-dependent goal-directed behavior. *Neuropsychopharmacology*, **38**, 1566–1574.

Parkes, S.L. & Balleine, B.W. (2013) Incentive memory: evidence the basolateral amygdala encodes and the insular cortex retrieves outcome values to guide choice between goal-directed actions. *J. Neurosci.*, **33**, 8753–8763.

Parkinson, J.A., Cardinal, R.N. & Everitt, B.J. (2000) Limbic cortical-ventral striatal systems underlying appetitive conditioning. *Prog. Brain Res.*, **126**, 263–285.

Paus, T., Keshavan, M. & Giedd, J.N. (2008) Why do many psychiatric disorders emerge during adolescence? *Nat. Rev. Neurosci.*, **9**, 947–957.

Paxinos, G. & Watson, C. (2007). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, London.

Pecina, S. & Berridge, K.C. (2005) Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? *J. Neurosci.*, **25**, 11777–11786.

Pecina, S., Cagniard, B., Berridge, K.C., Aldridge, J.W. & Zhuang, X. (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. *J. Neurosci.*, **23**, 9395–9402.

Richard, J.M. & Berridge, K.C. (2011) Nucleus accumbens dopamine/glutamate interaction switches modes to generate desire versus dread: D(1) alone for appetitive eating but D(1) and D(2) together for fear. *J. Neurosci.*, **31**, 12866–12879.

Robinson, M.J., Warlow, S.M. & Berridge, K.C. (2014) Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another. *J. Neurosci.*, **34**, 16567–16580.

Robinson, M.J., Burghardt, P.R., Patterson, C.M., Nobile, C.W., Akil, H., Watson, S.J., Berridge, K.C. & Ferrario, C.R. (2015) Individual differences in cue-induced motivation and striatal systems in rats susceptible to diet-induced obesity. *Neuropsychopharmacology*, **40**, 2113–2123.

Roitman, M.F., Wheeler, R.A. & Carelli, R.M. (2005) Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron*, **45**, 587–597.

Roitman, M.F., Wheeler, R.A., Wightman, R.M. & Carelli, R.M. (2008) Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. *Nat. Neurosci.*, **11**, 1376–1377.

Schultz, W. (2000) Multiple reward signals in the brain. *Nat. Rev. Neurosci.*, **1**, 199–207.

Smith, K.S. & Berridge, K.C. (2005) The ventral pallidum and hedonic reward: neurochemical maps of sucrose "liking" and food intake. *J. Neurosci.*, **25**, 8637–8649.

Smith, K.S. & Berridge, K.C. (2007) Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *J. Neurosci.*, **27**, 1594–1605.

Smith, K.S., Berridge, K.C. & Aldridge, J.W. (2011) Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *P. Natl Acad. Sci. USA*, **108**, E255–E264.

Spear, L.P. (2000) The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. R.*, **24**, 417–463.

Stanley, B.G., Willett, V.L. 3rd, Donias, H.W., Ha, L.H. & Spears, L.C. (1993) The lateral hypothalamus: a primary site mediating excitatory amino acid-elicited eating. *Brain Res.*, **630**, 41–49.

Steiner, J.E. (1973) The gustofacial response: observation on normal and anencephalic newborn infants. *Symp. Oral. Sens. Percept.*, 254–278.

Stratford, T.R. & Kelley, A.E. (1997) GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. *J. Neurosci.*, **17**, 4434–4440.

Stratford, T.R., Swanson, C.J. & Kelley, A. (1998) Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. *Behav. Brain Res.*, **93**, 43–50.

Taha, S.A. & Fields, H.L. (2005) Encoding of palatability and appetitive behaviors by distinct neuronal populations in the nucleus accumbens. *J. Neurosci.*, **25**, 1193–1202.

Talbot, J.N., Happe, H.K. & Murrin, L.C. (2005) Mu opioid receptor coupling to Gi/o proteins increases during postnatal development in rat brain. *J. Pharmacol. Exp. Ther.*, **314**, 596–602.

Vendruscolo, L.F., Gueye, A.B., Darnaudery, M., Ahmed, S.H. & Cador, M. (2010a) Sugar overconsumption during adolescence selectively alters motivation and reward function in adult rats. *PLoS One*, **5**, e9296.

Vendruscolo, L.F., Gueye, A.B., Vendruscolo, J.C., Clemens, K.J., Mormede, P., Darnaudery, M. & Cador, M. (2010b) Reduced alcohol drinking in adult rats exposed to sucrose during adolescence. *Neuropharmacology*, **59**, 388–394.

Volkow, N.D. & Wise, R.A. (2005) How can drug addiction help us understand obesity? *Nat. Neurosci.*, **8**, 555–560.

Wang, Y.C., Bleich, S.N. & Gortmaker, S.L. (2008) Increasing caloric contribution from sugar-sweetened beverages and 100% fruit juices among US children and adolescents, 1988–2004. *Pediatrics*, **121**, e1604–e1614.

Wassum, K.M., Ostlund, S.B., Maidment, N.T. & Balleine, B.W. (2009) Distinct opioid circuits determine the palatability and the desirability of rewarding events. *P. Natl Acad. Sci. USA*, **106**, 12512–12517.

Wassum, K.M., Cely, I.C., Balleine, B.W. & Maidment, N.T. (2011) Micro-opioid receptor activation in the basolateral amygdala mediates the learning of increases but not decreases in the incentive value of a food reward. *J. Neurosci.*, **31**, 1591–1599.

Wheeler, R.A., Twining, R.C., Jones, J.L., Slater, J.M., Grigson, P.S. & Carelli, R.M. (2008) Behavioral and electrophysiological indices of negative affect predict cocaine self-administration. *Neuron*, **57**, 774–785.

Willner, P., Towell, A., Sampson, D., Sophokleous, S. & Muscat, R. (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology*, **93**, 358–364.

Wilmouth, C.E. & Spear, L.P. (2009) Hedonic sensitivity in adolescent and adult rats: taste reactivity and voluntary sucrose consumption. *Pharmacol. Biochem. Be.*, **92**, 566–573.

Zhang, Y., Hoon, M.A., Chandrashekar, J., Mueller, K.L., Cook, B., Wu, D., Zuker, C.S. & Ryba, N.J. (2003) Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell*, **112**, 293–301.

Zheng, H., Corkern, M., Stoyanova, I., Patterson, L.M., Tian, R. & Berthoud, H.R. (2003) Peptides that regulate food intake: appetite-inducing accumbens manipulation activates hypothalamic orexin neurons and inhibits POMC neurons. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **284**, R1436–R1444.