Psychoneuroendocrinology 104 (2019) 300–307

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Sucrose exposure in juvenile rats produces long-term changes in fear memory and anxiety-like behavior

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ARTICLE INFO

Keywords:
Open field
GABA
Medial prefrontal cortex
Hippocampus
Calretinin

ABSTRACT

Sugar consumption has increased dramatically in our society, a phenomenon that is primarily associated with obesity and diabetes appearance. However, whether this overconsumption of sugar has an impact on the developing CNS remains unknown. This study investigated the long-term effects of unlimited access to sucrose using the two-bottle choice paradigm and the juvenile and adult effects were compared. Male Sprague Dawley rats had free access to water containing 10% sucrose and water during youth (PD 25–50) or adulthood (PD 75–100). Rats in the sucrose group, privileged to take sugary solution over the water. No weight differences were observed between the sucrose groups and their age-matched water controls. After treatment all animals drank only water for another 25 days. Frustration, measured as the amount of water drank after the sucrose period, was higher in young-exposed animals compared to adults. In addition, rats that consumed sucrose during youth travelled less the central zones of an open field. Sucrose consumption during youth also affected fear behavior as animals exhibited impaired extinction of fear memory compared to control, indicating that prefrontal and hippocampal function is impaired. In contrast, rats exposed to sucrose during adulthood did not behave significantly different from control on either task. The calretinin and parvalbumin GABAergic interneurons go through extensive remodeling during youth in the medial prefrontal cortex and the ventral hippocampus. Here, we found that rats exposed to sucrose during youth presented an increased expression of calretinin-immunoreactivity in the medial prefrontal cortex, but not in the ventral hippocampus, indicating that early sucrose consumption produces enduring effects on the GABA system. Altogether these results indicate that sugar overconsumption at early stages of life induces long-term effects on behaviors related to fear and anxiety in adulthood.

1. Introduction

Sugar intake in the modern society has increased dramatically over the past years, with an estimated rise in *per capita* consumption from 5 kg to 70 kg per year from 1800 to 2006 (Tappy, 2012). This augmentation is largely attributable to the rising consumption of sugar-sweetened beverages which contributed to 80% of the increase in added sugar consumption (Popkin and Nielsen, 2003) being the largest single source of added sugar consumption (Yang et al., 2014).

Of particular interest are the youngest groups of population since they are the ones with the highest sugar consumption compared to any other age group (Harnack et al., 1999). Argentina is not exempt from this phenomenon, according to CESNI (Center for Studies on Child Nutrition) the Argentinian children have the highest consumption rate of soft drinks in Latin America with very low water consumption (of every ten acts of fluid intake, only two are water) (Carmuega, 2015).

The transition between childhood and adulthood represents a critical developmental period in the central nervous system (CNS) characterized by increased plasticity as well as active physical and emotional maturational changes. Therefore, this is a period highly susceptible to environmental influences (Spear, 2000; Andersen, 2003; Paus et al., 2008). Even if the metabolic effects of high caloric diets are well known on obesity (Schmidt, 2014) type-2 diabetes (Malik and Hu, 2012) and cardiovascular diseases (Malik et al., 2010; Yang et al., 2014) little is known about the sugar effects on mental health and the impact on the developing CNS.

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https://doi.org/10.1016/j.psyneuen.2019.03.016
Received 11 September 2018; Received in revised form 12 March 2019; Accepted 19 March 2019

Unlimited access to 10% sucrose solution during adolescence has been shown to reduce the motivation for sweet drinks in adulthood (Vendruscolo et al., 2010a, b) as well as the hedonic response to sweets in rats (Naneix et al., 2016). More recently the same group reported that sucrose consumption during adolescence caused anhedonia, decreased motivation for saccharin, increased immobility in the forced swim test and exacerbated anxiety-like behavior in adulthood (Gueye et al., 2018). Altogether these results suggest that excessive sugar intake during adolescence induces a long-term chronic depression associated with reward processing.

Intermittent sucrose consumption during adolescence has also shown to affect higher neurocognitive functions, such as decision making and memory, in adulthood (Reichelt et al., 2015; Wong et al., 2017). Recently, an epidemiological study showed adverse effects of sugar intake from sweet food/beverage on long-term psychological health and suggests that lower intake of sugar may be associated with better psychological health (Knuppel et al., 2017).

However, to date, no studies investigating the long-term effects of early sucrose exposure on emotional learning (e.g., fear conditioning) have been published. In the current study we examined the effect unlimited access to sucrose during youth on exploratory activity as well as on fear-related learning in the rat. The sugar exposure period selected ranges from early juvenile to adolescence, since sugar consumption has also risen in the youngest group of the society (Carmuega, 2015). We hypothesized that chronic sucrose intake during youth may later alter the emotional behavior and brain function, specifically, on anxiety-like behavior. The levels of the calretinin-positive interneurons were also assessed in the medial prefrontal cortex (mPFC) and ventral hippocampus (vHIP) as these brain areas undergo extensive remodeling of the GABAergic system during early stages of the development, specially the calretinin and parvalbumin-positive interneurons (Caballero et al., 2013, 2014; Caballero et al., 2016). Moreover, it has recently been shown that parvalbumin-positive interneurons are affected by early sucrose consumption (Reichelt et al., 2015), but it is still not known if it could also affect calretinin-positive interneurons. In parallel, the effects of sugar intake on adult rats were also analyzed, in order to assess whether the behavioral changes produced by excessive consumption of sucrose are due to a specific action during the juvenile stage, or if it also produces similar effects in adulthood.

2. Material and methods

2.1. Experimental animals

Animal procedures were approved by the Animal Care and Ethical Use Committee of the Experimental Medicine and Biology Institute, IByME, Argentina, in accordance with guidelines defined by the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, or the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). Sprague Dawley male rats were maintained on a 12 h light: 12 h darkness cycle with food and water available ad libitum. Over a 25-day period, 25 days-old rats (juvenile groups) and 75 days-old rats (adult groups) had unrestricted access to tap water (control groups) or the choice to drink a 10% sucrose solution (w/v made up on tap water, sucrose groups) ad libitum. At the end of this period, all animals drank water for another 25 days (Fig. 1A). Fresh sucrose was prepared every second day and consumption was recorded daily by weighing the bottles. Once a week the rats were also weighed. A total of 25 animals were assigned per group. At the end of the protocol, 12 animals from each group were tested in the open field while the 13 remaining rats were used for fear conditioning studies. This last set of animals was euthanized by exposure to CO_2 5 days later (Day 7 Fig. 1A). Animals that underwent the open field test were euthanized 30 days after the test by decapitation (Day 32 Fig. 1A). Western blot samples of the mPFC and the vHIP were rapidly dissected out, quickly frozen on dry ice and stored at −80∘C (Fig. 1A). In this last set of animals (n = 12) the water consumption was measured along a 30-days period after sucrose removal. For the rest of the animals, the consumption was measured for only a 25-days period after sucrose removal (n = 13).

2.2. Open field

Behavioral testing occurred between 08:00 and 12:00 h. The device, made of opaque wood, consists of a square box of 75 cm with walls 30 cm high in a dimly lit room with controlled temperature 21 °C and humidity (40–70%) (Rey et al., 2016). The animals were always placed in the testing room and allowed to acclimate for 20 min before testing. In day 1, the animals were placed in the center of the device and allowed to freely explore the arena for 15 min (preconditioning). A solution of 70% EtOH was used between animals to clean the apparatus. The next day the animals returned to the arena for 5 min and the session was video-recorded and analyzed with the ANY-maze© software (Stoelting Co., USA). The arena was divided into 3 zones, the central zone 1, the intermediate zone 2 and the peripheral zone 3 (Fig. 2A). The behavioral parameters evaluated were activity time, distance traveled, number of areas visited, distances and time of activity in the central areas of the arena and rearing as a sensitive measure of vertical exploration (Lever et al., 2006).

2.3. Fear conditioning

The test was performed over two days. On the first day (conditioning), the rats were transported to a testing room and placed in a conditioning chamber (0.6″ x 10.6″ x 14.1″ height) previously cleaned with 1% vinegar. This cabin was sound isolated, had an audio speaker, a dim house light, an infrared (IR) LED light and a digital camera sensitive to the IR range. Continuous background white noise (72 dB) was supplied by a fan in the cabinet. The trace fear conditioning phase (Chowdhury et al., 2005; Zhang and Rosenkranz, 2013) consisted of 2 min habituation followed by 5 pairings of a neutral tone (10 s, 1500 Hz, 85 dB) paired with a footshock (1 s, 0.4 mA) at a delay of 20 s from the end of the tone. Conditioning trials were presented at 240–280 s intervals. Upon completion of conditioning, rats were returned to their home cage. After 24 h (testing day) rats were returned to the testing room, and placed in a novel chamber (black-walled, oval shaped 12″ x 9″ peak diameters x 12″ height, simple green odor) inside of a different sound attenuation cabinet. After a 2 min habituation phase, conditioned tones were presented to the rat without discharges (15 trials, 60 s interval). The test was video recorded and freezing was measured manually by 2 independent observers naïve to group allocation. The correlation between freezing scores of the 2 observers was high ($r^2 > 0$ 0.90).

2.4. Western blot

Samples obtained from 7 rats that underwent the open field study were randomly selected (Day 32, Fig. 1A). Homogenates were prepared by sonication in ice-cold lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 2 mM EDTA, 1 mM phenylmethylsulphonylfluoride, 1 mM Na3VO4, and 1% Triton 100, pH 7.4) containing a protease inhibitor cocktail (Roche Diagnostics) as previously described (Kruse et al., 2012, 2017, 2018). A total of 20 mg of protein was separated by 10% SDS–PAGE in Tris–glycine electrophoresis buffer at 120 V for 90 min. Proteins from gels were transferred onto PVDF membranes (Bio-Rad), and the membranes were blocked with TBS-T (20 mmol/l Tris, pH 7.5; 150 mmol/l NaCl; and 0.1% Tween-20) containing 5% fat free milk for 1 h. Blocked membranes were incubated with the primary antibody in TBS-T containing 5% fat-free milk at 4 °C overnight. The primary antibodies used were calretinin (1:1000, Swant) and F-actin (1:1000, Santa Cruz Biotechnology). Immunoblots were then washed with TBS-T 3 times and

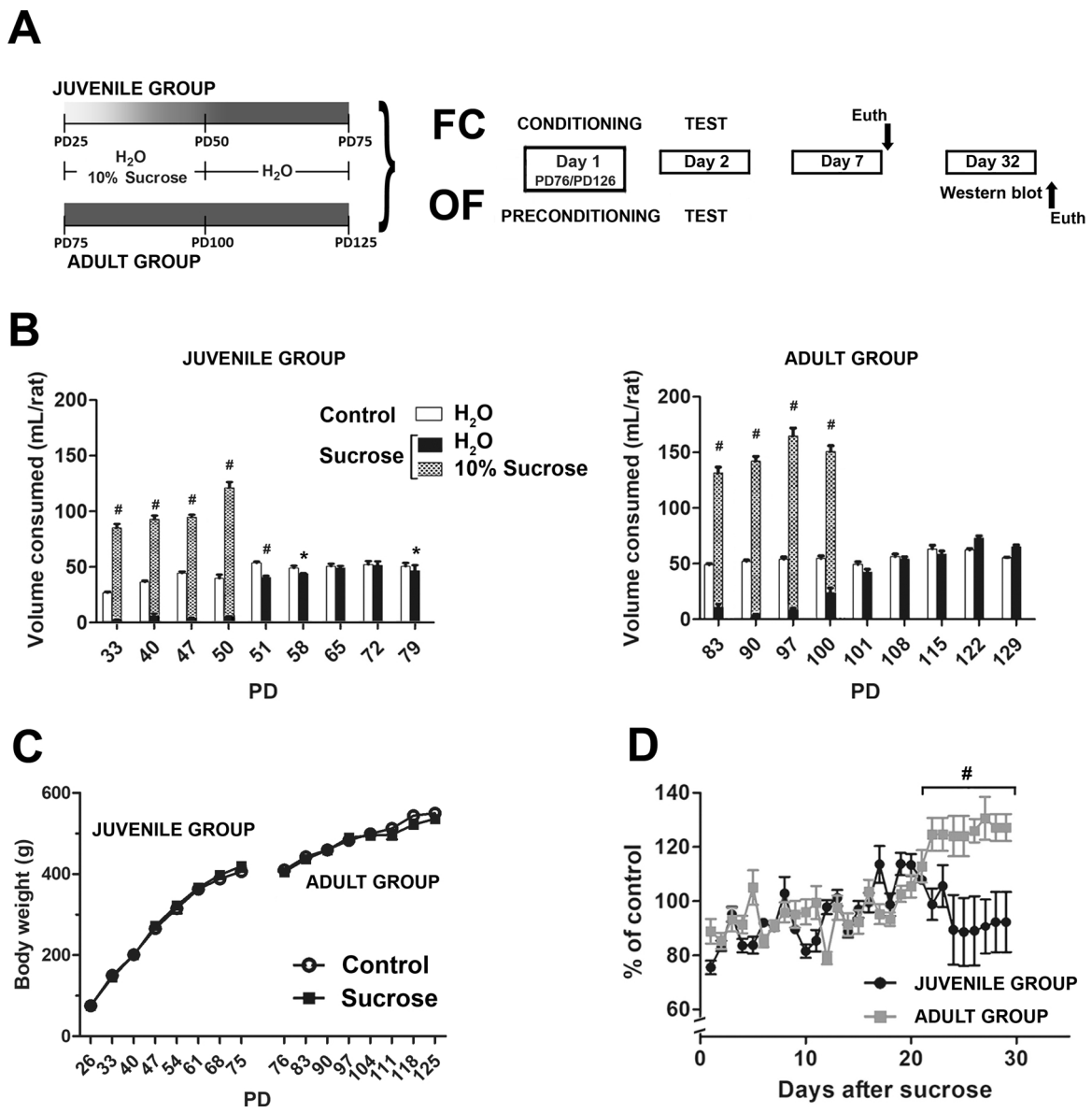

Fig. 1. A. Time line of experimental manipulations. The transition zone (soft grey to solid dark grey) represents the early juvenile-adolescent transition to adulthood (solid color). B. Sucrose and water intake. C. Weights of rats in control and sucrose groups. D. Percentage of water consumed by the sucrose groups (juvenile and adult) compared to their age control after removal of sucrose (PD 51-79/101-129). Values are expressed as mean ± SEM, for B and C, n = 25; for D, n = 12–25. Bonferroni post hoc test, #$p < 0.001$, *$p < 0.05$ (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

incubated at room temperature for 1 h with the respective HRP-conjugated secondary antibodies (1:5000, GE Healthcare Life Sciences, Buenos Aires, Argentina). Chemiluminescence was detected with the ECL system (GE Healthcare Life Sciences) and exposure to hyperfilm (GE Healthcare Life Sciences). All membranes were then stripped and reprobed for F-actin as a loading control. Signals in the immunoblots were scanned and analyzed by Scion Image Software (National Institutes of Health, Washington DC, USA). The amount of target protein was indexed to F-actin in all cases to ensure correction for the amount of total protein on the membrane. The results were reported as percentages of values obtained from expression of target proteins compared to controls.

2.5. Statistical analysis

The significances among variables were evaluated by ANOVA or repeated measures ANOVA (RM ANOVA), using the IBM® SPSS® Statistics 21 Software followed by Fisher's LSD post hoc test or GraphPad Prism 5.0 software followed by Bonferroni post hoc test, respectively. For fear conditioning, two-way RM ANOVAs were conducted on the trials following each cue presentation, using the IBM® SPSS® Statistics 21 Software followed by Fisher's LSD post hoc test. Significance was assumed at $p < 0.05$. Means are reported ± S.E.M.

3. Results

3.1. Sucrose consumption

The rats of the sucrose groups preferred the sweet solution rather than tap water as only 3–15% of the total volume consumed was water (RM ANOVA, juvenile group: $F(1,1152) = 412.4$; $p < 0.0001$ and adult group: $F(1,1152) = 1599$; $p < 0.0001$). Moreover, they drank a larger volume comparing to their control of age (+2.2-3.2 fold, RM ANOVA, juvenile group: $F(1,1152) = 155.3$; $p < 0.0001$ and adult

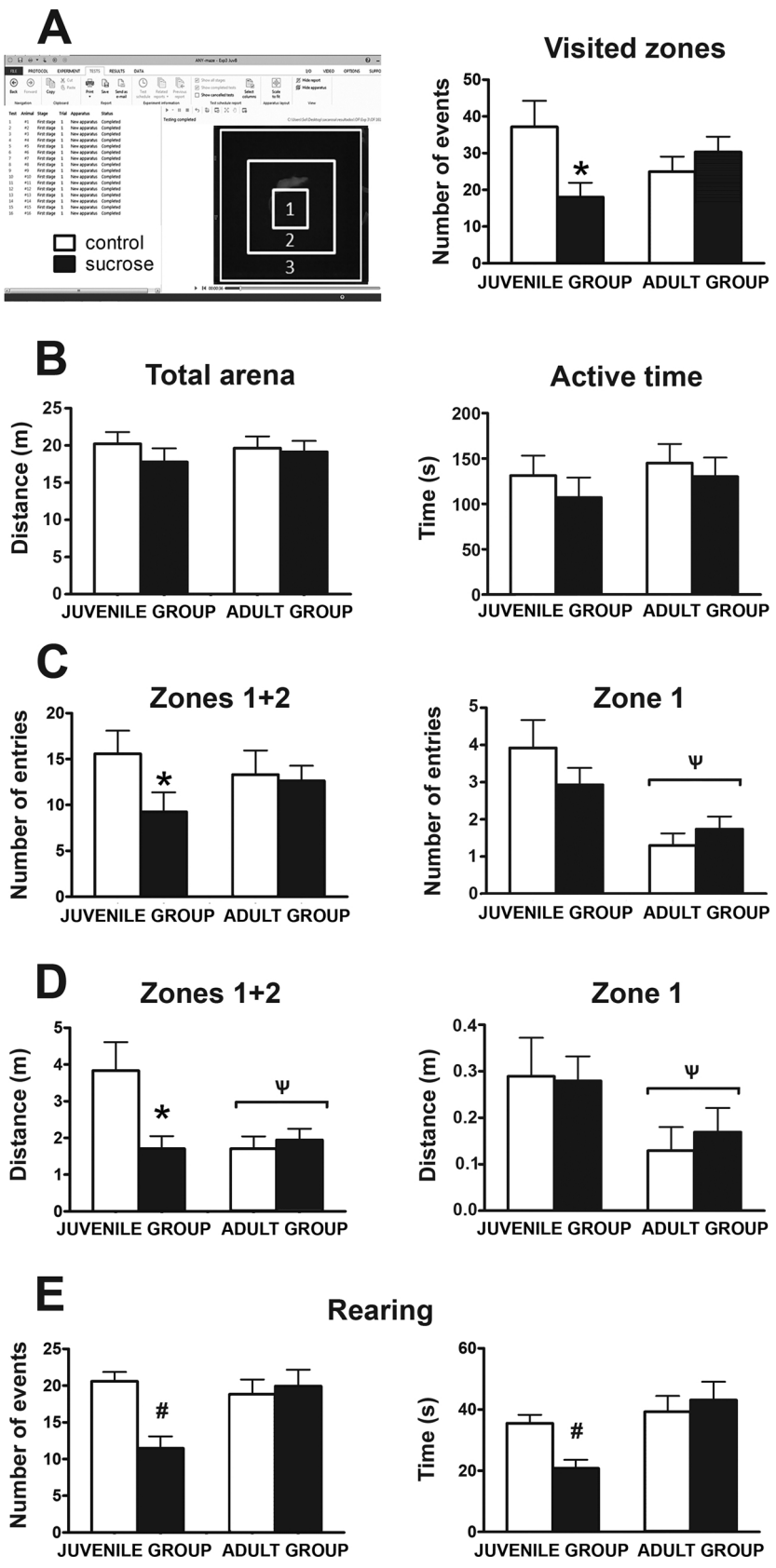

Fig. 2. A. Open field test was analyzed by the ANY-maze© software in animals exposed during youth or adulthood to only water (control, white bars) or water and sucrose (sucrose, black bars). The arena was divided into 3 zones, the central zone 1, the intermediate zone 2 and the peripheral zone 3. The graph shows the number of visited zones in the chamber. B. Total distance traveled and total activity time. C. Number of entries to zone 1 + 2 or zone 1 alone. D. Distance traveled by the animals in zone 1 + 2 or zone 1 alone. E. Number and time of rearing. Data is presented as mean ± SEM, n = 12. Fisher's LSD post hoc test, #p < 0.0001, * p < 0.05. Difference by age, ψp < 0.001.

group: F(1,1152) = 674.3; p < 0.0001; Fig. 1B). After the sucrose period all groups of animals consumed only water for another 25 days (Fig. 1A). The animals exposed to sucrose during youth showed frustration over this period as they drank less volume of water compared to their control (RM ANOVA, juvenile group: F(1,1152) = 6.174; p = 0.0165 and adult group: F(1,1152) = 0.2630; p = 0.6104; Fig. 1B). Moreover, after 20 days of sucrose removal, the percentage of water consumed by the sucrose group compared to their age control was

significant different between juvenile and adult groups (RM ANOVA, $F(1,162) = 4.828$; $p = 0.0413$; Fig. 1D). The animal weights were also registered weekly. All rats gained weight over time (RM ANOVA, $F(1,672) = 723.6$; $p < 0.0001$) but no differences between control and sucrose groups were observed (RM ANOVA, $F(1,672) = 0.6744$; $p = 0.4156$; Fig. 1C).

3.2. Open field

No differences were found in the total distance traveled or the total active time among the 4 groups (juvenile control-sucrose and adult control-sucrose; two-way ANOVA, total distance traveled: $F_{Treatment}(1,44) = 0.811$; $p = 0.3712$; $F_{Age}(1,44) = 0.053$; $p = 0.8189$; $F_{Treatment\text{-}Age}(1,44) = 0.359$; $p = 0.089$ and total active time: $F_{Treatment}(1,44) = 0.846$; $p = 0.3612$; $F_{Age}(1,44) = 0.765$; $p = 0.3850$; $F_{Treatment\text{-}Age}(1,44) = 0.046$; $p = 0.8303$; Fig. 2B). Nonetheless, significant differences were found by treatment-age interaction in the number of visited zones (two-way ANOVA, $F_{Treatment}(1,44) = 1.792$; $p = 0.1869$; $F_{Age}(1,44) = 0.0008$; $p = 0.9928$; $F_{Treatment\text{-}Age}(1,44) = 5.688$; $p = 0.0210$; Fig. 2A) and by treatment, age and age-treatment interaction, in the distance traveled in the central zones 1 + 2 (two-way ANOVA, $F_{Treatment}(1,44) = 4.367$; $p = 0.0413$; $F_{Age}(1,44) = 4.347$; $p = 0.0417$; $F_{Treatment\text{-}Age}(1,44) = 6.886$; $p = 0.0112$; Fig. 2D). Fisher's LSD post hoc analyses revealed that only the animals that consumed sucrose during youth visited less number of zones (control: 37.1 ± 7.1 vs. sucrose: 18.9 ± 4.7, $p = 0.012$; Fig. 2A), evidenced by a decrease of entries to the central zones 1 + 2 (ANOVA, control: 13.2 ± 01.4 vs. sucrose: 7.1 ± 1.0, $p = 0.020$; Fig. 2C). Moreover the distance traveled in the central zones 1 + 2 was decreased in these animals compared to controls of the same age (ANOVA, control: 3.8 ± 0.8 vs. sucrose: 1.7 ± 0.3, $p = 0.002$; Fig. 2D), indicating that these animals are more anxious and less exploring.

Two-way ANOVA showed no differences by treatment or age-treatment interaction in the entries to zone 1 or the distance travelled in there ($F_{Treatment}(1,44) = 0.198$; $p = 0.6577$; $F_{Treatment\text{-}Age}(1,44) = 1.697$; $p = 0.1980$ and $F_{Treatment}(1,44) = 0.360$; $p = 0.5506$; $F_{Treatment\text{-}Age}(1,44) = 0.602$; $p = 0.4409$, respectively), but only by age ($F_{Age}(1,44) = 18.503$; $p < 0.0001$ and $F_{Age}(1,44) = 7.249$; $p = 0.0091$, respectively; Fig. 2C and D).

Rearing behavior was also compromised in animals exposed to sucrose in the early stages of development. Two-way ANOVA showed significance by treatment age and interaction both in the number of times they performed this action ($F_{Treatment}(1,44) = 4.647$; $p = 0.0351$; $F_{Age}(1,44) = 3.240$; $p = 0.0769$; $F_{Treatment\text{-}Age}(1,44) = 7.556$; $p = 0.0079$) and in the time invested in this behavior ($F_{Treatment}(1,44) = 1.389$; $p = 0.2434$; $F_{Age}(1,44) = 3.989$; $p = 0.0640$; $F_{Treatment\text{-}Age}(1,44) = 4.043$; $p = 0.0408$; Fig. 2E). Fisher's LSD post hoc analysis shows that only juveniles exposed to sucrose present a decrease of this behavior compared to their controls ($p = 0.001$) and in the time invested in rearing ($p = 0.034$; Fig. 2E). Altogether these results suggest that individuals exposed to sucrose consumption during their youth, but not during adulthood, explore less in the adult stage and present an anxious profile.

3.3. Fear conditioning

For trace fear conditioning phase (day 1), no difference in conditioned stimulus induced freezing was observed either by sucrose exposition or age (two-way RM ANOVA, $F_{Trial\text{-}Treatment}(4,192) = 0.357$; $p = 0.754$; $F_{Trial\text{-}Age}(4,192) = 1.213$; $p = 0.306$; $F_{Trial\text{-}Treatment\text{-}Age}(4,192) = 0.116$; $p = 0.932$; $F_{Trial}(4,192) = 61.852$; $p < 0.0001$). On day 2, all 4 groups of rats showed conditioned freezing to the tone, which diminished over repeated trials, consistent with acquisition of fear extinction (two-way RM ANOVA, $F_{Trial}(4,192) = 20.700$; $p < 0.0001$; Fig. 3A). Significant differences were also found by age ($F_{Trial\text{-}Age}(4,192) = 3.833$; $p = 0.013$) and trial-treatment-age interaction ($F_{Trial\text{-}Treatment\text{-}Age}(4,192) = 2.787$; $p = 0.039$), but not by treatment ($F_{Trial\text{-}Treatment}(4,192) = 1.978$; $p = 0.124$). Fisher's LSD post hoc analyses revealed that youth-treated sucrose group freeze more than their control group at the trials 4–6 ($p = 0.003$), trials 7–9 ($p < 0.0001$), trial 10–12 ($p = 0.004$) and trials 13–15 ($p < 0.045$; Fig. 3A). In addition, a significant effect of age between controls was also noted at the trials 7–9 and 10–12 (Fisher's LSD post hoc test, $p = 0.0002$ and $p = 0.011$, respectively).

The sucrose effect was also evidenced by calculating the total freezing time for each group. Significant differences were found only by treatment but not by age or by treatment-age interaction (Fig. 3B; two-way ANOVA, $F_{Treatment}(1,48) = 5.157$; $p = 0.0276$; $F_{Age}(1,48) = 0.696$; $p = 0.4081$; $F_{Treatment\text{-}Age}(1,48) = 1.101$; $p = 0.2992$). Animals exposed to sucrose in youth had longer total freezing time compared to their controls (Fisher's LSD post hoc test, $p = 0.024$). No differences were observed between the adult groups (Fisher's LSD post hoc test, $p = 0.388$).

3.4. Calretinin immunoblotting

The GABAergic system goes through extensive remodeling during youth especially in the prefrontal cortex and the hippocampus (Caballero et al., 2013, 2014). Here we studied whether the excessive sucrose consumption exerts long-term alterations on the calretinin (CR) expression, one type of interneurons present in these brain regions (Caballero et al., 2016). The expression of CR in the ventral hippocampus (vHIP; Fig. 4A) and the medial prefrontal cortex (mPFC; Fig. 4B) was analyzed by Western blot. Two-way ANOVA showed significance only by treatment in the mPFC ($F_{Treatment}(1,24) = 5.871$; $p = 0.0227$; $p = 0.0276$; $F_{Age}(1,24) = 2.587$; $p = 0.1198$; $F_{Treatment\text{-}Age}(1,24) = 2.612$; $p = 0.1181$), but not in vHIP ($F_{Treatment}(1,24) = 0.009$; $p = 0.9233$; $p = 0.0276$; $F_{Age}(1,24) = 0.199$; $p = 0.6596$; $F_{Treatment\text{-}Age}(1,24) = 0.211$; $p = 0.6498$). Fisher's LSD post hoc analyzes revealed a significant increase in CR expression in the mPFC of animals that consumed sucrose during youth compared to their age control ($p = 0.011$; Fig. 4B).

4. Discussion

In this study we found that early juvenile-adolescent exposure to 10% sucrose in the beverage produces anxiety-like behaviors in adulthood, while comparable sucrose exposure in adult rats fails to generate such effects. We included an adult group which received the same sucrose and water treatment, showing that lasting effects in the open field and fear conditioning behavior and the calretinin expression are specific to exposure during youth.

In the open field animals that consumed sucrose during youth avoid being exposed to open space evidenced by a decreased number of entries to the central zones and the distance traveled there. They also presented less time and number of rearing events. Together these results suggest that unlimited consumption of sucrose at early stages of development increases anxiety level demonstrated by a reduced exploration of the center of the open field while not vertically exploring the arena indicated by their reduced rearing.

There are some studies reporting that acute withdrawal from diets high in sucrose can induce acute anxiety and depression-like behaviors (Avena et al., 2008; Cottone et al., 2009; Iemolo et al., 2012). More recently, it has also been shown that unlimited consumption of sucrose during adolescence causes a depressive-like phenotype (anhedonia, decreased motivation for a sweet reward and a passive coping strategy) and a higher anxiety-like behavior (increased latency to reach food in a novel environment) in the adulthood (Vendruscolo et al., 2010a, b; Naneix et al., 2016; Gueye et al., 2018), in consistent with our findings. Interestingly, all these effects were counteracted by chronic treatment with the antidepressant imipramine (Gueye et al., 2018).

Rats exposed to sucrose during youth also demonstrated frustration

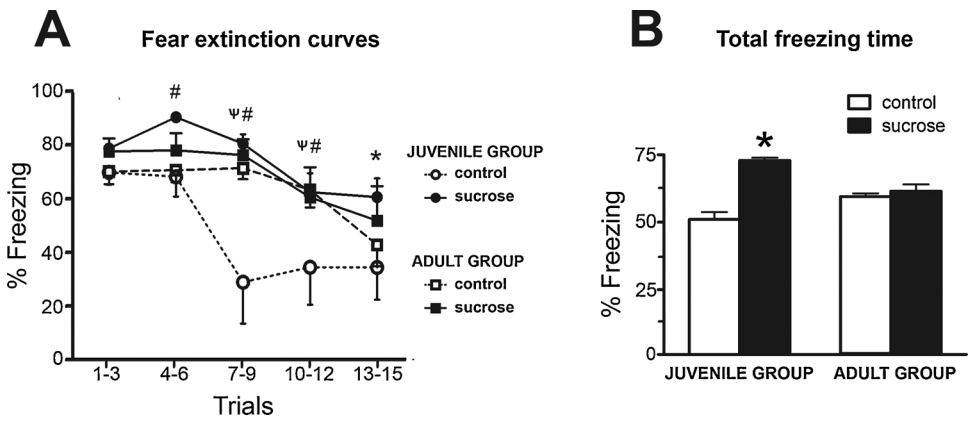

Fig. 3. A. Extinction curves of sucrose-exposed animals and their age-matched controls. Open and filled circles correspond to control and sucrose treated animals from the juvenile group, respectively. Open and filled squares correspond to control and sucrose treated animals from the adult group, respectively. A significant impairment of fear extinction was found in the animals exposed to sucrose during youth relative to the control group (Fisher's LSD post hoc test, #$p < 0.001$ and *$p < 0.05$. Difference by age, ψ$p < 0.05$). B. Total freezing time shows that sucrose-exposed rats during youth present an altered fear behavior. Fisher's LSD post hoc test, *$p < 0.05$. Data is presented as mean ± SEM, n = 13.

as they consumed less water respective to their age control group. Similarly, rats exposed to a sudden downshift in sucrose concentration (e.g. from 32% to 4%) display a reduced consummatory behavior (Williams, 1997; Justel et al., 2012a, b) a situation that can be reversed by anxiolytic compounds administration (Becker and Flaherty, 1982; Justel et al., 2012a, b). This phenomenon was much less dramatic in rats that drank sucrose during adulthood that even increased their long-term water consumption after 20 days of finalized the sucrose period (Fig. 1D) suggesting that pre-exposure to sucrose in the adulthood could ameliorate the reduction in water intake induced by age (Begg et al., 2012).

We extended the long-term emotional effects following early sucrose consumption by examining fear learning. Our data reveals that adult animals exposed to sucrose during the juvenile stage fail to show normal extinction of the freezing behavior. Even when increased freezing may indicate a better aversive memory recall, it has also been used to model anxiety disorders (Likhtik et al., 2005). Recent assessments of the neuronal bases of extinction indicate the involvement of the amygdala, hippocampus and frontal cortex in the contextual retrieval of fear memories after extinction (Maren et al., 2013). The mPFC projections to both excitatory and inhibitory neurons in the amygdala have been implicated in anxiety (Sigurdsson et al., 2010; Adhikari et al., 2011; Gordon, 2011) and fear expression (Sotres-Bayon et al., 2012). On the other hand, the vHIP projections to both the basolateral amygdala (BLA) and mPFC are essential for fear renewal (Orsini et al., 2011; Knapska et al., 2012) suggesting that the network of these structures is involved in contextual regulation of associative fear retrieval.

Importantly, also these brain areas undergo drastic anatomical and functional changes during development, including the GABAergic system (Bosch and Ehrlich, 2015; Caballero et al., 2016). Analysis of GABAergic populations shows that the interneurons expressing the Ca_2^+- binding proteins parvalbumin (PV) or CR varies during development. Specifically, PV expression increases in the mPFC and the vHIP while CR expression decreases only in the mPFC over the childhood transition to adulthood (Caballero et al., 2013, 2014).

The results of this study suggest that at least one of the possible mechanisms underpinning sucrose effects on anxiety could be a deficient GABA system. We observed CR expression is increased in the mPFC of rats exposed to sucrose during youth while not affecting the vHIP. In accordance to this theory Reichelt et al. (2015) found that PV expression decreases in the mPFC and hippocampus of adult rats that were exposed to intermittent 10% sucrose consumption during their adolescence. Interestingly, all these results show an opposite developmental trajectory of the PV and CR interneurons in rats with a history of sucrose consumption during youth suggesting that sucrose interferes with the normal development of the GABAergic system keeping it to an immature state. Consistent with these findings, other studies have reported correlations between an altered GABAergic system and impairments in fear conditioning (Mao et al., 2009) and attentional set

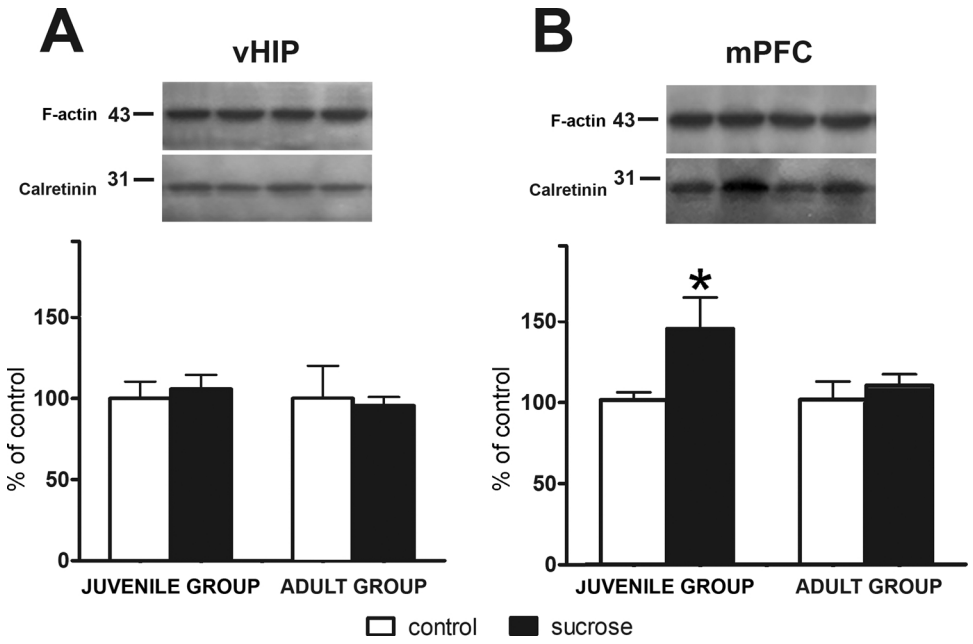

Fig. 4. Western blot of CR in the vHIP (A) and mPFC (B) of control (white bars) and sucrose (black bars) exposed animals during youth or adulthood, respectively. Data was quantified by densitometric analysis and corrected with reference to the F-actin loading control. Representative pictures of CR expression and the F-actin loading control are shown in the upper panel. Data is presented as mean ± SEM, n = 7. Fisher's LSD post hoc test,*$p < 0.05$.

formation (Canetta et al., 2016).

The results of this study show that rats under unlimited access to sugary beverages during youth present lasting behavioral detriments including increases anxiety-like behavior, impaired fear extinction and frustration in the adulthood. These deleterious effects on emotional behavior are accompanied by increased CR neurons in the mPFC, which together with previous reports, indicate that an impaired GABAergic system is at least one of the mechanisms by which high-sucrose diets alter emotional behavior. Our data provides evidence that the chronic sucrose intake in children and adolescents is prejudicial to normal mental development and could favor the onset of mental illness in adulthood, especially those related to anxiety and depression. Considering that soft drinks consumption has increased dramatically among children and adolescents and that sugary beverages are normalized in our day-by day life, strong nutritional education messages encouraging limited consumption of these drinks should be addressed to children and/or their parents and educators.

5. Conclusions

Free access to sucrose in drinking water during youth is detrimental to mental health and produces lasting effects observable in the adulthood. Specifically, rats expose to 10% sucrose during youth show frustration when the sugary drink is removed. They also present an anxiety-profile and an increase in fear memory in the adulthood. All these disturbances are probably related to at least a poor maturation of the GABAergic system in the vHIP and the mPFC.

Author disclosures

Funding for this study was provided by CONICET (National Research Council of Argentina) Grant PIP-0243 and partially supported by Fundación Rene Barón. The funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

All authors declare that they have no conflicts of interest.

Acknowledgements

The authors thank Dr. Sergio Lew for his help with the fear conditioning studies and Dr. Tuire Karaharju-Huisman for fruitful comments on the manuscript.

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