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# INTRAUTERINE GROWTH RESTRICTION MODIFIES THE HEDONIC RESPONSE TO SWEET TASTE IN NEWBORN PUPS – ROLE OF THE ACCUMBAL μ-OPIOID RECEPTORS

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**Abstract—Intrauterine growth restriction (IUGR) is associated with increased preference for palatable foods. The hedonic response to sweet taste, modulated by the nucleus accumbens μ-opioid-receptors, may be involved. We investigated hedonic responses and receptor levels in IUGR and Control animals. From pregnancy day 10, Sprague–Dawley dams received either an *ad libitum* (Control), or a 50% food restricted (FR) diet. At birth, pups were cross-fostered, and nursed by Adlib fed dams. The hedonic response was evaluated at 1 day after birth and at 90 days of life, by giving sucrose solution or water and analyzing the hedonic facial responses (within 60 s). Control pups exposed either to water or sucrose resolved their hedonic responses after 16 and 18 s, respectively, while FR hedonic responses to sucrose persisted over 20 s. FR pups had deceased phospho-μ-opioid-receptor ($p = 0.009$) and reduced phosphor:total mu opioid receptor ratio compared to controls pups ($p = 0.003$). In adults, there was an interaction between group and solution at the end of the evaluation ($p = 0.044$): Control decreased the response after sucrose solution, FR did not change over time. There were no differences in phosphorylation of μ-opioid-receptor in adults. These results demonstrate IUGR newborn rats exhibit alterations in hedonic response accompanied by a decrease in μ-opioid-receptor phosphorylation, though these alterations do not persist at 3 months of age. Opioid system alterations in early life may contribute to the development of preference for highly palatable foods and contribute to rapid weight gain and obesity in IUGR offspring.** 

**Key words: intrauterine growth restriction, hedonic response, μ-opioid receptor, palatable foods, opioid system.**

## INTRODUCTION

The Developmental Origins of Adult Disease concept proposes that environmental factors act early in life in order to program the risk of chronic diseases in adulthood (Hales and Barker, 1992). Epidemiological studies have provided evidence for the association between disturbances of the fetal nutritional environment (with consequent low birth weight) and increased prevalence of cardiovascular disease (Barker et al., 2002), hypertension (Barker et al., 1989) and type 2 diabetes (Eriksson et al., 2002), among many other adult chronic non-communicable diseases. Most of such conditions have increased adiposity as a common factor in their trajectory, and indeed being born small for a given gestational age (intrauterine growth restricted or IUGR) alters adult body composition (Ravelli et al., 1976, 1999; Bettiol et al., 2007; Pilgaard et al., 2011).

Simply put, obesity results in cases where the energy intake exceeds the expenditure. Therefore, the chronic consumption of calorie-enriched foods (high fat, high sugar, highly palatable) often precedes the development of overweight and its metabolic consequences (Sampey et al., 2011; Desai et al., 2014). Interestingly, human studies from our group and others show that IUGR is associated with a natural preference for palatable foods over the life course (Lussana et al., 2008; Barbieri et al., 2009; Stein et al., 2009; Perala et al., 2012; Crume et al., 2013; Migraine et al., 2013), and IUGR girls at 3 years of age are more impulsive when facing a sweet reward (Silveira et al., 2012). We also demonstrated that the hedonic response (taste reactivity) to a sweet solution delivered into the newborns' mouth in their first day of life varies according to their degree of IUGR (Ayres et al., 2012).

Taste reactivity to different solutions is an automatic behavior that demonstrates homology between humans and animals (Berridge, 2000; Steiner et al., 2001) and therefore shares underlying brain circuits. Hedonic feeding typically involves the consumption of highly palatable foods and has been behaviorally defined in two processes: "wanting" and "liking" (Berridge, 1996; Berridge and Robinson, 1998). "Wanting" is ultimately related to

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*Abbreviations:* ANOVA, analysis of variance; FR, food restricted; GEE, generalized estimating equations; IUGR, intrauterine growth restriction; NAcc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area.

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the motivation to obtain the food reward, mostly dependent on mesolimbic dopamine circuits (Berridge et al., 2009). The "liking" component is the neural substrate of the elicited pleasure per se, and has the accumbal opioid system as its main neurobiological basis (Berridge, 2009).

Opioids are a family of peptides (β-endorphin, enkephalin, and dynorphins), each of which is cleaved from three prepropeptides (proopiomelanocortin, proenkephalin and prodynorphin, respectively), that differentially interact with three receptor classes (μ, δ, κ, in addition to the novel ORL 1 receptor) (Akil et al., 1984; Mollereau et al., 1994; Waldhoer et al., 2004). Studies show that opioid antagonists decrease, while agonists increase feeding in rats (Holtzman, 1974; Grandison and Guidotti, 1977; Glass et al., 1999). Sugar intake may lead to an increased number of and/or affinity for opioid receptors, which in turn leads to further ingestion of sugar and may contribute to obesity (Fullerton et al., 1985). The nucleus accumbens (NAcc) is an important site for opioid regulation of palatability (Pecina et al., 2006; Castro and Berridge, 2014). Opioid neurotransmission in this structure contributes to reward motivation (Pecina and Berridge, 2005) and is able to amplify the hedonic impact of sensory pleasure (Richardson et al., 2005).

Considering that IUGR influences the hedonic response to palatable flavors in newborn humans (Ayres et al., 2012; Rotstein et al., 2015), we aimed to (1) determine if similar findings are seen in a rodent model of IUGR; (2) investigate if these behavioral differences are stable over the life-course and (3) study the putative role of accumbal μ-opioid receptor in this behavior. Our hypothesis was that IUGR would affect the hedonic response to the sweet flavor in a persistent fashion over the life-course, and this would map onto levels of accumbal μ-opioid receptor phosphorylation.

## EXPERIMENTAL PROCEDURES

### Animals

Primiparous Sprague–Dawley rats (CEMIB Laboratory Animal Reference Center, Campinas, SP, Brazil), at approximately 80 days old, were time-mated at our animal facility by vaginal smear visualization. During pregnancy they were single-housed in Plexiglas home cages (49 × 34 × 16 cm) and maintained in a controlled environment: altered dark/light cycle (lights on between 09:00 h and 19:00 h, due to maternal behavior observations in the dark phase of the cycle), temperature of 22 ± 2 °C, cage cleaning once a week, and food and water provided *ad libitum* (*Adlib*). At 10 days of gestation, dams were randomly allocated to two groups: control (*Adlib*; $n = 21$), receiving an *ad libitum* diet of standard laboratory chow (Nuvilab®) and 50% food restricted (FR; $n = 14$), receiving 50% of the *ad libitum* fed dam's intake (determined by quantification of normal intake in a cohort of pregnant Sprague–Dawley rats) (Desai et al., 2005). These diets were provided from day 10 of pregnancy to birth. Within 24 h after birth, pups were weighed and limited to eight per litter (four males and four females) and cross-fostered to other dams, forming the following groups (examining the biological/adoptive mother, i.e., gestation/lactation maternal diet: Adlib_Adlib, FR_Adlib). The excess pups were used in the hedonic test at 1 day of life ($n = 90$ pups), and were immediately decapitated thereafter (see below). Eight pups/litter were left undisturbed until adulthood and a portion of them were submitted to hedonic test in adulthood ($n = 67$; 16–18 animals per group and sex). Remaining animals not used for the adult hedonic test were used in other projects. On postnatal day 21, pups were weaned, separated by sex, and allocated four to a cage. All animals were fed standard lab chow and water *ad libitum* and were kept in a controlled environment similar to that described above, except for the light cycle (lights on between 07:00 h and 19:00 h). From day 21 onward, at the time of cage cleaning once a week, body weight was measured using a digital scale with 0.01 g precision (Marte®, Canoas, Brazil).

All animal procedures were approved by the Research Ethics Committee of Hospital de Clínicas de Porto Alegre (GPPG/HCPA, projects numbers 11-0053, 12-0353). Tasks were performed in climate-controlled behavioral rooms within our animal research facility (Unidade de Experimentação Animal/HCPA).

### Taste reactivity test

Pups, at 1 day of life, were randomly divided and tested for hedonic response by receiving either a droplet (10 μl) of distilled water or sucrose solution (0.3 M). After dripping the droplet on pup's mouth, using a 100-μl micropipette, facial responses were filmed for 90 s. Films were watched frame by frame (30 frames/s) using the KMPlayer® software and scored by an observer blinded to the study allocation. According to Ganchrow et al. (1986), tongue protrusions (licking) and rhythmic mouth movements were the most salient features elicited by the sweet stimuli. Therefore the frequency of these positive facial responses (here called hedonic responses) (Berridge, 2000) were analyzed during the first 60 s, as proposed by Berridge (2000). After being filmed for 90 s, rat pups from the two test conditions (water or sucrose) were immediately decapitated, as brain responses are seen a few minutes after the intake of sucrose (Hajnal et al., 2009; McCutcheon et al., 2012).

In adults, at 90 days of life, the experiment occurred in the same way, except for the amount of solution delivered (200 μL) and the sucrose solution concentration (1 M) (Berridge, 2000; Silveira et al., 2010). The test occurred over 2 days, whereby the same animal received both water (day 1) and sucrose (day 2). Similarly, the animals were decapitated immediately after the last test. Therefore, neurochemical findings in adulthood were observed only in the "sucrose condition".

Hedonic taste reactivity response patterns were scored using time bin scoring procedures developed to assess hedonic vs. aversive taste valuations (Berridge and Grill, 1984; Berridge, 2000); this is an objective evaluation done by counting the number of frames in which tongue protrusions are exhibited by the animal after the sucrose stimulus. A time bin scoring procedure was used to ensure that taste reactivity components of different relative frequencies were balanced in their contributions