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Obese Rats with Deficient Leptin Signaling Exhibit Heightened Sensitivity to Olfactory Food Cues

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Abstract

The Zucker rat is used as a model of genetic obesity, and while Zucker rats have been well studied for their reduced sensitivity to leptin signaling and subsequent weight gain, little work has examined their responses to environmental signals that are associated with “hedonic” feeding. This study evaluated the effects of a high-fat food olfactory cue (bacon) in stimulating nose-poke food-seeking behavior upon first exposure (novel) and after a period of access for consumption (familiar) in lean and obese Zucker rats at either 4 or 12 months of age, and under ad-lib fed (unrestricted; U) or chronically food-restricted (70% of ad-lib; R) conditions.

Baseline nose-poke levels were comparable amongst all groups. At 4 months of age, only ObU rats displayed increased behavioral activation to familiar food cues. Twelve month-old Ob rats, regardless of diet, exhibited substantially greater food-seeking behavior when exposed to both the novel and familiar olfactory cues. A strong positive correlation between body weight and nose-poke entries for the familiar food cue was observed at both ages, while this correlation for the novel food cue was significant in 12 month old rats only. Similarly, there were strong positive correlations between food intake and poke entries for the familiar food cue was observed at both ages, while this correlation for the novel food cue was significant in 12 month old rats only. Although it is possible that differences in olfactory sensitivity contribute to these behavioral effects, our findings support the interactions between food intake, obesity, and food-seeking behavior, and are consistent with leptin inhibiting the brain’s reactivity to food cues and suggest that the enhanced sensitivity to the food cues with leptin deficiency is likely to contribute to overeating and weight gain.

Keywords

novelty-seeking; food restriction; olfactory stimulus; leptin; obesity

1. Introduction

Eating is regulated by the complex interaction of biological and environmental factors that modulate nutrient and caloric requirements, as well as food’s reinforcing properties, including its ability to serve as conditioned rewarding stimuli (Berthoud 2004). Two systems for regulating feeding behavior have been theorized, the homeostatic and the hedonic

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pathways, which have complementary functions. The homeostatic pathway regulates energy balance by prompting food consumption in times when energy stores are low, while food intake is controlled via the inhibition of feeding with satiety (Blundell and Gillett 2001). The hedonic pathway is capable of overriding the homeostatic pathway, even in times when energy stores are sufficient, and increases the eating of highly palatable food (Lutter and Nestler 2009). Therefore, unrestrained eating and obesity could be consequences of decreased neuronal sensitivity to satiety signals (Powley 2000) and/or increased sensitivity to reward signals (Rothmund, Preuschhof et al. 2007; Stoeckel, Weller et al. 2008).

Previous studies have shown neurobiological differences between obese and lean subjects that may link an individual's responsiveness to environmental cues associated with food and the development of obesity. Obese patients have been shown to exhibit increased resting activity of oral (mouth, lips, and tongue) somatosensory processing regions of the brain, suggesting that overeating may result from increased sensitivity to food palatability and its rewarding effects (Wang, Volkow et al. 2002). Obese and obesity-prone humans and rodents have reduced levels of D2R in the striatum, as well as blunted striatal responses to food cues, which modulate the incentive properties of food (Wang, Volkow et al. 2001; Fetissov, Meguid et al. 2002; Primeaux, Blackmon et al. 2007; Stice, Spoor et al. 2008; Thanos, Michaelides et al. 2008; Volkow, Wang et al. 2008; Davis, Michaelides et al. 2009). Obese subjects have also displayed increased brain reward circuit activation in response to food cues (Rothmund, Preuschhof et al. 2007; Stoeckel, Weller et al. 2008), while another imaging study showed decreased sensitivity to the rewarding effects of food, but enhanced motivational responses (Stice, Spoor et al. 2008). Finally, obese humans report that some food odors have a higher hedonic value than their lean counterparts (Trellakis, Tagay et al. 2011). These differences in response to food and food cues between lean and obese subjects may help explain in part, the differences in susceptibility to weight gain observed in the general population.

Little is known about how differences in hormonal responsiveness may lead to differences in the incentive value of food cues. This could represent a major point of interaction between the proposed homeostatic and hedonic regulatory systems. Leptin, an anorexigenic protein that moderates food intake and energy balance is believed to participate in both homeostatic and hedonic food regulatory mechanisms (Bates and Myers 2003; Hommel, Trinko et al. 2006; Davis, Choi et al. 2011). Leptin signaling in the hypothalamus inhibits overconsumption of food (homeostatic), while leptin signaling in the midbrain decreases the hedonic properties of food (Davis, Choi et al. 2011). Leptin also reduces brain activation in other regions associated with hunger (insula, parietal, and temporal cortex) and increases activation in regions associated with inhibition and satiety (prefrontal cortex) (Baicy, London et al. 2007). Additionally, reduced leptin activity is associated with enhanced behavioral and neural reactivity to food cues, which is reversed by leptin administration (Baicy, London et al. 2007; Farooqi, Bullmore et al. 2007; Rosenbaum, Sy et al. 2008). Moreover, leptin was shown to be associated with the hedonic value of olfactory food cues, (Trellakis, Tagay et al. 2011).

The Ob Zucker rat (fa/fa) has a mutation in the leptin receptor gene that prevents the long form of the receptor (Ob-Rb) from being expressed (Chua, Chung et al. 1996). This form of the receptor is localized in the brain (hypothalamus, thalamus, cortex, midbrain, and hippocampus) (Mercer, Hoggard et al. 1996; Hommel, Trinko et al. 2006) and in peripheral organs (pancreas, liver, kidney, spleen, and heart) (Emilsson, Liu et al. 1997). Zucker rats exhibit decreased leptin signaling that results in hyperphagia, decreased energy expenditure, and severe obesity by adolescence (Iida, Murakami et al. 1996). Previously, we found that Zucker Ob rats displayed altered brain metabolic responses to food olfactory stimuli (bacon

scent) in several brain regions including the hippocampus, frontal cortex, superior colliculus, and thalamus (Thanos, Michaelides et al. 2008).

Since differences in brain metabolic responses to olfactory food cues were observed between lean (Le) and Ob rats, we sought to determine whether Le and Ob rats also exhibit differences in behavioral responses to the same food cue (bacon scent), as well as whether these responses are dependent on dietary restriction (ad-lib fed versus food-restricted) and familiarity with the food cue (novel versus familiar). In the present study, we examined nose-poke food seeking behavior at baseline and in response to a high-fat food olfactory stimulus (bacon scent) during first time exposure (novel) and after repeated exposure and consumption (familiar). Lastly, to determine whether patterns of behavior are age-dependent, both 4 and 12 month old subjects were tested. We chose these ages to test behavioral responses across development, and because it has been shown that D2R binding and availability in the striatum are differentially affected by genetic obesity and diet at these ages (Michaelides, Piyis et al. 2006).

2. Materials and Methods

2.1 Animals

Male Obese (Ob) (fa/fa; n=26) and Lean (Le) (Fa/?; n=30) Zucker rats (Harlan, Indianapolis, IN) were single-housed under controlled conditions and maintained on a 12h reverse light cycle (0800h lights off). Rats were divided into 4 groups: i) Ob rats with ad-libitum (unrestricted; U) food access, ii) Ob rats with restricted (R) food access, iii) LeU rats, and iv) LeR rats. The rats placed on restricted food access were given a daily amount of food limited to 70% of that consumed by similarly aged ad-libitum fed animals of the same strain. Rats were fed a standard (Purina) laboratory rat chow, and food intake and body weight were monitored. Behavioral tests were performed at 4 and 12 months of age, with different rats used at each age to prevent carryover effects. All experiments were approved by and conducted in conformity with the Brookhaven National Laboratory Institutional Animal Care and Use Committee (IACUC) protocols.

2.2 Baseline activity and novel and conditioned responses to a food olfactory stimulus

An open-field arena fitted with a photobeam activity monitoring system (TruScan, Coulbourn Instruments, Allentown, PA) (dimensions 40.64 cm × 40.64 cm × 40.64 cm) and equipped with a nose-poke floor (16 holes, 4 × 4 array) was used to detect baseline nose-poke activity, as well as this behavior in response to a novel and conditioned food olfactory stimulus (bacon) (Figure 1). Animals were tested for a total of three days in the nose-poke arena, with each session lasting 30 minutes and occurring between 1200h and 1600h. This olfactory stimulus and paradigm were chosen to compare behavioral results to microPET results obtained from a previous imaging study (Thanos, Michaelides et al. 2008). Rats were habituated to the nose-poke arena for a 30 minute period on the day prior to the first experimental session. Rats were then tested for baseline (B; day 1) activity, as well as during novel (N; day 2) and familiar (F; day 8) stimulation sessions. During the N session, a novel olfactory food stimulus (5g piece of cooked bacon wrapped in a 5cm × 5cm cotton piece of gauze) was placed under one nose-poke hole per arena, inaccessible to the rat behind a stainless steel grid. Placement was randomized for each animal. The conditioning period occurred on days 3 through 6, during which 5g of cooked bacon were placed in each rat's home cage, where it was consumed between 1200h and 1300h each day. Rats were not put in the nose-poke arena on these days. On day 7, rats were not presented with bacon, nor were they put in the arena. The food stimulation (familiar) session occurred on day 8, during which animals were put in the arena, with bacon placed inaccessibly in the same hole as in the N session (day 2). During the B, N, and F sessions, nose-poke entries were recorded.

2.3 Statistical Analysis

A two-way ANOVA was used to determine the effects of strain (Ob versus Le) and diet (U versus R) on body weight, and a Kruskal-Wallis one-way ANOVA on Ranks was used to determine the effects of strain on food intake in ad-lib fed rats (due to determined non-normality and/or unequal variance), both at 4 and 12 months of age. Three-way repeated measures ANOVAs were used to determine the effects of strain, diet, and session (B versus N versus F) on nose-poke entries at each age. ANOVAs were followed by pairwise multiple comparisons using the Holm-Sidak method ($\alpha=0.05$ used to determine significance), except for the Kruskal-Wallis one-way ANOVA on Ranks used to analyze food intake, which was followed by Dunn's method (significance set at $p<0.05$). Linear regression analyses were used to assess relationships between food intake and nose-poke activity, as well as between body weight and nose-poke activity, at both ages. All statistical analyses were performed using SigmaStat v. 3.5 software.

3. Results

3.1 Body weight and food intake

At both 4 and 12 months of age, a two-way ANOVA revealed significant main effects of strain [4 months: $F(1,16)=265.508$; 12 months: $F(1,32)=167.790$, $p<0.001$ for both] and diet [4 months: $F(1,16)=120.170$; 12 months: $F(1,32)=87.508$; $p<0.001$ for both] on body weight (Figure 2). The interaction of strain $\times$ diet was significant for 4 month old [$F(1,16)=14.487$, $p<0.01$], but not 12 month old [$F(1,32)=0.107$, $p=0.746$], rats. Pairwise comparisons revealed that at both ages, Ob rats weighed more than Le rats on both diets ($p<0.001$ for all), and food restriction decreased body weight in both strains ($p<0.001$ for all). A Kruskal-Wallis one-way ANOVA on Ranks showed a significant main effect of strain on ad-lib food intake at 4 [$H=6.818$, $p<0.01$] and 12 [$H=6.518$, $p<0.05$] months of age. Pairwise comparisons revealed that Ob rats consumed more food than Le rats at both ages ($p<0.05$ for both). Mean $\pm$ SEM for daily food intake (g): Ob 4 months (42.5 ± 2.3), Ob 12 months (34.4 ± 2.1), Le 4 months (21.5 ± 0.8), Le 12 months (29.6 ± 0.7).

3.2 Nose-poke Activity

Nose-poke activity was measured to determine the effects of strain, diet, and session [baseline (B), novel food olfactory stimulus (N) and familiar food olfactory stimulus (F); Figure 3]. A three-way repeated measures ANOVA at 4 months of age revealed that session had a significant effect on nose-poke entries [$F(2,32)=5.697$, $p<0.01$], such that rats made more nose-poke entries in the familiar session compared to the B ($p<0.01$) and N ($p<0.05$) sessions. The strain $\times$ diet interaction was also significant [$F(1,32)=4.762$, $p<0.05$]; however, no pairwise comparisons were significant. All other effects were not significant ($p>0.05$).

Since the main effects seen in 4 month old rats were being driven by increased activity of the ObU (ad-lib fed) rats in the F session, a one-way repeated measures ANOVA examining the effect of session within the ObU rats was performed. This ANOVA found that session did in fact have a significant effect on nose poke entries [$F(2,8)=7.304$, $p<0.05$], such that ObU rats displayed more nose-pokes during the F session compared to the B and N sessions ($p<0.05$ for both). Subsequently, a two-way ANOVA found that within the F session, there was a strain $\times$ diet interaction [$F(1,16)=10.368$, $p<0.01$], such that Ob U rats exhibited a greater number of nose-poke entries compared to all other groups ($p<0.01$ for all).

A three-way repeated measures ANOVA performed for rats at 12 months of age found a significant main effect of strain on nose-poke entries [$F(1,64)=32.236$, $p<0.001$], with Ob rats performing a greater number of nose-pokes compared to Le rats overall ($p<0.001$). The

main effect of session was significant [$F(2,64)=6.271$, $p<0.01$], with rats being more active in the F session compared to B ($p<0.01$). The session $\times$ strain interaction was also significant [$F(2,64)=5.485$, $p<0.01$], such that Ob rats nose-poked more than Le rats in the N ($p<0.05$) and F sessions ($p<0.001$). Ob rats also exhibited increased nose-poke behavior in the F compared to the B session ($p<0.001$). No other parameters were found to be significant ($p>0.05$).

3.3 Correlations between body weight and nose-poke activity

Relationships between food intake and nose-poke entries (Figure 4) and body weight and nose-poke entries (Figure 5) in all groups were assessed during each session at 4 and 12 months of age using linear regression models. There was a significant positive relationship between body weight and nose-poke entries during the N session at 12 months of age ($R=0.58$, $p<0.05$) and in the F session at both ages (4 months: $R=0.88$, $p<0.001$; 12 months: $R=0.66$, $p<0.01$). Similarly, there was a significant positive relationship between body weight and nose-poke entries during the N session at 12 months of age ($R=0.56$, $p<0.001$) and in the F session at both ages (4 months: $R=0.65$, $p<0.01$; 12 months: $R=0.66$, $p<0.001$).

4. Discussion

Here, we show that Ob rats (regardless of dietary condition), when compared with Le rats, displayed an enhanced behavioral response to both novel and familiar food cues at 12 months of age, and these responses were positively correlated with their food intake and body weight. At 4 months, only Ob rats on an unrestricted diet exhibited increased seeking behavior in response to a food cue, and only when the food cue was familiar. At this younger age, there was also a positive correlation between seeking behavior in response to a familiar food cue and both food intake and body weight. This suggests that there is a developmental shift in the expanded reactivity to food cues from familiar to novel in the transition from young adulthood into mature adulthood, and that food restriction no longer suppresses high-fat food seeking behavior in Ob rats in mature adulthood as it did in young adulthood. The positive correlations between both food intake and body weight with nose-poke entries when rats were exposed to the familiar olfactory stimulus (4 and 12 months) provides evidence of the interactions between sensitivity to food cues, amount of food consumed, and subsequent weight gain.

Ob rats displayed greater (+144%) seeking behavior compared to Le rats when exposed to the novel food olfactory cue, and there was a strong positive correlation of both food intake and body weight with nose-poke entries, at 12 months of age (but not at 4 months). Previous studies have reported that novelty-seeking is associated with increased self-administration and responsivity to palatable foods in rodents (Dellu, Piazza et al. 1996; Klebaur, Bevins et al. 2001; Alsiö, Pickering et al. 2009), and binge-eating and obesity in humans (Fassino, Leombruni et al. 2002; Hwang, Lyoo et al. 2006; Grucza, Przybeck et al. 2007; Sullivan, Cloninger et al. 2007; Davis, Levitan et al. 2008). To our knowledge, our data is the first evidence to suggest increased novelty-seeking behavior in a rodent model of leptin deficient signaling. Impaired leptin signaling is likely to underlie the behavior exhibited by Ob rats since leptin decreases exploratory behavior (Buyse, Bado et al. 2001), and leptin-resistant rats were previously shown to exhibit increased novelty-seeking in a hole board task (Fraga-Marques, Moura et al. 2009). The lack of an effect of the novel cue in the 4 month old Ob rats could reflect differences in the role of leptin at these two developmental stages and/or an interaction between impaired leptin signaling and weight (HorlickK, Rosenbaum et al. 2000), which was lower in the 4 than the 12 month old rats. It should be noted that levels of nose-poke activity during the novel session did not apparently change for obese rats on either diet between 4 and 12 months of age, and that this difference between obese and lean rats at 12 months may be due to neophobia or decreased preference for novelty expressed by

the lean rats that is not observed in the obese strain at this age. This argument is also supported by the fact that although there was an increase in nose-poke activity between the baseline and novel sessions for the obese rats at this age, this was not statistically significant.

At 12 months of age, both Ob groups (unrestricted and restricted diet) of rats exhibited similarly increased nose-poke entries (+173%) compared to Le rats in the familiar session, and Ob rats displayed a significant increase (+90%) in nose-poke behavior in the familiar session compared to baseline. These results demonstrate that olfactory cues for a high-fat food elicit heightened behavioral responses in the Ob compared to Le rats. During the familiar stimulation session at 4 months of age, ObU rats (but not ObR) exhibited more nose-poke entries compared to the baseline (+48%) and novel sessions (+65%), and also displayed greater nose-poke behavior than any other group during this session. This suggests that there is an interaction between leptin dysfunction and diet, such that food restriction may protect Ob rats from some forms of hyperresponsivity to food cues at this age. One possible explanation is that food restriction blocks the obesity-related deficit in striatal D2R at 4 months of age (Thanos, Michaelides et al. 2008), whereas such an effect is not observed at 12 months of age (unpublished data). Striatal D2 receptors modulate motivational operant responding and their transient overexpression results in attenuated lever pressing for food reward (Drew, Simpson et al. 2007). Also supporting the link between diet, obesity and sensitivity to food cues, we found a positive correlation of both food intake and body weight with nose-poke entries when rats were exposed to the familiar olfactory stimulus, which was highly significant at both ages.

The lack of an effect in lean rats across sessions within age cohorts should be noted, suggesting no observed behavioral responses to food olfactory cues. Although one may hypothesize that this high fat food would be rewarding and stimulate seeking behavior in all rats, one of our recent studies found that in a group of another non-obese strain of rat (Sprague Dawley), bacon did not produce a significant conditioned place preference (unpublished data).

The hole board task, when not baited with a stimulus, is generally utilized as a measure of exploratory/novelty-seeking behavior (File 2001; Abreu-Villaça, Queiroz-Gomes et al. 2006; Fraga-Marques, Moura et al. 2009), and thus the lack of differences between groups in baseline nose-poke entries (4 and 12 months) suggests that the enhanced response of the Ob rats in the novel (12 month old) and familiar (12 month old, and 4 month old ObU) sessions reflect a specific enhancement of the saliency of the food cue. Therefore, these results provide evidence that olfactory cues for a high-fat food stimulus elicit heightened behavioral responses in the Ob compared to Le rats, and that leptin is likely to mediate these responses. These findings are in agreement with preclinical and clinical studies that have found links between leptin and sensitivity to food-reward (Saper, Chou et al. 2002; Figlewicz, Evans et al. 2003; Figlewicz, Bennett et al. 2004; Baicy, London et al. 2007; Rothmund, Preuschhof et al. 2007; Stoeckel, Weller et al. 2008; Davis, Choi et al. 2011; Trellakis, Tagay et al. 2011).

We previously reported that exposure to the same olfactory cue (bacon) under a similar conditioning regimen resulted in greater hippocampal deactivation in Ob than Le rats; greater medial thalamic activation in Ob than Le rats; superior colliculus activation in Ob and deactivation in Le rats; and frontal cortex deactivation in Ob rats and activation in Le rats (Thanos, Michaelides et al. 2008). We hypothesized that the distinct patterns of response to the food cue was likely to reflect differences in conditioning and sensitivity to the cues between Ob and Le rats. Here, we document that indeed there are differences in sensitivity to food cues between Ob and Le rats. Though we cannot infer causality between the neurobiological substrates that differentiated Ob and Le rats, it is likely that they might

underlie some of the observed differences in behavioral responses to food cues. Indeed, the hippocampus is involved in behaviors linked with memories of food, and seeking and consuming food (Davidson, Kanoski et al. 2005); the medial thalamus is involved in conditioned learning and memory for reward value (Gaffan and Parker 2000; Li, Inoue et al. 2004; Mitchell and Dalrymple-Alford 2005), and the frontal cortex bridges the functions of the hippocampus and medial thalamus with that of the superior colliculus, which is involved in the execution of goal-directed locomotor choices elicited by olfactory (and other sensory) cues (Felsen and Mainen 2008).

There are a few other factors that must be explored and discussed when interpreting the findings of this study. Leptin is synthesized, and leptin receptors are expressed, in the olfactory mucosa of rats, and the transcription of both is increased during periods of fasting (Baly, et al., 2006). Subsequently, leptin signaling modulates olfactory sensitivity (Julliard, et al., 2007) and olfactory-mediated behavior (Getchell, Kwong et al. 2006); therefore, the lack of leptin receptor function in the Ob rats would be expected to result in an enhanced activation of olfactory cells by the olfactory stimuli. Since odor threshold detection studies were not performed, differences in olfactory sensitivity between the obese and lean rats may have influenced the behaviors measured, and cannot be distinguished from differences in motivation to seek out the high fat food. While clinical studies suggest that obesity is associated with deficits in olfaction possibly attributable to metabolic disturbances (Obrebowski, Obrebowska-Karsznia et al. 2000; Richardson, Vander Woude et al. 2004), obese, leptin signaling deficient ob/ob and db/db mice perform a food-finding task ten times more quickly than wildtypes, which could be due to increased olfaction and/or increased motivational responses (Getchell, Kwong et al. 2006). Follow-up studies are necessary to tease out the role of each of these potential contributing factors.

Zucker obese rats have been shown to have impairments in long term potentiation in the hippocampus, hippocampal-dependent learning, and spatial memory (Li, Aou et al. 2002; Gerges, Aleisa et al. 2003; Winocur, Greenwood et al. 2005). Our findings that obese rats exhibit heightened behavioral responses to conditioned food cues suggest that at least some types of memory/learning are indeed intact in this strain. Because behavioral tests were performed in the test arena and bacon exposure (conditioning) occurred in the home cage, it is possible that differences in test performance may reflect differences between groups in their sensitivity of conditioned food cues to context change rather than conditioning mechanisms. We chose this particular protocol to mimic that from our previous imaging study (Thanos, Michaelides et al. 2008) for comparative purposes, but it would be interesting to explore other types of conditioned responses to cues for a high fat olfactory food (e.g. Pavlovian or other associative learning protocols).

In summary, these findings provide evidence that deficits in leptin signaling are associated with an enhanced sensitivity to food cues that is modulated in part by age, food availability, and familiarity with the cues, and that these responses are correlated with food intake and body weight. Thus, the resistance to leptin seen with clinical obesity could increase overeating in part by modulating one's sensitivity to food cues.

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Figure 1.

Timeline of experimental procedure. Baseline (B; day 1): rats placed in nose-poke arena to record baseline nose-poke activity. Novel food stimulation (N; day 2): rats exposed to olfactory stimulus (bacon) in nose-poke arena, nose-poke behavior measured. Conditioning (days 3–6): rats received bacon in their home cage, available for consumption. Familiar food stimulation (F; day 8): rats exposed to conditioned olfactory stimulus (bacon) in nose-poke arena, nose-poke behavior measured.

Figure 2.

Mean (+SEM) body weight of rats at 4 and 12 months of age. At both ages, Ob rats weighed more than Le rats (* $p < 0.001$), and food restriction decreased body weight of rats of the same strain (# $p < 0.001$).

Figure 3.

Mean (+SEM) nose-poke entries at 4 and 12 months of age at baseline (B) and when rats were exposed to a novel (N) and familiar (F) food olfactory stimulus. At 4 months of age, ObU rats nose-poked more than any other group in the familiar session, and ObU rats were the only group to exhibit a significant increase in nose-poke behavior from the B and N sessions to the familiar session. At 12 months of age, Ob rats performed more nose-pokes than Le rats in the N and F sessions, and only Ob rats showed an increase in seeking behavior between the B and F sessions. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 4.

Linear regression analyses performed between food intake and nose-poke entries. Analyses were performed separately for each session (baseline, novel, and familiar) at each age (4 and 12 months old). At 4 months of age, there was a significant positive correlation between these measures in the F session ($p<0.001$). At 12 months of age, there were significant positive correlations between body weight and nose-poke entries in the N ($p<0.05$) and F ($p<0.01$) sessions.

Figure 5.

Linear regression analyses performed between body weight and nose-poke entries. Analyses were performed separately for each session (baseline, novel, and familiar) at each age (4 and 12 months old). At 4 months of age, there was a significant positive correlation between these measures in the F session ($p<0.01$). At 12 months of age, there were significant positive correlations between body weight and nose-poke entries in the N and F session ($p<0.001$ for both).