INTRODUCTION

Binge Eating (BE) episodes are characterized by uncontrollable urge to obtain and consume food, which is similar to that exhibited by addicted individuals towards drug of abuse. They represent a central feature of eating disorders, such as the binge eating disorder and bulimia nervosa [1]. Using a well-characterized animal model of BE, we investigate the epigenetic regulation of the Adenosine Receptor A2A gene, receptor already known to have an effect on food intake and BE (2).

MATERIAL AND METHODS

Animals
Female Sprague-Dawley rats (Charles River, Calco, Como, Italy) were used. They were 52-day-old at the beginning of the experiment.

Diets
Animals were offered standard rat food pellets (4RF18, Mucedola, Settimo Milanese, Italy; 2.6 kcal/g) and a HPF. The HPF was a paste in texture, prepared by mixing: (a) Nutella (Ferrero, Alba (TO), Italy) chocolate cream (5.33 kcal/g; 56%, 31%, and 7% from carbohydrate, fat, and protein, respectively), (b) grounded food pellets (4RF16), (c) water (52% Nutella, 33% food pellets, and 15% water).

Stress
Acute stress was elicited by exposing rats to HPF, but preventing them from access to it for 15 min, while rats were able to see and smell it.

Workflow of the molecular study
- Rat brain regions dissection
- Genomic DNA and total RNA isolation
- Real-time RT-PCR and MSP-PCR
- Data analysis

Drugs
VT 7, A2A AR agonist (5’-N-ethylcarboxamido-2’(2-phenethylamino) adenosine, was dissolved by adding dimethylsulfoxide (DMSO), polyethylene glycol (PEG) 400 and water in ratio (50:150:850), and vortexing vigorously.
ANR 94, A2A AR antagonist ANR 94 (8-ethoxy-9-ethyladendine) was dissolved by adding DMSO, PEG 400 and water in the ratio 50:350:650 and vortexing vigorously.

EXPERIMENT 1

BE evoked by cycles of food restriction an exposure to acute stress

After a week of recovery from surgery, 36 rats were divided in 4 groups of 9 animals, matched for body weight and daily food intake:

1. not restricted and not exposed to stress rats (NR + NS)
2. restricted and not exposed to stress rats (R + NS)
3. not restricted and exposed to stress rats (NR + S)
4. restricted and exposed to stress rats (R + S)

Rats were submitted to 3 consecutive 8-day cycles followed by the final test on day 25.

CONCLUSIONS

- The combination of stress and repeated episodes of food restriction is able to induce a pronounced BE response for HPF in rats. A2A AR agonists exert a rather general effect on food intake inhibiting HPF intake, whereas A2A AR selective antagonist reverses these effects.
- We observed a consistent selective significant increase of A2A gene expression in the amygdala complex of R + S rats.
- A2A AR antagonist administration completely reversed the alterations in receptors gene expression.
- This result is further supported by the epigenetic regulation of this receptor gene transcription, evident by the reduction in DNA methylation at gene promoter, which might be responsible of the increase in gene expression.

This results suggest that A2A AR activation is effective in reversing HPF intake via D2 receptors due to the interaction between these two systems. Stress, associated with food restriction, promotes alterations in genes critical in feeding and reward circuitry, that influence food intake and stress-related behaviours.

ACKNOWLEDGMENTS
The work was supported in part by the Italian Ministry of University and Research under grants FIRB-RBFR12DELS to CC and CDA .

Disclosure: No potential conflict of interest.