EPIGENETIC REGULATION OF A_{2A} GENE TRANSCRIPTION IN A MODEL OF BINGE EATING IN THE AMYGDALA COMPLEX OF FEMALE RATS

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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 A_{2A} gene, receptor already known to have an effect on food intake and BE (2).

[1] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: APA2013.
[2] Micioni Di Bonaventura MV, Cifani C, Lambertucci C, Volpini R, Cristalli G, Massi M. A(2A) adenosine receptor agonists reduce both high-palatability and low-palatability food intake in female rats. Behav Pharmacol. 2012; 23: 567-574.

MATERIAL AND METHODS

Animals

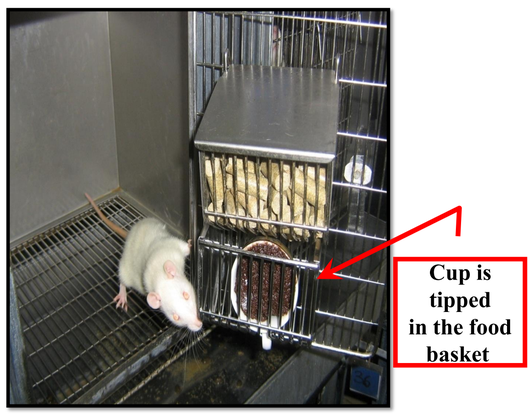
Female Sprague-Dawley rats (Charles River, Calco, Como, Italy) were used. They were 52-day-old at the beginning of the experiment.

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 (4RF18), (c) water (52% Nutella, 33% food pellets, and 15% water).

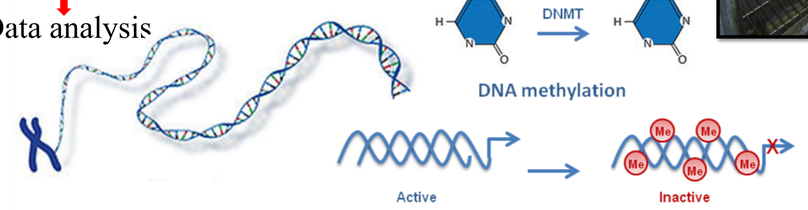
Stress

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

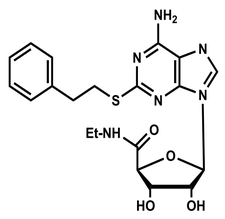


Drugs

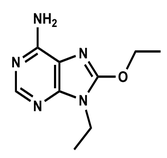
VT 7, A_{2A}AR agonist (5'-N-ethylcarboxamido-2-(2-phenethylthio) adenosine, was dissolved by adding dimethylsulfoxide (DMSO), polyethylene glycol (PEG 400) and water in ratio (50:150:800), and vortexing vigorously.

ANR 94, A_{2A}AR antagonist ANR 94 (8-ethoxy-9-ethyladenine), was dissolved by adding DMSO, PEG 400 and water in the ratio 50:350:600 and vortexing vigorously.

Both compounds were injected intraperitoneal in a volume of 0.2 ml/kg, 30 min before access to HPF.



VT 7



ANR 94

Affinities of VT 7 and ANR 94 in radioligand binding assays at A_1AR, A_{2A}AR, and A_3AR

Cpd	K_i (A_1AR)[a]	K_i (A_{2A}AR)[a]	K_i (A_3AR)[a]
VT 7	380 r 189 h	15 r 24 h	46 r 86 h
ANR 94	4600 r 2400 h	643 r 46 h	34%[b] r 21000 h

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)**

Group	Days 1-4	Days 5-6	Days 7-8	Days 9-12	Days 13-14	Days 15-16	Days 17-20	Days 21-24	Day 25
NR + NS	Ad lib chow	Ad lib chow + HPF(2 h)	Ad lib chow	Ad lib chow	Ad lib chow + HPF(2 h)	Ad lib chow	Ad lib chow	Ad lib chow	No stress+ ad lib chow+HPF(2h)
NR + S	Ad lib chow	Ad lib chow + HPF(2 h)	Ad lib chow	Ad lib chow	Ad lib chow + HPF(2 h)	Ad lib chow	Ad lib chow	Ad lib chow	Stress+ ad lib chow+HPF(2h)
R + NS	Restricted chow to 66%	Ad lib chow + HPF(2 h)	Ad lib chow	Restricted chow to 66%	Ad lib chow + HPF(2 h)	Ad lib chow	Restricted chow to 66%	Ad lib chow	No stress+ ad lib chow+HPF(2h)
R + S	Restricted chow to 66%	Ad lib chow + HPF(2 h)	Ad lib chow	Restricted chow to 66%	Ad lib chow + HPF(2 h)	Ad lib chow	Restricted chow to 66%	Ad lib chow	Stress+ ad lib chow+HPF(2h)

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

Fig. 1A

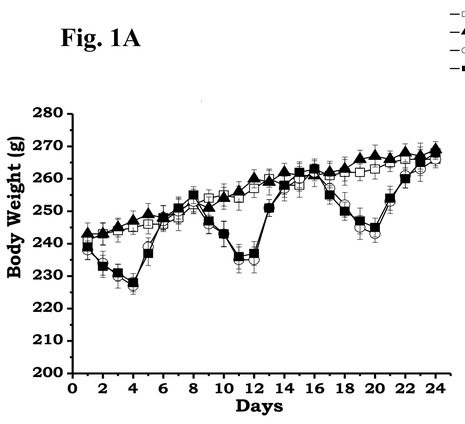
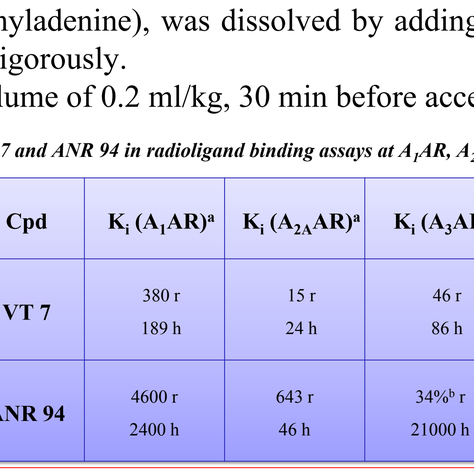


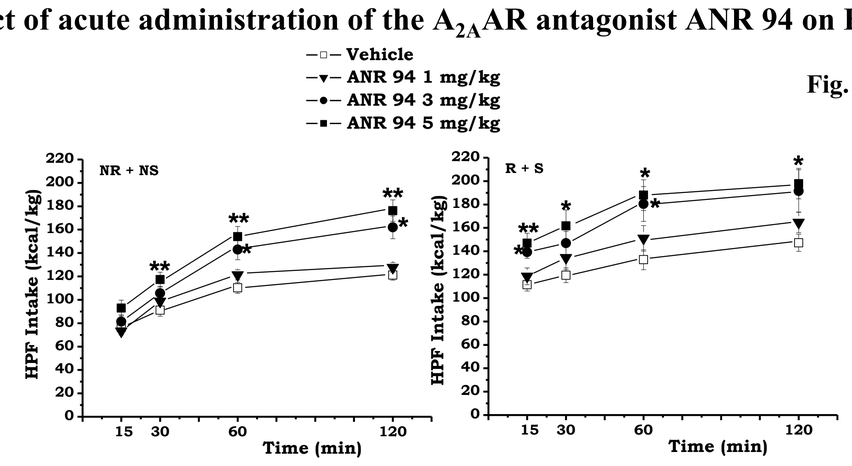
Fig. 1B



EXPERIMENT 2

Effect of acute administration of the A_{2A}AR antagonist ANR 94 on BE

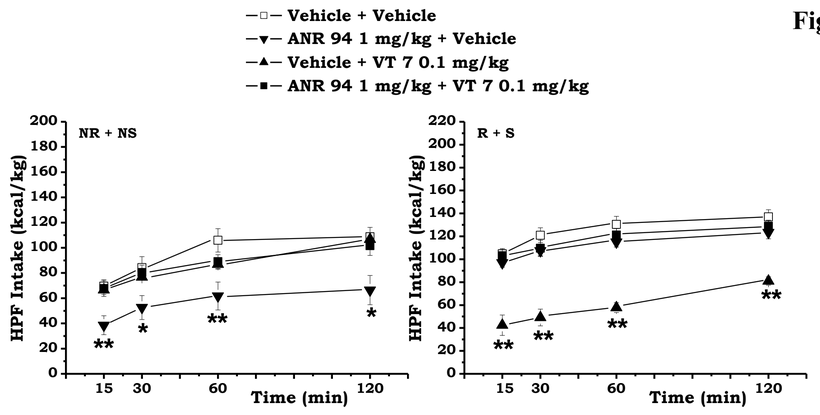
Fig. 3



EXPERIMENT 3

Effect of the A_{2A} agonist VT 7 after pre treatment with ANR 94 on BE

Fig. 4



EXPERIMENT 4

A_{2A} regulation of A_{2A} gene transcription in a model of BE in the amygdala complex of female rats

Fig. 5A

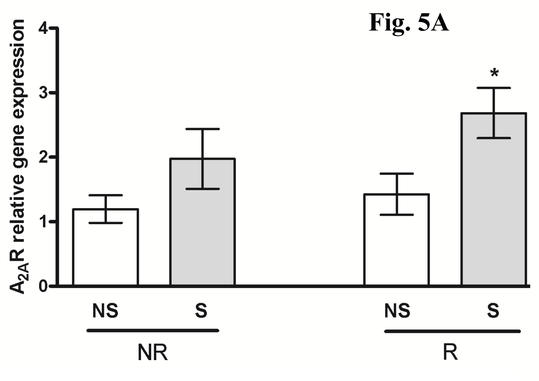
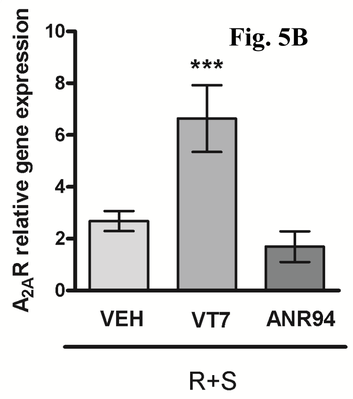


Fig. 5B



RT-qPCR analysis of A_{2A}R gene expression. A) Level of A_{2A}R mRNA in AM in NR + NS, NR + S, R + NS, R + S). **B)** A_{2A}R level R + S treated with VT 7 or ANR 94.

Fig. 6A

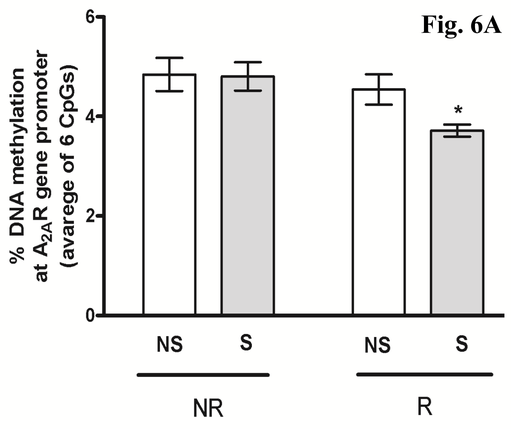
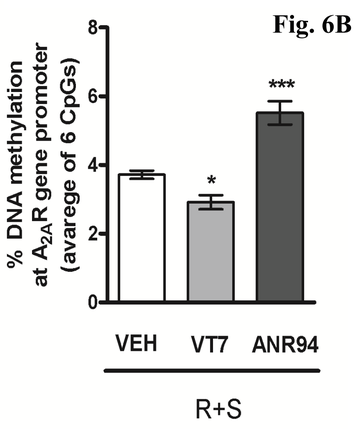


Fig. 6B



Pyrosequencing analysis of DNA methylation at A_{2A}R promoter region in the AM of food restricted and exposed to acute stress rats.

	CpG 1	CpG 2	CpG 3	CpG 4	CpG 5	CpG 6	CpGs AVE
NR+NS	6.69 ± 0.46	8.82 ± 0.57	7.60 ± 0.54	1.96 ± 0.19	2.33 ± 0.19	2.42 ± 0.12	4.97 ± 0.33
NR+S	6.57 ± 0.45	8.37 ± 0.53	7.77 ± 0.55	1.93 ± 0.22	2.20 ± 0.24	2.49 ± 0.19	4.89 ± 0.28
R+NS	6.61 ± 0.49	7.53 ± 0.46	6.87 ± 0.45	1.84 ± 0.15	2.52 ± 0.37	2.50 ± 0.24	4.64 ± 0.38
R+S	5.60 ± 0.40	6.32 ± 0.25	6.00 ± 0.36	1.72 ± 0.09	2.27 ± 0.14	2.46 ± 0.14	3.71 ± 0.13
R+S+VT7	4.85 ± 0.59	5.43 ± 0.09	4.93 ± 0.35	1.55 ± 0.16	1.88 ± 0.20	2.01 ± 0.21	3.07 ± 0.17
R+S+ANR94	9.28 ± 0.99	10.58 ± 0.87	10.29 ± 1.01	2.30 ± 0.24	1.91 ± 0.43	2.68 ± 0.36	6.17 ± 0.54

DNA methylation at A_{2A}R gene promoter (6 CpG sites evaluated by pyrosequencing)

CONCLUSIONS

- **The combination of stress and repeated episodes of food restriction is able to induce a pronounced BE response for HPF in rats. A_{2A}AR agonists exert a rather general effect on food intake inhibiting HPF intake, whereas A_{2A}AR selective antagonist reverts these effects.**
- **We observed a consistent selective significant increase of A_{2A} gene expression in the amygdala complex of R + S rats.**
- **A_{2A} antagonist administration completely reversed the alterations in receptors genes expression.**
- **These 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 A_{2A}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.

A_{2A}R agonists may potentially be useful pharmacological agents to control BE via modulation of A_2AR and D2R gene transcription.

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