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Epigenetic regulation of endocannbinoid system in Activity-based model of Anorexia nervosa

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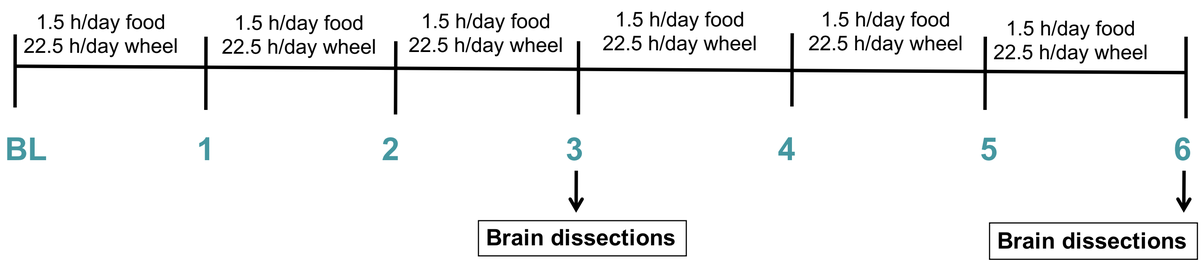
Introduction

Anorexia nervosa (AN) is a psychiatric disorder characterized by a dramatic reduction in caloric intake by excessive dieting, which is accompanied by physiological, biochemical, and behavioral disturbances. Up to now, there is no proof of efficacy of any drugs [1, 2]. Animals housed with running wheels and subjected to daily food restriction show paradoxical reductions in food intake and increases in running wheel activity. This phenomenon, known as activity-based anorexia (ABA), leads to marked reductions in body weight and provides an imortant tool for investigating the neurobiological underpinnings of AN-like behaviour, which is of a great need given the reductions in quality of life, high mortality rate and lack of pharmacological treatment for AN [3].

Activity-based Anorexia rat model



Control: food ad libitum, no access to wheel
Restricted: food restriction 90 min/day, no access to wheel
Anorexic: food restriction 90 min/day, free access to wheel

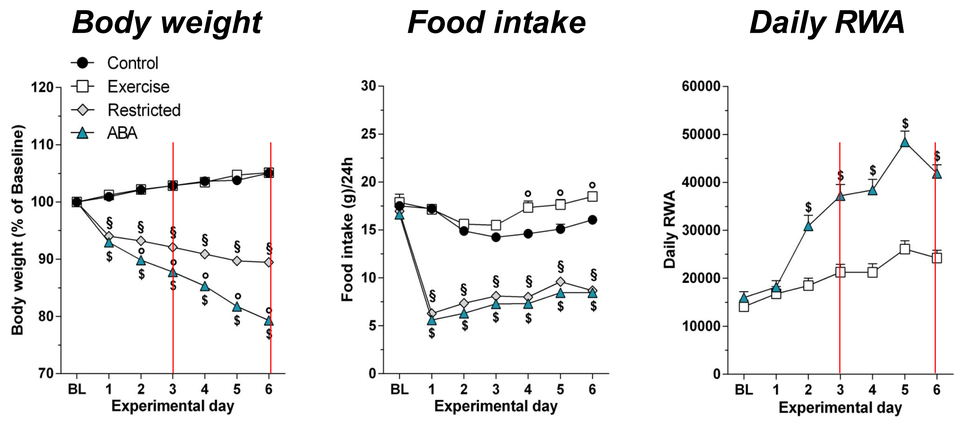
Methods

Sample preparation
- RNA and DNA extraction
- Isolated nucleic acids quality control

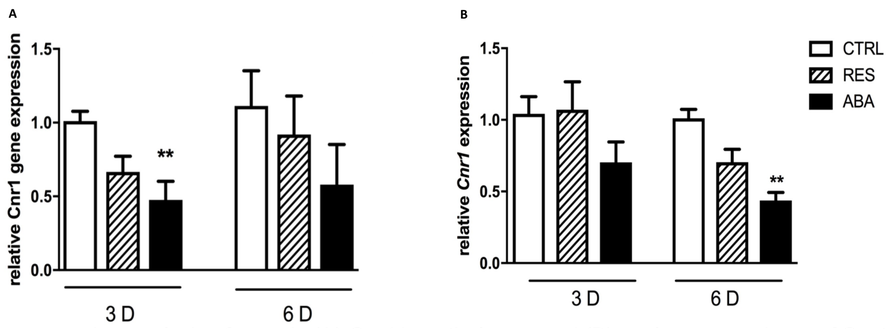
Gene expression studies
- Retrotranscription (cDNA synthesis)
- Quantitative real-time PCR
- Statistical analysis

DNA methylation analysis
- Bisulfite conversion of DNA
- PCR amplification of DNA
- Pyrosequencing
- Statistical analysis

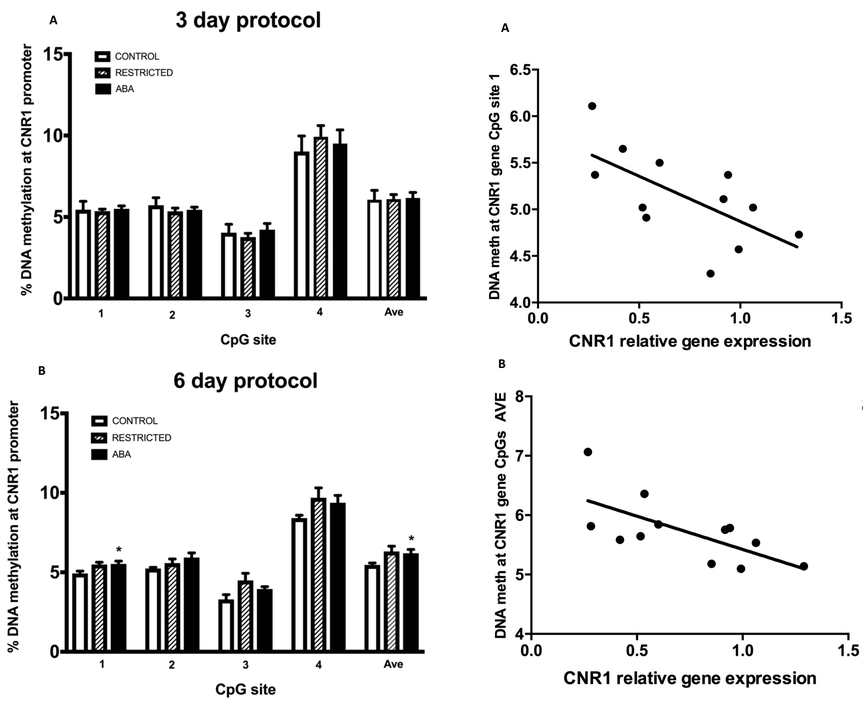
Results



Data on animal body weight, daily food intake and running wheel activity upon ABA induction in Control, Restricted, ABA and Excercise (not included in further analysis) group.



Gene expression analysis of gene in (A) *Cnr1* hypothalamus and (B) nucleus accumbens of Control (CTRL), Restricted (RES) and Anorectic (ABA) rats after 3 and 6 days of experiment induction



Left: Percentage of DNA methylation assessed with bisulfite pyrosequencing in the nucleus accumbens of Control (CTRL), Restricted (RES) and Anorectic (ABA) rats for *Cnr1* gene promoter at each single CpG site (A) and all sites combined (Ave, B) after 3 and 6 days of experiment induction

Right: Correlation between downregulation of *Cnr1* gene expression and DNA hypermethylation in its promoter region at first CpG site (A) and all CpG sites combined (B).

Gene expression studies have shown decreased expression of *Cnr1* gene in ABA rats nucleus accumbens (6D) and hypothalamus (3D) (Fig 1a e Fig 1b)). Consistently we observed an increase in DNA methylation at *Cnr1* promoter region in rats sacrified after 6 days in the nucleus accumbens (Fig 2 b). Correlation analysis has shown a relationship between gene expression and DNA methylation results at first CpG site and all sites combined (Fig 3a and Fig 3b)

Reference

1. Casper, R.C., et al., *Total daily energy expenditure and activity level in anorexia nervosa.* The American journal of clinical nutrition, 1991. **53**(5): p. 1143-1150.
2. Davis, C., *Eating disorders and hyperactivity: a psychobiological perspective.* The Canadian Journal of Psychiatry, 1997. **42**(2): p. 168-175.
3. Klenotich, S.J. and S.C. Dulawa, *The activity-based anorexia mouse model*, in *Psychiatric Disorders*2012, Springer. p. 377-393.

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Conclusions

- ✓ We identified selective and time-dependent epigenetic modulation of CNR1 in ABA rats in selected relevant brain regions
- ✓ Our data support the role central role played by CNR1 in food intake
- ✓ It is of relevance the identification of gene transcription regulation at the beginning of anorexia development
- ✓ The study of epigenetic mechanisms might be of help to predicte disease trajectories and choose effective therapies