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

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Conclusion

- ✓ We identified selective and time-dependent epigenetic modulation of cannabinoid receptor 1 (CB_1) gene (*Cnr1*) regulation in ABA rats nucleus accumbens along with reduced *Cnr1* expression in hypothalamus, whereas genetic *anx/anx* model exhibited *Cnr1* down-regulation in prefrontal cortex
- ✓ Selectivley observed molecular alterations in the two animal models account for differences in behavioural and genetic contribution to Anorexia nervosa onset and development
- ✓ Our data support the central role played by CB_1 in food intake

Background and aims

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]. It has the highest mortality rate among psychiatric disorders and lacks efficient pharmacological treatment [3].

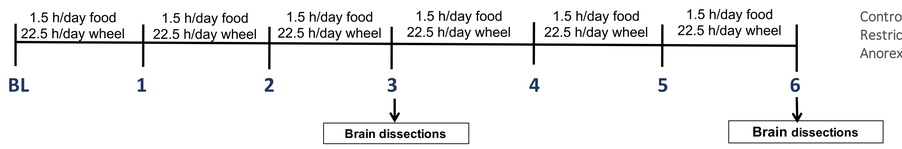
Animals housed with running wheels and subjected to daily food restriction show paradoxical reductions in food intake and increases in running wheel activity. This phenomenon, known as activity-based anorexia (ABA), leads to marked reductions in body weight and provides an imortant tool for investigating the neurobiological underpinnings of AN-like behaviour.

The anorectic genetic model, *anx/anx* mice, arose by spontaneous mutation (anorexia, allele symbol *anx*) and die three to five weeks after birth. They exhibit distrubed feeding behavior, neurodegeneration in appetite regulating hypothalamic arcuate nucleus and dysfunction of the mitochondrial oxidative phosphorylation system [4].

The aim of the present study was to interrogate the possible transcriptional regulation of ECS components, eventually via DNA methylation changes at gene promoters, in two animal models of AN, behavioural and genetic one, in order to validate whether behavioural or genetic component drives AN onset and development.

Methods

Activity-based Anorexia rat model

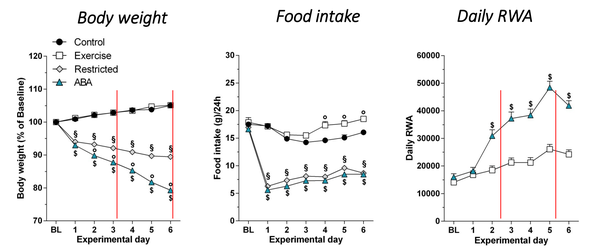


Control: food ad libitum, no access to wheel
Restricted: food restriction 90 min/day, no access to wheel
Anorexic: food restriction 90 min/day, free access to wheel

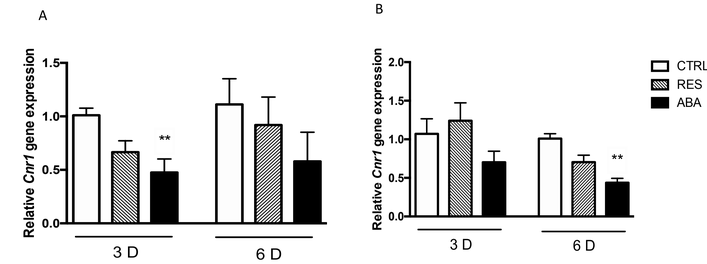
Wet-lab Workflow:

- Sample preparation: • DNA extraction • RNA extraction
- Gene expression studies: • Retrotranscription (cDNA synthesis) • Quantitative real-time PCR • Statistical analysis
- DNA methylation assessment: • Bisulfite conversion of DNA • DNA amplification by PCR • Pyrosequencing • Statistical analysis

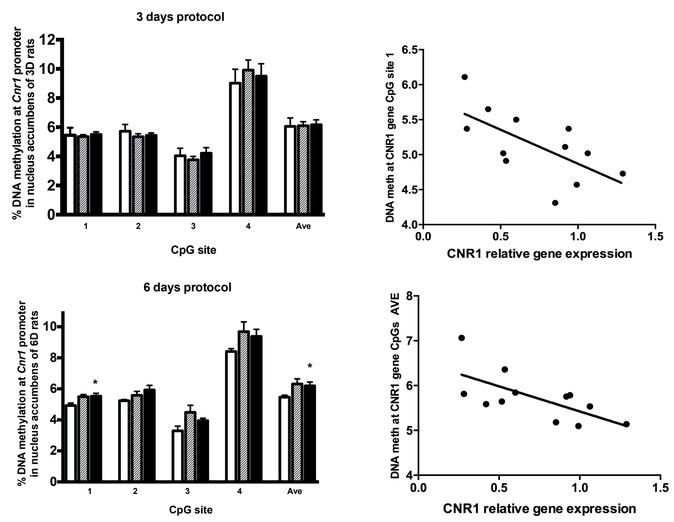
Results



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

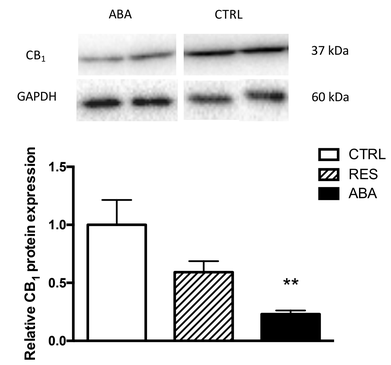


Gene expression analysis of gene in (A) *Cnr1* hypothalamus and (B) nucleus accumbens of Control (CTRL), Restricted (RES) and Anorectic (ABA) rats after 3 and 6 days of experiment induction



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).



CB_1 protein levels in nucleus accumbens of 6 day rats compared to GAPDH as an internal control.

	Hypothalamus		Prefrontal cortex		Nucleus accumbens	
	wt	*anx/anx*	wt	*anx/anx*	wt	*anx/anx*
Cnr1	1,133 ± 0,248	1,000 ± 0,182	1,043 ± 0,126	**0,609 ± 0,053**	1,159 ± 0,262	0,967 ± 0,222
Cnr2	1,077 ± 0,179	1,600 ± 0,179	1,112 ± 0,206	1,230 ± 0,183	1,088 ± 0,210	1,077 ± 0,436
Faah	1,036 ± 0,117	1,072 ± 0,092	1,178 ± 0,257	0,955 ± 0,152	1,331 ± 0,485	1,259 ± 0,164
Magl	1,020 ± 0,084	0,943 ± 0,104	1,084 ± 0,158	1,040 ± 0,116	1,028 ± 0,116	1,348 ± 0,074
Dagl	1,057 ± 0,128	1,457 ± 0,294	1,075 ± 0,164	0,714 ± 0,141	1,079 ± 0,187	1,880 ± 0,449
Trpv1	1,024 ± 0,089	0,764 ± 0,131	1,056 ± 0,129	1,028 ± 0,107	1,099 ± 0,249	1,397 ± 0,268
Nape-pld	1,060 ± 0,154	0,974 ± 0,144	1,052 ± 0,121	1,143 ± 0,158	1,095 ± 0,212	1,233 ± 0,241

Expression levels of ECS genes in hypothalamus, prefrontal cortex and nucleus accumbens of the *anx/anx* mouse, a genetic model of anorexia, and wild type (wt) mice

Reference

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