# UNRAVELLING THE MEDIATING ROLE OF NUCLEUS ACCUMBENS FUNCTIONAL CONNECTIVITY AND CIRCULATING ENDOCANNABINOIDS ON ANOREXIA NERVOSA SEVERITY

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## Introduction and Aims

Anorexia nervosa (AN) is a psychiatric disorder characterized by neurobiological alterations affecting eating behaviour. Despite the health risks, individuals with AN persistently engage in starvation, resulting in severe and harmful low weight [1]. DSM-5 classifies severity based on body mass index (BMI), from mild (BMI ≥17 kg/m²) to extreme (BMI <15 kg/m²). Research suggests that NAcc and circulating eCBs, like AEA and 2-AG, may contribute to AN severity and maladaptive behaviours [2,3], warranting an examination of the central reward circuitry and eCBs interaction. For this purpose, we investigated the intrinsic functional architecture of the NAcc and examine the fasting circulating concentrations of AEA and 2-AG in individuals with AN compared to healthy controls (HC). The main objective was to investigate whether NAcc functional connectivity and circulating eCBs had an impact on body mass index (BMI) in the AN and HC groups.

## Methods and Materials

Thirty-six women (18 with AN; 18 HC) underwent resting-state functional magnetic resonance imaging (rs-fMRI) and fasting blood collection for eCBs.

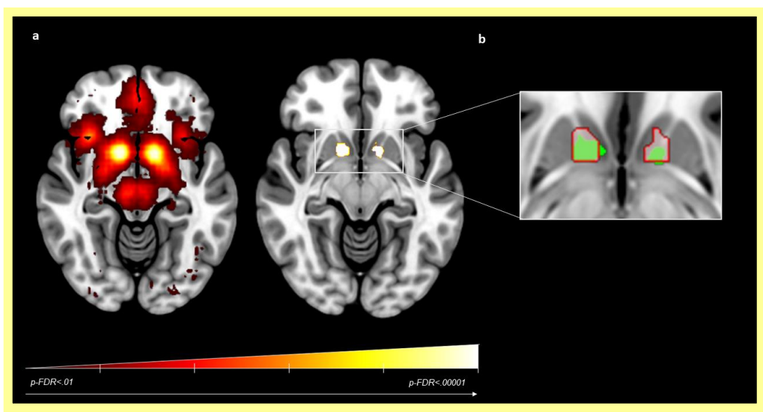
**Table 1.** Sample description

| | HC (n = 18) | | AN (n = 18) | | | |
|---|---|---|---|---|---|---|
| | *Mean* | *SD* | *Mean* | *SD* | *p* | *\|d\|* |
| Age (years old) | 34.22 | 7.78 | 22.89 | 4.66 | **<.001*** | **1.77†** |
| BMI (kg/m²) [1] | 21.63 | 2.06 | 16.28 | 1.40 | **<.001*** | **3.04†** |
| Duration (years) | -- | -- | 5.78 | 4.58 | **--** | **--** |
| Onset (years old) | -- | -- | 17.11 | 3.98 | **--** | **--** |
| 2-AG[1] | 6.16 | 4.73 | 6.34 | 4.18 | .930 | 0.04 |
| AEA[1] | 0.24 | 0.09 | 0.20 | 0.08 | .285 | **0.50†** |

**Note**. HC: healthy controls. AN: anorexia nervosa. SD: standard deviation. BMI: body mass index. SD: standard deviation. 2-AG: 2-arachidonoylglycerol. AEA: anandamide. *Bold: significant comparison (p < .05). †Effect size in the moderate to high range. [1] Comparison between groups: ANCOVA adjusted for age

We compared whole-brain connectivity maps between groups using seed-based functional connectivity analysis focused on the NAcc obtained using Neurosynth a meta-analytic neuroimaging database that uses a large series of previous studies to create empirical maps based on the probability that activation in specific brain regions would be associated with a specific term, such as “reward”. . Analyses were controlled for age and corrected for multiple comparisons (pFWE<0.05). Finally, we used a multigroup Structural Equation Model (SEM) to investigate the effect of circulating eCBs and NAcc functional connectivity on BMI.

**Figure 1.** NAcc bilateral seed from a reward brain mask obtained Neurosynth inference maps (https://www.neurosynth.org)




**a.** Considering a total of 922 studies reporting the “reward” term, several brain regions such as striatal regions, midbrain, the hippocampus, amygdala, insula, cingulate, frontal, and intraparietal cortices were included in the empirical mask obtained from Neurosynth inference maps (family discovery rate (FDR) correction of pFDR<.01). To obtain the nucleus accumbens (NAcc) mask, statistical significance was restricted to pFDR<.00001. **b.** The final NAcc mask, comprised of 219 voxels, overlapped with the automated anatomical labeling (AAL3) atlas to confirm the location of the obtained seed. The green seed represents the NAcc anatomical template from the AAL and the white mask with red boundaries represents the NAcc Neurosynth mask.

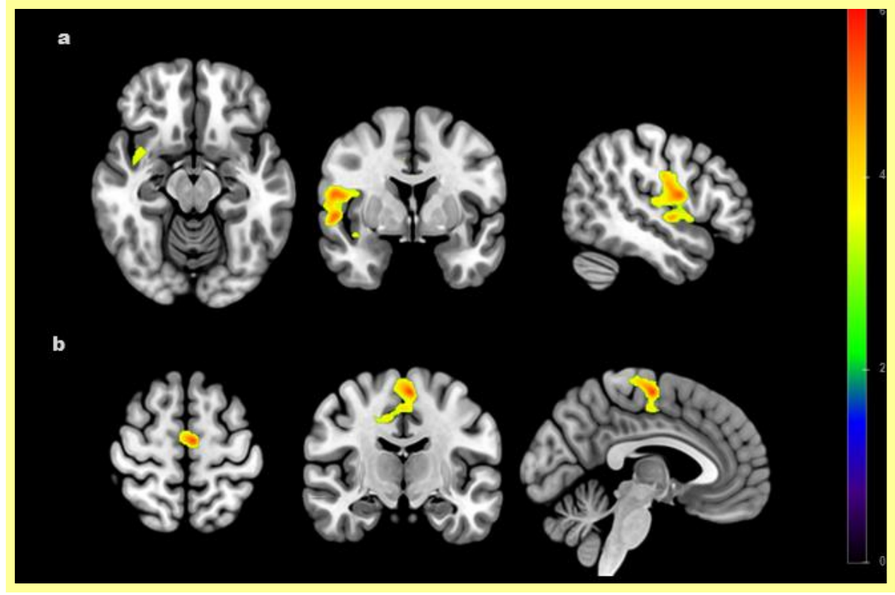
## Results (I)

The AN group was younger (M = 22.89 ± 4.66) than the HC group (M = 34.22 ± 7.78; t= 5.30; p<.001). Likewise, the AN group had a lower BMI (M = 16.28 ± 1.40) than the HC group (M = 21.63 ± 2.06; p<.001). In the AN group, the mean age of onset was 17.11 years old and the mean illness duration was 5.78 years. An ANCOVA (adjusted for age) compared differences between groups in the 2-AG and AEA concentrations. No significant differences in 2-AG ($F(1,33) = .008$; p=.930; |d|= 0.04) and AEA ($F(1,33) = 1.18$; p=.285; |d|= 0.50) concentrations between the AN and the HC group were observed, although a moderate size effect was observed in AEA concentrations differences.

## Results (II)

In comparison to HCs, Individuals with AN demonstrated significantly lower NAcc functional connectivity with the insula (NAcc-insula; pFWE <0.001) and the supplementary motor area (NAcc-SMA; pFWE <0.001).

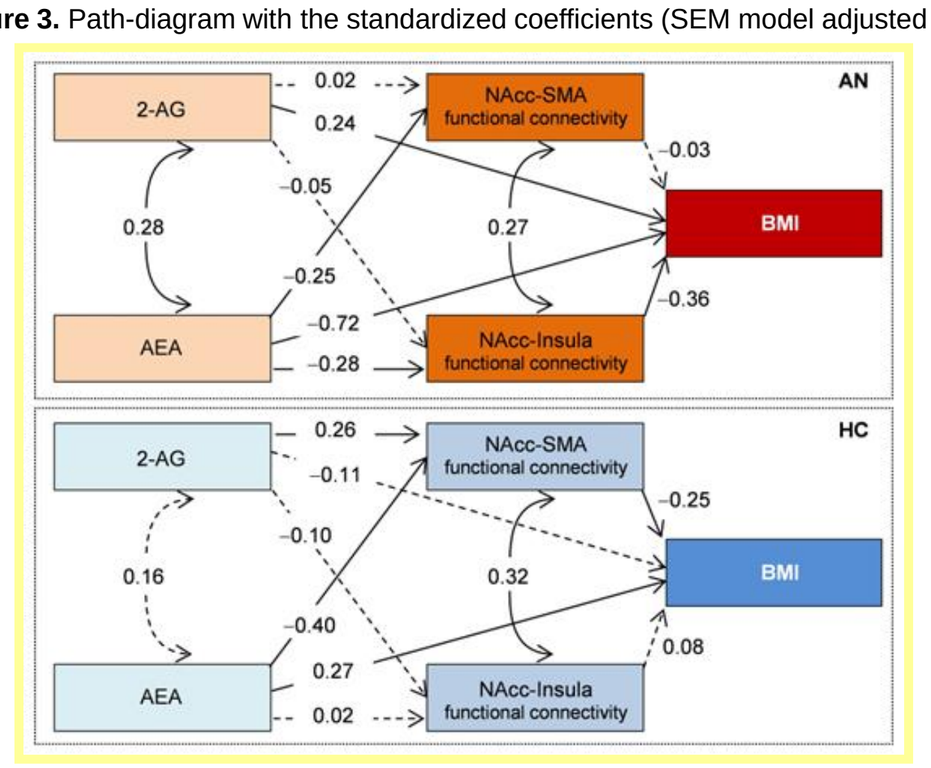
**Figure 2.** Between-group differences (AN<HC) in the functional connectivity of bilateral nucleus accumbens seeds




**Figures a and b display lower functional connectivity between the nucleus accumbens and a. the right insula (NAcc-Insula; pFWE<.001) b. the left supplementary motor area (NAcc-SMA; pFWE<.001) in the AN group compared to HC. Color bar represents t-values. Results are corrected and displayed at family-wise error (FWE) probability pFWE<.05 threshold, cluster-extent..**

Path diagrams in the AN group showed that AEA and 2-AG directly influenced BMI, while NAcc-insula functional connectivity played a dual role by directly affecting BMI and mediating the relationship between AEA and BMI. In the HC group, AEA directly influenced BMI, and NAcc-SMA functional connectivity acted both as a direct contributor to BMI and a mediating link in the relationship between AEA, 2-AG, and BMI.

**Figure 3.** Path-diagram with the standardized coefficients (SEM model adjusted for age)

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## Discussion and conclusion

Altered functional connectivity between the NAcc-insula and NAcc-SMA in individuals with AN can disrupt the integration of interoceptive, somatosensory, and motor planning information related to rewarding stimuli. The results highlight the influential role of eCBs and brain network profiles in AN severity [2–4]. Multivariate SEM analyses revealed differences between the AN and HC groups in the pathways associating intrinsic functional architecture of the NAcc, eCBs, and BMI, emphasizing potential clinical implications for the development of therapeutic strategies focused on maintaining a healthy weight.

## Keywords

2-arachidonoylglycerol, anandamide, anorexia nervosa, endocannabinoids, nucleus accumbens

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