

Differences in orexin-A level in the functional brain network of HUD patients undergoing harm reduction therapy

Wan-Ru You, MD^a, Liang-Chun Lin, MS^b, Wei-Che Lin, MD^c, Meng-Chang Tsai, MD^{a,*}

Abstract

Orexins regulate the reward-seeking pathway and also play a role in drug addiction. The aim of this study was an investigation of the changes in serum level of orexin-A as well as changes in the functional brain network in heroin use disorder (HUD) patients undergoing harm reduction therapy (HRT).

Twenty-five HUD patients undergoing HRT that included methadone and buprenorphine, and 31 healthy control (HC) subjects, were enrolled for this study. Serum orexin-A levels and brain-derived neurotrophic factor were measured with assay kits. The functional brain network in HUD patients and HC was investigated and assessed using seed-based analysis and functional brain MRI scans.

† Tested orexin-A levels were found to be significantly higher in HUD patients undergoing HRT than in HCs ($P < .05$). Analysis showed the functional activity of the right ventral anterior insula (RAVI) in HUD patients to be significantly lower than in HCs ($P < .05$, Family-Wise Error) corrected). In addition, the internetwork functional connectivity was significantly lower in the left nucleus accumbens and left dorsal anterior insula in the HUD subjects than in HCs ($P < .05$, Family-Wise Error corrected).

In this study, no significant correlation between orexin-A levels and functional brain networks was found. However, the results suggest that HRT might increase orexin-A levels and decrease functional activity in RAVI in HUD patients.

Abbreviations: BDNF = brain-derived neurotrophic factor, CSF = cerebrospinal fluid, ELISA = enzyme-linked immunosorbent assays, EPI = echo planar imaging, FC = functional connectivity, FWE = Family-Wise Error, HC = healthy control, HRT = harm reduction therapy, HUD = heroin use disorder, MNI = Montreal Neurological Institute, NAc = nucleus accumbens, RAI = right anterior insula, VTA = ventral tegmental area.

Keywords: functional brain network, harm reduction therapy, heroin, HUD, orexin-A

1. Introduction

Orexins play a role in the reward-seeking pathway. This includes the ventral tegmental area (VTA) and nucleus accumbens (NAc)^[1,2] and may be related to drug addiction.^[3–6] Also, orexins modulate dopaminergic, GABAergic, glutamatergic, and cholinergic neurons.^[7–9]

Orexins (hypocretins) including orexin-A and orexin-B are neuropeptides synthesized by neurons in the lateral and dorso-medial hypothalamus and perifornical areas.^[10,11] Two orexin receptors (OX1R and OX2R) mediated with orexins A and B can trigger different effects. In addition, a higher affinity for OX1R was found in orexin-A than in orexin-B. However, OX2R has similar affinities for both orexins A and B.^[12–14]

Some studies have shown that OX2R plays a role in the regulation of energy homeostasis and the sleep/wake cycle,^[15–17] while OX1R modulates reward-seeking and motivated behavior.^[18,19] Previous reports showed that orexin-A is involved in drug-seeking.^[18,20]

Harm reduction therapy might play a role in risky and injected substance use.^[21] Methadone maintenance treatment (MMT), frequently used in harm reduction, might increase orexin-A levels in heroin use disorder (HUD) patients.^[22]

Brain-derived neurotrophic factor (BDNF) a brain neurotrophin may be related to drug addiction severity^[23] and may even regulate drug addiction-related behavior.^[24–26] Some reports have shown that BDNF levels are lower in substance-dependent subjects than in healthy controls (HCs).^[23,27,28]

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Informed consent was obtained from all individual participants included in the study.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Numerous MRI studies have shown that chronic drug exposure may be associated with a smaller frontal cortex volume, as well as with cognitive and decision-making problems in drug abusers,^[29–31] enlarged basal ganglia,^[32–34] and more extensive gray matter deficit in the cingulate, limbic, and paralimbic cortices^[35] than in normal subjects.^[36] Some reports showed that the persistent craving for drugs may be positively correlated with activity in the NAc, inferior frontal/orbitofrontal gyrus, and anterior cingulate.^[37–40] Previous study has also revealed that some brain regions regulate reward systems that are associated with treatment response in subjects undergoing addiction intervention.^[41]

Some research results suggest that gray matter reduction in the bilateral hypothalamus, thalamus, NAc, anterior cingulate cortex, left mid-orbital and rectal gyri, right inferior frontal, and superior temporal gyri may be related to the orexin pathway in narcolepsy.^[42] However, there are few reports of the correlation of orexin levels with the functional brain network in HUD patients undergoing harm reduction treatment (HRD). Therefore, the aim of this study was an investigation of changes in serum levels of orexin-A and the functional brain network in HUD patients undergoing harm reduction therapy (HRT).

2. Method

2.1. Patients and study design

Twenty-five HUD (22 males, 3 females) patients who were undergoing HRT at the Kaohsiung Chang Gung Memorial Hospital were enrolled as subjects for the study. Twenty-four of them were on methadone maintenance treatment and 1 was undergoing buprenorphine therapy. Inclusion criteria were as follows:

1. A diagnosis of HUD based on DSM-5 (The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition);
2. Age from 20 to 65 years;
3. No history of psychotic, bipolar, major depressive, or substance use disorder except heroin or nicotine use;
4. Seronegative for human immunodeficiency virus; and
5. Stable physical condition. Data collected included age, body mass index (kg/m²), methadone or buprenorphine dose, onset age, duration of HRT, serum orexin-A, and BDNF levels. A single board-certified psychiatrist made the diagnosis of HUD for each participant. Blood was drawn from each subject during the no-heroin withdrawal period.

Thirty-one HC subjects (27 males, 4 females) were also recruited. No subjects with any medical or mental disorder were included. All were healthy and none were taking medication.

All participants underwent functional brain MRI scans to compare the functional brain networks of the 2 groups. All participants signed informed consent agreements after the aims and procedures of the study had been explained to them. This study had the prior approval of the Institutional Review Board of the hospital (IRB number: 201801378A0C601A3) and was carried out at the Kaohsiung Chang Gung Memorial Hospital in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

2.2. Laboratory data

Firstly, blood samples (15 mL) were drawn from a forearm vein of each subject after a fast of at least 8 hours. The samples were immediately centrifuged at 3000g for 10 minutes to separate the serum which was stored at –80°C (1–3 months) for analysis. Enzyme-linked immunosorbent assays were carried out using commercially available assay kits for orexin-A (MyBioSource) and BDNF (Promega Corporation, Wisconsin, WI). One trained laboratory technician performed all the analyses in the same laboratory.

2.3. MR image acquisition and processing

Functional imaging data were acquired using a 3.0 T GE Signa MRI scanner (Milwaukee, WI). Resting state images were gathered using an echo planar imaging (EPI) sequence (repetition time: 2500 ms; echo time: 27 ms; field of view: 1540 × 1540 mm; flip angle: 77°; matrix size: 64 × 64; slice: 3.4 mm). For the resting state experiment, the scanner room was darkened and the subjects were required to relax, with their eyes closed, and to think of nothing without falling asleep. 3D T1-weighted anatomic images were obtained using an inversion recovery fast spoiled gradient-recalled echo pulse sequence (repetition time: 2.5 ms; echo time: 7.5 ms; flip angle: 24°; field of view: 192 × 256 mm; matrix size: 288 × 384).

2.4. Image preprocessing of rs-fMRI dataset

Preprocessing of resting state functional MRI datasets was carried out using the FMRIB Software Library.^[43,44] The following standard preprocessing pipeline was applied for each subject:

1. Removal of the first 10 volumes from the whole-time series to allow for T1-equilibration effects;
2. Correction of temporal shifts in rs-fMRI data acquisition (slice timing correction);
3. Realignment of the rs-fMRI data to the first volume of the whole-time series dataset using MCFLIRT (the FMRIB Motion Correction Linear Image Registration Tool);^[45]
4. Removal of non-brain tissue using the BET Brain Extraction Tool;^[46]
5. Spatial smoothing with a 6 mm full width at half maximum Gaussian kernel;
6. Grand-mean intensity normalization;
7. Band-pass temporal filtering (0.01–0.1 Hz) to remove low-frequency drift and high-frequency noise; and
8. Removal of nuisance signals to minimize nonneural noise (head motion, white matter, and cerebrospinal fluid [CSF] signals).

In addition, 3-dimensional rigid-body motion correction (realigned to the first EPI acquisition) was carried out using MCFLIRT. Any subject showing a maximum rotation of 2° or displacement of 2 mm in any direction was excluded from further analysis. To ensure that the final functional connectivity (FC) results were not biased by head motion, the 3 rotational and translational displacement parameters from MCFLIRT were also used to calculate the mean frame displacement of each subject, the criterion was 0.5 mm. After preprocessing, residual RS-fMRI datasets were entered into the Montreal Neurological Institute (MNI) EPI template space and interpolated to a voxel size of 2 m³ (2 × 2 × 2). The preprocessed RS-fMRI data were used in the following seed-based FC analysis.

2.5. Seed-based functional connectivity

To compute the resting state FC of the insula and NAc, 8 spherical seed regions of interest (radius = 6 mm) were defined using previously published NAc and insular subdivisions,^[47,48] each corresponding to the left and right NAc (MNI coordinates: –8, 8, –8 and 10, 8, –8), the left and right ventral anterior insula (MNI coordinates: –33, 13, –7 and 32, 10, –6), the left and right dorsal anterior insula (MNI coordinates: –38, 6, 2 and 35, 7, 3), and the left and right posterior insula (MNI coordinates: –38, –6, 5 and 35, –11, 6, see Fig. 1).

Pearson's correlation analysis was carried out for between-group comparisons of seed time series and the time series of all voxels in the brain for each subject. To improve the normality of the correlation coefficients, Fisher's *r*-to-*z* transformation was used to convert correlation maps to *Z*-value maps. Group analyses were performed for the correlation maps of each seed region.

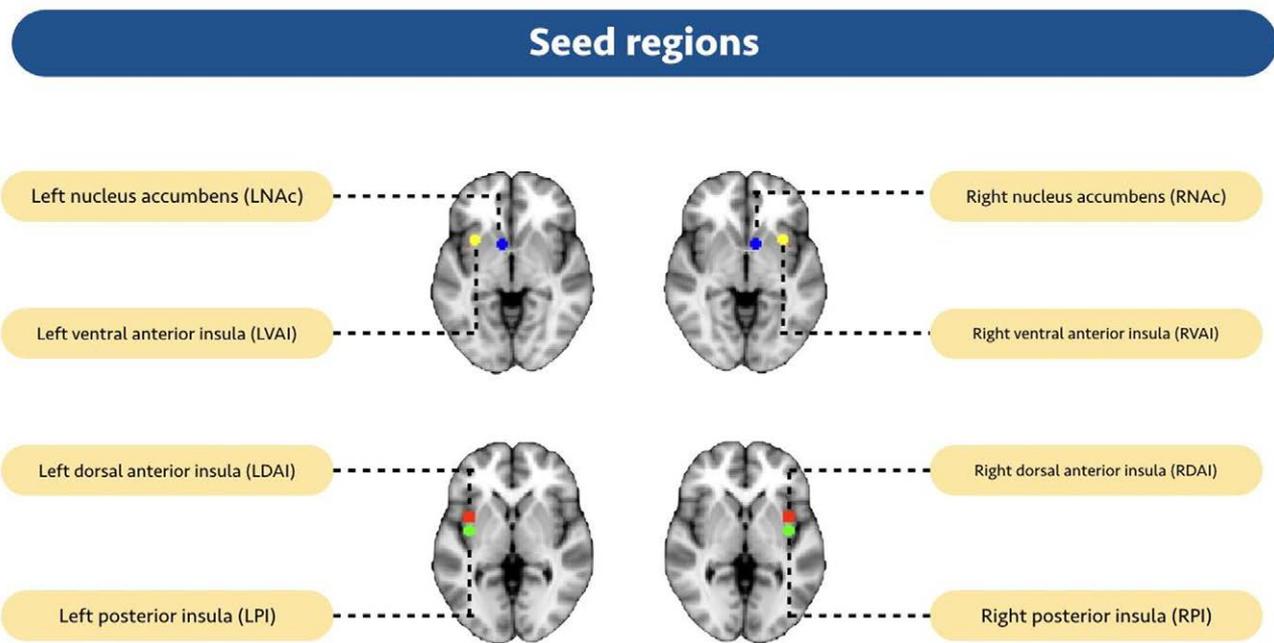


Figure 1. Functional brain network seed points. Four areas in the bilateral brain were defined as seed points. LDAI = left dorsal anterior insula, LNAc = left nucleus accumbens, LPI = left posterior insula, LVAI = left ventral anterior insula, RDAI = right dorsal anterior insula, RNAc = right nucleus accumbens, RPI = right posterior insula, RVAI = right ventral anterior insula.

The correlation maps of HC and HUD were *t* tested and used separately to demonstrate brain regions with significant positive correlations with the NAc and subregions of insula, see Figure S1, Supplemental Digital Content, <http://links.lww.com/MD/H13>.

2.6. Within network connectivity analysis

For between-group comparisons, all individual subject correlation maps were subjected to analysis of covariance with age, sex, and mean FD as covariates ($P < .05$, Family-Wise Error [FWE] corrected).

2.7. Between network connectivity analysis

Pearson's correlation analysis was used to compute the correlation coefficient among the correlation maps which had been derived from the 8 seed regions. For normality correlation, coefficients were transformed to *z*-scores using Fisher's *r*-to-*z* transformation. The correlation coefficients were then used in an analysis of covariance with age, sex, and mean FD as covariates to compare difference of FC between the groups ($P < .05$, FWE corrected).

2.8. Statistical analysis

The results were expressed as the mean \pm standard deviation. Comparisons between the orexin-A and BDNF in both HUD patients undergoing HRT and the control group were assessed using the *t* test. Pearson correlation was used to estimate the relationships between the FC of brain networks, and orexin-A and BDNF in patients with HUD. A *P* value of <0.05 was considered statistically significant.

3. Results

3.1. Characteristics of the included sample

The samples included 25 HUD patients undergoing HRT and 31 healthy subjects.

Table 1 shows the demographic data of the HUD patients that underwent HRT. The average age of the subjects was 46.03 ± 4.38 years, their average body mass index was 25.11 ± 4.22 , average age at first time heroin use was 23.24 ± 7.1 , the average dose of methadone was 58.12 ± 29.8 mg per day, and average length of treatment was 38.22 ± 38.8 months.

3.2. Orexin-A level in HUD patients

The (*t* tested) serum levels of orexin-A were significantly higher in HUD patients undergoing HRT than in the HC ($P < .001$). However, changes in BDNF levels were insignificant, see Table 2.

3.3. Functional connectivity between groups

Figure 2 shows the difference in intranetwork FC between the groups. HUD subjects showed significantly lower FC in the right ventral anterior insula functional network ($P < .05$, FWE corrected) than those in the HC group. However, no significant differences were observed in the intranetwork FC in left ventral anterior insula, bilateral NAc, bilateral dorsal anterior insula, and bilateral posterior insula, between the 2 groups.

Figure 3 shows the difference of internetwork FC between the groups and more analyses were conducted to determine internetwork FC differences. These showed that FC between left NAc and left dorsal anterior insula ($P < .05$, FWE corrected) was significantly lower in the HUD group subjects than in those of the HC group.

Further correlations were analyzed to assess the relationship between the level of orexin-A and strength of the network. However, no significant correlation between orexin-A level and the functional brain network was found in this study.

4. Discussion

One of important finding in this study was that HUD patients undergoing HRT had significantly higher serum levels of

Table 1
Demographic data of HUD patients undergoing harm reduction therapy.

Variable	Patients (n = 25)
Age (yr)	46.03 ± 4.38
Sex	22 males, 3 females
BMI (kg/m ²)	25.11 ± 4.22
Age at first time of heroin use (yr)	23.24 ± 7.1
Methadone dosage in a recent single month (mg/d) (n = 1, buprenorphine 2 mg/d)	58.12 ± 29.8
Duration (mo) of harm reduction therapy	38.22 ± 38.8

Plus-minus values are given as mean ± standard deviation.
BMI = body mass index, HUD = heroin use disorder.

Table 2
Orexin-A and BDNF of HUD and healthy controls.

Variable	Patients (n = 25)	Controls (n = 31)	t Test
Age (yr)	46.03 ± 4.38	49.35 ± 10.06	
BMI (kg/m ²)	25.11 ± 4.22	25.66 ± 3.82	
Orexin-A (pg/mL)	537.95 ± 123.8	404.21 ± 99.63	<i>P</i> < .001
BDNF conc (pg/mL)	20,709.93 ± 6326.21	21,676.58 ± 4698.65	<i>P</i> = .528

Plus-minus values are given as mean ± standard deviation.
BDNF = brain-derived neurotrophic factor, BMI = body mass index, HUD = heroin use disorder.

orexin-A than the HC group subjects. In addition, the functional brain networks indicated significant differences in RVAI between 2 groups. Although the correlation between orexin-A and functional brain network was not significant, further study of their association was needed. There may be some undefined reason(s) to explain why the correlation was not significant. The limited sample size could be 1. Orexins commonly exist in CSF and levels fluctuate slightly with the diurnal cycle, where the highest levels are seen in the middle of the night.^[49] Orexin is also present in human plasma with an uncertain secretion source^[50] and the level may be about 30% lower than that observed in the CSF.^[51] In addition, a previous study showed no significant difference in plasma orexin-A level over a period of 1 day.^[50] This means that plasma orexin-A level may not be a true reflection of its function in the central nervous system.

As we know, of all the neurotransmitters, dopamine is most closely related to the mechanism of drug addiction. Orexin-A

could activate the VTA dopamine neurons by mediating with OX1R and the dopamine would then be released from VTA.^[52] A previous study revealed that addictive substances originating from the VTA act on the mesocorticolimbic dopamine system and cause elevated dopamine concentrations in the NAc.^[53]

It has been shown that the NAc might play a role in addiction-related behavior. The dopamine transmission in the NAc shell could be stimulated by the addictive drug and cause drug-conditioned behavior.^[54] Furthermore, the glutamatergic projections within the NAc might regulate drug-seeking and cause a relapse to substance use.^[55]

Data from the present study suggest that functional RVAI activity in HUD subjects undergoing HRT show less significant differences in the intranetwork in FC than in HC. Another finding was that HUD group subjects showed significantly lower internetwork FC between left NAc and left dorsal anterior insula compared to those in a HC group (*P* < .05).

For years, neuroscience research has supported the idea that the anterior insula is associated with emotional experience,^[56] bodily sensation,^[57] and affective feelings.^[58] A ventral anterior insula region connected with the limbic system participates in affective processes, and a dorsal anterior insula subdivision with connections to frontal, anterior cingulate, and parietal regions is involved in cognitive control processes.^[59-61] Some studies highlight the important role of the left anterior insula in social function, such as empathy.^[61-63] More recent functional imaging studies of the left anterior insula also present its function in language, especially the motor aspects of speech production.^[64-66] The right anterior insula (RAI) might participate in sympathetic nervous system function, because lesions in the RAI may cause elevated heart rate^[67] and peripheral noradrenergic transmitter levels.^[68]

Recent evidence indicates that the insula plays a role in drug addiction.^[69] The probable role in conscious urges to take drugs was noted in the insula.^[64,70] In addition, elevated RAI activity is seen in subjects with anxiety disorders^[71] and might regulate decision making that involves uncertain risk and reward.^[72,73] Some reviews indicated that decreased insula activity may cause some of the abnormal decision making that may lead to relapse and further drug use.^[74,75] Also, previous research showed decreased gray matter in the insula in substance-dependent patients.^[76,77] The left insula was more affected in methamphetamine users, whereas in cocaine users it was the right insula.^[78] Those findings were consistent with our results showing lower FC in RVAI in HUD patients undergoing HRT than in HC.

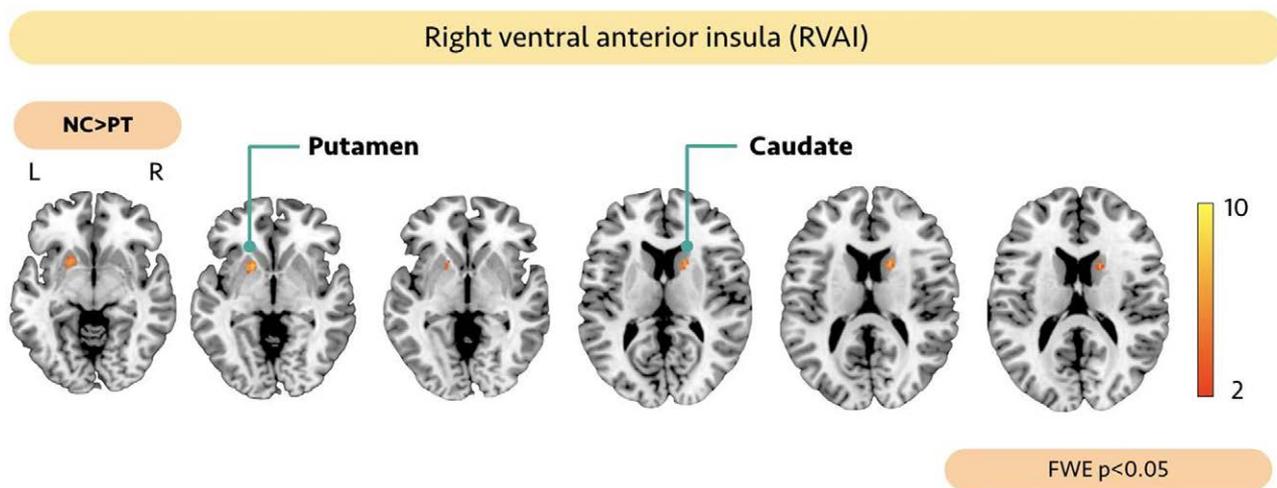


Figure 2. Group comparison of RVAI functional network. The HUD group subjects showed significantly lower FC in the right caudate (*x*, *y*, *z* = 16, 12, 14) and left putamen (*x*, *y*, *z* = -18, 12, -4) in the RVAI functional network (*P* < .05, FWE corrected) than those of the control group. FC = functional connectivity, FWE = Family-Wise Error, HUD = heroin use disorder, RVAI = right ventral anterior insula.

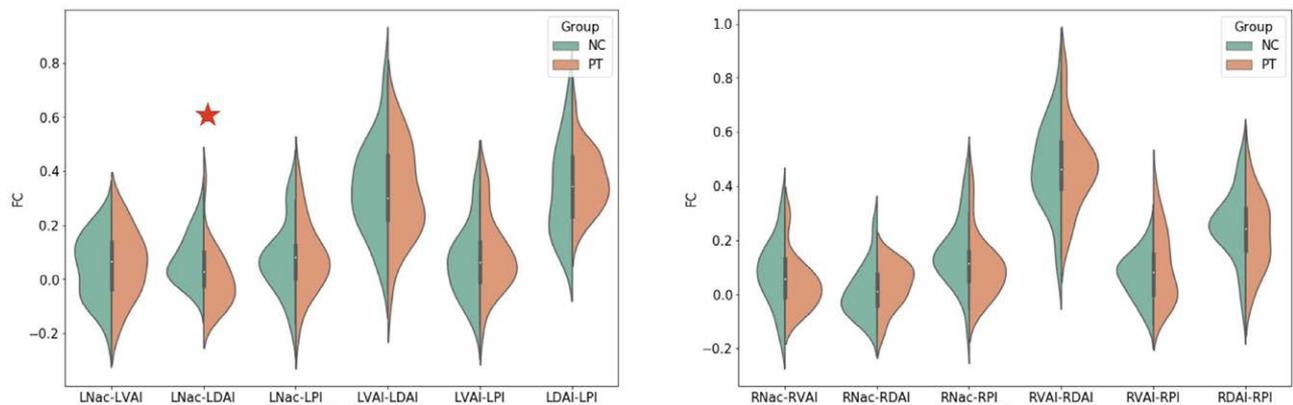


Figure 3. Group comparison of interaction functional networks among seed points. The HUD group subjects showed significantly lower FC between LNAc and LDAI ($P < .05$, FWE corrected) than those in the control group. FC = functional connectivity, FWE = Family-Wise Error, HUD = heroin use disorder, LDAI = left dorsal anterior insula, LNAc = left nucleus accumbens, LPI = left posterior insula, LVAI = left ventral anterior insula, RDAI = right dorsal anterior insula, RNac = right nucleus accumbens, RPI = right posterior insula, RVAI = right ventral anterior insula.

Our study was limited by sample size. It was also not clear if there was a significant correlation between orexin-A and the functional brain network. Future study will involve far more subjects.

5. Conclusions

In conclusion, the orexin-A levels in HUD subjects undergoing HRT showed significantly greater differences than those in the HC group. However, the functional brain network showed lower FC in HUD patients undergoing HRT than in HC. Our results suggest that HRT might increase orexin-A levels and decrease functional activity in RVAI in the HUD subjects.

Author contributions

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