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**Functional connectivity changes in Insomnia disorder: a systematic review**

Elisabetta Fasiello<sup>1</sup>, Maurizio Gorgoni<sup>1</sup>, Serena Scarpelli<sup>1</sup>, Valentina Alfonsi<sup>2</sup>, Luigi Ferini

Strambi<sup>3,4</sup>, Luigi De Gennaro<sup>1</sup>

<sup>1</sup>*Department of Psychology, Sapienza - University of Rome, Rome, Italy*

<sup>2</sup>*IRCCS Fondazione Santa Lucia, Rome, Italy*

<sup>3</sup>*Vita-Salute San Raffaele University, Milan, Italy.*

<sup>4</sup>*Sleep Disorders Center, Division of Neuroscience, San Raffaele Scientific Institute, Milan,*

Corresponding Author:

Prof. Luigi De Gennaro

E-mail address: [luigi.degennaro@uniroma1.it](mailto:luigi.degennaro@uniroma1.it)

Tel: +39-06-49917647; fax: +39-06- 49917711

Department of Psychology

University of Rome “Sapienza”

Via dei Marsi, 78

00185 Rome

Italy

## SUMMARY

Insomnia (ID) is the most common sleep disorder; however pathogenetic mechanisms underlying ID symptoms are not fully understood. Adopting a multifactorial view and considering ID a condition that involves interregional neuronal coordination would be useful to understand the ID pathophysiology. Functional connectivity (FC) may help to shed light on functional processes and neural correlates underlying ID symptoms. Despite a growing number of studies assessing FC anomalies, insight into ID pathophysiology is still fragmentary. This systematic review aims to search empirical evidence regarding FC changes in ID during resting-state. Thirty-one studies involving 1052 ID participants met the inclusion criteria for this review. Results suggested several associations between ID symptoms and impaired intra- and inter-hemispheric interactions of principal resting-state networks. Overall, evidence supported the hypothesis that a disrupted organization of the brain functional connectome characterizes ID, resulting in a decline in sleep, cognition, emotion, and memory. However, the wide methodological heterogeneity between reviewed studies and limitations in terms of study protocols and statistical approaches raised from this systematic review, makes it difficult to provide a univocal framework of ID pathophysiology. Future researches in this field should lead towards shared and rigorous search designs to ensure solid research evidence in the ID pathophysiology.

**Keywords:** Insomnia disorder; Functional connectivity; Hyperarousal; fMRI; RSNs; Node-based analyses; Voxel-based analyses.

**Abbreviation box**

Anterior cingulate cortex, ACC	Inferior temporal gyrus, ITG
Anterior default mode network, aDMN	Insomnia disorder, ID
Anterior midcingulate cortex, aMCC	Insomnia severity index, ISI
Basal ganglia, BG	International classification of sleep disorder, ICSD
Cerebellum anterior lobe, CAL	Intrinsic connectivity contrast, ICC
Cerebellum posterior lobe, CPL	Local functional connectivity density, lFCD
Cingulate gyrus, CG	Medial prefrontal cortex, mPFC
Default mode network, DMN	Middle frontal gyrus, middleFG
Degree centrality, DC	Middle occipital gyrus, middleOG
Diagnostic and statistical manual of mental disorders, DSM	Middle temporal gyrus, middleTG
Dorsal attentional network, DAN	Orbital part of frontal lobe, ORB
Dorsolateral prefrontal cortex, dlPFC	Orbitofrontal cortex, OFC
Electroencephalogram, EEG	Pittsburgh sleep quality index, PSQI
Executive-control network, ECN	Polysomnography, PSG
False discovery rate, FDR	Postcentral gyrus, PoCG
Family wise error rate, FWE	Posterior cingulate cortex, PCC
Fronto-parietal network, FPN	Posterior default mode network, pDMN
Functional connectivity, FC	Psychomotor vigilance task, PVT
Functional connectivity density, FCD	Regions of interest, ROI
Functional connectivity strength, FCS	Resting-state networks, RSNs
Functional magnetic resonance, fMRI	Saliency network, SN
Fusiform gyrus, FG	Seed-based correlational analysis, SCA
Gaussian random field theory, GRF	Self-rating scale of sleep, SRSS
Generalized anxiety disorder, GAD	Sensory-motor network, SMN
Global functional connectivity density, gFCD	Superior frontal gyrus, SFG
Hamilton depression rating scale, HAMD	Superior parietal lobe, SPL
Healthy controls, HCs	Superior temporal gyrus, STG
Independent component analyses, ICA	Supplementary motor cortex, SMA
Inferior frontal gyrus, IFG	Voxel-mirrored homotopic connectivity, VMHC.
Inferior parietal lobe, IPL	

## Introduction

Insomnia disorder (ID) is a highly prevalent sleep disorder [1] that implicates a perceived difficulty in falling or staying asleep and obtaining refreshing sleep, as well as heightened arousal in bed [2,3]. In addition to classical sleep complaints, ID patients often report diurnal symptoms that make it a 24-hours disorder. Indeed, ID can be explained through a psychobiological model called hyperarousal theory, considering the neurocognitive factors that characterise the disorder [4,5]. Biological factors refer to cortical arousal associated with higher electroencephalogram (EEG) activity and enhanced sensory processing that interferes with the wake-sleep transition and nightly sleep processes. On the other hand, psychological factors pertain to affective, behavioural, and cognitive alterations [4], making ID a neuropsychiatric syndrome. Indeed, ID presents high comorbidity with mood and anxiety disturbances, emotional dysregulation, and a cognitive style characterised by rumination and neuroticism [6]. A bidirectional influence between sleep disturbances and psychopathological traits can be assumed: emotional reactivity is a risk and a perpetuating factor to develop ID [7], and disrupted sleep may be a potential factor in developing psychiatric traits [8]. Moreover, as a consequence of insufficient sleep, ID is linked with cognitive and memory impairments, specifically in psychomotor performance, problem-solving, episodic and working memory [9].

Given these considerations, a multifactorial view is needed to understand the ID pathophysiology, considering the disorder as global rather than focal, involving interregional neuronal coordination. Even though many neuroimaging studies described specific neurobiological alterations in ID [10], unanimous conclusions about the neurophysiology of the disorder have yet to be reached. In this perspective, functional connectivity (FC) may help establish functional processes and correlates underlying ID symptoms. Indeed, the study of brain connectivity makes it possible to shed light on the inherent organization and functioning at the basis of a rich set of behaviours and thoughts of the human being. Moreover, further

insight into brain communication processes may help understand specific disorders associated with aberrant communications between brain regions [11]. Consistently with this view, the number of studies assessing FC anomalies and their relations with diurnal functioning and clinical symptoms in ID is progressively growing. In particular, many recent studies focus on resting-state FC, assessed when subjects are not engaged in specific tasks. Resting-state FC gives information about spontaneous fluctuations in neural activity, allowing an evaluation of the relationship between different brain regions.

The scope of the present systematic review is to collect and analyse empirical evidence regarding FC changes in ID during resting wake and their possible functional significance.

We will consider the different methods used to assess resting-state FC in this context, and the relation between FC anomalies, cognitive-behavioural functioning and clinical symptoms.

Finally, we will describe the main reviewed findings considering the functional networks potentially involved in ID in light of the theoretical framework represented by hyperarousal theory.

## **Methods**

### ***Search method***

The systematic review was performed according to the Preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [12]. One author (EF) systematically searched for literature up to April 2020 using the professional databases PubMed, PsyArticles, Scopus, and Web of Science. Search terms in the abstract or title were: (((‘Insomnia’) OR Primary Insomnia) AND “Resting-State Functional Connectivity”). The search was limited to studies written in English or Italian, to primary studies (books, abstracts, comments, reviews, or meta-analyses were rejected) and human species, without age, gender, or ethnicity restrictions.

After removing duplicates, the search returned 236 articles overall.

### ***Studies selection***

The articles screening was conducted through a two-step evaluation. The first assessment evaluated how the title and the abstract are relevant to the aim of the review. After the screening procedure, 190 papers were excluded, and 46 full-text articles were assessed for eligibility through the following qualitative criteria, in order to provide a more exhaustive evaluation of the selected articles:

1. The attendance of healthy controls (HCs) and ID patients' sample.
2. Diagnosis of ID had to be based on the presence of nocturnal and diurnal symptoms, assessed through clinical interviews and/or questionnaires.
3. Studies including exclusively subjects with ID in comorbidity with other disease conditions were rejected.

Moreover, most of the studies that emerged from the initial bibliographic research was conducted in functional magnetic resonance (fMRI), employing two macro-categories of analyses: node-based and voxel-based (see Table S1). Only four studies employed EEG to assess FC. Due to the heterogeneity of the experimental features and the paucity of data, we decided to exclude EEG studies from the systematic evaluation and consider exclusively papers that performed FC thorough fMRI during a resting wake protocol.

At the end of the eligibility stage, 31 articles published between 2008 and 2020 were included in this systematic review.

An outline of the selection procedure is illustrated via the PRISMA flow-chart (see Figure S1).

### ***Qualitative assessment***

As shown in Figure S2, studies that passed the eligibility step underwent a qualitative assessment employing the Critical appraisal skills program for cohort studies [13] modified *ad hoc* according to the aim of the systematic review. This is a tool consisting of 12 questions

which ensure a qualitative evaluation of each study with a systematic method. We considered nine questions pertaining to the aim of this review, and for each item, the quality of the study was categorized as “YES”, “NO”, or “CAN’T TELL” (see Figure S2).

### ***Data collection***

From each study, the following methodological dimensions have been extracted, according to the PICOS approach [14]: authors and publication year, sample characteristics (see Table 1), diagnostic confirmation methods (Figure S3), connectivity metrics extracted (see Table S1), presence of clinical data, cognitive measures (see Table S2), the fMRI acquisition protocols adopted in the studies (see Table S3) and statistical methods applied to correct for multiple comparisons (see Table S4 and S5).

## **Results**

### ***Demographic data***

Overall, reviewed studies evaluated FC in 1052 IDs (age range: 18–69 y) compared to 1047 HCs (age range: 21–70 y). One study also included 12 generalized anxiety disorder (GAD) patients [24], and another study included 15 ID patients with depression [28]. Without considering one study that did not report gender information [34], 60% of participants in the ID sample were females, a representative proportion of the prevalence of insomnia symptoms among women [1].

### ***Clinical and methodological features***

#### ***Illness duration and insomnia severity***

Of the reviewed studies [17-21,25–30,32,33,35–42], 62% reported the ID duration, ranging from  $\geq 1$  month to 20.2 years above all studies (see Table 1). Seventeen studies [17,18,21,24,25,27,29,32–34,36,37,41–45] assessed the ID severity by the Insomnia severity



index (ISI) questionnaire [46], three studies [15,35,38] used the Pittsburgh sleep quality index (PSQI) [47], although this questionnaire is not accurate in measuring the degree of ID severity. Lastly, one study [23] reported the ID score to identify ID severity without clarifying the instrument used to assess this index.

#### *Insomnia diagnosis criteria and insomnia symptoms medications*

The criteria applied for the ID diagnosis are heterogeneous (see Table S2, Figure S3). Two studies used the second version of the International classification of sleep disorder (ICSD-2) [48] criteria [17,40]; three studies based the diagnosis on ICSD-3 criteria [27,32,34]; 15 studies [15,16,18,19,21,26,28,29,35–38,42,43,45] used criteria reported in the fourth edition of Diagnostic and statistical manual of mental disorders (DSM-IV) [49], and 8 used DSM-V criteria [20,24,25,30,33,39,41,44]. Three studies [22,23,31] assessed insomnia symptoms among HCs according to the score of the 3-item sleep subscale on the 17-item Hamilton depression rating scale (HAMD-17) [50] and one of these studies [31] included also insomnia items on the Hamilton anxiety rating scale [51].

As shown in Table 1, such heterogeneity explains differences between the reviewed studies concerning the definition of the disorder (i.e., primary insomnia [15-18,24,26,28,29,32–34,36–38,40,42,43,45], chronic primary insomnia [19,20,25,30,39] or ID [21,27,35,41,44]). Patients were not drug-free for all studies (see Table 1). Specifically, three studies [21,23,38] did not provide information on subjects' pharmacological conditions, and two studies involved IDs not completely drug-free [17,27].

#### *Sleep assessment and exclusion of other sleep disorders*

The reviewed studies used different tools to subjectively or objectively assess participants' sleep (see Table S2).

Subjective sleep parameters were collected through sleep diaries [17,24,32,34,40,41] and various questionnaires to assess sleep quality [15–21,24–30,32–40,42,43,45], insomnia severity [17,18,24,25,27,29,32–34,36,37,40–45] and daytime sleepiness [18,24,33].

Furthermore, three studies [22,23,31] evaluated sleep disturbances by the sum of three HAMD-17 sleep questions, that examined the difficulty in initiating and maintaining sleep and early morning awakenings. Other three studies [17,32,40] employed the Self-rating scale of sleep (SRSS) [52].

Moreover, subjective sleep-related indices were collected: two studies [18,24] defined the participants' chronotype, two studies [18,44] assessed cognitive and somatic manifestations of pre-sleep arousal, and one study [18] evaluated sleep-related cognitions.

Objective sleep parameters were considered by 12 studies in all. Specifically, five papers [15,18,25,41,44] employed polysomnography (PSG) as objective tool to evaluate sleep measures, three studies [17,32,40] adopted actigraphic recordings, and one study [24] used both objective tools.

All these subjective and objective tools were combined differently depending on the individual study (see Table S2).

Another methodological feature extracted from the revised studies concerned the procedures adopted to exclude other sleep disorders (see Table S2). Despite all studies reporting the presence of other sleep disorders as exclusion criteria, the majority of them [22,23,26–29,31,33–37,42,43,45] did not mention the tools used to determine the presence of sleep disorders in addition to ID. However, to ensure the absence of other sleep disorders, eight studies had carried out a clinical interview conducted by specialized neurologists [17,19,20,30,32,38–40], one study [21] administered a semi-standardized sleep-related interview, and six studies [15,18,24,25,41,44] employed PSG monitoring.

*Psychological, cognitive, and behavioural assessment*

The majority of the reviewed studies have performed a psychological assessment for the ID and HC samples through self-reported questionnaires.

Employing various questionnaires (see Table S2), the majority of the studies evaluated depression [15,17–23,25–40,42,43,45] and anxiety [15,17–33,35–40,42,43,45]. Moreover, three papers [17,32,40] identified and quantified specific mood states, one study [24] explored personality traits, and another study [27] evaluated affectivity and thought control ability.

Only a small percentage of the studies included in the review used tests to assess cognitive and behavioural functioning (see table S2).

Huang et al.[15] used a neuropsychiatric interview during the preliminary screening to ensure the absence of psychiatric diseases, but these data were not used for correlational analysis with FC. Pang et al. [25] carried out a complete neuropsychological assessment. Dong et al. [27] explored visuospatial and executive functions, also investigating daytime alertness and sustained attention using the Psychomotor vigilance task (PVT) [53]. Finally, Liu et al.[31] assessed executive functions, sustaining and selective attention.

### *fMRI acquisition protocols*

The reviewed studies applied different protocols for fMRI acquisition (see Table S3). The scans duration ranged from four minutes to ~20 minutes among studies. However, eight papers [15,19,27,30,36,37,43,45] did not report the duration of the single acquisition scans. The basic instructions given to participants before the scans were partially similar among papers. Before the fMRI scan, many authors recommended participants to relax, lie quietly, as motionless as possible, and not systematically think about anything during the functional scan, being careful to remain awake [15-20,22-31,33-39,41-45]. In addition, in several studies the investigators asked subjects to avoid assuming activating (i.e., caffeine, alcohol, strong tea) or psychoactive substances for 6- [41,44], 24- [16,28], 48-hours [19,20,24,38,39] or one week [25] before the fMRI scan, to avoid daytime napping for the two days prior to the

acquisition [24] and to rest 30 minutes before the experiment [16]. The majority of the authors asked the participants to keep their eyes closed [15,16,18-23,25,26,28-31,33,35-39,42,43,45]. At the same time, one study [17] instructed subjects to wear black blinders and sponge earplugs to avoid audio-visual stimulus during the scan. On the other hand, in five studies [24,27,34,41,44] participants were encouraged to keep their eyes open and look at a fixation cross for the entire fMRI acquisition duration. Finally, two studies [32,40] did not report any specific instructions given to participants.

The reviewed studies applied different methods to control the vigilance state during the fMRI acquisition. Some authors required an oral report [19,20,24-26,29,32,35,38,42,45] or a short questionnaire [17,22,40] after the scans to control for the awake state and the degree of cooperation during the acquisitions. In three studies that asked the subjects to keep their eyes open [34,41,44], the participant' state during the scan was monitored via a camera. Only Regen et al. [18] recorded EEG data simultaneously to the fMRI acquisition in order to objectively verify the wakefulness state. In addition, Zhou et al.[39] evaluated the sleepiness state after the scan through the Epworth Sleepiness Scale (ESS). However, a large part of the revised studies [15,16,21,23,27,28,30,31,33,36,37,43] did not specify the tools and methods applied to control the vigilance state during the acquisition.

Concluding, Li M. et al.[43], after the fMRI acquisition, asked the subjects how they felt during the scan to control the effect of anxiety during the scan.

### ***Multiple comparisons methods***

Different statistical approaches can be applied to correct FC data when between-groups comparisons are performed. The two most appropriate and common methods applied in the reviewed studies were the False discovery rate (FDR) [17,25,27,33,34,44,38,41] and the Family-wise error rate (FWE) [19,24,29,45] corrections (see Table S4 and S5). Moreover,

four revised studies [20,35,37,39] used the Gaussian random field theory (GRF) approach, a widely applied method to control the FWE rate in fMRI studies.

Moreover, fMRI data can be corrected using simulation programs, such as the Monte Carlo simulation, adopted in one reviewed study [22], or the AlphaSim [15,26,28,31,36,43,42].

It is worth noting that not all reviewed studies applied multiple comparisons corrections for the between-group FC comparisons. Specifically, three papers reported both corrected and uncorrected results [30,32,40], and other three papers did not perform correction for multiple comparisons [18,21,23] (see Table S4 and S5). These data have been approached with great caution in the manuscript: uncorrected results have been underlined in the “Results” section and excluded from the “Discussion” section.

### *Connectivity analyses*

Methodologically, reviewed studies employed a wide variety of connectivity analyses that can be divided into node-based and voxel-based methods (see Table S1 for a description of the specific methods).

Node-based connectivity analyses were applied in 8 studies [20,23,30,32,33,37,42,44]. These methods are graph-based connectivity models represented by two concepts: nodes (i.e., different brain areas) and edges (i.e., connections between considered regions). On the other hand, voxel-based connectivity analyses, applied in 26 reviewed studies [15-22,24-29,31,32,34-41,43,45], estimate FC values between specific regions of interest (ROI) and all the other voxels in the whole brain, performing the spatial organization of large-scale resting-state networks (RSNs) [54]. Although these two methods share similar steps to define FC patterns, they differ in spatial scale. Voxel-based analyses allow the extraction of few but extended spatial networks, investigating differences between subjects (or groups) within the extracted networks. Instead, node-based analyses examine connections between a large number of nodes in a smaller space, comparing the functional connections between edges

across subjects. Information derived from these two approaches is different but complementary. Therefore, both are often applied in individual studies [55]. Specifically, three papers included in the systematic revision used node-based analyses followed by voxel-based analyses [20,32,37].

In the following paragraphs, we will report findings that emerged from the reviewed studies (see Tables S4 and S5), according to the FC analyses conducted.

### ***FC changes in ID: findings with node-based connectivity analyses***

#### *Small-world and nodal centrality indices*

Three reviewed studies [23,30,33] adopted the small-world and nodal centrality indices. The small-world network is one of the topological properties of brain networks. It may be described as the optimal network organization characterised by nodes highly connected locally with few long-distance connections [56] (see Table S1). One study [23] did not show significant differences in the small-world efficiency between IDs and HCs. However, another research [30] indicated a decreased number of modules and hierarchy in IDs on one hand and an increased network assortativity on the other, with a significant relationship between decreased network modularity and depression in IDs. Nevertheless, it should be noted that these results are not corrected for multiple comparisons.

Moreover, the study conducted by Ma et al. [33] reported increased network density and global efficiency (positively correlated with daytime sleepiness) but decreased local efficiency related to increased daytime sleepiness and ID severity. These indices describe the small-world network: modularity reflects the optimal functional segregation of brain networks into smaller modules, and nodal efficiency reflects the level of information spread out of a node with all other nodes in the network [57]. Nodal efficiency decreased in the left inferior frontal gyrus (IFG) triangularis, and it increased in the right fusiform gyrus (FG) [23], superior frontal gyrus (SFG), middle frontal gyrus (middleFG), superior temporal gyrus

(STG), cingulate gyrus (CG)/precuneus, thalamus, superior parietal lobe (SPL) and supramarginal gyrus. Moreover, subsequent analyses showed increased FC between insula and parietal prefrontal regions [33].

These findings in ID point to heterogeneity of changes in the small-world network and nodal centrality indices, suggesting dysfunctions in the global and regional topological and functional organization. As already underlined, the interpretation of small-world and nodal centrality results is limited due to the methodological and statistical weakness of most of the reviewed studies [23,30].

#### *Voxel-wise degree centrality analyses and functional connectivity strength*

As shown in Table S1 and S4, two studies [32,37] applied the degree centrality (DC) metric. This is another graph-theoretic measurement, useful to assess the topology of the architecture of the brain functional connectome at the voxel level, measuring the centrality of single nodes and reflecting information flow characteristics [58].

Evidence highlighted increased DC values in IDs within the right visual association cortex, right cerebellum posterior lobe (CPL) [32], and precuneus [37]. Instead, a decreased DC was observed within insula, left medial prefrontal cortex (mPFC) [32], left IFG [37], middle temporal gyrus (middleTG). Moreover, in both studies only decreased DC values showed significant correlations with clinical variables [32,37]. Nevertheless, using FWE or FDR corrections, the study conducted by Liu and collaborators [32] did not reveal any significant between-group difference in the DC values, which have emerged only using a more liberal uncorrected statistical threshold. In other words, the reported results were significant only if uncorrected for multiple comparisons.

A peculiar data-driven DC approach is the FC strength (FCS), useful to investigate strength abnormalities in connections across the whole brain [58] and applied in two studies [20,42] (see Table S1, S4). Both studies observed in IDs FCS reduction within left basal ganglia

(BG)/insula, right dorsolateral PFC (dlPFC), right mPFC, right cerebellum anterior lobe (CAL) [20] and right caudate nucleus [42]. Moreover, the reduced FCS within mPFC and right CAL were related to worse sleep quality [20], and reduced FCS within the right caudate nucleus was related to higher depressive symptoms [42]. However, only one study [42] showed an increased FCS in IDs localized within the right anterior insular cortex and left middleFG, positively correlated with PSQI score. FCS results showed disconnections (i.e., decreased FCS) between areas that composed principal RSNs, such as the fronto-parietal network (FPN) [20,42] and default mode network (DMN) [20]. At the same time, increased FCS was found in the insular cortex [42], a principal hub of the salience network (SN) and involved in vigilance and arousal processes [59]. However, it is necessary to underline that Huang and colleagues [20] adopted a weak, non-conservative and unusual voxel level p-value threshold (i.e.,  $< .01$ ). This represents a statistical limitation in the results interpretation.

#### *Dynamic functional connectivity*

Only one reviewed study [44] applied dynamic FC analysis, a metric to study how interactions between RSNs evolve over time (see Table S1). Results showed in IDs less FC variability between anterior SN/dorsal DMN and between anterior SN/left executive-control network (ECN). Moreover, decreased FC variability between anterior SN/left ECN was significantly related to more severe ID symptoms (see Table S4). This result highlighted in IDs aberrant FC between RSNs.

#### ***FC changes in ID: findings with voxel-based connectivity analyses***

##### *Seed-based correlational analysis*

Seed-based correlational analysis (SCA) is a hypothesis-driven approach aimed to estimate the whole-brain connectivity pattern of a ROI and how this pattern might change across



subjects (see Table S1) [55]. It has been applied in 20 reviewed papers [15–21,24,26,28,31–37,39-41,43].

In the first published SCA study in this field [15], amygdala-based connectivity analysis was performed in a small sample of patients compared to HCs. Results showed decreased FC between the amygdala and other brain areas, such as the insula, striatum, thalamus, right IFG, STG, caudate, lentiform nucleus. On the other hand, the authors showed increased FC between amygdala, sensorimotor and premotor cortex (correlated with worse sleep quality), precentral gyrus, left IFG, left postcentral gyrus (PoCG), left middleTG and middle occipital gyrus (middleOG), right cuneus, STG, and left globus pallidus.

Li and colleagues [16] used the parietal lobe as ROI showing in IDs a decreased connection between SPL/SFG and an increased resting-state FC between SPL and anterior/posterior cingulate, right splenium of the corpus callosum, right middleFG, and right claustrum. It is worth noting that the authors did not apply a correction for multiple comparisons on the FC analyses.

Two SCA studies [17,18] used regions included in DMN as ROI. Nie and co-workers [17] showed in ID decreased region-to-region FC between the mPFC/right medial temporal lobe and between the left medial temporal lobe/left inferior parietal cortices, without correlations of abnormal FC patterns with clinical features. On the other hand, Regen and colleagues [18] did not detect any differences in waking resting-state DMN connectivity between IDs and HCs. Still, the objective disturbances of sleep continuity and architecture were associated with higher waking FC between the retro-splenial cortex, hippocampus, and several DMN nodes. Zhou and colleagues [19] showed three main connectivity changes in IDs compared to HCs. The first finding was a significant FC decrease within the right temporal–occipital junction, left anterior midcingulate cortex (aMCC), right dorsal posterior cingulate cortex (PCC). Secondly, the authors observed a significant FC increase within the right hippocampus. Moreover, simultaneous enhancement and reduction of connections were observed within the

right PoCG, right BG, left orbitofrontal cortex (OFC), and right frontal operculum/insula. ID duration was negatively related to decreased FC between aMCC/right supplementary motor cortex (SMA), and PSQI was negatively correlated with decreased FC between OFC/STG, right BG/SFG, and left/right aMCC. PSQI also positively correlated with FC in the hippocampus and between right PoCG/bilateral SMA [19].

Huang and colleagues [20] used brain regions that revealed a FCS reduction in IDs (see the *Voxel-wise degree centrality analyses and functional connectivity strength* paragraph) as ROIs to explore FC differences between IDs and HCs. Taking right CAL as seed, authors demonstrated in IDs a significant FC decreased with CPL, bilateral BG/thalamus, SFG. Secondly, compared to HCs, left BG/insula showed decreased FC with right BG, left IFG/frontal operculum, SMA/SFG in IDs. Moreover, the right dlPFC exhibited a decreased FC with the right ventral premotor area/middleFG, while the right mPFC showed decreased FC with SFG and right IFG/middleFG. However, the authors did not carry correlational analyses between FC evidence and clinical variables and, as already mentioned in the *Voxel-wise degree centrality analyses and functional connectivity strength* paragraph, authors adopted an unusual voxel-level p-value ( $< .01$ ).

Li and colleagues [21] reported FC changes among diffuse brain regions in ID, in particular between the main RSNs (i.e. DMN, SN, sensory-motor network [SMN]). Moreover, these changes were associated with worse sleep quality, higher depressive traits and ID severity. However, it should be considered that results were uncorrected for multiple comparisons [21]. Using SCA, Pace-Schott and co-workers [24] showed a decreased resting-state FC between the left amygdala and the rostral portion of the anterior cingulate cortex (ACC) in a small sample of IDs. The IDs connectivity pattern was intermediate between HCs and GADs, suggesting that ID pathophysiology may be an intermediate condition between HC and GAD. Moreover, this FC decreased in ID was negatively correlated with ISI, PSQI, anxiety scores, and objective sleep latency but positively correlated with subjective sleep efficiency.

Using insula as seed, Wang and co-workers [26] found an increased FC between left insula/controlateral ACC, superior orb, bilateral thalamus, left precuneus, and decreased FC with left middleTG and right FG. Increased connectivity changes in ID were associated with depression (i.e., ACC, thalamus) and anxiety traits (i.e., ACC) [26]. This evidence was partially confirmed by another study [31] investigating insula FC patterns in subjects with sleep complaints as a subclinical category of ID. The study showed an increased FC between the right posterior insula/left PoCG in HCs with sleep complaints compared to HCs without sleep complaints. Considering all participants, the enhanced right posterior insula/left PoCG connectivity was related to more sleep disturbances and anxiety traits and negatively correlated with random errors during the executive functions assessment. However, results have to be considered with caution due to several limitations (i.e., small sample size, participants without ID diagnosis, absence of sleep questionnaires).

Two SCA [28,36] studies considered ACC as ROI to explore FC. Comparing ID patients with and without depression to HCs, Li G. and co-workers [28] showed positive FC correlations between ACC and CAL, culmen, lingual gyrus, and negative FC correlation between ACC and right inferior temporal gyrus (ITG), superior parietal gyrus/SPL, inferior parietal lobe (IPL), left limbic lobe/CG. An abnormal FC between ACC and left PCC positively correlated with more depressive traits in IDs without a depression diagnosis. On the other hand, comparing ID patients in comorbidity with depression and HCs, the authors showed a negative FC between ACC and middleTG, CPL, cerebellum Crus1 area, subcortical areas, which was negatively correlated with PSQI. Also, compared to IDs, patients with depression showed a negative correlation between ACC and left corpus callosum/PCC and a positive correlation with the midbrain. Yan and co-workers [36] conducted SCA analyses using ACC as ROI after voxel-mirrored homotopic connectivity (VMHC) analysis (see the *Voxel-mirrored homotopic connectivity analyses* paragraph). The authors reported an increased FC

between left ACC/right thalamus and between right ACC/left OFC in ID, without significant correlations with clinical variables.

Two other studies [39,40] performed SCA to illustrate FC changes between brain regions exhibiting VMHC abnormal values and the whole brain (see the *Voxel-mirrored homotopic connectivity analyses* paragraph). Zhou and colleagues [39] showed in ID a significant FC reduction between right and left middleOG/posterior middleTG, as well as between right middleOG/posterior middleTG and left precuneus/cuneus, that negatively correlated with state anxiety. Left middleOG/posterior middleTG showed decreased FC with right CAL, left lingual gyrus, left middleOG, left precuneus/cuneus, and increased FC with IPL. Such FC changes were negatively correlated with anxiety. Dai and colleagues [40], using mPFC as seed-region, showed in IDs decreased FC with CG and precuneus (bilateral mPFC/precuneus: negatively correlated with subjective sleep efficiency and total sleep time; right mPFC/precuneus: positively correlated with ID duration and SRSS). Moreover, considering left ITG as ROI, IDs exhibited a decreased FC with visual network and ECN; considering right ITG, a decreased FC with left middleFG and SFG was reported, with the latter positively correlated with depression traits. The bilateral PoCG was also taken as seed, showing decreased FC with lingual gyrus and cuneus (both positively correlated with ID duration), and with right CAL, left ITG (positively correlated with ID duration) and SFG.

Leerssen and collaborators [41] showed stronger FC between hippocampus/left middleFG in ID. Across all participants, individual differences in hippocampus/left middleFG connectivity strength were associated with more severe ID symptoms, lower subjective sleep efficiency, and higher wakefulness during the night. However, considering only ID participants, correlations between sleep parameters and FC disappeared when correction for multiple comparisons was applied.

Liu and colleagues [32] performed a SCA using regions exhibiting abnormal DC (see the *Voxel-wise degree centrality analyses and functional connectivity strength* paragraph) as ROI.

Authors reported increased FC between middleTG (located in the auditory-language comprehension region) and two ECN sub-regions (i.e., left temporal lobe, IPL) in IDs. Decreased FC in ID was observed between left middleTG, middleFG, and IFG, between two DMN sub-regions (i.e., left SFG, precuneus), as well as between left/right insula (positively correlated with anxiety score). FC between middleTG/left frontal lobe was positively correlated with ISI and negatively with negative mood states. It is important to underline that these results may be confounded by the significant levels of depression in IDs included in the study. With the same method, another study [37] showed decreased FC between middleTG/left PCC correlated with ISI. On the other hand, increased FC between the right precuneus/lateral occipital cortex was reported in ID.

Santarnecci and colleagues [34] used the occipital lobe as ROI, revealing increased FC with superior occipital lobe and decreased FC with bilateral temporal pole regions. Furthermore, positive connectivity between DMN/SMA was significantly correlated with the age of ID onset.

Wang and co-workers [35] focused on striatal FC in ID. Results demonstrated signs of both increased positive and negative FC within dorsal caudate and ventral striatum. Moreover, dorsal caudal putamen presented increased negative FC with IPL, negatively correlated with PSQI. However, these authors corrected the results of between-group comparisons for each subregion and not for the subregion number (i.e., 12). This choice entails results less conservative compared to a conventional correction for multiple comparisons and constitutes a limitation in the results interpretation.

One study [43] focused on reduced thalamus resting-state FC and several brain regions (i.e., ACC, orbitofrontal cortex, hippocampus, caudate, putamen) in ID. Moreover, decreased FC between left thalamus and ipsilateral putamen, caudate, hippocampus, and contralateral thalamus was negatively correlated with PSQI scores. Instead, decreased FC between the right thalamus/ACC was related to the self-reported depression severity.

As can be seen from SCA evidence, it is arduous to extract an unambiguous conclusion, probably due to the intrinsic nature of SCA. Indeed, this analysis considered only one system at the time and results strictly depend on focused seeds, basing on *a priori* assumptions. However, most of the results highlighted diffuse FC changes between brain areas that composed the main brain RSNs.

#### *Functional connectivity density analysis*

Another data-driven method performed in two studies [38,45] is the FC density (FCD). Contrary to other voxel-based analyses, which calculate one-to-one connections between regions or networks, FCD can estimate one-to-many relationships without regional hypotheses. From an interpretative perspective, higher FCD values indicate a more crucial role of the considered voxel in brain information processing. Furthermore, it is possible to measure two indices of FCD for each voxel: the local one (lFCD), assessing the size of the local cluster of correlated voxels, and the global one (gFCD), indicating the total number of correlated voxels [60] (see Table S1).

Yu and colleagues [38] reported increased gFCD in ECN, SN, dorsal attentional network (DAN), visual path, and within anterior DMN (aDMN) in ID. Increased gFCD within SN was associated with more severe insomnia symptoms, whereas increased gFCD in the visual network was associated with greater anxiety symptoms. Moreover, results revealed decreased gFCD in the posterior DMN (pDMN), specifically in the precuneus associated with more depressive traits in ID. However, the correlation between gFCD values and clinical variables disappeared after multiple comparison corrections. The second study [45] that used FCD analysis reported lower lFCD and gFCD in IDs located in the thalamus, insula, ACC, OFC, caudate, putamen. Moreover, the reduced gFCD between right insula/controlateral ACC was related to anxiety symptoms and might partially mediate the relation between ID severity and anxiety.

These results showed in IDs major FCD changes, characterised by a substantial decrease between the brain regions that composed DMN and SN.

### *Independent component analyses*

Pang and colleagues [25] adopted independent component analyses (ICA), a data-driven exploratory method able to describe different multiple RSNs with the advantage to reveal hidden factors not depending on *a priori* seed definition (see table S1) [55]. The authors showed 105 pairs of significant FC differences in IDs compared to HCs. Among these differences, decreased positive correlations in ID were mostly located in the frontal and prefrontal lobe, subcortical areas, and in key nodes of DMN (i.e., mPFC and PCC), and two pairs (i.e., left SFG/hippocampus; left orbital middleFG/right middleTG) demonstrate increased positive correlations. Moreover, the authors found increased negative correlations in IDs between the frontal lobe and mPFC, middleTG, orbital IFG, precuneus, ITG, cerebellum, SMA. Conversely, decreased negative correlation was observed between the temporal lobe and middleTG, thalamus, FG, cerebellum. Moreover, the authors demonstrated that the magnitude of the decreased positive correlation between left IFG/SMA was associated with worse sleep quality, and increased FC between left amygdala/cerebellum was related to ID severity. This study also showed a relationship between FC changes in ID and cognitive variables. Specifically, the global cognitive functioning in ID was positively correlated with the strength of positive FC decrease between IFG/vermis, SFG/cerebellum, positive FC increase between SFG/hippocampus, and negative FC increase between SFG/ITG [25]. However, while the ICA can be applied on the single-subject level, group-ICA analyses (see Table S1) are more appropriate to assess group differences [55]. This approach has been applied in two reviewed studies [27,29]. Dong and collaborators [27] showed in ID a significant FC increase between DAN/FPN associated with a decline in sustained attention performance and decreased FC between aDMN/pDMN. The second study [29] compared IDs

and HC FC within 10 RSNs, assuming significant differences between two groups. In contrast to Dong et al., [27], authors reported reduced FC within right FPN in IDs and reduced FC between FPN and right middleTG, lateral occipital cortex positively correlated with ID duration and anxiety, respectively. However, a statistical limitation in the analyses should be considered: besides a FWE correction for each RSN was adopted controlling for multiple comparisons, results were not corrected for the fact that 10 RSNs were analysed [29].

Similar to the ICA approach, another FC metric adopted in a single study [34] is the intrinsic connectivity contrast (ICC) that allows taking into account the strength of connections between brain regions and producing voxel-based connectivity maps without the need for *a priori* assumptions [61]. Authors highlighted in IDs compared to HCs increased ICC patterns in the bilateral occipital lobe. Moreover, increased ICC values in DMN were predicted by the age of ID onset [34].

Evidence in studies adopting ICA approaches is heterogeneous and contradictory, probably due to the methods' limitations. Indeed, results with ICA may change depending on the components extracted in the single analysis.

#### *Voxel-mirrored homotopic connectivity analyses*

Four reviewed studies [22,36,39,40] employed VMHC analysis, a recent validated approach that allows correlating each voxel in one hemisphere with its mirrored counterpart in the opposite one, reflecting the interhemispheric coordination [62].

Results in healthy subjects with ID symptoms reported higher VMHC values within bilateral thalamus/posterior insula, FG, and middle CG, compared with HCs without ID symptoms. A significant positive correlation was found between VMHC values in ACC and sleep disturbance in all participants, with and without ID symptoms [22].

Moreover, in ID, higher VMHC values emerged within ACC [36,39] (associated with increased sleep latency and depression [36]), PCC, precuneus, primary visual cortex,



precentral gyrus [39] and within the visual network (i.e., PoCG), negatively correlated with positive mood states [40]. On the other hand, decreased VMHC values were observed within middleOG/posterior middleTG [39], DMN and ECN [40]. However, in the study conducted by Dai and colleagues [40] any significant between-group differences in the VMHC values were revealed using the GRF correction. The reported results were significant only if uncorrected for multiple comparison.

These results suggest that in ID, in addition to the intraemispheric FC aberrations, disturbance in the interhemispheric functional coordination involving the spatial organization of large-scale RSNs may occur.

## **Discussion**

In the light of available results, and according to the view of ID as a global disorder involving the interregional neuronal coordination of multiple RSNs, evidence from the reviewed studies will be discussed focusing on FC changes within and between the main RSNs investigated. Moreover, we will examine the meaning of these findings in light of hyperarousal theory to explain ID mechanisms and symptoms [4].

As previously observed, some of the studies included in this systematic review did not correct for multiple comparisons FC analysis between IDs and HCs, making data interpretation difficult (see the Multiple comparisons methods and Limitations sections). For this reason, findings affected by this limitation have been excluded from the discussion.

### ***Default-mode network***

Most of the reviewed studies focused on the DMN, a RSN involved in many cognitive processes, including mind-wandering, self-referential mental activity, and autobiographical memory retrieval [63]. The interest concerning DMN-connectivity can be explained by the

overlap between cortical and subcortical brain areas involved in the ID pathophysiology and regions that constitute the DMN.

Overall, the present review points to heterogeneous findings about the direction of FC changes involving DMN in ID (see Figure 1). Several studies showed decreased FC borne by several DMN subregions [17,19,25,28,32,45], particularly in the direction of a relative PFC disconnection [17,20,25,32]. This evidence is consistent with findings reporting structural impairments of the PFC in ID [64,65] and the key role of this area mediating healthy sleep processes [66].

Moreover, many studies suggest distinguishing anterior and posterior components of DMN involved in different cognitive functions. According to this view, the balance between these components sustains healthy sleep, emotion, and cognition [67]. Reviewed studies highlighted a significantly decreased aDMN-pDMN connectivity in IDs [20,27,38]. Previous evidence points to damaged FC between aDMN-pDMN in depressed individuals [68], suggesting partially similar pathological mechanisms in ID and depression. Thus, this finding may disclose impairments in affective and emotion regulatory processes consistent with the relationship between ID and depression or anxiety disorders [7]. Although these studies are methodologically robust for the most part, three of them included medicated patients [17,27,40]. Moreover, the study conducted by Huang and colleagues [20] as already discussed, adopted a weak and non-conservative cluster level p-value threshold. Indeed, this cluster–extent high threshold provides low spatial specificity producing ambiguous neuroscientific maps and inferences. To avoid this pitfall, a cluster-extended thresholding of  $p < .001$  is recommended [69]. These are relevant aspects that could affect FC results.

On the other hand, seven reviewed studies reported increased FC between DMN subregions [19,22,33,34,36,39,41] (see Figure 1). It is worth noting that this RSN is deactivated in the presence of specific tasks when attention is directed to external stimuli. At the same time, it is activated during internal-oriented attention conditions [63]. In line with the view of a

physiological hyperarousal state, increased FC within DMN in IDs might explain the disorder's self-referential nature, indicating heightened sensitivity and self-awareness, which could potentially affect the processes of falling asleep [1].

It is worth noting that among the reviewed studies showing increased FC within DMN, only three papers have received a good qualitative assessment score [19,33,41] (see Figure S2). Instead, one of these studies [22] was conducted with a subclinical category of IDs and three studies [34,36,39] had a small sample size.

Summarizing, despite the numerous findings regarding FC changes within the DMN, the available data in ID are not enough to reach an unambiguous conclusion about their significance. We can only conclude that the hyperactivity states of the DMN observed in ID seems to be directly associated with higher levels of self-referential and introspection processes, worry, and rumination [20,22,33,34,36,39,41].

### ***Salience network***

The SN is a RSN consisting of limbic brain regions (i.e., amygdala, insula, ACC) engaged in a broad spectrum of cognitive processes, such as salience filtering and executive control, and maintaining vigilance and arousal [59]. In line with the relationship between increased activity within the SN and higher stress and arousal levels in healthy adults [70], most of the reviewed studies showed increased FC within SN [22,26,33,38,42] (see Figure 1). Due to the key role of this network in the sustained sensory processes of environmental and proprioceptive stimuli, the increased SN connectivity observed in ID may implicate a reduced ability of this RSN to disengage from information processing of external stimuli.

Anterior insula, the SN's principal hub, plays a crucial role in cognitive and emotional processes [71]. Insula aberrations may be considered markers of pathological anxiety [72], and insular cortex activity has a key role in integrating emotional and bodily states [73].

Impaired connectivity patterns between this area and other regions could underlie cognitive,

vigilance, and perception dysfunctions, as well as subjective distress and sleep complaints [74]. Reviewed studies showed that elevated FC within SN correlated with anxiety and depression traits in ID [26,31,38], supporting the notion of emotional hyperarousal in ID. Consequently, the significance of these results suggests that increased FC within SN may be part of the functional substrate for the observed emotional and interoceptive awareness in ID, which leads to more severe insomnia. However, ID subjects enrolled in these studies [26,31,38] showed significantly higher anxiety and depression levels than HCs and above the clinical cut-off. As a consequence, since between-groups FC differences were not controlled for anxiety and depression levels, we cannot exclude that the elevated FC within SN is a direct consequence of the anxiety and depression and that it is not due to ID. Despite these notable findings, three of these studies are methodologically weak due to the participation of HCs with ID symptoms [22,31], and this issue does not allow to generalize their results to the clinical population. Moreover, one study reported conflicting results, highlighting signs of decreased FC in the SN. However, according to previous evidence, the study proved the mediator role played by the insula in the relation between anxiety traits in ID patients and sleep disorders severity, suggesting that sleep complaints probably affect the insula's role in emotional processes, leading to anxiety and depression [45].

### ***Fronto-parietal or executive-control network***

FPN, also called ECN, is composed of a set of brain areas (i.e., dlPFC, middleFG, middleTG, parietal lobe, CG, ACC) involved in working memory, decision making, top-down goal-directed control processes [75], and it is central in anxiety disorders [76].

Despite two studies showed increased FC indices within the FPN [38,42], other findings highlight reduced connectivity [20,29,32,39] (see Figure 1). Specifically, these studies showed a relation between the reduced intrinsic FPN connectivity and depression, anxiety [29,39], and post-insomnia negative emotions [32]. These findings suggest that impaired FC

within FPN may be a determining factor in ID aetiology. This pattern of impaired connectivity may be linked to the emotion dysregulation observed in these patients. Moreover, several authors suggested that impaired connectivity within FPN may be the functional substrate of daytime cognitive and memory impairments reported in ID [20,29,32,39]. Indeed, the dlPFC and the middleTG, two hot nodes of FPN that exhibited decreased FC in IDs, are critically involved in executive functions, decision-making, working memory, and attentional processes [77]. These hypotheses are speculative since cognitive functions were never directly assessed in these studies. Moreover, three of these studies [20,29] have made statistical choice of results corrections poorly conservative. As consequence these results should be read and interpreted with caution. Despite this, FC results are broadly in agreement to proposed decreased FC between FPN sub-areas as the functional substrate of cognitive and emotional impairments reported in ID.

### ***Sensory-motor network***

SMN comprises motor (i.e., precentral gyrus), somatosensory (i.e., PoCG) regions, and SMA, playing a role in the sustained sensory processes of environmental stimuli and proprioceptive information [78]. Several reviewed studies agreed to report increased FC within SMN in ID [19,39,40]. Moreover, a relationship between increased connectivity in specific areas located in SMN (i.e., PoCG) and longer ID duration [40] has been reported (see Figure 1). These findings may indicate that suffering from sleep complaints for a longer time is closely associated with higher FC in the SMN.

The reviewed studies also suggest ID-related increased FC in the visual pathway [15,22,34,38,39], canonically belonging to SMN [78]. Specifically, increased gFCD [38] and VMHC [22] values have been observed in FG, a brain area involved in the processing of visual stimuli and modulation of mood in depression [79]. Although these findings are in line with previous evidence of altered fusiform activity in ID [80], they should be considered with

caution since two of these studies included healthy participants with poor sleep quality [22,23].

Overall, this neurophysiological framework suggests a reduced ability of brain areas to disengage from information processing of external stimuli. Indeed, the increased FC within SMN in ID may be linked to the greater sensitivity to external sensory stimulation during rest or sleep, connected to difficulty in initiating or maintaining sleep, supporting the notion of hyperarousal as predisposing and perpetuating factor in ID.

### ***Dorsal-attentional network***

DAN is a RSN activated during attention-demanding tasks and composed of several regions responsible for top-down control and goal-directed attentional processes [81]. Three studies evidenced in ID hyperconnectivity (i.e., increased FC) within the DAN [19,28,38] and one node-based study showed decreased nodal centralities in opercular and triangular IFG [30] (see Figure 1). These two DAN sub-regions are involved in slow-wave sleep regulation, with a key role in healthy sleep [82]. Therefore, these findings might also suggest a hyperarousal state that involves DAN affecting sleep processes and resulting in cognitive daytime impairments observed in IDs. However, these conclusions remain speculative.

### ***Between resting-state networks***

Together with results showing aberrant FC within RSNs, several studies highlighted between-networks changes in ID [25–27, 34,35,37,44] (see Figure 1). Interesting findings suggest that brain patterns related to wakefulness, attention, introspection, saliency, and sensory-motor functions showed increased FC with each other in ID, resulting in a state of hyperemotion, hypersensorimotor, and a general hyperarousal [26,27,32,34,35,37]. Dong and colleagues [27] showed increased DAN-FPN connectivity associated with worse performance at the PVT, one of the most used tasks in sleep-related research to investigate sustained attention [53]. Poorer

PVT performances are associated with sleep loss and daytime sleepiness [83]. Consequently, insufficient nightly sleep, daytime sleepiness, and impaired sustained attention complained by ID patients may be a consequence of increased DAN-FPN connectivity. A relevant limitation in the evidence reading is represented by the non-conservative results of between-group comparisons applied in the study conducted by Wang et al., [35].

On the other hand, these regions that play inhibition mechanisms between them showed decreased FC in ID patients [37,44]. Particularly, the SN modulates the activation and deactivation of other RSNs, such as DMN and FPN. Reduced FC between SN and these RSNs in ID may result in hyperactivation of DMN and FPN. This pattern may represent the functional base of increased self-referential and attentional processing during rest. Moreover, the same findings may disclose ID-related impairments in switching between RSNs in response to changing needs [44]. This hypothesis is sustained by previous fMRI studies showing an unsuccessful reduction of activity within the FPN and DMN in ID during working memory tasks [84].

### **Limitations**

It is difficult to determine the causal relationship between ID and aberrant FC. Indeed, the cross-sectional design of all reviewed studies does not give any information on the temporal relationship between FC reorganization and the progression of the disorder. Moreover, most studies included in our systematic revision did not evaluate cognitive and emotional functions (see Table S2). Consequently, it is difficult to indicate the relationship between FC alterations in cognitive and emotional pathways and functional deficits observed in these patients.

Also, only a few studies employed objective sleep measures, which could be fundamental to better understand the subjects' sleep status and disclose potential relations with FC alterations revealed in ID.

Despite all the studies adopting similar and substantially appropriate fMRI acquisition protocols, some criticism can be directed to the methods applied to control the vigilance state during the scans. Indeed, the most reliable method to ensure the awake state during the scan is the simultaneous EEG recording during the fMRI acquisition, applied only in one study [18]. During the scan in eyes-open condition, video monitoring can be considered a fairly adequate method, which was used only in three studies [34,41,44]. However, most of the revised studies adopted methods that do not objectively ensure wakefulness during the scans. Vigilance state during the scan is a potentially relevant source of great variability among subjects, and it should always be considered for the interpretation of fMRI findings. Indeed, the onset of sleep during the scan may impact the findings because sleep alters the FC measures [85,86]. This procedural bias precludes any generalization concerning fMRI findings due to the lack of objective discrimination between wake and sleep. Moreover, the emotional state during the scans and the high variability among subjects also potentially affected the fMRI data and the brain FC. However, only one study [43] asked how the subjects felt during the scan to eliminate the effects of anxiety. No revised study administered a structured and standardized questionnaire to evaluate the emotional state during fMRI acquisition.

A relevant research issue in the insomnia field is whether the observed FC changes may be primarily related to connectivity differences in patients and healthy controls or whether these changes may be ascribed to depressive and anxiety symptoms in insomnia. Although most of the reviewed studies ruled out patients with depressive/anxiety disorder, some included patients with anxiety and depressive levels significantly higher than HCs. Moreover, reviewed studies have not always considered this issue during the connectivity analyses to exclude the effect of depressive/anxiety traits on FC changes. Therefore, it is difficult to establish if and to what extent anxiety/depressive symptoms may have affected the observed FC pattern in



insomnia. For these reasons, future research should investigate the relationship between brain connectivity and psychological traits in ID, especially through longitudinal studies.

The heterogeneity in the ID definition is evident in the reviewed studies (see Table 1), which may be explained by an uneven definition of the disorder reported in the various versions of principal nosological classifications of mental and sleep disorders. Indeed, ICSD-2 [48] delineated numerous primary and secondary insomnia subtypes. Later, the ICSD-3 [3], according to DSM-V [2], has followed the decision not to subtype ID, simplifying its classification in more inclusive diagnostic labels. The specific methods and criteria employed to assess the ID diagnosis are relevant since they drive patients' phenotyping. The ID phenotyping is more than a formal manner to define the disorder since it is driven by the symptoms, which might be the basis for differences in neurophysiological brain mechanisms. Moreover, PSG represents the only objective method to exclude insomnia which is secondary to other sleep disorders. However, only six studies [15,18,24,25,41,44] included in this systematic review were able to objectively exclude the presence of other sleep disorders using PSG recordings. This lack of objective sleep assessment could have produced relevant biases since the reported FC findings may be due to other sleep disorders.

Several statistical limitations have arisen from the revised studies. The most critical issue is the presence of connectivity results partially or totally uncorrected for multiple comparisons [16,18,21,23,30,32,40]. Moreover, as already mentioned in the results and discussion sections, three studies [20,29,35] adopted inappropriate methods and non-conservative p-value thresholds to correct for multiple comparisons. The multiple testing comparison is a common statistical problem in neuroimaging studies due to the large number of tests performed. Results without proper corrections lead to problematic interpretations because it is hard to monitor false positives among the findings and determine which results reflect true connections.

Finally, no revised studies preregistered the ROIs selected for the analyses and the research designs, a good and useful scientific practice which is spreading to avoid the risk to produce inflated or false positive results [87]. This is a relevant gap in the field of FC study in ID that future studies should bridge.

## **Conclusions**

ID has been studied in depth in recent years, as shown by the large amount of fMRI FC studies that emerged from this systematic review. Overall, FC evidence suggests that ID symptoms are associated with aberrant intra- and inter-hemispheric interactions of brain areas related to the hyperarousal, salience, sensory-motor, cognitive and self-referential processes. These results support the hypothesis that ID is characterised by a disrupted topological organization of the brain functional connectome, and it may result in a functional decline in sleep, cognition, emotion, and memory. However, we observed a wide methodological heterogeneity between the reviewed studies, making it difficult to provide a univocal framework of ID pathophysiology. Moreover, this systematic revision of the literature revealed serious limitations in methodological and statistical terms (i.e., lack of cognitive and objective sleep assessment; absence of objective vigilance state control during the fMRI scans; no exclusion of other sleep disorders or depressive/anxiety symptoms in the ID samples; heterogeneity in the ID definition and diagnosis between studies; unsuitable methods and thresholds to control for multiple comparisons) in the field of the FC research in ID. We highlight that further efforts in this field are needed to increase our knowledge about the functional significance of the observed FC alterations by longitudinal observations, larger sample sizes, appropriate statistical approaches, objective sleep assessment, and FC-EEG measures.

## **Practice Points**

- Increased functional connectivity within default-mode network observed in insomnia patients might explain the disorder's self-referential nature, indicating the heightened sensitivity and self-awareness which could potentially affect the processes of falling asleep.
- Increased functional connectivity observed in insomnia disorder within salience and sensory-motor networks may implicate a reduced ability of brain areas to disengage from information processing of external stimuli, and it could underlie cognitive, vigilance, and perception dysfunctions.
- Decreased connections between fronto-parietal network sub-areas seem to be the functional substrate of cognitive and emotional impairments reported in insomnia disorder.
- Increased dorsal-attentional network connectivity seems to affect sleep processes and result in cognitive daytime impairments observed in insomnia patients.
- Brain patterns related to wakefulness, attention, introspection, saliency and sensory-motor functions showed increased connections with each other, resulting in a state of hyperemotion, hypersensorimotor and a general hyperarousal.
- Data agree with the hyperarousal theory of insomnia: a chronic state that pervades patients around the clock and interferes with sleep processes.
- Functional connectivity aberrations during rest seem to be closely associated with sleep complaints and daily cognitive and emotional dysfunctions observed in insomnia patients.
- Insomnia disorder should be considered a global disorder involving the interregional neuronal coordination of the main resting-state brain networks.
- The inconsistency among some of the results seems to be caused by the clinical heterogeneity of insomnia disorder, differences in functional connectivity analyses, and experimental designs adopted in the different studies.

## Research agenda

- Longitudinal designs are needed to outline the possible temporal relations between insomnia symptoms and functional connectivity changes.
- The use of neuropsychological batteries, cognitive-behavioural tasks, and objective sleep measures would help clarify the functional significance of the observed functional connectivity changes in insomnia disorder.
- Future researchers in insomnia disorder should use quantitative electroencephalographic measures to investigate functional connectivity during wakefulness and sleep useful for exploring peculiar changes in brain functionality in this condition.
- Future studies in the field of functional connectivity in insomnia disorder should preregister their protocols to improve the quality and transparency of the research and to reduce false-positive findings.
- Limitations and heterogeneity in terms of acquisition protocols in the functional magnetic resonance and clinical characterization of the samples emerged from this systematic review, suggest the need for common research designs and protocols to provide homogenous evidence in the insomnia disorder framework.

## Conflicts of interest

The authors do not have any conflicts of interest to disclose.

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\*The most important references are denoted by an asterisk.

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**Table 1. Samples characteristics**

Study	Participants					
	Group = n	Age (mean±SD)	Gender (M/F)	Medications for ID	ID duration (mean±SD)	ID severity (ISI score) (mean±SD)
Huang et al. 2012 <sup>15</sup>	PI=10 HC=10	37.5±12.3 35.5±8.67	5/5 5/5	X	--	13.70±2.63* 2.10±1.10*
Li et al. 2014 <sup>16</sup>	PI=15 HC=15	41.3±8.9 39.8±11.2	7/8 7/8	X	--	--
Nie et al. 2015 <sup>17</sup>	PI=42 HC=42	49.2±12.2 49.1±10.2	15/27 18/24	23: ✓/19: X	>2 months	15.3±3.77 --
Regen et al. 2016 <sup>18</sup>	PI=20 HC=20	42.7±13.4 44.1±10.6	8/12 8/12	X	9.4 ± 10.0 years	12.8±3.1 1.6±1.9
Zhou et al. 2016 <sup>19</sup>	CPI=29 HC=29	43.1±11.4 41.6±11.5	10/19 --	X	9.51 ± 2.93 years	--
Huang et al. 2017 <sup>20</sup>	CPI=27 HC=26	40.0±11.6 41.1±11.6	10/17 10/16	X	10.68 ± 8.27 years	--
Li C et al. 2017 <sup>21</sup>	ID=50 HC=40	39.6±10.4 39.2±8.4	21/29 10/30	--	40.3 ± 44.09 months	12.56±2.96 5.78±2.34
Li X et al. 2017 <sup>22</sup>	IS=27 NIS=27	38.3±11.8 38.1±11.6	12/15 17/10	X	--	--
Lu et al. 2017 <sup>23</sup>	IS=30 NIS=62	38.0±11.8 37.4±11.9	15/15 26/36	--	--	--
Pace-Schott et al. 2017 <sup>24</sup>	PI=13 GAD=12 HC=13	35.2±15.7 30.2±10.3 35.0±15.0	2/11 2/10 2/11	X	--	18.83±3.79 -- 0.77±1.67
Pang et al. 2017 <sup>25</sup>	CPI=39 HC=28	52.2±11.7 51.2±12.4	6/33 5/23	X	>3months	15.52±0.52 0.59±0.62
Wang et al. 2017 <sup>26</sup>	PI=57 HC=46	39.5±10.7 39.9±9.2	20/37 14/32	X	>1months	--
Dong et al. 2018 <sup>27</sup>	ID=31 HC=32 PI=15	44.8±11.6 42.0±11.6 37.1±2.5	14/16 14/16 6/9	9: ✓/22: X X	≥6 months - ≤1year ≥1 year	17.07±3.86 1.87±1.59

Li G et al. 2018 <sup>28</sup>	PID=15 HC=15	40.5±1.9 32.60±2.5	7/8 7/8		≥1 year	--
Li S et al. 2018 <sup>29</sup>	PI=59 HC=53	39.7±10.6 39.7±8.8	27/32 21/32	X	23.81 ± 29.73 months	19.59±3.22 5.90±1.07
Li Z et al. 2018 <sup>30</sup>	CPI=45 HC=32	41.4±10.8 38.1±9.9	38/7 22/10	X	≥3 months	--
Liu CH et al. 2018 <sup>31</sup>	IS=25 NIS=25	37.1±11.4 34.3±13.4	15/10 14/11	X	--	--
Liu X et al. 2018 <sup>32</sup>	PI=48 HC=28	46.4±12.6 45.6±12.5	16/32 23/25	X	4.96 (years) ± 5.25	17.83±2.94 1.54±1.4
Ma et al. 2018 <sup>33</sup>	PI=36 HC=38	38.6±9.5 37.7±9.9	12/24 12/26	X	28.61 (months) ± 43.58	17.28±6.70 1.39±2.40
Santarnecci et al. 2018 <sup>34</sup>	PI=17 HC=17	18 - 45y (range) Not specified	-- --	X	--	18.45±5.16 --
Wang et al. 2018 <sup>35</sup>	ID=18 HC=16	21.9±3.3 22.4±3.1	5/13 6/10	X	≥1 month	17.3±1.6* 1.3±1.7*
Yan et al. 2018 <sup>36</sup>	PI=26 HC=28	48.8±12.0 49.07±11.8	11/15 12/16	X	17.62 (months) ± 3.80	14.38±2.48 --
Yan et al. 2018 <sup>37</sup>	PI=26 HC=28	48.8±12.0 49.07±11.8	11/15 12/16	X	17.62(months) ± 3.80	14.38±2.48 --
Yu et al. 2018 <sup>38</sup>	PI=48 HC=51	38.9±11.7 41.3±13.6	19/29 29/29	--	12.61 (months) ± 12.70	13.97±2.01* --
Zhou et al. 2018 <sup>39</sup>	CPI=29 HC=30	42.0±11.3 40.0±11.2	19/10 20/10	X	11.74 (years) ± 9.38	--
Dai et al. 2019 <sup>40</sup>	PI=48 HC=48	46.4±12.6 45.6±12.5	16/32 23/25	X	4.96 (years) ± 5.25	--
Leerssen et al. 2019 <sup>41</sup>	ID=65 HC=65	48.3±14.0 44.1±15.2	17/48 23/42	X	20.2 (years) ± 15.9	16.3±5.2 4.2±4.3
Li C et al. 2019 <sup>42</sup>	PI=38 HC=48	40.6±9.4 39.9±9.4	16/22 11/33	X	12.61 (months) ± 12.70	19.32±3.09 5.43±2.46
Li M et al. 2019 <sup>43</sup>	PI=27 HC=39	42.1±9.3 41.0±9.1	9/18 16/23	X	--	17.30±6.44 --
Wei et al. 2020 <sup>44</sup>	ID=65 HC=64	21–69y(range) 22–70y(range)	17/48 23/42	X	--	16.29±5.16 4.19±4.28
Zhu et al. 2020 <sup>45</sup>	PI=27 HC=32	41.8±9.6 40.9±8.7	9/18 12/20	X	--	17.28±6.74 --

Abbreviations: Chronic primary insomnia (CPI); Female (F); Generalized anxiety disorder (GAD); Healthy controls (HC); Healthy subjects with insomnia symptoms (IS); Healthy subjects without insomnia symptoms (NIS); Insomnia disorder (ID); Male (M); Paradoxical insomnia (PARA-I); Primary insomnia (PI); Primary insomnia with depression (PID); Psycho-physiological insomnia (PSY-I).

Note: \*ID severity assessed by PSQI

X = absence of medications; ✓ = presence of medications.

**Figure 1. Main FC changes within and between principal RSNs in ID.** The figure shows the main evidence of FC changes in ID. Data referring to subjects with sleep complaints (a subclinical category of ID) and findings not corrected for multiple comparisons were excluded from the figure.

Legend: The green rings represent the principal RSNs affected in the ID: Default-mode network; Salience network; Fronto-parietal network; Sensory-motor network; Dorsal-attentional network.

Red circles point to areas with increased FC; blue circles point to areas with decreased FC. Circles within the green rings represents sub-regions of the RSN; circles out from the green rings represents brain regions outside the RSN.

Red thin arrows mark the direction of the increased FC within the RSNs; blue thin arrows mark the direction of the decreased FC within the RSNs.

Red large arrows mark the direction of the increased FC between RSNs; blue large arrows mark the direction of the decreased FC between the RSNs.

Abbreviations: Anterior cingulate cortex (ACC); Anterior default-mode network (a-DMN); Cerebellum anterior lobe (CAL); Default-mode network (DMN); Dorsal-attentional network (DAN); Dorsolateral prefrontal cortex (dlPFC); Frontal gyrus (FG); Fronto-parietal network (FPN); Hippocampus (Hipp); Inferior frontal gyrus (IFG); Inferior frontal gyrus opercularis (IFGoperc); Inferior frontal gyrus triangularis (IFGtri); Inferior temporal gyrus (ITG); Midcingulate cortex (MCC); Occipital gyrus (OG); Occipital lobe (OL); Orbital part of frontal lobe (ORB); Orbitofrontal cortex (OFC); Post-central gyrus (PoCG); Posterior default-mode network (p-DMN); Pre-central gyrus (PrCG); Prefrontal cortex (PFC); Premotor area (PM); Salience network (SN); Sensory-motor network (SMN); Superior frontal gyrus (SFG); Superior parietal lobe (SPL); Temporal gyrus (TG).

