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A WORKING MODEL ON LARGE-SCALE SPATIO-TEMPORAL ORGANIZATION OF BRAIN FUNCTIONING AND ITS IMPLICATIONS FOR BIPOLAR DISORDER

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SUMMARY

PART B

A WORKING MODEL ON LARGE-SCALE SPATIO-TEMPORAL ORGANIZATION OF BRAIN FUNCTIONING AND ITS IMPLICATIONS FOR BIPOLAR DISORDER

PART A

FUNCTIONAL ORGANIZATION OF THE SENSORIMOTOR SYSTEM AND PSYCHOMOTRICITY IN BIPOLAR DISORDER

INTRODUCTION

Background

Bipolar Disorder (BD) is defined by the occurrence of recurrent episodes of mania and depression characterized by opposite constellations of affective, cognitive and psychomotor symptomatology [1-2]. Among other dimensions, psychomotor disturbances assume a central clinical role in BD [3-7]. In the typical patterns, mania is characterized by excitation of psychomotricity, as manifest in tendency to act, impulsivity and hyper-activity, while depression is characterized by inhibition of psychomotricity, as manifest in poor motricity and retardation; however, mixed features can frequently occur, mainly in the depressive phase [1-2, 6, 8].

Physiologically, at a neuronal level, sensorimotor and psychomotor functions are related to the sensorimotor system [9]. Coherent neuronal oscillations across distributed brain areas in the low frequency band <0.1Hz (which are indirectly investigated in resting state functional magnetic resonance imaging - fMRI) consistently organize the large-scale resting state networks, such as the sensorimotor network (SMN) [10-23]. The SMN comprises the strongly interconnected sensory, motor and premotor cortical areas, and is involved in sensorimotor functions [10, 20-23]. The cortical areas of the SMN are reciprocally connected, both structurally and functionally, with the thalamus [9, 24]. Since synchrony in neuronal oscillations are fundamental to coordinate the communication between different brain regions and to information processing [10-12, 16, 23, 25], the functional coupling between SMN and thalamus, as measured by fMRI functional connectivity (FC), can be central in the transfer and integration of sensory inputs and motor outputs and thus in the sensorimotor processing, and it has been shown to be behaviorally relevant [26].

The thalamo-cortical sensorimotor system is connected with and modulated by the basal ganglia within the cortico-basal ganglia-thalamo-cortical sensorimotor loop [27]. In turn, basal ganglia and thalamic regions of the loop are strongly connected

with and differently modulated by the brainstem neurotransmitter systems, such as the dopamine (DA) and serotonin (5HT) systems - which are key neuromodulators involved in the sensorimotor/psychomotor functions. In particular, both preclinical and clinical data quite consistently suggest that impulsive behavior - that can be defined as the tendency to act in relation to sensory stimuli and, thus, a core dimension of psychomotricity – is enhanced by DA (although possibly with a non-linear relationship), and inhibited by 5HT [28]. The DA-related substantia nigra (SN) shows strong connections, both anatomically and functionally, with regions of the subcortical-cortical sensorimotor loop and SMN, such as the striatum, globus pallidus, and sensory and motor cortices (and these functional connections, in turn, are also related to impulsive behaviors) [29-38]. Moreover, the administration of pro-dopaminergic substances increases the FC between subcortical and cortical sensorimotor regions, as well as the activity of SMN (as measured by the related increase in temporal variability of BOLD signal); while the administration of anti-dopaminergic substances decreases the FC between the same sensorimotor areas [39-41]. On the other hand, the 5HT-related raphe nuclei (RN) shows strong FC (coherently with its anatomical connections) with the subcortical-cortical sensorimotor areas [42-44]. Interestingly, RN shows a positive FC with basal ganglia and thalamus while negative FC with somatosensory and motor cortices [44]. Coherently, 5HT and SMN activity are inversely related, i.e. the decrease in 5HT availability is associated with an increase in activity of the SMN (as measured by a related increase in the amplitude of low frequency fluctuations of BOLD signal at rest and by increased activation during tasks) [45-46]. However, the physiological functional relationship between the cortical SMN and thalamus, as well as its interactions with the DA/5HT-related subcortical-cortical loop connectivity, need to be further investigated.

On the other hand, on a pathophysiological level, sensorimotor and psychomotor disturbances, as manifested in BD, can be related to functional alterations of the sensorimotor system. In BD patients, resting state fMRI alterations in the SMN [47-49], as well as functional disconnectivity (mainly increased FC) between sensorimotor cortical areas and thalamus [50-52], have been demonstrated. Moreover, considering the phases of BD separately, the SMN (in relation to the default mode network) showed an opposite pattern of functioning (as measured by temporal variability of BOLD signal) in mania (increased) and depression

(decreased) [53]. However, the functional relationship between the cortical SMN and thalamus remains to be investigated specifically in the manic and depressive phases of BD.

By stepping to the subcortical-cortical loops and neurotransmitters level, some evidences suggested an involvement of especially DA and 5HT systems alterations in BD. With regard to DA, preclinical and clinical studies have shown similarities between the behavioral effects of pro-dopaminergic substances (such as cocaine and amphetamine) and manic symptomatology, as well as an anti-manic and/or pro-depressant effect of anti-dopaminergic substances (such as antipsychotic drugs) [28, 54-60]. These evidences have led to a DA hypothesis of BD, supposing increased DA activity in mania and decreased DA activity in depression [59-60]. However, data on alterations of DA activity in BD are sparse and conflicting, but the most consistent reported finding is a decrease in DA transmission in the depressive phase [61]. On the other hand, also 5HT has been implicated in BD pathophysiology [62]. Preclinical and clinical evidences have shown strong association between low 5HT activity and behavioral impulsivity [28, 63], which in turn is a core feature of BD (especially in the manic phase) [28, 64-65]. Coherently, quite consistent data reported a decrease in 5HT transmission in BD [61]; in particular, a deficit in 5HT has been supported in mania especially [62], while both decreased and increased 5HT activity has been supposed in depression [66]. However, a potential functional disconnectivity of the DA-related SN and 5HT-related RN within the context of the sensorimotor loop still needs to be investigated in BD and specifically in its manic and depressive phases.

Aims of the study

Therefore, focusing on the sensorimotor/psychomotor dimension and related spatio-temporal organization of the sensorimotor system, the general aim of the study was to investigate the functional relationships between large-scale resting state networks (i.e. SMN), subcortical-cortical loops (i.e. basal ganglia-thalamo-cortical loop) and neurotransmitters nuclei (i.e. DA-related SN and 5HT-related RN) in physiology and pathophysiology (i.e. BD).

Our specific aims were the following. Firstly, in a dataset of healthy subjects (and in other two independent datasets that served to replicate our findings), we aimed to investigate: (1A) the FC between the cortical SMN and thalamus; (1B) the

relationship between the thalamus-SMN FC and the FC of SN or RN via basal ganglia/thalamic loop. According to the current data (see above) we hypothesized that the thalamus-SMN FC is differently related to SN- and RN-related FC with basal ganglia/thalamic loop.

Secondly, in a dataset of BD (and in an independent dataset that served to replicate our findings), we aimed to investigate the same measures - i.e. (2A) the thalamus-SMN FC, as well as (2B) the SN-basal ganglia/thalamus FC and RN-basal ganglia/thalamus FC. Considering our focus on sensorimotor/psychomotor dimension and system, we compared specifically manic patients (characterized by psychomotor excitation) and inhibited depressed patients (characterized by psychomotor inhibition), with respect to healthy subjects (we also controlled the same measures in agitated, or mixed, depressed patients, with less homogenous features). Basing on current data on BD (see above) and on our results on healthy subjects (see the results section), we hypothesized: in mania, increased thalamus-SMN FC, as well as decreased RN-related FC (and/or increased SN-related FC); in inhibited depression, decreased thalamo-SMN FC, as well as decreased SN-related FC (and/or increased RN-related FC).

METHODS

Subjects and clinical assessment

Healthy controls (HC) were recruited from the Genoa (Italy) metropolitan area, and BD patients from the in-patient and out-patient services of the Psychiatric Clinic of Genoa (San Martino Polyclinic Hospital and Department of Neuroscience at the University of Genoa). The Ethical Committee of San Martino Polyclinic Hospital approved the study, and written informed consent was obtained from all the participants. The study was conducted on 67 HC and 100 BD patients - 34 in manic phase, 37 in depressive phase (subdivided in 21 inhibited depressed and 16 agitated depressed), and 29 in euthymic phase (see **Table 1** for a description of the sample). Each participant was evaluated with standardized structured and/or semi-structured clinical instruments in order to obtain information on clinical and diagnostic features, course of illness, family history, and actual and past pharmacotherapy. The instruments were: the Mini International Neuropsychiatric Interview (MINI) [67]; the Structured Clinical Interview for DSM Axis-I Disorders/Patient edition (SCID-I/P) [68]; the Structured Clinical Interview for DSM Axis II Personality Disorders (SCID-II) [69]; the Structured Interview for Mood Disorder – Revised (SIMD-R) [70]; the Hamilton Depression Scale (HAM-D) with 17 items [71]; the Young Mania Rating Scale (YMRS) [72]. General, physiologic, pathologic and psychopathologic history was also investigated.

Inclusion criteria for HC were as follows: absence of any psychiatric disorder according to DSM criteria [1], either at the time of study participation or in the past; scores of HAM-D<8 and YMRS<8; age between 18 and 60. Inclusion criteria for patients were as follows: diagnosis of BD type I according to the DSM criteria (for manic, depressed and euthymic patients) [1]; score of YMRS≥13 for manic patients; score of HAM-D≥18 for all depressed patients (subdivided in: inhibited depressed with score at HAM-D item8 “retardation” > score at HAM-D item9 “agitation”, and agitated depressed with score at HAM-D item9 “agitation” > score at HAM-D item8 “retardation”); scores of HAM-D<8 and YMRS<8 for euthymic patients; age between 18 and 60; ability to provide written informed consent. Exclusion criteria for all the subjects included: diagnoses of schizophrenia, mental retardation, dementia, or other cognitive disorders; history of severe or decompensated somatic diseases, neurological diseases (stroke, cerebral vascular malformations, or epilepsy), previous

head injury with loss of consciousness (for 5 or more minutes); current alcohol and substance abuse (during the preceding 3 months); history of alcohol or substance dependence; history of abuse of synthetic and/or new drugs; pregnancy and lactation; left-handedness; the inability to undergo a magnetic resonance imaging MRI (claustrophobia, metal implants, etc); previous treatment with electroconvulsive therapy, chemotherapy or brain radiotherapy.

Data acquisition

Images were acquired using a 1.5-T GE scanner with a standard head coil. Foam pads were used to reduce head motion and scanner noise. fMRI scanning was carried out in darkness, and the participants were explicitly instructed to keep their eyes closed, to relax without sleeping, and to move as little as possible. Functional images were collected by using a gradient echo Echo Planar Imaging (EPI) sequence sensitive to BOLD contrast (TR/TE=2000/30 ms, flip angle=90 degree, FOV=24 cm). Whole-brain volumes were acquired in 33 contiguous 4-mm thick transverse slices, with a 1 mm gap and 3.75 x 3.75 mm^2 in-plane resolution. For each participant, fMRI scanning lasted 5 min and acquired a total of 150 scans.

In addition, three-dimensional T1-weighted anatomical images were acquired for all participants in a sagittal orientation by means of a 3D-SPGR sequence (TR/TE=11.5/5 ms, IR=500 ms, flip angle=8°, FOV=25.6 cm) with an in-plane resolution of 256 x 256, and slice thickness of 1 mm.

Data processing

Resting-state fMRI data were preprocessed and analyzed using tools from the FMRIB software library (FSL 5.0, http://www.fmrib.ox.ac.uk/fsl/) [73].

A standard preprocessing was performed including: (1) slice timing correction; (2) volume realignment; (3) brain extraction; (4) regression out of linear and non-linear drift, head motion and its temporal derivative, and mean time-series from the white matter and cerebrospinal fluid to control for non-neural noise [11, 74]; (5) non-linear alignment and normalization of anatomical and functional images with the FSL MNI152 2mm T1 standard space template; (6) spatial smoothing with a 6mm full-width at half-maximum isotropic Gaussian kernel.

Since head motion can affect FC estimates, we sought to minimize this effect in various ways. The motion parameters from the volume realignment step were used to

exclude participants who had translations greater than 2mm or rotations greater than 2° in each direction. The FSL motion outlier tool was then used to identify individual volumes that may be influenced by excessive movement (using an intensity difference metric thresholded at the 75^{th} percentile + 1.5 times the interquartile range). These volumes plus the ones immediately before and after them were excluded from the FC calculation. Mean head motion showed no significant difference between groups (all $p>0.05$; see **Supplemental Figure 1**). Finally, mean head motion was entered as covariate in all the subsequent correlation and comparison analyses.

The data were filtered within the standard frequency band of 0.01–0.08Hz, which is thought to reflect mainly neuronal fluctuations, and is less affected by physiological variables like respiration and aliased cardiac signals that fall in the other frequency ranges [12, 16, 75-77].

Data analysis in healthy subjects

We investigated the functional relationship between the SMN and thalamus, as well as its interaction with the SN- and RN-related connectivity via basal ganglia (BG)-thalamo-cortical loop, in healthy subjects, in order to characterize the spatio-temporal organization of the sensorimotor system from a physiological perspective.

Firstly, we calculated the FC between thalamus and SMN, by using a region of interest (ROI)-to-ROI approach. Specifically, a bilateral thalamus mask from the Harvard-Oxford subcortical atlas and a bilateral mask of the cortical SMN as defined by Yeo et. al [20] were used as ROIs (**Supplemental Figure 2**). The seed reference time-series of each ROI was obtained by averaging the fMRI time-series of all voxels within. Pearson's correlation coefficient was calculated between the two ROI timecourses and transformed to z-value by means of the Fisher r-to-z transformation, in order to improve normality. The obtained thalamus-SMN FC, which represents the correlation of BOLD signal oscillations between thalamus and SMN, showed high variability between subjects - with values ranging from high negative values to around-zero values up to high positive values (see below in the results section). Thus, we also calculated the absolute value (or modulus) of this parameter - i.e. the non-negative value |FC thalamus-SMN| without regard to its sign – which represents the absolute strength of correlation of BOLD signal oscillations between thalamus and

SMN, ranging from zero (i.e. no correlation) to high positive values (i.e. strong correlation, both positive or negative).

Secondly, we calculated the FC between SN and BG/thalamus and between RN and BG/thalamus by using the same approach and methodology as above. A bilateral SN mask and a bilateral RN mask from the ATAG-Atlas (http://www.nitrc.org/projects/atag) [78], as well as a bilateral BG/thalamus mask (composed of bilateral striatum, putamen and thalamus) from the Harvard-Oxford subcortical atlas, were used as ROIs (**Supplemental Figure 2**). We calculated the SN-BG/thalamus FC and RN-BG/thalamus FC, as measures of correlation of BOLD signal oscillation between the DA-related SN or 5HT-related RN and the BG-thalamic loop. Then we performed a partial correlation analysis between the thalamus-SMN FC and SN-BG/thalamus FC as well as between the thalamus-SMN FC and RN-BG/thalamus FC (with age, gender, and head motion as covariates), in order to investigate if and how the SN-related FC and/or RN-related FC could affect the thalamus-SMN FC via BG/thalamic loop.

Finally, we performed the same analyses by using the default mode network (DMN) [20], to control for the specificity of SMN.

Data analysis in bipolar disorder

We then investigated the thalamus-SMN FC, as well as the SN- and RN-related FC via BG-thalamic loop, in BD subjects, focusing on manic and inhibited depressed patients, in order to characterize the spatio-temporal organization of the sensorimotor system in these opposite phases of BD which present the extremes poles of psychomotor dimension, i.e. excited and inhibited psychomotricity respectively.

Considering the clinical features of BD patients, as well as previous data on this disorder and our results on healthy subjects (see the introduction and results sections), a-priori hypothesis-driven analyses on both mania and inhibited depression were performed (with agitated depression as control group). We thus calculated the same measures of thalamus-SMN FC, SN-BG/thalamus FC and RN-BG/thalamus FC in the BD subjects, by using the same ROI-to-ROI approach, masks and method as in the HC sample. Then we compared these measures (with age, gender, and head motion as covariates) between HC and manic or inhibited depressed patients (as well as agitated depressed patients as control). Finally, potential clinical correlations were explored. Statistical analyses were performed in SPSS version19.

RESULTS

Results in healthy subjects

Firstly, we calculated the thalamus-SMN FC in HC. As shown in **Figure 1, part A**, the mean value of thalamus-SMN FC among the group was slightly negative, but a high inter-subject variability for this parameter, with values ranging from high negative values to around-zero values up to high positive values, was observed.

Secondly, we calculated the SN-BG/thalamus FC and RN-BG/thalamus FC and correlated them with the thalamus-SMN FC (with age, gender and motion as covariates).

The thalamus-SMN FC did not show a significant linear correlation with SN-BG/thalamus FC. Interestingly, as shown in **Figure 1 part B**, the values distribution of this correlation showed a U-shape pattern, and a significant quadric correlation between the two parameters was found (R^2=0.221; p=0.000). Accordingly, low values of SN-BG/thalamus FC is associated with around-zero values of thalamus-SMN FC; by contrast, high values of SN-BG/thalamus FC is associated with high thalamus-SMN FC values, both positive and negative (see both plot and histogram in the figure). As confirmation, as shown in **Supplemental Figure 3**, we detected a significant linear and positive correlation between the SN-BG/thalamus FC and the absolute values (or modulus) of thalamus-SMN FC (|FC thalamus-SMN|) (r=0.460; p=0.000), so that the SN-BG/thalamus FC values is positively linearly associated with the absolute FC value between thalamus and SMN (i.e. independently from the sign of thalamus-SMN FC).

The pattern was different for RN. As shown in **Figure 1 part B**, the thalamus-SMN FC showed a significant linear and negative correlation with RN-BG/thalamus FC (r=-0.425; p=0.000). Accordingly, low values of RN-BG/thalamic FC is associated with high positive values of thalamus-SMN FC, while high values of RN-BG/thalamus FC is associated with high negative values of thalamus-SMN FC (see both plot and histogram in the figure).

This pattern of correlations was specific for SMN, since the thalamus-DMN FC showed no significant correlations with both SN-BG/thalamic and FC RN-BG/thalamic FC, as shown in **Supplemental Figure 4**.

Results in bipolar disorder

We then investigated the same measures, i.e. the thalamus-SMN FC, as well as the SN-BG/thalamus FC and RN-BG/thalamus FC, in BD subjects, focusing on manic and inhibited depressed patients, since they present the extremes poles of psychomotor dimension.

In order to characterize our BD sample according to psychomotor behavior, we selected the psychomotor items in both YMRS (i.e. item2 "increased motor activity-energy") and HAM-D (i.e. item8 "retardation" and item9 "agitation"). As represented in **Figure 2a**, manic patients showed increased motor activity-energy (score at YMRS item2≥1) in almost 100% of the sample, and agitation (score at HAM-D item9≥1) in more than half (while almost none of them showed retardation). By contrast, inhibited depressed patients (who scored at HAM-D item8>item9) showed retardation (score at HAM-D item8≥1) in 100% of the sample (while a very low percentage of them showed agitation). Finally, agitated depressed patients (who scored at HAM-D item 9>item8) showed agitation (score at HAM-D item9≥1) in 100% of the sample (while a low percentage of them showed retardation or increased motor activity).

Thus, considering the described clinical features of our BD sample and our results on healthy subjects (see above), as well as previous data on BD (see the introduction section), we hypothesized: in mania, increased thalamo-SMN FC, as well as decreased RN-BG/thalamus FC (and/or increased SN-BG/thalamus FC); in inhibited depression, decreased thalamo-SMN FC, as well as decreased SN-BG/thalamus FC (and/or increased RN-BG/thalamus FC). Agitated depressed patients were used as control group.

Firstly, we calculated the thalamus-SMN FC in BD patients and compared it between groups (with age, gender and motion as covariates). As shown in **Figure 2b part A**, the FC values showed high inter-subject variability within each group, but the mean value was progressively shifted, with respect to the slightly negative value in HC, to around-zero value in inhibited depressed patients, up to more positive value in agitated depressed and manic patients. Accordingly, as shown in **Figure 2b part B**, manic patients specifically showed a significant increase in thalamus-SMN FC with respect to HC ($F=4.554$; $p=0.035$), while inhibited depressed patients specifically showed a significant decrease in the absolute value or modulus of |thalamus-SMN

FC| with respect to HC (F=6.093; p=0.016). Agitated depressed patients showed a thalamus-SMN FC pattern similar to mania, albeit not statistically significant.

Secondly, we calculated the SN-BG/thalamus FC and RN-BG/thalamus FC and compared them between groups. Coherently with the previous results, manic patients showed a significant decrease in the RN-BG/thalamus FC with respect to HC (F=6.392; p=0.013), but no significant changes in the SN-BG/thalamus FC. On the other hand, inhibited depressed patients showed a significant decrease in the SN-BG/thalamus FC with respect to HC (F=4.222; p=0.043), but also a significant decrease of the RN-BG/thalamus FC (F=5.086; p=0.027). No significant changes were observed for agitated depressed patients.

Finally, we explored potential clinical correlations of the investigated FC measures (with age, gender, movement and group as covariates). None of them showed significant correlations with total scores of YMRS and HAM-D. However, the thalamus-SMN FC correlated with item2 (hyper-activity) of YMRS (r=0.242; p=0.017), while |thalamus-SMN FC| inversely correlated with item8 (retardation) of HAM-D (r=-0.201; p=0.049).

REPLICATION STUDY

In order to confirm and replicate our findings on HC and BD, we conducted the same analyses on independent datasets: one independent datasets of HC and BD and another independent dataset of HC.

Methods

Independent dataset I

The independent dataset I consisted of 108 HC (HC-I) and 40 BD (BD-I), 20 in active phases and 20 in euthymia (**Table 1**). It is part of an openly available fMRI resting-state dataset (OpenfMRI database, UCLA Consortium for Neuropsychiatric Phenomics: https://openfmri.org/dataset/ds000030/). MRI functional and structural data were acquired on 3-T Siemens scanners. The resting fMRI data were collected using a EPI sequence with a TR=2000 ms, and lasted 304s. See the website for a detailed description of subjects and clinical assessment, inclusion and exclusion criteria, and MRI acquisition information and parameters.

We applied the same preprocessing procedures and FC analyses to these data as were used in our main analysis (see above for a systematic description). Pre-processing and resting state data analyses were conducted using FSL. Briefly, the pre-processing steps were: slice timing, regression out of motion, white matter and cerebrospinal fluid, non-linear alignment and normalization, 6mm smoothing, standard 0.01-0.08Hz filtering. All selected participants showed no processing artifacts and head motion of less than 2 mm. We then calculated the same measures of thalamus-SMN FC, SN-BG/thalamus FC and RN-BG/thalamus FC, by using the same ROI-to-ROI approach, masks and methods in all the subjects of this independent dataset as were used in our main analysis.

Independent dataset II

The independent dataset II consisted of 19 HC (HC-II) and was recruited from the Taipei (Taiwan) metropolitan area **(Table 1).** The study was approved by the Taipei Medical University Institutional Review Board. Participants were screened for psychiatric disorders (either at the time of study participation or in the past), neurological and somatic illnesses, and other standard MRI exclusion criteria (e.g.,

claustrophobia, metal implants, etc.). All participants gave their written informed consent. MR images were acquired on a 3-T GE MR750 scanner using a body-coil for transmission and a standard 8-channel head-coil for reception. A high-resolution T1-weighted anatomical was acquired (FSPGR; resolution = 1 x 1 x 1 mm^3), followed by resting-state functional images. During the resting-state period, participants were instructed to keep their eyes shut, to not fall asleep, and to not focus on any thoughts in particular. Resting-state BOLD sensitive images were acquired using a T2*-weighted EPI sequence (TR=1000 ms; TE=30 ms; flip angle=90°; FOV=22 cm; matrix = 64 x 64; slice thickness = 6 mm; slice gap = 0 mm; 21 slices). 360 volumes were acquired in a total time of 6 minutes.

We applied the same preprocessing procedures and FC analyses to these data as were used in our main analysis (see above for a systematic description). After preprocessing (all selected participants showed no processing artifacts and head motion of less than 2 mm), we calculated the same measures of thalamus-SMN FC, SN-BG/thalamus FC and RN-BG/thalamus FC, by using the same ROI-to-ROI approach, masks and methods as in our main analysis.

Results

Results on healthy subjects

We again calculated the thalamus-SMN FC and its partial correlation with SN-BG/thalamus FC and RN-BG/thalamus FC (with age, gender and movement as covariates) in HC-I and HC-II datasets, in order to confirm and replicate our findings in other two independent samples that were acquired on 3-T scanners from different sites with different acquisition parameters (see above).

Firstly, as in our main dataset, the thalamus-SMN FC both in HC-I and HC-II samples was characterized by negative mean value and high inter-subject variability (with values ranging from high negative values to around-zero values up to high positive values) (**Supplemental Figure 5**).

Secondly, as in our main dataset, both in HC-I and HC-II samples, the thalamus-SMN FC showed a significant quadric correlation with SN-BG/thalamus FC (without showing any significant linear correlation) (**Supplemental Figure 5**). This was confirmed in both samples by a significant linear and positive correlation between SN-BG/thalamus FC and the absolute values (or modulus) of |FC thalamus-SMN| (**Supplemental Figure 3**). On the other hand, again as in our main dataset, the

thalamus-SMN FC showed a significant linear and negative correlation with RN-BG/thalamus FC (**Supplemental Figure 5**).

Results on bipolar disorder

Then we compared the thalamus-SMN FC, SN-BG/thalamus FC and RN-BG/thalamus FC (with age, gender, and motion as covariates) between HC-I and BD-I samples, in order to replicate our BD findings in an independent dataset (see above).

We grouped together the BD-I patients in active phases - since the heterogeneous features (mania, hypomania, depression, mixed states) and small number of subjects for each subgroup did not allow us to investigate mania and inhibited depression separately - and compared them to HC-I for the FC measures. Accordingly, we also grouped together all the patients in active phases (i.e. manic, inhibited depressed and agitated depressed patients) in our main BD dataset, and analogously compared them with HC for the FC measures, in order to make the replication results comparable between our main dataset and the BD-I/HC-I independent dataset.

Firstly, in both datasets, the thalamus-SMN FC was significantly increased in BD patients in active phases when compared to HC (**Supplemental Figure 6** and **Table 2**). Secondly, in both datasets, the RN-BG/thalamus FC showed a significant reduction in BD patients in active phases with respect to HC, while no significant differences were detected for SN-BG/thalamus FC between groups (**Supplemental Figure 6** and **Table 2**).

Finally, as explorative analysis, we investigated the FC measures between BD patients in euthymia and HC. In both datasets, the thalamus-SMN FC showed no significant differences between euthymic patients and HC (**Supplemental Figure 6** and **Table 2**). In our main dataset, both SN-BG/thalamus FC and RN-BG/thalamus FC were significantly reduced in euthymia; however, this finding was not replicated in the BD-I/HC-I dataset (**Supplemental Figure 6** and **Table 2**).

DISCUSSION

Main findings

The main findings of the study are the following.

At the physiological level (A), in healthy subjects, the thalamus-SMN FC showed: (1) mean negative value with high inter-subject variability (ranging from high negative up to high positive values); (2) a quadric correlation with SN-BG/thalamus FC (confirmed by its positive linear correlation with the modulus of |thalamus-SMN FC|) and a linear and negative correlation with RN-BG/thalamus FC. These results were replicated in two other independent HC samples.

At the pathophysiological level (B), manic and inhibited depressed patients, as characterized by excited vs. inhibited psychomotricity, showed a different pattern of alterations in (1) thalamus-SMN FC and (2) SN/RN-BG/thalamus FC. Specifically, mania showed an increase in thalamus-SMN FC with a concomitant reduction of RN-BG/thalamus FC. By contrast, inhibited depression showed a decrease in the modulus of |thalamus-SMN FC| with a concomitant reduction of SN-BG/thalamus FC and RN-BG/thalamus FC. The FC results on active phases of BD were replicated in an independent BD sample.

Functional organization of sensorimotor system in healthy subjects

In our study, the thalamo-SMN FC showed a high inter-subject variability, ranging from high negative to high positive values (and a resulting slightly negative mean). The FC between thalamus and SMN may reflect the communication pattern in the intrinsic activity of the thalamo-cortical sensorimotor complex, playing a role in the sensorimotor processing [9-10, 13, 18, 23-24]. In particular, since sensory stimuli reach the cortical SMN through the thalamus, different pattern of thalamus-SMN FC may underlie different patterns of communication between these two brain regions and, consequently, different patterns of modulation and integration of sensory inputs into the thalamo-cortical ongoing intrinsic activity, and finally, to different patterns of related motor and behavioral outputs (i.e., different patterns in the sensorimotor and psychomotor dimension). Thus, the thalamo-SMN FC and its high inter-subject variability could be behaviorally relevant, potentially reflecting different sensorimotor and psychomotor patterns, at least in the extreme pathological poles of excited or inhibited psychomotricity (see below).

In turn, in our study, the thalamus-SMN FC showed a non-linear (i.e. quadratic) correlation with the DA-related SN-BG/thalamus FC. Accordingly, the increase in coherence of BOLD signal oscillations between the DA-related SN and BG-thalamic regions from low to high values is associated with the increase in the strength itself of the correlation of BOLD signal oscillations (independently from the sign of correlation) between the thalamus and SMN, from around-zero values to both strong correlation or strong anti-correlation between thalamus and SMN signals. These findings are coherent and complementary with previous data on the strong relationship between the DA system and sensorimotor system. In particular, our finding of a functional relationship between SN, BG/thalamic regions and cortical SMN could represent a bridge between the previous evidences showing the strong FC of SN to especially BG and thalamic regions (coherently with its structural connectivity) [29-38], and the increase of FC, mediated by pro-dopaminergic substances, between subcortical and cortical sensorimotor regions [39-41]. Accordingly, we speculate that DA signaling, via the widespread projections of brainstem neurotransmitter SN nucleus to BG and thalamic regions, allows the coherence (or synchronization) of low-frequency oscillations between different regions of BG and thalamus, as different subcortical relay stations of the cortico-BG-thalamo-cortical sensorimotor loop. This, in turn, would favor the functional coupling of low-frequency oscillations (i.e. communication) between thalamus and cortical SMN, thus potentially enhancing the influence of sensory inputs (via the thalamic pathways) on the ongoing intrinsic thalamo-cortical activity, and finally, to the related motor and behavioral patterns.

On the other hand, the thalamus-SMN FC showed a linear and negative correlation with the 5HT-related RN-BG/thalamus FC. Thus, the increase in coherence of BOLD signal oscillations between the RN and BG-thalamic regions from low to high values is associated with the shift from positive correlation to negative or anti-correlation of BOLD signal oscillations between the thalamus and SMN (thus affecting the sign of correlation). This finding is coherent with previous evidences of inhibitory effects of 5HT signaling on the sensorimotor system. In particular, the RN (following its structural connections) showed a positive FC to BG/thalamic regions but a negative FC to somatosensory and motor cortices [42-44]. Coherently, an association of 5HT signaling with reduction of SMN activity was reported [45-46]. We thus speculate that 5HT signaling, via the brainstem RN-mediated projections and opposite

modulation of subcortical BG/thalamic regions and sensorimotor cortical regions, favors the anti-correlation of low-frequency oscillations between thalamus and cortical SMN, thus modulating the influence of sensory inputs on the ongoing intrinsic thalamo-cortical activity and related motor and behavioral outputs.

In summary, the functional coupling of low-frequency oscillations between thalamus and SMN seems to be differentially modulated by the DA-related SN and 5HT-related RN, hypothetically via synchronization of low-frequency oscillations between BG/thalamic relay stations within the cortico-BG-thalamo-cortical loop. The SN-mediated FC seems to be related to the absolute strength of correlation between thalamus and SMN, while the RN-mediated FC seems to modulate the sign of correlation, i.e. favoring the anti-correlation between thalamus and SMN. Thus, high levels of SN-related FC is associated with high absolute values of thalamus-SMN FC. If concomitantly the RN-related FC is high, the high thalamus-SMN FC would result in a negative correlation (or anti-correlation) of low-frequency oscillations between thalamus and SMN. By contrast, if concomitantly the RN-related FC is low, the high thalamus-SMN FC would result in a positive correlation of low-frequency oscillations between thalamus and SMN (and, potentially, a more direct integration between resting state activity and sensorimotor processing). On the other hand, low levels of SN-related FC, independently of the RN-related FC, would be associated with around-zero values of thalamus-SMN FC, i.e. disconnectivity or dissociation of low-frequency oscillations between thalamus and SMN (and, potentially, dissociation between resting state activity and sensorimotor processing) (**Figure 3**).

Functional alterations of sensorimotor system in manic and depressive phases of bipolar disorder

In our sample, we grouped the BD subjects in manic patients and inhibited or agitated depressed, according to the psychomotor dimension (see the results section), which is a fundamental feature of BD [3-7]. In this regard, mania is clinically characterized by increased level of activity/energy, distractibility and impulsive behaviors, representing the extreme pole of excited psychomotricity [1-2, 6, 8]. By contrast, depression in its typical inhibited form is characterized by poor motricity, rigid ruminations and motor retardation, representing the extreme pole of inhibited psychomotricity [1-2, 6, 8]. In between, mixed features can frequently occur, mainly in the depressive phase, resulting in mixed forms as the agitated depression; since

agitation can be related both to inner tension/anxiety and/or psychomotor hyper-activity, this group shows more heterogeneous features (but can be possibly more shifted toward to the excited pole of psychomotricity) [1-2, 6, 8]. Considering the clinical features of the extreme poles of psychomotor disturbances, it is conceivable that in mania sensory stimuli more directly and easily affect the motor/behavioral patterns, while the opposite would occur in inhibited depression, with a dissociation between sensory inputs and motor/behavioral patterns.

Coherently, according to our a-priori hypotheses, we found in BD patients an opposite pattern of thalamus-SMN FC, which was shifted toward positive values (i.e. abnormal coupling between thalamus and SMN oscillations) in mania, and toward around-zero values (i.e. dissociations between thalamus and SMN oscillations) in inhibited depression. However, considering the slightly negative mean value of HC, manic and depressed (both inhibited and agitated) patients together showed a relative general increase in thalamus-SMN FC. Thus, our results are in accordance with previous evidences of mainly increased FC between thalamus and sensorimotor areas in BD patients considered as a whole (regardless of the phases of illness) [50-52], and extended them by showing specific differences in BD phases. In mania the shifting toward a positive correlation of low-frequency oscillations between thalamus and cortical SMN might allow an over-influence of sensory inputs (via the thalamic pathways) on the ongoing intrinsic thalamo-cortical sensorimotor activity and related increased motor/behavioral patterns. Accordingly, increased activity of the SMN, both in low frequencies (as evidenced by increased temporal variability of fMRI BOLD signal in the SMN in relation to the default mode network) [53] and in high frequencies (as evidenced by increased power of beta oscillations in EEG signal in sensorimotor areas) [79], was found in the manic phase, hypothetically resulting in the excited pattern of psychomotor dimension of mania. By contrast, in inhibited depression, the shifting toward a dissociation of low-frequency oscillations between thalamus and cortical SMN might lead to a reduced influence of sensory inputs on the ongoing intrinsic thalamo-cortical sensorimotor activity and related decreased motor/behavioral patterns. Accordingly, decreased activity of the SMN, both in low frequencies (as evidenced by decreased temporal variability of BOLD signal in the SMN in relation to the default mode network) [53] and in high frequencies (as evidenced by decreased power of beta oscillations in EEG signal in sensorimotor

areas) [79], was found in the depressive phase, hypothetically resulting in the inhibited pattern of psychomotor dimension of inhibited depression.

Concomitantly, we found a reduction of RN-BG/thalamus FC in mania, while a reduction of SN-BG/thalamus FC (as well as RN-BG/thalamus FC) in inhibited depression. These findings are coherent with previous data on the association between decreased 5HT activity and impulsivity [28, 63], which is characteristic of BD especially in the manic phase [64-65], as well as the association between decreased DA activity and depressive-like behaviors [28, 54-60]. Notably, our findings are also coherent and complementary with previous evidences on alterations of DA and 5HT signaling in BD specifically, which showed decreased DA transmission in the depressive phase and decreased 5HT transmission in BD as the most consistent neurotransmitters findings in this illness [61]. In turn, deficit of 5HT has been shown to induce a decrease in RN-related FC in patients [80-82]. Thus, our data are partially in line with the neurotransmitters hypotheses of mania and depression [28, 59-60, 66]: accordingly, we found a decrease in DA-related SN FC (together with the RN FC decrease) in depression, while a decrease in 5HT-related RN FC, rather than an increase in DA signaling, in mania.

In summary, mania and inhibited depression, as the extreme poles of excited and inhibited psychomotor dimension, may be characterized by different patterns in the spatio-temporal organization of the sensorimotor system. In mania, decreased correlation of low-frequency oscillations between the 5HT-related RN and BG/thalamic loop regions occurs. This, in turn, is associated with abnormal positive functional coupling of intrinsic low-frequency oscillations between thalamus and SMN, hypothetically resulting in psychomotor excitation. On the other hand, in inhibited depression (in addition to decreased RN-BG/thalamus FC), decreased correlation of low-frequency oscillations between the DA-related SN and BG/thalamic loop regions also occurs. This, in turn, is associated with functional dissociation of intrinsic low-frequency oscillations between thalamus and SMN, hypothetically resulting in psychomotor inhibition (**Figure 3**).

LIMITATIONS

The main limitation of the present study was the possible confounding effects of medication. Indeed, almost all of the bipolar patients in our sample were taking medications, including mood stabilizers, antipsychotics, antidepressants and benzodiazepines, which could interfere with the BOLD signal. Following recent suggestions and standards, we examined the potential impact of the psychotropic medication load - the number and dosage of different medications - on FC in BD [83]. This was done by converting antipsychotics into chlorpromazine dose-equivalents [84], mood stabilizers into lithium dose equivalents [84], antidepressants into imipramine dose-equivalents [84], and benzodiazepines into diazepam dose-equivalents [85]. We then used the codes 0, 1, 2 and 3 to indicate no medication, and dose-equivalents below, equal or above the mean effective daily dose, respectively [86]. We generated a composite measure of the medication load by summing all individual medication codes for each category and each individual BD patient [83]. We investigated the potential impact of medications on imaging data by correlating the resulting pharmacological load with the investigated FC measures, and no significant results were obtained. Nevertheless, we cannot exclude some influence of treatment on our findings, especially for antipsychotics on SN-relared FC and antidepressants on RN-related FC, since subgroup differences could be affected in a degree of complexity that is not captured by simple correlation analyses. However, the SN-related FC was significantly reduced in inhibited depression, but depressed patients were taking less antipsychotic than manic patients. On the other hand, the RN-related FC was significantly reduced both in manic and depressed patients, while antidepressant therapy was more frequently taken by depressed than manic patients. All together, the considerations suggest that these FC results were not a mere consequences of pharmacotherapy.

On the other hand, strengths of our work were the replication results in HC of three different and independent samples (acquired on different 1.5-T or 3-T scanners, with different parameters such as TR of 2s or 1s) and the replication results on the active phases of BD in two different samples, as well as an a-priori hypothesis driven approach.

CONCLUSION

In conclusion, our results suggest a functional link between resting state large-scale networks, cortico-BG-thalamo-cortical loops and brainstem neurotransmitters-related areas. This needs to be further investigated in future studies in order to shed further light in the spatio-temporal organization of brain resting state activity and its relation to stimulus-induced activity and phenomenal features. Moreover, our findings in mania and inhibited depression may represent potential biomarkers according to the dimension of psychomotricity rather than BD in general. This may prompt for further studies that specifically investigate these functional correlates in the sensorimotor system (as well as other features, included combined studies with neurotransmitters direct monitoring) in different disorders, such as schizophrenia, using a dimensional approach on psychomotricity (among other psychopathological dimensions). This approach could help to better understand the pathophysiology of different psychiatric disorders and indentify more specific diagnostic features.

FIGURES

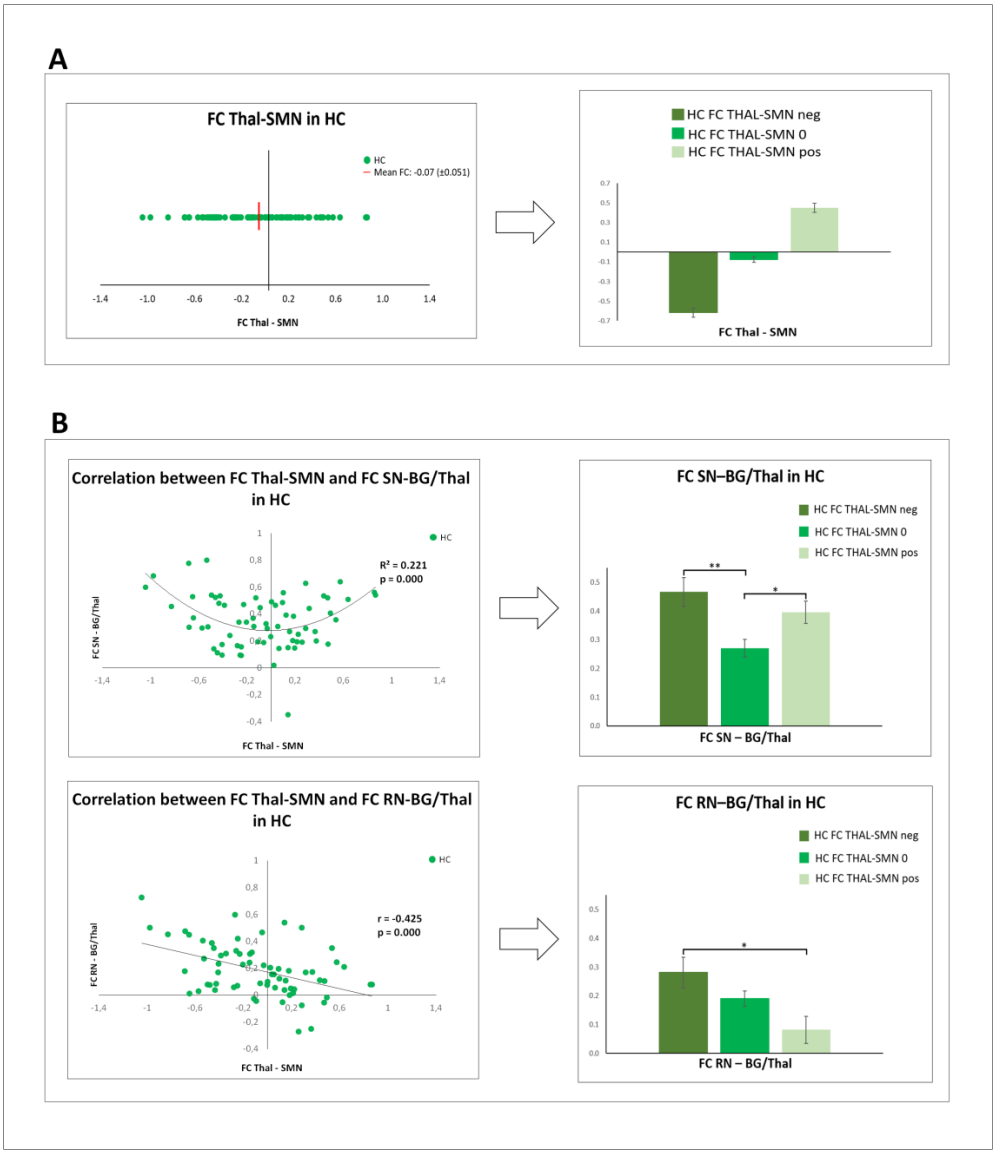


Figure 1. FC results in HC

A. FC Thal-SMN
Left part: distribution of FC Thal-SMN values among HC subjects.
Right part: split of the HC sample in 3 percentiles in order to better visualize the different distribution of FC Thal-SMN values in HC (HC showing a negative mean value of FC Thal-SMN; HC showing an around-zero mean value of FC Thal-SMN; HC showing a positive mean value of FC Thal-SMN).

B. Relationships of FC Thal-SMN with FC SN-BG/Thal and FC RN-BG/Thal
Left part: correlations of FC Thal-SMN with FC SN-BG/Thal (quadratic correlation) and FC RN-BG/Thal (linear correlation).

Right part: comparison between the 3 group of HC (percentiles) spitted according to FC Thal-SMN in order to better visualize the distribution of FC SN-BG/Thal and FC RN-BG/Thal values (in relationship to FC Thal-SMN values).
The ANOVA and post-hoc analysis of FC SN-BG/Thal between the 3 groups revealed a significant group difference ($F=7.032$; $p=0.002$) with a significant reduction of FC SN-BG/Thal in the group showing an around-zero mean value of FC Thal-SMN with respect to both the group showing a negative mean value ($p=0.007$) and a positive mean value ($p=0.047$). The ANOVA and post-hoc analysis of FC RN-BG/Thal between the 3 groups revealed a significant group difference ($F=4.914$; $p=0.010$) with a significant increase of FC RN-BG/Thal in the group showing a negative mean valued of FC Thal-SMN with respect to the group showing a positive mean value ($p=0.024$).
Abbreviations: HC, healthy controls; FC, functional connectivity; SMN, sensorimotor network; Thal, thalamus; BG, basal ganglia; SN, substantia nigra; RN, raphe nucleus; neg, negative mean value; 0, around-zero mean value; pos, positive mean value.

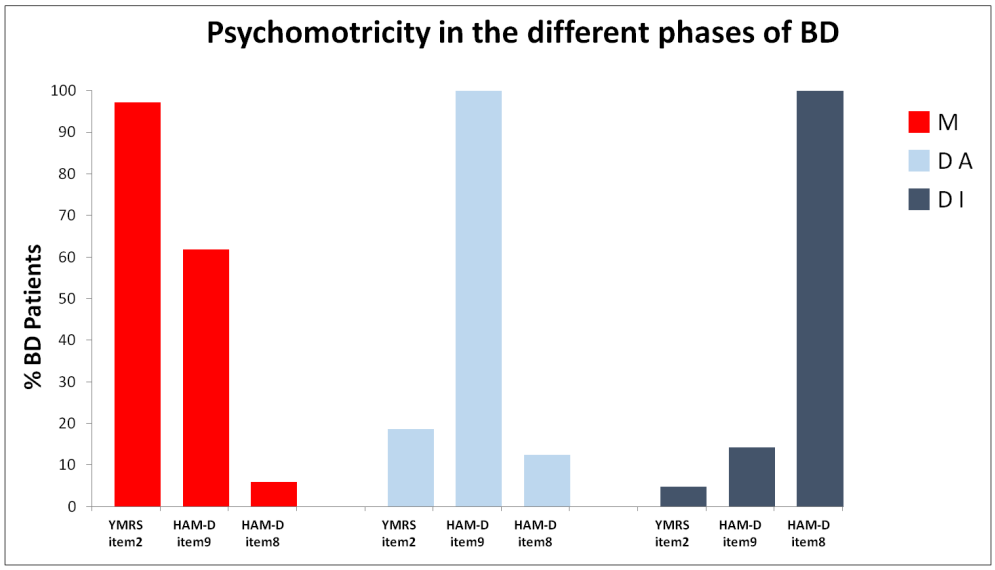


Figure 2a. Psychomotricity in the different phases of BD

Percentage of BD patients scoring ≥1 at psychomotor-relevant items of YMRS (i.e. item2 "increased motor activity-energy") and HAM-D (i.e. item8 "retardation" and item9 "agitation"), as divided in the groups of manic, agitated depressed and inhibited depressed patients.
Abbreviations: BD, bipolar disorder; M, manic patients; D A, agitated depressed patients; D I, inhibited depressed patients; YMRS, Young mania rating scale; HAM-D, Hamilton depression scale.

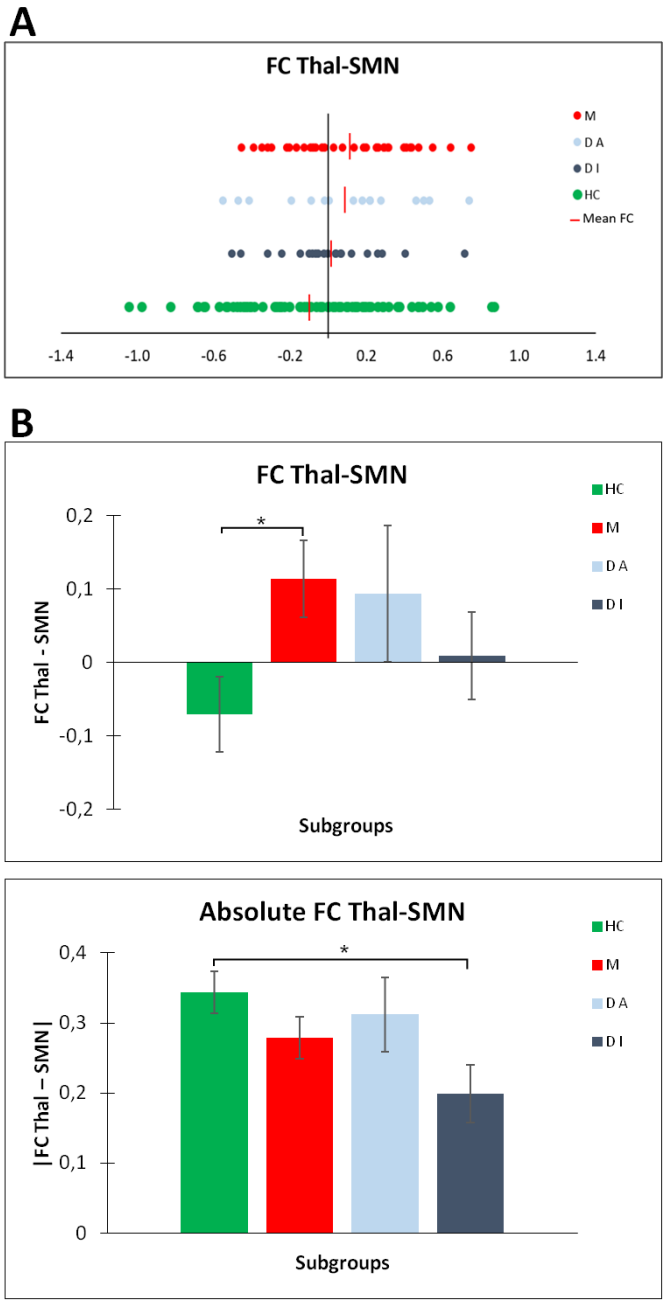


Figure 2b. FC results in BD: FC Thal-SMN

A. Distribution of FC Thal-SMN among single subjects, as divided in manic, agitated depressed and inhibited depressed patients, with respect to HC.
B. Comparison of FC Thal-SMN and its absolute value (or modulus) |FC Thal-SMN| between manic, inhibited depressed and agitated depressed patients, with respect to HC.
* $p<0.05$
Abbreviations: BD, bipolar disorder; M, manic patients; D A, agitated depressed patients; D I, inhibited depressed patients; HC, healthy controls; FC, functional connectivity; SMN, sensorimotor network; Thal, thalamus.

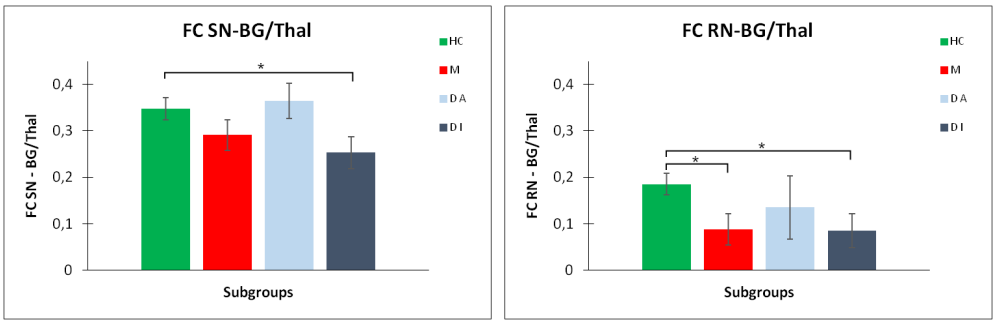


Figure 2c. FC results in BD: FC SN-BG/Thal and FC RN-BG/Thal

Comparison of FC SN-BG/Thal and FC RN-BG/Thal between manic, inhibited depressed and agitated depressed patients, with respect to HC.
* $p<0.05$
Abbreviations: BD, bipolar disorder; M, manic patients; D A, agitated depressed patients; D I, inhibited depressed patients; HC, healthy controls; FC, functional connectivity; Thal, thalamus; BG, basal ganglia; SN, substantia nigra; RN, raphe nucleus.

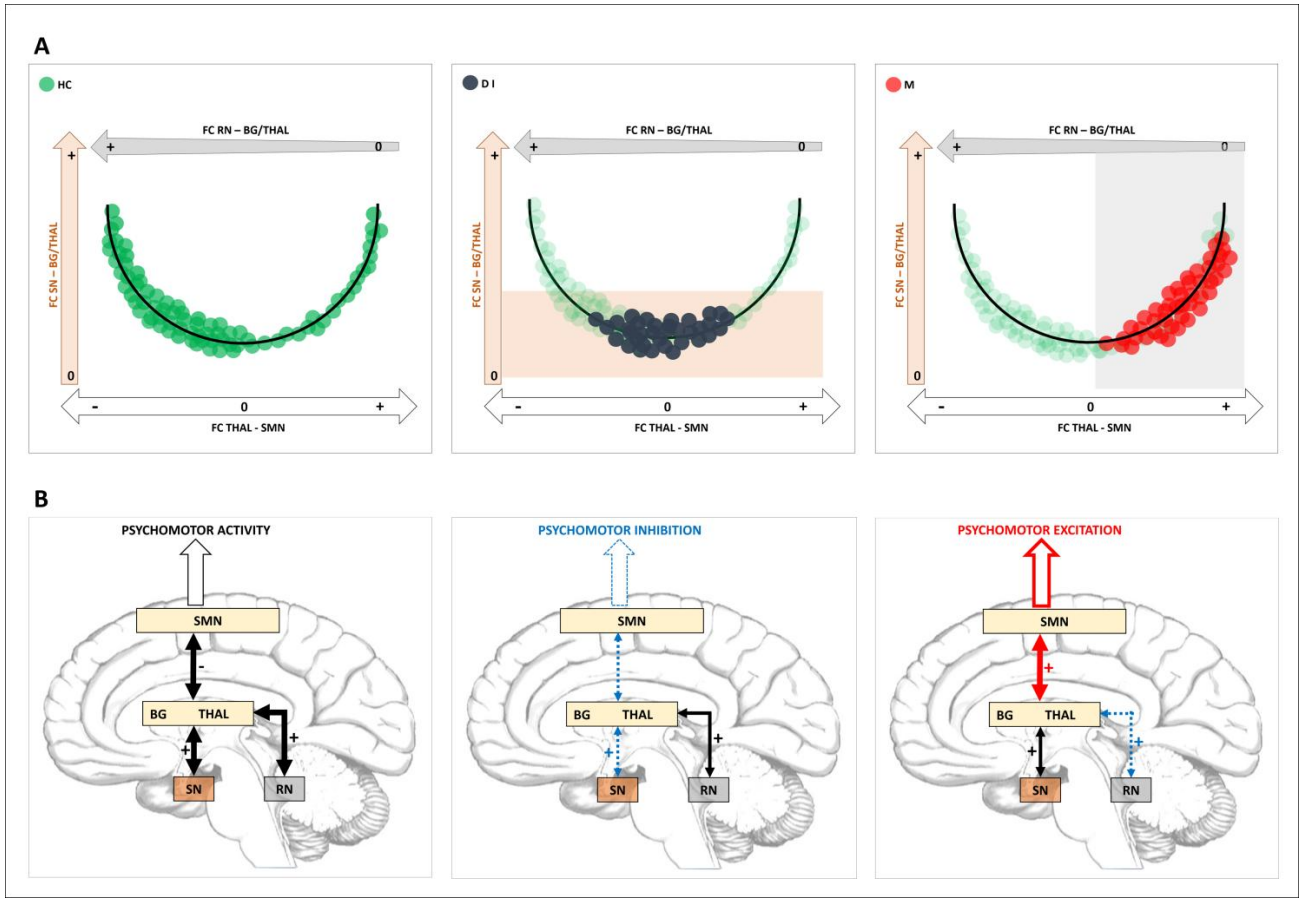


Figure 3. Schema

A. Schematic representation of the relationship between FC Thal-SMN, FC SN-BG/Thal and FC RN-BG/Thal, with the subject distribution of HC, manic and inhibited depressed patients.

B. Schematic representation of the alterations of FC Thal-SMN, FC SN-BG/Thal and FC RN-BG/Thal in manic and inhibited depressed patients with respect to HC.
In HC, the FC Thal-SMN shows high inter-subjects variability, ranging from high negative to high positive values (but with a relative shift toward negative values), and different relationships with FC SN-BG/Thal (i.e. quadratic relationship) and FC RN-BG/Thal (linear negative relationship). In mania, psychomotor excitation could be related to increased FC Thal-SMN toward positive values, which is associated with reduced FC RN-BG/Thal. In inhibited depression, psychomotor inhibition could be related to decreased FC Thal-SMN toward around-zero values, which is associated with reduced FC SN-BG/Thal.
Abbreviations: M, manic patients; D I, inhibited depressed patients; HC, healthy controls; FC, functional connectivity; SMN, sensorimotor network; Thal, thalamus; BG, basal ganglia; SN, substantia nigra; RN, raphe nucleus

TABLES

Table 1a. Healthy Controls Demographic Information

	HC (study dataset)	HC-I (independent dataset I for replication)	HC-II (independent dataset II for replication)
Sample Size *n*	67	108	19
Age *mean* (SD)	41.5 (13.3)	30.9 (8.5)	13 (68.4)
Female *n* (%)	43 (64.1%)	49 (45.4%)	34.3 (5.6)

Table 1b. Bipolar Disorder Patients Demographic and Clinical Information

	BD (study dataset)					BD-I (independent dataset I for replication)		
	BD TOT	M BD	D I BD	D A BD	E BD	BD TOT	M + D BD	E BD
Sample Size *n*	100	34	21	16	29	40	20	20
Age *mean* (SD)	46.0 (9.9)	46.1 (9.4)	48.8 (9.3)	43.4 (9.6)	45.4 (11.0)	34.7 (8.6)	34.7 (7.7)	34.7 (9.6)
Female *n* (%)	60 (60%)	22 (64.7%)	10 (47.6%)	12 (75%)	16 (55.1%)	18 (45%)	11 (55%)	7 (35%)
HAM-D *mean* (SD)		6.6 (5.6)	21.1 (4.3)	21.8 (3.1)	3.1 (3.3)		15.4 (8.3)	8.2 (7.2)
YMRS *mean* (SD)		18.8 (5.4)	2.7 (2.9)	5.0 (2.2)	3.1 (2.9)		12.5 (11.9)	7.3 (8.6)

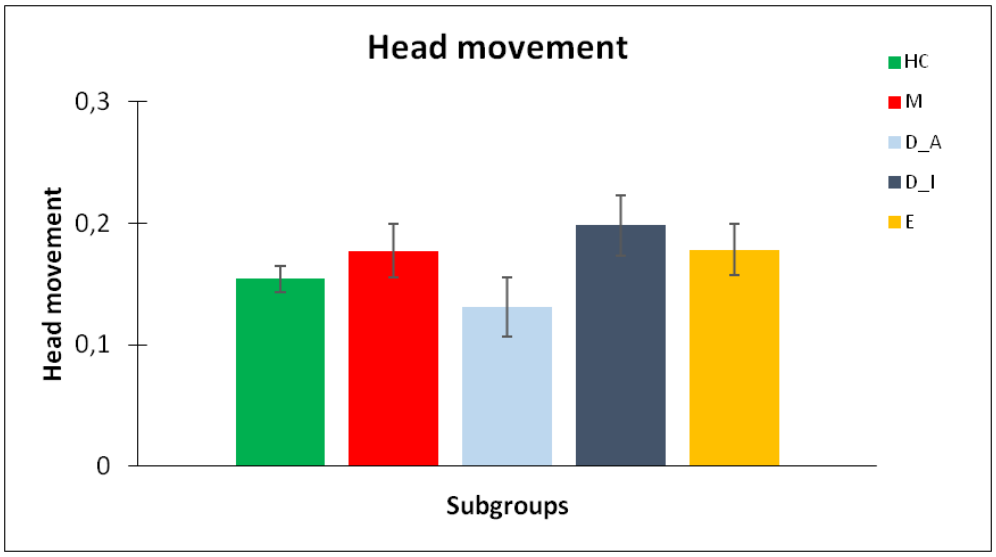
Abbreviations: HC, healthy controls; BD, bipolar disorder; M, manic patients; D A, agitated depressed patients; D I, inhibited depressed patients; E, euthymic patients; HAM-D, Hamilton Depression Scale; YMRS, Young Mania Rating Scale

Table 2. Replication results of FC in BD

	BD (study dataset)		BD-I (independent dataset I for replication)	
	M+D vs. HC	E vs. HC	M+D vs. HC	E vs. HC
FC Thal - SMN	4.934 (0.028)	ns	4.670 (0.033)	ns
FC SN - BG/Thal	ns	13.228 (0.000)	ns	ns
FC RN - BG/Thal	6.682 (0.011)	5.338 (0.023)	5.493 (0.021)	ns

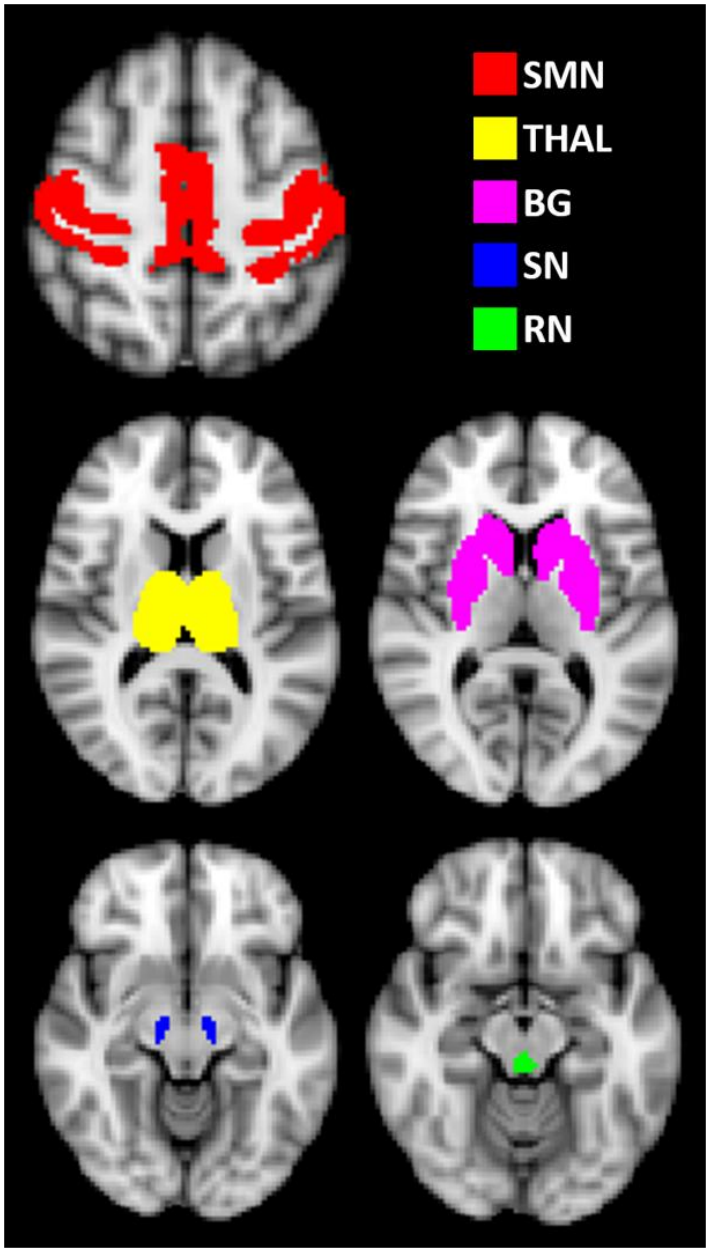
Abbreviations: BD, bipolar disorder; M, mania; D, depression; E, euthymia; HC, healthy controls; FC, functional connectivity; Thal, thalamus; SMN, sensori motor network; SN, substantia nigra; BG, basal ganglia; RN, raphe nuclei

SUPPLEMENTAL FIGURES



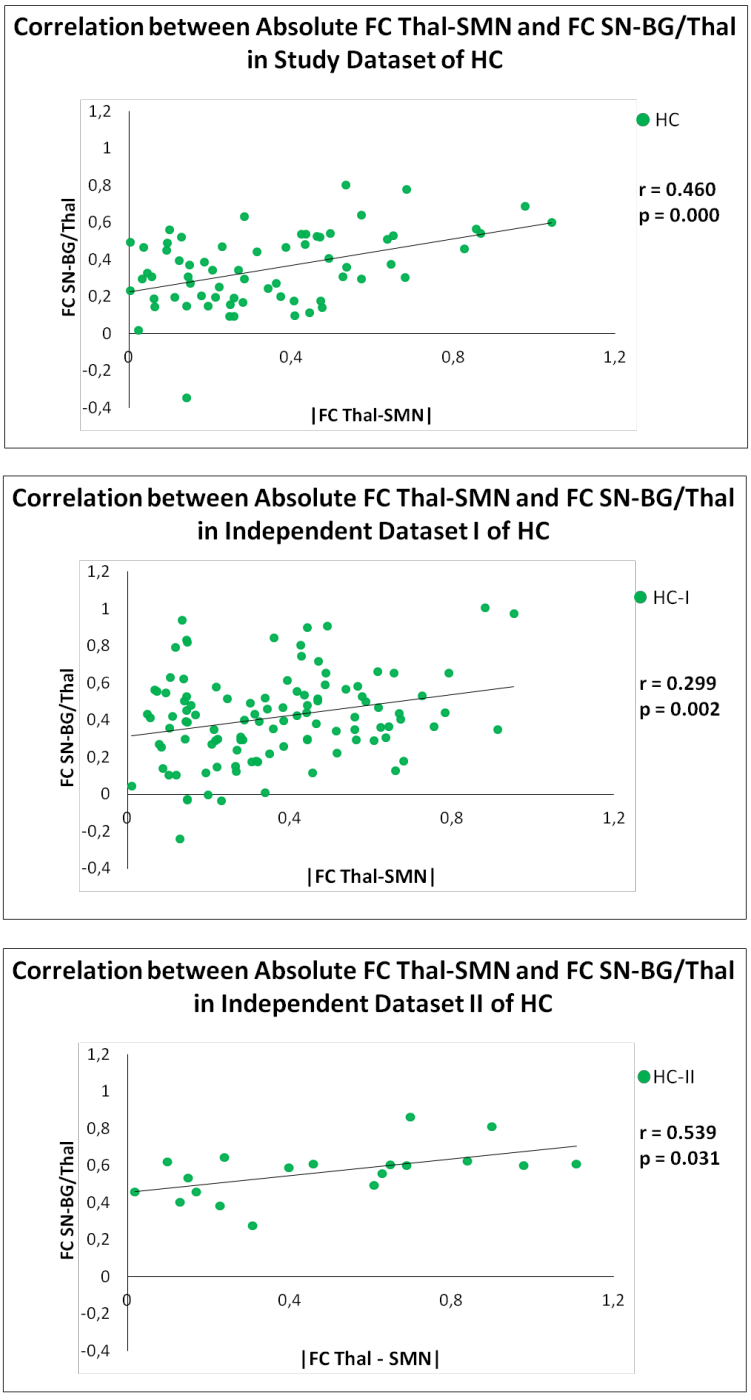
Supplmental Figure 1. Head movement

Comparison of head movement between manic, inhibited depressed, agitated depressed and euthymic patients with respect to HC. Head movement showed no significant differences between HC and manic patients (t=-1.01; p=0.31), inhibited depressed patients (t=-1.78; p=0.07), agitated depressed patients (t=0.86; p=0.39), and euthymic patients (t=-1.00; p=0.27).
Abbreviations: BD, bipolar disorder; M, manic patients; D A, agitated depressed patients; D I, inhibited depressed patients; E, euthymic patients; HC, healthy controls.



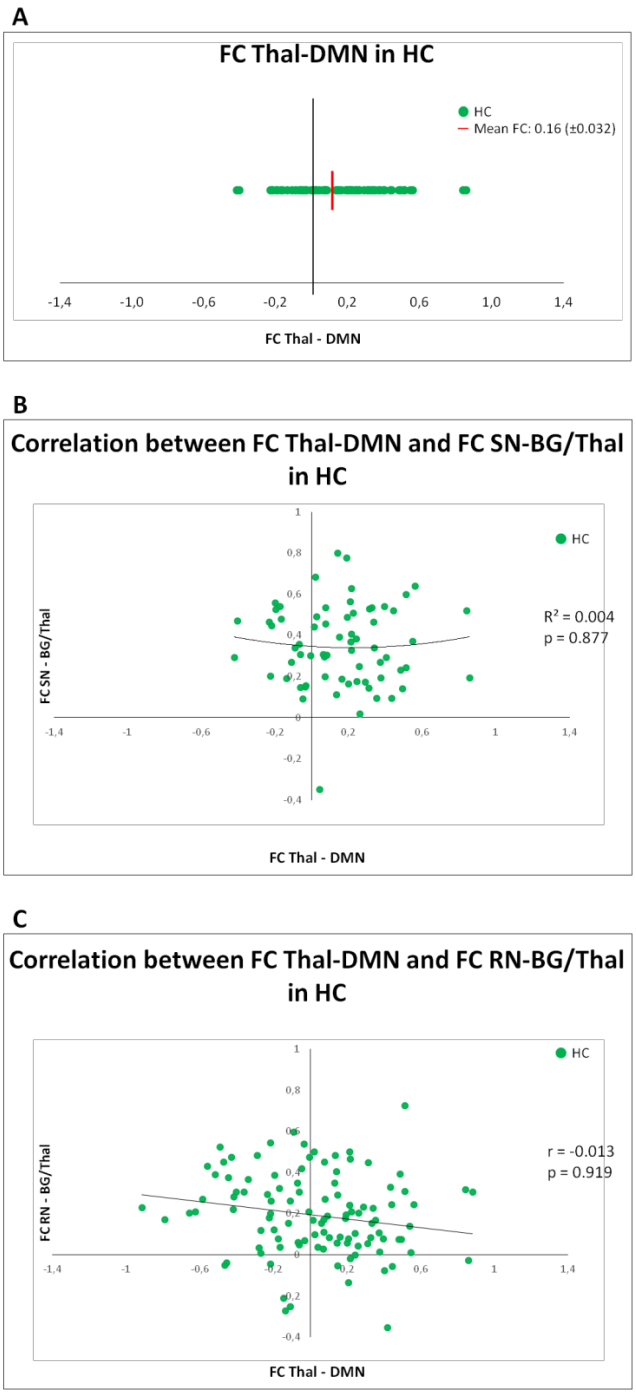
Supplemental Figure 2. ROIs

Abbreviations: ROIs, regions of interests; SMN, sensorimotor network; Thal, thalamus; BG, basal ganglia; SN, substantia nigra; RN, raphe nucleus



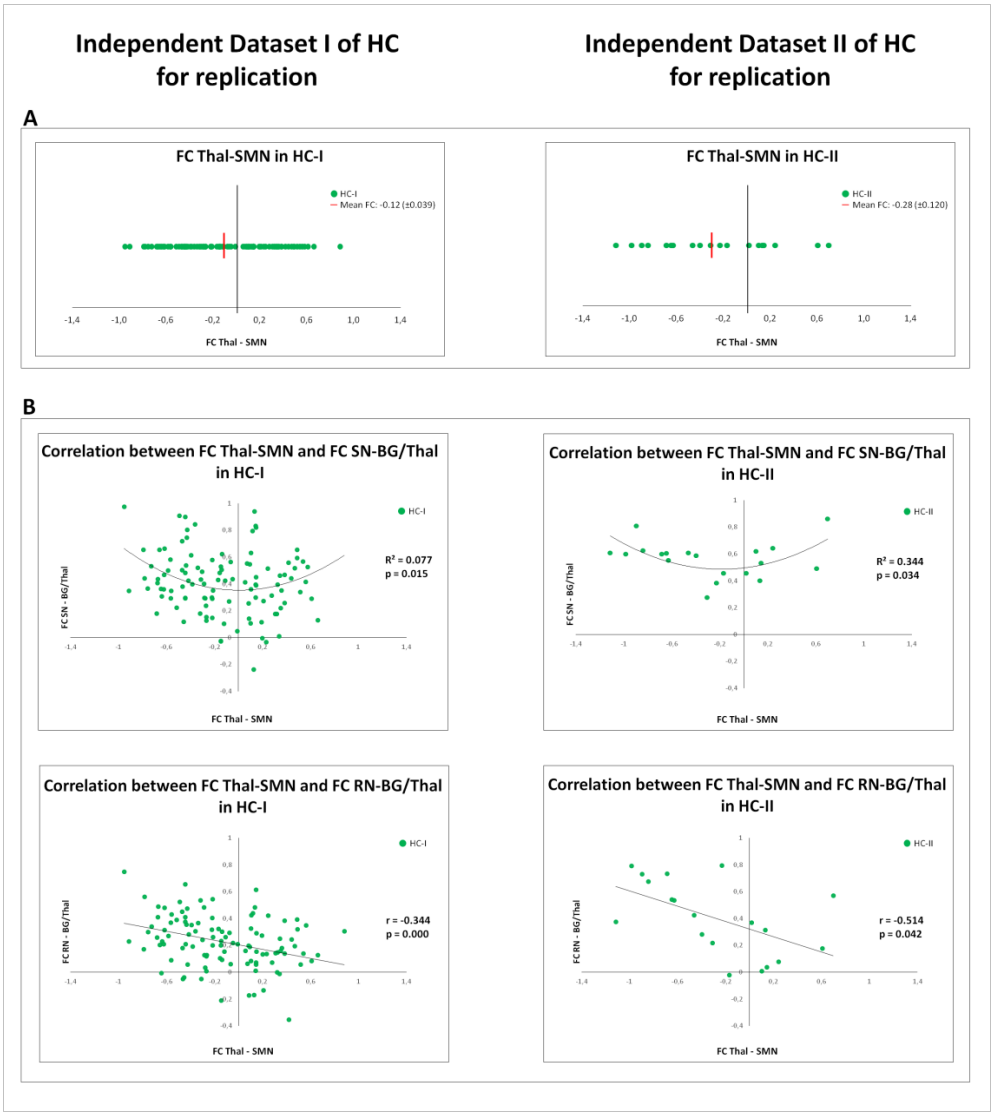
Supplemental Figure 3. Supplemental correlation analysis in HC and replication

Correlation between the absolute value, or modulus, of |FC Thal-SMN| and FC SN-BG/Thal in HC, in the study dataset and in the 2 independent datasets for replication
Abbreviations: HC, healthy controls; FC, functional connectivity; SMN, sensorimotor network; Thal, thalamus; BG, basal ganglia; SN, substantia nigra.



Supplemental Figure 4. Control analysis for DMN in HC

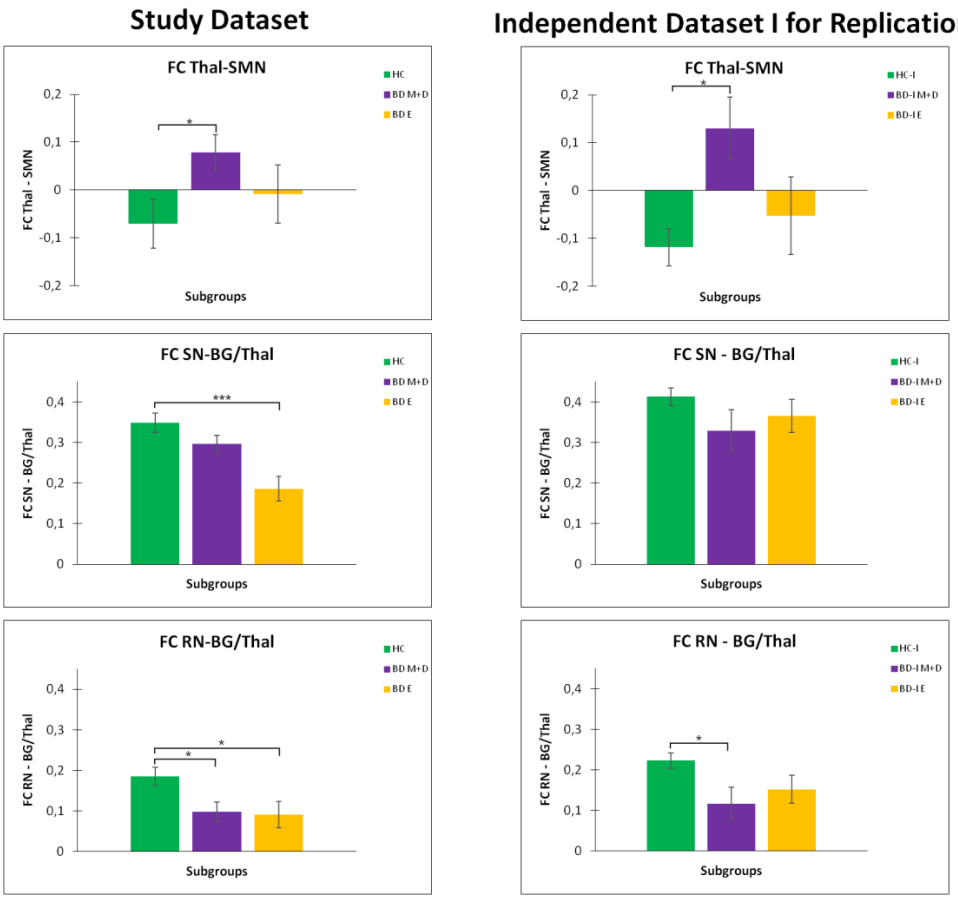
Distribution of FC Thal-DMN values among single subjects and correlations of FC Thal-DMN with FC SN-BG/Thal and FC RN-BG/Thal.
Abbreviations: HC, healthy controls; FC, functional connectivity; DMN, default mode network; Thal, thalamus; BG, basal ganglia; SN, substantia nigra; RN, raphe nucleus.



Supplemental Figure 5. Replication results of FC in HC

A. Distribution of FC Thal-SMN values among single subjects in the 2 independent datasets for replication.

B. Correlations of FC Thal-SMN with FC SN-BG/Thal (quadratic correlation) and FC RN-BG/Thal (linear correlation) in the 2 independent datasets for replication.
Abbreviations: HC, healthy controls; FC, functional connectivity; SMN, sensorimotor network; Thal, thalamus; BG, basal ganglia; SN, substantia nigra; RN, raphe nucleus.



Supplemental Figure 6. Replication results of FC in BD

Comparison of FC Thal-SMN, FC SN-BG/Thal and FC RN-BG/Thal between patients in active phases of illness and in euthymia, with respect to HC, in the study dataset and in the other independent dataset for replication.
* $p<0.05$
*** $p<0.001$
Abbreviations: BD, bipolar disorder; M, manic patients; D A, agitated depressed patients; D I, inhibited depressed patients; E, euthymic patients; HC, healthy controls.

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APPENDIX A

Relationship between functional connectivity and neuronal variability of sensorimotor network in mania and depression

We investigated the relationship between thalamo-SMN FC and neuronal variability of SMN in manic and inhibited depressed patients, as well as potential clinical correlations.

We performed resting state fMRI analyses on our study dataset, by using AFNI. After standard pre-processing (slice timing, alignement, normalization, smoothing and regression out of motion, white matter and cerebrospinal fluid, detrending, and filtering within 0.01-0.08Hz), the temporal variability (fractional standard deviation – fSD) of BOLD signal was extracted, and the mean values of fSD were calculated for SMN and DMN, as well as their ratio (SMN/DMN).

Then, we performed a correlation analysis between thalamo-SMN FC and fSD SMN (with age, gender and movement as covariates). Moreover, we compared the fSD SMN/DMN ratio between mania and inhibited depression. Finally, we calculated the correlation of fSD SMN/DMN ratio with YMRS and HAM-D scores.

Firstly, the thalamo-SMN FC showed a significant quadratic correlation with fSD SMN. Thus, around-zero values of FC are associated with low fSD, while high values of FC (both positive and negative) are associated with high fSD. Secondly, the fSD SMN/DMN ratio was found to be significantly higher in mania with respect to inhibited depression (confirming our previous work). Finally, the fSD SMN/DMN ratio was significantly correlated with the total scores of YMRS (positively) and HAM-D (negatively). See **Figure 1**.

The fSD can be considered as an index of intrinsic activity. Accordingly, the coherence of low-frequency oscillations between thalamus and SMN is associated to the level of intrinsic activity in SMN. In turn, the intrinsic activity in SMN is balanced with DMN. Thus, in mania (which shows an abnormal coupling between thalamus and SMN) the SMN/DMN balance is tilted toward the SMN. By contrast, in depression (which shows a decoupling between thalamus and SMN) the SMN/DMN balance is tilted toward the DMN. Finally, the SMN/DMN disbalancing is correlated with manic/depressive symptomatology.

Figures

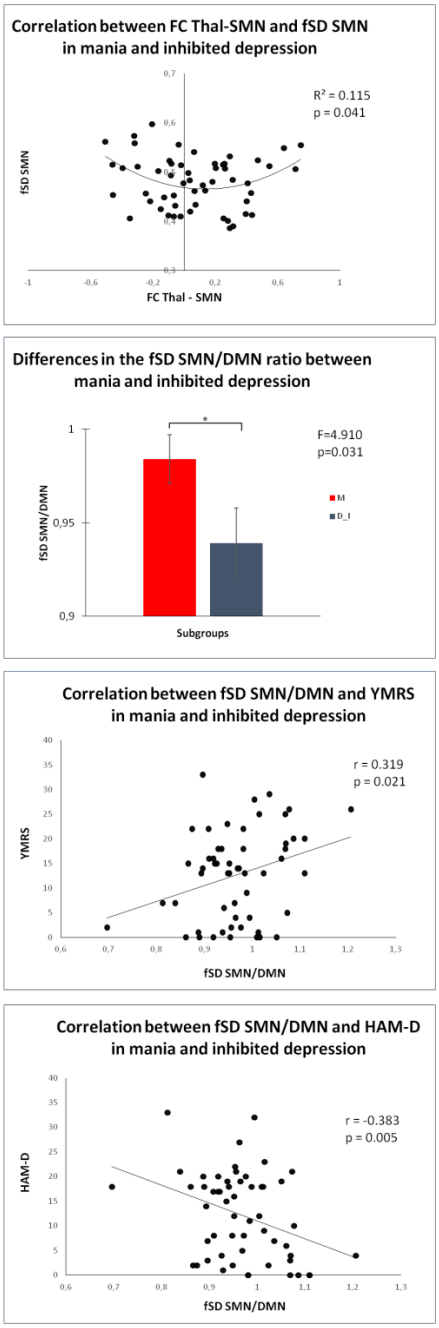

Figure 1. Relationships between thalamo-SMN FC, fSD in SMN, fSD SMN/DMN ratio, and clinical symptoms

Abbreviations: FC, functional connectivity; Thal, thalamus; SMN, sensorimotor network; fSD, fractional standard deviation; DMN, default mode network; M, manic patients; D I, inhibited depressed patients; YMRS, Young mania rating scale; HAM-D, Hamilton depression rating scale.

APPENDIX B

Sensorimotor network and psychomotor alterations in bipolar disorder and schizophrenia

We investigated the functional relationships between psychomotricity, SMN, subcortical-cortical loops and DA/5HT-related nuclei, in schizophrenia (SCZ).

We performed the same FC analyses on two samples of SCZ patients, belonging to the independent dataset I (OpenfMRI database, UCLA Consortium for Neuropsychiatric Phenomics: https://openfmri.org/dataset/ds000030/) and independent dataset II (from Taipei Medical University, Taiwan). We applied the same preprocessing procedures and FC analyses as were used in our main analysis (see above for a systematic description). Pre-processing and resting state data analyses were conducted using FSL. Briefly, the pre-processing steps were: slice timing, regression out of motion, white matter and cerebrospinal fluid, non-linear alignment and normalization, 6mm smoothing, standard 0.01-0.08Hz filtering. All selected participants showed no processing artifacts and head motion of less than 2 mm. We then calculated the same measures of thalamus-SMN FC, SN-BG/thalamus FC and RN-BG/thalamus FC, by using the same ROI-to-ROI approach, masks and methods in all the subjects of this independent dataset, as were used in our main analysis.

By using a dimensional approach, we investigated these FC measures in SCZ patients with excited psychomotricity or inhibited psychomotricity. According to the clinical scales, the SCZ sample of the independent dataset I was composed by patients with high level of psychomotor activity and patients with no alterations in psychomotor activity. By contrast, the SCZ sample of the independent dataset II was characterized by low level of psychomotor activity. See **Figure 1**.

SCZ patients with excited psychomotricity showed a significantly increase in thalamo-SMN FC when compared to HC ($t=2.240$; $p=0.027$), as well as a significant decrease in RN-BG/thalamus FC when compared to both HC ($t=2.356$; $p=0.020$) and SCZ patients without psychomotor disturbances ($t=2.397$; $p=0.021$). This was exactly the same pattern of FC alterations which has been detected in manic patients. No significant alterations were detected in SCZ patients without psychomotor disturbances. See **Figure 2a**.

By contrast, SCZ patients with inhibited psychomotricity showed a significant decrease in the absolute value (or modulus) of |thalamo-SMN FC| ($t=3.516$; $p=0.002$) and a significant decrease in SN-BG/thalamus FC ($t=5.353$; $p=0.000$), as well as a significant decrease in RN-BG/thalamus FC ($t=3.236$; $p=0.003$), when compared to HC. This was exactly the same pattern of FC alterations which has been detected in inhibited depressed patients. See **Figure 2b**.

Thus, independently from the diagnosis of BD or SCZ, psychomotor abnormalities were related to specific functional alterations in the sensorimotor system.

Figures

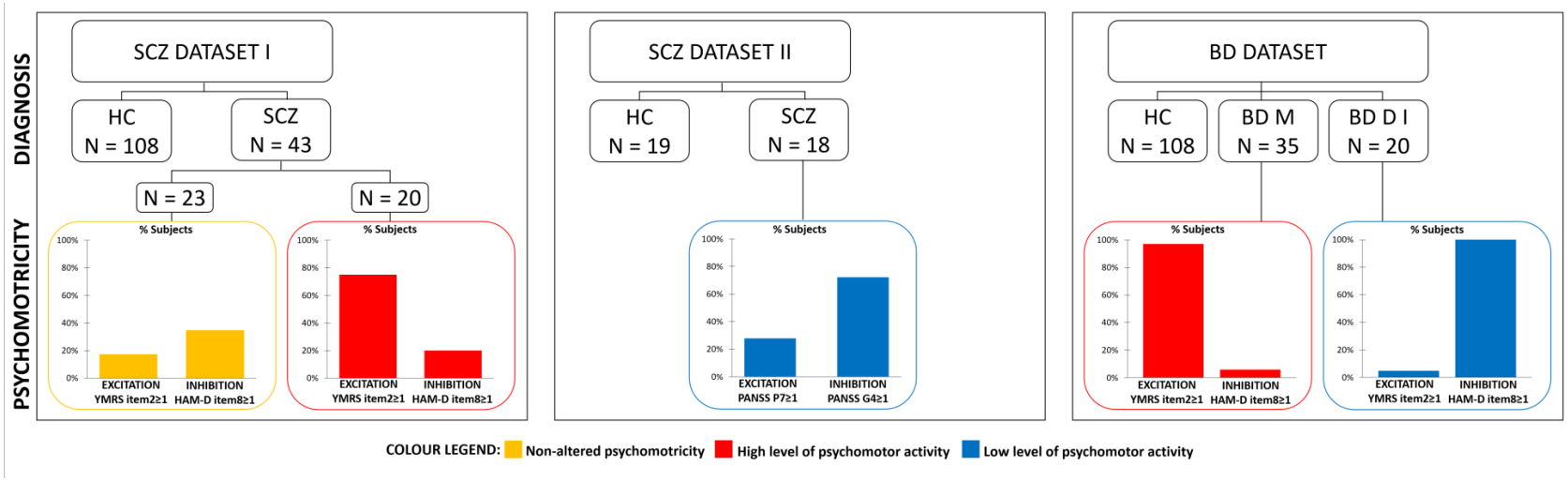

Figure 1. Psychomotricity in SCZ and BD

Abbreviations: HC, healthy controls; SCZ, schizophrenia; BD M, bipolar disorder manic phase; BD D I, bipolar disorder inhibited depressive phase
Psychomotor exctitation = YMRS item2≥1 or PANSS P4≥1; Psychomotor inhibition = HAM-D item8≥1 or PANSS G7≥1

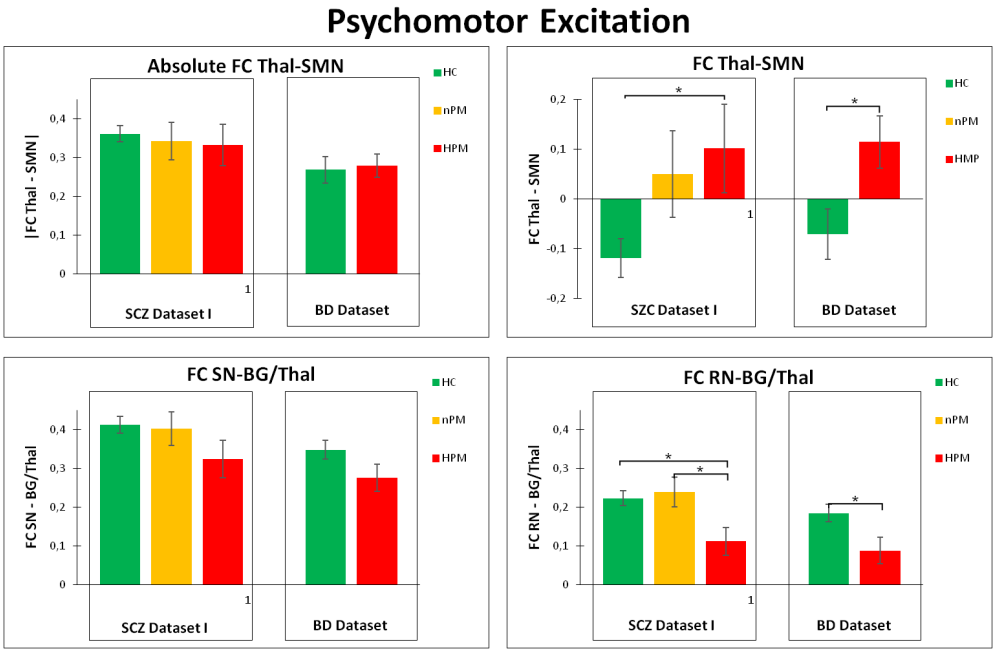

Figure 2a. Psychomotor excitation in SCZ and BD

Abbreviations: FC, functional connectivity; Thal, thalamus; SMN, sensorimotor network; BG, basal ganglia; SN, substatia nigra; RN, raphe nuclei; nPM, non-altered psychomotricity; HPM, high level of psychomotor activity; HC, healthy controls; SCZ, schizophrenia; BD, bipolar disorder

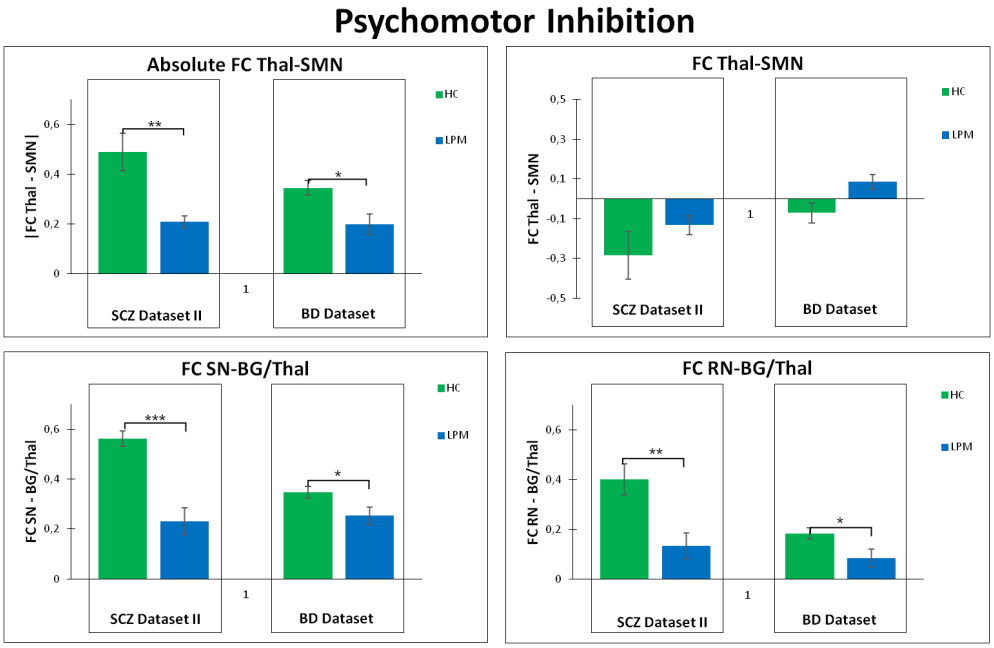

Figure 2b. Psychomotor inhibition in SCZ and BD

Abbreviations: FC, functional connectivity; Thal, thalamus; SMN, sensorimotor network; BG, basal ganglia; SN, substatia nigra; RN, raphe nuclei; LPM, low level of psychomotor activity; HC, healthy controls; SCZ, schizophrenia; BD, bipolar disorder

PART B

A WORKING MODEL ON LARGE-SCALE SPATIO-TEMPORAL ORGANIZATION OF BRAIN FUNCTIONING AND ITS IMPLICATIONS FOR BIPOLAR DISORDER

PART I - PHYSIOLOGY

Introduction

We propose a working model on the large-scale spatio-temporal organization of brain functioning, which integrates resting state networks, subcortical-cortical loops, neurotransmitters, and behavioral/phenomenological patterns.

1. Spatio-temporal structure of neuronal oscillations

Brain can be considered a generator of oscillations with complex spatio-temporal configurations [1]. Neurons, because of their intrinsic properties and their interactions via large- to small-scale anatomical circuitry, produce complex oscillatory activity [1]. Neuronal oscillations, which are self-generated by the dynamic balancing between excitation and inhibition in the intrinsic neuronal activity, are organized in a complex spatio-temporal structure and are fundamental to coordinate the communication between different brain regions and to information processing [1-3].

The neuronal activity is arranged in different spatial configurations of neuronal assemblies and networks, via temporal coherence and synchronization of oscillations between different brain regions (as measured, for instance, by functional connectivity – FC) [1, 4-7]. Within a network, a brain region or a neuronal assembly, the neuronal signal dynamically changes across time, showing different complexity in temporal

and spatial domains and representing the level of excitability and activity (as measured, for example, by the amplitude of signal oscillations - ALFF - or its variability across different points in time – SD) [1, 8-11]. A higher or lower spatio-temporal complexity of intrinsic neuronal activity enhances or reduces the detection of weak signals, and allows or inhibits sub-threshold neurons to fire, thus affecting the subsequent neuronal processing of incoming stimuli and neuronal outputs [11-15].

Neuronal oscillations are organized in different frequency bands which dynamically engage and disengage each other giving rise to perpetual fluctuation between unstable and stable phase synchrony in transient spatial distributed patterns [1]. The different frequency bands and their interaction can play a specific role in the activity synchronization of various brain areas and the level of excitability within different neuronal assemblies, being involved in different functions [1].

Neuronal oscillations in the low frequency bands (<0.1Hz) - which are typically investigated in resting state functional magnetic resonance imaging (fMRI) studies - consistently structure the spatio-temporal organization of intrinsic activity into large-scale resting state networks, as temporally coherent (or synchronous) slow oscillations across spatially-distributed brain areas [2-7, 16-19]. Moreover, the standard low frequency band is further subdivided into two ultra-slow frequencies: Slow5 (0.01-0.027Hz) shows stronger power and longer cycle duration, representing the "spatio-temporal basement" of brain functioning; while Slow4 (0.027-0.073Hz) is strongest throughout the neurotransmitters areas, basal ganglia, thalamus and sensorimotor cortical areas, potentially playing a role in subcortical-cortical loop communication at a network level [1, 3, 19-21].

On the other hand, neuronal oscillations in the higher frequency ranges – which are typically investigated by electroencephalography (EEG) - construct sub-networks up to the small-scale of local neuronal assemblies, which are more instable and dynamically adaptive via synaptic plasticity [1]. In particular, delta (1-4Hz) and theta (5-8Hz) waves are mainly involved in the deep stages of sleep and memory functions respectively, while alpha (8-12Hz) waves are stronger during resting wakefulness, and beta (12-30Hz) and gamma (30-180Hz) waves are mainly related to sensorimotor functions, cognitive functions and consciousness [1].

Importantly, changes in the low-frequency oscillations affect the coupling of slower to higher frequencies, i.e. cross-frequency coupling, and reverberate on higher

frequency oscillations [1, 22]. It has been proposed that neuronal activity in the lower frequency range is central for tonic activity of the brain (fluctuating, ongoing activity), which provides the substrate (or baseline) for the brain's default activity; phasic or stimulus-driven activity in the higher frequency ranges appears to operate on the basis of this existing tonic activity to permit specific behaviors [11, 23].
Accordingly, the spatio-temporal structure of intrinsic neuronal oscillations in the lower frequencies, representing the basal architecture of brain functioning, has been supposed to shape and set the correspondent general spatio-temporal structure (or form) of behavioral patterns and phenomenological experience [24]. On the other hand, during specific stimulus/task-related activity, high-frequency neuronal oscillations occurring in transient specific configurations may be related to the specific contents of behavioral patterns and subjective experience [1, 23-24].

2. Data on networks, subcortical-cortical loops and neurotransmitters

The large-scale organization of low-frequency neuronal oscillations (as well as its relationship with input/output processing) is shaped by the large-scale anatomical circuitry and biochemical organization [1].

2.1. Cortex and large-scale networks: anatomical and functional data

At the large-scale anatomical organization, cortical areas can be subdivided in sensorimotor and associative regions [25]. The sensorimotor cortex is composed of: the posterior sensorial regions – i.e. visual areas (occipital cortex as well as the dorsal parietal ("where") and ventral temporal ("what") visual pathways), auditory areas (superior temporal gyrus) and somatosensory areas (postcentral gyrus and associated parietal areas); the anterior motor regions (precentral gyrus and frontal premotor areas); and the interoceptive/limbic regions (insular cortex) [25-26]. The posterior sensorimotor cortex receives sensory inputs from the external and internal environment (visual, auditory, olfactory, gustative, somatic and visceral stimuli) via projections from the sensory peripheral systems [25]. In turn, the anterior sensorimotor cortex originates motor projections to the peripheral motor and visceral/autonomic/endocrine systems [25]. On the other hand, the associative cortices – i.e. posterior temporo-parietal cortex (such as the temporoparietal junction)

and anterior prefrontal cortex (such as the dorsolateral prefrontal cortex) – are not directly connected to the peripheral systems [25]. Finally, the cingulate cortex contains both sensorimotor areas (middle cingulate cortex - MCC) and limbic areas (supragenual anterior cingulate cortex - SACC), as well as associative areas (perigenual anterior cingulate cortex – PACC – and posterior cingulate cortex – PCC) [27]. Its anterior pole is connected to the medial prefrontal cortex (including the orbital prefrontal cortex), which, in turn, projects to all the neurotransmitters (brainstem) nuclei [28]. Its posterior pole is instead connected with the hippocampal formation, which is diffusively connected with all cortical areas (mainly the posterior and anterior associative regions) [29].

On the functional level, cortical neuronal oscillations in the low frequencies originate different large-scale resting state networks, which correspond to the structural subdivisions of the cortex [3, 18, 30]. Among them, the sensorimotor networks (SMN) [30-32], salience network (SN) [30, 33], default mode network (DMN) [30, 34-35], and central executive network (CEN) [30, 33] represent the most consistent networks in the brain architecture. The SMN comprises the strongly interconnected somatosensory (postcentral gyrus), motor (precentral gyrus) and premotor areas, as well as the MCC, and is involved in somatosensory awareness and motor/behavioral functions [30-32]. Furthermore, the visual and auditory networks, which mainly comprise the visual areas (occipital cortex) and auditory areas (superior temporal gyrus), and are involved in visual and auditory awareness [30-32]. The SN mainly comprises the insula and SACC, and is involved in interoceptive awareness, including pain and pleasure, and visceral and emotional responses, as well as salience attribution [26, 30, 33, 36]. The DMN mainly comprises the posterior associative areas (such as the temporoparietal junction and middle temporal gyrus) and PCC, as well as the anterior associative prefrontal areas and PACC, and is involved in mind wandering, remembering and imagination [37]. Finally, the CEN comprises mainly the anterior associative cortices (such as the dorsolateral prefrontal cortex) and posterior associative parietal cortices, and is involved in behavioral programming and executive functions [30, 33]. Importantly, intrinsic neuronal oscillations between specific networks, i.e. the SMN and DMN, are anti-correlated, suggesting that resting state networks are organized in different balances [16].

2.2. Thalamus and thalamo-cortical loops: anatomical and functional data

The different portions of the cortex (mainly via its deep layers IV-V) are reciprocally connected, through the thalamic radiations, with specific portions of the thalamus (via glutamatergic projections), constituting different thalamo-cortical loops [25]. The sensorimotor cortices are connected to the ventral and lateral part of the thalamus (ventral anterior/lateral nuclei, ventral posterolateral/posteromedial nuclei) as well as lateral and medial geniculate nuclei [25]. The insular cortex (and ACC) is mainly connected with the dorsal part of the thalamus (dorsomedial nucleus) and hypothalamus [38-40]. The posterior associative cortices are connected with the posterior part of the thalamus (pulvinar) [25], and the anterior associative cortices are connected with the anterior part of the thalamus (anterior thalamic group) and the mediodorsal nucleus [25, 38, 41]. The sensorimotor component of thalamo-cortical loops is directly connected with the external/internal environment: sensory cortices receive inputs from the environment through (and exclusively) the sensorial thalamic nuclei, while motor and other specific cortices send outputs to the effector systems [25]. On the other hand, the associative component of the thalamo-cortical complex is not directly connected with the environment and effector systems [25].

On the functional level, the resting state networks are functionally connected with different regions of the thalamus, coherently with the structural connections [42]. In particular, the SMN is connected with the ventral thalamic nuclei, while visual and auditory networks are connected with the geniculate nuclei [42]. The SN is connected with the mediodorsal thalamic nucleus [33] (and/or the anterior thalamic nuclei [42]). The DMN and CEN show more overlapped functional connections: the CEN and anterior DMN are mainly connected with the anterior thalamic nuclei and the anterior part of mediodorsal thalamic nucleus [33, 42], while the posterior DMN is mainly connected with the pulvinar and the posterior part of mediodorsal thalamic nucleus [42].

2.3. Basal ganglia and cortico-striatopallido-thalamo-cortical loops: anatomical and functional data

The thalamo-cortical loops are connected with and modulated by the basal ganglia, and these structures together constitute different in-parallel cortico-striatopallido-thalamo-cortical loops [43]. In particular, different regions of the cortex project to the striatum (via excitatory glutamatergic signaling), which projects to the globus

pallidum (via inhibitory gabaergic signaling), which in turn projects to the thalamus (via inhibitory gabaergic signaling), which finally projects back to the cortex (via excitatory glutamatergic signaling), mainly in the frontal regions [43]. These circuits are segregated into different in-parallel cortico-striatopallido-thalamo-cortical loops.

The most studied and anatomically well-defined basal ganglia circuitry is the sensorimotor loop, which involves the dorsal parts of basal ganglia [43]. Specifically, in the sensorimotor loop, the sensorimotor cortex (and MCC) projects to the dorsal striatum (in the putamen), which projects to the dorsal regions of globus pallidum, which then project to the ventral anterior and ventral lateral nuclei of thalamus, which finally project back to the cortical motor areas [43]. Basal ganglia, through this sensorimotor loop, seem to modulate the excitability of the correspondent cortical motor areas (as shown by the well-known occurrence of hyper- or hypo-kinetic movement disorders when basal ganglia are anatomically or biochemically altered) [43].

Another basal ganglia circuitry is the limbic loop, which involves the ventral parts of basal ganglia [43]. In the limbic loop, the ACC and other regions project to the ventral striatum (including the nucleus accumbens), which projects to the ventral regions of globus pallidum, which then project to the dorsomedial nucleus of thalamus, which finally projects back to the ACC and other regions (including the insula) [38-40, 43]. Other strictly related limbic areas include the amygdala (which is part of the "extended amygdala" together with the ventral striatum), hypothalamus, and insular cortex [38, 44]. Finally, an associative loop has been described: it connects the head of caudate and dorsal part of pallidum, the dorsomedial nucleus of thalamus, and the dorsolateral prefrontal cortex [43].

On the functional level, the regions of cortico-striatopallido-thalamo-cortical sensorimotor loop – i.e. dorsal striatum, ventral anterior/lateral nuclei of thalamus, and postcentral/precentral gyri, premotor areas and MCC - are functionally connected (coherently with the structural connections), and are functionally integrated into the SMN [42, 45].

On the other hand, the regions of cortico-striatopallido-thalamo-cortical limbic loop – i.e. ventral striatum and amygdala, dorsomedial nucleus of thalamus and hypothalamus, as well as insula and SACC - are functionally connected (coherently with the structural connections), and are functionally integrated into the SN [33, 45].

Finally, the associative anterior cortices (such as the dorsolateral prefrontal cortex),

the caudate head and different portions of the striatopallidum, as well as the anterior thalamic nuclei (and/or the anterior part of dorsomedial nucleus), are functionally connected and part of the CEN (and DMN) [33, 42, 45].

2.4. Brainstem and neurotransmitters systems: anatomical and functional data

In turn, basal ganglia, thalamus and cortex are connected with and differentially modulated by the brainstem neurotransmitter systems, such as dopamine (DA) and serotonin (5HT) systems which are key neuromodulators involved in the sensorimotor and affective functions [46].

The dopaminergic mesencephalic system is composed by the substantia nigra compacta (SNC) and ventral tegmental area (VTA) [47-52]. These nuclei give rise to the nigrostriatal, mesocorticolimbic and mesothalamic pathways, which show widespread but organized connections to different relay stations of the sensorimotor and limbic loops [47-52]. In particular, the SNC neurons mainly project to the dorsal striatum (including the dorsolateral portions of caudate and putamen), globus pallidum and ventral thalamic nuclei, thus connecting different relay stations of the sensorimotor loop and being mainly involved in sensorimotor functions [47-48, 50-52]. The VTA neurons mainly project to the ventral striatum (including the nucleus accumbens and ventral parts of caudate and putamen), dorsomedial thalamic nucleus and medial prefrontal cortex (including the ACC and medial orbitofrontal cortex), thus connecting different relay stations of the limbic loop and being involved in affective functions [47, 49-51, 53].

On the other hand, the 5HT neurons of the raphe nuclei (RN) show structural connections with distributed subcortical and cortical regions [54-57]. These nuclei mainly project to the striatum and thalamus (including the ventral anterior/lateral nuclei, dorsomedial nucleus, and posterior complex), as well as different cortical regions (including sensorimotor cortices and cingulum), thus connecting different relay stations of the sensorimotor/limbic loops and being involved in sensorimotor/affective functions [54-57].

On the functional level, coherently with the structural projections, the DA-related SN shows functional connections mainly with regions of the subcortical-cortical sensorimotor loop and SMN, such as the dorsal striatum and pallidum, ventral thalamus, and sensory and motor cortices [58-62]. The VTA shows functional connections mainly with regions of the subcortical-cortical limbic loop and SN, such

as the nucleus accumbens, dorsomedial thalamus and ACC [58-59, 62]. The administration of pro-dopaminergic substances increases the FC between subcortical and cortical sensorimotor regions (such as between striatum and sensorimotor cortex), as well as the activity (i.e. increase in SD) of SMN [63-65]. By contrast, the administration of anti-dopaminergic substances decreases the FC between the same sensorimotor areas [63-65]. Moreover, pro- and anti-dopaminergic substances increase and decrease, respectively, the FC between subcortical and cortical regions of SN (such as between ventral striatum and insula) [64, 66]. Finally, dopaminergic substances reduce the FC between regions of the DMN, while a decrease in dopaminergic availability reduces the task-related suppression of DMN and the anti-correlation between task-positive networks and DMN [64, 67-68].

On the other hand, according to the anatomical projections, the RN shows functional connections with regions of the subcortical-cortical sensorimotor loop and SMN (such as striatum and sensorimotor cortices), as well as regions of the limbic loop and SN (such as dorsomedial thalamus, SACC and insula) [62, 69]. Interestingly, RN signal is positively correlated with basal ganglia and thalamus while it is negatively correlated with somatosensory and motor cortices [69]. Coherently, 5HT and SMN activity are inversely related, i.e. the decrease in 5HT availability is associated with an increase in activity (i.e. increase in ALFF) and reactivity (increase activation during tasks) of SMN [70-71]. Pro-serotonergic substances reduces the intra-network FC of SN/insula [72]. Moreover, a decrease in serotonergic availability reduces the activity (i.e. decrease in ALFF) of DMN [71, 73].

Finally, we found that the FC of DA-related SNC and 5HT-related RN with the basal ganglia and thalamus (i.e. the subcortical regions of the cortico-striatopallido-thalamo-cortical loops) differentially modulate the functional coupling of low-frequency oscillations between thalamus and cortical SMN. In particular, a non-linear (i.e. quadratic) correlation between the SNC-striatopallidum/thalamus FC and thalamus-SMN FC was found (with a U-shaped distribution). Accordingly, low SNC-related FC is associated with around-zero values of thalamus-SMN FC, while high SNC-related FC is associated with high absolute values of thalamus-SMN FC (i.e. both positive or negative). On the other hand, a linear and negative correlation between the RN-striatopallidum/thalamus FC and thalamus-SMN FC was found. Accordingly, low RN-related FC is associated with high positive values of thalamus-SMN FC, while high RN-related FC is associated with high negative values of

thalamus-SMN FC. Thus, the SNC-related FC seems to be associated to the absolute strength of correlation between thalamus and SMN, while the RN-related FC seems to modulate the sign of correlation. Altogether, low SNC-mediated loop FC is associated to disconnectivity between thalamus and SMN, while high SNC-mediated loop FC is associated with high functional coupling between thalamus and SMN, showing anti-correlation (if the RN-mediated loop FC is high), or positive correlation (if the RN-mediated loop FC is low). According to these and previous data, a similar pattern between VTA/RN-mediated loop FC and thalamus-SN FC can be supposed, but this needs to be tested in future studies.

3. A working model on the large-scale spatio-temporal organization of brain functioning

On the basis of the reported data, we propose a working model on the large-scale spatio-temporal organization of brain functioning, and its relationship with behavioral/phenomenological patterns. According to our model, the large-scale low-frequency intrinsic oscillations of neuronal activity, as shaped by the large-scale anatomical organization and circuitry, can be divided in different levels. a) The large-scale resting state networks and their balances organize the basal architecture of intrinsic brain functioning. b) The thalamo-cortical loops modulate the relationship of intrinsic activity with sensory inputs and outputs processing. c) The cortico-striatopallido-thalamo-cortical loops modulate the transition pattern from sensory inputs to motor/visceral outputs. d) The brainstem neurotransmitter nuclei modulate the coupling by synchronization of subcortical-cortical loops.

In turn, the configuration of large-scale low-frequency intrinsic activity reverberates and affects the processing of specific inputs and outputs during high-frequency stimulus/task-related activity.

Finally, the spatio-temporal structure of large-scale low-frequency intrinsic activity shapes the different dimensions and core features of behavioral/phenomenological patterns, while the specific input/output processing during high-frequency activity arranges the correspondent specific contents.

3.1. Large-scale cortical networks

The large-scale resting state networks and their balances shape the basal architecture of brain functioning. According to the patterns of connections and the relationship of cortical areas with processing of sensory external/internal inputs and motor/visceral outputs, we can differentiate: (a) the sensorimotor networks, which comprise (1) the SMN (sensorimotor cortex) and visual/auditory networks (visual/auditory cortex) as well as (2) the SN (insular cortex); and (b) the associative networks, which comprise (3) the DMN (mainly posterior associative cortex) and (4) the CEN (mainly anterior associative cortex).

In the sensorimotor networks, the ongoing intrinsic neuronal oscillations produce ongoing motor and visceral outputs, as processed by the SMN and SN respectively, and are directly modulated by environmental changes via sensory inputs both from: (i) the external world (such as somatosensory, visual and auditory stimuli), as processed by the SMN and visual/auditory networks; and (ii) the internal world (such as visceral stimuli, including pleasure and pain), as processed by the SN.

On the other hand, in the associative networks, the ongoing intrinsic neuronal oscillations are detached from the environment, and are involved in: (i) associative sensorial functions, i.e. associations between actual and memorized sensorial stimuli in the posterior cortices, as prevalently processed by the DMN; and (ii) associative executive functions, i.e. associations between actual outputs and memorized behavioral programs in the anterior cortices, as prevalently processed by the CEN.

In general, neuronal oscillations in sensorimotor and associative networks are anti-correlated, so that the global intrinsic activity is organized in different network balances.

The spatial distribution of coherent neuronal oscillations in the lower frequency bands that changes across networks (as measured for example by SD) organize the basal spatio-temporal structure of ongoing intrinsic activity, which is then associated with different patterns of inputs and outputs processing.

Moreover, the cingulum could be involved in the integration of activity of different networks (considering that the different portions of the cingulum are part of the different networks). Based on this, the cingulum activity could then allow the pre-setting of neurotransmitters basal activity (via its anterior pole and related medial prefrontal cortex-brainstem interactions) and the modulation of neural plasticity

within networks, i.e. memorization (via its posterior pole and related hippocampus-associative cortex interactions).

3.2. Thalamo-cortical loops

The thalamo-cortical loops, via segregated bidirectional connections between the cortical areas and thalamic nuclei, mediate the relationship of ongoing intrinsic oscillations in the cortical networks with processing of external/internal sensory inputs and motor/visceral outputs. Accordingly, the SMN is connected with the ventro-lateral thalamus, while the visual/auditory networks are connected with the geniculate thalamic nuclei; the SN is connected with the dorso-medial thalamus; the DMN (in particular the posterior DMN) is mainly connected with the posterior thalamus; and the CEN (and the anterior DMN) is mainly connected with the anterior thalamus.

The functional coupling of low-frequency oscillations between thalamus and cortex (as measured for example by FC) directly affects the modulation and integration of sensory inputs into the ongoing intrinsic low-frequency oscillations of the sensorimotor cortices and related processing of output patterns. This would be associated with changes in the spatio-temporal complexity of the intrinsic low-frequency activity within the sensorimotor areas. At the same time, considering the anti-correlation of neuronal oscillations between sensorimotor and associative areas, changes in the thalamo-sensorimotor coupling can also affect, indirectly, the global ongoing low-frequency oscillatory activity via the sensorimotor/associative areas balancing.

3.3. Cortico-striatopallido-thalamo-cortical loops

The cortico-striatopallido-thalamo-cortical loops, via inhibition or excitation of thalamo-cortical loops mediated by basal ganglia, modulate the transition pattern from external/internal sensory inputs to motor/visceral outputs, which is processed by cortical networks (mainly in the frontal areas). The loops are differentiated in: (a) the cortico-striatopallido-thalamo-cortical sensorimotor loop (mainly involving the dorsal basal ganglia and ventro-lateral thalamus), which modulates the thalamo-cortical activity of SMN; and (b) the cortico-striatopallido-thalamo-cortical limbic loop (mainly involving the ventral basal ganglia and dorso-medial thalamus), which modulates the thalamo-cortical activity of SN.

The coordination of intrinsic low-frequency neuronal oscillations through the different inhibitory and excitatory areas of basal ganglia and thalamus within the sensorimotor loop can finally modulate and set the level of basal excitability and the coupling between thalamus and cortical SMN. In turn, the level of intrinsic activity and excitability of cortical motor areas of the SMN (and its relative balancing with associative networks) is associated with a different threshold for external sensory inputs to initiate or change correspondent motor outputs, resulting in a facilitation or inhibition of the transition from sensory inputs to motor outputs. Analogously, the activity pattern within the limbic loop could modulate the level of excitability and coupling between thalamus and SN, thus modulating the threshold for internal sensory stimuli to initiate or change correspondent visceral outputs.

3.4. Neurotransmitters systems

The brainstem DA and 5HT systems, via their widespread but organized projections, synchronize and coordinate the activity within the different cortical-striatopallido-thalamo-cortical loops and relative sensorimotor networks. (a) the DA system seems to be the main neuromodulator, favoring the activity in: (i) the subcortical-cortical sensorimotor loop and SMN, via the connections of SNC with dorsal basal ganglia and thalamic regions; and (ii) the subcortical-cortical limbic loop and SN, via the connections of VTA with ventral basal ganglia and thalamic regions. On the other hand, (b) the 5HT system seems to act as a secondary neuromodulator, affecting the activity in: (i) sensorimotor loop and SMN, and (ii) limbic loop and SN, via the connections of RN with basal ganglia, thalamic and cortical regions.

The intrinsic low-frequency oscillations of DA neurons (i.e. tonic activity) in the SNC would mediate the synchronization of low-frequency oscillations between the different subcortical relay stations within the cortico-striatopallido-thalamo-cortical sensorimotor loop, i.e. the dorsal regions of basal ganglia and ventro-lateral regions of thalamus. This in turn allows the basal ganglia to modulate the excitability and functional coupling (i.e. the absolute strength of correlation) of low-frequency oscillations between thalamus and cortical SMN, thus affecting the basal activity of SMN and its processing of external inputs and motor outputs (as well as its balancing with the associative networks). Analogously, the low-frequency oscillations of DA neurons in the VTA would mediate the synchronization of neuronal oscillations between ventral regions of basal ganglia and dorso-medial thalamus within the

limbic loop. This modulates the excitability and functional coupling (strength of correlation) between thalamus and SN, its basal network activity and its processing of internal inputs and visceral outputs (as well as its balancing with the associative networks). Thus, overall, the DA activity on subcortical-cortical loops would favor an enhancement of basal excitability with a direct coupling of neuronal oscillations between thalamus and sensorimotor cortex. This, in turn, would result in enhanced modulation of intrinsic neuronal activity in the sensorimotor networks by external/internal sensory inputs with direct and facilitated transformation into motor/visceral outputs.

On the other hand, the low-frequency oscillations of 5HT neurons in the RN would modulate the pattern of (DA-related) synchronization of low-frequency oscillations between basal ganglia, thalamus and cortex within the sensorimotor and limbic loops. In particular, 5HT neuronal oscillations would transform the (DA-related) synchronous in-phase oscillations between thalamus and cortex into a counter-phase pattern (possibly by exciting the thalamus and simultaneously inhibiting the sensorimotor cortex). Accordingly, when the thalamus is excited, the sensorimotor cortex is inhibited and the associative cortex is excited; subsequently, when the thalamus is inhibited, the sensorimotor cortex is excited and the associative cortex is inhibited. Thus, it could be assumed that 5HT activity would favor a greater contribution from the associative networks into the input/output processing by the sensorimotor networks, and a more indirect transition pattern of sensory stimuli into motor/visceral outputs.

Finally, we can assume that the activity of other neurotransmitters, such as noradrenalin and histamine or acetylcholine, by differently coordinating the various thalamo-cortical loops and cortico-striatopallido-thalamo-cortical loops, could favor other specific patterns of networks functioning and balancing, with relative functions and behaviors.

3.5. Large-scale low-frequency intrinsic activity and high-frequency stimulus/task-related activity

Thus, as suggested, the spatio-temporal organization of the large-scale low-frequency neuronal oscillations provides the substrate (or baseline) of intrinsic activity, as the basal architecture of brain functioning.

This configuration, in turn, reverberates and affects the neuronal processing of specific inputs and outputs during stimulus/task-related activity. In particular, the resulting spatial distribution of coherent low-frequency oscillations across cortical networks shapes (via low-high cross-frequency coupling) correspondent transient spatial synchrony of high-frequency oscillations (such as in beta and gamma bands) within dynamically changing neuronal assemblies, with consequent processing of specific inputs and outputs.

3.6. Behavioral/phenomenological dimensions and features

According to the model, the specific spatio-temporal structure of large-scale low-frequency intrinsic neuronal oscillations shapes the general organization (or form) of different dimensions and core features of behavioral/phenomenological patterns. On the other hand, this configuration affects the neuronal processing in the higher frequencies, so that the general behavioral/phenomenological organization assumes specific contents in relation to specific inputs and outputs

Thus, considering the patterns of connections and functioning, the different networks can be linked to the classical dimensions of psychomotricity, affectivity and thought [74]. (1) The SMN functioning (together with visual/auditory networks) could be related to psychomotricity, which, accordingly, can be defined as the modulation of motor/behavioral patterns by external environmental sensory stimuli. (2) The SN functioning could be related to affectivity, which, accordingly, can be defined as the modulation of visceral effector patterns by internal visceral/body stimuli, importantly including pleasure and pain [36]. On the other hand, (3) the DMN functioning could be related to sensorial thought, which, accordingly, can be defined as the association of actual external/internal sensory stimuli (directly present in the environment) with memorized sensory external/internal inputs (not directly present in the environment). (4) Finally, the CEN functioning could be related to executive thought, which, accordingly, can be defined as the association of actual motor/visceral outputs with memorized motor/effector programs.

The pattern of excitability and functional coupling between thalamus and cortical areas (as well as the related balancing between networks) affects the balance between sensory inputs and associative networks activity in the modulation of the ongoing intrinsic activity in the sensorimotor networks and consequent outputs. This would

affect the spatio-temporal patterns of both behavioral and phenomenological basal features.

Specifically, the balancing of intrinsic activity between sensorimotor and associative networks is respectively associated with: (i) a SMN-mediated enhanced or reduced transition from external sensory inputs to motor outputs, reflecting in excited or inhibited psychomotricity; (ii) a SN-mediated enhanced or reduced transition from internal sensory inputs to visceral outputs, reflecting in excited or inhibited affectivity; (iii) a reduced or increased contribution of DMN/CEN, with consequent direct or indirect transformation of actual stimuli into outputs (i.e., with lower or greater contribution of memorized stimuli), reflecting in a sensorimotor or associative thought.

On the phenomenological level, accordingly, the subjective experience is spatially focalized toward external/internal environmental contents, as characterized by perceptions (which are localized in the objective external/internal space, and are defined and vivid, constant and independent from the will [75]), or toward associative memorized contents, as characterized by representations (which are unlocalized, and are undefined and not vivid, inconstant and dependent from the will [75]). The phenomenal contents change rapidly according to sensorial stimuli (the time perception become faster) or can tend to reiterate and change more slowly, if they are partially detached from the environment and linked only indirectly to sensorial stimuli (the time perception become slower).

Thus, according to the spatio-temporal structure of brain functioning, behavior and phenomenal experience can be more tuned or detuned on the external/internal environment.

3.7. Sensorimotor vs. associative patterns

In summary, according to our model, the DA/5HT low-frequency activity modulates the synchronization of low-frequency oscillations between different (mainly subcortical) regions within the cortico-striatopallido-thalamo-cortical sensorimotor/limbic loops. This allows the dorsal/ventral basal ganglia to modulate and set the basal excitability and functional coupling of low-frequency oscillations between ventrolateral/dorsomedial thalamus and cortical SMN/SN. The different setting of excitability and coupling within the thalamo-cortical loops manifests in a different level of spatio-temporal complexity of neuronal oscillations within the

sensorimotor networks, and relative balancing with the associative networks (DMN/CEN). The resulting intrinsic spatial distribution of coherent low-frequency oscillations across networks reverberates and affects the neuronal processing of specific inputs and outputs in the higher frequencies during stimulus/task-related activity. This spatio-temporal configuration of intrinsic activity changes the detection, influence and integration of external/internal sensory inputs into the ongoing intrinsic activity of the sensorimotor cortex, and modifies the related transition pattern from sensory stimuli to motor/visceral outputs.

Accordingly, the spatio-temporal structure of large-scale low-frequency intrinsic activity shapes the general organization (or form) of dimensions and core features of behavioral/phenomenological patterns (psychomotricity, affectivity and thought), which then assume, during high-frequency activity, specific contents in relation to specific inputs and outputs. See **Figure 1**.

The large-scale spatio-temporal organization of brain functioning can assume a multitude of shades and combinations of different neuronal and related behavioral/phenomenological configurations, ranging between two opposite poles, the sensorimotor and associative patterns.

3.8. *Sensorimotor pattern*

According to the model, high basal or intrinsic low-frequency activity of brainstem DA nuclei (with concomitant low intrinsic activity of 5HT nuclei) synchronizes and couples the low-frequency oscillations in the basal ganglia and thalamic regions within the cortico-striatopallido-thalamo-cortical loops. In particular, SNC activity synchronizes the neuronal oscillations in the dorsal striatum-pallidum and ventro-lateral thalamus within the subcortical-cortical sensorimotor loop, while VTA activity synchronizes the neuronal oscillations in the ventral striatum-pallidum and dorso-medial thalamus within the subcortical-cortical limbic loop.

The DA-mediated synchronization of low-frequency oscillations in the subcortical regions of the loops is associated with an increase in excitability of the thalamus and its coupling with the respective cortical areas. Within the sensorimotor loop, the increased inhibitory activity of dorsal striatum on inhibitory activity of dorsal pallidum favors the disinhibition of ventro-lateral thalamic activity, which manifests in increased excitability and positive (in-phase) functional coupling of low-frequency oscillations between thalamus and cortical SMN. Analogously, within the limbic

loop, the increased coordination of neuronal oscillations between ventral basal ganglia and dorsomedial thalamus favors increased excitability and positive (in-phase) functional coupling between thalamus and cortical SN.

The loops-mediated functional coupling (i.e. high positive correlation) of low-frequency oscillations between thalamus and sensorimotor cortex is associated with increased basal excitability and spatio-temporal complexity of ongoing intrinsic oscillatory activity within both the SMN and SN. This in turn favors a balance between sensorimotor networks (SMN-SN) and associative networks (DMN-CEN) shifted toward the sensorimotor pole.

The resulting resting state spatial distribution of temporally coherent low-frequency oscillations then reverberate and affect the configuration of stimulus/task-related activity during specific input/output processing, favoring the high-frequency neuronal oscillations in the sensorimotor regions at the expense of associative areas.

This spatio-temporal structure of brain functioning predisposes an alignment of intrinsic activity with sensory inputs and peripheral outputs. The ongoing intrinsic oscillatory activity in the sensorimotor areas is more affected by incoming sensory stimuli (rather than by the activity in the associative areas), and the transition pattern from sensory stimuli to outputs is enhanced and direct (i.e. the external/internal sensory stimuli can easily and directly initiate or change the motor/visceral outputs patterns).

This may manifest in a correspondent behavioral/phenomenological sensorimotor pattern. In particular, psychomotricity is excited, with enhanced transformation of external sensory inputs into motor outputs. Affectivity is excited, with enhanced transformation of internal sensory inputs into visceral outputs. Thought is shifted toward a sensorimotor pattern, as manifest in a direct transition from sensory inputs to outputs with minor contribution of associative memorized stimuli.

Phenomenologically, in this state, the subjective experience is spatially focalized and unbalanced toward external or internal environmental contents with respect to associative or memorized contents, i.e. it is characterized by perceptions. The contents change rapidly according to sensorial stimuli, resulting in a faster time perception. Thus, behavior and subjective experience are tuned on the external/internal environment. See **Figure 2a** and **Figure 2b**.

3.9. Associative pattern

On the opposite side, low (basal) intrinsic low-frequency activity of DA nuclei (independently from the 5HT activity) is associated with desynchronization and decoupling of low-frequency oscillations in the basal ganglia and thalamic regions within the cortico-striatopallido-thalamo-cortical loops, both the sensorimotor (low SNC activity) and limbic (low VTA activity) loops.

Desynchronization within the sensorimotor loop results in decreased excitability and functional decoupling between thalamus and SMN, while descynchronization within the limbic loop results in decreased excitability and functional decoupling between thalamus and SN.

The resulting functional decoupling (i.e. low absolute correlation) between thalamus and sensorimotor cortex is associated with decreased basal excitability and spatio-temporal complexity of ongoing intrinsic oscillatory activity within both the SMN and SN. This in turn favors a balance between sensorimotor networks (SMN-SN) and associative networks (DMN-CEN) shifted toward the associative pole.

This resting state configuration of low-frequency oscillations affects the stimulus/task-related activity during specific input/output processing, favoring the high-frequency neuronal oscillations in the associative regions at the expense of sensorimotor areas.

This spatio-temporal structure of brain functioning predisposes a partial detachment of intrinsic activity from sensory inputs and peripheral outputs. The ongoing intrinsic oscillatory activity in the sensorimotor areas is more affected by the activity in the associative areas, and the transition pattern from sensory stimuli to outputs is reduced and indirect (i.e. the external/internal sensory stimuli can difficulty and only indirectly initiate or change the motor/visceral outputs patterns).

This may manifest in a correspondent behavioral/phenomenological associative pattern. In particular, psychomotricity is inhibited, with reduced transformation of external sensory inputs into motor outputs. Affectivity is inhibited, with reduced transformation of internal sensory inputs into visceral outputs. Thought is shifted toward an associative pattern, as manifest in an indirect transition from sensory inputs to outputs, with major contribution of associative memorized stimuli.

Phenomenologically, in this state, the subjective experience is focalized and unbalanced toward associative or memorized contents with respect to external/internal environmental contents, i.e. it is characterized by representations.

The contents, detached from the environment, tend to reiterate and change more slowly, resulting in a slower time perception. Thus, behavior and subjective experience are detuned from the external/internal environment, while are more tuned on imagination. See **Figure 2c** and **Figure 2d**.

Conclusions

In conclusion, our working model on the spatio-temporal structure of large-scale brain functioning could provide a framework for the investigation of the specific functional relationships between resting state networks, subcortical-cortical loops and neurotransmitters, as well as their transformation into dimensions and core features of behavioral/phenomenological patterns.

On the other hand, this model could provide specific hypotheses on the neurobiological background of different psychopathological states of major psychiatric disorders, such as bipolar disorder.

PART II – PATHOPHYSIOLOGY

Introduction

We apply our working model on bipolar disorder (BD), investigating the relationship between specific alterations in the large-scale spatio-temporal organization of brain functioning (i.e. networks, subcortical-cortical loops and neurotransmitters) and specific psychopathological patterns.

1. Manic-depressive cycle and bipolar disorder

According to our model, manic and depressive states can be considered the extreme polarizations of brain functioning and related behavioral/phenomenological patterns. Thus the manic-depressive cycle can be considered a core alteration, while other factors and conditions (such as mixed states, basal temperament and psychosis) can then play a role in the final expression of psychopathology.

1.1. Data on functional alterations of networks, subcortical-cortical loops and neurotransmitters in the manic and depressive phases of bipolar disorder

BD is clinically defined by the occurrence of recurrent episodes of mania and depression, which are characterized by opposite constellations of symptoms, and represent the extreme poles of excitation and inhibition in the different psychopathological dimensions, i.e. psychomotricity, affectivity and thought [74, 76]. Longitudinally, mania and depression can be linked together in the manic-depressive cycle (mania tends to occur first, followed by depression) [77], and different patterns of illness course are described. Among different clinical presentations, a well-defined phasic course (where cycles are separated by clear periods of asymptomatic euthymia) characterizes BD type I, while, on the opposite side, a more unstable course (where no defined periods of euthymia occurs) is typical of the cyclothymic disorder [76].

On the neuronal level, mania and depression show, coherently with their opposite psychopathology, some opposite or different functional alterations, especially in large-scale networks, subcortical-cortical loops and neurotransmitters systems.

In particular, mania is clinically characterized by: excited psychomotricity, as manifest in tendency to act, impulsivity, hyper-activity, logorrhea, increased energy and motor agitation; excited affectivity, as manifest in irritability, internal agitation, emotional lability and excessive salience especially toward environmental stimuli, and/or mood biased toward positive affect; thought focused on environmental contents, which can manifest in distractibility and flight of ideas [74, 76].
In our resting state fMRI work on BD, an abnormal spatio-temporal organization of intrinsic low-frequency oscillations was found in manic patients. At a networks level, manic patients show altered topographical distribution in temporal variability of low-frequency oscillations across the sensorimotor and associative cortex: specifically, a relative increase in SD in the SMN at the expense of DMN was found, so that the balance between SMN and DMN is tilted toward the SMN [78]. Coherently, mania is also characterized by a predominance of global signal over the sensorimotor cortical areas (mainly motor cortex). Moreover, the degree of centrality is increased in sensorimotor areas in this phase of illness. Additionally, in these patients, the intra-network FC of DMN (between PACC and PCC) is reduced [79-80]. At the subcortical-cortical loops level, manic patients show an abnormal functional coupling of low-frequency oscillations between thalamus and sensorimotor cortex: specifically, the FC between thalamus and SMN is increased and abnormally shifted toward positive values. Finally, at the neurotransmitter level, manic patients show a functional decoupling of low-frequency oscillations of 5HT-related RN with basal ganglia and thalamic regions, as manifest in reduced FC between these regions.
Coherently, in biochemical studies on neurotransmitters systems in BD, an alteration of 5HT signaling was found in manic patients. In particular, decreased 5HT signaling has been associated with impulsivity (a core symptoms of mania), and, notably, a decreased 5HT transmission in BD, and especially in the manic phase, has been reported as the most consistent neurotransmitters finding in BD [46, 81-83]. On the other hand, even if similarities between the behavioral effects of pro-dopaminergic substances (such as cocaine and amphetamine) and manic symptomatology have

been observed (suggesting a DA hypothesis of BD), data on altered DA transmission in the manic phase of BD are negative or conflicting [83-84].
By stepping to the investigation of the higher frequencies of neuronal oscillations in BD by means of EEG studies, greater beta frequencies activity across sensorimotor cortical areas was reported in manic patients [85].

On the opposite side, depression (in its typical inhibited form) is clinically characterized by: inhibited psychomotricity, as manifest in difficulty to act, indecisiveness, poor motricity, loss of energy and motor retardation; inhibited affectivity, as manifest in anhedonia, apathy, emotional blunting and reduced salience especially toward environmental stimuli, and/or mood biased toward negative affect; thought detached from environmental contents, which can manifest in concentration deficits and ruminations [74, 76].
In our resting state fMRI work on BD, an opposite or different pattern of alterations in the spatio-temporal organization of intrinsic low-frequency oscillations was found in depressed patients. At a networks level, depressed patients show a relative decrease in SD in the SMN at the expense of DMN, so that the balance between SMN and DMN is tilted toward the DMN [78]. Coherently, depression is also characterized by a predominance of global signal over the associative cortical areas (such as PCC). Moreover, the degree of centrality is increased in associative areas (such as PCC) in this phase of illness. At the subcortical-cortical loops level, patients in the depressive phase, in particular the inhibited form, show a functional decoupling of low-frequency oscillations between thalamus and SMN, as manifest by a decrease of absolute FC shifting toward around-zero values. Finally, at the neurotransmitter level, inhibited depressed patients show a functional decoupling of low-frequency oscillations (i.e. reduced FC) of DA-related SNC with basal ganglia and thalamic regions, together with reduced FC of RN with the same subcortical regions.
Coherently, in biochemical studies on neurotransmitters systems in BD, alterations of DA and 5HT signaling were found in depressed patients. Along with the observation of anti-manic and/or pro-depressant effects of anti-dopaminergic substances (such as antipsychotic drugs), a decreased DA transmission in the depressive phase of BD has been consistently detected (in accordance with the DA hypothesis of BD) [83-84].

On the other hand, decreased 5HT transmission is reported in BD, but both decreased and increased 5HT activity has been supposed in depressive states [83, 86].
Finally, by stepping to the EEG investigations of the higher frequencies in BD, reduced beta frequencies activity across sensorimotor cortical areas was reported in depressed patients [85].

1.2. A working model on the large-scale spatio-temporal organization of brain functioning of psychomotor alterations in mania and depression

The reported data on functional alterations in BD are in accordance with our working model on the large-scale spatio-temporal organization of brain functioning, as mania and depression can be considered the pathological side of sensorimotor and associative patterns, respectively. In particular, the line of evidences is consistent and coherent especially with regard to the alterations of the psychomotor dimension in manic and depressive phases.
Thus, according to our model, in mania a deficit in 5HT transmission occurs, with related functional disconnection of RN (as manifested by decreased FC between RN and basal ganglia/thalamic regions). Consequently, the DA/SNC-related synchronization of intrinsic activity within the subcortical-cortical sensorimotor loop is no more modulated by 5HT, so that thalamus and SMN are abnormally coupled (as manifested by increased FC between thalamus and SMN toward positive values). In turn, this is associated with increased excitability and complexity of intrinsic low-frequency activity within the SMN at the expense of the associative networks (as manifested by increased ratio in SMN/DMN SD, predominance of global signal and increased degree of centrality in cortical areas of SMN). This reverberates on high-frequency activity, which is consequently stronger in the SMN (as manifested in greater beta frequencies in areas of SMN). Thus, the ongoing intrinsic activity in SMN is over-affected by incoming external sensory stimuli, which are easily and directly transformed into motor outputs. This manifests clinically in excited psychomotricity, resulting in the correspondent psychopathological symptoms, such as impulsivity and hyper-activity. Coherently, thought is shifted toward an external sensorimotor pattern, with minimal contribution of associative stimuli (as manifest for example in distractibility). The subjective experience is focalized toward external environmental contents and time perception is faster. Overall, this results in over-tuning on the external environment.

On the opposite side, in depression, along with the 5HT signaling alteration, a deficit in DA transmission also occurs, with related functional disconnection of SNC (as manifested by decreased FC between SNC and basal ganglia/thalamic regions). The related de-synchronization of intrinsic activity within the subcortical-cortical sensorimotor loop is then associated with functional decoupling between thalamus and SMN (as manifested by a reduction of absolute FC between thalamus and SMN toward around-zero values). In turn, this is associated with decreased excitability and complexity of intrinsic low-frequency activity within the SMN with relative shifting toward the associative networks (as manifested by decreased ratio in SMN/DMN SD, predominance of global signal and increased degree of centrality in cortical areas of DMN). This reverberates on high-frequency activity, which is reduced in the SMN (as manifested in reduced beta frequencies in areas of SMN). Thus, the ongoing intrinsic activity in SMN is under-affected by incoming external sensory stimuli, which are more difficulty and only indirectly transformed into motor outputs. This manifests clinically in inhibited psychomotricity, resulting in the correspondent psychopathological symptoms, such as difficulty to act and motor retardation. Coherently, thought is shifted toward an associative pattern, with excessive contribution of associative stimuli (as manifest for example in ruminations). The subjective experience is focalized toward associative or memorized contents and time perception is slower. Overall, this results in excessive de-tuning from the external environment. See **Figure 3**.

1.3. Hypotheses on affective alterations in mania and depression

We suppose that analogue alterations of functional organization at level of VTA, subcortical-cortical limbic loop and SN would result in alterations of the affective dimension in manic and depressive phases.

Thus, in mania the deficit in 5HT transmission and related RN functional disconnection would result in unmodulated VTA-related synchronization within the subcortical-cortical limbic loop, abnormal thalamus-SN coupling, and increased excitability/activity in SN (at the expense of associative networks). Consequently, the SN intrinsic activity would be over-affected by incoming internal sensory stimuli (including pleasure and pain), which are easily and directly transformed in visceral outputs. This would manifest in excited affectivity, with thought shifted toward an

internal sensorimotor pattern, resulting in irritability, emotional lability and focalization of subjective experience toward actual internal bodily contents.

By contrast, in depression, the DA transmission deficit and related VTA functional disconnection would result in de-synchronization within the subcortical-cortical limbic loop, abnormal thalamus-SN decoupling, and decreased excitability/activity in SN (favoring the associative networks). Consequently, the SN intrinsic activity would be under-affected by incoming internal sensory stimuli, which are difficultly or only indirectly transformed in visceral outputs. This would manifest in inhibited affectivity, with thought shifted toward an internal associative pattern, resulting in anhedonia, emotional blunting, and focalization of subjective experience toward associative or memorized internal bodily contents.

1.4. Hypotheses on mania, depression and mixed states

Clinically, the typical form of mania presents concomitant alterations in psychomotricity, affectivity and thought in the same polarity, while the typical form of depression shows the opposite polarity for all these dimensions; between them, different mixed states have also been described, when a co-presence of dimensions with opposite polarity occurs [74].

We suppose that the typical form of mania is associated with a deficit in 5HT signaling that results in concomitant and similar patterns of synchronization and increased activity in both the SNC/sensorimotor loop/SMN and VTA/limbic loop/SN systems. This would manifest in excitation of both psychomotricity and affectivity, with thought shifted toward a sensorimotor pattern.

On the opposite side, the typical depressive phase would be associated with an adjunctive deficit in DA signaling that results in concomitant and similar patterns of de-synchronization and decreased activity in both the SNC/sensorimotor loop/SMN and VTA/limbic loop/SN systems. This would manifest in inhibition of both psychomotricity and affectivity, with thought shifted toward an associative pattern.

We then hypothesize that mixed states are related to dissociation of synchronization and activity patterns between these systems. Thus, synchronization and increased activity in the SNC/sensorimotor loop/SMN system with concomitant de-synchronization and decreased activity in the VTA/limbic loop/SN system would be associated with excited psychomotricity and inhibited affectivity with thought focalized on external contents. By contrast, de-synchronization and decreased

activity in the SNC/sensorimotor loop/SMN system with concomitant synchronization and increased activity in the VTA/limbic loop/SN system would be associated with inhibited psychomotricity and excited affectivity, with thought focalized on internal contents.

This dissociation could depend on different concomitant factors, such as other neurotransmitters activity or their combinations. For instance, the activity of the endorphins system is able to differently modulate the SNC and VTA activity patterns, as well as to inhibit the SMN and to enhance the SN resting state activity [87-90]. Thus, differential levels of endorphins activity could be associated with different levels of dissociation between the psychomotor and affective systems.

Therefore, a differential contribution of alterations within the psychomotor and affective systems, up to opposite and dissociated changes, would lead to a corresponding predominance of psychomotor or affective symptomatology, with the same or opposite polarity, finally resulting in different combinations and shades of behavioral/phenomenological features, from full-blown manic or depressive to different mixed states of BD. See **Figure 4a** and **Figure 4b**.

1.5. Working hypotheses on temperament

The temperament can be defined as the physiological individual baseline in psychomotor, affective and thought functioning, and, consequently, could play an important role in the expression of pathological manic-depressive states and their longitudinal course [91-92].

Classically, cyclothymic, irritable, depressive and hyperthymic temperaments have been described [91-92]. The cyclothymic and irritable temperaments are characterized by behavioral and emotional instability and hyper-reactivity, impulsivity and susceptibility to addictive or phobic behaviors; while the depressive and hyperthymic temperaments are more stable and reflective [91-92]. Moreover, the cyclothymic temperament has been associated with bipolar illnesses with unstable course, such as cyclothymic disorder, while the hyperthymic temperament to bipolar illnesses with more defined and separated phases in manic-depressive-interval cycles, such as BD type I [91-93].

At a neuronal level, we found that intrinsic activity (SD) in the SMN correlates with the cyclothymic trait and is increased in cyclothymic temperament with respect to depressive temperament. On the other hand, the intrinsic activity in the SN correlates

with the irritable trait and is increased in irritable temperament with respect to hyperthymic temperament.

Thus, the physiological basal setting of the SNC/sensorimotor loop/SMN system at the opposite poles may underlie the excited or inhibited psychomotor dimension that characterize the cyclothymic or depressive temperaments, respectively. On the other hand, the physiological setting of the VTA/limbic loop/SN at the opposite poles may underlie the excited or inhibited affective dimension that characterize the irritable or hyperthymic temperaments, respectively. According to the model, the excited (sensorimotor) temperaments (i.e. cyclothymic and irritable) would be over-sensitive to external/internal environmental stimuli, which are easily transformed into motor/visceral outputs. Speculatively, this hyper-reactivity to stimuli could favor circuitry plastic changes; this might result in addictive and phobic behaviors, which could be considered as pleasure- and pain-related memories. By contrast, the inhibited (associative) temperaments (i.e. depressive and hyperthymic) would be under-sensitive to environmental stimuli, which could modulate behavioral patterns only indirectly and with the high contribution of associative stimuli. Finally, we speculate that other conditions such as the attention deficit hyperactivity disorder (ADHD) and Asperger syndrome could be considered at the extreme sensorimotor and associative (maladaptive) ends of this temperamental spectrum.

We hypothesize that a pathological shift from the individual temperamental baseline setting of the physiological spatio-temporal organization of brain functioning (i.e. sensorimotor, associative or combined patterns within the temperamental spectrum) might result in the pathological re-organization of brain functioning (and related reduction in adaptability to environmental changing conditions) which characterizes mania, depression and mixed states. Thus, for instance, the occurrence of a deficit in 5HT signaling would produce a re-organization of brain functioning with a similar pattern (and direction) of change between individuals, but occurring on the pre-existing temperamental setting (different from individual to individual). This would reflect in a manic shift in the brain functioning and related behavioral/phenomenological patterns, but showing different neuronal-clinical features with respect to the different temperamental baseline. Thus, a manic shift in subjects with associative (i.e. hyperthymic and depressive) temperaments would result in pathological episodes with a more defined clinical change with respect to baseline and a potential wider range of severity (in relation to the amplitude of the

shift). Furthermore, this could result in a course of illness characterized by clear and separated manic-depressive-interval cycles, typical of the BD type I. On the other side, a manic shift in subjects with sensorimotor (i.e. cyclothymic and irritable) temperaments would result in pathological episodes with blurred changes with respect to baseline, a disorganized pattern, and an instable course of illness, typical of cyclothymic disorders.

1.6. Working hypotheses on psychosis

Psychosis is defined by delusions and hallucinations, which can frequently occur in association with manic, depressive and mixed episodes of BD (and schizophrenia) [76, 94].

Clinically, psychosis includes a wide range of patterns: from an over-interpretativeness and excessive salience attribution to irrelevant incoming sensory stimuli, up to dreaming states dissociated from the environment.

At a neuronal level, psychosis has been associated with various alterations in the organization of intrinsic activity in networks and their balancing, including: a relative increase in DMN intrinsic activity at the expense of CEN; an increase of intrinsic activity in SN or sensory networks, and their increased coupling with DMN [95-97]. Psychotic symptoms have also been associated with alterations in the thalamo-cortical loops, resulting in both excessive opening or excessive closing of the thalamic filtering to incoming sensory inputs [98]. Finally, signaling abnormalities of various neurotransmitters have been detected in psychotic patients. In particular, an increase in DA signaling has been found in both psychotic mania (but not in non-psychotic mania) and schizophrenia (especially with positive symptoms) [83-84]. Moreover, dysregulations of histamine and noradrenaline or acetylcholine systems (via opening or closing the thalamic sensorial filtering) have been supposed in psychotic symptoms [98-101].

Thus, we speculate that alterations in various neurotransmitters signaling may differently affect the intrinsic neuronal activity in the thalamo-cortical loops, modulating the flow of incoming sensory inputs to the sensorial cortices via the thalamic filter. An excessive opening of the thalamic sensorial filtering with increased excitability and intrinsic activity in the sensorial networks (auditory, visual, somatosensory or insular areas) would lead to an over-flow and over-processing of actual sensory stimuli. This would result in psychotic states shifted

toward a sensorial pattern, which manifest in abnormal perceptions, delusional interpretations and excessive salience to irrelevant actual sensorial stimuli. On the other hand, an excessive closing of the thalamic sensorial filtering with relative shifting of excitability and intrinsic activity toward the associative sensorial networks (posterior areas of DMN) would stop the flow and processing of actual sensory stimuli in favor of memorized stimuli. This would result in psychotic states shifted toward an associative pattern, which manifests in abnormal representations (more similar to dreams) and dissociation from the actual sensory stimuli, which are unable to modify delusional thoughts. Concomitant alterations in the sensorimotor/affective and thalamic filtering systems would then result in disturbances of psychomotricity, affectivity and thought with psychotic features. This manifests in a different combination of manic, depressed or mixed states with sensorial or associative psychosis, which characterizes the psychotic BD.

2. Working hypotheses on manic-depressive cycle and schizophrenia

Schizophrenia is clinically defined by the occurrence of positive symptoms (i.e. delusions, hallucinations, and disorganized behavior), negative symptoms (i.e. blunted affect and psychomotor inhibition) and cognitive impairment [76, 94].

At the neuronal level, several alterations in the spatio-temporal organization of intrinsic brain activity have been detected in schizophrenic patients, including: disruption of intrinsic activity and connectivity within the sensorimotor networks, hyper-connectivity within the DMN and reduced connectivity within the CEN; increased connectivity between thalamus and sensorimotor networks while reduced connectivity between thalamus and prefrontal areas; reduced connectivity between SN and basal ganglia/thalamic regions [61, 95, 102-104]. Moreover, biochemical studies have revealed consistent alterations of DA signaling (suggesting a DA hypothesis of schizophrenia) [105].

However, these functional alterations tend to overlap between schizophrenia and BD. Accordingly, by using a dimensional approach on psychomotricity, we observed similar changes in the intrinsic functional organization of SNC/sensorimotor loop/SMN system between bipolar and schizophrenic patients. In particular, both manic patients and schizophrenic patients with excited psychomotricity show

increased FC between thalamus and SMN as well as decreased FC between RN and basal ganglia/thalamic regions. By contrast, both inhibited depressed patients and schizophrenic patients with inhibited psychomotricity show reduced absolute FC between thalamus and SMN as well as decreased FC of SN (and RN) with basal ganglia/thalamic regions. It is conceivable that, by using an analogue dimensional approach on excited or inhibited affectivity, similar alterations in the VTA/limbic loop/SN system between bipolar and schizophrenic patients may be also detectable.

On the other hand, schizophrenia is also characterized by a relevant loss of grey matter (already present in the early stages of illness and mainly involving the frontal regions), which correlates with cognitive deficits [106]. This represents the most consistent difference from BD, which instead is associated with a low degree of neuronal loss mainly occurring in late stages of illness (with high variability between subjects) [106]. Accordingly, it has been suggested that schizophrenia is mainly related to alterations in neurodevelopmental processes (e.g. genetic abnormalities, prenatal/perinatal infections and injuries, alterations of synaptic plasticity) [107-108]. Then, potential additional neurodegenerative processes may superimpose onto the developmental impairment after illness onset, finally leading to the neuronal loss and consequent cognitive deficits [107-108].

Thus, we suppose that different factors are involved and combined in the final expression of BD and schizophrenia, including the manic-depressive cycle (in relation to the temperamental baseline), psychosis, and structural cortical alterations. According to our model, changes in intrinsic activity within the RN-SNC/sensorimotor loop/SMN and RN-VTA/limbic loop/SN, as well as their balance with the associative networks DMN-CEN, would result in psychomotor, affective and thought alterations, manifesting in manic, depressive or mixed states. These pathological episodes tend to organize into manic-depressive cycles with a phasic course, which characterizes the typical form of BD. The combined alteration in the sensorimotor/affective systems and the thalamic sensorial filtering would result in concomitant psychomotor/affective/thought and psychotic alterations, which differently combine into manic, depressive or mixed states with different psychotic features, characterizing the psychotic form of BD. On the other hand, the concomitant changes in the sensorimotor/affective systems, dysfunctions in thalamic sensorial filtering, and structural (develpmental/degenerative) cortical alterations would result in psychomotor/affective/thought and psychotic alterations as well as

cognitive deficits. In particular, the subcortical-mediated changes in excitability/activity on structurally altered cortical sensorimotor/associative networks could lead to psychomotor, affective, thought and psychotic symptoms with odd, or disorganized, or poor contents. The psychotic onset is usually characterized by a pathological episode with full-blown symptomatology, often with excited psychomotricity, excited affectivity, thought shifted toward a sensorimotor pattern, and related sensorial psychosis with odd and disorganized contents. The active phase is usually followed by a chronic course (even if potential new re-exacerbation/episodes can also occur). During the chronic course, the psychopathological state tends to manifest inhibited psychomotricity, inhibited affectivity, thought shifted toward an associative pattern, and related associative psychosis with poor contents and cognitive deficits. This course characterizes the typical form of schizophrenia. Finally, structural neurodevelpmental/degenerative brain alterations in absence (or with minimal contribution) of sensorimotor/affective alterations would result in different neuropsychiatric disorders such as mental retardation, autism or dementia (according to the relative weight of neurodevelopmental or neurodegenerative processes).

3. Working hypotheses on manic-depressive cycle, immunology and white matter

The functional disconnection of intrinsic low-frequency oscillations between the 5HT-related RN and subcortical-cortical areas could play a key role in the manic shift, which typically represents the BD onset. The deficit in 5HT transmission could be linked, in turn, to the other core alterations that are consistently associated with BD, i.e. immune activation and white matter (WM) damage.

In particular, an immune activation (e.g. shifting toward Th1 responses and increased levels of pro-inflammatory cytokines like IL-6) has been consistently demonstrated in BD patients, especially during the active phases of mania and depression [109]. In turn, pro-inflammatory factors, such as Th1 cells and IL-6 or IFN-γ cytokines, can enhance the activity of the indoleamine 2,3-dioxygenase (IDO) enzyme, which diverts tryptophan away from 5HT synthesis and favors the kynurenine pathway (with immune-modulatory functions) [110]. Moreover, activation of the

hypothalamic-pituitary-adrenal (HPA) stress system is also consistently associated with BD; in turn, glucocorticoids can enhance the activity of the tryptophan 2,3-dioxygenase (TDO) enzyme, which increases tryptophan degradation [110]. Thus, the immune and/or stress response can reduces the 5HT availability [110]. In turn, depletion of 5HT can cause a decrease in functional connectivity of RN, but only in patients with affective disorders (and not in healthy subjects) [111].

On the other hand, widespread alterations of WM have been consistently reported in BD patients, especially in the active phases of illness again [79, 112-113]. The WM changes seem to reflect alterations in oligodendroglial and myelin microstructure, which have been related to CD8+ T cell-mediated pathogenesis in various diseases [114].

Coherently, we found a combined occurrence of WM changes (decrease in FA and increase in RD, mainly in the corpus callosum) and immunological alterations (increase in peripheral IL-6 as well as decrease in peripheral CD8+CD28-CD45RA+ and CD8+IFNγ+ T cells) especially in mania. Moreover, WM abnormalities correlated with reduction in circulating CD8+ T cell subpopulations that are terminally differentiated effector cells prone to tissue migration, suggesting that these T cells could exert a pathogenic role through migration into the brain.

Thus, we hypothesize that, when an immune activation occurs in subjects with genetic susceptibility (e.g., in immunological dysregulation or myelin damage), the pro-inflammatory response would induce: widespread microstructural WM damage (e.g. alterations in oligodendroglial and myelin microstructure); and enhancement of IDO activity with 5HT depletion. We suppose that the widespread WM damage would induce a functionally-relevant alteration proportionally greater on those fibers emerging from the brainstem neurotransmitters nuclei (since those fibers are relatively few but largely ubiquitous). Consequently, the combined effects of structural damage of neurotransmitters-related fibers and depletion of 5HT would result in a reduction of functional connections of RN. In turn, this would induce a functional re-organization of intrinsic brain activity and related manic shift. It is conceivable that an adjunctive WM damage on DA-related fibers (among other potential alterations, e.g. mania-related excitotoxicity) would then result in functional disconnections of SNC/VTA and in a consequent depressive switch. Finally, these structural-functional alterations would make the 5HT system more susceptible to

further decreases in 5HT level, for instance due to activation of the stress system, which could thus trigger new manic-depressive cycles.
In summary, we provisionally hypothesize that BD is an inflammatory disease which compromises the microstructure of WM, leading to functional disconnections of neurotransmitters systems and related changes in the spatio-temporal organization of neuronal intrinsic activity at levels of subcortical-cortical loops and networks, finally resulting in disturbances of psychomotricity, affectivity and thought.

Conclusions

Our working model might provide a provisional framework for testing specific hypotheses on the pathophysiology of major psychiatric disorders, such as BD (and schizophrenia). Confirmation or refutation of these hypotheses can improve or change the model. A better understanding of the pathophysiology of the psychiatric disorders could open the door to potential new strategies of therapy.

FIGURES

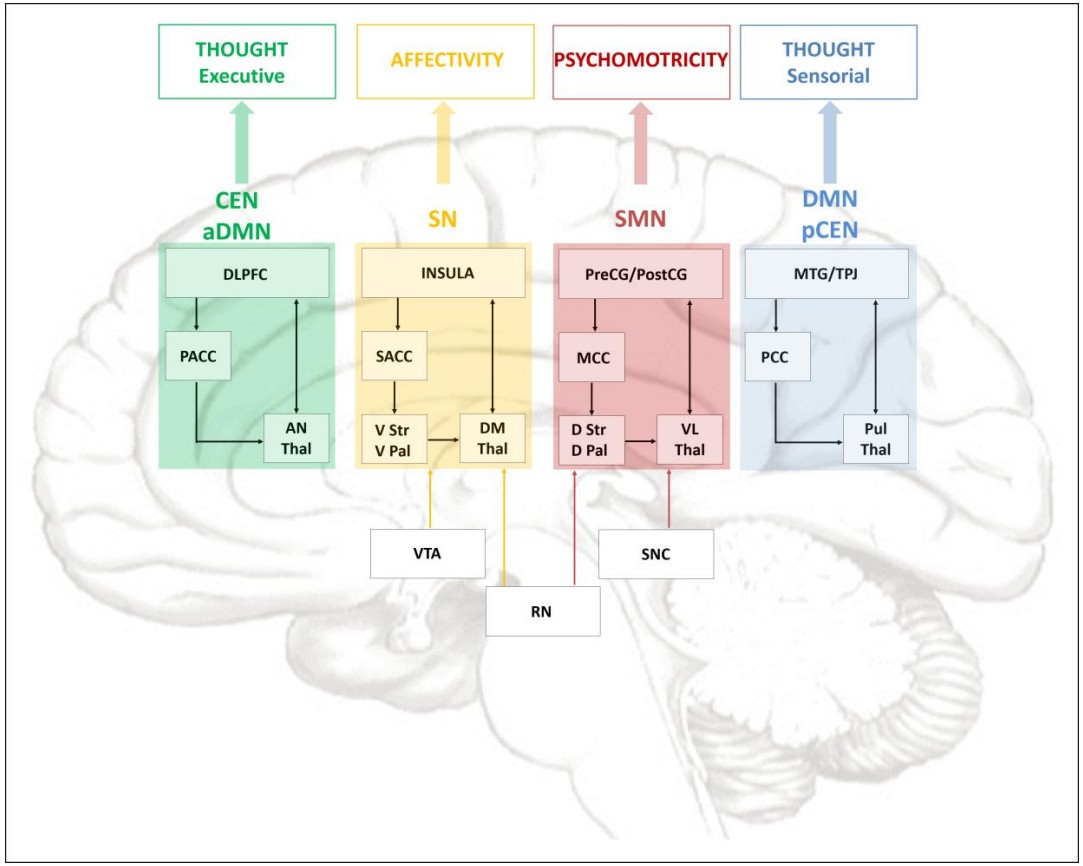

Figure 1. Working model – Networks, subcortical-cortical loops and neurotransmitters

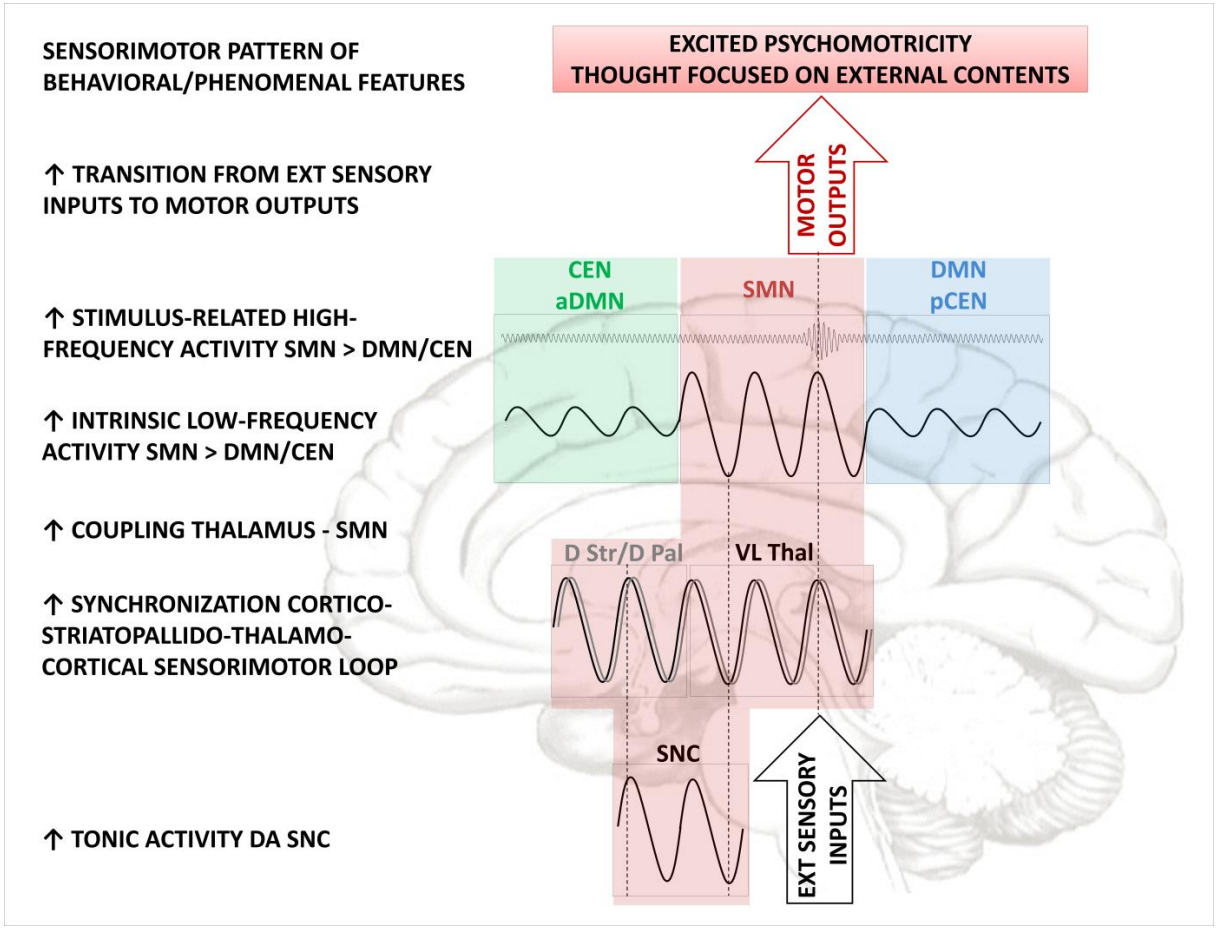

Figure 2a. Working model – Sensorimotor pattern: psychomotricity

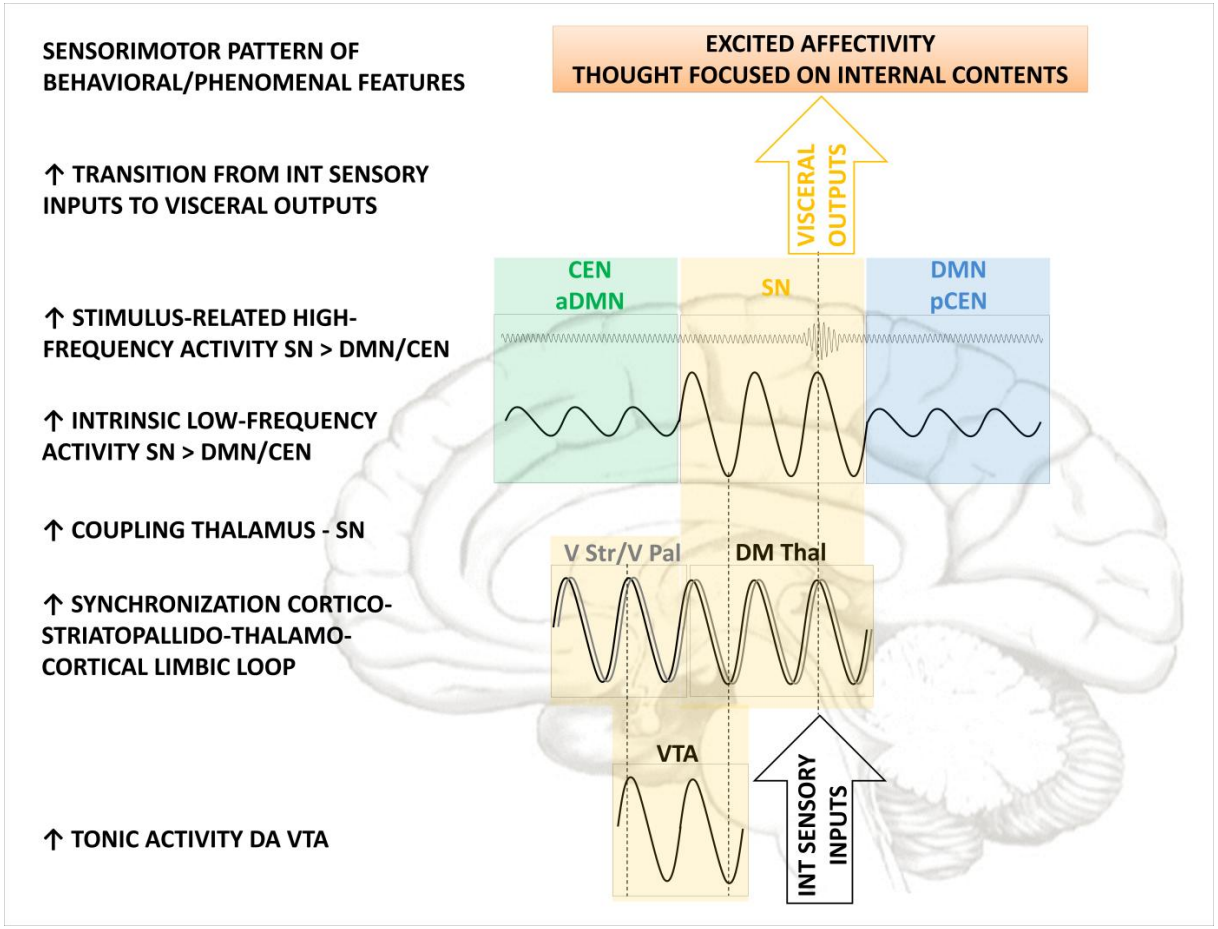

Figure 2b. Working model – Sensorimotor pattern: affectivity

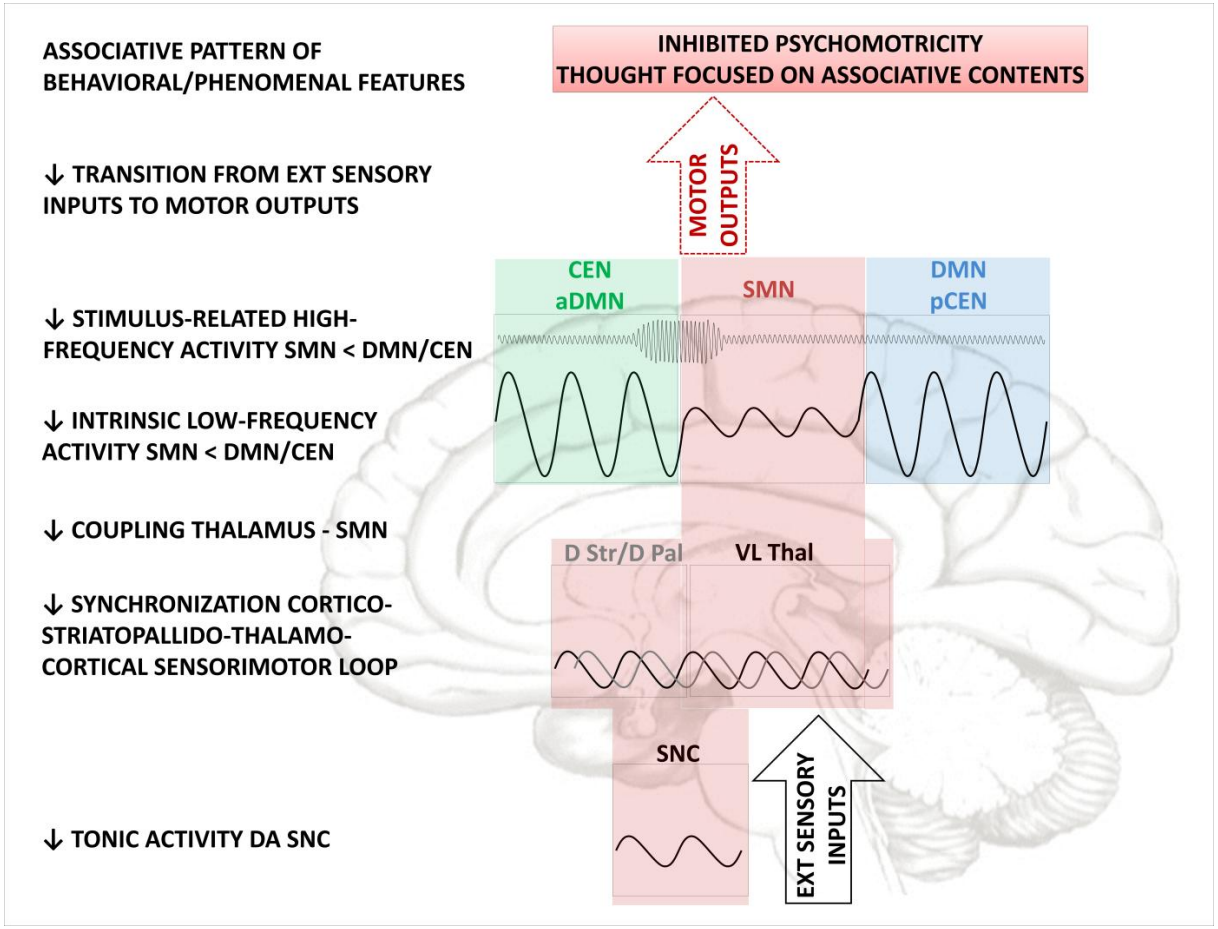

Figure 2c. Working model – Associative pattern: psychomotricity

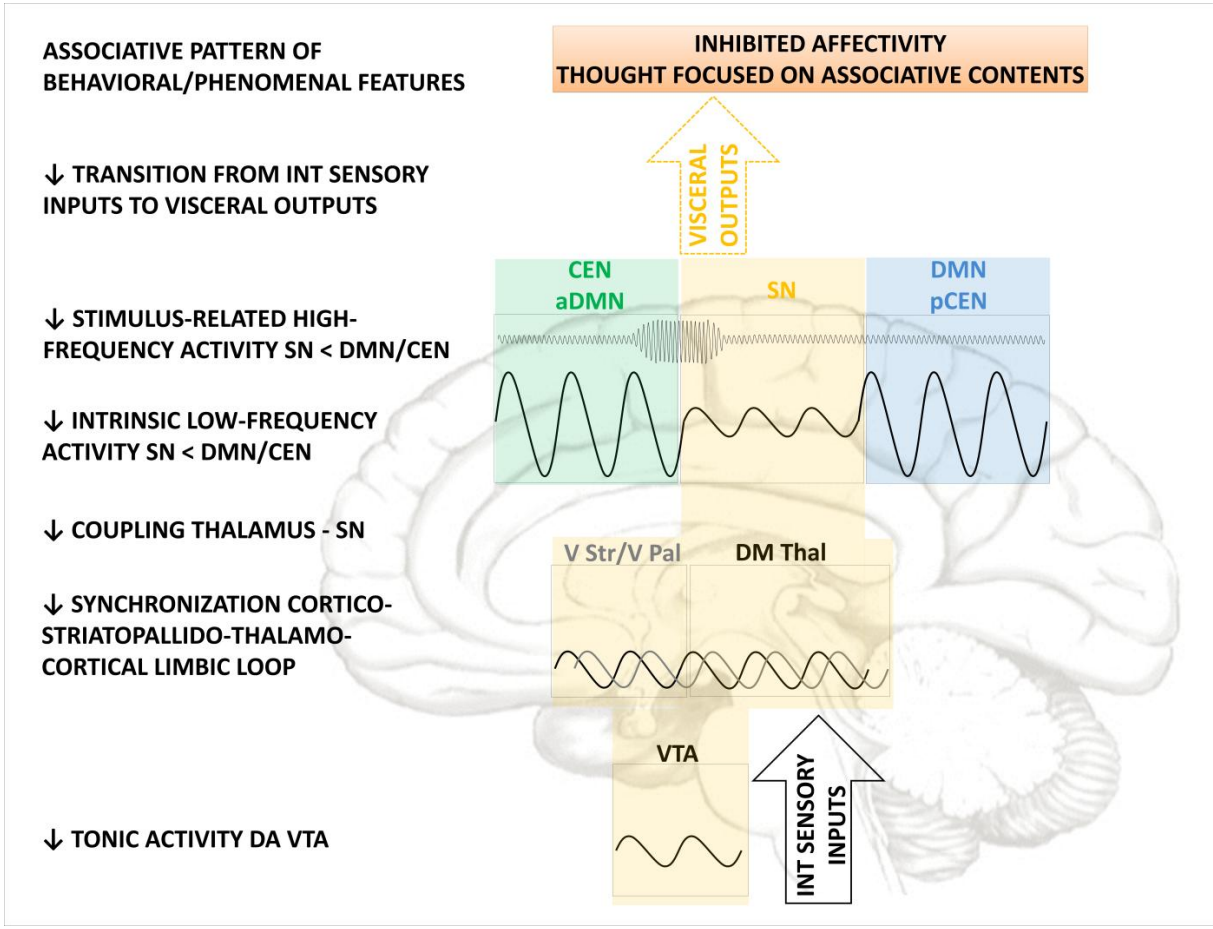

Figure 2d. Working model – Associative pattern: affectivity

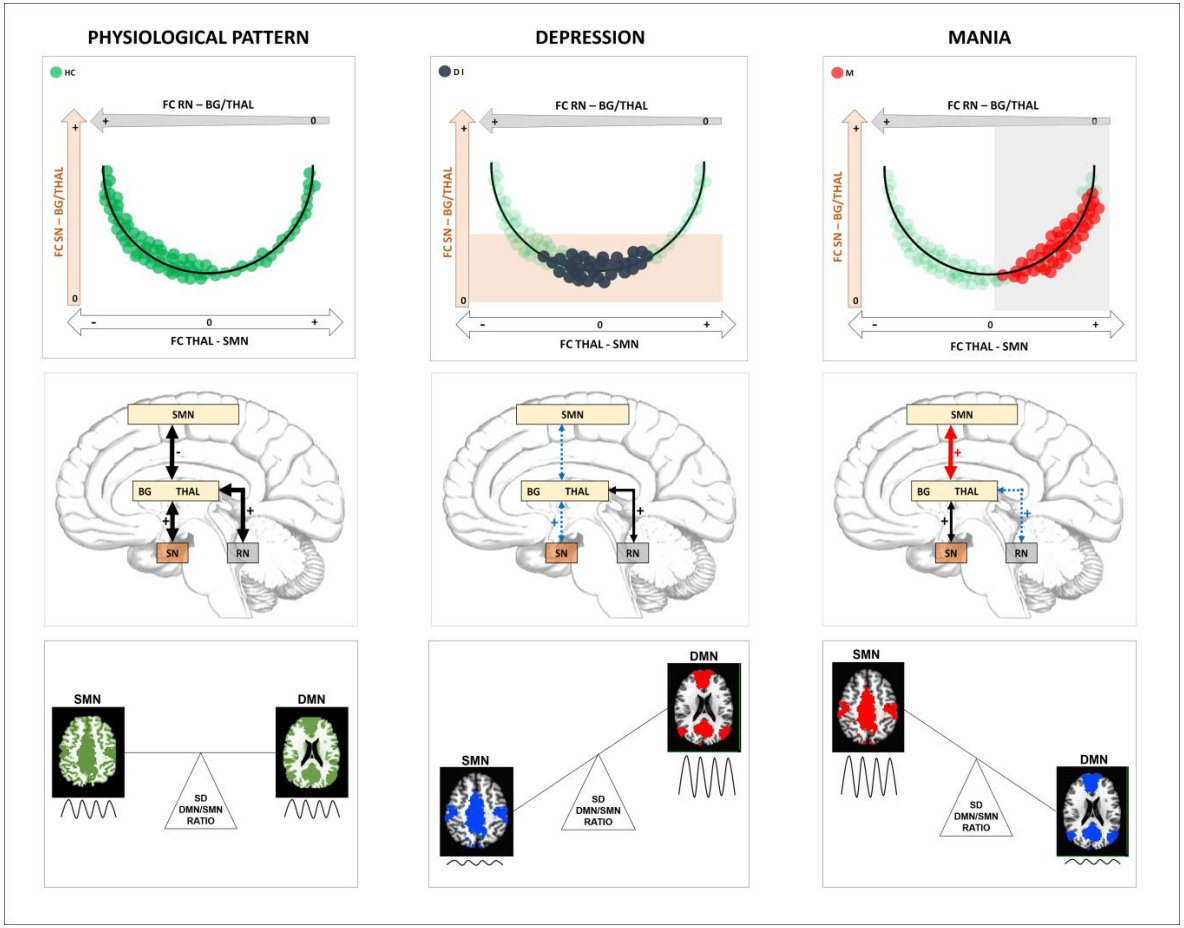

Figure 3. Working model – Psychomotricity in mania and depression

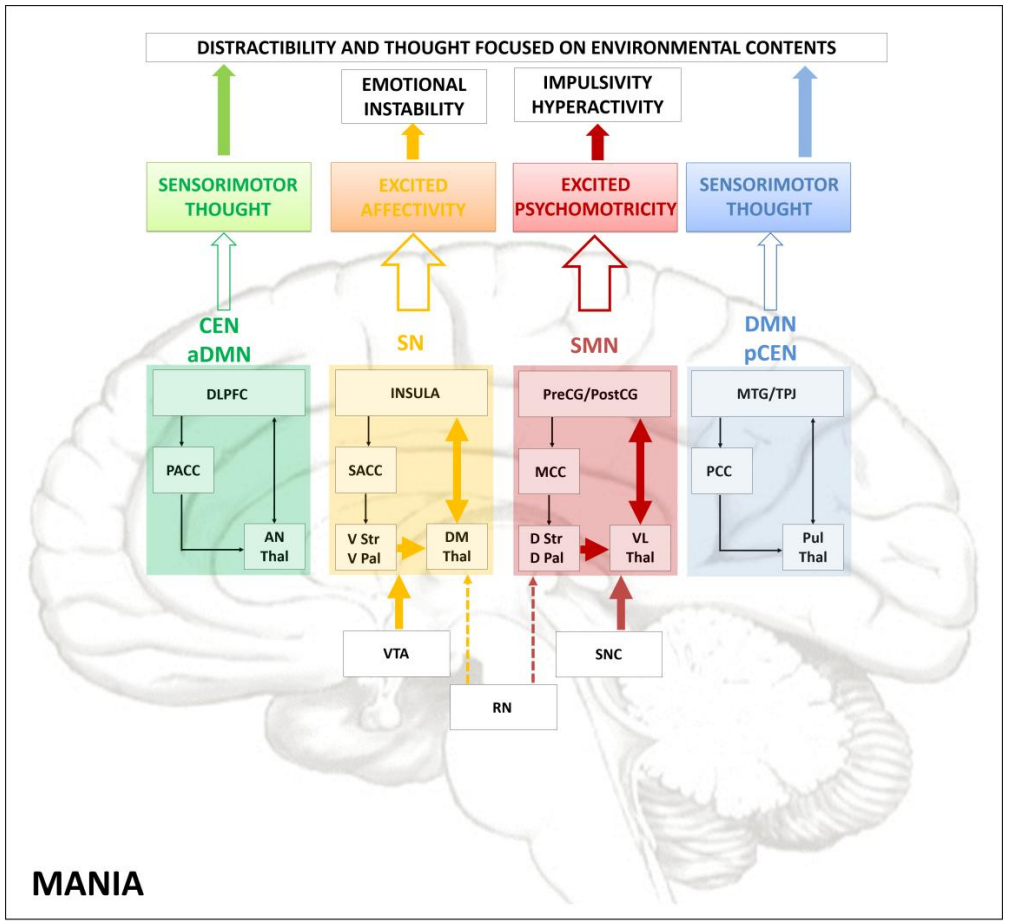

Figure 4a. Working model – Mania: psychomotricity, affectivity and thought

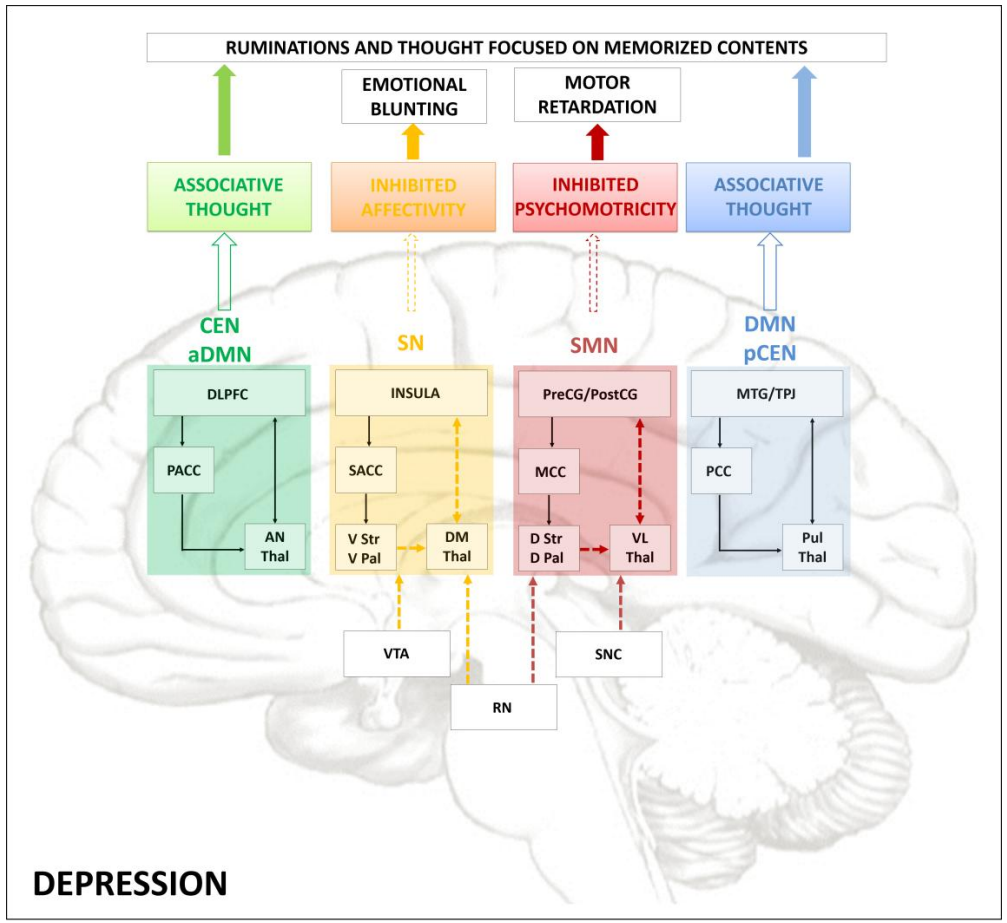

Figure 4b. Working model – Depression: psychomotricity, affectivity and thought

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