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CHANGES IN *BDNF* METHYLATION PATTERNS AFTER COGNITIVE REMEDIATION THERAPY IN SCHIZOPHRENIA: A RANDOMIZED AND CONTROLLED TRIAL

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INTRODUCTION

Cognitive impairment is a critical issue in schizophrenia as it is a key contributor to disability and is strongly related to poor social and functional outcomes (Green 2016). Cognitive remediation therapy (CRT) is a learning-based intervention that targets cognitive difficulties (attention, memory, executive function), with the goal of improving functional outcomes (Bowie et al. 2020). Meta-analyses have demonstrated that CRT is effective, with improvements ranging from small to medium effects in cognition and functioning (Wykes et al. 2011; Vita et al. 2021). In fact, it has been considered with the highest degree of recommendation according to the guidance provided by international experts (Vita et al. 2022).

Some have assumed that CRT effects are related to neuroplasticity (Matsuda et al. 2019); however, the precise neurobiological processes underlying cognitive improvement in schizophrenia have rarely been investigated, and even when investigated, no markers of response have been identified. Neuroimaging studies provide preliminary evidence suggesting that CRT could be facilitating neuroplasticity processes as some changes in brain functioning have been described after CRT, including improvements in patterns of activation (Wykes et al. 2002 ; Mothersill, Donohoe 2019), changes in neuroconnectivity (Ramsay et al. 2017; Penadés et al. 2020) and even some structural changes in white matter (Penadés et al 2013; Matsuoka et al. 2019) and gray matter (Eack et al. 2010; Morimoto et al. 2018).

From a different perspective, brain-derived neurotrophic factor (BDNF) is a neuropeptide that plays a central role in neuronal growth, synapse formation and maturation, and refinement of developing brain circuits (Park, Poo, 2013; Kowiański 2018). It is also a key regulator of synaptic plasticity and late-phase long-term potentiation (Lu et al. 2014). BDNF has been involved in the pathophysiology and treatment of schizophrenia, as well as other psychiatric disorders (Dombi et al. 2022; Wang et al. 2022). Accordingly, several efforts have been made to assess its potential role as a biomarker of different psychiatric disorders. In schizophrenia, some meta-analytic data have shown the association of *BDNF* gene variants (mainly the Val66Met polymorphism) with the risk for the disorder and specific cognitive and neuroimaging phenotypes (Ahmed et al. 2021; Kheirollahi et al. 2016). Similarly, different case-control studies have linked the diagnosis of schizophrenia with changes in BDNF plasma or serum levels (Ahmed et al. 2015; Fernandes et al. 2015; Skibinska et al. 2018). In addition, BDNF has been proposed as a putative biomarker for cognitive recovery in schizophrenia (Penadés et al. 2015; Nieto et al. 2021) based on its therapeutic effects in improving synaptic markers, stimulating neuronal function, and preventing cell death, making it a potentially promising therapeutic option (Nagahara, Tuszynski, 2011; Colucci-D'Amato et al. 2020). However, only two studies testing BDNF in the context of CRT have been published. In one, CRT showed a significant increase in BDNF serum levels (Vinogradov et al. 2009) and in the other, the CRT group showed a significant positive interaction effect between the serum BDNF levels and the different *BDNF* gene genotypes (Penadés et al. 2018).

Epigenetic evidence suggests that DNA methylation changes are implicated in the etiology of schizophrenia (Castellani et al. 2015). While the physiopathological components are poorly understood, it is well known that methylation is a dynamic mechanism critically involved in the regulation of transcriptional activity and plasticity (Li, Zhang 2014). The changes in DNA

methylation throughout the lifespan affect and modulate crucial processes such as gene expression (Moore et al. 2013; Jung, Pfeifer 2015). DNA methylation occurs mainly in cytosines that precede guanine, known as CpG sites. Some regions of the genome exhibit a high CpG content and are called CpG islands (CGI) and the methylation of CGIs within the gene promoters has the potential to silence gene expression, while promoter regions from highly expressed genes are usually hypomethylated (Mohn et al. 2008). There is also evidence that changes in DNA methylation in gene regulatory regions or the gene body might be related to both induction and repression of gene expression (Arechederra et al. 2018; Rauluseviciute et al. 2020) and also to alternative splicing regulation (Shayevitch et al. 2018). In the case of BDNF, this gene's multidirectional methylation patterns have also been shown (Fuchikami et al. 2011), adding to the complex relationship between DNA methylation and gene expression regulation in the context of neuropsychiatric disorders.

Different studies have connected BDNF gene methylation alterations to various psychiatric disorders in DNA extracted from blood, buccal tissue, or postmortem brain samples (Teroganova et al. 2016; Treble-Barna et al. 2023). Focusing on schizophrenia, both increased and decreased *BDNF* methylation levels have been identified in patients as compared to healthy subjects (Ikegame et al. 2013; Kordi-Tamandani et al. 2012); while other studies have not detected significant differences (Mill et al. 2008). Such heterogeneity reflects, on the one hand, the need for new studies on the biological underpinnings relating *BDNF* methylation and expression and the correlation of such patterns with the clinical outcomes. On the other hand, the current results highlight the diversity in methodological approaches while pointing towards the need for additional experimental designs that allow a more precise control of different confounding variables.

Based on data about the role of environmental influences on epigenetic changes (Gluckman et al. 2007; Toraño et al. 2016) it is likely that treatment, including psychological therapies, could influence methylation patterns (Penadés et al. 2020). In fact, synaptic plasticity mechanisms, elicited by experience, modify brain function through changes in gene expression, which ultimately rely on modifications of the epigenetic landscape, including DNA methylation (Gu et al. 2011). Differential DNA methylation has been linked to cognitive alterations both through candidate gene and epigenome-wide association studies (Levenson et al. 2008; Marioni et al. 2018) and, pharmacological and psychological treatments have been shown to modify the DNA methylation of the *BDNF* gene promoters (Lopez et al. 2013; Perroud et al 2013). Therefore, DNA methylation changes could be relevant in understanding the biological effects of different psychotherapies in different disorders, including CRT.

The main objective of the current study is to investigate whether *BDNF* methylation levels are associated with the cognitive changes that usually follow cognitive remediation treatment in people with a diagnosis of schizophrenia. The principal hypothesis is that some changes in the methylation pattern of some CpG units of the *BDNF* gene will be found in the cognitive treatment group. Secondly, we hypothesized that those changes will be correlated with improvement in different cognitive domains.

METHODS AND MATERIALS

Design

A two-arm randomized controlled trial (RCT) (registration NCT04278027) with sixty participants who were randomized to receive either Cognitive Remediation Therapy (CRT) or treatment as usual (TAU). The primary outcome was global cognition, and the secondary outcomes were cognitive domains. *BDNF* methylation was measured by a blood test. Outcomes were assessed at T0 (0 weeks), T1 (16 weeks) and all were assessed blind to group assignment. The local Research Ethics Committee prepared and approved a study information sheet with a cover letter and consent form and ethical approval has been obtained to carry out the trial. (HCB/2016/0536).

Randomization was independently conducted by a psychiatrist who took no part in the implementation of assignments. A randomized sequence was performed using a free, web-based program (www.randomizer.org) that generated lots. The lots were drawn as sealed envelopes whereby the patients were assigned to either the experimental (CRT) or the control group (TAU), with a CRT group having 40 participants and a TAU group of 20 patients (ratio 2:1). We used sequentially numbered, opaque sealed envelopes using carbon paper as described in the SNOSE procedure (Doig & Simpson 2005). All the details are displayed in Figure 1, in the CONSORT diagram.

Participants

Participants were recruited by the Barcelona Clinic Schizophrenia Unit (BCSU) staff at the Hospital Clinic of Barcelona, which serves part of the Barcelona area. Nearly 90 patients with schizophrenia disorder (according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) classification, determined by The Structured Clinical Interview for DSM-5 (SCID-5), were recruited. Recruitment took place in the last quarter of 2018. Interventions were carried out from January 2019 to December 2021. This phase was interrupted for months by the restrictions of the COVID-19 pandemic. Moreover, symptoms were assessed using the Spanish version of the Positive and Negative Syndrome Scale (PANSS) at baseline (Kay 1990; Peralta, Cuesta 1994).

The inclusion criteria were a) Diagnosis of schizophrenia according to DSM-5 and confirmed by the semi-structured interview (SCID) for axis 1; b) Stability of symptoms at least during the last six months and no proposed changes in pharmacological antipsychotic treatment. Exclusion Criteria were a) Presence of organic-cerebral syndrome due to neurological or traumatic conditions; b) Antipsychotic dose change during trial >10%; c) Abuse of psychotropic substances.

Interventions

1. Cognitive Remediation Therapy (CRT)

The experimental group (n=40) received a CRT based on the software CIRCuiTS^{TM} (Computerised Interactive Remediation of Cognition and Thinking Skills; Reeder and Wykes, 2010). CIRCuiTS^{TM} is a therapist-supported web-based CRT, supplemented with independent sessions to facilitate massed practice. CRT was offered at a rate of delivery between a maximum of three and at least twice a week (over a 16-week period) for up to 40 sessions lasting up to sixty minutes. Each session includes a series of tasks (usually 4 to 8) that target different cognitive domains: memory, working memory, attention, problem solving, speed of processing, comprehension,

flexibility of thought, and planning. Two trained therapists, graduates in psychology and experts in psychosocial rehabilitation techniques were in charge of the CRT delivery. Therapists facilitate the development of cognitive strategies and provide additional scaffolding for CRT tasks to ensure consistent successful performance on tasks that become progressively more complex with each session. Therapists also support participants' motivation, use of metacognitive skills, and generalization of learning by encouraging the participant to learn about and regulate their cognitive performance and transfer this learning to meet real-world goals (Drake et al. 2014; Reeder et al. 2017).

2. Treatment as Usual (TAU)

The control group (n=20) received their usual treatment with antipsychotic medication and did not follow any cognitive remediation program or other psychological treatment during the trial period. To control the psychopathological course and pharmacological treatment, participants from both the CRT and TAU groups received four visits, one per month, by the psychiatrist in charge, according to the BCSU treatment guide.

Outcome measures

Cognitive assessment included a battery of tests called the Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery (MATRICS Consensus Cognitive Battery - MCCB) (Nuechterlein et al. 2008; Kern et al. 2008). The MCCB subtests are organized into six domains: Speed of processing, Attention/Vigilance, Working Memory, Verbal Learning and Reasoning, and Problem Solving. T-scores were calculated for each cognitive domain. Following the MCCB recommendation, a global composite score was calculated by averaging the t-scores of all tests and called Global Cognition. We have used a cognitive composite score which excludes the Social Cognition as we were focused mainly on basic cognition. That composite score has been previously validated showing excellent psychometric characteristics (Georgiades et al. 2017).

Methylation of the BDNF gene

DNA extractions from peripheral blood samples were performed using high throughput nucleic acid isolation technology (REALPURE "SSS" Kit) and the obtained DNA samples were quantified with the Qubit dsDNA HS (High Sensitivity) assay kit and the Qubit fluorometer.

Our interest was to obtain the methylation status of the *BDNF,* so three CpG Islands (CGI1, CGI2, and CGI3) covering the *BDNF* gene were selected according to data obtained from the Genome Browser (Human GRCh38/hg38 version).

Methylation was assessed using the EpiTYPER® method (Ehrich et al. 2005), carried out at the Centro Nacional de Genotipado - Fundación Pública Galega de Medicina Xenómica (CEGEN-FPGMX; http://usc.es/cegen/). EpiTYPER® is a mass spectrometry-based method that allows for quantitative DNA methylation analysis in a high-throughput manner. The technology utilizes bisulfite treatment to convert unmethylated cytosines to uracil, leaving methylated cytosines unaffected. The treated DNA is then subjected to polymerase chain reaction (PCR) to amplify the regions of interest, and the resulting PCR products are used for mass spectrometry analysis. The resulting fragments are analyzed using the MALDI-TOF technology integrated into the Agena

Bioscience MassArray platform, which directly correlates with the DNA methylation status at specific CpG sites.

Therefore, this technology enables the quantitative assessment of DNA methylation levels at single-nucleotide resolution across targeted genomic regions ranging from 100 to 600 base pairs. The obtained methylation values can range between 0 and 1, being 0 the value corresponding to a 0% methylation state and 1 the value associated with a 100% methylated state.

EpiTyper methodology does not allow the determination of methylation if specific CpG sites are too closely located so the methylation status of a fragment reflects the mean of all sites within the fragment. We will refer to these fragments as CpG units that can comprise one or more CpG sites. They have been numbered consecutively within each CGI and are detailed in the Supplementary Material (Supplementary Table S1). We have used each CpG unit's scores as methylation variables.

Statistical Analyses

Two multivariate logistic regressions were executed as part of the experimental design to control and detect potential heterogeneous distribution of variables at the initial stage, contrasting the two intervention conditions. One regression scrutinized clinical and demographic factors, while the other focused on cognitive variables.

Effectiveness of CRT and Methylation analysis.

To assess CR effects on Global cognition and cognitive domains we carried out intention-to-treat analyses without any ad hoc imputation, used Mixed models (CRT n=40; TAU n=20) and considered the interaction between time (pre-post) and treatment (CRT/TAU) to understand variations in outcome. Methylation analyses were carried out with only those participants retained in the two assessments of the study.

A significant interaction means that slopes observed between pre-post measures are different between treatments. Random intercepts were retained in the model. Age, sex, and years of formal education were found to exhibit significant correlations with computed changes in certain neuropsychological variables. Given their significance in the context of cognitive impairment in schizophrenia (Lee et al. 2020), these factors were included in all analyses. The same approach was considered for each methylation variable, to analyze whether methylation changes differed between treatment groups.

Effects of changes in methylation and cognition.

To identify significant associations between changes in neuropsychological and methylated variables, we first calculated the changes (delta) between pre/post measures (T1-T0) for those neuropsychological (delta-NP) and methylated (delta-methyl) variables. This was conducted for those CpG units with a significant interaction effect observed in the previous Mixed model analyses.

As age, sex and years of formal education are associated with cognition, in a second step, each neuropsychological variable was considered as an output in a regression analysis, including age, sex, and years of formal education as predictors. The residuals of these regression models were saved. In the third step, the new adjusted scores were correlated (Spearman correlation) with delta-methyl. We performed the correlation analysis only in the CRT group because of the low sample size of the TAU group and its effect on the statistical power of the analyses conducted in that group.

Effect size estimated.

Effect sizes were estimated with Cohen's *d* index by calculating the mean difference between the two groups and dividing the result by the pooled standard deviation (mean (group 1) – mean (group 2) / standard deviation).To have a medium effect size (Cohens' *d* = 0.5) when contrasting pre and post measures of cognition and methylation in the CRT group, assuming a power of 80% and an alpha error of 5%, it was estimated that the necessary sample size of the treated group would be n=34.

RESULTS

Data were gathered from a representative sample of outpatients from our clinic who serves a geographical district of Barcelona. The sociodemographic and clinical characteristics of participants at basal moment are described in Table 1, including bivariate contrasts between the two study conditions. None of sociodemographic and clinical variables (sex, age, years of formal education, age onset of illness, duration of illness, and number of hospitalizations) showed a significant effect in a multivariate logistic regression analysis distinguishing between the two study conditions (all variables p>.523). A second logistic regression analysis, in these case for cognitive variables, showed also a non-statistical differentiation between the two study groups at the basal moment (all variables p>.075).

CRT benefits

Primary outcome: There was a significant interaction between the type of treatment (CRT, TAU) on Global Cognition (Coef. -39.00; z = 9.33; p <0.001; 95% CI –47.19 to -30.81). As expected, cognitive remediation produced significant changes in cognition.

Secondary outcomes: Table 2 shows how cognitive remediation significantly affected all cognitive domains but to different extents. CRT showed the greatest improvement in Attention /Vigilance (Cohen's d = 0.75; t = -5.43; <0.001) and Reasoning and Problem Solving (Cohen's d = 0.86; t = -6.11; <0.001). Verbal Learning (Cohen's d = 0.66; t = -4.73; <0.001) and Visual Learning (Cohen's d = 0.66; t = -4.74; <0.001) also showed relevant changes. Finally, a more modest change was found in Speed of Processing (Cohen's d = 0.34; t = -2.48; 0.017) and Working Memory (Cohen's d = 0.29; t = -2.09; 0.041).

Changes in BDNF methylation

Six cases dropped out of the study (CRT 3, TAU 3) and 11 refused another blood test (CRT 4, TAU 7). Participants with full methylation data (n=43) were comparable to those without these data (n=17) on age, gender, and years of formal education (all tests p>.178). In addition, when comparing the demographic and clinical description at baseline of the CRT and TAU groups with

full methylation data (33 and 10, respectively) no differences were found (Supplementary Table S2).

Methylation values for each CpG unit in both groups before and after treatment are included in Supplementary Table S3. There were differential changes in 5 CpG units over time, 4 in island 1 (CpG1.2, CpG1.7, CpG1.10, CpG1.17) and 1 in island 3 (CpG3.2). For CpG1.2, while both CRT and TAU displayed an increase in methylation, this was more prominent in the CRT group (Coef. 0.015; z = 2.28; p=0.022; 95% CI 0.002 to 0.028). For CpG1.7, the TAU group showed a decrease in methylation while the CRT increased their methylation status (Coef. -0.005; z = 2.48; p=0.013; 95% CI -0.015 to -0.002). For CpG1.10 there was a significantly decreased methylation only in the CRT group (Coef. 0.005; z = 2.01; p=0.045; 95% CI 0.0001 to 0.009). For CpG1.17, both groups showed a decrease in methylation levels, but this was more evident in the CRT group (Coef. -0.009; z = 2.25; p=0.025; 95% CI -0.017 to -0.001). Finally, the unit CpG3.2 displayed contrary changes with TAU showing an increase in methylation while CRT showed a decrease (Coef. 0.013; z = 3.66; p<0.001; 95% CI 0.006 to 0.021). No other significant results for other CpG units were detected.

Relationship between CRT-driven changes in cognition and methylation

We did not observe any significant correlations between changes in global cognition and changes in methylation (p> 0.152). However, we detected a significant correlation between speed of processing and CpG1.2 and CpG1.10 (r=-.41 p=0.017 and r=-.36 p=0.038, respectively; Figure 2A and 2B). The same was observed for verbal learning and CpG1.7 (r=.38 p=0.030; (Figure 2C).

In summary, these results show that an improvement in speed of processing and verbal learning domains is associated with a decrease in methylation in these CpG units, while no change or increase in methylation is related to no effects of CRT on cognitive improvement. No other significant correlations were observed in the CRT group.

DISCUSSION

This is the first study to consider the methylation in the *BDNF* gene and its relationship to therapy-driven cognitive improvement. Our data suggest that cognitive rehabilitation is associated with some detectable changes in the methylation pattern of some CpG units of the *BDNF* gene in patients with a diagnosis of schizophrenia. Those changes are correlated with improvement in cognitive performance following the intervention.

Epigenetic mechanisms regulate important brain functions such as neurogenesis, neurodegeneration, neuronal activity, and cognition (Ma et al. 2010). The brain has remarkable abilities to adapt to the environment and, therefore, cognitive training would be a strong positive stimulus for adaptive neural mechanisms (Kaneko, Keshavan 2012; Medalia, Choi 2009). However, research based on DNA methylation and prognosis after psychological therapy is a new field of study in which few results have been published (Gooding 2022). It has been suggested that CRT-induced cognitive recovery could be interconnected with changes in the methylation of certain genes that play an important role in different systems, particularly in the neurotrophic, dopaminergic, glutaminergic, and serotonergic (Penadés et al. 2019).

Among these genes, the *BDNF* gene plays a regulatory role in the development, plasticity, and survival of neurons in these systems. A recent review has compiled current literature relating *BDNF* gene, methylation, and several brain conditions such as neurodevelopmental, psychiatric, and neurological disorders (Treble-Barna et al. 2023). However, studies linking methylation changes with psychological interventions are scarce, although some preliminary data are available (Penadés et al. 2020). For instance, Perroud et al. (2013) observed significant effects on *BDNF* methylation patterns after psychological treatment in patients with borderline personality disorder. More recently, the effect of CRT implementation on *BDNF* gene methylation in patients with schizophrenia was directly tested. However, Ho et al. (2020) did not detect significant changes, unlike our study. That study had a different methodology: i) the sample size of the patients in therapy was smaller, ii) they did not have a comparator patient group (TAU) but rather included healthy controls as a comparison group, iii) on average the patients were younger than in our study and, finally, iv) the dose of clozapine used are much higher than those in our study.

Of all the CpG positions analyzed in our study, only three correlated with cognitive functioning. These CpG sites depending on the *BDNF* isoform (long isoforms or short isoforms) are located in the promoter zone of the gene, exonic regions, or both (see Table -S1 of the Annex). A decrease in methylation levels of these CpG sites has been related to an improvement in Speed Processing (CpG1.2 and CpG1.10) or Verbal Learning (CpG1.7). Evidence concerning epigenetic processes suggests that methylation near gene promoters is correlated with gene silencing while within the gene body is associated with gene activation (Suzuki, Bird 2008; Rojas et al. 2015). The impact that an increase of methylation in these specific sites can have on *BDNF* gene expression and the improvement of some cognitive domains is hard to predict. In this sense, methylation changes in CpG positions could block the binding of repressors to the promoter region, allowing enhancers and other transcription factors to exert their action (Fu et al. 2020), or could influence the appearance of different alternative splicing and in the location and functionality of the BDNF protein (Zheleznyakova et al. 2016; Ferrer et al. 2019). Therefore, these changes could ultimately regulate the expression of the different isoforms of the *BDNF* gene, which are expressed in greater or lesser concentrations at the central nervous system level (Shayevitch et al. 2018). While there is still a knowledge gap in the connection of mRNA and protein levels with behavioral phenotypes, some studies have provided enlightening data. For instance, data from case-control postmortem analyses indicate that an association between the imbalance or reduction of certain BDNF isoforms in the hippocampus and the dorsolateral prefrontal cortex with the risk for schizophrenia (Wong et al. 2010) and the depletion of the same isoforms has been related to schizophrenia-like cognitive phenotypes through animal-based approaches (Chen 2022). Such complex *BDNF* expression patterns are linked to its downstream signaling cascades and, in turn, to the significant role in brain network development and synchronization of network activities that sustain neurophysiological and neurocognitive functions (Lu et al. 2014).

Our results are consistent with some basic research suggesting that DNA methylation and de-methylation levels can show very fast responses to behavioral stimuli (Levenson et al. 2006; Miller, Sweatt 2007). Studies in the area of epigenetics of cognition can significantly improve our basic understanding of critical aspects of cognitive recovery processes in the context of cognitive

treatments (Rudenko, Tsai 2014). Eventually, a better understanding of epigenetic mechanisms could lead to discoveries that identify response biomarkers and help clinicians in providing more personalized treatments (Lisoway et al. 2021) or even monitor those who need to change treatment to achieve benefits.

However, caution is needed in interpreting these findings, and some aspects should be considered. First, the sample size was relatively small, particularly when considering the subsample with methylation data. Second, the levels of BDNF protein were not determined, and changes in the concentration of the different protein isomorphism or genetic variation were not evaluated. Third, we must consider that methylation is a dynamic mechanism, and we do not have a complete understanding of the factors that can impact the stability of the methylation patterns in the *BDNF* gene. Therefore, despite our experimental design being focused on assessing the methylation changes derived from CRT, we cannot completely rule out the effect of other factors. Fourth, the unavailability of human brain samples compelled us to use blood as a surrogate tissue, complicating interpretation in a brain context. However, insights from animal models exposed to various environmental stimuli, such as stress, suggest that psychotherapy might exert its effects by inducing methylation changes in neurons (Quevedo et al. 2022; Martinez-Aran, Vieta 2022), with blood potentially serving as a predictor of methylation changes occurring in the brain (Kundakovic et al. 2015). Hence, though direct extrapolation of the identified blood-based methylation changes to brain effects is not possible, our findings linking peripheral methylation data with cognitive improvements in a clinical trial context are suggestive of the potential involvement of the BDNF gene in neuronal changes related to CRT. Fifth, the relationship between changes in cognition, methylation, and neuroplasticity is not limited only to this gene. Expanding the scope to encompass additional genes like the BDNF receptors (TrkB or p75 neurotrophin receptor) and considering the involvement of various neurotransmitter pathways (such as GABAergic, dopaminergic, etc.) could offer a more comprehensive understanding of these intricate interactions (Hing et al. 2018).

In all, our study contributes a piece to the puzzle, but new research aiming for a comprehensive analysis of BDNF methylation patterns is needed to establish its role in CRT-derived cognitive improvement. Also, upcoming research may need to focus on specific epigenetic changes associated with different subtypes of cognitive profiles that might lead to more targeted and effective treatments. Another future line of research might explore the benefits of combining cognitive remediation with pharmacological interventions, physical exercise, or other psychosocial interventions. Lastly, longitudinal studies tracking cognitive remediation over an extended period, examining both immediate gains and sustainability, are also warranted.

Future research may need to focus on specific epigenetic changes associated with different subtypes of cognitive profiles that might lead to more targeted and effective treatments. Another future line of research might explore the benefits of combining cognitive remediation with pharmacological interventions, physical exercise, or other psychosocial interventions. Finally, longitudinal studies tracking cognitive remediation over an extended period, examining both immediate gains and sustainability are also warranted.

CONCLUSION

Improvements in cognitive functioning that follow cognitive remediation therapy are associated with differential changes in the methylation levels for different CpG positions of the *BDNF* gene. Our study provides promising data for future research that will help to better understand the

role of the neurobiological mechanisms associated with cognitive remediation. This potential biomarker of methylation patterns and gene expression profiles could help clinicians to provide more personalized treatments.

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DISCLOSURES

Rafael Penadés has received honoraria/travel support (unrelated to the present work) from Angelini. C Garcia-Rizo has served as consultant, advisor or received honoraria/travel support (unrelated to the present work) from Adamed, Angelini, Casen-Recordati, Janssen-Cilag, Lundbeck, Otsuka and Newron. The rest of the authors reported no biomedical financial interests or potential conflicts of interest.

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TABLES

Table 1. Demographic and clinical variables at baseline assessment.

	CRT (n = 40)	**TAU (n = 20)**	**F**	***p***
Sex (M/F)	24/16 (60%/40%)	12/8 (60%/40%)		
	Mean (SD)	**Mean (SD)**		
Age (years)	38.70 (12.89)	45.70 (12.29)	4.474	0.039
Education (years)	10.30 (2.80)	10.50 (3.27)	0.061	0.806
Age onset of illness	24.88 (8.38)	23.50 (7.79)	0.376	0.542
Duration of illness (years)	14.23 (10.16)	22.95 (10.90)	9.371	0.003
Number of hospitalizations	2.30 (1.14)	2.80 (1.44)	2.158	0.147
Antipsychotic dosage (clozapine mg/d)	265.00 (145.64)	283.75 (127.03)	0.240	0.626
PANSS- Positive	13.74 (5.57)	13.74 (4.92)	2.02	0.996
PANSS-Negative	23.21 (6.28)	21.05 (3.88)	1.83	0.181
PANSS- General Psychopathology	44.26 (11.67)	40.79 (9.51)	1.26	0.266

Table 2. Interaction of experimental group (CRT, TAU) and time (T0 Baseline, T1 Post-treatment)

	CRT Mean (Standard Deviation)		**TAU Mean (Standard Deviation)**		**Interaction Group (CRT, TAU) x Time (T0, T1)**		
	T0	**T1**	**T0**	**T1**	**Coefficient (Standard Error)**	**95% Confidence Interval**	**Z**
Speed Processing	33.76 (10.76)	40.24 (10.52)	42.26 (11.13)	40.84 (12.04)	-4.789 (1.32)	(-7.381, -2.193)	-3.62 (<0.001)
Attention Vigilance	38.45 (10.44)	43.37 (10.63)	40.68 (12.14)	40.53 (12.12)	-5.437 (1.14)	(-7.674, -3.200)	-4.76 (<0.001)
Working Memory	34.13 (9.70)	40.11 (10.55)	42.42 (15.35)	39.11 (13.72)	-9.583 (2.45)	(-14.392, -4.774)	-3.91 (<0.001)
Verbal Learning	36.18 (7.02)	42.05 (8.20)	39.42 (12.45)	38.63 (10.96)	-6.905 (1.48)	(-9.799, -4.010)	-4.68 (<0.001)
Visual Learning	39.03 (11.72)	46.87 (10.85)	50.53 (1.53)	50.89 (11.12)	-8.143 (2.34)	(-12.726, -3.560)	-3.48 (<0.001)
Problem Solving	35.63 (7.94)	37.95 (8.36)	44.26 (8.36)	43.11 (5.23)	-3.884 (1.24)	(-6.315 -1.453)	-3.13 (0.002)

FIGURES

Figure 1. CONSORT diagram.

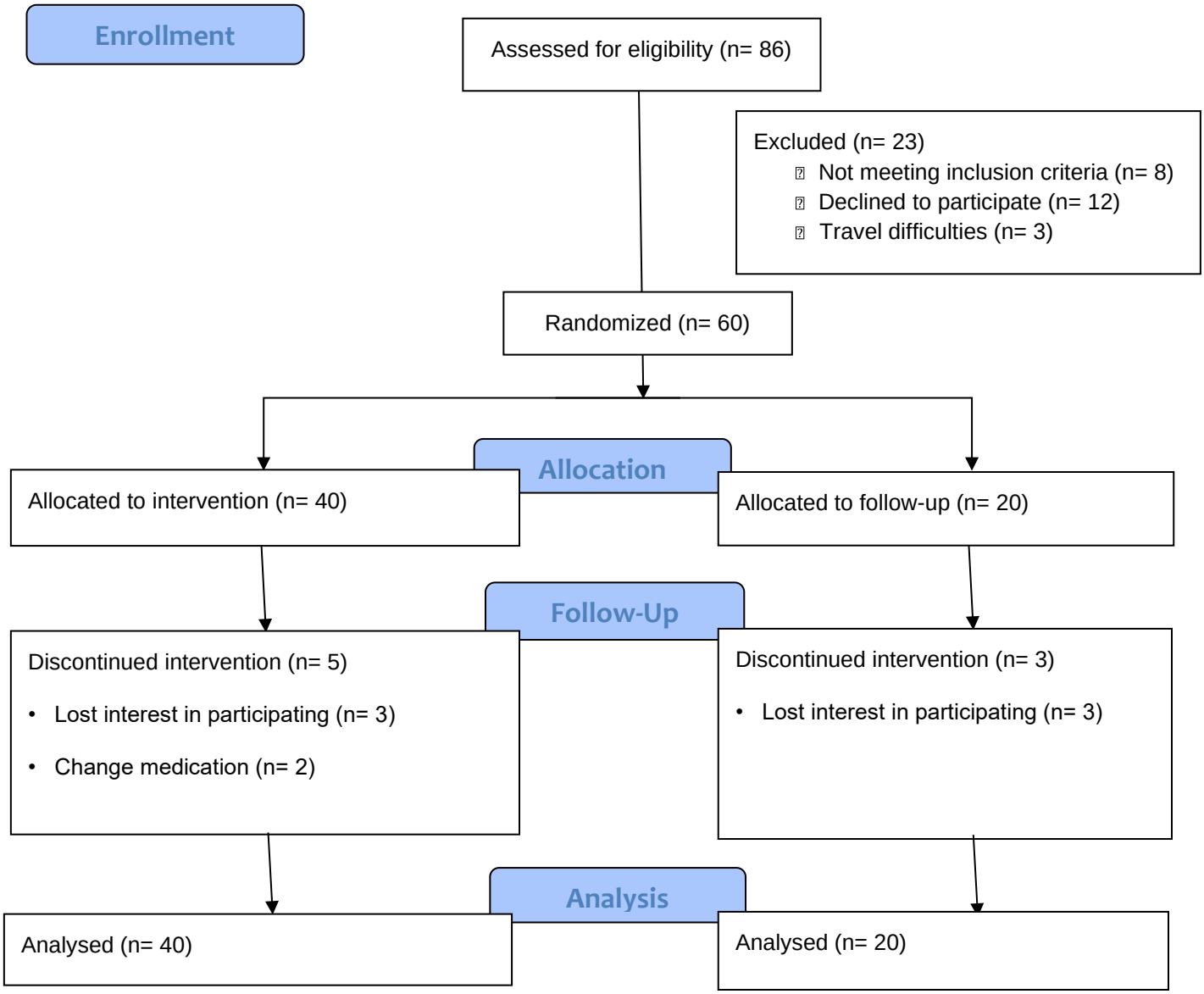
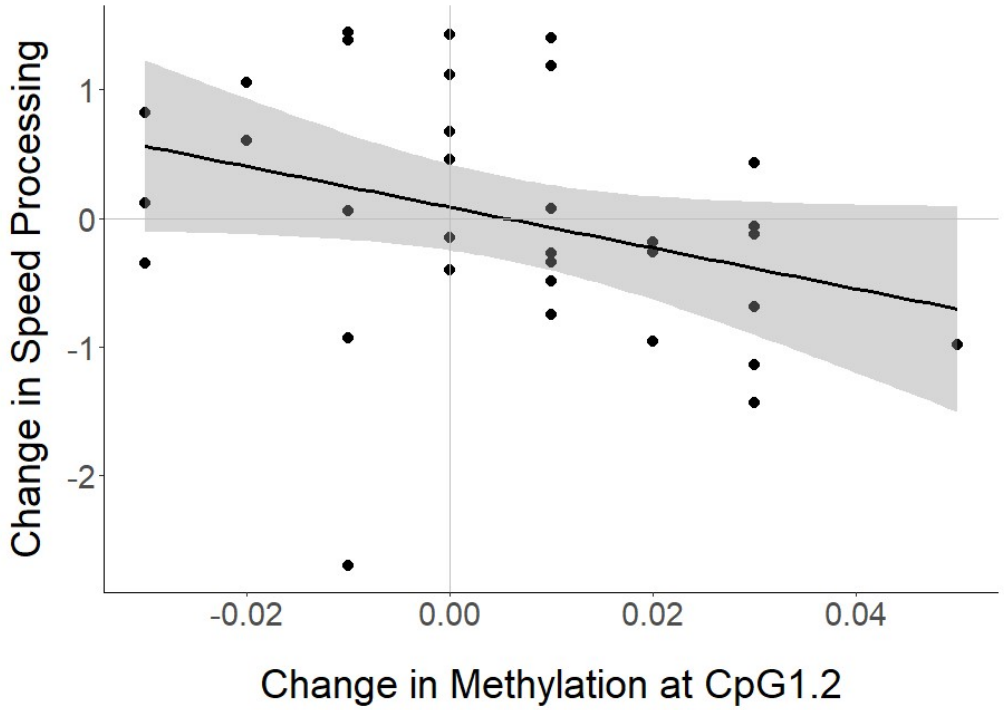
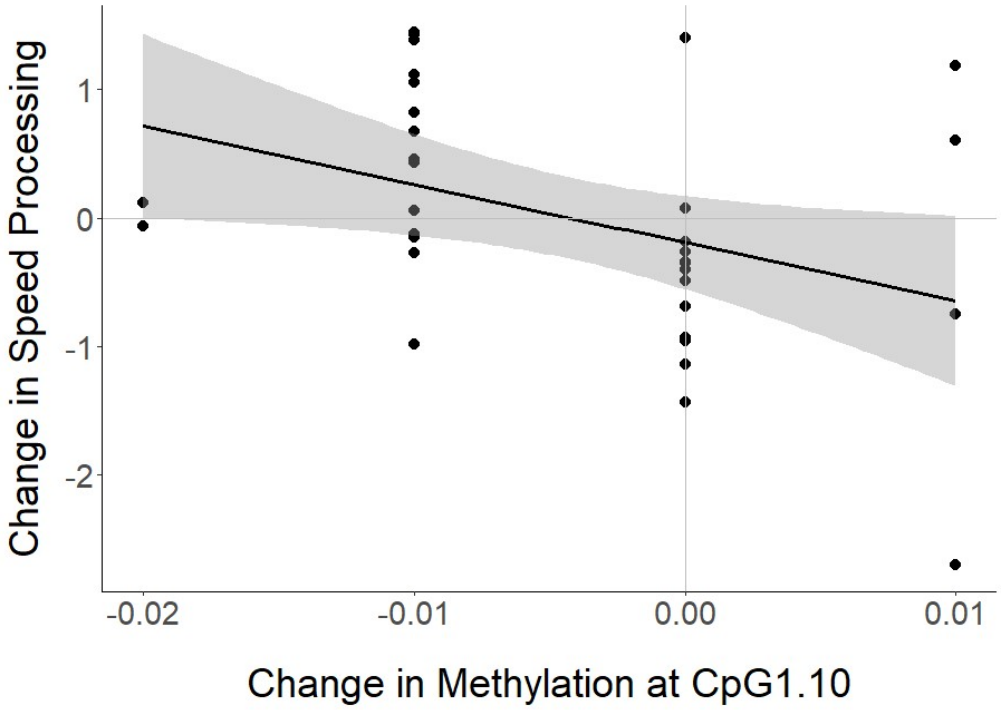
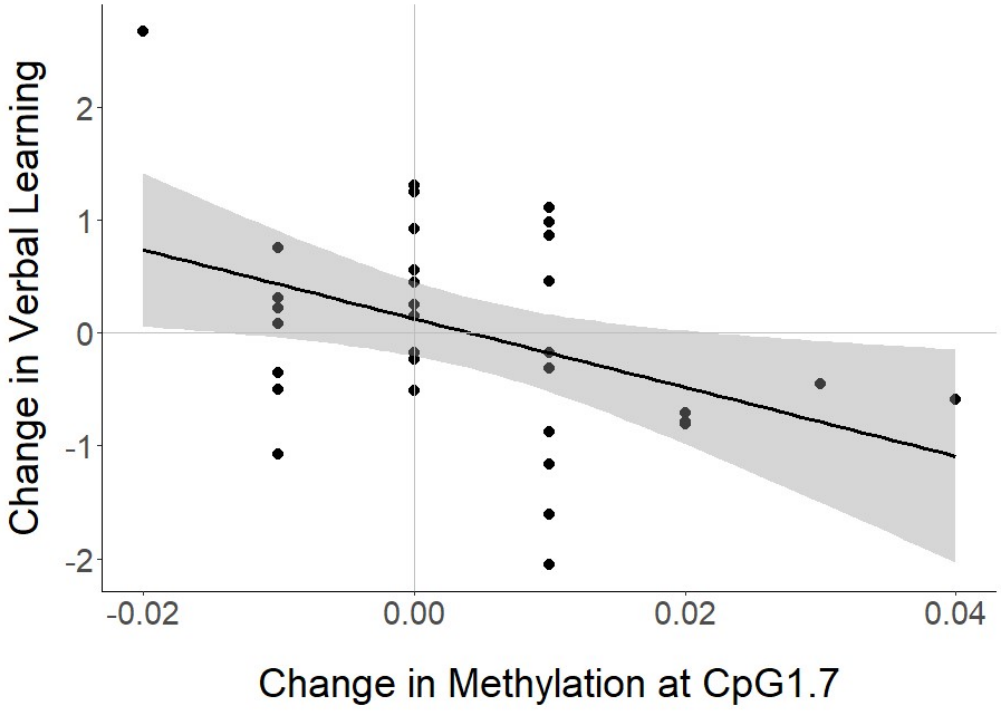


Figure 2. Scatter plots of the correlation between methylation changes and cognitive performance changes after CRT. **A)** In the X axis, the scores correspond to T1-T0 methylation at CpG1.2. In the Y axis, the scores represent the subtraction of the Speed Processing Domain baseline assessment (T0) to the values after CRT (T1). **B)** In the X axis, the scores correspond to T1-T0 methylation at CpG1.10. In the Y axis, the scores represent the subtraction of the Speed Processing Domain baseline assessment (T0) to the values after CRT (T1).**C)** In the X axis, the scores correspond to T1-T0 methylation at CpG1.7. In the Y axis, the scores represent the subtraction of the Verbal Learning Domain baseline assessment (T0) to the values after CRT (T1).




Change in Speed Processing
1
0
-1
-2
-0.02
-0.01
0.00
0.01
Change in Methylation at CpG1.10


Change in Verbal Learning
2
1
0
-1
-2
-0.02
0.00
0.02
0.04
Change in Methylation at CpG1.7

SUPPLEMENTAL INFORMATION

SUPPLEMENTARY TABLE S1. CpG units' information. Each CpG unit can include one or more CpG sites. The locus of each CpG site is given. The table includes the chromosome location and gene position (located within promoter (P), exonic (E) or intronic (I) regions) of each CpG site (UCSC Genome Browser on Human Mar. 2006 Assembly (NCBI36/hg18)

BDNF

CGI 1				**CGI 2**				**CGI 3**		
CpG unit	**CpG site locus**	**Gene position**		**CpG unit**	**Locus**	**Gene position**		**CpG unit**	**Locus**	**Gene position**
1.1	chr11:27700717	P, E		2.1	chr11:27719121	I		3.1	chr11:27722502	I
1.2	chr11:27700744	P, E			chr11:27719131	I		3.2	chr11:27722507	I
	chr11:27700748	P, E		2.2	chr11:27719143	I		3.3	chr11:27722587	I
	chr11:27700750	P, E			chr11:27719149	I		3.4	chr11:27722616	I
1.3	chr11:27700756	P, E		2.3	chr11:27719201	I			chr11:27722622	I
1.4	chr11:27700786	P, E		2.4	chr11:27719245	I		3.5	chr11:27722637	I
	chr11:27700796	P, E			chr11:27719252	I		3.6	chr11:27722649	I
	chr11:27700798	P, E			chr11:27719255	I			chr11:27722652	I
1.5	chr11:27700803	P, E			chr11:27719258	I			chr11:27722655	I
	chr11:27700805	P, E		2.5	chr11:27719274	I		3.7	chr11:27722663	I
1.6	chr11:27700823	P, E			chr11:27719278	I		3.8	chr11:27722672	I
1.7	chr11:27700833	P, E		2.6	chr11:27719288	I			chr11:27722674	I
1.8	chr11:27700862	P, E			chr11:27719291	I			chr11:27722677	I
	chr11:27700866	P, E			chr11:27719297	I		3.9	chr11:27722698	I
	chr11:27700871	P, E		2.7	chr11:27719309	I		3.10	chr11:27722705	I
1.9	chr11:27700944	E		2.8	chr11:27719322	I				
1.10	chr11:27700955	E			chr11:27719329	I				
1.11	chr11:27700976	P, E			chr11:27719331	I				

1.12	chr11:27700993	P, E		2.9	chr11:27719360	I				
1.13	chr11:27701002	P, E			chr11:27719363	I				
	chr11:27701006	P, E			chr11:27719367	I				
	chr11:27701008	P, E		2.10	chr11:27719392	I				
1.14	chr11:27701025	P, E		2.11	chr11:27719402	I				
1.15	chr11:27701070	I		2.12	chr11:27719416	I				
	chr11:27701073	I			chr11:27719420	I				
1.16	chr11:27701089	I			chr11:27719425	I				
	chr11:27701091	I		2.13	chr11:27719445	I				
1.17	chr11:27701162	I		2.14	chr11:27719469	I				
				2.15	chr11:27719497	I				
				2.16	chr11:27719506	I				
				2.17	chr11:27719530	P, E				
					chr11:27719535	P, E				
				2.18	chr11:27719546	P, E				
					chr11:27719551	P, E				
					chr11:27719553	P, E				
				2.19	chr11:27719559	P, E				

SUPPLEMENTARY TABLE S2. Demographic and clinical variables at baseline assessment of the individuals with methylation data.

	CRT (n = 33)	**TAU (n = 10)**	**X^2/F**	***p***
Sex (M/F)	19/14 (58%/42%)	7/3 (70%/30%)	0.496	0.481
	Mean (SD)	**Mean (SD)**		
Age (years)	39.91 (12.40)	40.70 (9.58)	0.034	0.854

Education (years)	10.55 (2.96)	11.20 (3.52)	0.344	0.561
Age onset of illness	24.82 (8.93)	21.10 (3.84)	1.621	0.210
Duration of illness (years)	15.39 (9.94)	19.70 (8.59)	1.524	0.224
Number of hospitalizations	2.24 (1.12)	2.50 (1.51)	0.345	0.560
Antipsychotic dosage (clozapine; mg/d)	260.61 (156.13)	302.50 (153.86)	0.556	0.460

SUPPLEMENTARY TABLE S3. The table shows the mean value and standard deviation of methylation for each CpG unit. The table includes the values in each group before and after the intervention.

CpG unit	PRE-CRT		PRE-TAU			POST-CRT		POST-TAU	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD
ISLAND 1									
1.1	0,0097	0,00984	0,009	0,00876		0,0164	0,01388	0,009	0,01101
1.2	0,0873	0,02516	0,078	0,01751		0,0927	0,02809	0,099	0,02846
1.3	0,0361	0,01499	0,037	0,02111		0,0276	0,01146	0,04	0,017
1.4	0,0333	0,00495	0,034	0,00459		0,0329	0,0082	0,032	0,00856
1.5	0,015	0,00433	0,014	0,00516		0,0158	0,00561	0,0175	0,00425
1.6	0,0279	0,0096	0,024	0,00843		0,0273	0,00801	0,025	0,00707
1.7	0,005	0,00586	0,006	0,00568		0,0091	0,00972	0,0015	0,00242
1.8	0,07	0,01837	0,073	0,01418		0,0736	0,01617	0,074	0,01955
1.9	0,0198	0,01057	0,014	0,00876		0,0239	0,0095	0,0225	0,00979
1.10	0,0667	0,00736	0,064	0,00738		0,0618	0,00481	0,064	0,00516
1.11	0,0138	0,00559	0,013	0,00856		0,0189	0,00693	0,02	0,00745
1.12	0,0158	0,00936	0,015	0,01841		0,0152	0,01004	0,012	0,00919
1.13	0,0417	0,00863	0,039	0,00775		0,0395	0,00814	0,0385	0,00851
1.14	0,0191	0,00579	0,016	0,00516		0,0212	0,00984	0,019	0,00699
1.15	0,0214	0,00472	0,0205	0,00497		0,0253	0,00847	0,0195	0,0055
1.16	0,003	0,0081	0	0		0,0009	0,00292	0	0
1.17	0,0303	0,00984	0,033	0,00949		0,0276	0,00936	0,021	0,00568
ISLAND 2									
2.1	0,0688	0,01516	0,061	0,01197		0,0733	0,02131	0,065	0,00972
2.2	0,0727	0,02004	0,07	0,02749		0,0655	0,01986	0,062	0,02348
2.3	0,0606	0,01144	0,061	0,01197		0,0636	0,01432	0,056	0,0135
2.4	0,0867	0,01689	0,082	0,01135		0,0848	0,02167	0,08	0,01764
2.5	0,0839	0,02692	0,076	0,01174		0,0833	0,02665	0,077	0,02214
2.6	0,0997	0,02481	0,093	0,01252		0,0979	0,02924	0,091	0,01853
2.7	0,0488	0,01728	0,047	0,01059		0,047	0,01741	0,045	0,01269
2.8	0,0912	0,02571	0,09	0,01826		0,0921	0,03059	0,084	0,0143
2.9	0,067	0,01928	0,06	0,00471		0,0658	0,01871	0,06	0,01333
2.10	0,0539	0,01784	0,049	0,01197		0,0576	0,02525	0,057	0,03773
2.11	0,0667	0,01614	0,063	0,00483		0,0655	0,01502	0,062	0,01033
2.12	0,1064	0,00822	0,106	0,00516		0,1009	0,01308	0,101	0,00738
2.13	0,0433	0,01555	0,038	0,00422		0,0427	0,0179	0,036	0,01075
2.14	0,1339	0,02499	0,134	0,01713		0,1279	0,02329	0,128	0,01989
2.15	0,0682	0,01648	0,064	0,01647		0,0715	0,02093	0,07	0,0216

2.16	0,0212	0,0074	0,019	0,00738		0,0215	0,0146	0,027	0,02058
2.17	0,063	0,02215	0,056	0,01265		0,0606	0,01936	0,054	0,00699
2.18	0,1	0,02449	0,091	0,01449		0,1015	0,02123	0,088	0,01549
2.19	0,0458	0,01786	0,043	0,00949		0,0421	0,01654	0,04	0,01563
ISLAND 3									
3.1	0,0461	0,01171	0,043	0,00675		0,0485	0,01603	0,044	0,00966
3.2	0,0373	0,01206	0,029	0,00876		0,0327	0,01281	0,038	0,00789
3.3	0,0339	0,01345	0,033	0,00823		0,0321	0,01867	0,03	0,01054
3.4	0,0661	0,02207	0,06	0,01633		0,0582	0,01944	0,067	0,02003
3.5	0,0276	0,013	0,024	0,01578		0,0242	0,01251	0,021	0,00738
3.6	0,0248	0,00834	0,026	0,00966		0,0252	0,00906	0,026	0,00843
3.7	0,0327	0,00876	0,029	0,00738		0,0282	0,01211	0,028	0,00919
3.8	0,0461	0,01116	0,049	0,01101		0,0458	0,00936	0,044	0,00966
3.9	0,0194	0,00496	0,017	0,00675		0,02	0,00612	0,022	0,00632
3.10	0,0245	0,00794	0,022	0,00632		0,0224	0,00936	0,021	0,00738