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Paving the way for precision treatment of psychiatric symptoms with functional connectivity neurofeedback

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Treatment for Major Depressive Disorder (MDD) remains relatively homogeneous, despite patients having heterogeneous subsets of symptoms with differing underlying neural aberrations. Demonstrating potential for a more individualised treatment approach, we recently showed that normalisation of a neural network and a corresponding reduction in related symptoms can be achieved using real-time fMRI functional connectivity neurofeedback (FCNef). Specifically, we showed that brooding rumination but not anxiety symptoms decreased as functional connectivity between the dorsolateral prefrontal cortex (DLPFC) and posterior cingulate cortex/precuneus (PCC) normalised with FCNef. However, the robustness of this effect, how localised it is in the brain, and the best parameters for optimising therapeutic outcomes remained unknown. We therefore ran additional participants (final N = 68) in our FCNef protocol. We replicated our original findings and ran new analyses that better highlighted the precision of this effect to rumination symptoms. For the first time we also demonstrated that connectivity between the Executive Control and Default-Mode networks reduced significantly with FCNef. Finally, we manipulated core FCNef parameters between participants and found that the most effective protocol involved consecutive training days with greater external reward. These results highlight the potential of FCNef for precision medicine in psychiatry and underscore the importance of optimising parameters to enhance feasibility of BMI-based clinical interventions.

Translational Psychiatry (2026)16:291 ; https://doi.org/10.1038/s41398-026-04040-3

INTRODUCTION

The World Health Organisation estimates that major depressive disorders will become the top cause of global disease burden by 2030 [1]. Of patients who do receive treatment, an estimated 30–50% do not respond fully [2, 3]. Patients with the same clinical diagnosis can have heterogeneous subsets of symptoms that relate to different underlying neural mechanisms [4]. Nonetheless, they usually receive relatively homogenous treatment, such as selective serotonin reuptake inhibitors as first-line treatment for depression. To improve response rates, individual differences clearly need to be considered. Future treatment may become more individualised using Brain-Machine Interfaces (BMIs) to identify and target a patient's underlying neural aberrations.

FCNef is a promising form of BMI, where functional magnetic resonance imaging (fMRI) neurofeedback is used to give participants real-time feedback about the current state of functional connectivity between two of their brain regions (measured as the correlation between time-courses of BOLD activity from these regions). This feedback is used to train participants to make a targeted functional connection more positive or negative, a result that has been demonstrated in multiple studies [5–12]. FCNef success can be operationalised as a targeted shift in functional connectivity and a corresponding change in behaviour. In line with this, showing promise for precision medicine, recent FCNef studies have reported precise correspondence between normalisation of functional connections and reductions in specific symptoms [9–11, 13]. We ourselves previously found that, with FCNef, functional connectivity between the dorsolateral prefrontal cortex (DLPFC) and the precuneus/posterior cingulate cortex (PCC) normalised and this normalisation was related to reductions in brooding rumination, but not anxiety symptoms [9]. Importantly, these effects persisted at least 1–2 months after FCNef [9].

Our previous results showed proof-of-concept, but they were reported from a small sample. We have since continued to collect data, and here we examine the robustness of our aforementioned results by testing for their replication in our newly collected data. Combining the new and old data provides sufficient statistical power so that we are now able to directly examine the specificity of this effect. Finally, here we statistically examine changes in

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Received: 14 October 2025 Revised: 16 March 2026 Accepted: 9 April 2026
Published online: 22 April 2026

Table 1. Sample sizes for different groups of participants.

Group	Main Experiment Sample Size	1-Month Follow-up Sample Size	2-Month Follow-up Sample Size
Consec/ High-Rew	21	11	9
Consec/ Low-Rew	23	20	17
Non/ Low-Rew	24	22	20
Total	68	53	46

This table shows the sample sizes for three groups of participants: (1) those run in consecutive days of FCNef with the high reward schedule (the "Consec/ High-Rew group"), (2) those run in consecutive days of FCNef with the low reward schedule (the "Consec/Low-Rew group"), and (3) those run over non-consecutive days of FCNef with the low reward schedule (the "Non-Consec/Low-Rew group"). Note that there were no participants run over non-consecutive days of FCNef with the high reward schedule (no "Non-Consec/High-Rew" group). Sample sizes are shown for the main experiment and for the one- and two-month follow-up tests. There are fewer participants for the follow-up tests for the Consec/High-Rew group because the long-term tests were not included in the earliest stages of this experiment and so only 12 of the participants from this group were invited back.

resting-state connectivity at the network-level. We speculated that FCNef may have not just normalised the connectivity between the two targeted brain regions (DLPFC and precuneus/ PCC), but between the two greater networks to which these belong (the Executive Control and Default Mode networks, respectively).

To date, only a handful of BMIs have received approval from local medical regulatory agencies for human trials [14–17], and even fewer have received full market authorisation [18, 19]. A key step toward approval of a given BMI technique by regulatory agencies is demonstrating the optimality of chosen parameters. An additional objective of this study was to find parameters that would best enhance FCNef success, while minimising patient burden (e.g., fatigue, which caused one out of six clinical patients to drop out in preliminary testing [20]). To accomplish this, we focused on (1) Reward schedule: Recent evidence suggests that target neural activity may be better reinforced during neurofeedback when monetary reward is used in combination with visual display of feedback scores [21]. We therefore manipulated monetary reward by assigning it differently to feedback scores for different groups of participants. (2) Experimental schedule: Consecutive days of experimentation, as often seen in neurofeedback studies [12], can be exhausting and require motivation and organisation skills that can be diminished in psychiatric disorders [22]. We therefore tested whether a more flexible schedule, over non-consecutive days, could yield similar results to the consecutive training schedule.

Overall, we ran 68 participants in our FCNef for depression paradigm while manipulating reward schedule (low/high) and experimental schedule (consecutive/non-consecutive training days). Our goals were: (1) to examine the precision and robustness of the FCNef effect using a larger sample size, (2) to extend analyses to the network-level, and (3) to fine-tune underlying FCNef parameters.

METHODS

Participants

Participants were screened twice using the Beck Depression Inventory-II (BDI) questionnaire [23] (see Supplementary Methods for more details). Only those with an average score ≥ 8, who indicated no intention of committing suicide, who spoke Japanese, who held no current clinical diagnosis, and who were not currently receiving treatment for a psychiatric illness were invited to participate. Overall, 69 people passed the screening and participated in the main experiment. However, experimental data of one participant was subsequently excluded from data analysis because it was revealed, subsequent to experimentation, that that subject held a current clinical diagnosis. Of the 68 participants whose data were used for analyses, the average BDI score over the two screening measurements was 14.33, with a standard deviation (STD) of 5.26. This puts them generally in the category of "mild depression" [23]. For this reason and because these participants lacked current clinical diagnoses, we considered them "subclinical".

Experimental conditions

Our goal was to examine the overall success of our paradigm and to determine whether it is influenced by reward and experimental schedules. However, due to financial and time constraints (the full protocol takes 10 days of experimentation to screen and run one participant), we could only run participants in three experimental groups: those with consecutive days of experimentation and a high-reward schedule (hereafter, "Consec/ High-Rew"), those with consecutive days of experimentation and a low-reward schedule (hereafter, "Consec/Low-Rew"), and those with non-consecutive days of experimentation and a low-reward schedule (hereafter, "Non-Consec/Low-Rew") (see Table 1 for sample size details). Within each of these three groups, about half of the participants were run with an induction time-window of 20 s and the other half with an induction time-window of 40 s (see Supplementary Table 1), but this had no major impact on FCNef success (see Supplementary Results, Supplementary Figure 1, and Supplementary Tables 1–5 for details).

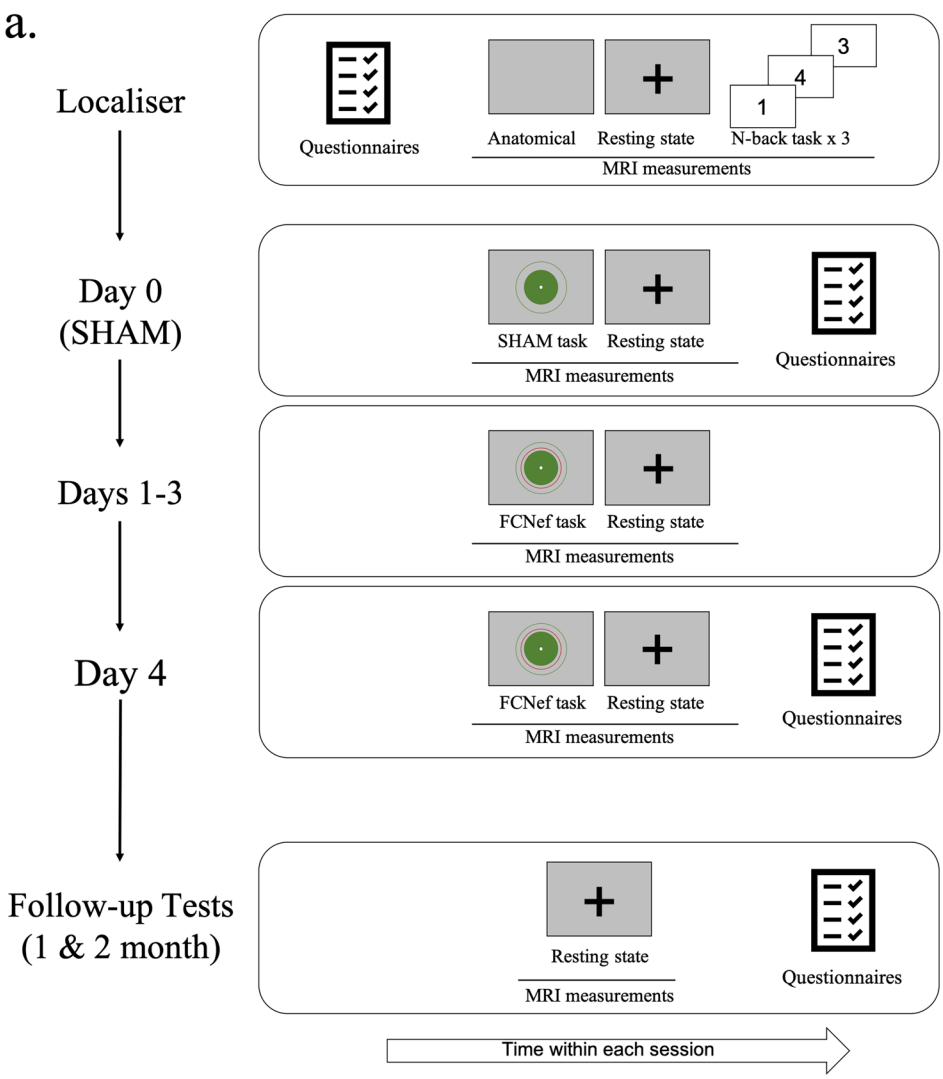
Participants in the three groups did not differ significantly in baseline levels of our main measures of interest: (1) DLPFC-PCC resting-state functional-connectivity (rs-FC), (2) BDI scores, and (3) brooding scores (measured on a subscale of the Rumination Response Scale [24, 25]) (see Supplementary Table 7). However, we found that baseline anxiety levels (measured with the trait anxiety subscale of the State-Trait Anxiety Inventory [26]) differed significantly between the Consec/Low-Rew and Non-Consec/Low-Rew groups (see Supplementary Table 7). This is unlikely to have had a large impact on results because: (a) the rs-FC we targeted is not thought to relate to anxiety, and (b) results showed that differences in FCNef success were not greatest between these two groups (as can be seen further down).

Participant group assignments were determined by concurrent availability of participants, experimenters, and MRI machine facilities. This allocation method reflects real-world constraints in neuroimaging research, though we acknowledge it could have introduced potential self-selection effects. To address possible allocation bias, we conducted analyses to compare baseline demographic measurements between groups (age and sex): No meaningful differences were found (see Supplementary Table 7). It should be noted that data of 19 participants (9/21 from the Consec/High-Rew group and 10/23 from the Consec/Low-Rew group) have been reported elsewhere [9]. Further details about recruitment, participant demographics, and payment can be found in the Supplementary Methods.

Experimental procedure, materials, and imaging data acquisition

An outline of the experimental procedure is shown in Fig. 1 alongside details of the FCNef task. This procedure, and related analyses, were not pre-registered. Experimental procedure details are largely the same as in our previous report [9], except for specific experimental conditions of interest. These experimental conditions are described in the sub-section below entitled "Differences in experimental conditions." The protocol and imaging data acquisition details are identical to those in our previous report [9] and are summarised in Supplementary Methods.

Details of the symptom questionnaires can be found in the Supplementary Methods, but overall general depressive symptoms were measured with the BDI [23]. Brooding rumination symptoms were measured with a subscale of the Rumination Response Scale (RRS) [24, 25], and trait anxiety symptoms were measured with the trait anxiety subscale of the State-Trait Anxiety Inventory (STAI-Y2) [26]. As can be seen in Fig. 1, because there would be limited clinical meaning, these symptom



questionnaire scores were not measured on all days of the main experiment. Instead, they were only measured on the first day (Day 0) and last day (Day 4) of the main experiment and during one- and two-month follow-up tests.

Differences in experimental conditions

The following parameters were manipulated, such that some participants were run under conditions different from those reported in our previous papers [9, 12].

Fig. 1 Experimental procedure and example FCNef trial. a Overall experiment design. Questionnaires = the Beck Depression Inventory-II [23], the Rumination Response Scale [24], and the State-Trait Anxiety Inventory [26]. Anatomical = T1-weighted structural MRI. N-back = a well-known executive control task [60], used here as a functional localiser [9]. **b An example FCNef trial**. During the rest period, participants should simply relax. During the induction period (20 s/40 s; manipulated between participants, see Supplementary Materials), they should "somehow" manipulate their brain activity to get the best possible feedback. Participants were told that different strategies of brain activity manipulation might work for different people. Unbeknown to participants (nothing changed on screen), there was a 2 s calculation period at the end of the induction period. During FCNef, DLPFC-PCC connectivity (from the induction period) was calculated during the calculation period and this determined the feedback presented during the feedback period. During SHAM, however, the feedback was just random. Feedback was presented on screen as a green circle and participants had been clearly instructed that the larger this was, the more monetary reward they would receive on that trial. During FCNef, they were instructed to try to make the green circle bigger than the red circle that was additionally presented on screen. The circumference of this red circle represented the participant's baseline DLPFC-PCC connectivity (the average from SHAM). During SHAM, there was no red circle and participants were simply instructed to try to make this green circle as big as possible. Modified with permission from Taylor et al. [9].

Reward schedule. All participants received a baseline reward bonus of ¥500 on each day of the SHAM and FCNef sessions. This is the maximum reward they could receive in the SHAM task, but they could get an additional reward bonus in the FCNef task depending on their average FCNef scores from that day. Participants were shown random scores on the SHAM day, displayed as feedback circles in the feedback period of each trial. Their FCNef scores on subsequent days were calculated based on their DLPFC-PCC functional connectivity from the induction period of each trial, ranging from 0 (no green circle; awarded when functional connectivity = average from SHAM + 1 STD) to 100 (full green circle; awarded when functional connectivity = average from SHAM - 1 STD). FCNef scores were calculated in this way to reinforce a more negative DLPFC-PCC functional connectivity. Although the scores themselves were calculated in an identical manner across conditions, the way additional reward bonus was assigned to these differed. Specifically, participants in the low-reward schedule conditions could achieve an additional reward bonus of ¥100 for each average FCNef score point over 75. Instead, participants in the high-reward schedule condition could achieve an additional reward bonus of ¥50 for each average FCNef score point over 50. Participants in each group were unaware of this between-subject manipulation. See Supplementary Table 6 for specific examples. These schedules resulted in participants in the high-reward group receiving higher reward overall and so the groups were named accordingly. See Supplementary Results and Supplementary Figure 2 for more details.

Experimental schedule. Two groups of participants completed Days 0–4 over 5 consecutive days (Monday-Friday). The third group completed Days 0–4 over 5 non-consecutive days, which took place over a period of several weeks to months (mean of 18.5 days ± STD of 15.3 days).

Data analyses

Correlations to extend previously reported results. We previously reported correlations between rs-FC and symptom changes using data from 19 participants who were run over consecutive days of FCNef [9]. Ten of these participants were run with the high-reward schedule and the other nine were run with the low-reward schedule, but overall their combined data showed promising results. Since then, we have collected data from 25 more participants under the same experimental conditions (consecutive days of FCNef with low/high-reward schedules), which gives us an overall dataset with 44 participants run under these conditions (see Table 1). Note that this does not include the data of the Non-Consec/Low-Rew group, which has also been collected since our previous report. This is because this group was run with a different experimental parameter to our previous report (non-consecutive days of experimentation) and so its data cannot contribute directly to our replication test. One goal of collecting this additional data was to assess the robustness of previously reported results in a larger dataset. Before doing this in the 44 participants, however, we first re-ran relevant correlations using only data of the additional 25 participants to ensure that we were not introducing bias by adding new data to the existing pool. After this test, we then re-ran the previously reported correlations with the larger pooled dataset. To check the adequacy of the sample size for statistical tests, we conducted post-hoc power analyses with G*Power version 3.1.9.7 (Franz Faul, Kiel University, Germany). The significance threshold was set at $\alpha = 0.05$, and we input relevant correlational values to calculate statistical power.

Network-level analyses to extend previously reported results. In our previous publication [9], we speculated that the correlation that we found between normalisation of the DLPFC-PCC FC and a reduction in brooding rumination may have arisen alongside changes at the network level. Specifically, we speculated that FCNef may have not just normalised the connectivity between the two targeted brain regions (DLPFC and precuneus/PCC), but between the two greater networks to which these belong (the Executive Control and Default Mode networks, respectively). Here, we investigated this using the same 44 participants as mentioned above. As above, these participants were selected for this analysis because they were run under the conditions described in our previous publication where this speculation was made. These analyses were run using the CONN Toolbox [27–29] (22.v2407) in conjunction with SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). Specifically, the CONN Toolbox default pipelines [30–32] were used to preprocess and denoise the resting-state whole-brain data of each participant from Days 0 (pre-FCNef) and 4 (post-FCNef). This data was then band-pass filtered in the 0.008-0.1 Hz range and time series were extracted from the 7 Yeo Network parcellation networks [33]. Using the CONN Toolbox [27–29], ROI-to-ROI connectivity (measured as Fisher-transformed bivariate correlations) was then calculated for both pre- and post-FCNef measurements. Finally, the difference between connectivity at the pre- and post-FCNef stages was examined using second-level F-Tests.

Linear Mixed Effect (LME) models to examine parameters of interest. We ran LME models to examine how manipulated parameters impacted measures related to FCNef success (see Table 2). Dependent variables (DVs) in these models were taken from the task (e.g. average task score) or from the resting-state scans (e.g., changes in resting-state functional connectivity from before to after FCNef). All 3 groups of participants (total N = 68) were included in these analyses.

Note that we could not run full factorial analyses because there was no Non-Consec/High-Rew group (see Table 1). Regardless, we had no reason to expect interactions between parameters. We instead analysed data with "Group" as a categorical Independent Variable (IV) with 3 levels. We set the Consec/High-Rew group as the first level because this group had parameters that we used in the initial pilot experiment [12] and we wanted to examine whether changes to these initial parameters would improve or worsen FCNef success.

In addition to Group, LME models also included other relevant IVs, which differed between models depending on the DV. In models examining how brain changes predicted symptom changes, this was changes in resting-state functional connectivity from before to after FCNef. In other models, this was the relevant experimental days.

For each DV, we first built a random-intercept only model [34]. We then built LME models that included additional IVs with and without interaction terms.

Random-intercept only: *'DV ~ 1 + (1|Subject)'*

No interaction term: *'DV ~ 1 + X + Group + (1|Subject)'*

With interaction term: *'DV ~ 1 + X * Group + (1|Subject)'*

Likelihood ratio tests were used to compare all three models for each DV. If there was a significant difference between models, the best-fit model was always selected based on the lowest Akaike Information Criterion (AIC) [35]. If there was no significant difference between models, then the simplest model (with fewer degrees of freedom) was selected as best-fit. Detailed information about the best-fit of each of these pairs of models can be found in tables in the Supplementary Results (Supplementary Tables 8, 9, 11–16, 18–23), with all significant main effects and interactions from the best-fit models additionally being reported in the Results Section below.

Table 2. The models used to examine different dependent variables from the FCNef experiment.

Analyses	Formulae					AICs		
	DV	IV1		IV2	Random Intercept	int. only	+	x
FCNef Scores	Mean FCNef Score	~ FCNef Day	+ or x	Group	+ (1|Subject)	2118.2	**2109.2**	2108.7
	FCNef Score Variance	~ FCNef Day	+ or x	Group	+ (1|Subject)	1608.3	1599.8	**1593**
Symptoms	General Depressive Scores	~ first/last FCNef Day	+ or x	Group	+ (1|Subject)	791.8	**776.72**	779.13
	Brooding Scores	~ first/last FCNef Day	+ or x	Group	+ (1|Subject)	694.2	**681.4**	682.68
	Anxiety Scores	~ first/last FCNef Day	+ or x	Group	+ (1|Subject)	929.46	**913.86**	914.62
	General Depressive Score Changes (D4, 1 M, 2 M)	~ Post-Day	+ or x	Group	+ (1|Subject)	**921.33**	926.03	925.51
	Brooding Score Changes (D4, 1 M, 2 M)	~ Post-Day	+ or x	Group	+ (1|Subject)	**764.71**	766.01	769.07
	Anxiety Score Changes (D4, 1 M, 2 M)	~ Post-Day	+ or x	Group	+ (1|Subject)	**1090.6**	1095.7	1095
rs-FC	rs-FC	~ first/last FCNef Day	+ or x	Group	+ (1|Subject)	**−23.97**	−22.99	−23.67
	rs-FC Changes (D4, 1 M, 2 M)	~ Post-Day	+ or x	Group	+ (1|Subject)	−56.56	**−58.77**	−55.47
Symptom Change/ rs-FC Change Relationship	General Depressive Score Changes (D4)	~ rs-FC Change	+ or x	Group	+ (1|Subject)	317.92	318.44	**303.87**
	Brooding Score Changes (D4)	~ rs-FC Change	+ or x	Group	+ (1|Subject)	302.8	**293.07**	294.37
	Anxiety Score Changes (D4)	~ rs-FC Change	+ or x	Group	+ (1|Subject)	410.18	**406.97**	410.29

Models with and without interactions between independent variables (IVs) were compared with each other and with random-intercept only models to see which would best predict each dependent variable (DV). The Akaike Information Criterion (AIC) for each model are displayed in the columns titled: 'int. only' for the random-intercept only models, '+' (for the models without the interactions), and 'x' (for the models with the interactions). The AICs of the best-fit models are highlighted in bold font. FCNef Day = Days 1–4; Subject = experimental participant; STD = standard deviation; First/last FCNef Day = Days 0 and 4; Post-Day = Day 4, and the 1- and 2-month follow-up test days; Changes = data from the day indicated in brackets (e.g. D4) minus data from Day 0; rs-FC = resting state functional connectivity between the DLPFC-PCC.

Follow-up statistical testing. Significant main effects and interactions from the best-fit models, as well as effects that we had specific hypotheses about, were followed up with t-tests or correlations. We applied False Discovery Rate (FDR) correction [36] whenever there were multiple comparisons. We had directional hypotheses that symptoms would decrease from before to after FCNef and that rs-FC would become more negative, aligning with patterns observed in healthy individuals. Therefore, we conducted the related t-tests using a one-tailed approach. Similarly, based on our directional hypothesis that symptoms would decrease as rs-FC became more negative (indicating a positive correlation between changes in symptoms and changes in rs-FC), we also used a one-tailed approach for the related correlation analyses. Other tests were run with two-tails.

RESULTS

Extension of past results

Previously reported data. We previously reported data of 9 participants from the current Consec/High-Rew group and 10 participants from the current Consec/Low-Rew group [9]. A significant positive relationship with rs-FC change was found for brooding change ($r = 0.43$, $p_{FDR} = 0.048$, 95% CI [−0.27, 0.74]), but not for anxiety change ($r = 0.11$, $p_{FDR} = 0.32$, 95% CI [−0.36, 0.54]) (note that we previously only reported correlation coefficients and uncorrected *p*-values). Here we calculated a Z-test to compare these two correlation coefficients [37]. This was run with one tail due to our directional hypothesis. No significant difference was found ($z = 0.99$, $p = 0.16$). A post-hoc power analysis, run with G*Power 3.1.9.4 [38], revealed a post-hoc power of 0.25, suggesting that the sample size (N = 19) was statistically insufficient to detect an effect of this magnitude.

Replication analysis with newly collected data. We have since collected 12 more participants for the Consec/High-Rew group and 13 more participants for the Consec/Low-Rew group. Before testing results for the pooled larger dataset, we first ran a replication test using just this newly collected data. We found a positive relationship with rs-FC change for brooding change ($r = 0.40$, $p_{FDR} = 0.08$, 95% CI [−0.01, 0.69]), although this only trended towards significance here. We found a non-significant negative relationship between anxiety change and rs-FC change ($r = -0.17$, $p_{FDR} = 0.79$, 95% CI [−0.53, 0.24]). Confidence intervals for these correlations overlap with those shown above for previously reported data. Furthermore, there were no significant differences between old and new correlation coefficients for the relationships between rs-FC change and brooding change ($z = 0.13$, $p = 0.90$), or between rs-FC change and anxiety change ($z = 0.91$, $p = 0.36$). We therefore considered the previously reported effects to be replicated in the newly collected data [39].

Pooled data. When we pooled the old and new data (total N = 44), we found a significant correlation between rs-FC change and brooding change ($r = 0.42$, $p_{FDR} = 0.004$; 95% CI [0.13, 0.64]), but not between rs-FC change and anxiety change ($r = -0.03$, $p_{FDR} = 0.57$; 95% CI [−0.32, 0.27]). Note that, we used trait anxiety here because its measurement has a comparable timeframe to the BDI and RRS, but similar results were found with state anxiety as well (no significant correlation between rs-FC change and state anxiety change; $r = 0.21$, $p_{FDR} = 0.17$; 95% CI [−0.09, 0.48]). Demonstrating the specificity achieved when targeting the DLPFC/PCC functional connection, we found a significant difference between coefficients from brooding change/rs-FC change and anxiety change/rs-FC change correlations ($z = 2.12$, $p = 0.017$) (see Fig. 2b). A post-hoc power analysis, run with G*Power 3.1.9.4 [38], revealed a power of 0.79, suggesting that the sample size (N = 44) was sufficient to detect an effect of this magnitude with high probability.

Second-level F-Tests were used to examine pre-to-post changes in ROI-to-ROI Fisher-transformed correlations between the seven

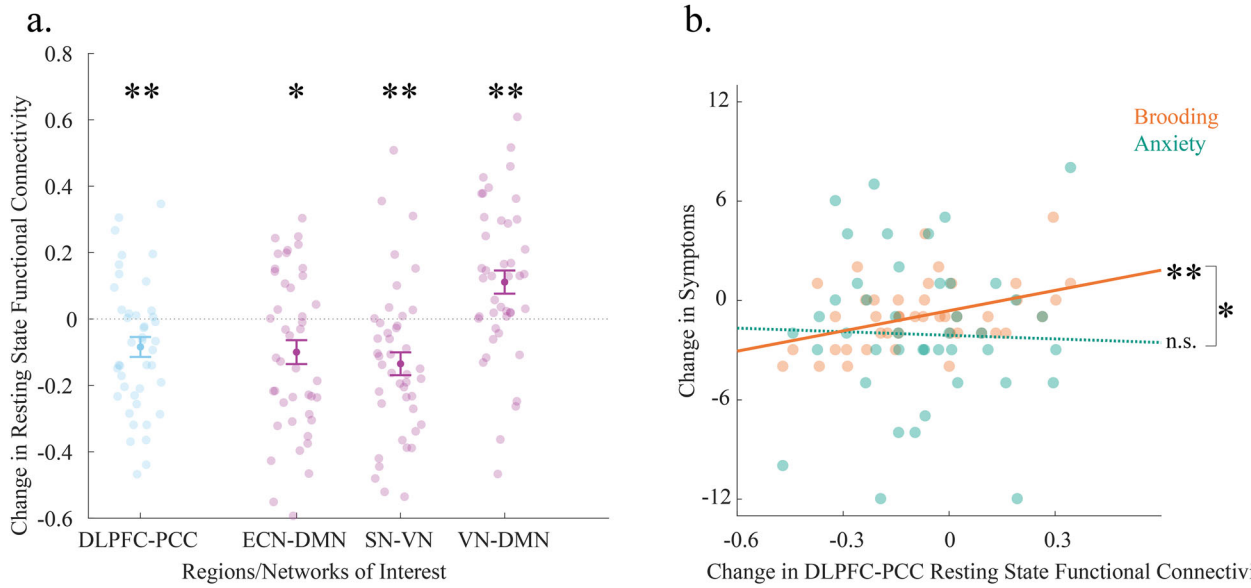


Fig. 2 ROI-level changes show precision to changes in brooding rumination symptoms. a Network-level functional connectivity changes driven by FCNef. Coefficients from pre-FCNef were subtracted from those from post-FCNef to display changes in functional connectivity. Paired-sample t-tests revealed that, as hypothesized, functional connectivity between the Executive Control (ECN) and Default Mode (DMN) networks became significantly more negative with FCNef. The same was seen for functional connectivity between Salience (SN) and Visual (VN) networks, whereas functional connectivity between the Visual and Default Mode networks became significantly more positive. For comparative purposes, changes in functional connectivity between the targeted regions (DLPFC-PCC) is also shown here, although this same data, but split by reward condition, is presented again in Fig. 4. ** = $p_{FDR} < 0.01$, * = $p_{FDR} < 0.05$ (**b**) Changes in DLPFC-PCC resting state functional connectivity from before- to after-FCNef correlated significantly with changes in brooding but not anxiety symptoms. The significant difference found highlights the precision of FCNef targeting this functional connection. Note that this same data, but split by reward condition, is presented again in Fig. 5a and b. ** = $p_{FDR} < 0.01$, * = $p < 0.05$, n.s. = not significant. Error bars reflect standard error of the mean.

Yeo Networks [33]. Changes in three connections were found to survive corrections for multiple comparisons (see Fig. 2a). These were between the following networks: Executive Control- Default Mode: $t(1,43) = -2.86$, $p_{FDR} = 0.039$; Salience - Visual: $t(1,43) = -3.87$, $p_{FDR} = 0.002$; Visual - Default Mode: $t(1,43) = 3.15$, $p_{FDR} = 0.009$. No significant correlations were found between these changes in network connectivity and changes in symptoms. Note that the 'FrontoParietal network' of Yeo is equivalent to the 'Executive Control network'; We refer to this as the 'Executive Control network' in this paper for consistency with our previous work [9].

Parameter investigations

FCNef scores. In the total dataset (N = 68), while comparing groups, we first examined whether mean FCNef scores increased significantly across training days, because this would indicate that participants successfully modified the target functional connection in the trained direction. Additionally, we assessed whether variance in FCNef scores decreased significantly across training days, because this would indicate that participants had gained better control of the DLPFC-PCC functional connection. As can be seen in Fig. 3, over time mean daily FCNef scores only reliably improved, and daily FCNef score variation only reliably reduced, for the Consec/High Rew group. See Supplementary Results and Supplementary Tables 8 and 9 for detailed LME analysis.

Self-report symptom scores. We did not include symptom changes from before to after FCNef and into the long-term in our operationalisation of FCNef success. This was because symptom reduction could occur for multiple reasons, including the Hawthorne effect. Symptom reduction would be meaningful here only if specifically related to changes in the targeted brain activity (examined further down). Nonetheless, we analysed symptom questionnaire scores (shown on Supplementary Table 10) and compared these between the three groups to ensure they had not worsened over the course of the study. Based on previous results [9], we expected overall symptoms to improve. Consistent with this, results showed that all types of symptoms reduced from before- to after- FCNef (Supplementary Tables 11–13) and that these all remained reduced in the long-run (i.e. did not subsequently change over the post-days; Supplementary Tables 14–16). There were no significant effects indicating that symptoms changed differently for different groups.

Resting-state functional connectivity. If FCNef is successful, then we predict that participants' rs-FC should become more negative with FCNef, which was the trained direction, and that it should remain so across long-term tests. Therefore, we used separate LME models to examine rs-FC initial and long-term effects for the three groups of participants.

rs-FC: The initial effect: Note that we measured rs-FC on all days (0–4) of the main experiment. However, we only included data from days 0 and 4 in LME models to examine the initial effect. This is because rs-FC on days 1–3 may be subject to homeostatic and/or compensatory mechanisms, causing overshoots [9] or rebounds [40] in brain activity. Average rs-FC for each group for each day of experimentation can be seen in Supplementary Table 17. Likelihood ratio testing showed that models to predict rs-FC from days 0 and 4 did not differ significantly. Therefore, the random-intercept only model can be considered as best-fit, because it is simplest (see Table 2 and Supplementary Table 18). Nonetheless, Fig. 4 clearly shows that rs-FC on day 4 was more negative relative to baseline for the Consec/High-Rew group, but not for the Non-Consec/Low-Rew group. rs-FC changes from day 0 to day 4 for the Consec/Low-Rew group lay in between those of the other 2 groups (see Fig. 4). Evidence for these between-group differences may be found at the LME model level if this experiment is run with greater sample sizes; This is suggested by the fact that the

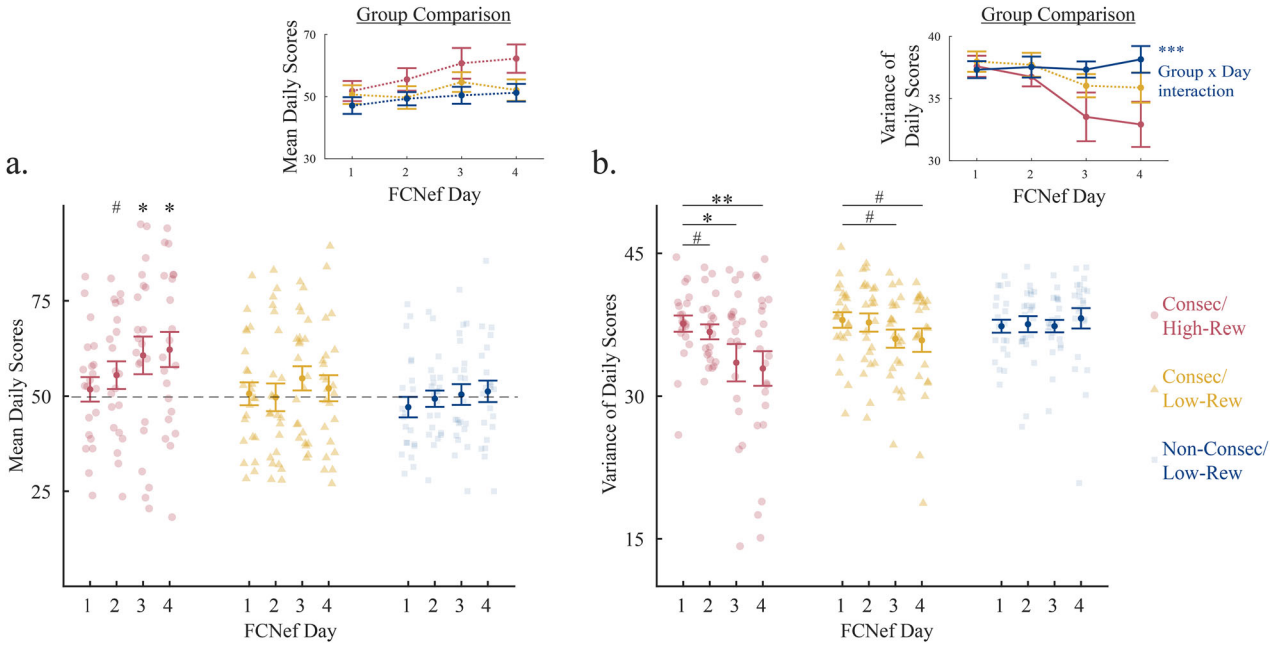


Fig. 3 Mean and standard deviation of FCNef scores over days of FCNef. a Mean daily FCNef scores. Exploratory t-tests, for each group separately, were conducted to examine whether scores on each FCNef day were higher than a baseline score of 50 (which was the average score given during SHAM on Day 0). Scores were found to significantly improve over days of FCNef only for the Consec/High-Rew group. The Group Comparison inset shows the mean ± standard error of the mean daily scores overlaid for the different groups. **b** Variance (standard deviation) of daily FCNef scores. Exploratory t-tests, for each group separately, were conducted to examine whether variance in scores on each subsequent day of FCNef was lower than that on the Day 1 of FCNef. Score variance was found to significantly reduce over days of FCNef only for the Consec/High-Rew group, but a similar trend was found for the Consec/Low-Rew group. The Group Comparison inset shows the mean ± standard error of the STD daily scores overlaid for the different groups; this highlights the Day x Group interaction found in the best-fit model for the Non-Consec/Low-Rew group relative to the reference Consec/High-Rew group (see Supplementary Results).). ***represents $p_{FDR} = 0.001$, **represents $p_{FDR} < 0.01$, *represents $p_{FDR} < 0.05$, # represents $p_{FDR} < 0.1$. Error bars reflect standard error of the mean.

interaction model trended toward being better than the other two models, despite this not reaching full significance ($\chi^2(2) = 4.69$, $p = 0.096$ when compared to the no interaction model; $\chi^2(5) = 9.70$, $p = 0.084$ when compared to the random-intercept only model). The interaction model had a significant first/last FCNef day x Non-Consec/Low-Rew group interaction ($p = 0.036$) which indicates that the trajectory for rs-FC from day 0 to day 4 differed for the Non-Consec/Low-Rew group compared to the reference Consec/Low-Rew group (see Supplementary Table 19 and for visualisation of this see the Group Comparison inset in Fig. 4).

rs-FC: Changes in the long-term: We next examined whether any changes in DLPFC-PCC rs-FC from before-to after- FCNef subsequently remained stable in the long-term. The no interaction model was best-fit (see Table 2 and Supplementary Table 20). This showed no significant main effect of post-day. This indicates that changes that occurred with FCNef subsequently remained stable across post-days. A significant main effect of the Non-Consec/Low-Rew group was found ($p = 0.007$), indicating that post-changes in rs-FC differed for the Non-Consec/Low-Rew group and the reference Consec/High-Rew group. Specifically, post-changes were more negative, which was the trained direction, for the Consec/High-Rew group versus the Non-Consec/Low-Rew group (see Fig. 4). Post-changes for the Consec/Low-Rew group lay between those of the other two groups (see Fig. 4).

Relationship between changes in self-report symptom scores and changes in rs-FC. Finally, in the three groups of participants, we examined how changes in participant DLPFC-PCC rs-FC from before to after FCNef related to changes in general depressive, brooding, and anxiety symptoms. Here, changes were defined as day 0 data subtracted from day 4 data. If the FCNef paradigm was successful, then we would expect changes in the DLPFC-PCC rs-FC to be positively related to changes in general depressive symptoms. This would mean that the more negative the rs-FC became, the more depressive symptoms decreased. If, as previously hypothesised [9], the targeted functional connection (FC) is specifically related to maladaptive symptoms of rumination, then we would expect changes in the DLPFC-PCC rs-FC to be positively related to changes in brooding symptoms, but not to changes in anxiety symptoms (which we used as a control). Importantly, by including Group as a factor in the models, we examined whether these effects were impacted by manipulated parameters.

Relationships between symptom changes and rs-FC changes: The best-fit model to explain changes in general depressive scores (see Table 2 and Supplementary Table 21) had a significant interaction between rs-FC change and Non-Consec/Low-Rew group ($p < 0.0001$). This interaction indicates that the relationship between changes in general depressive scores and rs-FC change differed for this group and the reference Consec/High-Rew group. A trend for a similar interaction was seen for the Consec/Low-Rew group ($p = 0.061$). Follow-up correlations revealed a significant positive correlation between general depression change and rs-FC change for the Consec/High-Rew group ($r = 0.66$, $p_{FDR} = 0.002$), a positive, but non-significant relationship for the Consec/Low-Rew group ($r = 0.30$, $p_{FDR} = 0.13$), and a negative, non-significant relationship for the Non-Consec/Low-Rew group ($r = -0.41$, $p_{FDR} = 0.98$).

The best-fit model to explain changes in brooding scores (see Table 2 and Supplementary Table 22) showed no significant main effects. However, hypothesis-driven follow-up correlations revealed a significant positive correlation between brooding change and rs-FC change for the Consec/High-Rew group ($r = 0.63$, $p_{FDR} = 0.003$), but nothing for the other two groups ($r = 0.18$, $p_{FDR} = 0.31$ for the Consec/Low-Rew group; $r = 0.05$, $p_{FDR} = 0.40$ for the Non-Consec/Low-Rew group).

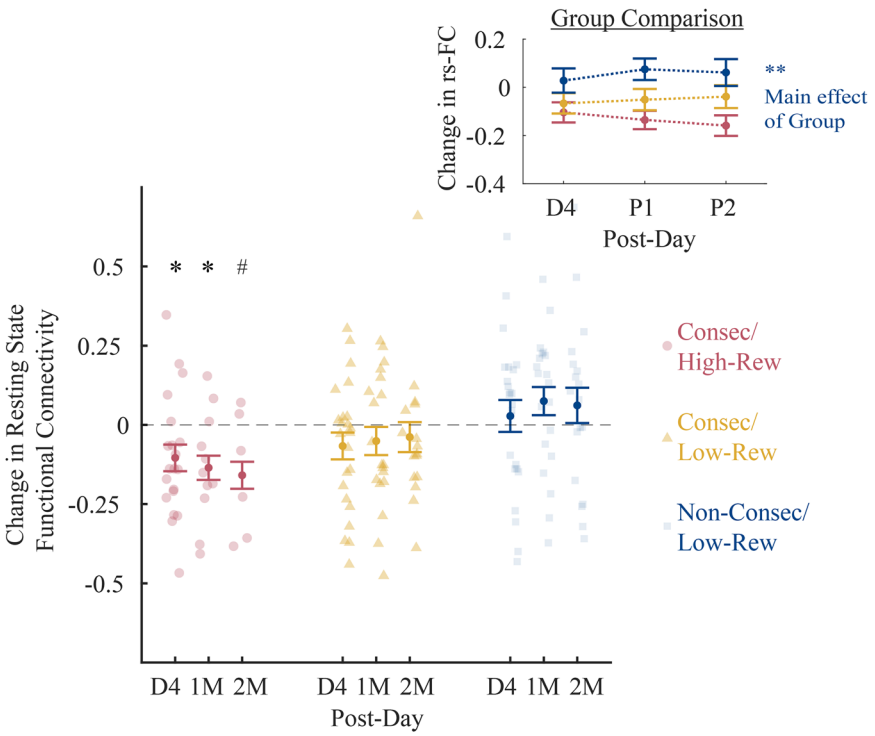


Fig. 4 Long-term changes in rs-FC. For descriptive purposes, here the coefficients from pre-FCNef were subtracted from those from post-FCNef to display changes in functional connectivity. Exploratory t-tests revealed that for the Consec/High-Rew group, overall DLPFC-PCC resting state functional connectivity (rs-FC) was significantly more negative than baseline immediately after FCNef had been completed (D4) and one-month later (M1), and that it trended towards this even two-months later (M2). Nothing of significance was found for either of the other groups. Note that the different bars on each subplot represent different sample sizes because not all participants were invited/ able to come back for long-term testing (see Table 1). The Group Comparison inset shows the mean ± standard error of the change in rs-FC, over different post-days, overlaid for the different groups. Changes = data from each post-day minus data from Day 0; D = Day, M = Month, *represents $p_{FDR} < 0.05$, # represents $p_{FDR} = 0.051$. Error bars reflect standard error of the mean.

The best-fit model to explain changes in anxiety scores (see Table 2 and Supplementary Table 23) also showed no significant main effects. Follow-up correlations also showed nothing of significance across groups ($r = 0.05$, $p_{FDR} = 0.63$ for the Consec/High-Rew group; $r = -0.08$, $p_{FDR} = 0.65$ for the Consec/Low-Rew group; $r = 0.09$, $p_{FDR} = 0.63$ for the Non-Consec/Low-Rew group).

The relationship between symptom changes and rs-FC changes for all groups can be seen in Fig. 5. Data of one participant whose general depression change was more than 3 STD lower than the group general depression change mean was excluded from the general depression change analysis (and Fig. 5a). Data of another participant whose brooding change was more than 3 STD lower than the group mean was excluded from the brooding change analysis (and Fig. 5b). Note that inclusion of these outliers did not change the results.

Evidence for specificity of the targeted functional connection: We specifically hypothesised that changes in DLPFC-PCC rs-FC from before to after FCNef should relate to changes in brooding, but not anxiety symptoms. Z-tests to compare correlation coefficients [37], run with one tail due to our directional hypothesis, demonstrated a significant difference for the Consec/High-Rew group ($z = 2.08$, $p = 0.019$), but not for the other groups ($z = 0.85$, $p = 0.20$ for the Consec/Low-Rew group; $z = 0.11$, $p = 0.46$ for the Non-Consec/Low-Rew group).

DISCUSSION

Overall, we replicated previous results showing that the more participants' DLPFC-PCC rs-FCs normalised over consecutive days of Functional Connectivity Neurofeedback (FCNef), the greater their corresponding decrease in brooding rumination. No such correlation was shown for changes in DLPFC-PCC rs-FC and anxiety, which is thought to relate to different underlying neural circuitry [41, 42]. Combining newly collected data with the previously reported data provided us with sufficient statistical power for direct comparison of these correlation coefficients and a significant difference was found. We also examined functional connectivity changes at the network level. Functional connectivity between the Executive Control and Default Mode networks, to which our targeted regions belong, became significantly more negative with FCNef. Finally, when we compared data from three groups of participants (see Table 3), we found the most promising results for the group run with a higher-reward schedule to reinforce the targeted shift in functional connectivity, and an experimental schedule with consecutive days. Weaker results were found for the group of participants that completed FCNef over consecutive days, but who were reinforced with a lower reward schedule. FCNef did not seem to have any effect on the group of participants who attended over non-consecutive days and that were reinforced with a lower reward schedule.

FCNef for precision medicine

The current results strengthen our previous finding that when FCNef is run over consecutive days, normalisation of a target functional connection relates to a specific reduction in only related symptoms (see Fig. 2). This replication of previous results with an independent sample of newly collected data shows their robustness and indicates that previous results were unlikely to have been spurious [9]. Furthermore, the novel finding that changes in the targeted rs-FC correlated significantly more with changes in related than unrelated symptoms highlights the precision of the FCNef

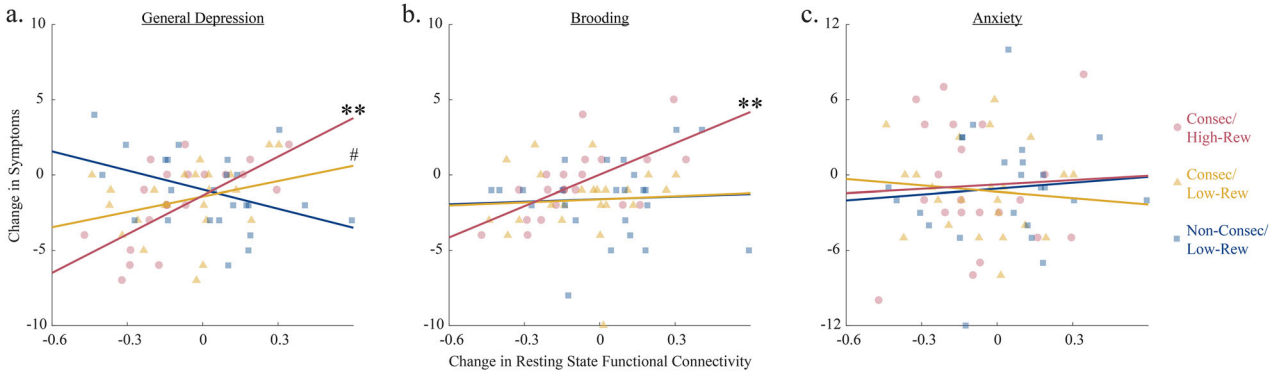


Fig. 5 How changes in rs-FC relate to changes in symptomatology. Changes in DLPFC-PCC resting state functional connectivity (rs-FC) are shown plotted against (**a**) changes in General Depression Scores, (**b**) changes in Brooding scores, and (**c**) changes in Anxiety scores. Overall, promisingly for precision medicine, the Consec/High-Rew group showed significant positive correlations between changes in rs-FC and changes in related (General Depression and Brooding) but not unrelated (Anxiety) symptoms. These effects were in the same direction (reaching a trend with the uncorrected p-value for the General Depression change correlation) for the Consec/Low-Rew group. On the contrary, promising results were not found for the Non-Consec/Low-Rew group, for whom the relationship between rs-FC changes and General Depression score changes was actually numerically in the opposite direction to expectation. **represents $p_{FDR} < 0.01$, # represents $p_{UNCORR} = 0.085$.

Table 3. Effect sizes summarised.

Expected Effects	Consec/ High-Rew	Consec/ Low-Rew	Non-Consec/ Low-Rew
Increase in mean FCNef score	*d* = -0.58	*d* = -0.09	*d* = -0.31
Decrease in FCNef score STD	*d* = 0.72	*d* = 0.42	*d* = -0.19
Normalisation of rs-FC	*d* = 0.47	*d* = 0.30	*d* = -0.11
Positive relationship between BDI changes and rs-FC changes	*r* = 0.66	*r* = 0.30	*r* = -0.41
Positive relationship between Brooding changes and rs-FC changes	*r* = 0.62	*r* = 0.21	*r* = 0.05

Large	Medium	Small	Trivial	Trivial	Small	Medium	Large

Hypothesised direction ⟷ Non-hypothesised direction

"Expected effects" are effects that we would expect to see if FCNef training was successful. Comparisons for "Increase in mean FCNef scores" and "Decrease in FCNef score STD" were t-tests comparing data from the first and the last day of FCNef (Days 1 and 4). Comparisons for "Normalisation of rs-FC" were t-tests comparing rs-FC from before to after FCNef (Days 0 and 4). The two "Positive relationship…" comparisons were correlations calculated between changes in rs-FC and changes in symptoms, where "changes" were defined as Day 4 - Day 0 data. Effect sizes for each comparison are shown in the relevant cell. These are shown here as Cohen's d (d) for t-tests and Pearson's correlation coefficient (r) for correlations. Following convention, these can be described on a scale ranging from trivial ($|d| < 0.2$ or $|r| < 0.1$), small ($0.2 \leq |d| < 0.5$ or $0.1 \leq |r| < 0.3$), medium ($0.5 \leq |d| < 0.8$ or $0.3 \leq |r| < 0.5$), to large ($|d| \geq 0.8$ or $|r| \geq 0.5$). Effects in the hypothesised direction are shown in shades of blue and effects in the non-hypothesised direction are shown in shades of orange. The larger the effect size, the stronger the shade of the colour in the relevant cell.

technique. This brings us one step closer to a future in which patients may one day enter a clinic, have their brains scanned, and then have targeted treatment to normalise specific neural aberrations related to their own subset of symptoms.

The DLPFC-PCC functional connection and rumination

Not only do our results have implications for precision medicine in general, but also specifically for understanding and treating brooding rumination symptoms. We previously hypothesised, based on both data- and hypothesis-driven evidence from past studies, that the DLPFC-PCC functional connection is likely to relate to brooding rumination [9]. Our initial study found preliminary support consistent with this proposal. Specifically, we found that changes in connectivity between these regions relate to changes in brooding rumination [9]. There has since been at least one other report that also showed increased connectivity

between the PCC and PFC related to ruminative symptoms [43]. We here find further support that brooding rumination relates to the DLPFC-PCC functional connection, by showing that the correlation between changes in these is significantly stronger than the correlation between changes in the same functional connection and a type of symptom thought to be unrelated (anxiety). Evidence from previous studies using repetitive transcranial magnetic stimulation to target the DLPFC [44] and using real-time neurofeedback to target the PCC [45], has also shown promising results for amelioration of depressive and brooding rumination symptoms. This could be because targeting these regions affects the functional connection between them, which if true, could mean that targeting the functional connection itself more directly (as we did here) could be of even further advantage.

FCNef effects extend to the network-level

Although an anticorrelation is typically found between DLPFC/PCC activity in healthy controls, this is reduced (closer to zero) in people with depressive symptoms [46]. We previously proposed that this anticorrelation might move closer to zero because rumination involves extended periods of time spent in a DMN-active state, which would result in fewer fluctuations between reciprocally inhibitory DMN- and ECN-active states [9]. FCNef may restore the balance between DMN- and ECN- active state switching, and thereby restore the anticorrelation between these networks and our targeted regions of interest. Here, consistent with this, we show that the functional connectivity between these networks does become significantly more negative with FCNef. Whether or not this is born as a product of changes in state switching remains to be determined. Interestingly, we also found significant changes in connectivity between the Salience and Visual networks, and between the Visual and Default Mode networks. However, none of these significant changes in functional connectivity at the network level correlated significantly with changes in symptoms from before- to after- FCNef. This may indicate that, although network-level changes are detectable, they are less precise than the changes to the specifically targeted regions of interest. Overall, while many questions remain to be answered, this result fits nicely with previous studies that have also shown FCNef effects to extend to broader brain networks than just the targeted functional connection [7, 8].

Parameter testing

The current report extends our previous data by clarifying some of the parameters under which FCNef for depression can best be achieved. These results should help guide the design of future neurofeedback and other BMI studies. When selecting parameters for neurofeedback, past studies have tended to follow convention or have gone with what seemed best in terms of cost/benefit trade-offs and/or in terms of making things easy for participants. Often, this is the only feasible way to design a BMI study because the cost of testing all possible parameters is enormous. However, our current results show that certain parameters can make a difference in BMI effectiveness. This means that without knowing the optimal parameters for a given BMI design, researchers may be finding null results simply because they are not running their designs in the most effective way. There is no simple solution to this problem, especially because optimal parameters may differ for different populations, target neural activities, experimental goals, etc. Nonetheless, the current results provide initial evidence that can be used to help future designs. Below, we discuss specific results for these parameter analyses, as well as their implications.

Reward schedule. Participants run with the high-reward schedule had better FCNef success than those run with the low-reward schedule (see Table 3 and Figs. 3, 4, and 5). These results support the proposal that external reward might work as reinforcement that is additional to that provided by feedback scores during neurofeedback [47]. However, this cannot be fully concluded with the current data because we were unable to test a group using high reward with a non-consecutive experimental schedule. Including a non-consecutive/high-reward condition in future work would directly test whether higher reward can compensate for a non-consecutive training schedule. Furthermore, because BMIs generally do not use external rewards for reinforcement, this might be worthy of consideration beyond the realm of neurofeedback. Whether greater success occurs as a direct result of the magnitude of the reward, the reward's contingency on performance, or both, should be more thoroughly examined and considered before such future applications are implemented.

Of course, disturbances to reward circuitry and disturbances in reward processing (usually reductions) are commonly reported in depressive and other psychiatric disorders [48–55]. This means that the effect of external reward on reinforcement of the target neural activity might be diminished when neurofeedback is conducted in patients with such disorders. Here, our results with subclinical patients did not corroborate this, but it remains worthy of further investigation in clinically depressed patients.

Experimental schedule. FCNef appears to be more effective when participants come for consecutive, as opposed to non-consecutive, days of FCNef. All expected effects were strongest in the consecutive condition with high reward, in the same direction (albeit with less strength) in the consecutive condition with low reward, and weak or in the opposite direction for the non-consecutive condition (see Table 3). While these results are not fully conclusive (as mentioned above, we cannot rule out the possibility that high reward can compensate for a non-consecutive experimental schedule), they do not appear promising for using non-consecutive days of FCNef. They indicate that, although possibly more tiring for participants, consecutive days of FCNef may be necessary to achieve positive outcomes. It is possible that consecutive days of reinforcement are needed to drive learning effectively and/or that more non-controllable confounding personal circumstances can occur between non-consecutive days of training (an idea that researchers designing future BMIs and clinical treatments ought to consider).

Another point to consider is the possibility that neural plasticity related to learning might cause dynamic rs-FC fluctuations in strength and direction that occur before settling into a new pattern (similar to the rebound effect documented by Kluetsch et al. [40]). If so, then our analyses may not have fairly tested consecutive versus non-consecutive conditions. Learning could begin from Day 1; but, post-FCNef measurements (from Day 4, and 1- and 2- months later) differed in the number of days after Day 1 for consecutive/non-consecutive conditions (and even for different participants in the non-consecutive condition). This may mean that measurements were taken at different points during ongoing dynamic rs-FC fluctuations for these different conditions. If so then comparisons between these conditions may actually have compared different snapshots of learning effects. Other researchers using neurofeedback over non-consecutive days should also consider this possibility.

Limitations of the current design

One limitation of our study is that it involved participants who had only subclinical levels of depression. Nonetheless, examining these effects with subclinical participants is important when FCNef is considered as holding potential for early intervention. Furthermore, preliminary studies using this FCNef technique with patients diagnosed with MDD have shown promise [12, 20]. Results with clinical patients may be improved if the right parameters are employed. The most effective parameter that we found was high(er) monetary reward. We used money because we wanted to test the effects of reward schedule with a type of

reward that is well known to strongly activate the human reward system. However, now that proof-of-concept has been provided, this idea should be further tested more creatively with other types of reward that might be more appropriate for a clinical setting. For example, revealing consecutive puzzle pieces for each successful trial of neurofeedback (see Ramot et al. [8]).

A second limitation of our study is the absence of a control group or a within-subject control condition. Therefore, it is possible that our target rs-FC changed and that symptoms improved for reasons such as the placebo or Hawthorne effects. However, only changes in relevant (depressive and brooding rumination, but not anxiety) symptoms changed in parallel to the targeted FC, which would be unlikely to occur merely from such nonspecific effects. A third limitation of our study is the incomplete factorial design due to practical constraints, as discussed in the Experimental Conditions section of the Methods. While this limits our ability to examine potential interaction effects between the experimental schedule and reward schedule parameters, the current design still allowed us to investigate main effects of these parameters separately, which was our primary aim. Future research could include all factorial combinations to provide a comprehensive understanding of parameter interactions.

Future directions

Although different symptoms are likely to arise from aberrations in wider brain networks involving multiple FCs, our current FCNef approach can directly target only one FC. In that sense, connectome-based FCNef [56] or neurofeedback targeting an estimation of the dynamic weighted linear sum of FCs might be more effective. Some promise for such types of neurofeedback has been found, including when targeting an estimation of the dynamic weighted linear sum of FCs from the greater biomarker from which we identified the DLPFC-PCC FC [46, 57]. However, the authors have also reported increased difficulty for participants with regard to the credit assignment (they report that it is difficult to target multiple functional connections and to know what actually worked) and overall experimental interpretability [T. Ogawa, personal communication, 27th January, 2025]. Furthermore, consistent with past results [7, 8], we found FCNef to affect broader brain networks than just the targeted functional connection anyway, so it remains possible that our simple FCNef approach might be efficient at ameliorating symptoms without the need for added complexity and burden for patients. Future studies should attempt to directly compare effectiveness of these types of neurofeedback.

Our current results are promising for precision medicine, but they were shown with functional connectivity in fMRI, which can be costly and impractical (but not impossible) for real clinical treatment. In the future, we expect FCNef to evolve further so that it may be conducted using electroencephalogram (EEG) signatures (see Keynan et al. [58]) of target FCs or so that it may be conducted using EEG signatures of weighted linear sums of multiple FCs. If successful, then this would allow neurofeedback targeting functional connections to eventually be conducted with portable EEG headsets, possibly even away from the clinic in the privacy of the patient's own home (for more detailed discussion see Taylor et al. [59]).

Conclusion

Overall, we replicated and extended previous results to show that normalisation of the targeted neural network (DLPFC-PCC) correlated significantly more with reductions in symptoms thought to relate to this neural circuitry (brooding rumination) than to changes in symptoms thought to relate to different neural circuitry (anxiety). This highlights the precision of the FCNef technique and brings us one step closer to a future where psychiatric treatment might be tailored to the individual patient. We show, for the first time, that these results extend beyond the targeted brain regions, to the greater networks to which these belong. Finally, we extended our previous work by investigating parameters under which our FCNef for depression paradigm is most effective. We found that FCNef effectiveness changes depending on those parameters with which it was run. Specifics and implications of some parameter-related results may be relevant beyond neurofeedback to BMIs in general. Furthermore, some of our results highlight benefits of testing conventional parameters. Overall, these results should be informative for design of future BMI testing and for inspiring new interpretations of existing data. For example, previously found null results should be considered in the context of the parameters under which the BMI was run. More broadly, by documenting how parameter optimisation can increase beneficial outcomes and reduce patient burden, we hope to inspire more of this in the future, with the ultimate goal of bringing optimised BMIs to the medical clinic.

CODE AVAILABILITY

Data and code supporting this study's findings will be publicly available on our GitHub at publication.

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ACKNOWLEDGEMENTS

We thank Kaori Nakamura for help in scheduling and conducting the experiments, Rumi Yorizawa for help in screening participants, and Toshinori Yoshioka for continued support with regard to experimental scripts. Furthermore, we thank the team from the ATR Brain Activity Imaging Center (BAIC), in particular Akikazu Nishikido and Ichiro Fujimoto, for their technical support. Last, but not least, we thank Talma Hendler and Guy Gurevitch for their valuable comments on this work.

AUTHOR CONTRIBUTIONS

JET: Conceptualization, experimental design and methodology, data acquisition, formal analysis, visualization, project administration, original manuscript preparation, reviewing and editing of manuscript. TO: Data acquisition, visualization, original manuscript preparation, reviewing and editing of manuscript. MM: Data acquisition, visualization, reviewing and editing of manuscript. TMo: Conceptualization, data acquisition. TY: Conceptualization, experimental design and methodology, data acquisition, formal analysis, reviewing and editing of manuscript. TK: Resources, methodology, reviewing and editing of manuscript. YK: Resources, methodology, reviewing and editing of manuscript. YY: Resources, methodology, reviewing and editing of manuscript. JM: Resources, methodology, reviewing and editing of manuscript. TMu: Resources, methodology, reviewing and editing of manuscript. MK: Resources, conceptualization, funding acquisition, experimental design and methodology, project administration, reviewing and editing of manuscript. AC: Funding acquisition, methodology, visualization, project administration, original manuscript preparation, reviewing and editing of manuscript.

FUNDING

This research was supported by the Japan Agency for Medical Research and Development (AMED) under Grant Numbers JP24wm0625502, JP24wm0625204, JP18dm0307008 and JP17dm0107044, by the Japan Society for the Promotion of

Science (JSPS) KAKENHI under Grant Number 24K22822, and by the Innovative Science and Technology Initiative under Security Grant Number JPJ004596, ATLA, Japan.

COMPETING INTERESTS

MK is an inventor of patents related to functional connectivity neurofeedback. The original assignee of the patents is ATR, with which the authors are affiliated. We have no other conflicts of interest.

ETHICS

Participants all provided written informed consent on each day of screening and experimentation prior to commencement. This research was approved by the Ethics Committee of the Review Board of Advanced Telecommunications Research Institute International, Japan (Ethics No. 132, 172) and by the Kyoto University Certified Review Board (YC0849) and the Committee on Medical Ethics of Kyoto University (C0849). All experiments were performed in accordance with the guidelines and regulations of these Ethics Committees. This research was conducted in association with research registered with the Japan Registry of Clinical Trials (jRCTs052180169) and the University Hospital Medical Information Network Clinical Trials Registry (UMIN000015249).

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41398-026-04040-3.

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