Escitalopram modulates learning content-specific neuroplasticity of functional brain networks

Manfred Klöbl, René Seiger, Thomas Vanicek, Patricia Handschuh, Murray Bruce Reed, Benjamin Spurny-Dworak, Vera Ritter, Godber Mathis Godbersen, Gregor Gryglewski, Christoph Kraus, Andreas Hahn, Rupert Lanzenberger*

Department of Psychiatry and Psychotherapy, Medical University of Vienna, Spitalgasse 23, Vienna 1090, Austria

A R T I C L E   I N F O

Keywords:
Neuroplasticity
Learning
Selective serotonin reuptake inhibitor
Effective connectivity
Permissive casualty

A B S T R A C T

Learning-induced neuroplastic changes, further modulated by content and setting, are mirrored in brain functional connectivity (FC). In animal models, selective serotonin reuptake inhibitors (SSRIs) have been shown to facilitate neuroplasticity. This is especially prominent during emotional relearning, such as fear extinction, which may translate to clinical improvements in patients. To investigate a comparable modulation of neuroplasticity in humans, 99 healthy subjects underwent three weeks of emotional (matching faces) or non-emotional learning (matching Chinese characters to unrelated German nouns). Shuffled pairings of the original content were subsequently relearned for the same time. During relearning, subjects received either a daily dose of the SSRI escitalopram or placebo. Resting-state functional magnetic resonance imaging was performed before and after the (re-)learning phases. FC changes in a network comprising Broca’s area, the medial prefrontal cortex, the right inferior temporal and left lingual gyrus were modulated by escitalopram intake. More specifically, it increased the bidirectional connectivity between medial prefrontal cortex and lingual gyrus for non-emotional and the connectivity from medial prefrontal cortex to Broca’s area for emotional relearning. The context dependence of these effects together with behavioral correlations supports the assumption that SSRIs in clinical practice improve neuroplasticity rather than psychiatric symptoms per se. Beyond expanding the complexities of learning, these findings emphasize the influence of external factors on human neuroplasticity.

1. Introduction

Learning constitutes an evolutionary indispensable process allowing for adjustment to an ever-changing environment. It is accompanied by adaptations in structure and function of the brain, reflected in changes of gray and white matter morphology (Taubert et al., 2012; Valkanova et al., 2014), structural (Sampaio-Baptista and Johansen-Berg, 2017) and functional connectivity (FC) (Guerra-Carrillo et al., 2014). The ability to learn, and thus, neuroplasticity per se, is modulated by the individual’s mental condition (Ehlers, 2012; Taylor Tavares et al., 2008) and the information acquired (Delon-Martin et al., 2013; Draganski et al., 2004; Draganski et al., 2006; Hyde et al., 2009; Maguire et al., 2000). The former is prominently affected in neurological (Filoteo et al., 2007; Grober et al., 2019; Schraegle et al., 2016; Vicari et al., 2005) and psychiatric disorders (Hartmann-Riener et al., 2017; Marin et al., 2017; Taylor Tavares et al., 2008). A crucial mediating neurotransmitter in these processes is serotonin, which plays a major role in structural (re)modeling of the brain (Daubert and Condon, 2010; Gaspar et al., 2003) and consequently in the pathophysiology and treatment of many psychiatric conditions (e.g., major depression (Kraus et al., 2017; Spies et al., 2015), obsessive-compulsive disorder (Fineberg et al., 2012), generalized anxiety disorder (Goodman et al., 2005; Spindelegger et al., 2009)). Furthermore, pharmacological modulation of the serotonin system using selective serotonin reuptake inhibitors (SSRIs) was shown to counteract learning deficits in temporal lobe epilepsy (Barkas et al., 2012) but also interact with other neurotransmitter systems (Spurny et al., 2021). However, it was recently shown that there is a common mechanism of action for different antidepressant agents, not unique to SSRIs (Casarotto et al., 2021): By binding to the tyrosine receptor kinase B (TrkB) synaptic localization and, hence, activation via brain-derived neurotropic factor (BDNF) is facilitated. BDNF, in turn, is known as major regulator of synaptic plasticity (Kowiański et al., 2018) and to be influenced by antidepressant therapies (Kraus et al., 2017).

Beyond its involvement in learning processes, serotonin plays a role in extinction and relearning. This is well established in animals (Furr et al., 2012; Lapiz-Bluhm et al., 2009; Masaki et al., 2006) but...
much less so in humans. In contrast, a link between impaired fear extinction due to a BDNF polymorphism was indeed established between mice and humans (Soliman et al., 2010). Faster fear extinction was shown in healthy human subjects after a two-week treatment with the SSRI escitalopram compared to placebo (Bui et al., 2013). This effect is suspected to be linked to a positivity bias induced by SSRIs (Harmer and Cowen, 2013; Pringle et al., 2011). Related to altered neuroplasticity in major depression, patients showed deficits in memory consolidation and enhanced fear acquisition (Nissen et al., 2010), but also enhanced fear extinction of the eye-blink startle response as measure of increased amygdalar neuroplasticity (Kuhn et al., 2015). Acute single-dose SSRI application already enhanced the recognition of emotional faces (Browning et al., 2007; Harmer et al., 2003) and induced FC changes predictive of treatment response within hours (Klöbl et al., 2020a). Also the acute effects of SSRIs on connectivity depend on the individual mental condition (e.g., presence or absence of depression, Dutta et al. (2019)). Considering the minimum of one week that is needed for an antidepressant effect (Taylor et al., 2006), these acute findings suggest two things: First, SSRI-modulated neuroplasticity facilitates but not necessarily implies improvements in depressive symptoms (Alboni et al., 2017). Second, since most likely an accumulation of the drug in the brain is needed for the effect on TrkB and subsequently BDNF, there might be additional routes of effect (c.f., Casarotto et al. (2021)). Thus, serotonergic pharmacological agents can induce widespread and substantial alterations of the brain FC (Armone et al., 2018; Klaassens et al., 2015; Schaefer et al., 2014; Schrantee et al., 2018).

Since learning induces task-dependent functional network adaptations (Horga et al., 2015; Kang et al., 2018; Lefebvre et al., 2017; Woolley et al., 2015; Zhao et al., 2019), FC provides a convenient surrogate for neuroplasticity. Furthermore, variations in memory and extinction task outcomes in patients with major depression indicate behavioral correlates of altered plasticity (Kuhn et al., 2015; Nissen et al., 2010). However, several aspects remain unknown. These concern the interactions between learning content and setting (learning vs. relearning) as well as how the effect of SSRIs on emotional learning in humans (Bui et al., 2013) relates to neuroplastic changes.

In order to map these learning-dependent network adaptations under SSRI intake, neuroplastic changes in FC after learning and relearning were investigated. Since connectivity changes induced by learning depend on the exact task and such induced by SSRI intake were shown to be spread throughout the brain, global functional connectivity (GFC) was used as basic measure of connectedness. Seed-based correlation then allowed for identifying the origin (Tagliazucchi et al., 2016) and dynamic causal modelling for inferring the direction of the affected connections (Friston et al., 2016). Thus, the analysis was focused on uncovering novel rather than confirming known interactions. A dedicated learning paradigm and online training platform were specifically developed for this purpose, comprising an emotional (matching face pairs) and a non-emotional (matching Chinese characters to unrelated German nouns) condition. To assess the modulatory effects of an SSRI on neuroplastic changes in humans, participants received escitalopram or placebo during the relearning phase. Resulting from this design, functional network adaptations depending on the learning content and setting were expected with stronger effects of escitalopram on emotional compared to non-emotional relearning. These adaptations were further assumed to correlate with the individual learning behavior.

2. Materials and methods

The study was conducted according to the Declaration of Helsinki including all current revisions and the good scientific practice guidelines of the Medical University of Vienna. The protocol was approved by the institutional review board (EK Nr.: 1739/2016) and the study was registered at clinicaltrials.gov (NCT02753738).

2.1. Experimental design

The overall study followed a randomized, double-blind, placebo-controlled longitudinal design. Three MRI examinations with 21 days of (re-)learning between each session were conducted (i.e., the MRIs were performed on the 1st, 22nd and 43rd day). For a subsample, a test-retest scan was performed 21 days before the baseline, to mitigate the chance of misinterpreting time- as learning-related changes. The subjects were randomized upon recruitment to one of four groups learning to match either Chinese characters to random German nouns or faces to faces and subsequently relearn new associations while receiving placebo or escitalopram (Fig. 1A). During the latter phase, the previous associations (character-noun / face-face) were shuffled and had to be relearned following the same time schedule. The study medication consisting of a daily oral dose of 10 mg escitalopram (Cipralex; Lundbeck A/S, Copenhagen, Denmark; provided by the pharmacy of the Medical University of Vienna) or placebo. To monitor the proper intake, the escitalopram blood plasma levels were assessed around day 7, 14 and 21 of the re-learning phase.

2.2. Learning paradigm

Throughout the course of the study, the subjects had to perform an association-learning task with emotional (face pairs) or non-emotional (Chinese characters – German nouns) content. In both cases they had to learn 200 pairs of images via a daily online training at home (i.e., 21 learning and 21 relearning sessions). Each session contained a pseudorandom selection of 52 image pairs (i.e., the same sequence for all participants). These were presented sequentially for 5 s each. After the training, a pseudorandom selection of 52 images out of all previously seen had to be matched to the correct counterpart without time limit. No feedback was given to keep learning and retrieval strictly separated. All pseudorandomizations were conducted with replacement. The subjects were given personal credentials for the online learning platform and instructed to complete one session per day at approximately the same time. In case sessions were missed, they could be done on the next day. However, subjects were excluded in case of generally irregular learning. During each MRI session, learning and retrieval tasks similar to those on the online platform were performed in the scanner (Reed et al., 2021). In order to minimize additional influences on neuroplasticity, subjects were told not to travel during their participation in the study.

2.3. Participants

In total, 138 healthy volunteers were recruited using advertisements at message boards on the campus of the Medical University and General Hospital of Vienna as well as in libraries, pharmacies and local supermarkets (target sample size for subgroups was 20 subjects). Inclusion criteria comprised general health based on medical history, physical and psychiatric examination (structured clinical interview (SCID-I) for DSM-IV), being 18 to 55 years of age, right-handedness, not smoking and signing the informed consent form. Subjects were excluded in case of psychiatric or neurologic conditions (also in first-degree relatives), MRI contraindications and knowledge of Mandarin, Cantonese or Japanese, positive drug-urine tests, not complying with the study schedule, reported side effects possibly related to the study medication, technical issues and structural anomalies or upon their own request. The distributions of sex, participation in the test-retest session and highest finished level of education between groups were tested using Fisher’s exact (SPSS 25, IBM, Amonk, New York) and that of age with a Kruskal-Wallis test (MATLAB 2018b, Natick, Massachusetts, as all other statistics; all two-sided).
2.4. Statistical analysis – learning behavior

Since the subjects saw only parts of the overall learning content in each session, the respective training results followed a u-shaped curve (Spurny et al., 2020). To correct for this effect, the raw retrieval success was scaled by the relative number of pairs that had already been seen. Weighting the sessions in the modelling process compensated for an overestimation of the training results if two sessions were conducted temporally closer together and an underestimation if further apart (Eqs. (2) and (1)):

\[
\begin{align*}
W_{\text{time}}(s, d) &= \begin{cases} 
T_A(s, d) & \text{if } T_A(s, d) \leq 24[h] \\
\frac{24[h]}{T_A(s, d)} & \text{if } T_A(s, d) > 24[h]
\end{cases}
\end{align*}
\]  
(1)

with

\[
t_A(s, d) = t_s - t_d + (d - 2) \cdot 24[h]
\]  
(2)

Here, \(s\) denotes the current and \(d\) a previous session, \(T_A\) is the time difference between expected and actual learning time. Linear discounting was used to reduce the influence of earlier learning times. The total weight \(w\) for each session was calculated as dot product of the time and discount weights (3).

\[
w(s) = W_{\text{disc}}(s) \ast W_{\text{time}}(s)
\]  
(3)

with the discounting weight

\[
w_{\text{disc}}(s, d) = \frac{d}{\sqrt{s(s+1)}}
\]  
(4)

To keep the weighting \(W_{\text{time}}\) for a specific session causal, only previous learning times were taken into account, i.e., \(d < s\) in (1) to (4). Using MATLAB, an exponential (5) and a hyperbolic model (6) were fit for each learning phase per subject

\[
y_{\exp}(x(s)) = k \ast \left(1 - e^{-\frac{x(s)}{a}}\right)
\]  
(5)

\[
y_{\text{hyp}}(x(s)) = k \ast \frac{x(s) + p}{x(s) + p + r}
\]  
(6)

where \(x\) is the adjusted training success, \(k\) the “learning capacity” (theoretical maximum of pairs that can be memorized), \(r\) the “learning rate” (determining how fast \(k\) is approached, lower values meaning steeper increase) and \(p\) the “previous knowledge” from the varying in-scanner session (Anzanello and Fogliatto, 2011). Due to equal complexity, the models were compared by a paired t-test over the Fisher-transformed model fits \(R^2_{\exp} = 88.13\%, \ R^2_{\text{hyp}} = 87.53\%, t_{164} = 3.97, p = 1.1E-4\) two-sided; the exponential model was preferred. The integral of the fitted learning curve (7) from day 1 to 21 was used to calculate the overall “performance” \(Y\) (adjusted fraction of correctly retrieved pairs) corrected for irregularities in learning. For further statistical analyses, \(Y\) was rescaled and Fisher-z-transformed to an unbound distribution (8) (Klöbl et al., 2020b).

\[
y_{\exp}(x(s)) = k \ast r \ast e^{-\frac{x(s)}{a}} + k \ast x(s)
\]  
(7)

\[
y_{z} = \text{atanh}\left(\frac{2 \ast Y_{\exp} - 100}{100}\right)
\]  
(8)

For behavioral analysis, the learning capacity and rate, as well as the adjusted performance were tested for interaction and main effects of the fixed factors “group”, “drug” and “phase” (learning, rehearsing; as opposed to the three measurements) using linear mixed effects models (LMEs) with a random intercept per subject and an additional random “phase” slope for performance. Covariance structures and random factors were chosen as to minimize the Akaike information criterion. Analyses of variance (ANOVA) were used to further investigate the effect of “drug” on rehearsing alone. The learning capacity was rank- and the rate log-transformed as indicated by the residual plots (Conover and Iman, 1981). Values were excluded as outliers if located further than...
three standard deviations from the mean after transformation. Correlations between the parameters were investigated via partial Spearman correlation, corrected for repetitions over subjects, “group”, “phase” and “drug” conditions. Multiplicity was controlled for using the Sidak correction. All inferences were two-sided.

### 2.5. MRI acquisition and processing

The RS data was recorded using a Siemens Prisma 3T scanner (Siemens, Erlangen, Germany) equipped with a 64-channel head coil before the in-scanner learning with the following parameters: gradient echo, echo-planar imaging, TE/TR = 30/2050 ms, GRAPPA 2, 210 x 210 mm field of view, 100 x 100 pixel in-plane resolution, 35 axial slices of 2.8 mm (25% gap), flip angle 90°, oriented parallel to the anterior-posterior commissure line, 201 frames (6.9 min). The subjects were instructed to keep their eyes open, lie still and let their mind wander.

The data was preprocessed primarily using Statistical Parametric Mapping, version 12 (SPM12) and custom MATLAB 2018b scripts. Slice-timing correction was performed to the temporally middle slice, followed by two-pass realignment. Images were normalized to the standard space defined by the Montreal Neurological Institute and a custom brain mask was applied. The BrainWavelet toolbox (Patel et al., 2014) was used for nonlinear artifact correction with the parameters “chain search” set to “harsh” and “threshold” to “20” to adjust for the application to unsmoothed data and GRAPPA acceleration. The images were then gray-matter-masked and smoothed with a Gaussian kernel of 8 mm full-width at half-maximum.

### 2.6. Statistical analysis – whole-brain functional connectivity

Network changes were identified in a step-wise approach: First, changes in GFC were calculated as a measure of brain-wide connectedness (Klaassens et al., 2015; Schaefer et al., 2014). Second, the specific connections underlying these changes were tracked using seed-based correlation analyses (Tagliazucchi et al., 2016). Third, spectral dynamic causal models (DCMs) were constructed to infer the directionality of the connections in the network discovered in step 2 (Friston et al., 2016).

Nuisance regression was performed utilizing the Friston-24 model (Friston et al., 1996), an adapted version of the CompCor method with an automated staircase approach (Klöbl et al., 2020b) and sine/cosine terms limiting the passband to 0.01–0.10 Hz (Hallquist et al., 2013). GFC maps were calculated by correlation with the standardized average gray matter signal after applying a group mask, which is a parsimonious equivalent of the average correlation to all voxels (Saad et al., 2013). See Fig. 1B for a flowchart of the analysis.

The GFC maps were Fisher-transformed and entered into a flexible factorial 2nd-level model in SPM. The model included factors for “group”, “drug” and “measurement” and the results were familywise-error-corrected to \( P_{\text{cluster}} \leq 0.025 \) (two inverse one-sided contrasts, as implemented in SPM12 (Chen et al., 2019)) at peak- or cluster-level (primary peak-level threshold \( p = 0.001 \)). The interaction effects were estimated and post-hoc comparisons adjusted again using the Sidak method.

For deducing which regions had the strongest influence on the changes in GFC, the analysis was repeated in a 10-fold cross-validation (the clusters were visually identified disregarding significance due to the reduced sample size). This way, the inherent circularity of inferences on the results is reduced. The first temporal eigenvariate from each significant cluster was extracted via the MarsBaR toolbox and used for a seed-based correlation analysis (SBCA). The Fisher-transformed SBCA maps were fed into the same model as used for the GFC data. Results were corrected for the number of seeds and post-hoc comparisons using the Sidak method.

### 2.7. Statistical analysis – dynamic causal models

For the DCMs, the smoothed data was reprocessed using the 1st-level GLM in SPM12 again correcting for the Friston-24 and CompCor regressors (Esnémio et al., 2019). Autocorrelation was set to “FAST” (Olszowy et al., 2019). The first temporal eigenvarianes of clusters from the GFC and the SBCA analyses surviving multiplicity correction were extracted from the data preprocessed for the DCM analysis. With these, fully connected linear spectral two-state DCMs were estimated. The parametric empirical Bayes (PEB) framework in SPM12 was used for group inference. A flat model was compared to a hierarchical PEB-of-PEBs approach in terms of free energy using only the subjects that completed all three scans from baseline to relearning. The former model has the advantage to allow for inclusion of partially available datasets whereas the latter can better account for within-subject effects by first creating PEB models for the individual subjects which are then fed into a group analysis. Since the flat model was favored the terms of free energy, this model was employed. To control for potential purely temporal effects, the test-retest scans were included as additional measurement and a correction factor for subjects that participated in these. Bayesian model averaging (Friston et al., 2016) was finally utilized to prune connections with high uncertainty. Since placebo application and character learning constitute reference conditions, reference coding was used.

In order to assess the dependencies between learning behavior and changes in effective connectivity, a PEB-of-PEBs model was set up with the differences between the scans after to before the respective phases on the lower level. The PEB-of-PEBs approach was here used to account for parameter certainty when calculating the difference. Since varying results for the different conditions were expected, interactions of the single learning parameters with “phase”, “group” and “drug” were investigated. The learning parameters were transformed as before. No outliers were excluded at this stage. Final Bayesian model reduction was applied as above. Unless otherwise mentioned, standard settings were used in the DCM analysis.

### 3. Results

Out of 138 subjects recruited, 99 subjects participated in the first MRI session. Of those, 87 completed the second and 78 also the third MRI scan. Additionally, 55 participants partook in the initial test-retest session (Fig. 1A). Dropouts were replaced but blinding inevitably led to slightly varying subgroup sizes. The subjects that at least completed the baseline MRI were 25 ± 5 years old (median ± interquartile range) and comprised 56 women and 43 men. There were no significant group differences regarding age, sex, highest finished level of education or participation proportions in the test-retest session (all \( p > 0.2 \)).

#### 3.1. Learning behavior

Fig. 2A shows example learning curves and fitting details for two subjects. An overview of the parameter distributions is given in Fig. 2B. The significant results are presented in Table 1. The models show that learning was more difficult for the “faces” condition and during the “re-learning” phase. The relationships between the parameters imply that higher learning capacities were reached later and drove performance. No significant behavioral interactions or drug effects were found, indicating no influence of escitalopram on modeled learning behavior.

#### 3.2. Whole-brain functional connectivity

A significant interaction effect in GFC between “group”, “drug” and “measurement” (post-relearning compared to baseline) was found in Broca’s area (BA; Table 2, Fig. 2C), showing a marked decrease during relearning of character-noun associations under escitalopram. A second interaction of “group” and “measurement” but without influence of
Fig. 2. Overview of learning and functional connectivity results. A: Example learning data and model fits over 21 days for two subjects. The percentages of correct answers, cumulative image pairs and model fitting weights calculated from the regularity of learning are shown in the upper row. The lower row shows the adjusted learning curves, model fits and derived learning parameters. B: Box plots of the learning parameters for the single conditions (the exponential learning rate was log-transformed for better visibility). Center line: median, box limits: quartiles, whiskers: most extreme non-outlier points, points: outliers further than 1.5 times the interquartile range from the quartiles. C: Means and 95%-confidence intervals of all available scans for the significant global (GFC) and inferential functional connectivity (FC) differences between sessions and conditions. D: Influences of the learning parameters (transformed due to outliers and skewed distributions) on the changes in GFC of 78 subjects. The different correlations and overlaid slopes demonstrate a varying influence of the learning parameters on GFC * indicates significant tested differences.

“drug” was found after relearning in the left lingual gyrus (ILG) with an increase for characters and a decrease for faces. No content-independent effect of escitalopram on GFC was detected.

Re-estimating the statistical model above with the SBCA maps revealed changes in the right inferior temporal gyrus (rITG) and the medial prefrontal cortex (mPFC) for the BA. No SBCA results for the ILG seed survived multiplicity correction. As the placebo and SSRI groups were not treated differently between the baseline and the post-learning session, any such temporal variations arose due to external factors not examined in this study.

Since both GFC results suggest an influence of learning in general rather than relearning alone, the effect of behavior on the GFC changes was further investigated on an exploratory basis. For this, the median values for the significant regions were extracted using the MarsBaR toolbox and changes in GFC modeled via LMEs (p < 0.05, two-sided) depending on the interaction of “group”, “drug”, “phase” and the transformed learning parameters. Associations with learning parameters substantiated the assumption for BA by a significant condition-dependent influence of capacity and rate (p = 0.0249, t130 = 2.27, N = 87). Further, the GFC change in the ILG could be similarly modeled by either performance (p = 0.0133, t149 = 2.50, N = 87) or capacity (p = 0.0142, t149 = 2.48, N = 87; see Fig. 2D). These relationships indicate that the influence of learning on GFC changes with content, setting and drug. No significant changes in GFC between directly consecutive scans (including test-retest) were found.

3.3. Effective connectivity

The DCMs explained 86.51 ± 3.96% of the single-subject variance (median ± interquartile range) indicating an adequate fit. Fig. 3 shows the learning-specific effects (posterior probability > 99%) of the final Bayesian model averaging after PEB inference. Since the model was reference coded, the effects need to be interpreted as additive. Connections
generally increased from the test-retest to the baseline session and decreased throughout learning.

Emotional relearning under escitalopram led to a drastic increase in the otherwise comparably stable connectivity from the mPFC to BA. During learning, the connections between mPFC and ILG showed an increase for emotional and a decrease for non-emotional content. These effects were inverted after relearning with a marked increase during non-emotional relearning under escitalopram. A strong decrease in connectivity during non-emotional relearning was observed between the ILG and the rITG. However, the different time courses of the “faces” subgroups from baseline to post-learning do not allow for the interpretation of prolonged learning effects. Similar unsystematic deviations over time affect the connections between rITG/ILG and BA.

3.4. Relationships between learning behavior and connectivity changes

In order to allow for conclusions on the influence of learning capacity, rate and performance, the dependence of effective connectivity changes on these parameters was estimated (Fig. 4, shown for a posterior probability > 99%). Correlations for learning per se were mostly weak compared to the changes related to the experimental conditions.

Learning capacity and performance showed similar dependencies including negative correlations with the connectivity changes between ILG and mPFC for non-emotional learning, which increased for relearning. The intake of escitalopram during relearning led to a strong positive dependency for emotional, and negative for non-emotional learning content. The connections between ILG and rITG showed similar correlations but were positive for relearning character-noun associations under placebo and negative for face pairs under escitalopram. Variations lower in magnitude were also found for the dependence of the rITG-BA connectivity changes. The connection from BA to the rITG was anti-correlated with learning rate. This relationship was further enhanced during re-learning and for the “faces” group. On the contrary, the connections between the ILG and BA showed an increased positive relationship with the rate for emotional (re-)learning, which is suppressed by the application of escitalopram.

4. Discussion

A functional brain network sensitive to the interaction of learning content (emotional, non-emotional), setting (learning, relearning) and citalopram intake was identified. Contrary to the initial assumption, the application of the SSRIs also had a limited influence on the connectivity changes induced by non-emotional relearning.

4.1. Context-dependent communication between medial prefrontal cortex and lingual gyrus

In mice, the chronic application of the SSRIs was found to induce dendritic spine growth in the mPFC (Guirado et al., 2014) and promote plasticity of the visual cortex (Chen et al., 2011; Maya Vetencourt et al., 2008). After acute citalopram administration in humans, an increase in mPFC FC with the dorsolateral prefrontal and posterior cingulate cortex was discovered (Arnone et al., 2018). Given reported reductions in global (Schaefier et al., 2014) and network-specific FC (Klaassen et al., 2015) following single SSRI doses, the prominent increases after 21 days of relearning under escitalopram point towards regionally specific changes of neuroplasticity for the mPFC.

Activity of mPFC and rITG has been related to the facial expression observed in others (Zaki et al., 2012). Both regions are also important for durable memory encoding (Wagner et al., 2016; Wagner et al., 2019). Furthermore, the mPFC plays an explicit role in memorizing emotional faces (Keightley et al., 2011). Thus, conflicting emotional memories could be mirrored in the opposed connectivity changes of mPFC and ILG when learning and relearning faces.

Table 1

| Parameter | Contrast | Estimate | 98.3%-CI | DoF | t-value | p-value |
|-----------|----------|----------|----------|-----|---------|---------|
| k         | faces-char | -16.15 | -29.74, -2.57 | 161 | -2.87   | 0.0140  |
| k         | relearn-learn | -11.17 | -16.32, -6.02 | 161 | -5.23   | 1.5E-6  |
| r         | relearn-learn | 0.77 | 0.65, 0.92 | 158 | -2.53   | 0.0016  |
| Y         | faces-char | -14.90 | -21.97, -7.83 | 161 | -5.15   | 2.2E-6  |
| Y         | relearn-learn | -2.98 | -5.40, -0.57 | 161 | -2.58   | 0.0318  |

Table 2

| Connectivity | Contrast | Level | Psidak | Cluster size [voxel] | Peak coordinate [mm] | Region |
|--------------|----------|-------|--------|----------------------|----------------------|--------|
| F-Cpx(S-P)x(M3-M1) | cluster | 0.0372 | 251 | -50 18 -2 | BA |
| F-Cpx(S-P)x(M3-M1) | peak | 0.0167 | 22 | 50 30 28 | rITG |
| F-Cpx(M3-M1) | cluster | 0.0045 | 484 | 0 56 -4 | mPFC |
| F-Cpx(M1-M3) | cluster | 0.0025 | 566 | -8 -68 -14 | ILG |
The LG has been related to extinction learning (Klass et al., 2017; Lissek et al., 2015a; Lissek et al., 2015b) and structural alterations to panic (Pang et al., 2021) and posttraumatic stress disorders (Kunimatsu et al., 2020). Both conditions are suspected to be based on dysfunctional fear learning and extinction. The central role in extinction processes is further backed by the correlation found between LG-mPFC and LG-rITG connectivity changes and relearning capacity and performance. An extinction-related network comprising the mPFC, hippocampus and right amygdala was previously identified in fear conditioning (Lang et al., 2009). There is also clear evidence for a cross-species role of the mPFC in extinction processes (see Giustino and Maren (2015) for a review).

Under escitalopram, the connections from ILG to mPFC further showed a distinct relationship to learning performance, with a strong positive association for relearning faces and a negative one for characters. Activation in the frontal gyrus was also previously shown to be stronger correlated with directed forgetting performance for emotional than non-emotional words (Wierzba et al., 2018). Given the increase in connectivity for the “characters” group under escitalopram, higher performance at a negative correlation implies a stronger reduction, also supporting the extinction perspective. This context-dependent increase between mPFC and ILG under assumedly enhanced neuroplasticity could highlight elementary differences between the learning contents: Where a direct connection to visual regions is mainly strengthened for the simpler (i.e., higher performance) word-character matching, the more difficult task involves a route over BA.

4.2. The role of Broca’s area in learning

BA is involved in numerous aspects of speech (Fujii et al., 2016), including inner speech (Morin and Hamper, 2012), and mnemonic strategies (Love et al., 2006). Even though reading words was shown to lead to electrical activity in BA (Magrassi et al., 2015) no relationship to nouns as linguistic objects was found (Farooqi-Shah et al., 2018). Theories of a topologically distinct representation of nouns in temporal regions (Vigliocco et al., 2011) have also failed to gain meta-analytical
Fig. 4. Correlations between changes in effective connectivity ($\Delta EC$) and the learning parameters capacity, rate and performance. Strengthened correlations are indicated in red, weakened in blue, line thickness represents the expected value of the relationship change. The initial learning phase, matching nouns to Chinese characters and placebo were used as reference conditions (left column). Effects with a posterior probability > 99% are shown. The correlations of selected effective connection changes with learning behavior for the 78 subjects are detailed below. Different relationships are visible for learning / relearning, the “faces” and “characters” groups and pharmacological modulation via the selective serotonin reuptake inhibitor (SSRI) escitalopram. Learning capacity, rate and performance were rank-, log- and Fisher-transformed beforehand to avoid skewed distributions and outliers. Figure created with BrainNet Viewer 1.7 (Xia et al., 2013).

Support (Crepaldi et al., 2013) making it unlikely that changes in these regions stem from the learning content alone. Under acute tryptophan depletion, BA showed a decrease and the mPFC an increase in activation for frontal- compared to side-viewed faces (Williams et al., 2007). This could point towards serotonergic modulability of BA since neither did acute tryptophan depletion significantly alter BDNF levels in rats (Cahir et al., 2008) nor transcranial direct current stimulation over BA lead to elevated serum BDNF levels in aphasic patients (Marangolo et al., 2014). It must, however, be noted that there are still other potential mechanisms of action for tryptophan depletion and missing evidence does not exclude an influence of BDNF (van Donkelaar et al., 2011). After relearning faces under escitalopram, a strong increase in connectivity from the mPFC towards BA was detected. For the same learning content, a bidirectional decrease in connectivity between BA and ILG was found independent of the drug condition. As briefly reasoned above, the enhanced connectivity might indicate serotonergic facilitation of emotional relearning and provides support for the importance of BA in emotion processing (Williams et al., 2007) and the context for
neuroplastic changes (Alboni et al., 2017; Chiarotti et al., 2017). The connection between mPFC and BA being affected especially under these conditions might further be related to a more difficult learning task and a stronger need for mnemonic strategies.

The connectivity changes between the ILG and BA showed a positive dependence on the learning and relearning rate of face pairs under placebo. Since by definition of the model (see Eq. (5)) a higher rate corresponds to a flatter slope and is accompanied by decreases in dependency on performance, this finding might be indicative of slower learning (see Fig. 2D). The positive correlation of the connectivity changes between ILG and BA with performance when initially learning character-noun associations could be expected based on the role of BA in language. In this light, the strong increase of the correlation of ILG-BA connectivity changes and capacity / performance for emotional relearning under escitalopram points towards a facilitating effect of serotonin on learning-related neuroplasticity.

4.3. Modulation along the ventral visual stream

The connections between the ILG and the rITG run along the ventral visual stream (VVS). This pathway is implicated in object recognition and identification (Goodale and Milner, 1992). Its plasticity and the modulatory effects of transcranial direct current stimulation on memory encoding were recently shown (Zhao and Woodman, 2021). The inferior temporal cortex itself is also involved in short-term (Ranganath et al., 2004) and long-term memory (Wagner et al., 2016; Wagner et al., 2019), object naming and identification (Acres et al., 2009).

For relearning, a bidirectional decrease in connectivity between ILG and rITG could be observed, being much more pronounced for non-emotional content. Escitalopram modulated the dependency of connectivity changes on capacity / performance. The positive correlation for relearning character-noun associations was not present under escitalopram. The dependency became even negative for face pairs.

The importance of the LG for visual memory is well-established (Bogousslavsky et al., 1987) together with its involvement in facial (Puce et al., 1995) and word form processing (Mechelli et al., 2000; Xiao et al., 2005). Hemispheric differentiation was previously suggested with the ILG being more active during memorizing faces. This is also reflected in the current results as increased connectivity from ILG to mPFC after emotional learning. Besides visual memory and processing, the LG and inferior frontal gyrus, where BA is located, were shown to be important for the analysis of novelty and spatial information (Menon et al., 2000). This might explain the differences in effective but not global connectivity between the test-retest and baseline scan.

The connection from ILG to rITG is the only one showing an increase after initial non-emotional learning. A possible explanation might lie in object identification or memory formation processes related to the rITG. The more wide-spread reductions could be related to the decreasing novelty or repetition suppression (Prêkowska et al., 2017). Serotonergic modulation of the behavioral dependence of connections along the VVS might be based on long-term effects of escitalopram on the rITG (Kaichi et al., 2016) or facilitation of neuroplasticity of the ILG as part of the visual cortex (Chen et al., 2011; Maya Vetencourt et al., 2008).

4.4. Learning and treatment under enhanced neuroplasticity

The supportive effects of SSRIs on fear extinction learning have been previously shown in animals (Furr et al., 2012; Karpova et al., 2011; Lapiz-Bluhm et al., 2009; Masaki et al., 2006) and healthy humans (Bui et al., 2013). Moreover, an increase in dendritic spine growth was observed in animal models after chronic fluoxetine administration (Chen et al., 2011; Guirado et al., 2014). Also a shift in visual cortical neurons could be observed after monocular deprivation and fluoxetine treatment, accompanied by elevated BDNF levels. The neuronal changes were further observed after direct application of BDNF (Maya Vetencourt et al., 2008). This can be seen as earlier indicator of the common effect of antidepressants on TrkB and BDNF (Casarotto et al., 2021). However, chronic administration of antidepressants (citalopram / tianeptine) was also reported to impede fear extinction learning in rats (Burghardt et al., 2013). This finding was related to a common glutamatergic rather than citalopram’s serotonergic effect. As tianeptine was shown to increase BDNF levels (Della et al., 2012), in light of a common antidepressant mechanism of action and enhanced neuroplasticity, this now points towards downstream effects of the glutamatergic system to be responsible. Especially in exposure therapy it might hence be important to take medication and environment into account. Such influences were formalized in the concepts of instructive and permissive causality (Branchi and Giuliani, 2021). Where the former promotes manifestation of a particular effect (e.g., symptom improvements) the latter does this for a range of different effects (e.g., any change in symptoms). This argument further partly contradicts a general positivity bias of SSRI treatment (c.f. Harmer and Cowen (2013); Pringle et al. (2011)).

The clinical relevance of a suitable therapeutic environment becomes evident when reconsidering comparisons of psycho- and pharmacotherapy in depression and the combination thereof. Additional cognitive-behavioral therapy was shown to be significantly more effective in the treatment of depression (Cuijpers et al., 2013; Pampallona et al., 2004) but also for cases of treatment-resistant depression compared to standard medication management (Nakagawa et al., 2017). For often difficult to treat chronic forms of depression, combination therapy was repeatedly shown to be superior to psycho- or pharmacological monotherapies (Schramm et al., 2020). Importantly, combined therapy might not only foster condition-specific recovery but also general quality of life (Ishak et al., 2011).

Besides the large variation in outcomes of antidepressant trials (Branchi, 2011), an increased susceptibility to change related to enhanced neuroplasticity also explains dissimilar learning results between studies and patient populations (Barkas et al., 2012; Chamberlain et al., 2006; Chantiluke et al., 2015). Since human subjects cannot be controlled to the extent it is possible in animal studies, comparably more variation must be expected in the learning data (e.g., arising from individual life events). This might underlie the missing drug effect on the behavioral data despite clear correlations with the induced connectivity changes. Indeed, the in-scanner sessions, which took place in a constrained environment and at the end of the escitalopram application, showed a positive drug effect (Reed et al., 2021). Another way to reduce variance in the data would have been to use word-matching tasks for both learning groups. It is however not clear whether emotional pictures and words elicit comparable responses and potential differences might be valence-dependent (Houwer and Hermans, 1994; Schlochtermeier et al., 2013). Hence, pictures of faces were chosen as they are known to at least evoke implicit emotion processing (Hariri et al., 2002; Spies et al., 2017). It cannot be excluded that differences in stimuli complexity influenced the results (Bayer and Schacht, 2014; Schlochtermeier et al., 2013). From this perspective, the content-specific drug effects not only relate to emotional aspects but also the difficulty of memorizing the content. The negative correlations with learning capacity accompanying the connectivity increases between mPFC-BA (faces) / mPFC-ILG (characters) might indeed be interpreted this way.

4.5. Limitations

Despite the comparatively large sample, the dropout rate led to slightly imbalanced subgroups. Models allowing for missing values were utilized where possible to mitigate this problem. Caution is needed when interpreting certain results in light of accidental subgroup differences at baseline and after initial learning. The test-retest session performed to differentiate temporal and general learning effects probably had an effect on the identified learning network due to shared processing of novelty. Despite correcting for such effects, the in-depth discussion thus
concentrated on the interactions of the experimental conditions which should not be affected so easily.

5. Conclusion

A learning context-, setting-sensitive and serotonergic modulated functional brain network and its behavioral correlates were mapped. Between the mPFC and the ILG, the intake of escitalopram during relearning potentiated the bidirectional connectivity increase for character-noun associations. For relearning face pairs, the directed connection from mPFC to BA was drastically strengthened only under escitalopram. Prominent context-dependent correlations of the relearning-induced connectivity changes with behavior might be related to content-specific enhancement of extinction processes. Where the mPFC is known to play a central role in extinction, the simpler character-noun association learning task might directly involve visual regions and the more difficult faces matching required mnemonic strategies. These findings match the theory of SSIs – likely via elevation of BDNF levels (Casarotto et al., 2021) – improving neuroplasticity rather than mood leading to permissive causality (Alboni et al., 2017; Branchi, 2011; Chiarotti et al., 2017). This would make patients more susceptible to environmental influences, which ideally provide a setting that supports the therapeutic endeavor, such as accompanying psychotherapy, and necessitates increased attention to factors toward external factors in treatment studies using serotonergic medication.

Declaration of competing interest

There is no conflict of interest to declare with relevance to this work. R. Lanzenberger received travel grants and/or conference speaker honoraria within the last three years from Bruker BioSpin MR, Heel, and support from Siemens Healthcare regarding clinical research using PET/MR. He is a shareholder of the start-up company BM Health GmbH since 2019.

Credit authorship contribution statement

Manfred Klöbl: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. René Seiger: Conceptualization, Software, Investigation, Writing – review & editing, Project administration. Thomas Vainicek: Conceptualization, Investigation, Writing – review & editing, Project administration. Patricia Handschuh: Investigation, Writing – review & editing. Murray Bruce Reed: Software, Validation, Investigation, Data curation, Writing – review & editing. Benjamin Spurny-Dworak: Investigation, Writing – review & editing. Vera Ritter: Writing – review & editing, Project administration. Godber Mathis Godbersen: Investigation, Writing – review & editing. Gregor Gryglewski: Conceptualization, Writing – review & editing, Funding acquisition. Christoph Kraus: Conceptualization, Writing – review & editing, Andreas Hahn: Methodology, Validation, Writing – review & editing, Supervision. Rupert Lanzenberger: Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Acknowledgments

This research was funded in part, by the Austrian Science Fund (FWF) [KLI 516, PI: Rupert Lanzenberger]. For the purpose of open access, the author has applied a CC BY public copyright license to any Author Accepted Manuscript version arising from this submission. This work was further supported by the Medical Imaging Cluster of the Medical University of Vienna, and by the grant „Interdisciplinary translational brain research cluster (ITHC) with highfield MR” from the Federal Ministry of Science, Research and Economy (BMWFV), Austria. Vienna. Manfred Klöbl and Murray Bruce Reed are recipients of a DOC fellowship of the Austrian Academy of Sciences at the Department of Psychiatry and Psychotherapy of the Medical University of Vienna. The funding agencies had no role in conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript. We want to thank Dietmar Winkler for providing clinical supervision, Leo Silberbauer, Jakob Unterholzer, Paul Michenthaler, Alim Basaran and Alexander Kautzky for medical support. We also want to express our gratitude towards the diploma students of the Neuroimaging Labs, especially Hannah van Abeek who was responsible for recruiting a large proportion of the volunteers for the study.

Availability statement

Due to data protection and the necessity of a formal data sharing agreement, only the preprocessed data underlying the analyses in this work is available from the corresponding author upon reasonable request.

Data analysis was conducted using SPM12 v7771 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The BrainWavelet Toolbox version 2 (https://www.brainwavelet.org/) was additionally used for preprocessing.

References

Acres, K., Taylor, K.L., Moss, H.E., Stamatakis, E.A., Tiley, L.K., 2009. Complementary hemispheric asymmetries in object name and recognition: a voxel-based correlational study. Neuropsychologia 47, 1836–1843.
Alboni, S., van Dijk, R.M., Pogginì, S., Millór, G., Ferrotta, M., Drenth, T., Brunello, N., Wolfer, D.P., Llamasola, C., Atrein, I., Cirulli, F., Maggi, L., Branchi, I., 2017. Fluxoxide effects on molecular, cellular and behavioral endophenotypes of depression are driven by the living environment. Mol. Psychiatry 22, 552–561.
Anzanello, M.J., Fogliatto, F.S., 2011. Learning curve models and applications: literature review and research directions. Int. J. Ind. Ergon. 41, 573–583.
Arnone, D., Wise, T., Walker, C., Covey, P.J., Howes, O., Selvarani, S., 2018. The effects of serotonin modulation on medial prefrontal connectivity strength and stability: a pharmacological fMRI study with citalopram. Prog. Neuropsychopharmacol. Biol. Psychiatry 84, 152–159.
Barkas, L., Redhead, E., Taylor, M., Shtaya, A., Hamilton, D.A., Gray, W.P., 2012. Fluoxetine restores spatial learning but not accelerated forgetting in mesial temporal lobe epilepsy. Brain 135, 2585–2594.
Bayer, M., Schacht, A., 2014. Event-related brain responses to emotional words, pictures, and faces-a cross-domain comparison. Front. Psychol. 5, 1106.
Bogossianl, T., Mikhosy, J., Deruz, J.P., Assal, G., Regli, F., 1987. Lingual and fusiform gyri in visual processing: a clinico-pathologic study of superior atrophic hemianopia. J. Neurol. Neurosurg. Psychiatry 50, 607–614.
Branchi, I., 2011. The double edged sword of neural plasticity: increasing serotonin levels leads to both greater vulnerability to depression and improved recovery to treatment. Psychoneuroendocrinology 36, 339–351.
Branchi, I., Giuliani, A., 2021. Shaping therapeutic trajectories in mental health: Instructive vs. permissive causality. Eur. Neuropsychopharmacol. 43, 1–9.
Browning, M., Reid, C., Covey, P.J., Goodwin, G.M., Harmer, C.J., 2007. A single dose of citalopram increases fear recognition in healthy subjects. J. Psychopharmacol. 21, 684–690.
Bui, E., Orr, S.P., Jacoby, R.J., Keshaviah, A., LeBlanc, N.J., Milad, M.R., Pollack, M.H., Simon, N.M., 2013. Two weeks of pretreatment with escitalopram facilitates extinction learning in healthy individuals. Hum. Psychopharmacol. 28, 447–456.
Burghardt, N.S., Sigurdsson, T., Gorman, J.M., McLennan, B.S., Le Doux, J.E., 2013. Chronic antidepressant treatment impairs the acquisition of fear extinction. Biol. Psychiatry 73, 1078–1086.
Cahill, M., Ardiz, T.C., Elliott, J.J., Kelly, C.B., Reynolds, G.P., Cooper, S.J., 2008. Acute tryptophan depletion does not alter central or plasma-derived neurotrophic factor in the rat. Eur. Neuropsychopharmacol. 18, 317–322.
Casarotto, P.C., Giryh, M., Fred, S.M., Kovala, V., Moliner, R., Enkawi, G., Bojone, C., Cannarrazo, C., Sahu, M.P., Kauriankois, K., Brunello, C.A., Steinag, A., Winkel, F., Patil, S., Veering, S., Serchov, T., Diniz, C.R.A., Laukunlin, L., Cardon, I., Antila, H., Rog, T., Piepponen, T.P., Bramham, C.R., Normann, C., Lau, S.E., Saarma, M., Vatulanien, I., Castrén, E., 2021. Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. Cell 184, 1299–1313 e1219.
Chamberlain, S.R., Müller, U., Blackwell, A.D., Clark, L., Robbins, T.W., Sahakian, B.J., 2006. Neurochemical modulation of response inhibition and probabilistic learning in humans. Science 311, 861–863 New York, N.Y.
Chastule, K., Barrett, N., Giampietro, V., Brammer, M., Simmons, A., Murphy, D.G., Rubins, K., 2015. Inverse effect of fluoxetine on medial prefrontal cortex activation during reward reversal in ADHD and autism. Cereb. Cortex 25, 1757–1770.
Chen, G., Cox, G.R., Glen, D.K., Rajendrak, J.K., Reynolds, R.C., Taylor, P.A., 2019. A tail of two sides: Artificially enabled false positive rates in fMRI due to the sparseness choice with t-tests. Hum. Brain Mapp. 40, 1037–1043.
Chen, J.J., Lin, W.C., Chua, J.W., So, P.T., Kubota, Y., Nedivi, E., 2011. Structural basis for the role of inhibition in facilitating adult brain plasticity. Nat. Neurosci. 14, 587–594.
