Serotonin modulates learning content-specific neuroplasticity of functional brain networks

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Abstract

Learning-induced neuroplastic changes, further modulated by content and setting, are mirrored in brain functional connectivity. Animal models emphasized the crucial role of serotonin in neuroplasticity particularly for emotional relearning, but comparable studies in humans are scarce. Assessing the translation of learning effects from animals to humans, 99 healthy subjects underwent six weeks of emotional or semantic learning and subsequent relearning and three resting-state acquisitions for functional connectivity estimation. During relearning, subjects received either a daily dose of the selective serotonin reuptake inhibitor escitalopram or placebo. The influence of escitalopram on functional connectivity was connection- and learning content-dependent, with potentiation of decreases during emotional and increases during semantic learning. The directedness of these effects indicates serotonergic modulation of emotional feedback routes. These results demonstrate that escitalopram intake during relearning facilitates content-dependent network adaptations and support the conclusion that enhanced neuroplasticity might be the major underlying mechanism in psychiatric therapies.

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, Villringer, & Ragert, 2012; Valkanova, Eguia Rodriguez, & Ebmeier, 2014), structural (Sampaio-Baptista & Johansen-Berg, 2017) and functional connectivity (FC) (Guerra-Carrillo, Mackey, & Bunge, 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, Plailly, Fonlupt, Veyrac, & Royet, 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, Maddox, Ing, & Song, 2007; Grober, An, Lipton, Kawas, & Resnick, 2019; Schraegle, Nussbaum, & Stefanatos, 2016; Vicari et al., 2005) and psychiatric disorders (Hartmann-Riemer 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 & Condron, 2010; Gaspar, Cases, & Maroteaux, 2003) and consequently in the pathophysiology and treatment of psychiatric conditions (e.g., major depression (Kraus, Castren, Kasper, & Lanzenberger, 2017), obsessive-compulsive disorder (Fineberg, Brown, Reghunandanan, & Pampaloni, 2012), generalized anxiety disorder (Goodman, Bose, & Wang, 2005)). 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). Animal experiments demonstrated that these effects enhance neuroplasticity in the brain (Chen et al., 2011; Guirado, Perez-Rando, Sanchez-Matarredona, Castrén, & Nacher, 2014; Maya Vetencourt et al., 2008).

Beyond its involvement in learning processes, serotonin plays a role in extinction and relearning. This is well established in animals (Furr, Lapiz-Bluhm, & Morilak, 2012; Lapiz-Bluhm, Soto-Piña, Hensler, & Morilak, 2009; Masaki et al., 2006) but much less so in humans. A noteworthy finding is faster fear extinction after a two-week treatment with the SSRI escitalopram compared to placebo in healthy subjects (Bui et al., 2013). This effect is suspected to be linked to a positivity bias induced by SSRIs (Harmer & Cowen, 2013; Pringle, Browning, Cowen, & Harmer, 2011). Interestingly, such changes were also reported already after a single SSRI dose. Acute application was shown to enhance the recognition of emotional faces (Browning, Reid, Cowen, Goodwin, & Harmer, 2007; Harmer et al., 2003) and induce FC changes predictive of treatment response within hours (Klöbl, Gryglewski, et al., 2020). Also the acute effects of SSRIs on connectivity depend on the individual mental condition (Dutta et al., 2019). Considering the minimum of one week that is needed for an antidepressant effect (34), these acute findings suggest that serotonin-modulated neuroplasticity facilitates but not necessarily implies improvements in depressive symptoms (27). Thus, serotonergic pharmacological agents can induce widespread and substantial

alterations of the brain FC (Arnone et al., 2018; Klaassens et al., 2015; Schaefer et al., 2014; Schrantee, Lucassen, Booij, & Reneman, 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; H. Zhao et al., 2019), FC provides a convenient surrogate for neuroplasticity. However, several aspects remain unknown. These concern the interactions between learning content and setting (learning vs. relearning) as well as the role of serotonin, especially how the effect of serotonergic agents on emotional relearning (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 21 days of learning and relearning were investigated. A dedicated learning paradigm and online training platform were specifically developed for this study, comprising an emotional (matching face pairs) and a semantic (matching Chinese characters to unrelated German nouns) condition. To assess the modulatory effects of serotonin on neuroplastic changes, participants received a daily dose of 10 mg escitalopram or placebo in a double-blind, randomized fashion during the relearning phase. Here, they had to memorize new associations contradictory to the previous ones. To further differentiate merely temporal from learning effects, a subgroup also attended an additional test-retest scan before the first regular appointment.

Network changes were identified in a step-wise approach. First, changes in global functional connectivity (GFC) were calculated as a measure of brain-wide connectedness since SSRIs were shown to induce widespread changes thereof (40, 41). Second, the specific connections underlying these changes were tracked using seed-based correlation analyses to identify the origins of the GFC change (49). Third, dynamic causal models (DCMs) were constructed to infer the directionality of the connections (Karl J. Friston et al., 2016). Finally, the learning behavior was modeled based on the 21 days of learning and relearning (Anzanello & Fogliatto, 2011) and related to the directed connectivity changes.

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 semantic relearning. These adaptations were further assumed to correlate with the individual learning behavior.

Results

Upon enrollment, subjects were assigned to the group learning either associations between Chinese characters and unrelated German nouns or face pairs. An initial resting-state (RS) scan was conducted to derive the individual baseline FC. Afterwards, subjects learned online for 21 days. A second RS scan was then conducted to derive the learning-inducted FC changes. Thereafter, subjects were randomized within the previous groups to receive either

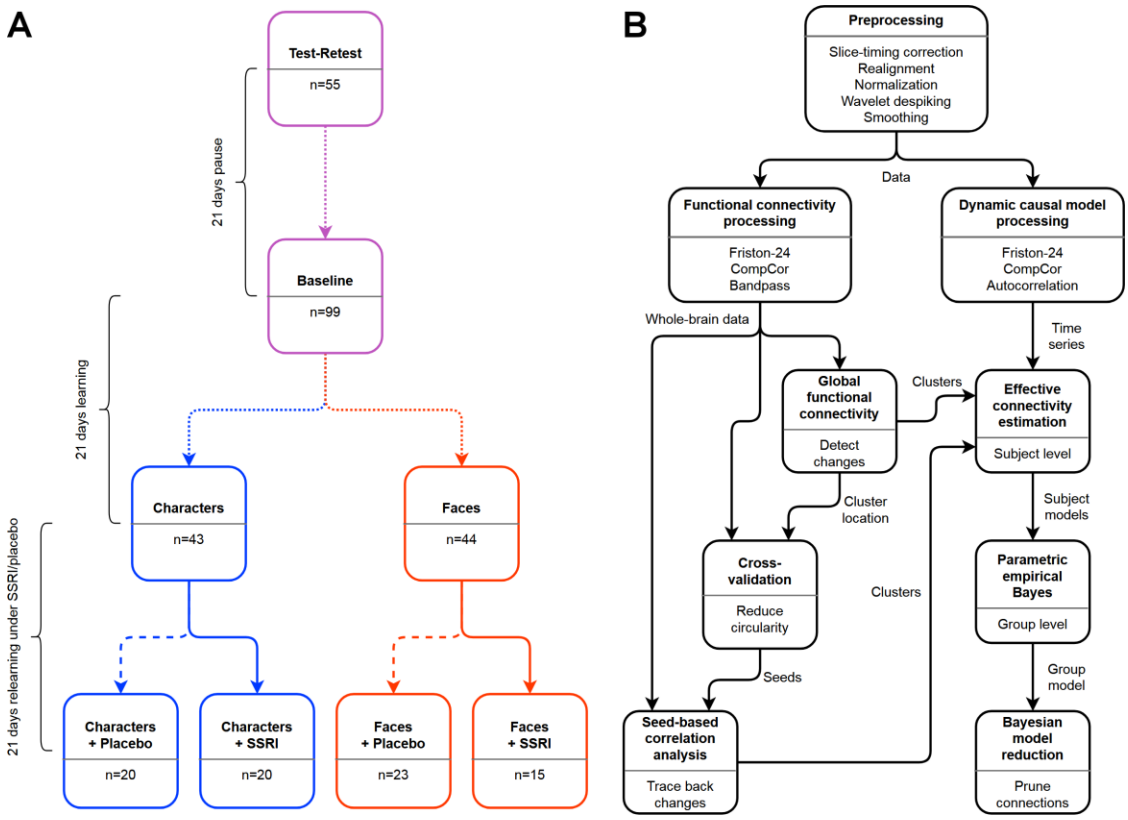

Figure 1: Study workflows. A: Study overview with the number of measurements included at each time point for each group. B: Analysis workflow from the raw data to the final effective connectivity analysis. SSRI: Selective serotonin reuptake inhibitor.

10 mg/day escitalopram or placebo for 21 days of relearning new associations. Participation was concluded with a final RS scan after relearning (Figure 1A).

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 (see Figure 1A). The subjects that at least completed the baseline MRI were 26.68 ± 4.75 years old (median ± interquartile range) and comprised 56 women and 43 men. There were no significant group differences regarding age, sex or participation proportions in the test-retest session (all $p > 0.2$).

<u>Learning behavior</u>

The learning parameters "capacity" k, (theoretical maximum of samples that can be memorized), "rate" r (determining how fast k is approached, lower values meaning steeper increase) and "performance" Y (adjusted amount of correctly retrieved pairs) were investigated for influences of the experimental factors (Figure 2A-B). Capacity significantly correlated with rate ($\rho = 0.61$) and performance ($\rho = 0.57$, both $p < 0.001$, Spearman partial correlation, corrected for repetition over subjects). These relationships imply that higher learning capacities were reached later and drove performance. Capacity and performance were significantly smaller for the "faces" condition ($p_{perm} = 0.010$ / < 0.001) and the relearning phase ($p_{perm} < 0.001$ / $= 0.036$) and the rate was significantly lower in the relearning phase

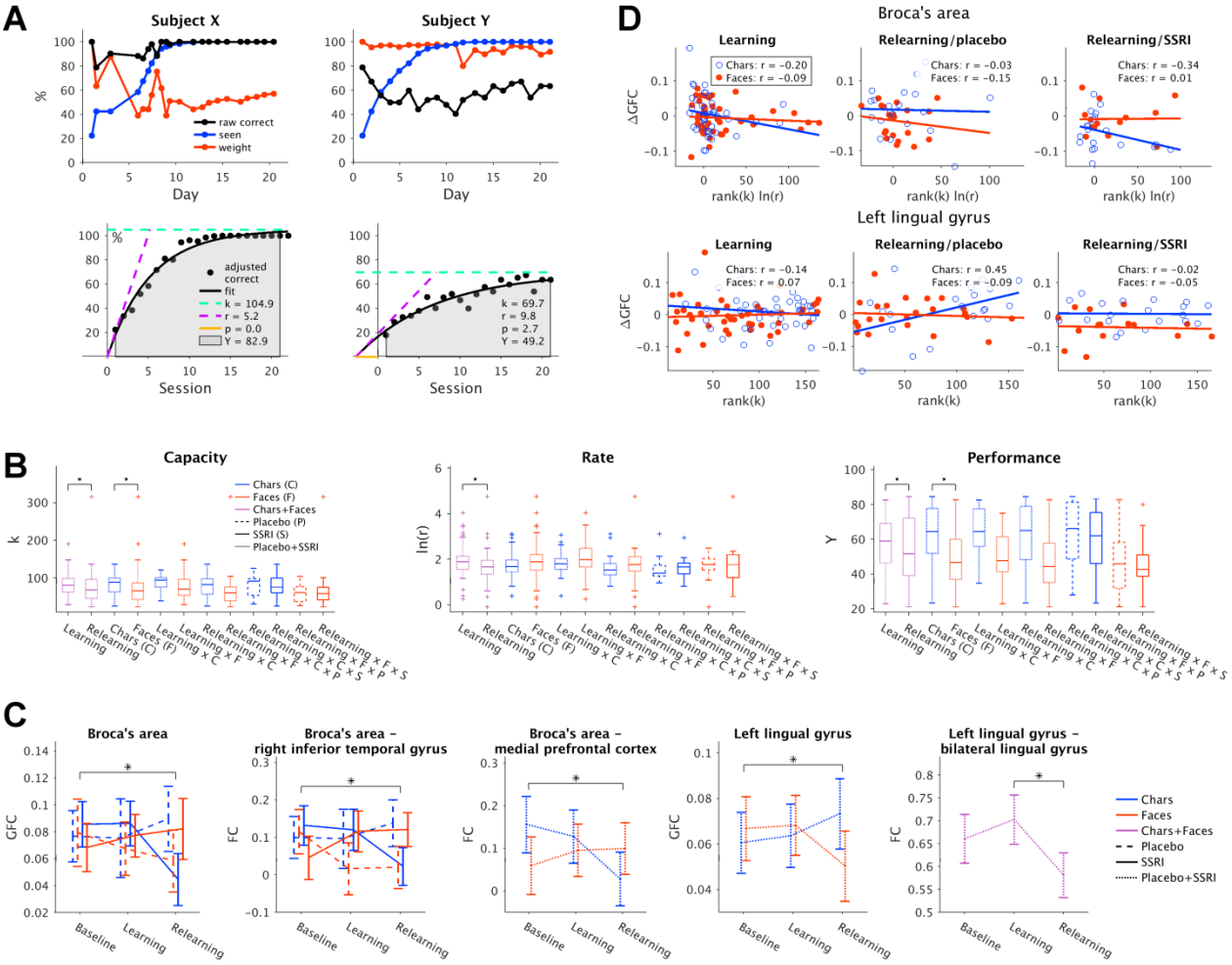

Figure 2: Overview of learning and functional connectivity results. A: Example learning data and model fits over 21 days for two subjects. The percentages of correct answers, cumulative image pairs and model fitting weights calculated from the regularity of learning are shown in the upper row. The lower 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). C: Means and 95%-confidence intervals of all available scans for the significant global (GFC) and inferred 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 differences.

(p_{perm} = 0.003). No significant behavioral interactions or substance effects were found, indicating no influence of escitalopram on learning behavior.

Whole-brain functional connectivity

RS data analysis is outlined in Figure 1B. Initial FC analysis was conducted to span the network influenced by learning and serotonergic modulation. A significant interaction effect in GFC between "group", "substance" and "measurement" (post-relearning compared to baseline) was found in Broca's area (BA; Table 1, Figure 2C), showing a marked decrease during relearning of character-noun associations and increase for face pairs under escitalopram. A second interaction of "group" and "measurement" but without influence of

Connectivity	Contrast	$p_{Cluster+Sidak}$	Cluster size [voxel]	Peak coordinate [mm]			Region
				x	y	z	
GFC	(F-C)x(S-P)x(M3-M1)	0.006	409	-48	20	0	BA
- SBCA	(F-C)x(S-P)x(M3-M1)	0.012	341	50	-30	-28	rITG
- SBCA	(F-C)x(M3-M1)	0.003	473	-6	62	10	mPFC
GFC	(F-C)x(M1-M3)	0.024	333	-14	-60	-8	lLG
- SBCA	M2-M3	0.024	320	-4	-70	8	bLG

Table 1: Whole-brain family-wise-error-corrected cluster-level results for the global functional connectivity (GFC) and the subsequent seed-based correlation analyses (SBCA) listed below. Factors: "group" (faces "F" / Chinese characters "C"), "substance" (placebo "P" / SSRI "S") and "measurement" ("M1…3"). BA: Broca's area, rITG, right inferior temporal gyrus, mPFC: medial prefrontal cortex, l/bLG: left/bilateral lingual gyrus.

"substance" was found after relearning in the left lingual gyrus (lLG) with an increase for characters and a decrease for faces.

To identify the connections driving differences in GFC, seed-based correlation analyses (SBCA) were conducted calculating the connectivity of the GFC clusters (Table 1). 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 and in the bilateral lingual gyrus (bLG) for the lLG seed.

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. Associations with learning parameters substantiated the assumption for BA by a significant condition-dependent influence of capacity and rate ($p = 0.018$). Further, the GFC change in the LG could be modeled by capacity ($p = 0.019$; see Figure 2D). Both relationships indicate that the influence of learning on GFC changes with content, setting and substance. No significant changes in GFC between directly consecutive scans (including test-retest) were found.

<u>Effective connectivity</u>

Dynamic causal modelling (DCM) was used to investigate the directionality of the connectivity changes between the GFC and SBCA clusters. Figure 3 shows the learning-specific effects of the final Bayesian model reduction after parametric empirical Bayes (PEB) inference. Temporal changes were observed for connections involving the LG, increasing from the test-retest session to the baseline and decreasing throughout learning.

Beyond the general increase in connectivity for semantic relearning under escitalopram, three parts of the network were prominently and differentially influenced by all experimental factors (i.e., group, substance measurement): the connectivity towards BA, between LG and mPFC, and between LG and rITG. In detail, the decrease in connectivity towards BA when relearning faces under placebo was inverted to a strong increase under escitalopram. On the other hand, SSRIs potentiated the decrease for relearning faces and increase for characters between mPFC and LG. The connection spanning from LG to rITG is

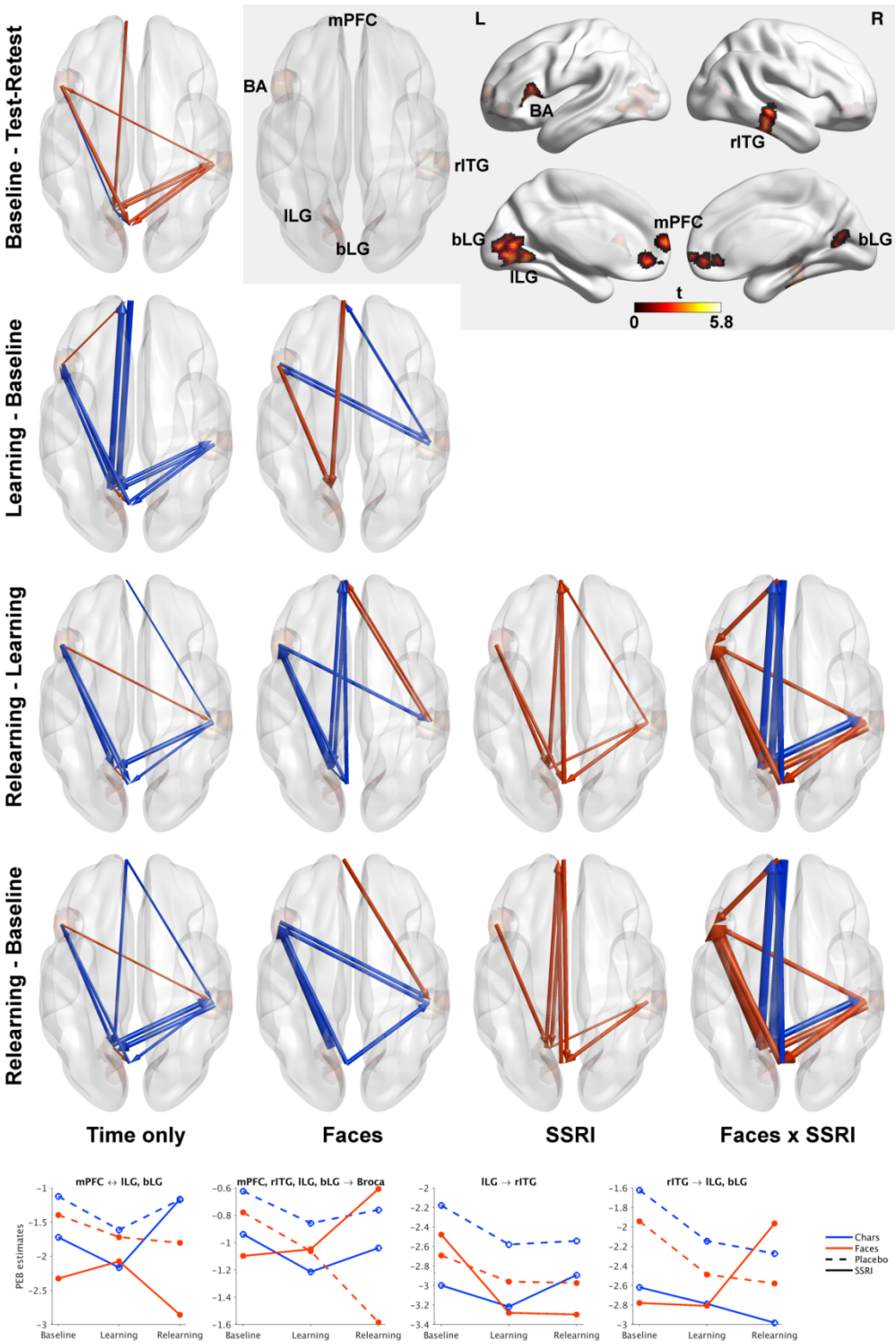

Figure 3: Effective connectivity time and time-related interaction effects relative to the reference conditions (learning Chinese characters, placebo application). Increases are indicated in red, decreases in blue, line thickness represents the expected value of change in effective connectivity. The shaded inlay shows the cluster positions and t-values of the preceding global and seed-based functional connectivity analyses that were used to define the regions of interest (primary threshold $p \leq 0.001$ – equivalent $t \geq 3.15$, $p_{Cluster} \leq 0.05$). Effects with a posterior probability > 99% are shown. The temporal changes of selected estimates of the preceding parametric empirical Bayes (PEB) analysis are displayed below (estimates were averaged where multiple connections are shown at once). BA: Broca's area, tITG: right inferior temporal gyrus, mPFC: medial prefrontal cortex, lLG: left lingual gyrus, bLG: bilateral lingual gyrus, SSRI selective serotonin reuptake inhibitor. Figure created with BrainNet Viewer 1.7 (Xia, Wang, & He, 2013).

increased again after a general decrease during learning, when relearning character associations under escitalopram. Lastly, a strong increase in connectivity in the reverse direction is observed for relearning faces under escitalopram.

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 (Figure 4). Connections including the LG show a correlation with learning behavior, changing with the experimental conditions. Whereas the connectivity changes between the LG, rITG and mPFC for learning characters depend negatively on learning rate and performance (i.e., stronger decreases in connectivity for better learning). This relationship was markedly increased towards a positive correlation during relearning under placebo. The connectivity from the LG to the mPFC shows a strong positive correlation with all learning parameters when relearning faces under SSRI. In contrast, the connectivity changes between mPFC / LG and BA were negatively correlated with the learning rate for relearning faces under placebo but not under escitalopram.

In summary, the changes in effective connectivity as well as their relationship to learning behavior imply that the connections between mPFC and LG and those towards BA are differentially affected by the learning content and serotonergic modulation. Depending on the direction, this also holds true for the connections between LG and rITG.

Discussion

A functional brain network in humans sensitive to the interaction of learning content (semantic, emotional), setting (learning, relearning) and serotonergic modulation was identified. Contrary to the initial assumption, the application of the SSRI escitalopram also had an influence on the connectivity changes induced by sematic learning. These effects were opposed to the modulations of emotional content.

Context-dependent communication between medial prefrontal cortex and lingual gyrus

Three weeks of escitalopram intake led to an increase in connectivity towards the LG and mPFC for semantic relearning, implying stronger integrative processing between these regions. 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). In contrast, reductions in global (Schaefer et al., 2014) and network-specific FC (Klaassens et al., 2015) were already reported after acute SSRI application. Given the increases after 21 days of

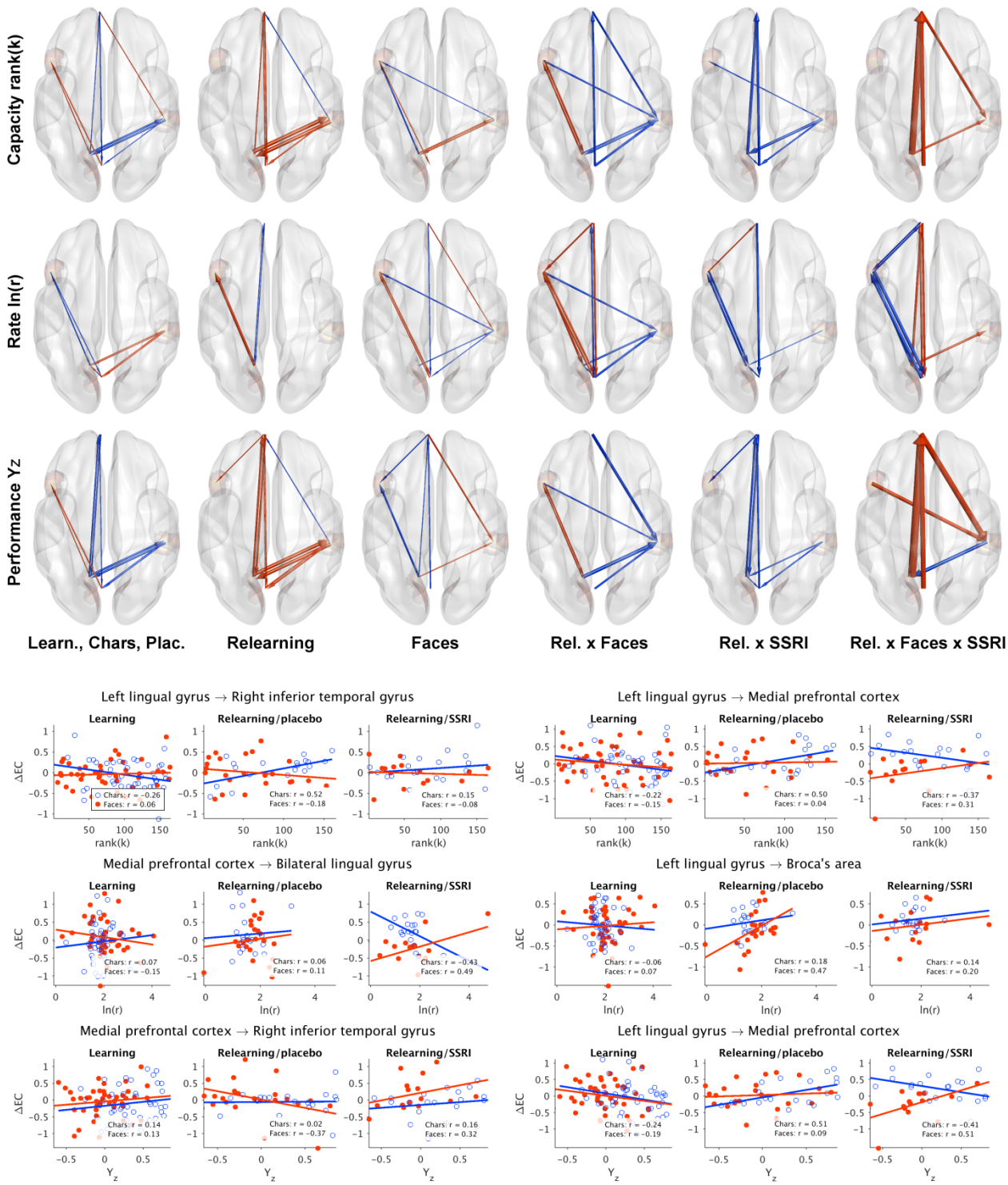

Figure 4: Correlations between changes in effective connectivity (ΔEC) and the learning parameters capacity, rate and performance. Strengthened correlations are indicated in red, weakened in blue, line thickness represents the expected value of the relationship change. The initial learning phase, matching 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).

relearning under escitalopram, this points towards content- and regional specific changes of neuroplasticity.

Citalopram has been previously linked to reduced activation in the bLG when viewing emotional faces (Henry et al., 2013). The current results imply an accompanying reduction in connectivity with the mPFC when associations with these faces should be relearned. Activity of mPFC and rITG have been related to the facial expression observed in others (Zaki, Weber, & Ochsner, 2012). Both regions are also important for durable memory encoding (Wagner, van Buuren, Bovy, & Fernández, 2016; Wagner, van Buuren, & Fernández, 2019). Furthermore, the mPFC plays an explicit role in memorizing emotional faces (Keightley, Chiew, Anderson, & Grady, 2011). Thus, conflicting emotional memories could be mirrored in the opposed connectivity of mPFC and rITG when learning and relearning faces as well as the inverse connectivity change of the mPFC and BA with the LG.

The LG has been linked to extinction learning (Klass, Glaubitz, Tegenthoff, & Lissek, 2017; Lissek, Glaubitz, Güntürkün, & Tegenthoff, 2015; Lissek, Glaubitz, Wolf, & Tegenthoff, 2015) and structural alterations to panic (Pang et al., 2019) and posttraumatic stress disorders (Kunimatsu, Yasaka, Akai, Kunimatsu, & Abe, 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 increased bidirectional LG-mPFC and LG-rITG connectivity and relearning performance. An extinction-related network comprising the mPFC, hippocampus and right amygdala was previously identified in fear conditioning (Lang et al., 2009).

Figure 3 reveals that the connections between mPFC and LG were strengthened when relearning characters and weakened for faces under placebo. This differential pattern extends previous results that SSRIs facilitate neuroplasticity not just in general but in a context-specific manner. Considering that the improvement of clinical symptoms in psychiatric disorders may actually be driven by the neuroplastic action of SSRIs (Chiarotti, Viglione, Giuliani, & Branchi, 2017), it seems particularly important to provide a well-designed environment for the treatment of these patients.

Under escitalopram, the connections from LG to mPFC also showed a distinct relationship to learning performance, with a strong positive association for relearning faces and a negative one for characters. Given a reduction in connectivity for the "faces" group under escitalopram, higher performance implies a smaller reduction. Thus, under escitalopram, a decreased connectivity between LG and mPFC is accompanied by an increased dependency of posterior-anterior communication on learning performance. The bidirectionality of the decrease might be related to a serotonergic modulation of affective feedback processes (Rudrauf et al., 2008).

<u>The role of Broca's area in learning</u>

BA is involved in numerous aspects of speech (Fujii et al., 2016), including inner speech (Morin & Hamper, 2012), and mnemonic strategies (Love, Haist, Nicol, & Swinney, 2006).

Even though reading words was shown to lead to electrical activity in BA (Magrassi, Aromataris, Cabrini, Annovazzi-Lodi, & Moro, 2015) no relationship to nouns as linguistic objects was found (Faroqi-Shah, Sebastian, & Woude, 2018). Theories of a topologically distinct representation of nouns in temporal regions (Vigliocco, Vinson, Druks, Barber, & Cappa, 2011) have also failed to gain meta-analytical 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, Perrett, Waiter, & Pechey, 2007), which ascribe BA also a serotonergic modulatable role in emotion processing.

For relearning faces, a strong decrease in connectivity towards BA was observed under placebo, which was inverted under escitalopram. This is in contrast to the connectivity changes between mPFC and LG, where escitalopram reversed the direction of the change for relearning faces. 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 connections between the LG and Broca's area showed a strong positive dependence on all three learning parameters only during relearning of faces under placebo. The application of SSRIs influenced this association by reducing the dependence of LG-Borca connectivity on the learning rate. The complexity of this relationship is also indicated by the interaction of learning capacity and rate on GFC (Figure 2D). In contrast, the connectivity changes between LG and BA when initially learning character-noun associations is positively correlated with learning capacity and performance, which might be expected based on the role of BA in language.

Modulation along the ventral visual stream

The connections between the LG and the rITG run along the ventral visual stream (VVS). It generally connects the visual and the inferior temporal cortex and is implicated in object recognition and identification (Goodale & Milner, 1992). The plasticity of this pathway and the effects of its modulation via transcranial direct current stimulation on memory encoding were recently shown (C. Zhao & Woodman, 2021). The inferior temporal cortex itself is also involved in short-term (Ranganath, Cohen, Dam, & D'Esposito, 2004) and long-term memory (Wagner et al., 2016; Wagner et al., 2019), object naming and identification (Acres, Taylor, Moss, Stamatakis, & Tyler, 2009).

Semantic relearning led to an increased connectivity from BA and a reduced one from the mPFC to the rITG. The opposite changes were observed for emotional relearning. This shows that the communication from the mPFC and BA towards the rITG is context-dependent during relearning, matching their roles in language (Fujii et al., 2016) and emotional processing (Etkin, Egner, & Kalisch, 2011; Parent et al., 2011; Zaki et al., 2012).

The importance of the LG for visual memory is well-established (Bogousslavsky, Miklossy,

Deruaz, Assal, & Regli, 1987) together with its involvement in facial (Puce, Allison, Gore, & McCarthy, 1995) and word form processing (Mechelli, Humphreys, Mayall, Olson, & Price, 2000; Xiao et al., 2005). Hemispheric differentiation was previously suggested with the lLG being more active during memorizing faces and the right LG during passive viewing (Kozlovskiy et al., 2014). This is also reflected in the current results as increased connectivity towards the lLG after learning to match face pairs. Besides visual memory and processing, the LG and IFG were shown to be important for the analysis of novelty and spatial information (Menon, White, Eliez, Glover, & Reiss, 2000). This might explain the differences in effective but not global connectivity between the test-retest and baseline scan.

Where the information flow for visual processing is directed from the visual to the temporal cortex, also feedback mechanisms from emotion-related structures were shown (Rudrauf et al., 2008). Moreover, plasticity in the visual cortex was demonstrated following emotional learning (Meaux, Sterpenich, & Vuilleumier, 2019). Escitalopram intake during relearning increases the communication in processing direction for characters and in feedback direction for faces. The latter is highly reasonable in light of the role of the rITG and the effects of SSRIs on emotion processing (Browning et al., 2007; Harmer et al., 2003; Pringle et al., 2011). Repetition suppression induced by daily learning could provide an explanation for the overall reduction from baseline to post-learning (Prčkovska et al., 2017). Serotonergic modulation of the 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 LG as part of the visual cortex (Chen et al., 2011; Maya Vetencourt et al., 2008). As before, strengthened connections along the VVS are related to decreased correlations with the relearning parameters and vice versa implying the same relationship.

Limitations

Despite the comparably 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 more likely baseline differences. 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. Even though serotonergic modulation of the correlations between connectivity and behavior was found, no direct influence of escitalopram on learning performance itself was detected. However, also previous findings on effects of SSRIs on learning performance were contradictory (Barkas et al., 2012; Chamberlain et al., 2006).

Conclusion

A learning content, setting sensitive and serotonergic modulated functional brain network and its behavioral correlates were mapped. The intake of escitalopram compared to placebo

during semantic relearning led to a general increase in connectivity pointing towards facilitated neuroplasticity.
Depending on the learning content, three network-specific scenarios were further identified: Between the mPFC and the LG, the intake of escitalopram during relearning potentiated the bidirectional decrease in connectivity for emotional and the increase for sematic learning. The decrease in connectivity towards BA for faces was inverted by the SSRI. Finally, along the VVS, escitalopram led to increased feedforward connectivity for relearning characters and increased feedback for faces. The affected connections towards the LG also indicate a general serotonergic modulation of emotional feedback processes.
These content-dependent changes match the theory that in depression SSRIs improve neuroplasticity rather than mood (Alboni et al., 2017; Chiarotti et al., 2017). This makes patients more susceptible to environmental influences, ideally providing a setting that supports the therapeutic endeavor. Moreover, context-dependent correlations of the relearning-induced connectivity changes with performance were found especially from the LG to the mPFC. This might be related to extinction of previously learned content. A challenge for future studies addressing the highly complex interactions between learning, network adaptations, serotonergic modulation and behavior will be to adequately control for phenomena with common characteristics, such as the recognition of novelty. Finally, the results on context-dependent neuroplasticity require consideration in treatment studies using serotonergic medication, as they necessitate increased attention towards external factors.

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).

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. 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 and that of age with a Kruskal-Wallis test.

Study design

The overall study followed a randomized, double-blind, placebo-controlled longitudinal design. Three MRI examinations with 21 days between each session were conducted (i.e., the MRIs were performed on the 1st, 22nd and 43rd day). For a subsample of 55 subjects, 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 two groups learning to match either Chinese characters to random German nouns or faces to faces over 21 days. After the first learning period, in each of these groups, subjects were further randomized to receive either SSRIs or placebo for the subsequent relearning phase. During this 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 relearning phase.

Learning paradigm

Throughout the course of the study, the subjects had to perform an association-learning task with facial / emotional or semantic content. In both cases they had to learn 200 pairs of images via a daily online training at home. 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 take one session every 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. The schedule for the learning and relearning phases was identical, just the pairings were shuffled. During each MRI session, learning and retrieval tasks similar to those on the online platform were performed in the scanner.

Modelling the 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 (equations (2) and (1)):

$$w_{time}(s,d) = \begin{cases} \frac{|t_\Delta(s,d)|}{24[h]} \Big| & t_\Delta(s,d) \leq 24[h] \\ \frac{24[h]}{|t_\Delta(s,d)|} \Big| & t_\Delta(s,d) > 24[h] \end{cases} \tag{1}$$

with

$$t_\Delta(s,d) = t_s - t_d + (d-2) * 24[h] \tag{2}$$

Here, s denotes the current and d a previous session, t_Δ 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).

$$w(s) = w_{disc}(s) * w_{time}(s) \tag{3}$$

with the discounting weight

$$w_{disc}(s,d) = \frac{d}{\frac{s*(s+1)}{2}} \tag{4}$$

To keep the weighting w_{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

$$y_{exp}\left(x(s)\right) = k * \left(1 - e^{-\frac{p+x(s)}{r}}\right) \tag{5}$$

$$y_{hyp}\left(x(s)\right) = k * \frac{x(s)+p}{x(s)+p+r} \tag{6}$$

where x is the adjusted training success, k the learning capacity, r the learning rate and p the previous knowledge from the varying in-scanner session (Anzanello & Fogliatto, 2011). Due to equal complexity, the models were compared by a paired t-test over the Fisher-transformed model fits ($\overline{R^2{}_{exp}}$ = 88.13%, $\overline{R^2{}_{hyp}}$ = 87.53%, p = 1E-4; 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 corrected for irregularities in learning. For further statistical analyses, Y was rescaled and Fisher-z-transformed to an unbound distribution (8) (Klöbl, Michenthaler, et al., 2020).

$$Y_{exp}\left(x(s)\right) = k * r * e^{-\frac{p+x(s)}{r}} + k * x(s) \tag{7}$$

$$Y_z = \operatorname{atanh}\left(\frac{2 * Y_{exp} - 100}{100}\right) \tag{8}$$

<u>MRI acquisition and processing</u>

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: 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) 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 SPM12 and custom MATLAB 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 (MNI) 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.

For the GFC analysis, the Friston-24 model (K. J. Friston, Williams, Howard, Frackowiak, & Turner, 1996), an adapted version of the CompCor method with an automated scree approach (Klöbl, Michenthaler, et al., 2020) and sine/cosine terms limiting the passband to 0.01-0.10 Hz were regressed out of the data (Hallquist, Hwang, & Luna, 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). For the subsequently built DCMs, the smoothed data was reprocessed using the 1st-level GLM in SPM12 again correcting for the Friston-24 and CompCor regressors (Esménio et al., 2019). Autocorrelation was set to "FAST" (Olszowy, Aston, Rua, & Williams, 2019).

Statistical inference

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", "substance" and "phase" (learning, relearning; as opposed to the three measurements) using linear mixed effects models (LMEs) with a random intercept per subject, an additional random "phase" slope for rate and "group"-slope for performance. Covariance structures and random factors were chosen as to minimize the Akaike information criterion. Analyses of variance were used to further investigate the effect of "substance" on relearning alone. The learning capacity was rank- and the rate log-transformed as indicated by the residual plots (Conover & Iman, 1981). Values were excluded as outliers if located further than three standard deviations from the mean. Multiplicity was controlled for using permutation tests with 1000 runs to account for dependencies between the variables.

For whole-brain analysis, the GFC maps were Fisher-transformed and entered into a flexible factorial 2nd-level model in SPM. The model included factors for "group", "substance" and "measurement" and the results were familywise-error-corrected to $p_{Cluster} \leq 0.05$ at the cluster-level with a primary peak-level threshold of $p = 0.001$. The interaction effects were estimated and post-hoc comparisons adjusted using the Sidak correction. The median GFC values for the significant regions were extracted using the MarsBaR toolbox and changes in GFC modeled depending on the interaction of "group", "substance", "phase" and the

learning parameters treated as above.
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 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) (Tagliazucchi et al., 2016). The Fisher-transformed SBCA maps were fed into the same model as the GFC ones. Results were corrected for the number of seeds and post-hoc comparisons using the Sidak method.

DCM analysis

The first temporal eigenvariates of clusters from the original 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 set up and estimated for all measurements. The 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 reduction (Karl J. Friston et al., 2016) was finally utilized to prune connections with high uncertainty.
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 “substance” were investigated. The learning parameters were transformed as before. No outliers were excluded at this stage. Final Bayesian model reduction was applied as above.

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Competing Interests

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.

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