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Title

Increased neural activity in mesostriatal regions after prefrontal transcranial direct current stimulation and L-DOPA administration

(Abbreviated title: Neural effects of tDCS and L-DOPA)

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Abstract

Dopamine dysfunction is associated with a wide range of neuropsychiatric disorders commonly treated pharmacologically or invasively. Recent studies provide evidence for a nonpharmacological and noninvasive alternative that allows similar manipulation of the dopaminergic system: transcranial direct current stimulation (tDCS). In rodents, tDCS has been shown to increase neural activity in subcortical parts of the dopaminergic system and recent studies in humans provide evidence that tDCS over prefrontal regions induces striatal dopamine release and affects reward-related behavior. Based on these findings, we used functional magnetic resonance imaging in healthy human participants and measured the fractional amplitude of low frequency fluctuations (fALFF) to assess spontaneous neural activity strength in regions of the mesostriatal dopamine system before and after tDCS over prefrontal regions (n=40, 22 females). In a second study, we examined the effect of a single dose of the dopamine precursor levodopa (L-DOPA) on mesostriatal fALFF values in male humans (n=22) and compared the results between both studies. We found that prefrontal tDCS and L-DOPA both enhance neural activity in core regions of the dopaminergic system and show similar subcortical activation patterns. We furthermore assessed the spatial similarity of whole-brain statistical parametric maps, indicating tDCS- and L-DOPA-induced activation, and more than one hundred neuronal receptor gene expression maps based on transcriptional data from the Allen institute for brain science. In line with a specific activation of the dopaminergic system, we found that both interventions predominantly activated regions with high expression levels of the dopamine receptors D2 and D3.

Significance Statement

Studies in animals and humans provide evidence that transcranial direct current stimulation (tDCS) allows a manipulation of the dopaminergic system. Based on these findings, we used functional magnetic resonance imaging (fMRI) to assess changes in spontaneous neural activity strength in the human dopaminergic system after prefrontal tDCS in comparison to the administration of the dopamine precursor and standard anti-Parkinson drug levodopa (L-DOPA). We found that prefrontal tDCS and L-DOPA both enhance neural activity in core regions of the dopaminergic system and show similar subcortical activation patterns. Using whole-brain transcriptional data of more than one hundred neuronal receptor genes, we found that both interventions specifically activated regions with high expression levels of the dopamine receptors D2 and D3.

Introduction

The functional diversity of the dopaminergic system explains the close relationship between dopamine dysfunctions and a multifaceted range of neuropsychiatric disorders, including Parkinson's disease (PD), addiction, and schizophrenia (Kalivas and Volkow, 2005; Goto and Grace, 2007; Galvan and Wichmann, 2008). Thus, therapeutic strategies for manipulating dopaminergic system activity are of great clinical relevance, but, despite major advances, they are frequently accompanied by sometimes severe side effects (Katzenschlager and Lees, 2002; Foster and Hoffer, 2004; Appleby et al., 2007).

There is now increasing evidence from studies in animals and humans that transcranial direct current stimulation (tDCS) might be an effective non-pharmacological and non-invasive way to activate deep brain regions of the dopaminergic system. tDCS is a form of sub-threshold brain stimulation that is based on a weak constant current, applied between an anodal and a cathodal electrode both placed on the scalp. Anodal stimulation causes the resting membrane potential to become slightly more positive, whereas cathodal stimulation slightly hyperpolarises the membrane. Hence, rather than causing neurons to fire, tDCS is supposed to modulate their excitability (Nitsche and Paulus, 2000; Rahman et al., 2013).

Takano et al. (2011) combined tDCS with fMRI in rats and observed increased fMRI signal intensities in the nucleus accumbens (NAcc) after anodal stimulation over the frontal cortex. Using the same electrode placement, Leffa et al. (2016) found elevated striatal dopamine levels after tDCS. Moreover, Lu et al. (2015) showed that anodal tDCS over the frontal cortex not only increased whole-brain dopamine levels but also relieved symptoms in a mouse model of PD, comparable in effect to L-DOPA, a standard anti-PD drug, which is converted to dopamine in the intracellular space of dopaminergic midbrain neurons (Volkow et al., 1996).

Several studies in humans have already demonstrated effects on neural activity in striatal areas during and after tDCS over prefrontal and motor cortical areas (Polanía et al., 2012; Chib et al., 2013; Hone-Blanchet et al., 2016) and a recent study provided first molecular evidence of elevated striatal dopamine levels after prefrontal tDCS in humans (Fonteneau et al., 2018). Based on these results, we examined the enhancement of neural activity in regions of the dopaminergic system before and after prefrontal tDCS and compared the effect to a pharmacological stimulation of dopamine synthesis.

We conducted two separate resting-state fMRI (rsfMRI) studies in healthy humans. In the first study (tDCS study), we applied a tDCS protocol developed by Chib et al. (2013), who provided first evidence for a causal manipulation of distant dopaminergic brain regions and associated dopamine-dependent functions after anodal stimulation over the frontopolar/ventromedial prefrontal cortex (fp/vmPFC, 10-20 electrode system: Fpz) and cathodal stimulation over the right dorsolateral prefrontal cortex (dlPFC, F4). In a parallel study design, we used the anodal/cathodal Fpz/F4 montage in the experimental group (main, n=20) and the same electrode locations but inverse polarity in a control group (inverse, n=20). We examined the fractional amplitudes of low-frequency signal fluctuations (fALFF), a proxy of spontaneous neural activity strength (Zou et al., 2008), in subcortical regions of the dopaminergic system before and after prefrontal tDCS. The same analysis was performed in a second study (L-DOPA study, n=22), in which the effect of a single dose of L-DOPA versus placebo was examined in a cross-over design.

The effect of tDCS and L-DOPA on fALFF was examined in a dopaminergic system mask and fALFF changes in predefined subcortical regions (subcortical activation profiles) were compared between both interventions. We furthermore compared tDCS- and L-DOPA-induced whole-brain activity patterns with >100 neuronal receptor gene expression maps, based on transcriptional data from the Allen institute for brain science, to assess similarities

between the interventions in receptor-specific activation patterns and to analyse the relative specificity for regions with high dopamine receptor expression.

To support L-DOPA-induced fALFF changes in the dopaminergic system from a cross-species perspective, we also report results from a study in which we analyzed fALFF in the dopaminergic system of medetomedine sedated rats (n=6).

Materials and Methods

Experimental Design and Statistical Analysis

Procedures tDCS study (humans). 42 healthy participants were enrolled. The Ethics Committee of the State Medical Board in Rheinland-Pfalz, Germany, approved the study, and all participants gave written informed consent. Regular use of illegal drugs was an exclusion criterion. Two participants were excluded due to technical problems (n=40).

In a single-blind parallel study design, participants were randomly assigned to the main (n=20, 11 females, mean age: 25.7 years, age range: 21-32 years) or the inverse group (n=20, 11 females, mean age: 25.1 years, age range: 19-32 years). There was no significant age difference between the groups (t_{38}=0.57, p=0.573, two-tailed *t*-test). rsfMRI data were acquired before (pre) and ~5min after (post) tDCS application. Prior to the first rsfMRI measurement, electrode positions were marked on the participant's head to allow a fast electrode placement after the first scan. The 10-20 international system for electroencephalography was used for electrode positioning. We employed a tDCS protocol developed by Chib et al. (2013) and placed a 3.5 cm x 3.5 cm (12.25 cm^2) anode with its center over electrode position Fpz and a 5 cm x 5 cm (25 cm^2) cathode over electrode position F4 in the main group. Using this specific protocol, Chib et al. were able to modulate

fronto-midbrain interactions and reported a correlation between tDCS-induced neural effects and reward-related behavioral changes. Hence, the authors found evidence for a causal manipulation of distant dopaminergic brain regions and associated dopamine-dependent functions. For the control condition, we also followed Chib et al., who, after an extensive series of testing, selected an active control condition with maximum similarity to the experimental condition in which the same electrode placement was used with inverse polarity. The electric field distribution was simulated using the SimNIBS software package (Thielscher et al., 2015).

tDCS was applied using a battery-driven constant-current stimulator (DC-Stimulator, neuroConn GmbH, Ilmenau, Germany). Constant current was delivered for 15 min at 2 mA intensity (20 s ramp in and 20 s ramp out) through conductive rubber electrodes inserted into saline-soaked sponge pockets. Controlled by the DC-Stimulator, the impedance was kept < 10 kΩ.

Procedures L-DOPA study (humans). 24 healthy male participants were enrolled. Participation was restricted to male participants because of potential estrogen-dopamine interaction effects on brain activity (Sánchez et al., 2012). A board-certified physician screened participants for contraindications of L-DOPA intake. Participants who reported to take illegal drugs on a regular basis were excluded. Abuse of illegal drugs was tested by urine drug screen (M10/3-DT; Diagnostik Nord). Participants were asked about their smoking habits but only three participants were smokers (each <5 cigarettes/day). The Ethics Committee of the State Medical Board in Rheinland-Pfalz, Germany, approved the study and all participants gave written informed consent. Two participants were excluded from rsfMRI analyses due to head motion and severe tiredness. Head motion was assessed based on realignment parameters (see rsfMRI data preprocessing [humans]) and tiredness was assessed by monitoring the right eye of the participant using an MRI-compatible camera (MR Cam Model 12M; MRC Systems, Germany). Participants who closed their eyes continuously

221 or repeatedly for more than ~5 sec during rsfMRI scans were excluded. Eventually, rsfMRI
222 data from 22 participants were analyzed (mean age: 29.3 years, age range: 25-39 years).
223
224 rsfMRI data were acquired on two measurement days (day 1 and 2), separated by at least 5
225 and not more than 14 days (Damoiseaux et al., 2006). In a cross-over design, each
226 participant received either L-DOPA at day 1 followed by a placebo treatment at day 2 or vice
227 versa. A third person randomly assigned participants to one of the two treatment sequences.
228 Experimenter and participants were both blinded. Participants were told not to eat 1.5 h prior
229 to the L-DOPA / placebo intake. Drugs were administered orally as capsules of 150 mg of L-
230 DOPA with 37.5 mg benserazide (Levodopa-Benserazid-ratiopharm®, Germany) or an
231 identically looking placebo capsule filled with mannitol and aerosil. Drugs were prepared and
232 provided by the pharmacy of the University Medical Center Mainz. On both days, L-
233 DOPA/placebo administration was directly followed by an rsfMRI baseline measurement at
234 which no L-DOPA effect can be expected (LD_{0min}/Plc_{0min}). Further rsfMRI scans were
235 performed after 45 (LD_{45min}/Plc_{45min}) and 90 min (LD_{90min}/Plc_{90min}), to capture the approximate
236 times of maximum L-DOPA plasma concentration (Benetello et al., 1997; Hilal-Dandan and
237 Brunton, 2014). Comparable time points have been chosen in other studies examining the
238 effect of L-DOPA on resting state activity (Flodin et al., 2012; Cole et al., 2013; Haaker et al.,
239 2013). Participants stayed under medical observation for the duration of the experiment,
240 including heart rate and blood pressure measurements and questionnaires on potential side
241 effects.
242
243 **Procedures L-DOPA study (rats).** In an additional animal rsfMRI study, we tested the effect
244 of L-DOPA on mesostriatal fALFF values in rats. Female Lewis rats (n=6; >12 weeks old;
245 160–180 g) were used in this experiment due to their limited growth in comparison to males.
246 Each animal was scanned under placebo (NaCl, 0.9%) and L-DOPA. The order was
247 randomized and the time interval between both measurements was at least one week.
248 Animals were anesthetized with isofluorane 1.5% (Forene, Abbott, Wiesbaden, Germany)

during the scanner placement procedure. Temperature and breathing rate were monitored during the entire experiment by an MRI compatible monitoring system (SA Instruments, NY, USA). After the placement of the animal and the intraperitoneal injection of L-DOPA (10 mg/kg)+benzeraside (20 mg/kg) or placebo, a bolus of 0.04 mg/kg of medetomidine was administrated in order to obtain a persistent state that shows neuronal and BOLD activity that resembles the awake state (Schwalm et al., 2017). We confirmed the persistent brain states based on an additional scan applying visual stimuli, yielding localized activation of the primary visual cortex, in sharp contrast to the cortex-wide activation in slow wave state. Five minutes later, isoflurane anesthesia was turned off and medetomidine 0.08 mg/kg/h was perfused until the end of the experiment. rsfMRI scans were performed 45, 60, 75, 90 and 120 min after L-DOPA/ placebo administration in line with previous rodent studies showing that striatal dopamine peaks between 60 and 90 min after L-DOPA administration (Fornai et al., 1999).

fALFF analysis (humans and rats). The amplitude of low-frequency fluctuations (ALFF) of the rsfMRI signal has been introduced to assess the intensity of regional spontaneous brain activity in humans (Zang et al., 2007). To reduce the sensitivity to physiological noise, Zou et al. (2008) developed fractional ALFF (fALFF), which is defined as the ratio of the low-frequency amplitudes (0.01–0.08 Hz) to the amplitudes of the entire frequency range (0–0.25 Hz). In humans, fALFF analyses on preprocessed rsfMRI data were performed using the REST toolbox (Zang et al., 2007). In rats, the same frequencies were analyzed using an in-house matlab script (The Mathworks, Inc., Natick, MA). Since small changes in anesthesia levels can modify dramatically the amplitude of low frequency fluctuations in rats (Maandag et al., 2007), the obtained values were then normalized by the mean fALFF of the cortical and subcortical structures.

ROI analysis (humans). To test our a priori hypothesis of dopaminergic system activation, ROI analyses in humans were performed for a combined bilateral dopaminergic system

277 mask (including the nucleus accumbens [Nacc], caudate [Caud], putamen [Put], substantia
278 nigra [SN] and the ventral tegmental area [VTA]). NAcc, Caud and Put masks were created
279 based on the HO (Harvard Oxford) brain atlas (Frazier et al., 2005; Desikan et al., 2006;
280 Makris et al., 2006; Goldstein et al., 2007) using a tissue probability cut-off threshold of 50%.
281 The SN and the VTA were combined in a single previously published mask (Bunzeck and
282 Düzel, 2006; Düzel et al., 2009).
283
284 In the tDCS and the L-DOPA study in humans, fALFF values of voxels within the
285 dopaminergic system mask were averaged and analyzed in a repeated measures ANOVA
286 using SPSS (Version 23). In the tDCS study, stimulation group (main, inv) and time (pre,
287 post) were entered as between-subject and within-subject factors, respectively. In the L-
288 DOPA study, both treatment and time (0 min, 45 min and 90 min after drug administration)
289 were entered as within-subject factors. Partial eta-squared values (η_p^2) are reported as effect
290 size measures. ANOVA results were further characterized by Bonferroni-corrected post hoc
291 two-tailed *t*-tests (tDCS study: post- vs. pre-tDCS in both groups; L-DOPA study: 45 vs. 0
292 min, 90 vs. 0 min in both conditions). Bonferroni-corrected p-values are denoted as p_{Bonf}. p_{Bonf}
293 values greater than 1 are reported as $p_{Bonf}=1$.
294
295 A potential baseline difference between the main and the inverse group in the tDCS study
296 and between the L-DOPA and placebo condition in the L-DOPA study was tested by two-
297 tailed *t*-tests. Gender-related effects in the tDCS study were tested by adding gender as
298 between-subject factor to the repeated measures ANOVA. In the L-DOPA study, effects
299 related to treatment order were tested by adding treatment order as between-subject factor.
300
301 A spectral analysis of mesostriatal rsfMRI time courses was performed in the tDCS and in
302 the human L-DOPA study using the REST toolbox (Zang et al., 2007) to inspect if non-
303 resting state frequencies were affected by the two manipulations. For each subject, power
304 spectra were calculated from unfiltered time courses of voxels within the dopaminergic

system mask. Next, power spectra were averaged across voxels, smoothed by a moving average and finally normalized.

ROI analysis (rats). A mask, covering the entire striatum and the SN was applied as dopaminergic system mask in the L-DOPA rat study using the atlas template from Valdés-Hernández et al. (2011). Averaged fALFF values were analyzed in a repeated measures ANOVA with treatment and time (45 min, 60 min, 75 min and 90 min after drug administration) as within-subject factors using SPSS (Version 23). Significant effects were further characterized by means of two-tailed *t*-tests (uncorrected).

Voxel-wise analysis (humans). To investigate the anatomical distribution of fALFF effects within the human dopaminergic system, voxel-wise analyses were performed for each subregion of the dopaminergic system mask (bilateral NAcc, Put, Caud and the SN/VTA) using the MatLab toolbox Statistical Parametric Mapping 8 (SPM8, Wellcome Trust Centre for Neuroimaging, UK). fALFF values were entered into a group analysis using SPM's flexible factorial design. In the tDCS study, stimulation group and time were entered as between-subject and within-subject factors, respectively. In the L-DOPA study, both treatment and time were entered as within-subject factors. tDCS-induced activation in the main as compared to the inverse group was tested by the following contrast: $[tDCS_{main,post} - tDCS_{main,pre}] > [tDCS_{inv,post} - tDCS_{inv,pre}]$. To test for L-DOPA-induced effects after 45 and 90 min, the following contrasts were calculated: 45 min = $[LD_{45min} - LD_{0min}] > [Plc_{45min} - Plc_{0min}]$, 90 min = $[LD_{90min} - LD_{0min}] > [Plc_{90min} - Plc_{0min}]$. Baseline differences between the tDCS groups and the L-DOPA / placebo condition were tested in all subregions of the dopaminergic system in both directions ($tDCS_{main,pre} > tDCS_{inv,pre}$, $tDCS_{main,pre} < tDCS_{inv,pre}$; $LD_{0min} > Plc_{0min}$, $LD_{0min} < Plc_{0min}$). Family-wise error (FWE) correction was performed for voxel-level inference as implemented in SPM8 at a threshold of $\alpha = 0.05$ for each region (SVC = small-volume correction).

In both studies, the peak voxel cluster was further characterized by a functional connectivity (FC) analysis. FC-maps were z-transformed and analyzed on a whole brain level using SPM's flexible factorial design (see above for contrasts).

Analysis of subcortical activation profiles (humans). To examine tDCS- and L-DOPA-induced effects in single subregions of the dopaminergic system (bilateral NAcc, Put and Caud) and to test if subcortical regions that are not part of the predefined dopaminergic system mask (bilateral hippocampus, amygdala, thalamus, pallidum and the brainstem) were significantly affected, we analyzed changes in averaged fALFF values for the entire set of 15 subcortical grey matter HO atlas regions. tDCS- and L-DOPA-related activation was analyzed by means of Bonferroni-corrected two-tailed *t*-tests (tDCS: $[tDCS_{main,post} - tDCS_{main,pre}]$ vs. $[tDCS_{inv,post} - tDCS_{inv,pre}]$; L-DOPA (45 min): $[LD_{45min} - LD_{0min}] - [Plc_{45min} - Plc_{0min}]$; L-DOPA (90 min): $[LD_{90min} - LD_{0min}] - [Plc_{90min} - Plc_{0min}]$). To assess the relationship of tDCS- and L-DOPA-induced subcortical activation profiles, corresponding effect sizes (Cohen's d) were calculated for each single region and compared between the two interventions by means of Pearson correlation (r, α=0.05).

Analysis of gene-fALFF similarity profiles (humans). We compared uncorrected group-level statistical parametric maps (*t*-maps) resulting from voxel-wise analyses with gene expression maps based on transcriptional data from the Allen institute for brain science (AIB) (Hawrylycz et al., 2012). Our aim was to examine similarities between brain activation maps and different neuronal receptor gene expression patterns, including those of the dopamine receptors D1-D5 (DRD1-DRD5). The AIB provides whole-brain-sampled transcriptional data of over 20000 genes based on six post-mortem brains. Averaged and smoothed (6 mm hard sphere) AIB gene expression maps were available from the Neurosynth database (Yarkoni et al., 2011). We restricted our analysis to genes encoding receptors of eleven preselected neurotransmitters and neuromodulatory ligands, resulting in a total number of 115 genes (adrenergic receptors: ADRA1A, ADRA1B, ADRA1D, ADRA2A-C, ADRB1-3; cholinergic

receptors: CHRM1-5, CHRNA1-5, CHRNA7-10, CHRNB1-4, CHRND, CHRNE, CHRNG; cannabinoid receptors: CRN1, CRN2; corticotropin releasing hormone receptors: CRHR1, CRHR2; dopamine receptors: DRD1-5; GABA receptors: GABARAP, GABBR1, GABBR2, GABARAPL1, GABARAPL2, GABRA1-6, GABRB1-3, GABRD, GABRE, GABRG1-3, GABRP, GABRQ, GABRR1-3; glutamate receptors: GRIA1-4, GRID1-2, GRIK1-5, GRIN1, GRIN2A-D, GRIN3A-B, GRINA, GRIP1-2, GRM1-8; 5-hydroxytryptamine receptors: HTR1A-B, HTR1D-F, HTR2A-C, HTR3A-E, HTR4, HTR5A, HTR6-7; glucocorticoid receptor: NR3C1; BDNF receptor: NTRK2; opioid receptors: OPRD1, OPRK1, OPRL1, OPRM1). Pearson correlations were calculated between *t*-maps (tDCS: [$tDCS_{main,post}$ - $tDCS_{main,pre}$] > [$tDCS_{inv,post}$ - $tDCS_{inv,pre}$]; L-DOPA: [LD_{45min} - LD_{0min}] > [Plc_{45min} - Plc_{0min}], 90 min = [LD_{90min} - LD_{0min}] > [Plc_{90min} - Plc_{0min}]) and gene expression maps. *t*-values of different voxels with the same gene expression intensity were averaged so that each unique gene expression intensity value was paired with a single averaged *t*-value in the correlation analysis. For each contrast we obtained a profile of correlation coefficients, indicating similarities between the induced fALFF pattern and 115 gene expression patterns (gene-fALFF similarity profile = GFS profile). GFS profiles were z-transformed and compared between the two studies using Pearson correlations (r, α=0.05) to analyse to what extent both interventions showed comparable receptor-specific activation patterns. To furthermore assess the relative specificity for dopaminergic target regions, we determined for both interventions the percentage of genes showing higher z-scores than a specific dopamine receptor gene (i.e., a small percentage indicates a high specificity). The analysis was performed for all dopamine receptors (DRD1-DRD5).

Data recording and processing

rsfMRI data recording (humans). In humans, rsfMRI data were obtained with a 3 Tesla MR scanner (MAGNETOM Trio; Siemens, Germany) by using a 32-channel head coil. Blood oxygenation-level dependent (BOLD) signal was acquired using a T2*-sensitive gradient echo-planar imaging (EPI) sequence with simultaneous multislice (SMS) acquisition

technique (TR: 1.0 s; TE: 29 ms; multiband acceleration factor: 4; field of view: 210×180 mm; 2×2 mm in-plane resolution). Each 3D-image comprised 60 contiguous axial slices (2.5 mm thick). The position of the slice package was individually adjusted for whole-brain image acquisition. Participants were instructed to remain awake with their eyes open. A fixation cross was presented on the screen center during scans. Time series of 600 rsfMRI images were acquired per session (10 min) in the tDCS study and 480 rsfMRI images per session (8 min) in the L-DOPA study. To account for T1 equilibrium effects, the first five images of each time series were discarded. This resulted in 595 and 475 rsfMRI images per session for the tDCS and the L-DOPA study, respectively. At the end of the experiment, a high-resolution T1-weighted structural image was further acquired.

rsfMRI data preprocessing (humans). rsfMRI data were preprocessed using SPM8. Preprocessing of the rsfMRI data first involved realignment to correct for head movements. rsfMRI data were then coregistered with corresponding T_1-weighted anatomical images and normalized to a standard template from the Montreal Neurological Institute (MNI) in order to allow for group comparisons. Finally, rsfMRI data were spatially smoothed with a 6 mm full-width-at-half-maximum (FWHM) isotropic Gaussian kernel. Participants showing head displacements greater than 2.5 mm (slice thickness; values were extracted from realignment parameters) were excluded. To further correct for possible effects of movement and other systemic effects, the six movement parameters resulting from realignment and mean cerebrospinal fluid (CSF) and white matter (WM) time series were regressed out.

Mean framewise displacements (humans). We analyzed mean framewise displacements (Power et al., 2012) to test if tDCS and L-DOPA had specific effects on head movement and if head movement differed between tDCS groups. Mean framewise displacements were calculated from movement parameters and analyzed in repeated measures ANOVAs (tDCS: stimulation x time interaction; L-DOPA: treatment x time interaction; see ROI analysis for a detailed description of the ANOVA design).

rsfMRI data recording and preprocessing (rats). rsfMRI data acquisition was performed on a 9.4 T small animal imaging system with a 0.7 T/m gradient system (Biospec 94/20, Bruker Biospin GmbH, Ettlingen, Germany). For rsfMRI measurements, T2*-weighted images were acquired with a single-shot gradient EPI sequence with TR = 1.5 s, TE = 14 ms, FA 65°, 320 × 290 µm, slice thickness 0.8 mm. Altogether, 600 images, each comprising 34 contiguous slices, were acquired per scan, resulting in a scan time of 15 min. Each scan of 600 images was preprocessed using Brain Voyager QX (vs. 2.8.4, Brain innovation, Maastricht, The Netherlands). rsfMRI data were realigned to correct for head movements and smoothed with a 0.7 mm FWHM isotropic gaussian kernel. Using the same software, rsfMRI data were manually aligned to a T1 template from (Valdés-Hernández et al., 2011) containing 150 cortical and subcortical regions. Finally, a high-resolution T1-weighted structural image (0.125×0.125×0.125mm) was acquired.

Results

Side effect reports and mean framewise displacements. All participants of the tDCS study reported a mild tingling under the electrodes during stimulation, irrespective of the group. However, no other somatic or psychological side effects that were attributable to the intervention were reported. Likewise, no treatment-related side effects were reported in the L-DOPA study. Analyzing mean framewise displacements revealed that tDCS and L-DOPA had no specific effects on head movement (tDCS: stimulation x time interaction: $F_{1,38}$=1.28, p=0.264; L-DOPA study: treatment x time interaction: $F_{2,42}$=0.7, p=0.503) and there was also no significant difference between tDCS groups ($F_{1,38}$=0.65, p=0.426).

Electric field simulation (tDCS study). A simulation of the electric field (anode: Fpz, cathode: F4) indicated maximal electric field strength (~0.3 V/m) in the right superior and middle frontal gyrus (Figure 1). Field strengths were comparable to those generated by other

443 prefrontal montages (see e.g. Saturnino et al., 2015) suggesting that a substantial amount of
444 current passed through cortical areas.
445
446 **ROI analysis (tDCS study).** We first tested our a priori hypothesis that stimulation in the
447 main group remotely activates brain areas of the subcortical dopaminergic system as
448 compared to stimulation with inverse polarity. When averaging across voxels in a
449 dopaminergic system mask (Figure 2A), we found significant main effects of time
450 ($F_{1,38}$=22.16, p<0.001, η_p^2=0.37) and stimulation ($F_{1,38}$=7.3, p=0.01, η_p^2=0.16), which however
451 could be explained by a significant stimulation x time interaction ($F_{1,38}$=13.77,
452 p<0.001, η_p^2=0.266; Figure 2B). A strong fALFF increase in the main as opposed to the
453 inverse tDCS group from pre to post stimulation was supported by post hoc *t*-tests (main:
454 t_{19}=5.08, p_{Bonf}<0.001; inv: t_{19}=0.89, p_{Bonf}=0.77). Power spectra, calculated from time courses
455 of masked mesostriatal voxels, indicated that tDCS most strongly amplified classical resting-
456 state frequencies ranging from 0.01 to 0.08 Hz (Figure 3A).
457
458 There was no significant fALFF difference between the two groups at baseline (t_{38}=-1.22,
459 p=0.231) and no significant effects of gender were found (main effect of gender: $F_{1,36}$=0.68,
460 p=0.416; time x stimulation x gender interaction: $F_{1,36}$=0.22, p=0.642).
461
462 **ROI analysis (L-DOPA study in humans).** We found a significant treatment x time
463 interaction indicating an L-DOPA-induced fALFF increase in the dopaminergic system
464 ($F_{2,42}$=4.17, p=0.022, η_p^2=0.166; Figure 2C). The interaction subsumed a main effect of time
465 ($F_{2,42}$=5.34, p=0.009, η_p^2=0.20). Post hoc *t*-tests revealed a significant fALFF increase 90 min
466 but not 45 min after L-DOPA administration (90 min: t_{21}=4.35, p_{Bonf}<0.001; 45 min: t_{21}=1.17,
467 p_{Bonf}=1). fALFF increases after placebo administration were not supported (90 min: t_{21}=0.66,
468 p_{Bonf}=1; 45 min: t_{21}=0.48, p_{Bonf}=1). As for tDCS, the spectral power analysis supported major
469 effects on resting-state frequencies (Figure 3B).
470

Treatment order had no significant effect on fALFF values (main effect: $F_{1,20}$=0.02, p=0.878, treatment x time x order interaction: $F_{2,40}$=1.96, p=0.154). There was no significant difference between the two conditions at baseline (t_{21}=0.95, p=0.354).

ROI analysis (L-DOPA study in rats). In an additional L-DOPA study, we conducted a comparable experimental approach in rats, masking the homologous brain regions, with recordings performed at 45, 60, 75, 90 and 120 minutes after L-DOPA / placebo administration. As in the human studies, we performed a ROI analysis on averaged fALFF values and found that L-DOPA led to a significant fALFF increase that reached its peak after 45 to 75 min (treatment x time interaction: $F_{4,20}$=3.62, p=0.023, η_p^2=0.43; Figure 4).

Voxel-wise analysis (tDCS study). Examining the voxel-wise spatial distribution of tDCS-induced effects within subregions of the dopaminergic system revealed significant activations in multiple striatal areas, including the bilateral Caud, NAcc, Put and the SN/VTA (Table 1). The right Put showed the strongest activation cluster, which was mainly located in dorsal parts of the striatum (Figure 5). None of the mesostriatal regions showed a baseline difference between the groups.

Extending the analysis of tDCS-induced effects to other subcortical HO atlas regions (bilateral hippocampus, amygdala, thalamus, pallidum and the brainstem) showed no further significant results and no significant FC changes were found for the right Put (peak voxel cluster).

Voxel-wise analysis (L-DOPA study in humans). In contrast to the analysis of averaged fALFF values, voxel-wise analyses revealed a significant activation 45 min after L-DOPA administration that was restricted to the SN/VTA. After 90 minutes, L-DOPA-induced effects were found in ventral parts of the striatum, where the strongest activation was observed at

499 the borderline between the left NAcc, Caud and Put (Table 2 and Figure 5). None of the
500 mesostriatal regions showed a significant baseline difference.
501
502 An exploratory analysis of all subcortical HO atlas regions furthermore indicated L-DOPA-
503 induced activations after 90 min in the brainstem (x,y,z: 6,-38,-44; p_{FWE}=0.009, SVC), left
504 pallidum (x,y,z: -24,-12,0; p_{FWE}=0.028, SVC), left thalamus (x,y,z: -12,-28,12; p_{FWE}=0.027,
505 SVC) and the left amygdala (x,y,z: -30,6,-22; p_{FWE}=0.049, SVC). No further activations were
506 found when analyzing the time window 45 minutes after treatment administration. The ventral
507 striatum peak cluster showed a significant L-DOPA-induced FC increase with the right
508 cerebellum after 90 min (x,y,z: 38,-52,-32; p_{FWE}=0.026, whole-brain analysis). No significant
509 L-DOPA-induced FC changes were found for the SN/VTA in the time window 45 min after
510 treatment administration.
511
512 **Analysis of subcortical activation profiles.** We examined tDCS-induced activity changes
513 (for each subcortical HO atlas region) on a region-wise anatomical scale and again found
514 peak activations in key regions of the mesostriatal dopamine system. The right and left Put
515 and Caud constituted the four most activated subcortical brain regions, of which the Put on
516 both sides reached the level of Bonferroni-corrected statistical significance ([$tDCS_{main,post}$ -
517 $tDCS_{main,pre}$] vs. [$tDCS_{inv,post}$ - $tDCS_{inv,pre}$]; right Put: t_{38}=3.93, p_{Bonf}=0.005; left Put: t_{38}=3.17,
518 p_{Bonf}=0.045). In the L-DOPA study, the right and left Caud constituted the most activated
519 subcortical brain regions 90 min after L-DOPA intake, of which the latter reached the level of
520 Bonferroni-corrected statistical significance ([LD_{90min} - LD_{0min}] - [Plc_{90min} - Plc_{0min}]; t_{21}=3.37,
521 p_{Bonf}=0.045). No significant activation was found 45 minutes after L-DOPA administration.
522
523 To compare subcortical activation profiles of both interventions, we next calculated effect
524 sizes (Cohen's d) for all subcortical HO atlas regions. No significant relationship was found
525 when comparing the effect size profiles for L-DOPA after 45 minutes with tDCS (r=-0.19,
526 p=0.504). However, there was a significant correlation when comparing the profiles for L-

527 DOPA after 90 minutes and tDCS (r=0.73, p=0.002; Figure 6) suggesting a potential analogy
528 of tDCS- and L-DOPA-induced subcortical effects.
529
530 **Analysis of gene-fALFF similarity profiles.** We finally calculated correlations between
531 tDCS-/L-DOPA-related brain activation maps (uncorrected whole-brain *t*-maps resulting from
532 voxel-wise analyses) and 115 receptor gene expression patterns (GFS profiles) to examine
533 the relative specificity of both interventions for regions with high dopamine receptor
534 expression and to compare GFS profiles between the two interventions. z-transformed GFS
535 profiles showed a strong correlation indicating that tDCS and L-DOPA activated brain regions
536 with similar receptor composition (tDCS vs. L-DOPA (90 min): r=0.88, p<0.001; tDCS vs. L-
537 DOPA (45 min): r=0.82, p<0.001; Figure 7).
538
539 Among all 115 preselected receptor genes, the DRD2 expression pattern most strongly
540 resembled the activation pattern induced by tDCS and only 1.74% of the gene expression
541 maps (i.e., two gene expression maps) correlated more with the tDCS-induced activation
542 pattern than the DRD3 expression map (DRD2 and the epsilon subunit of the GABA A
543 receptor [GABRE]). DRD2 and DRD3 expression maps also showed a strong correlation with
544 the L-DOPA-induced pattern after 90 min in comparison to other genes: As for tDCS, no
545 other gene expression map showed a stronger correlation than DRD2 and only four gene
546 expression maps (3.48%) correlated more with the L-DOPA-induced pattern than DRD3
547 (DRD2, 5-hydroxytryptamine receptor 1D [HTR1D], alpha-2B adrenergic receptor [ADRA2B],
548 alpha-2C adrenergic receptor [ADRA2C]; Figure 7). Hence, both interventions showed a high
549 similarity in their receptor-specific activation patterns and specifically activated regions with
550 high DRD2 and DRD3 expression levels.
551
552 Analyzing the L-DOPA effect 45 min after administration revealed that 5.21% and 7.83% of
553 all gene expression maps showed a stronger correlation with the brain activation pattern than
554 DRD2 and DRD3, respectively. In contrast to DRD2 and DRD3, other dopamine receptor

555 expression maps showed less similarity with tDCS- and L-DOPA-induced patterns, i.e., more
556 genes showed stronger correlations (tDCS: DRD1=14.78%, DRD4=75.65%, DRD5=33.04%;
557 L-DOPA (45 min): DRD1=40.87%, DRD4=60.87%, DRD5=51.3%; L-DOPA (90 min):
558 DRD1=8.7%, DRD4=49.57%, DRD5=57.39%). The difference between DRD2-3 on the one
559 hand and DRD1 and DRD4-5 on the other was also reflected when analyzing correlations
560 between the gene expression maps of the dopamine receptors indicating the strongest
561 correlation between DRD2 and DRD3 (r=0.67, Table 3).
562
563

564 ## Discussion

565 We tested the influence of prefrontal tDCS and L-DOPA on deep regions of the mesostriatal
566 dopamine system at rest in healthy humans. Regional neural activity strength was assessed
567 by means of fALFF analyses. Anodal/cathodal Fpz/F4 stimulation led to enhanced fALFF
568 values in key regions of the dopaminergic system. No effects were observed when the same
569 montage was used with inverse polarity. Increased activity in mesostriatal regions was also
570 found after L-DOPA administration. Both interventions showed distinct similarities in
571 subcortical and receptor-specific activation profiles, suggesting mechanistic commonalities
572 and a potential application of prefrontal tDCS in the treatment of dopamine dysfunctions.
573

574 To achieve better comparability between studies in animals and humans, we investigated
575 fALFF in task-free resting states. fALFF has been applied as a proxy measure of
576 spontaneous neural activity strength and has been used increasingly as a biomarker of
577 neuropsychiatric diseases, including Alzheimer's disease (Zhou et al., 2015), schizophrenia
578 (Xu et al., 2015) and depression (Liu et al., 2016). Several studies reported significant
579 correlations of fALFF values and symptom severity in patients (Chen et al., 2015; Fryer et al.,
580 2015) as well as behavioral performance in healthy humans (van Dam et al., 2015),
581 suggesting a close association between resting-state fALFFs and specific behavioral
582 parameters.

We employed a stimulation protocol developed by Chib et al. (2013) to remotely activate reward-related regions of the dopaminergic system. Chib et al. found increased task-related fMRI signal interactions between the vmPFC and the ventral midbrain after tDCS, and most importantly, participants with more enhanced fronto-midbrain interactions assigned higher attractiveness ratings when judging the attractiveness of computer-generated faces. These results indicated, for the first time, a tDCS-induced effect on midbrain parts of the dopaminergic system with direct behavioral consequences. However, an interpretation of Chib et al.'s results from a translational perspective is not straightforward, mainly because the authors investigated the effect of tDCS on reward-related brain activity in a task, which is only suited for human subjects.

Considering the neuroanatomical literature, anodal stimulation of glutamatergic projections from the vmPFC to the mesostriatal system may have evoked the observed fALFF increases (see e.g. Hedreen and DeLong, 1991; Frankle et al., 2006; Haber and Knutson, 2010). However, the electric field simulation indicated maximal field strength in right lateral cortical regions suggesting furthermore a crucial role of inhibitory cathodal stimulation over the right dlPFC (F4) in the remote activation of mesostriatal regions. Interestingly, Chib et al. tested multiple electrode montages but only observed effects on dopamine-dependent functioning when placing the cathode at F4 and the anode at Fpz. Furthermore, Fonteneau et al. (2018) reported elevated striatal dopamine levels after cathodal F4 and anodal F3 stimulation. Hence, one could speculate that the right dlPFC exerts inhibitory control over the dopaminergic system, which can be deactivated through cathodal stimulation. However, overall the electric fields generated by anodal/cathodal Fpz/F4 stimulation (used here and by Chib et al., 2013) and anodal/cathodal F3/F4 stimulation (used by Fonteneau et al., 2018) differ, which is against the hypothesis that both configurations activate the same neural pathway (see e.g. Austin et al., 2016; Bikson et al., 2018 for electric field maps of anodal/cathodal F3/F4 stimulation). An alternative explanation for striatal effects after both

anodal/cathodal Fpz/F4 and anodal/cathodal F3/F4 stimulation is provided by FC analyses in humans, showing a topological relationship between distinct cortical networks and specific striatal subregions (Choi et al., 2012). Hence, two different tDCS protocols, modulating activity in different parts of the prefrontal cortex (and thus possibly in different cortical networks), may both affect the striatum but through different cortico-striatal pathways and targeting different striatal subregions.

When comparing our results to those of Fonteneau et al. (2018), it should also be mentioned that Fonteneau et al. reported significant changes in dopamine release 20-35 min after tDCS but not directly after stimulation as shown for fALFF in the present study. To fully investigate this difference, it will be required to directly compare acute and late mesostriatal effects between the two tDCS protocols using both positron emission tomography (PET) and fMRI.

L-DOPA is converted to dopamine in the intracellular space of dopaminergic neurons, which form the neuroanatomical basis of dopamine-dependent neuromodulation (Volkow et al., 1996), while as previously stated, the effect of prefrontal tDCS is likely mediated by cortico-subcortical projections. Both interventions induced similar subcortical activation profiles on a region-wise anatomical scale, but however voxel-wise fALFF analyses also revealed intra-regional differences. This is not necessarily contradictory since ascending dopaminergic projections (activated by L-DOPA) and descending cortical projections (activated by tDCS) may target different parts of the same anatomical region. From a clinical perspective, this constitutes in fact a promising finding, as it points to a complementary and more effective stimulation of the dopaminergic system by combining both interventions in the treatment of dopamine dysfunctions.

So far there are only limited insights into the effects of L-DOPA on resting state fALFFs as most studies primarily focused on changes in FC (see e.g. Cole et al., 2013). One exception is the study by Flodin et al. (2012), who investigated L-DOPA-related fALFF changes in a set

639 of cortical and subcortical ROIs, but did not find significant striatal effects. However, it should
640 be mentioned that the authors employed a between subjects design and acquired data at
641 only 1.5 Tesla, which might have reduced the sensitivity to detect fALFF changes.
642
643 Using transcriptional data from the AIB, we found that both interventions manipulated neural
644 activity in dopaminergic target regions characterized by high DRD2 and DRD3 expression.
645 Although the analysis of gene expression maps may partly bridge the gap between
646 hemodynamic effects and molecular mechanisms, it is important to note that we do not
647 provide direct evidence for tDCS/L-DOPA-induced dopaminergic neurotransmission, which
648 can only be achieved with molecular imaging techniques. In particular, the combination of
649 fMRI and PET will finally help to understand the relationship between fALFF increases and
650 dopamine release.
651
652 In an additional study, we analyzed the effects of L-DOPA in lightly sedated rats, which
653 resembled those in awake humans. fALFF increases followed different temporal
654 characteristics as compared to humans, which however might be explained by several
655 factors that are difficult to control, most importantly differences in administration routes,
656 dosage, and metabolic rate. Recent rodent studies (Cha et al., 2016; Yan et al., 2017) as
657 well as our own results support fALFF as a suitable marker of intrinsic brain activity changes
658 in animals. Furthermore, it supports the notion, that a direct (back-)translation of a network
659 manipulation yields homologous oscillatory activity, provided that the brain state of the
660 animal is being carefully controlled (Schwalm et al., 2017).
661
662 Several methodological drawbacks of the present approach should be mentioned. In the
663 tDCS study, both genders were tested, whereas either only male humans or female rats
664 were tested in the L-DOPA studies. A careful control would have been conducive to the
665 comparability of both interventions and might have given additional information. Although
666 experimenter effects are unlikely to occur in task-free study designs, it should be mentioned

667 that the tDCS study was only single-blinded. In both studies, blinding efficacy was not
668 assessed, but none of the participants reported unexpected side effects or perceived
669 symptoms (apart from cutaneous sensations at the site of stimulation in both tDCS groups),
670 which at least reduces the risk of a significant experimental bias. Finally, our tDCS study
671 does not include a sham group to exclude unspecific time-dependent effects. However, we
672 assume that such effects only play a minor role as they were not observed in the inverse
673 group of the tDCS study and in the placebo condition of the L-DOPA study.
674
675 In summary, in line with animal studies and recent molecular imaging studies in humans, we
676 found spatially specific tDCS-related activity increases in subcortical parts of the
677 dopaminergic system, particularly in the striatum. tDCS and L-DOPA showed comparable
678 subcortical and receptor-specific activation profiles, suggesting mechanistic commonalities
679 between both manipulations. These results are promising in respect of restoring depleted
680 dopamine levels and may expand the repertoire of tDCS protocols that have been
681 successfully applied in therapeutic contexts (Brunoni et al., 2012; Kalu et al., 2012).
682 However, future studies, testing whether the approach presented here affects clinically
683 relevant neurochemical and, most importantly, behavioral parameters are required to finally
684 draw conclusions on its potential clinical value.
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864 Table legends

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866 **Table 1: tDCS-induced effects in the mesostriatal system (voxel-wise analysis).** Peak
867 voxels indicating tDCS-induced neural activity in the main as opposed to the inverse group in
868 mesostriatal subregions. Coordinates are denoted by x,y,z in mm (MNI space). R: right, L:
869 left, Caud: Caudate, NAcc: Nucleus Accumbens, Put: Putamen, SN/VTA: Substantia nigra /
870 ventral tegmental area. p_{FWE} indicates small-volume FWE-corrected p-values for voxel-level
871 inference.

872

873 **Table 2: L-DOPA-induced effects in the mesostriatal system (voxel-wise analysis).**
874 Peak voxels indicating enhanced neural activity after L-DOPA as opposed to placebo
875 administration in mesostriatal subregions after 45 and 90 minutes. Coordinates are denoted
876 by x,y,z in mm (MNI space). For abbreviations, see Table 1. p_{FWE} indicates small-volume
877 FWE-corrected p-values for voxel-level inference.

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879 **Table 3: Comparison of dopamine receptor gene expression maps.** Pearson correlation
880 coefficients (r) resulting from pairwise correlations of dopamine receptor gene expression
881 maps (DRD1-DRD5).

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Figure legends

Figure 1: tDCS electric field simulation. Anode placement over the frontopolar / ventro-medial prefrontal cortex (Fpz [red], left panel) and cathode placement over the right dorsolateral prefrontal cortex (F4 [blue], left panel) generated an electric field with maximal field strength in right lateral prefrontal areas (right panel).

Figure 2: tDCS- and L-DOPA-induced effects in the mesostriatal system (ROI analysis). Using a human dopaminergic system mask (A), averaged fALFF increases were observed after tDCS in the main but not in the inverse group (B) and 90 min after L-DOPA administration (C). Boxplots show median, quartiles (boxes), and range (whiskers). Whiskers extend 1.5 times the interquartile range. Outliers are represented by dots. Asterisks indicate levels of statistical significance for interaction contrasts (tDCS: [$tDCS_{main,post}$ - $tDCS_{main,pre}$] vs. [$tDCS_{inv,post}$ - $tDCS_{inv,pre}$], L-DOPA: [LD_{90min} - LD_{0min}] - [Plc_{90min} - Plc_{0min}]) and Bonferroni-corrected post hoc *t*-tests (*, p<0.05; **, p<0.01; ***, p<0.001).

Figure 3: Spectral analysis. Spectral analyses of the fMRI signal in the dopaminergic system revealed that both tDCS (A) and L-DOPA (B) amplified characteristic resting state frequencies (dashed lines: 0.01 to 0.08 Hz).

Figure 4: L-DOPA-induced effects in the mesostriatal system of rats (ROI analysis). In rats, we found L-DOPA-induced fALFF increases after 45, 60 and 75 min when averaging across voxels in a dopaminergic system mask. Box plots show median, quartiles (boxes), and range (whiskers). Whiskers extend 1.5 times the interquartile range. Outliers are represented by dots. Asterisks indicate levels of statistical significance for uncorrected post hoc *t*-tests (*, p<0.05; **, p<0.01; ***, p<0.001).

Figure 5: tDCS- and L-DOPA-induced effects in the mesostriatal system (voxel-wise analysis). tDCS-induced activation in the main as compared to the inverse group was restricted to areas of the dopaminergic system and most pronounced in the right putamen. L-DOPA as compared to placebo administration led to a significant activation of the SN/VTA after 45 min and of the left ventral striatum after 90 min. Green arrows indicate peak voxels. Small-volume FWE correction was performed for voxel-level inference. The display threshold was set to $p<0.01$ (uncorrected).

Figure 6: Subcortical activation profiles. tDCS and L-DOPA evoked similar subcortical activation profiles as revealed by a significant Pearson correlation of region-wise effect sizes (Cohen's d; yellow: tDCS-induced effect; blue: L-DOPA-induced effect after 90 min; $r=0.73$, $p=0.002$). Labels were taken from the HO atlas. R: right, L: left, Hipp: Hippocampus, Amy: Amygdala, NAcc: Nucleus Accumbens, Pall: Pallidum, Thal: Thalamus, Caud: Caudate, Put: Putamen

Figure 7: gene-fALFF similarity profiles. Each point indicates the z-transformed spatial correlation (similarity) of a particular gene expression map and the evoked activity pattern after tDCS (x-axis) and L-DOPA (y-axis, grey points: L-DOPA after 45 min; black points: L-DOPA after 90 min). Activity patterns induced by tDCS and L-DOPA after 90 min showed a pronounced similarity with gene expression patterns of the dopamine receptors D2 and D3 (DRD2, DRD3; red circles) in comparison to other neuronal receptor genes. There was a significant linear relationship between gene-fALFF similarity values of tDCS and L-DOPA (grey line: L-DOPA after 45 min, $r=0.82$; black line: L-DOPA after 90 min, $r=0.88$), indicating analoguous receptor-specific activation patterns for both interventions.

Tables

Table 1

ROI	MNI [mm]	Cluster size	t-value	z-value	p_{FWE}
R Put	32 -8 0	286	5.25	4.52	0.002
L Caud	-14 16 4	58	4.18	3.77	0.026
L Put	-26 2 0	192	4.26	3.83	0.035
R Caud	12 10 10	15	3.98	3.61	0.043
SN/VTA	-10 -22 -12	7	3.59	3.31	0.045

966 **Table 2**

ROI	MNI [mm]	Cluster size	t-value	z-value	p_{FWE}
L-DOPA-induced activation after 45 min					
SN/VTA	-6 -14 -12	12	3.79	3.66	0.013
	-8 -24 -18	16	3.76	3.64	0.015
	6 -22 -20	10	3.67	3.55	0.019
L-DOPA-induced activation after 90 min					
L NAcc/Caud	-14 16 -8	11	3.91	3.77	0.004
R NAcc/Caud	14 18 -6	3	3.41	3.32	0.014
L Put	-28 -6 10	55	4.03	3.88	0.025

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Table 3

	DRD1	DRD2	DRD3	DRD4	DRD5
DRD1	-	0.375	0.49	-0.07	0.39
DRD2	0.375	-	0.67	-0.06	0.1
DRD3	0.49	0.67	-	-0.16	0.04
DRD4	-0.07	-0.06	-0.16	-	0
DRD5	0.39	0.1	0.04	0	-