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Neurofeedback-mediated self-regulation of the dopaminergic midbrain

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ABSTRACT

The dopaminergic system is involved in reward encoding and reinforcement learning. Dopaminergic neurons from this system in the substantia nigra/ventral tegmental area complex (SN/VTA) fire in response to unexpected reinforcing cues. The goal of this study was to investigate whether individuals can gain voluntary control of SN/VTA activity, thereby potentially enhancing dopamine release to target brain regions. Neurofeedback and mental imagery were used to self-regulate the SN/VTA. Real-time functional magnetic resonance imaging (rtfMRI) provided abstract visual feedback of the SN/VTA activity while the subject imagined rewarding scenes. Skin conductance response (SCR) was recorded as a measure of emotional arousal. To examine the effect of neurofeedback, subjects were assigned to either receiving feedback directly proportional ($n = 15$, veridical feedback) or inversely proportional ($n = 17$, inverted feedback) to SN/VTA activity. Both groups of subjects were able to up-regulate SN/VTA activity initially without feedback. Veridical feedback improved the ability to up-regulate SN/VTA compared to baseline while inverted feedback did not. Additional dopaminergic regions were activated in both groups. The ability to self-regulate SN/VTA was differentially correlated with SCR depending on the group, suggesting an association between emotional arousal and neurofeedback performance. These findings indicate that SN/VTA can be voluntarily activated by imagery and voluntary activation is further enhanced by neurofeedback. The findings may lead the way towards a non-invasive strategy for endogenous control of dopamine.

Introduction

The mesencephalic dopaminergic brain regions, mainly substantia nigra (SN) and ventral tegmental area (VTA), are involved in various cognitive, motor and emotional functions, namely decision making (Pessiglione et al., 2006), reinforcement learning (Schultz, 1998), movement execution and motor skill learning (Flöel et al., 2005; Hosp et al., 2011; Molina-Luna et al., 2009; Reynolds et al., 2001). A dysfunction of these regions occurs in Parkinson's and related disorders as well as in various psychiatric conditions (Davis et al., 1991). Dopaminergic drugs may have beneficial effects temporarily, but lead to negative side effects after long-term use (Goodwin, 1971). As such, a reliable, non-invasive strategy for modulating the activity of these regions could be of great clinical and scientific value.

The substantia nigra and ventral tegmental area complex (SN/VTA) contains the highest concentration of dopaminergic neurons in the human brain (Francois et al., 1999). Neural activity in this region has been equated with dopamine release (Schultz, 1986) and identified as the source of the nigrostriatal, mesolimbic and mesocortical dopaminergic pathways. We group these two regions together since, in primates, their functions are very similar (Düzel et al., 2009).

Dopaminergic neurons that form the origin of the mesolimbic and mesocortical pathways fire if an unexpected reward occurs. Firing is modulated by the (inverse) variance of the probability of its occurrence (Bayer and Glimcher, 2005; Fiorillo et al., 2003; Friston et al., 2012; Hollerman and Schultz, 1998; Schultz et al., 1997; Tobler et al., 2005). Other studies have also found activation of SN/VTA during pleasant visual (Lane et al., 1997), erotic stimuli (Arnow et al., 2002; Paul et al., 2008; Redouté et al., 2000; Stark et al., 2005) or romantic love (Aron et al., 2005; Bartels and Zeki, 2004). Hence, imagery of romantic

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love or other pleasant scenes could be one strategy to up-regulate SN/VTA.

Endogenous regulation of neural activity through biofeedback (visualization of neural activity is known as neurofeedback) has been accomplished using invasive (Fetz, 1969) and non-invasive recordings (Birbaumer et al., 1990). Real-time functional magnetic resonance imaging (rtfMRI) neurofeedback can substitute direct recording of brain activity and is specifically suited to non-invasively access deep brain structures. A number of studies have shown self-regulation of functions in specific brain areas by changes in the BOLD signal, including the anterior cingulate cortex (deCharms et al., 2005), inferior frontal gyrus (Rota et al., 2009), amygdala (Posse et al., 2003), anterior insula (Caria et al., 2007), premotor cortex (Sitaram et al., 2012) and the limbic system (Sitaram et al., 2011) using operant conditioning techniques (for reviews, see (deCharms, 2008; Sitaram et al., 2009; Weiskopf, 2011)).

In this study, we examined the feasibility of endogenous up-regulation of SN/VTA and the potential beneficial effects of neurofeedback in this regard. We additionally investigated the likelihood that the up-regulation activated dopaminergic pathways and finally whether any learning was evident within a single session. The implications of such self-regulation apply to treatment of various neurological and psychiatric disorders.

Methods

Experimental setup

Thirty-two healthy male subjects, aged between 24 and 35 years, participated in this experiment, conducted according to the requirements of the Zurich Cantonal Ethics Commission (KEK 2010-0190). Each subject participated in the experiment in a single session in a Philips Achieva 3.0 T magnetic resonance (MR) scanner with an eight channel SENSE head coil (Philips, Best, The Netherlands) at the Laboratory for Social and Neural Systems Research (SNS), Zurich. MR-compatible electrocardiogram (ECG), respiration and skin conductance (PowerLab 4/25 T and Chart v5.5.2, ADInstruments, Bella Vista, Australia) measurements were collected from each participant. Individual brain volumes were converted from Philips PAR/REC format to ANALYZE DRIN using software from Philips and then placed on a server in real time. A laptop running Turbo BrainVoyager v3.0 (TBV — Brain Innovation, Maastricht, The Netherlands) extracted the BOLD signal from these files, and redirected to provide visual feedback of neural activation using custom-made software on the same laptop with Visual Studio 2008 (Microsoft, Redmond, WA, USA). The subjects viewed the visual feedback through a mirror mounted on the head coil reflecting a back-projected display behind the bore.

Instructions

The participants were instructed to attempt to gain self-control over the region of the brain activated by novel rewarding stimuli. Examples of rewards, such as food, romantic or sexual imagery, time with family and friends, and achievements were suggested. After our pilot studies had shown that romantic or sexual imagery was most effective in volitionally controlling the BOLD signal in the SN/VTA, the participants were informed of these results but were allowed to adapt their strategy according to neurofeedback success. The participants were asked to maximize the height of a vertically moving ball on the screen, representing their brain activity, when cued, and informed that the scanner acquisition time as well as the hemodynamic effects would cause approximately 5 s of delay between thought and the feedback signal. The participants were also asked not to move or change their breathing rate consciously, and especially not to change breathing as a strategy to self-regulate the feedback signal. The subjects were informed that they could stop the experiment at any time by pressing a pneumatic button.

Sequence

Anatomical data were acquired with an ultrafast gradient echo T1-weighted sequence in 301 sagittal plane slices of 250×250 mm^2 resulting in 1.1 mm^3 voxels, lasting approximately 5 min. The images were then transformed to 1 mm^3 voxel representations and in a standard sagittal plane orientation by BrainVoyager QX v2.3 (Brain Innovation, Maastricht, The Netherlands). Functional data were acquired in 27 ascending transverse plane slices using a gradient echo T2*-weighted echo-planar image sequence over the whole brain. Acquired in-plane resolution was 2×2 mm^2, 3 mm slice thickness and 1.1 mm gap width over a field of view of 220×220 mm^2, a TR/TE of 2000/35 ms and a flip angle of 82°. Slices were aligned with the anterior–posterior commissure and elevated by 15°. A single volume was first obtained to help TBV with online coregistration specific for Philips scanners. Five runs of 185 volumes were acquired afterwards.

Protocol

We used an anatomical localizer to identify the SN/VTA. The spatial extent of the SN/VTA was selected based on previous research (D'Ardenne et al., 2008; Düzel et al., 2009). The caudal edge of the SN was delineated by the cranial edge of the pons at the midline. The cranial border of the region coincided with the cranial border of the tegmentum, measured from the midline, representing the height of the midbrain. VTA was determined by the anterior connection between the two lateral SN structures. Both the SN and VTA were combined into a single anatomical region of interest (ROI) and automatically coregistered with the functional scans in TBV during the neurofeedback runs (Fig. 1). The participants received visual feedback of the SN/VTA BOLD signal in the form of a vertically moving ball with written instructions. When "Happy Time" was displayed on the screen, the subjects were asked to raise the position of the ball on the screen with a smiley face as high as possible using rewarding mental imagery (Fig. 2). The position and color of the ball were proportional to the BOLD signal extracted by TBV. As the ball was climbing, its color gradually changed from red to yellow. When "Rest" was displayed, the participants were asked to perform neutral imagery such as mental arithmetic or paper writing, thereby reducing the height of the ball and making it redder in color. The BOLD signal of the SN/VTA region of interest that determined the elevation of the ball, was first normalized based on the percent signal increase from the previous baseline condition (last five volumes), then three-point averaged (i.e. averaging the current value with the previous two) to reduce noise.

Groups were defined by the type of visual feedback presented in the neurofeedback condition. In the veridical feedback group (15 subjects), elevation of the ball and change in its color from red to yellow were proportional to the BOLD signal in the ROI. As feedback may act as a reward signal that could independently stimulate SN/VTA, we used a control group (inverted feedback) of 17 subjects to separate the recruitment of SN/VTA due to feedback from its recruitment through mental imagery. In this group, the participants were given the same instructions, hence, they used the same imagery to raise the ball. But, the feedback that they received was inverted: the elevation of the ball decreased and its color became red as the SN/VTA BOLD signal increased. The inverted feedback subjects were not made aware of this inverse proportional relationship, and we confirmed that these subjects remained unaware of this relationship in a post-experimental debriefing. In this manner, any differences between the performance of the two groups are caused by the information provided by the neurofeedback. Other control conditions were initially investigated, specifically yoked sham feedback (deCharms et al., 2005), but some subjects were able to identify the non-contingency

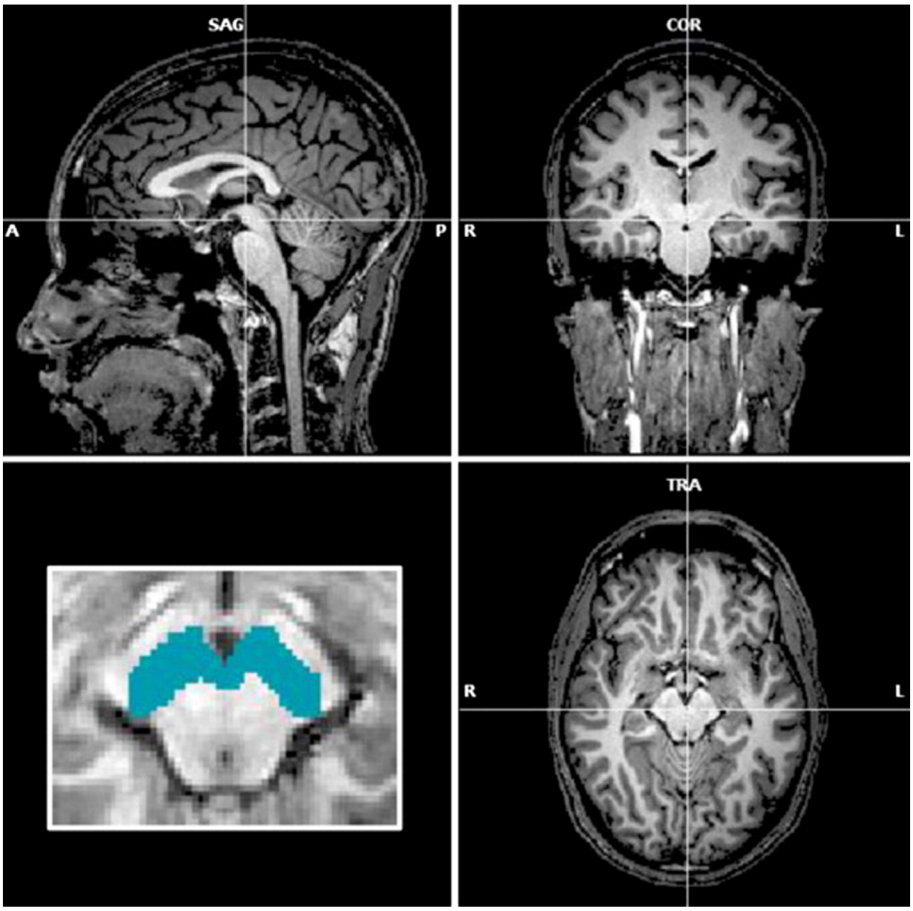

Fig. 1. Anatomical localizer for SN/VTA. The three radiological views are centered on the SN/VTA, shown in detail in the inset on the bottom left.

of the feedback during the experiment and thus this strategy was abandoned.

Each subject underwent five runs with approximately 2 min rest in between. Each run comprised nine blocks of alternating "Rest" (20 s) and "Happy Time" (20 s), totaling about 6 min (Fig. 2). Ten seconds was added to the initial rest block to allow enough time to load the initial parameters to TBV. In the first and last runs (i.e. baseline and transfer runs), only instructions were provided with no feedback. Following the baseline run, the next three runs provided feedback of BOLD signal to the participants. To explore whether the subjects had learned to self-regulate SN/VTA activity without feedback, the transfer run only showed the instructions. Following the experiment, the subjects were asked to report whether they remained with the initial suggested mental strategy, and if not, what strategy they found most useful.

Data postprocessing and statistical tests

Offline preprocessing of functional data was performed using BrainVoyager QX v2.6. The data were slice-time corrected using cubic spline interpolation, motion-corrected with sinc interpolation, and then temporally high-pass filtered using a discrete cosine set of three sines/cosines. The data were coregistered with the subject's own anatomical image. Correction for physiological noise was performed by RETROICOR (Glover et al., 2000) using Fourier expansions of different orders for the estimated phases of cardiac pulsation (3rd order), respiration (4th order) and cardio-respiratory interactions (1st order) (Harvey et al., 2008). The corresponding confound regressors were created using a custom in-house Matlab (version R2011a) implementation (Kasper et al., 2009).

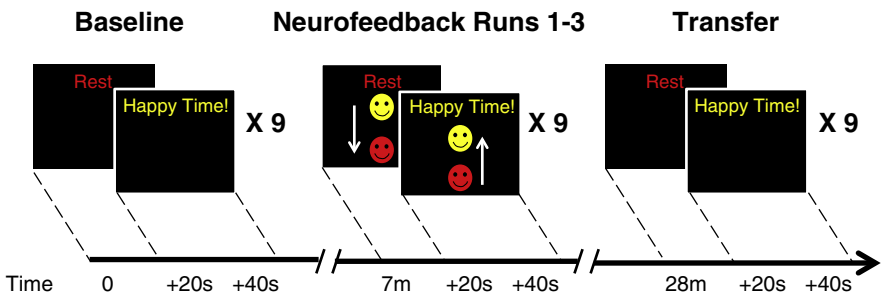

Fig. 2. Experimental protocol. Following an anatomical localizer, each participant was exposed to five runs, each one composed of "Rest" (20 s) followed by "Happy Time" (20 s), then repeated nine times per run over five runs. The first and last runs, (baseline and transfer, respectively), only showed instructions with no neurofeedback. During the three neurofeedback runs, subjects were asked to use rewarding imagery to raise the ball during Happy Time, and neutral imagery to lower the ball during Rest.

In first-level analysis of functional data, a standard general linear model (GLM) analysis was used. The design matrix included head movement regressors and additional regressors based on the aforementioned RETROICOR analysis. The percent-transformed, mean-corrected beta values from the predefined subject-specific SN/VTA ROI were extracted through BrainVoyager QX. The beta values in BrainVoyager represent the parameter estimates for the task regressor subtracted by a constant representing the mean whole brain parameter estimate. Skin conductance response (SCR) was de-trended and down-sampled to 10 Hz using SCRalyze (Bach et al., 2009). After fitting to a GLM based on task onset, the beta values of the SCR data were extracted from each run. The skin conductance data of two subjects in the veridical feedback group were excluded due to experimenter error.

Second-level analysis of beta values in baseline runs were compared using one-sample t-tests to examine whether SN/VTA could be activated without feedback, and two-sample t-tests to compare initial performance between groups. The beta values during neurofeedback runs were first subtracted from subject-specific baseline values (thus baseline-corrected), and then input into a 2×3 mixed effects repeated measures analysis of variance (ANOVA) over the three neurofeedback runs to examine differences between groups during neurofeedback training. Run number (three levels) was the random effect and group (two levels) was the fixed effect. Within group differences were determined using one-way repeated measures ANOVA for each group separately. One-sample t-tests of SN/VTA beta values during transfer runs of each group were used to determine whether SN/VTA remained active, and baseline-corrected beta values were used to compare between groups in a two-sample t-test. Analysis of Covariance (ANCOVA) was used to determine whether the covariation between SCR and SN/VTA beta varied between groups. We used SPSS v19 (IBM, Armonk, NY) for all aforementioned statistical tests.

Voxel-wise random effects group analysis of neurofeedback runs was performed first by coregistering functional data to Talairach-transformed anatomical images (Talairach and Tournoux, 1988). We used a summary statistic random effects approach where t-tests were applied to first-level contrast images to determine significance within or between conditions. Images were corrected for multiple comparisons using a false discovery rate of $p < 0.05$. Active regions were identified based on the nearest coordinate using a Talairach Daemon (Lancaster et al., 2000). Voxel-wise analysis focused on caudate nucleus (Strafella et al., 2001), putamen (York, 1970), nucleus accumbens (Salamone and Correa, 2002), hippocampus (Rossato et al., 2009), amygdala (Wilson et al., 1994), subthalamic nucleus (Limousin et al., 1998) and prefrontal cortex (Williams and Goldman-Rakic, 1995) — all brain regions with presumed involvement in reward processing.

We also conducted a functional connectivity analysis using the BrainVoyager QX plugin. The seed region (SN/VTA) was anatomically

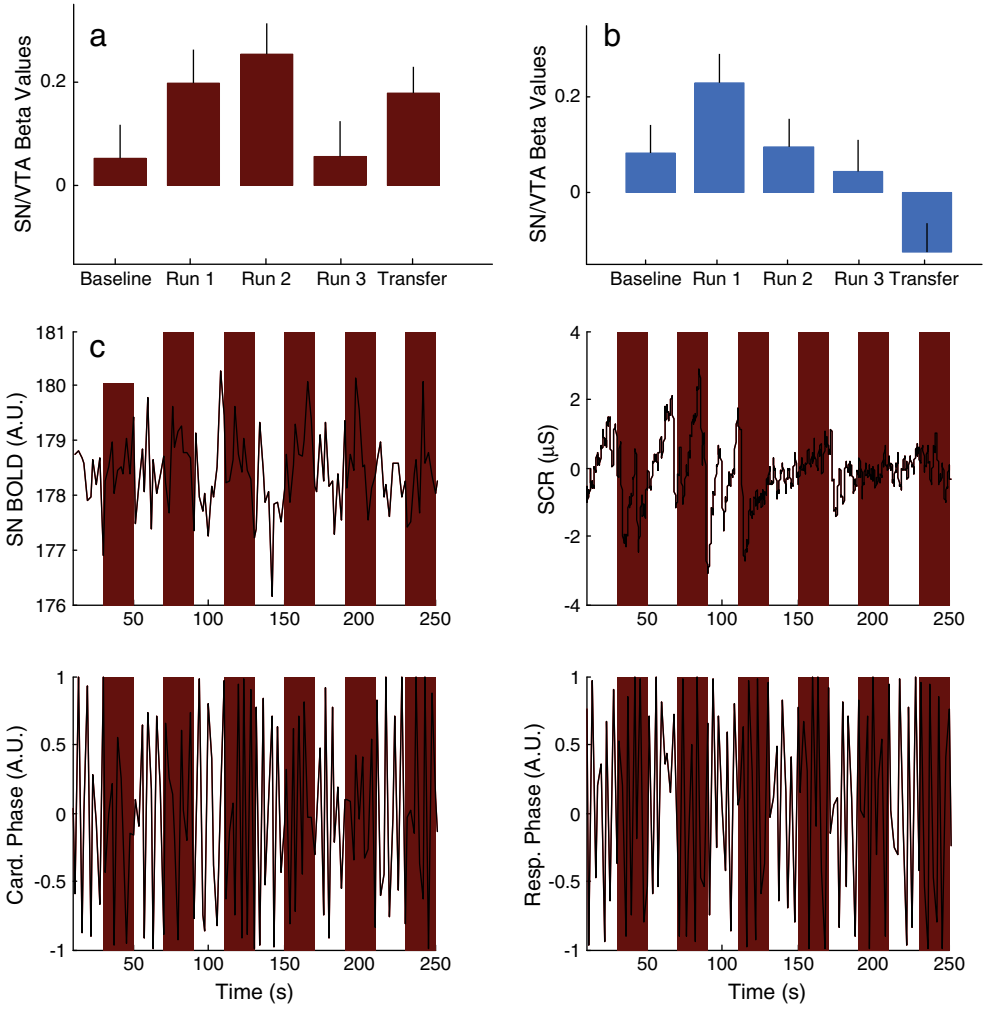

Fig. 3. Results from representative subject in veridical feedback group. In (a), SN/VTA activity is shown over runs for a representative veridical feedback participant, whereas a representative inverted feedback participant is shown in (b). Below, in (c), the time course of activity from the second neurofeedback run in the veridical feedback participant is shown along with skin conductance response and example cardiac and respiration regressors (first order cosine), respectively.

defined as described above, but based on the average Talairach-transformed anatomical scans of all subjects instead of subject-specific scans. Second-level analysis was performed using t-tests. Correction for multiple comparisons was performed at the cluster level. Assuming contiguous clusters rather than individual voxels, Monte Carlo simulations in BrainVoyager were used to adjust for false positives on a cluster level (Forman et al., 1995). We first set the statistical threshold of the contrast to $p < 0.05$, then conducted simulations over 1000 iterations, using a cluster-level threshold of $p < 0.05$.

Results

ROI analysis

Both groups showed an increase in SN/VTA activity during baseline (see Fig. 3 for representative raw data from both groups). The veridical feedback group mean beta values (mean difference = 0.19, $t(14) = 5.96$, $p < 10^{-4}$) during baseline were slightly less than the inverted feedback group (mean difference = 0.20, $t(16) = 3.69$, $p < 0.005$). However, this difference was not significant (mean difference = −0.01, $t(30) = -0.22$, $p = 0.83$).

During neurofeedback, repeated measures ANOVA revealed an overall increase in SN/VTA activity compared to baseline in the veridical feedback group, as expressed by the intercept ($F(1) = 8.54$, $p < 0.05$), with a significant increase between the first and second neurofeedback runs ($F(2,13) = 4.26$, $p < 0.05$). In contrast, the inverted feedback group showed neither an overall change in baseline-corrected SN/VTA activity ($F(1) = 1.46$, $p = 0.24$), nor a difference between runs ($F(2,15) = 1.50$, $p = 0.26$). Between the two groups, there was a significant interaction between group and run ($F(2,29) = 4.80$, $p < 0.05$), driven by higher SN/VTA activity in the second neurofeedback run in the veridical group compared to the inverted group. Fig. 4 shows the baseline-corrected SN/VTA beta values over all runs for both groups.

In transfer, both groups showed increased SN/VTA activity compared to rest, with the veridical group (one-sample t-test, mean difference = 0.20, $t(14) = 4.40$, $p < 0.001$) slightly higher than the inverted group (mean difference = 0.18, $t(16) = 3.21$, $p < 0.01$). When corrected for baseline performance, however, there was no change in the veridical (mean difference = 0.03, $t = 0.64$, $p = 0.53$), or inverted (mean difference = −0.02, $t = -0.33$, $p = 0.75$) feedback groups, nor a difference between them (two-sample t-test, mean difference = 0.05, $t(30) = 0.64$, $p = 0.53$).

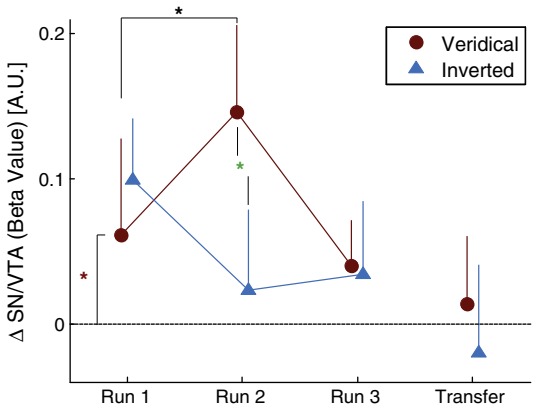

Fig. 4. Effect of neurofeedback. Baseline-corrected SN/VTA beta values over all runs in the veridical feedback group compared to the inverted feedback group. Vertical lines indicate standard error. Results show a significantly larger increase between Run 1 and Run 2 in the veridical feedback group ($p < 0.05$), which is significantly larger compared to inverted feedback ($p < 0.05$). The veridical feedback group also showed an overall increase compared to baseline ($p < 0.05$), whereas the inverted group did not ($p = 0.26$).

Random effects group analysis

In a secondary voxel-wise analysis, we investigated whether other dopaminergic regions were additionally activated. In the veridical feedback group we found activation of reward-related regions when comparing neurofeedback to rest. Both groups showed significant activation in SN/VTA, caudate body (Ca), hippocampus (Hi) and nucleus accumbens (NAcc) (Table 1 and Fig. 5). There were no significant differences found between groups.

Functional connectivity analysis

Both groups also showed increased functional connectivity of the SN/VTA with reward regions. As shown in Fig. 6 and Table 2, the veridical feedback group showed increased connectivity between SN/VTA and left NAcc and Putamen (Pu), whereas the inverted feedback group showed increased connectivity between SN/VTA and bilateral Ca and Pu. Comparing both groups, the veridical feedback group had greater connectivity with SN/VTA than the inverted feedback group in the Ca.

Behavioral measures

To account for inter-subject variations in performance, we acquired an independent measure of attention and emotional arousal (skin conductance response, SCR, for review see Critchley, 2002). There was no significant change in baseline-corrected beta values of SCR for either the veridical ($F(1) = 1.85$, $p = 0.20$) or inverted ($F(1) = 0.04$, $p = 0.85$) feedback groups, nor within runs ($F(2,11) = 2.45$, $p = 0.13$), ($F(2,15) = 0.50$, $p = 0.62$), or between groups ($F(2,27) = 1.42$, $p = 0.32$). However, the correlation between pooled beta values for SCR and SN/VTA activity showed a significant difference between groups ($F(1) = 5.00$, $p < 0.05$, see Fig. 7). This difference was driven by a positive trend in the veridical feedback group ($r = 0.45$, $p = 0.12$) and a negative trend in the inverted feedback group ($r = -0.35$, $p = 0.18$).

Table 1
Summary of random effects analysis of both groups over whole brain (peak activation, FDR-corrected, $p < 0.05$, cluster volumes > 160 mm^3). No significant clusters were found when comparing groups.

	Side	Veridical feedback				Inverted feedback			
		Talairach X	Y	Z	t	Talairach X	Y	Z	t
Reward areas									
SN/VTA		1	−18	−12	7.03	6	−22	−10	6.27
Caudate body	L	−19	−19	29	6.27	−18	−10	26	6.26
R	18	−11	27	6.97	17	0	22	6.61	
Hippocampus	R	32	−42	0	7.32	30	−39	0	4.67
Nuc. accumbens	L					−10	−3	12	4.64
	R	7	7	3	6.1	8	3	11	5.57
Other regions									
Cingulate gyrus	L	−3	0	23	8.43	−4	−1	22	5.47
Declive	L	−7	−78	−17	5.55				
	R					28	−60	−13	5.06
Inf. frontal gyrus	L	−29	10	−9	6.48				
Inf. occipital gyrus	L					−27	−87	−5	5.09
Inf. parietal lobule	L	−53	−30	38	4.85	−36	−34	34	4.74
Insula	L	−45	9	3	6.21	−33	26	12	5.58
	R					31	23	11	4.94
Lingual gyrus	L	−1	−75	2	6.86				
Med. frontal gyrus	L	−5	13	45	6.47	−6	46	25	5.03
Mid. frontal gyrus	L	−39	30	32	4.46	−48	29	27	5.77
Mid. Occipital Gyrus	L	−28	−77	5	4.94				
Mid. temporal gyrus	L	−50	−54	−8	5.97	−43	−54	9	4.94
Post. cingulate gyrus	L	−3	−48	21	5.86				
	R					26	−57	17	4.52
Precentral gyrus	L	−10	−13	73	5.85	−56	3	31	7.27
Precuneus	L					−13	−61	49	4.93
Sup. frontal gyrus	L					−18	37	44	5.34
Thalamus	L	−3	−31	11	6.96	−10	−3	12	4.64

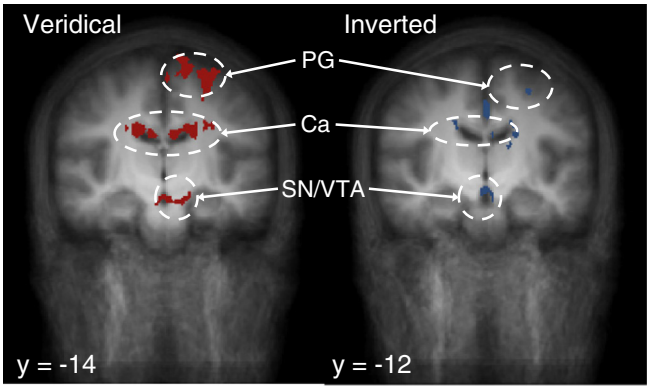

Fig. 5. Random effects GLM analysis. Both the veridical (left) and inverted (right) feedback groups showed significant activation in SN/VTA and other reward regions. Both of the views show FDR-corrected ($p < 0.05$) activity along the nigrostriatal pathway, from the SN/VTA to the dorsal caudate (Ca) to the precentral gyrus (PG). Table 1 provides a summary of other regions activated.

The groups also differed in their chosen imagery strategy, despite both groups being given the same instructions. In the veridical feedback group, all 15 subjects reported that their best mental strategy was sexual or romantic imagery, whereas in the inverted feedback group, six out of 17 reported that their best strategy was something other than the suggested imagery strategy. The alternate strategies were sports, holidays, family, friends, academic success and travelling (one subject used each strategy). Strategy did not correlate with the ability to self-regulate SN/VTA (binary logistic regression, $p = 0.29$).

Discussion

The primary goal of this study was to investigate whether one can self-regulate the SN/VTA activity and if so, if neurofeedback can assist. We found that the participants were able to increase the activity in the region during the baseline condition without feedback. The subjects with veridical feedback improved the ability to up-regulate SN/VTA, co-activated other dopaminergic regions, and showed increased connectivity along the nigrostriatal pathway compared to controls. Behavioral measures such as SCR correlation with SN/VTA and chosen imagery strategy also differed between groups, further showing the beneficial effect of veridical neurofeedback.

The first aim of this investigation was to determine whether any mental strategy could consistently up-regulate the SN/VTA. We built upon previous research of visual presentation of pleasant, erotic or romantic scenes to evoke activity in this region (Arnow et al., 2002; Aron et al., 2005; Bartels and Zeki, 2004; Lane et al., 1997). In this study, both groups showed increased SN/VTA activity using mental imagery of sexual and romantic scenes instead of explicit visual stimulation. These results suggest that such rewarding imagery is a robust method of SN/VTA self-up-regulation. To our knowledge, this is the first evidence of endogenous (i.e. without external stimulation) up-regulation of SN/VTA.

More interestingly, our results show that veridical neurofeedback positively affects control of SN/VTA. The veridical feedback group had significantly increased SN/VTA activity during neurofeedback compared to baseline whereas the inverted feedback group did not. Additionally, the veridical feedback group showed significantly higher activity in the second run than the inverted feedback group. Although both groups showed activity in both the nigrostriatal and mesolimbic pathways, we additionally found increased functional connectivity in the dorsal striatum in the veridical feedback group compared to controls. This indicates an advantageous effect of veridical neurofeedback within the nigrostriatal pathway. We expected a significant drop in the inverted feedback group, but instead observed a non-significant decreasing trend. We attribute this to the instructed explicit rewarding mental imagery strategy in opposition to the feedback.

Behavioral evidence also indicates a positive effect of veridical neurofeedback on SN/VTA up-regulation. There was a difference in emotional arousal due to the type of feedback provided, as the correlation between ability to self-regulate and SCR beta values was significantly different between the groups. It is notable that despite being given identical instructions, all of the subjects in the veridical feedback group remained with the suggested strategy, whereas over a third of the inverted feedback group found other strategies more effective. Taken together, results strongly suggest that neurofeedback does play a role in facilitating self-regulation of SN/VTA.

There is mixed evidence to suggest a learned ability to self-regulate SN/VTA was obtained in a single session. There was an increased SN/VTA activity in the veridical feedback group in the second run, but this improvement does not continue into the third run; instead an insignificant negative trend was observed. In addition, there was no significant increase in transfer compared to baseline. Whether this drop in performance later in the session was a consequence of habituation (attenuation) or cognitive fatigue remains to be investigated. Indeed, cognitive fatigue-related regions such as the cerebellum, cingulate cortex, insula, and lingual gyrus were activated (Cook et al., 2007; DeLuca et al., 2008). Since disruption of SN/VTA activity is a neural mechanism of central fatigue (for review, see (Chaudhuri and Behan, 2000)), it is possible that SN/VTA self-regulation could have an interactive effect. Hemodynamic delay could additionally impair learning, but this is unlikely given the greater learning earlier in the session instead of later. Additionally, there is strong evidence showing that delayed feedback can be used effectively as long as the delay is consistent (Miall et al., 1993). Learned self-regulation has been achieved using rtfMRI neurofeedback with delays as long as 60 s (Yoo and Jolesz, 2002). Habituation may have

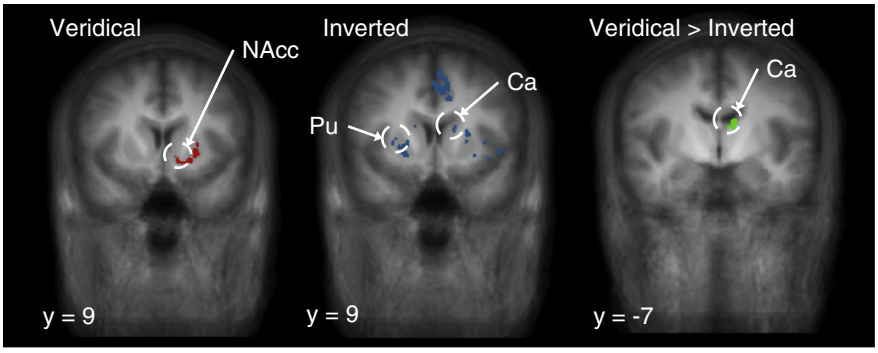

Fig. 6. Functional connectivity analysis. Functional connectivity using a SN/VTA seed region, veridical (left), inverted (middle) and veridical > inverted (right) all cluster-level corrected, $p < 0.05$, all cluster volumes >320 mm^3. Both groups show increased connectivity in the ventral and dorsal striatum. Veridical feedback group shows greater connectivity in the dorsal striatum than the inverted group. A summary is given in Table 2.

Table 2
Summary of functional connectivity analysis over whole brain with SN/VTA seed (cluster-level corrected, $p < 0.05$, all cluster volumes > 320 mm^3).

	Side	Veridical feedback				Inverted feedback				Veridical > inverted			
		Talairach X Y Z			t	Talairach X Y Z			t	Talairach X Y Z			t
Reward areas													
Caudate body	L					−14	11	15	5.1	−11	−10	19	3.86
	R					8	3	13	5.08				
Nuc. accumbens	L	−17	4	−7	5.41								
Putamen	L	−23	2	−5	5.09	−25	0	−5	5.49				
						31	14	7	5.19				
Other areas													
Culmen	L	−26	−52	−23	5.54								
	R					20	−52	−21	5.19				
Declive	L					−3	−73	−11	5.79				
Inf. frontal gyrus	L					−50	22	18	5.41				
Insula	L					−38	16	3	5.45				
Med. frontal gyrus	L					−4	−1	48	5.4				
Mid. temp. gyrus	L					−50	−63	22	5.46				
Precuneus	L	−4	−68	22	6.49	−9	−51	41	6.28				
Sup. frontal gyrus	L					−36	39	30	5.63				
Sup. temp. gyrus	L					−39	−50	29	5.28				
Thalamus	L					−15	−21	12	5.02				
	R					11	−10	13	5.53				

contributed towards the apparent drop in SN/VTA activity. It is well known that the SN/VTA responds to novel rewards (Bunzeck and Düzel, 2006; Schultz, 1998), and after a 30-minute succession of trials, self-generation of novel rewards likely becomes increasingly difficult. Therefore the drop in SN/VTA activity seen in both groups may be in part due to habituation, and veridical feedback may have delayed this drop.

Whether or not the increase in BOLD signal in SN/VTA reflects the activity of dopaminergic neurons cannot be directly answered with fMRI, although activity found within dopaminergic pathways offers indirect evidence. Voxel-based whole brain analysis in both random effects GLM and functional connectivity analyses showed that apart from SN/VTA, the NAcc, Ca and Hi were activated in both groups. BOLD activity in these areas has been previously correlated to dopamine levels using positron emission tomography (Schott et al., 2008), and they are known as target regions for dopaminergic projections originating in SN/VTA (Düzel et al., 2009). The fact that we found functional connectivity to increase between SN/VTA and ventral (veridical) as well as dorsal striatum (both groups) supports the validity of an assumption, i.e., activation of dopaminergic pathways. Therefore, the BOLD signal increases observed in our study may well reflect firing of dopaminergic neurons.

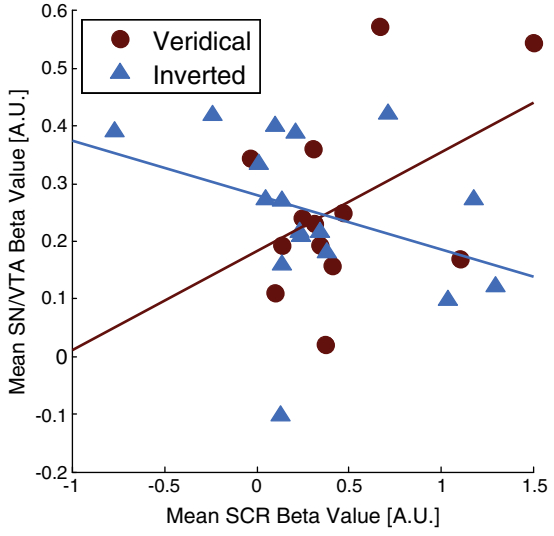

Fig. 7. Differential correlations between SCR and SN/VTA depend on neurofeedback. When correlating the beta values of the subjects from the two groups with their corresponding SCR beta values, the veridical feedback group (dark) shows a positive trend with SN/VTA beta ($r = 0.45$, $p = 0.12$), while the inverted feedback group (light) shows a negative trend ($r = -0.35$, $p = 0.18$), significantly less than the veridical feedback group (ANCOVA, $p < 0.05$).

When using subject-wise measures of SCR as a covariate, we found a positive trend between SCR and SN/VTA activity in the veridical feedback group, which was significantly larger than that of the inverted feedback group. On one hand, this could mean that the visual feedback affects SCR, which could be an orienting response (Maltzman and Boyd, 1984). However, this appears unlikely because an orienting response should affect both groups in the same manner, which was not evident. On the other hand, another way of interpreting this differential correlation is that self-regulation of SN/VTA is associated with emotional arousal and/or valence. Previous work has shown a strong relationship of SCR with mental imagery, including correlations with emotional arousal and valence (McTeague et al., 2009, 2010, 2012). Thus, it is more likely that the differential correlation of SCR with SN/VTA activity is due to a stronger relationship of self-regulation of SN/VTA with emotional arousal rather than with orienting response.

Given the supposed habituation, one of the main limitations of this study is lack of a direct measure of cognitive fatigue. We did not find reductions in SCR, but did find activity in some associated brain regions. Yet without a separate measure, relating this activity to cognitive fatigue or habituation remains somewhat speculative. Understanding the dynamics of self-regulation, especially as it relates to the ability to learn, is crucial to the development of neurofeedback as a clinical tool. While there does appear to be some intrasession learning, further study would be necessary to evaluate between session learning effects that other rtfMRI neurofeedback studies have shown (e.g. Bray et al., 2007; Caria et al., 2007; Shibata et al., 2011).

The SN/VTA is a difficult region to image (D'Ardenne et al., 2008), with challenges such as small size and proximity to the basilar artery leading to issues with magnetic susceptibility. It may be possible that neurofeedback from other regions along the dopaminergic pathways could be more effective. For instance, Subramaniam et al. have previously conducted a successful pilot study on Parkinson's patients using neurofeedback from the supplementary motor area, showing improved motor outcomes compared to controls with sham feedback (Subramanian et al., 2011). Another study by the same group used neurofeedback from target areas in the emotion network individually tailored to the subject, such as the amygdala and insula (Johnston et al., 2010). They found increased NAcc activity as a consequence of successful up-regulation. Other regions with a more direct connection to the SN/VTA could also have been used as targets, such as the striatum. However, the SN/VTA has the largest concentration of

dopaminergic neurons (Francois et al., 1999) and as such, BOLD signal there may be more likely to represent dopaminergic activity (Düzel et al., 2009). In addition, the SN/VTA is the source of multiple dopaminergic pathways and therefore could have a wider impact.

Conclusions

This study demonstrates that young healthy volunteers can voluntarily up-regulate SN/VTA by imagining pleasant scenes and receiving online neurofeedback information about their SN/VTA activation. We found that SN/VTA can be self-regulated through imagery and that neurofeedback can assist in this regard. Further research is required to develop strategies for persistent regulation and investigate behavioral consequences. If successful, such strategies could have far reaching applications from the treatment of addiction to Parkinson's disease.

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Conflict of interest statement

The authors declare no competing financial interests.

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