Delay Period Activity of the Substantia Nigra during Proactive Control of Response Selection as Determined by a Novel fMRI Localization Method

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Abstract

The ability to proactively control motor responses, particularly to overcome overlearned or automatic actions, is an essential prerequisite for adaptive, goal-oriented behavior. The substantia nigra (SN), an element of the BG, has figured prominently in current models of response selection. However, because of its small size and proximity to functionally distinct subcortical structures, it has been challenging to test the SN’s involvement in response selection using conventional in vivo functional neuroimaging approaches. We developed a new fMRI localization method for directly distinguishing, on echo-planar images, the SN BOLD signal from that of neighboring structures, including the subthalamic nucleus (STN). Using this method, we tested the hypothesis that the SN supports the proactive control of response selection. We acquired high-resolution EPI volumes at 3 T from 16 healthy participants while they completed the Preparing to Overcome Prepotency task of proactive control. There was significantly elevated delay period signal selectively during high-compared with low-control trials in the SN. The STN did not show delay period activity in either condition. SN delay period signal was significantly inversely associated with task performance RTs across participants. These results suggest that our method offers a novel means for measuring SN BOLD responses, provides unique evidence of SN involvement in cognitive control in humans, and suggests a novel mechanism for proactive response selection.

INTRODUCTION

The ability to prepare for and select optimal responses in the face of competing demands and choices is one of the critical requirements for the implementation of goal-oriented behavior. A particularly important challenge in this regard is when prepotent or automatic responses must be overcome in favor of more effortful and contextually appropriate actions (Schneider & Chein, 2003). Automatic responses, although quicker and less resource intensive, are inflexible and insensitive to contextual contingencies; controlled responses, although slower, more resource intensive, and capacity constrained, allow adaptive and contextually appropriate behavior to occur. A better understanding of the neural mechanisms supporting controlled response selection would have significant impact on our understanding of behavioral control as well as provide clues to the pathophysiology of neuropsychiatric conditions involving impairments in this process, such as substance abuse (Everitt et al., 2008).

Among the distributed network of brain regions that may be supporting controlled response selection, subcortical contributions to this process have been relatively understudied. Although fMRI has provided considerable evidence implicating the involvement of the lateral pFC in specific components of the control of behavior (Aron & Poldrack, 2006; Ridderinkhof, van den Wildenberg, Segalowitz, & Carter, 2004; O’Doherty, Critchley, Deichmann, & Dolan, 2003; Miller & Cohen, 2001; MacDonald, Cohen, Stenger, & Carter, 2000), including rule-guided or controlled response selection (Bunge, 2004; Bunge, Hazeltine, Scanlon, Rosen, & Gabrieli, 2002; Rowe, Toni, Josephs, Frackowiak, & Passingham, 2000), we know relatively little about the involvement of subcortical brain regions in this process in humans.

The BG have long been considered to play a central role in response selection (Frank, Scheres, & Sherman, 2007; Redgrave, Prescott, & Gurney, 1999; Mink, 1996; Graybiel, 1995). Computational models propose that these nuclei select among competing motor programs for further processing and execution (Frank, 2011; Redgrave et al., 1999). Within the BG, the substantia nigra (SN) is a critical regulator of information throughput in striato-thalamic circuits (Haber, Fudge, & McFarland, 2000; Alexander & Crutcher, 1990; Graybiel, 1990). The SN

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contains the largest dopaminergic nucleus, and its connectivity with associative and motor regions of the striatum (Haber et al., 2000) and neocortex (Williams & Goldman-Rakic, 1998) suggests that it is well positioned to efficiently communicate with the diverse brain regions necessary to mediate the control of responses. However, because of technical limitations, direct evidence from humans supporting the hypothesis of SN involvement in the control of response selection has been scant.

One of the major obstacles to the in vivo study of the SN in humans has been its small size and location adjacent to functionally distinct structures (Schafer et al., 2011; Coenen, Prescher, Schmidt, Piccozzi, & Gielen, 2008; Richter, Hoque, Halliday, Lozano, & Saint-Cyr, 2004). This means that even relatively small localization errors that may occur using conventional methods could prove to be significant relative to the size of the SN and its proximity of other midbrain structures. Thus, new, more accurate methods for the localization of SN activity are needed to test its involvement in response selection.

To address these challenges, we developed a new fMRI method for localizing and measuring the function of the SN. This method is an ROI-based approach, in which the SN is identified directly on task EPIs that have been minimally processed. The identification of the SN is made possible because of its high iron concentration (Haacke et al., 2005; Dexter et al., 1991; Sofic, Paulus, Jellinger, Riederer, & Youdim, 1991), which makes it appear as a region of relative hypointensity on T2*-weighted images (Haacke et al., 2005), including EPI volumes. Our SN localization procedures thus avoid image processing steps utilized by standard fMRI approaches, such as spatial normalization or coregistration of functional images onto structural images, which could contribute to localization errors.

Our method includes procedures for discriminating the SN from nearby structures, including the subthalamic nucleus (STN). Because of its location, morphological similarity, and image characteristics on EPI volumes, the STN is perhaps the most difficult structure to distinguish from the SN (Richter et al., 2004). This distinction may be critical, as the STN has a closely tied yet unique functional role in BG-thalamic circuitry (Temel, Blokland, Steinbusch, & Visser-Vandewalle, 2005). The failure to distinguish these two structures could lead to significant misestimation of true SN activity because of the misattribution of activity from the STN to the SN. The ability to separately localize and measure activity from the STN also provides a unique opportunity to test the specificity of SN findings. Furthermore, measurement of STN activity could provide information helpful for interpreting the neural source of the SN BOLD signal. The SN is composed of the pars compacta (SNc) and pars reticulata (SNr), which are functionally distinct compartments with differing cellular compositions, with dopaminergic neurons constituting a major component of the former (Björklund & Dunnett, 2007) and GABAergic neurons constituting a major component of the latter (Rajakumar, Elisevich, & Flumerfelt, 1994). fMRI lacks the spatial resolution to resolve these compartments, particularly in primates (Poirier, Giguere, & Marchand, 1983), which are interdigitated with each other. Because the SNr receives direct excitatory afferents from the STN, forming an important circuit within the indirect pathway (Parent & Hazrati, 1995), STN activity would be expected to show similar activity patterns as the SNr in particular. Therefore, the STN results could help interpret which of the two SN compartments are contributing to the SN findings.

With this method for localizing the SN, we tested the hypothesis that the SN is involved in the proactive control of response selection. During fMRI, a sample of healthy adult participants performed the Preparing to Overcome Prepotency (POP) task (Minzenberg et al., 2010), a well-validated cued alternative choice selection task in which the color of the cue signaled whether the upcoming response required high or low control. Thus, the delay period, which follows cue stimulus presentation and precedes presentation of the probe stimulus when response selection occurs, represents a key period in which brain regions supporting proactive control would be expected to display selective engagement in the high-control condition. Conforming to this prediction, the SN exhibited above-baseline delay period activity selectively in the high-control condition. This effect was region specific in that the SN did not show this condition-specific delay period activity, indicating a distinct functional role for the SN.

METHODS
Participants
Twenty (four were excluded, leaving 16 participants whose data were included in this study) right-handed adult participants (mean age = 30.7, SD = 3.9; 50% male) free of major neuropsychiatric illness (determined by the Structured Clinical Interview for DSM-IV-TR Disorders, Nonpatient version, administered by trained diagnosticians) participated in this study. Exclusion criteria for all participants were as follows: history of psychiatric or neurologic illness, IQ of less than 70, drug and/or alcohol dependence history or abuse in the previous 3 months or a positive urine drug screen on the day of testing, significant head trauma, use of any psychotropic medication, or any known contraindication to MRI. After complete description of the study, informed consent was obtained, and participants were compensated for their participation. This study was approved by the institutional review board at the University of California, Davis.

Cognitive Paradigm
Participants completed the POP (Minzenberg et al., 2010) task while being scanned (Figure 1). The POP task was...
presented using E-Prime (Psychological Software Tools, Pittsburgh, PA). A trial begins with the central visual presentation of a color cue for 500 msec (a green or red square that indicates a low- or high-control response selection to the subsequent probe, respectively). Following a 7500-msec delay period, the probe stimulus (a centrally presented arrow pointing right or left, randomized with 50% probability) is shown for 500 msec. The intertrial interval (time from probe onset to onset of cue for the next trial) was 12 sec; that is, total trial duration was 20 sec. In low-control (green cue) trials, participants were to respond with a button press in the congruent (prepotent) direction of the subsequent arrow (e.g., for a right-pointing arrow, press the right button, and for leftward arrow, press the left button). For the high-control condition (red-cued trials), participants responded in the incongruent (non-prepotent) direction (e.g., for a right-pointing arrow, press the left button, and vice versa). The POP task takes advantage of the Simon spatial incompatibility effect (Simon, 1990) in which the appearance of a probe stimulus opposite to the response side results in slower RTs compared with when stimulus and response appear on the same side. Participants were instructed to “go as fast as you can without making mistakes.” To increase the control requirements during the high-control trials, we reinforced the prepotency of the congruent stimulus–response mappings of the low-control condition by using a prevalence of green cues of 70%. This promoted the overlearning of the congruent stimulus response mapping. Participants received four blocks of 20 trials in each block. All participants responded with their dominant right hand.

fMRI

All data were collected at the University of California, Davis, Imaging Research Center. The visual stimuli were displayed on a projection screen and viewed by participants through a mirror attached to the head coil. Foam padding stabilized the participant’s head in the head coil to minimize head motion during the experiment. Functional scans (T2* weighted, gradient recalled echo-EPI, repetition time = 2000 msec, echo time = 34 msec, flip angle = 75°, field of view = 224 mm × 224 mm with 25 contiguous slices [zero gap] in the axial oblique plane, voxel size of 1.8 × 1.8 × 1.9 mm) were acquired on a 3-T Siemens Tim Trio MRI System (Siemens HealthCare, Erlangan, Germany) with an eight-channel head RF coil (In-Vivo, Inc., Gainesville, FL). Preprocessing, implemented in SPM5, included temporal and spatial realignment to correct for slice acquisition timing differences and head movement, respectively. Four participants exhibiting greater
than 2 mm within-run movement (approximately one voxel) were excluded. Note that images did not undergo spatial smoothing or spatial normalization to coregister with a template brain. Instead, all analyses were conducted in the participant’s “native space.”

To provide full coverage of the brainstem, the volume of acquisition was established with axial-oblique slices arranged at an angle that was approximately 45° from the AC–PC plane. The image volume was centered over the medulla to ensure full coverage of the midbrain (Figure 2).

**SN Localization**

We took an ROI-based approach for SN localization. This entailed identifying the SN on each participant’s task EPI volumes and generating participant specific binary masks of the SN using a semiautomated procedure described below. On task volumes, the SN appears as a discrete region of hypointensity (Figure 3) because of its high concentration of iron (Haacke et al., 2005; Dexter et al., 1991; Sofic et al., 1991), which dephases MR spins and shortens T2* relaxation times (Haacke et al., 2005; Ordidge, Gorell, Deniau, Knight, & Helpern, 1994) within structures containing iron. We derived estimates of task-evoked BOLD signal changes within these masks for each participant. These signal changes were group-averaged for hypothesis testing.

The first step in our procedure for creating a mask of the SN was to generate an optimal EPI volume for visualizing the SN for each participant. We maximized the visibility of the SN borders by creating an averaged EPI image from the spatially realigned task volumes. For the same reason, the task volumes did not undergo spatial smoothing or transformation to match a template or structural image. We selectively averaged the last two volumes of the intertrial interval of each trial because we

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**Figure 2.** fMRI volume. This figure displays the fMRI volume acquired for a representative participant. The volume was centered approximately on the brainstem with axial-oblique slice angle.

**Figure 3.** SN and STN BOLD signal localization. (A) A coronal section of a task EPI obtained from a representative participant is displayed. The black box outlines the midbrain region shown in B and C. (B) Close-up of the midbrain region showing the red nucleus, SN, and STN. The white arrow points to the separation between the SN and the STN that can be visualized in a coronal section. (C) Masks were created for the STN (yellow) and SN (blue). The red nucleus (purple) has also been identified here as an additional landmark. (D, E) Group averages of trial-averaged BOLD time series of the STN and SN show their activity during the three major phases of the task (cue, delay, probe). N = 16. *Delay period BOLD signal (Scans 5 and 6) was significantly elevated in the high-control condition in the SN but not in the STN, p < .05 for the interaction between Region × Condition interaction. (F, G) Beta estimates from the STN and SN showing of the delay period signal for the SN and STN, *p < .05, for the interaction between region and condition; *SN delay period betas were significantly higher in the high- compared with the low-control condition.
individuals, there were instances where clear boundaries identified by the automated segmentations for all individuals could not be unambiguously drawn. In consultation with one of the coauthors (PL), a neurosurgeon with extensive experience in localizing structures and is utilized by neurosurgeons to guide the targeting of BG nuclei during deep brain stimulation implants. However, some limitations of this atlas have been documented (Niemann & van Nieuwenhofen, 1999). The reslicing of images in AC–PC-aligned planes brought these coronal slices orthogonal to the AC–PC plane. These coronal slices orthogonal to the AC–PC plane were critical for distinguishing the SN from the STN, which also shows up a region of hypointensity on T2 weighted volumes (Richter et al., 2004) because of its high concentration of iron (Dormont et al., 2004). The coronal slices bring into view a hyperintense region that separates the SN and the STN, allowing us to distinguish and draw separate masks for these structures (Schafer et al., 2011; Coenen et al., 2008; see Figure 3). We utilized ITK-Snap (Yushkevich et al., 2006), a medical image segmentation software program, to view the midbrain coronal slices and to generate intensity-based segmentations of the SN. Image contrast for visualizing these regions and the hyperintense gap between them was optimized by setting the minimal (level) and maximal (window) voxel intensities to the 1st and 99th percentiles of voxel intensity distribution, modifying the control point for the function relating input versus output voxel intensities and filtering voxel intensities. Then, using ITK-Snap’s snake toolbox and default setting for the snake evolution equation, we generated the initial automated segmentations for the SN and STN.

These initial segmentations underwent manual inspection and editing. Although the SN and STN were readily identified by the automated segmentations for all individuals, there were instances where clear boundaries with neighboring structures could not be unambiguously drawn. In consultation with one of the coauthors (PL), a neurosurgeon with extensive experience in localizing midbrain structures using neuroimaging and intraoperative electrophysiological recordings (Larson et al., 2012), we developed a set of objective rules that conservatively limited the extent of SN and STN masks. These rules were developed using anatomic information provided by the Schaltenbrand and Wahren Brain Atlas (Schaltenbrand, Hassler, & Wahren, 1977). This atlas is widely considered to reliably depict the location of the SN and other BG structures and is utilized by neurosurgeons to guide the targeting of BG nuclei during deep brain stimulation implants. However, some limitations of this atlas have been documented (Niemann & van Nieuwenhofen, 1999). The reslicing of images in AC–PC-aligned planes brought them into register with the Schaltenbrand and Wahren Brain Atlas (Schaltenbrand et al., 1977). This allowed us to cross-reference our segmentations with anatomic information provided by the Schaltenbrand and Wahren Atlas.

On the basis of the information provided by the atlas, we applied the following rules to manually edit the automated segmentations of the SN and STN: The anterior extent of the SN and STN segmentations were limited to 3 mm anterior to the midcommissural point (the midpoint of the AC–PC line); to avoid the inclusion of the globus pallidus, which also appears hypointense because of its high iron content, in coronal slices anterior to the midcommissural point, we limited the lateral extent of segmentations to 10 mm left and right of the AC–PC defined midline; the inferior extent of the SN mask was limited to 6 mm inferior to the AC–PC plane; in instances in which the hyperintense region separating the STN and SN cannot be unambiguously identified, the horizontal plane defined by the inferior border of the red nucleus in the anteriormost coronal slice that includes the red nucleus served as the boundary defining the superior and inferior extents of the SN and STN, respectively; the posterior extent of the STN and SN was limited to 7 and 10 mm posterior to the midcommissural point, respectively. It should be noted that these bounding rules were intended to generate conservative masks, such that we would maximize the inclusion of true SN regions and the exclusion of non-SN regions. For example, recent structural MRI studies have demonstrated significant intersubject variability in the lateral extent of the SN beyond 10 mm from midline (Keukken et al., 2014). Therefore, limiting the lateral boundary of the SN ROIs in the manner we have would minimize the inclusions of non-SN regions.

We focused on the left SN and STN because of the well-recognized contralateral control of motor responses by these regions (Ouchi et al., 2002; Benazzouz et al., 1996; Bergman, Wichmann, & DeLong, 1990; Kempster, Gibb, Stern, & Lees, 1989; Schultz, Ruffieux, & Aebischer, 1983) and the fact that all participants provided right-handed responses.

**SN Masks Reliability**

We evaluated the test–retest reliability of our SN masking procedures by calculating the intraclass correlation coefficient of mask volumes. One of the coauthors drew masks of the left SN for the entire sample on one session and then redrew these masks 4 weeks later. We also calculated the percentage of overlap between the masks drawn on these two sessions.

**BOLD Time Series**

We derived BOLD time series estimates from each individual’s SN and STN mask. We generated the time series by trial-averaging the BOLD signal across all voxels within an ROI for all correct trials for each participant. Signal change was calculated by normalizing each value in the time series by the mean fMRI signal across the entire scan. We then averaged the time series across all participants within a group.
fMRI GLM

We conducted a slow event-related analysis of the BOLD time series by convolving the canonical hemodynamic response function with a series of delta functions, with covariates for cue, delay, and response for correct trials, and a separate set of nuisance covariates for incorrect/no-response trials. The three task phases were modeled in the following manner: cue—a single covariate during cue presentation at the beginning of the trial (t = 0 sec); maintenance—a single covariate in the middle of the delay period (t = 4 sec); and response—a single covariate during probe presentation (t = 8 sec). Previous work has demonstrated that trial events separated by 3–4 sec can be resolved using slow event-related fMRI (D’Esposito, Zarahn, & Aguirre, 1999; Kim, Richter, & Ugurbil, 1997; Zarahn, Aguirre, & D’Esposito, 1997). We included the first temporal derivative of covariates in the regression matrix to account for potential differences in BOLD signal temporal dynamics resulting from RT differences or other variables across individuals.

Statistical Testing

We conducted all analyses using SPSS (SPSS, Inc., Chicago, IL), utilizing two-tailed significance levels except when there were clear directional hypotheses. These instances have been indicated explicitly. We examined the relationship between the delay period signal of the SN and RTs with a linear mixed model; otherwise, t tests and ANOVAs were utilized for hypothesis testing.

RESULTS

Behavioral Results

Participants displayed lower accuracy, \( p = .05 \), and slower RTs, \( p < .01 \), in the high-control compared with the low-control condition (Figure 1).

Visualizing the SN on EPIs

We were able to visualize the SN and distinguish the SN from the STN in all 16 participants. Figure 3, from a representative participant, shows the characteristic hypointensity of the SN caused by its high iron concentration. In the coronal section, the SN is clearly distinguishable from its neighboring structures. These include the red nucleus and STN, which also possess relatively high iron content (Dormont et al., 2004) and appear as regions of hypointensity. For the sample, the average volume of the left SN was 209 mm\(^3\) (±62 mm\(^3\)). To evaluate the reproducibility of our procedure for drawing the SN mask, we measured the test–retest reliability of the volume of the masks drawn across two sessions. The intraclass correlation coefficient for these volumes derived from two separate sessions for all 16 participants was .87, \( p < .001 \). We also calculated the percentage overlap between the SN masks drawn in the two sessions. On average, 90% (±6.2%) of the SN mask drawn on the first session overlapped with the second SN mask.

SN BOLD Signal

We calculated the trial-averaged time series of the BOLD signal for the SN during high-control (red cue) and low-control (green cue) trials of the POP task. The group-averaged time series are displayed in Figure 3. On a qualitative basis, SN showed distinct activity pattern in the high- compared with the low-control condition. Although activity was elevated above baseline during cue and response phases in both conditions, SN activity during the delay period (Scans 5 and 6) was elevated only during high-control trials. In low-control trials, it returned to baseline during the delay period. We statistically tested the condition specificity as well as the region specificity of the SN delay period signal by means of a repeated-measures ANOVA of the BOLD signal from Scans 5 and 6 with factors of Condition (high and low control) and Region (SN and STN). The SN’s close proximity, similar size, as well as its high iron concentration and its effect on the BOLD signal makes the STN an ideal comparison region for the SN. Any regionally based confound such as movement-related artifacts induced by cardiovascular events or head movement would be expected to affect both the SN and STN. Thus, a Region × Condition interaction in this model would suggest that the difference in delay period signal in the SN was specific to this region. The repeated-measures ANOVA revealed a significant Region × Condition interaction, \( F(1, 15) = 7.33, p = .016 \). Post hoc t tests confirmed a significantly higher SN signal in the high compared with the low cognitive control condition during Scan 5, \( df = 15, t = 2.81, p = .013 \), and Scan 6, \( df = 15, t = 2.63, p = .019 \) and nearly equivalent activity across conditions in the STN. NB: For both Scans 5 and 6, the difference between conditions remains significant even after correcting for multiple comparisons because the corrected critical \( p \) value would be .025. These findings were further confirmed with parameter estimates (beta values) from the regression model for the delay period. ANOVA of delay period beta values from these two regions also showed a significant Region × Condition interaction, \( F(1, 15) = 6.40, p = .023 \). Post hoc t test of delay period betas also revealed significantly higher values in red cue trials compared with green cue trials, \( df = 15, t = 2.81, p = .013 \).

We further examined whether head movement could be contributing to the delay period activity difference across conditions in the SN. We quantified the total magnitude of scan-to-scan head movement in terms of geometric distance and found that movement did not significantly differ between the high- and low-control trials, \( p = .91 \). This was the case even when we limited the comparison with scans in and around Scan 5, where the significant group difference occurred (movement...
that this relationship was inverse, parameter estimate = .036, and that this relationship was inverse, parameter estimate = −7.83. In other words, a unit increase in SN activity was associated with a decrease in 7.83 msec of RT.

**DISCUSSION**

We applied a new method for localizing the BOLD signal from the SN and obtained novel evidence of this structure’s involvement in the control of response selection. During the delay period preceding response, we observed increased SN activity only in the high-control condition. This pattern of activity appeared to be region specific, in that the STN did not show above baseline delay period signal. The magnitude of SN delay signal was significantly inversely associated with task performance RT. Taken together, these results suggest that the SN is involved in the implementation of proactive control of response selection.

Our findings are consistent with prior theoretical and empirical studies that have implicated the SN in higher-order cognitive processes. Much of this work, however, has centered on working memory (WM). Theories have proposed that the SN helps to gate information into and out of cortico-BG-thalamo-cortical loops during encoding and response phases of WM (O’Reilly & Frank, 2006). Our results here are consistent with this hypothesized role. Although we have highlighted the controlled response aspect of our cognitive paradigm, the successful execution of our task also requires WM to properly encode cue information, maintain task rules, and act on response contingencies. Thus, the transient SN activity we observed during encoding and response is consistent with this structure’s hypothesized role in gating information in and out of WM during these task phases. This set of findings is consistent with a recent fMRI study, which also found transient, task-evoked activity of the SN (and ventral tegmental area) during the execution of a task with a trial structure and task demands that are quite similar to ours (D’Ardenne et al., 2012).

Our most novel result is the high-control condition-specific SN BOLD delay period signal, which, to the best of our knowledge, has not been documented previously in humans. A discussion of the interpretation of what this delay period activity represents would benefit first from a consideration of what this activity does not represent. The absence of delay period activity in the STN suggests that this activity is not a general feature of the BG nor is it likely due to methodological confounds affecting ventral midbrain structures, such as movement. Given the small size of the SN, even relatively minor amounts of head movement during scanning could contribute to significant error in estimating the signal from this region. We attempted to minimize the effects of head movement by using relatively stringent exclusion criterion for excess movement in this study. Moreover, we conducted post hoc analyses and did not find evidence of movement contributing substantially to our main findings. Another source of movement-related artifacts that could affect measurements of SN activity is the presence of large pulsatile blood vessels close to the ventral midbrain. Some investigators have instituted special procedures to mitigate pulsatile artifacts in measuring midbrain signal (D’Ardenne, McClure, Nystrom, & Cohen, 2008). However, this source of movement and artifacts attributable to them likely cannot explain the condition-specific increase in the delay period signal of the SN signal. The close proximity of the STN to the SN and the region specificity of our findings provide further evidence against this potential confound.

A consideration of the task requirement of the POP paradigm suggests that the condition-specific SN delay activity is likely not attributable to processes directly related to WM maintenance. This is because WM requirements during this task phase, either in terms of bits of information to be maintained or manipulated, are essentially equivalent between conditions during the delay period. The only information to be maintained in WM during this task period is the color cue and/or task rules. In the case of the latter, the information required to complete the task for the low condition would be something along the lines of “press button on the same side indicated by the probe arrow” whereas for the high-control condition it would be “press button on the opposite side indicated by the probe arrow.” Given the SN’s well-recognized involvement in motor functions, it would be reasonable to consider whether SN delay activity is facilitating a motor related process. However, critically, the selective enhancement of the signal in the high-control condition argues against a low level, purely motor function, for example, preparing to make a specific motor response, because the specific motor response to be executed is not known until the probe stimulus appears after the delay period.

The condition-specific upregulation of delay period signal in the high-control condition, along with its inverse relationship with task RTs, supports the hypothesis that the SN is involved in proactive control of response selection.
The interpretation that the SN delay signal, and by extension the BG, is serving a control function is consistent with recent propositions that the execution of overlearned or automatic responses relies on cortical networks, particularly sensorimotor cortices (Floyer-Lea & Matthews, 2005; Karni et al., 1995), whereas controlled responses are dependent on the associative regions of the BG (Ashby, Turner, & Horvitz, 2010). The proactive control of responses itself, however, is comprised of a number of different processes, including aspects of attention, inhibitory control, and effort. A goal of future research should be to combine our new method for SN localization with the appropriate paradigms capable of mapping specific component processes to the SN. Nonetheless, within the limitations of this study, a consideration of the possible neural source of the observed SN delay period activity, to be presented below, could inform this discussion.

The specificity of the neurobiological inferences we can draw from these results is limited by the uncertainty of the neural cell type source for the SN BOLD signal. A thorough consideration of this complex issue is beyond the scope of this discussion, and the reader is referred to a recent discourse on the potential neural sources for the fMRI signal from midbrain dopaminergic nuclei (Duzel et al., 2009). Our SN ROI likely included both SNc and SNr regions, which are enriched in dopaminergic (Björklund & Dunnett, 2007) and GABAergic (Rajakumar et al., 1994) neurons, respectively. Our ROI localization procedure identifies tissue with relatively high iron content, which both SN compartments have been shown to contain (Haacke et al., 2005; Dexter et al., 1991; Sofic et al., 1991). Furthermore, in primates, particularly in humans, these compartments are not spatially segregated as they are in lower animals such as rodents (Poirier et al., 1983). Thus, in as much as the BOLD signal can reflect unit output activity of neurons and not only inputs to a region (Mukamel et al., 2005; Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001), the SN BOLD signal we measured may reflect contributions from dopaminergic and/or GABAergic activity.

These issues notwithstanding, lines of evidence suggest the possibility that the SN delay period BOLD signal signifies the involvement of SNc and dopamine (DA) in the proactive control of response selection. This possibility is supported by the central role this structure plays in the regulation of DA neurotransmission (Duzel et al., 2009; Björklund & Dunnett, 2007) and the recent demonstration by a PET-fMRI study of a strong correlation between SN BOLD signal and the amount of DA release (Schott et al., 2008). Evidence from Parkinson disease literature also implicates SN DA in controlled response selection. If SN DA neurotransmission were involved in this process and because Parkinson disease involves the loss of dopaminergic SN neurons, then individuals with this condition should exhibit poor performance on tasks requiring controlled response selection. A recent meta-analysis has documented that the magnitude of the effect size for performance decrements in participants with Parkinson disease compared with control participants was greatest for the Stroop effect among tasks of executive functions surveyed (Kudlicka, Clare, & Hindle, 2011). The Stroop task is a prototypical paradigm that probes controlled versus automatic responses. Another indirect line of evidence from the Parkinson literature supporting a link between SN DA and controlled actions is the elicitation of paradoxically rapid and smooth movements by visual cues in some Parkinsonian patients who otherwise suffer from prominent akinesia (Asmus, Huber, Gasser, & Schols, 2008; Glickstein & Stein, 1991). These phenomena demonstrate that seemingly automatic responses elicited under visual guidance remain intact in Parkinson disease and suggests that the essential deficit in this condition lies within the willed or controlled components of motor responses.

A consideration of the STN results may provide useful information for speculating on the neural source of the observed SN delay period activity. As indicated earlier, the SNr and STN would be expected to exhibit similar activation patterns because they are directly connected and functionally closely related as central components of the indirect pathway (Parent & Hazrati, 1995). Consequently, the absence of delay period activity in the STN suggests that the indirect pathway and, by extension, the SNr are not engaged during the delay period. If the SNr were not involved, this would leave the other major component of the SN, the SNc, as the source of the delay period activity. This raises an intriguing possibility for the operation represented by the SN delay period. Recently, investigators have highlighted that effort in relation to decision-making is an important function of the DA system (Kurniawan, Guitart-Masip, & Dolan, 2011). It is tempting to speculate then that the SN delay period signal may represent the increased effort coded by DA signaling in the high-control condition. Likewise, in as much as STN activity signifies the involvement of the indirect pathway and response inhibition-related processes are thought to involve this pathway (Aron, 2011), the absence of STN delay activity suggests that proactive control of inhibition is not involved in this task period of the POP task.

To the best of our knowledge, this study’s strategy for localizing the SN BOLD signal differed from all prior investigations in at least one fundamental way. We identified the SN directly on task EPI volumes in “native space” and thus avoided procedures that could contribute to significant localization errors. We instituted procedures to distinguish the SN from the STN, which, due to their similar morphology, location, and signal properties, can easily be mistaken for the other (Schaefer et al., 2011; Coenen et al., 2008; Richter et al., 2004).

The most common approaches for localization of the BOLD signal from the SN as well as the VTA utilize a multistep procedure. The functional images are spatially normalized and coregistered with structural (Bunzeck, Guitart-Masip, Dolan, & Duzel, 2014; D’Ardenne et al.,...
significantly inversely associated with task performance in the SN but not in the STN. SN delay period signal was reduced by the SN. The scale of the spatial distortion in the ventral midbrain region we have observed on our EPI volumes is substantial when considering the small size of the SN and proximity of functionally distinct structures in this region (unpublished observation). Although spatial normalization of EPI volumes onto structural images can theoretically correct for spatial distortion, it is very challenging to verify the accuracy of SN normalization because, at present, there are no commonly accepted landmarks within functional images that can verify normalization accuracy of this and other midbrain regions.

Notable aspects of our method are that it is relatively straightforward to implement, it may be widely adopted by other investigators, and it may have widespread impact on the future study of SN function. It relies on fMRI sequences, software and hardware that are readily available in many modern fMRI neuroimaging centers. Consequently, investigators who are familiar with basic fMRI methods and have access to higher-field MR systems capable of producing small voxel images can readily implement the procedures described in this study. We anticipate that both basic and clinical investigators will be interested in applying our method given the important role the SN is thought to play in diverse cognitive and affective processes as well as in the pathophysiology of many neuropsychiatric conditions with significant public health impact, including Parkinson disease, substance abuse, and affective processes as well as in the pathophysiology of diverse neuropsychiatric conditions.

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