Baseline frontostriatal-limbic connectivity predicts reward-based memory formation

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Baseline Frontostriatal-Limbic Connectivity Predicts Reward-Based Memory Formation

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Abstract: Reward mediates the acquisition and long-term retention of procedural skills in humans. Yet, learning under rewarded conditions is highly variable across individuals and the mechanisms that determine interindividual variability in rewarded learning are not known. We postulated that baseline functional connectivity in a large-scale frontostriatal-limbic network could predict subsequent interindividual variability in rewarded learning. Resting-state functional MRI was acquired in two groups of subjects (n = 30) who then trained on a visuomotor procedural learning task with or without reward feedback. We then tested whether baseline functional connectivity within the frontostriatal-limbic network predicted memory strength measured immediately, 24 h and 1 month after training in both groups. We found that connectivity in the frontostriatal-limbic network predicted interindividual variability in the rewarded but not in the unrewarded learning group. Prediction was strongest for long-term memory. Similar links between connectivity and reward-based memory were absent in two control networks, a fronto-parieto-temporal language network and the dorsal attention network. The results indicate that baseline functional connectivity within the frontostriatal-limbic network successfully predicts long-term retention of rewarded learning. Hum Brain Mapp 00:000–000, 2014. © 2014 Wiley Periodicals, Inc.

Key words: reward learning, brain connectivity, learning, memory, resting state functional magnetic resonance imaging, ventral striatum

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INTRODUCTION

Humans vary in their capacity to acquire and retain memories [Forsmann et al., 2010; Zimerman et al., 2013], and one fundamental determinant for this variability is the degree to which reward facilitates learning [Bratagro et al., 2004; Ericson et al., 1993; Forsmann et al., 2010; Zimerman et al., 2013]. Previous studies focused on the contribution of specific brain regions to reward-based memory including the ventral striatum [Wächter et al., 2009], midbrain, and hippocampus [Adcock et al., 2006; Kuhl et al., 2010; Wittmann et al., 2005]. However, reward-related brain activity is present beyond this circuitry and throughout the human brain [Vickery et al., 2011], mainly in structures along the mesocortical and mesolimbic dopaminergic pathways [Liu et al., 2011; Sescousse et al., 2013].

While there has been increasing interest in the role of reward in procedural and declarative learning [Adcock et al., 2006; McGinty et al., 2013; Wittmann et al., 2005], a systems-level mechanistic understanding of the way in which reward modulates learning across individuals remains missing. This gap in knowledge is of translational relevance as deficits in rewarded learning have been well documented in neuropsychiatric disorders such as depression [Kumar et al., 2008; Vriese et al., 2012], schizophrenia [Gold et al., 2008; Strauss et al., 2011] and addiction [Hyman et al., 2006; Volkow et al., 2009] and in neurological conditions such as Parkinson’s disease [Bodi et al., 2009; Peterson et al., 2009] and Stroke [Rochet et al., 2013].

To address this question, we assessed functional interactions within a large-scale network of brain areas implicated in reward processing using resting-state functional magnetic resonance imaging (fMRI) [Biswal et al., 1995; Friston et al., 1994]. Resting-state fMRI provides valuable information on intrinsic interactions among local and distributed brain networks [Albert et al., 2009; Birn, 2007; Vahdat et al., 2011; van den Heuvel and Hulshoff Pol, 2010; Van Dijk et al., 2010]. We, thus, evaluated the extent to which baseline functional connectivity in a frontostriatal-limbic functional network [Di Martino et al., 2008] predicted memory in two groups of healthy subjects (n = 30) half of whom then trained on a visuomotor procedural learning task with and the other half without reward feedback. Since the neural substrates of immediate and long-term procedural memory differ [Dayan and Cohen, 2011; Doyon and Benali, 2005], and these forms of memory appear to be differentially affected by reward [Abe et al., 2011; Dayan and Cohen, 2011; Wächter et al., 2009] links between baseline network connectivity and memory measured at different time intervals after training were examined.

MATERIALS AND METHODS

Participants

Thirty-Six right-handed, healthy volunteers (20 males; mean age $24.3 \pm 3.1$ years) gave written informed consent approved by the Combined Neuroscience Institutional Review Board, National Institutes of Health. Inclusion criteria were normal physical and neurological examinations, no MRI contraindications and no use of psychoactive medication. We also required a minimum of 6 h sleep prior to every testing session. Data from six subjects (four from the rewarded and two from the unrewarded group, see below) were later excluded due to excessive head movements with predefined criteria of translation or rotation movements above 1.5 mm during fMRI acquisition. Therefore, our final pool of participants included 30 volunteers (16 males, mean age $24.2 \pm 2.7$ years).

Procedural Learning Task

Subjects were assigned to one of two groups: a rewarded (15 subjects; eight males, mean age $24 \pm 2.4$ years) or an unrewarded (15 subjects; eight males; mean age $24.5 \pm 2.9$ years) group, both of whom trained on a modified version of a previously published visuomotor learning task used for studying procedural learning and memory [Dayan et al., 2014; Reis et al., 2009; Schambra et al., 2011]. Sitting in front of a laptop computer (Dell, Latitude E5510, screen size 15.6") placed at comfortable viewing height and distance, subjects were asked to move a cursor through a numbered sequence of five targets (four gates and one thick line, Fig. 1A) horizontally arranged on the screen. Movement of the cursor was controlled by pinching a force-transducer with the distal phalanx of the thumb and the proximal interphalangeal joint of the index of the right dominant hand (Fig. 1B). When pressure was applied to the transducer, the cursor moved to the right, and by releasing pressure, the cursor moved back to the left. Subjects had to accurately move the cursor through the following sequence: Target 1, return, Target 2, return, Target 3, return, Target 4, return, and Target 5. In both groups, auditory feedback (a “beep” sound) was given whenever a target was reached successfully, that is, the cursor was moved between the lines of a gate (or on the line of Target 5) for a minimum of 0.2 sec. A trial was successful if it was completed within 8 sec without overshooting beyond the boundaries of any of the targets. A GO-Signal, which was displayed as a bright green circle below the target zones, indicated the beginning of each trial. The GO-signal was switched off when a trial was completed or the subject overshoot the target boundaries. Disappearance of the GO-signal signaled the subject to wait for the next trial. The sequence of targets was the same for all subjects and did not change over the course of the experiment. Subjects were instructed to be as accurate as possible while completing the sequence within the allocated time (8 sec). During training, the rewarded group received visual feedback, indicating “You Win: $0.6$,” after the completion of each successful trial. A “Total Winnings” display was shown beneath this section, accumulating winnings throughout the entire training session. If a trial was unsuccessful, subjects received no reward feedback (“You
Behavioral training on a visuomotor task. A: Subjects had to move a cursor through a sequence of five targets horizontally arranged on a computer screen (1-Return, 2-Return, 3-Return, 4-Return, 5). B: Pinching a force-transducer with the right hand moved the cursor to the right (i.e., toward a target) whereas releasing pressure moved it back to the left (toward the start position). C: Experimental setup of the 3-session experiment. Day one began with an 8 min resting state scan. Subjects then trained on the visuomotor task outside of the scanner for 30 min. Tests of procedural memory were administered immediately (Immediate), one day (Delayed24h) and 4 weeks (Delayed4wk) after training. D: While at baseline performance between these two groups was comparable, differences between the groups emerged after training at the Immediate, Delayed24h and Delayed4wk tests.

Win: $50$. Thus, in our training paradigm accuracy was rewarded, rather than a combination of accuracy and speed [Dayan et al., 2014; Reis et al., 2009; Schambra et al., 2011]. As a control, the unrewarded group trained on the same task in the absence of reward feedback at the end of each trial. Both groups received identical real time visual depiction of cursor movements and heard auditory tones indicating a successfully reached target and a completed trial. Unbeknownst to the subjects, both groups ultimately received an identical compensation for their participation in the study after the third session, when the experiment was completed.

Experimental Setup

Session 1 began with an 8-min resting-state fMRI scan (Fig. 1C). Subjects were instructed to lay still inside the scanner, close their eyes, and avoid falling asleep. Foam inserts were used to limit head movements. After scanning, baseline performance was measured with 20 trials of the visuomotor learning task. Subsequently, subjects trained on the visuomotor learning task (5 blocks of 20 trials each, approximately lasting 30 min) with or without reward feedback. After training, memory was measured [Dayan and Cohen, 2011] immediately after (Immediate), the following day (Delayed24h, $\pm$ 110 min) and a month later (Delayed4wk, $\pm$ 0.6 days). Testing on the Delayed24h and Delayed4wk consisted of 22 trials, where the first two trials were discarded to exclude the warm-up effect [Ajeim, 2010; Schmidt and Lee, 2011].

Baseline and test measurements (Immediate, Delayed24h, Delayed4wk) were administered in the absence of reward feedback in both groups. Two subjects in the unrewarded group dropped out prior to the third testing session and, thus, data in the Delayed4wk was obtained from $n = 13$ in this group.

Imaging Setup

Scanning was performed on a 3.0-T GE Signa HDx scanner using an 8-channel coil. T2*-weighted images were acquired using a gradient echo echoplanar imaging sequence (201 volumes; 38 ascending axial slices, 4 mm thickness, 2,400 ms repetition time, 35 ms echo time, 90° flip angle, $24 \times 24$ cm$^2$ field of view, $64 \times 64$ matrix size). In addition, high-resolution 3D magnetization prepared rapid gradient echo images ($1 \times 1 \times 1$ mm) were acquired from each subject to allow for volume-based statistical analysis and for visualization purposes.
fMRI Preprocessing

Data were preprocessed using Brainvoyager QX. Anatomical images were aligned to an ACPC plane and then transformed to Talairach space [Talairach and Tournoux, 1988]. The first two functional volumes were discarded to insure the experimental data were acquired after the scanner reached steady-state magnetization. The remaining 199 volumes were used for analysis. Preprocessing of functional data included slice scan time correction, head motion correction, spatial smoothing with a Gaussian filter of 4 mm full width half maximum (FWHM) and temporal filtering using low-pass frequency filter of 0.01 Hz, and high-pass Gaussian-FWHM filter of 4 sec, resulting in frequencies between 0.01 and 0.1 Hz and reducing low-frequency drifts and high-frequency noise [Meindl et al., 2010; Wang et al., 2006]. Each subject’s functional images were then coregistered to the corresponding 3-D anatomical image to produce a 4-D volume time course. Every coregistration was checked manually and adjusted if needed.

Network Identification

Seed-based functional connectivity analysis was performed with the data from all 30 subjects (i.e., collapsed across groups) to identify a network of areas showing functional connectivity with the right nucleus accumbens (NAcc), a subset of the ventral striatum crucial in reward processing. Further analysis compared the results with those obtained with a left NAcc seed. Because of its small size and variable localization, NAcc was identified anatomically for every subject individually using a published procedure based on anatomical landmarks [Neto et al., 2008]. A spherical volume of interest (VOI; 123 voxels, voxel size 1 × 1 × 1 mm) was placed within each subject’s localized NAcc. The average Talairach coordinates of the NAcc VOIs across the 30 subjects were 8/7/−4 (± 0.86/1.1/0.95), which is in agreement with a recent meta-analysis of reward-processing studies and with a previous study of ventral striatum functional connectivity [Di Martino et al., 2008; Liu et al., 2011]. Average time series were then extracted from each NAcc VOI and served as predictors in a multivariate general linear model. Heart rate and respiration data from every subject were used as nuisance covariates to account for the influence of these slowly fluctuating signals on the BOLD contrast. Statistical activation maps were corrected for multiple comparisons using a false discovery rate (FDR) correction at P < 0.01. VOIs with identical shape and size (spherical, 512 voxels, voxel size 1 × 1 × 1 mm) were placed at the center of each cluster of activity, of activity that showed connectivity with the NAcc. Beta-weights from these VOIs were then extracted and averaged to compute a subject specific index of network connectivity.

Two control networks, the dorsal attention network [Buckner et al., 2013; Fox et al., 2006; Thomas Yeo et al., 2011] and a fronto-parieto-temporal language network [Tomasi and Volkow, 2012] were defined following the same procedure as described earlier but using the right intraparietal sulcus (rIPS) and left inferior frontal gyrus (lIFG), respectively, as seeds. Seed coordinates were identical in each subject, and were 27/−58/49 for rIPS [Fox et al., 2006] and −49/25/18 for lIFG [Tomasi and Volkow, 2012]. In the dorsal attention network, where voxels showed highly significant correlation with the rIPS seed, a higher minimal statistical threshold was used (t = 12, compared to t = 7) to allow for the identification of distinct nodes within the network. These networks were chosen because we expected them to have little overlap with the reward circuits in the frontostriatolimbic pathway. Single region beta-weights were extracted and connectivity indices were again computed for each subject by averaging beta-weights across all nodes within each of the networks.

Data Analysis

Accuracy (number of correct trials in a block) served as the behavioral outcome measure. Group performance was analyzed using a two-way repeated-measures analysis of variance, where “Group” (rewarded, unrewarded) served as the between-subjects factor and “Testing Session” (all testing sessions) as the within-subject factor. Pearson’s correlations were used to examine links between memory scores and network connectivity within subjects. Between-group comparisons of correlations between network connectivity and memory were performed with a Fisher r-to-z transformation [Fisher, 1915]. Statistical analyses were performed with SPSS and MATLAB. Robust correlation tests were performed using the “Robust Correlation Toolbox” in MATLAB [Pernet et al., 2012]. Significance levels for all statistical tests were set at P < 0.05 and were corrected for multiple comparisons.

RESULTS

Baseline performance was comparable in the rewarded and unrewarded groups (1.7 ± 0.5 and 1.5 ± 0.5 correct trials, respectively). There was a significant effect of Group (F_{1,24} = 4.28, P < 0.05, Fig. 1D) in the absence of a Group x Testing session interaction (F_{3,72} = 1.48, ns). Thus, both groups’ performance in the task improved immediately post training (P < 0.01) reaching 14.6 ± 1 and 11.8 ± 0.6 correct trials in the rewarded and unrewarded groups, respectively. Memory then remained relatively stable at 24 h (14.4 ± 0.7 and 12.3 ± 0.6 correct trials in the rewarded and unrewarded groups, respectively) and 4 weeks (14.3 ± 1 and 12.3 ± 0.8 correct trials, respectively) testing times.

We then evaluated the relationship between baseline connectivity and memory. A seed-based functional connectivity analysis focusing on the brain regions showing functional connectivity with the right NAcc [Cauda et al., 2011; Di Martino et al., 2008] identified a fronto-cingulate-striato-limbic network (Fig. 2A and Table I, Methods). Further analysis averaged the beta-weights (regression
Frontostriatal-limbic network. A: Seed-based connectivity analysis with NAcc revealed a network composed of frontal, striatal, and limbic regions, visualized here on inflated and flattened brains (FDR-corrected at $P < 0.01$). B: Correlations of average network connectivity with memory at Immediate, Delayed24h, and Delayed4wk testing points in the rewarded group. Only long-term memory (Delayed4wk) showed significant correlations with average network connectivity. C: Correlations of average network connectivity with memory in the unrewarded group. No significant correlations were found.

coefficients) across all nodes in the network allowing computation of an index of network connectivity for each individual subject. Average connectivity in this network was comparable across the rewarded and unrewarded groups ($t_{28} = 1.88$, ns).

In the rewarded group, network connectivity correlated with memory at Delayed4wk ($r = 0.625$, $P < 0.01$, Bonferroni corrected for multiple comparisons; Fig. 2B; see also Supporting Information Fig. 1) but not at the Immediate or Delayed24h testing times ($r = 0.057$; ns and $r = 0.35$; ns,
TABLE I. Components of the frontostriatal-limbic network

<table>
<thead>
<tr>
<th>l/r</th>
<th>Region</th>
<th>BA</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>Middle Temporal Gyrus</td>
<td>21</td>
<td>49</td>
<td>5</td>
<td>-24</td>
</tr>
<tr>
<td>r</td>
<td>Parahippocampal Gyrus</td>
<td>21</td>
<td>40</td>
<td>7</td>
<td>-32</td>
</tr>
<tr>
<td>r</td>
<td>Insula</td>
<td>36</td>
<td>7</td>
<td>-6</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>Amygdala (ventral)</td>
<td>33</td>
<td>-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>Uncus</td>
<td>20</td>
<td>31</td>
<td>-4</td>
<td>-34</td>
</tr>
<tr>
<td>r</td>
<td>Cingulate Gyrus</td>
<td>32</td>
<td>3</td>
<td>22</td>
<td>28</td>
</tr>
<tr>
<td>r</td>
<td>Amygdala (dorsal)</td>
<td>26</td>
<td>-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>Medial Frontal Gyrus</td>
<td>10</td>
<td>13</td>
<td>52</td>
<td>15</td>
</tr>
<tr>
<td>r</td>
<td>Medial Pallidum</td>
<td>10</td>
<td>-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>Thalamus</td>
<td>7</td>
<td>-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>Midbrain</td>
<td>4</td>
<td>-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>Cerebellum (Culmen)</td>
<td>5</td>
<td>-54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l</td>
<td>Anterior Cingulate</td>
<td>24</td>
<td>-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l</td>
<td>Cerebellum (Culmen)</td>
<td>1</td>
<td>-55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l</td>
<td>Medial Frontal Gyrus</td>
<td>9</td>
<td>-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l</td>
<td>Medial Pallidum</td>
<td>-17</td>
<td>-15</td>
<td>-6</td>
<td></td>
</tr>
<tr>
<td>l</td>
<td>Amygdala</td>
<td>-34</td>
<td>-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>l</td>
<td>Middle Temporal Gyrus</td>
<td>21</td>
<td>-36</td>
<td>-4</td>
<td>-24</td>
</tr>
</tbody>
</table>

Talairach coordinates of the center of each cluster within the frontostriatal-limbic network. Every volume of interest was of identical shape and size (spherical; 512 voxels).

Significant correlations at Delayed4wk were not restricted to the network connectivity index but were also evident in pairwise correlations with multiple single regions within the network (Supporting Information Fig. 2A). In the unrewarded group, network connectivity did not correlate with memory at any testing time (Immediate: \( r = -0.001 \); ns, Delayed24h: \( r = 0.069 \); ns, Delayed4wk: \( r = -0.418 \); ns, Fig. 2C and Supporting Information Fig. 2B). Correlations between baseline connectivity and measures were significantly different between groups at Delayed4wk (\( z = 2.75, P < 0.01 \)) but not at the Immediate and Delayed24h tests (\( z = 0.14, n_s \) and \( z = 0.73, n_s \), respectively).

Additional analyses were carried out to confirm the reliability of the significant correlation between network connectivity and learning scores at Delayed4wk in the rewarded group. First, the correlation between network connectivity and scores at Delayed4wk was preserved after a leave-one-out cross validation procedure (with \( r \)-values ranging from 0.563 to 0.694, all significant at \( P < 0.02 \)). We next tested the correlation using two robust correlation procedures, where outliers are down weighted or removed and accounted for in significance testing [Pernet et al., 2012]. The first procedure, the percentage-bend correlation [Wilcox, 1994], is robust against the influence of marginal outliers and estimates linear associations without computing a Pearson’s correlation coefficient [Pernet et al., 2012]. The second procedure, skipped-correlations [Wilcox, 2004], directly reflects Pearson’s \( r \) and protects against the influence of bivariate outliers. The correlation between network connectivity and scores at Delayed4wk in the rewarded group was significant using both techniques (Bend correlation: \( r = 0.63, P < 0.02 \); skipped correlation: \( r = 0.62, P < 0.05 \)).

The literature points to the involvement of both right and left NAcc in reward processing [Liu et al., 2011], with some evidence for right lateralization [Cauda et al., 2011; Di Martino et al., 2008; Knutson et al., 2001]. While, for brevity, our focus here was on right NAcc, comparable results were obtained also when the network was defined using a left NAcc seed (Supporting Information Fig. 3). A significant correlation was found between network connectivity defined with left NAcc and learning scores at Delayed4wk for the rewarded (\( r = 0.532, P < 0.02 \)) but not for the unrewarded group (\( r = -0.425, n_s \)). These correlations were significantly different (\( z = 2.51, P < 0.02 \)). Moreover, average network connectivity between the networks based on right and left NAcc did not differ (\( t = 1.675, P = 0.105 \)) and showed a strong correlation across subjects (\( r = 0.678, P < 0.01 \)).

To evaluate the topographic specificity of the findings, we examined two control networks. The first network, referred to as the language network [Tomasi and Volkow, 2012], is composed of frontal parietal and temporal regions, all showing functional connectivity with left IPI (FDR-corrected at \( P < 0.01 \), Fig. 3A and Supporting Information Table 1). The second control network, known as the dorsal attention network [Buckner et al., 2013; Fox et al., 2006] is primarily composed of frontal and parietal regions, that show functional connectivity with the right IPS (FDR-corrected at \( P < 0.01 \), Fig. 3B and Supporting Information Table 2). Baseline connectivity within neither the language network (Fig. 3C), nor the dorsal attention network (Fig. 3D) correlated with any of the memory measures in the rewarded group (all \( P \) values > 0.091). Similarly, connectivity in neither of the networks correlated significantly with any of the memory measures in the unrewarded group (all \( P \) values > 0.089, see Supporting Information Fig. 4A, B). Consistently, no significant differences between the correlations obtained for the rewarded and unrewarded groups were found (all \( z \) tests < 1.2, all ns).

**DISCUSSION**

The results demonstrate that baseline functional connectivity within a frontostriatal-limbic network predicts the formation of long-term procedural memory acquired with rewarded feedback. The specificity of the frontostriatal-limbic network for predicting reward-based long-term memory was established by training a second group of subjects who performed the same learning task in the absence of reward feedback. There were significantly weaker correlations between network connectivity and procedural memory in this group. Similar links between connectivity and reward-based memory were absent in two control networks, confirming the topographic specificity of the findings.
Specificity of the memory-connectivity links to the frontostriatal-limbic network. **A:** The fronto-parieto-temporal language network, composed of regions showing functional connectivity with left IFG (FDR-corrected at $P < 0.01$) was used as a first control network. **B:** The dorsal attention network, composed of regions showing functional connectivity with right IPS (FDR-corrected at $P < 0.01$) was used as a second control network. **C:** In the rewarded group, no significant correlations were obtained between any of the memory measures and connectivity in the fronto-parieto-temporal language network or **D:** the dorsal attention network. Similar results were obtained for the unrewarded group (Supporting Information Fig. 4 A,B).

The frontostriatal-limbic network studied here is composed of regions implicated in reward-related computations. First, the NAcc, part of the ventral striatum, is engaged in reward anticipation [Knutson et al., 2001; Schoot et al., 2008], encoding of reward prediction errors [Abler et al., 2006; Rodriguez et al., 2006] and evaluation of reward [Liu et al., 2007]. In the context of reward-based learning, it has been proposed that the striatum integrates dopaminergic reward...
signals with sensory cues via corticostriatal and thalamo-
striatal afferents to modulate the generation of motor com-
mands [Hosp et al., 2011; Wickens et al., 2003]. This effect is
mediated by direct and indirect output pathways to the
motor cortex (M1) [Wickens et al., 2003], including a projec-
tion from the ventral tegmental area (VTA) to M1 [Hosp
et al., 2011]. Moreover, it was recently reported that activity
within a local neuronal network in the rat’s NAcc is strongly
predictive of performance of a locomotor task [McCanty
et al., 2013]. Other regions within the frontostriatal-limbic
network have also been implicated in reward-related func-
tions. Activity in ventromedial prefrontal cortex (vmPFC),
supports the formation of memory under predictable reward
[Bialleck et al, 2011]. The orbitofrontal cortex (OFC) is
engaged in encoding of reward value [Elliott et al., 2005]
and in processing motivational relevant variables [Elliott
et al., 2000; Fellows and Farah, 2005]. The anterior cingulate
cortex is essential for integrating reward and motor responses
[Williams et al., 2004], while other structures including the thalamic, amygdala, and vmPFC interact dur-
ing associative memory encoding acquired under reward
[Gaffan and Murray, 1990]. Additionally, medial temporal
lobe interactions with the prefrontal cortex (PFC) are
required for transitioning recently acquired memories into
long-term memories [Frankland and Bontempi, 2005; Simons
and Spiers, 2003]. Similarly, the amygdala is not only activat-
ed by rewarding stimuli [Canli et al., 2002] but also couples
with the striatum during learning [Popescu et al., 2009]
and interacts with the medial temporal lobe to coordinate
memory formation [Dolcos et al., 2004]. Anatomically, direct
projections from VTA to the hippocampus contribute to
reward-based formation of adaptive memory, as do disinhibitory projections from NAcc and globus pallidum and
excitatory projections from the PFC to VTA [Shohamy
and Adcock, 2010]. The amygdala, PFC and NAcc also
receive direct dopaminergic projections from VTA sending
disinhibiting projections back to VTA [Düzel et al., 2009].
Altogether, these findings support our current focus on the
frontostriatal-limbic network and fit in well with its role in
predicting interindividual variability in rewarded learning.

Interestingly, connectivity in the frontostriatal-limbic net-
work predicted procedural memory 4 weeks after but not
immediately post-training. These preliminary findings raise
the hypothesis that this network may play a more promi-
nent role in consolidation of rewarded learning over time,
taking place after the end of training [Dayan and Cohen,
2011]. Consistent with our findings, reward has been shown
to specifically facilitate long-term procedural memory [Abe
et al., 2011; Dayan et al., 2014], plausibly through engaging
similar circuitries as those included in the frontostriatal-
limbic network. While our results cannot specifically pro-
vide evidence as to whether different networks are involved
in the formation of memory immediately, one day or several
weeks after training, the strengthening of correlations over
time imply that the same network could be engaged in
memory formation, across all temporal windows, dynami-
cally reshaping through systems consolidation, a process
known to last between weeks to months [Dudai, 2004].
Additional work is needed to confirm this suggestion.

The results are consistent with the view that reward proc-
esting is distributed throughout the human brain [Liu et
al., 2011; Vickery et al., 2011]. Integrative processing within this
large-scale network is likely rooted in anatomical connec-
tions between core network nodes [Haber and Knutson,
2010]. For instance, projections from limbic areas [Friedman
et al., 2002; Fudge et al., 2002] and the OFC reach the ven-
tral striatum, and similarly from the ventral striatum to pal-
lidum and midbrain [Haber et al., 1995]. Human studies
demonstrated diffusion MRI-based structural connectivity
between the striatum, ventromedial frontal cortex and limbic
regions such as the uncus and the amygdala [Johansen-Berg
et al., 2008; Lehericy et al., 2004]. Links between functional
connectivity as detected at rest and structural network archi-
tecture have been widely reported [Grecius et al., 2009;
Tomasini et al., 2011], and may potentially provide the neu-
roanatomical basis for the results reported here. Namely,
stronger functional connectivity within the frontostriatal-
limbic network may facilitate long-term memory retention
by maximizing the efficiency of information processing in
both local and distant network interactions [Sepulcre et al.,
2010], conceivably through an underlying variation in struc-
tural connectivity [Fields, 2011].

Procedural learning is crucial for rehabilitation after focal
brain lesions such as stroke [Dimyan and Cohen, 2011].
Stroke often severely disrupts motor function, requiring
patients to relearn basic procedural skills. Various rehabilita-
tive interventions for stroke utilize reinforcement schemes
[Krakauer, 2006; Pulvermühler et al., 2001], and reward can
modulate specific functional abnormalities after stroke [Mal-
hotra et al., 2013]. However, stroke patients, particularly
those with lesions in the basal ganglia, thalamus, insula, and
PFC show deficits in their sensitivity to reward [Rochat
et al., 2013], necessitating tools that could potentially differ-
entiate among patients who are more and less likely to
respond to reward. Thus, it is conceivable that functional
connectivity within frontostriatal-limbic circuits may help in
predicting interindividual variability in the success of
reinforcement-based interventions after brain lesions.

CONCLUSIONS

In summary, we report that baseline task-free functional
connectivity in a frontostriatal-limbic network predicts for-
mation of long-term memories acquired under rewarded
training. Baseline variation in intrinsic task-free brain con-
nectivity may thus underlie the interindividual differences
that characterize reward learning.

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REFERENCES


2. DARSTELLUNG DER PUBLIKATION

2.1 INTRODUCTION AND CONTEXT OF THE DISSERTATION

The human ability to learn and remember is remarkable, yet a trait that remains puzzling and poorly understood. Only a small subset of our experiences are stored in long-term memory and become accessible for later retrieval. One key mediator of learning and formation of long-term memory is reward. Reward (e.g., food, juice, monetary incentives) has been shown to mediate a range of human and animal behaviors (O'Doherty, 2004; Schultz, 2006), in particular, long-term retention of procedural and episodic memory is enhanced by reward (Abe et al., 2011; Wittmann, Dolan, & Düzel, 2011; Wittmann, Schiltz, Boehler, & Düzel, 2008). The striatum is known to play a crucial role in reward-based learning and interacts with distinct brain systems during learning (Alexander, DeLong, & Strick, 1986; Breiter & Rosen, 1999; Shohamy, 2011). Understanding the way by which interactions between the striatum and other brain regions contribute to learning and memory formation has recently become a focus of increasing neuroscientific interest (Shohamy & Adcock, 2010). Long-term memory relies on activity in areas within the medial temporal lobe (Frankland & Bontempi, 2005; Simons & Spiers, 2003), with recent evidence also pointing specifically to the ventral striatum (Ferretti et al., 2010). Functional connectivity between ventral striatum, specifically nucleus accumbens (NAcc), and the hippocampus can be strengthened by reward further contributing to long-term memory (Tabuchi, Mulder, & Wiener, 2000). The neuronal systems underlying reward-based learning have been investigated extensively in neuroimaging studies. In particular, increased blood oxygenation level dependent (BOLD) signal, as measured with functional magnetic resonance imaging (fMRI), has been consistently documented in ventral striatum, with additional evidence for right side lateralization (Diekhof, Kaps, Falkai, & Gruber, 2012; Knutson, Adams, Fong, & Hommer, 2001a). Reward-based learning and memory formation also engages larger scale frontostriatal and striatolimbic circuits (Haber & Knutson, 2010; Liu, Hairston, Schrier, & Fan, 2011; Pennartz, Ito, Verschure, Battaglia, & Robbins, 2011; Shohamy, 2011). Notably, activity within mesolimbic circuits potentiates the formation of reward-based memory, implying that motivational states can modulate learning through this mesolimbic network (Adcock, Thangavel, Whitfield-Gabrieli, Knutson, & Gabrieli, 2006).
The ability to form and retain memories varies substantially across individuals (Grön et al., 2003; S. Robertson, Myerson, & Hale, 2006). Such inter-individual variability also characterizes reward-based learning (Avila et al., 2011; Samanez-Larkin, Hollon, Carstensen, & Knutson, 2008; Samanez-Larkin, Kuhnen, Yoo, & Knutson, 2010). The relationship between structural and functional neural activity of entire networks and behavior has not been fully understood. Previous work started to investigate such links by studying network connectivity with resting state and diffusion tensor MRI. Variability in resting state functional connectivity (RSFC) was found to be related to inter-individual differences in performance of memory and sensori-motor tasks (Sala-Llonch et al., 2011; Tomassini et al., 2011; Wang et al., 2010). The behavioral relevance of spontaneous neuronal activity is by now well established (Albert, Robertson, Mehta, & Miall, 2009; Bassett et al., 2011; Damoiseaux et al., 2006; M. D. Fox et al., 2005), and regions within networks identified with RSFC are active in association with performance of specific tasks (Greicius, Krasnow, Reiss, & Menon, 2003; Smith et al., 2009; Toro, Fox, & Paus, 2008). It is therefore feasible that RSFC within related networks could contribute to learn and form a memory (Wang et al., 2010). The extent to which network activity correlates with formation of long-term memories in the framework of reward-based learning is not known.

2.2 SUMMARY OF METHODS

In the present study, we measured fMRI at rest before training to identify the contribution of frontostriatal-limbic networks to reward-based memory formation by examining whether spontaneous neuronal activity within this network can account for inter-individual differences in learning a visuomotor task with reward feedback and with long-term retention of the associated procedural memory. Memory formation was tested in 15 subjects at three separate time points to account for the different stages in learning which are characteristic for procedural learning (Dayan & Cohen, 2011): immediately, one day and one month after training. We then correlated a measure of average network connectivity with each of the memory scores to determine connectivity-memory relations. A control group (n=15) that trained with no reward feedback and two control networks unrelated to reward processing were analyzed to assess the specificity of our results.
2.3 Further Discussion of the Results

The results of this study demonstrate novel evidence linking functional connectivity at rest and reward-based procedural memory. Connectivity within a widely distributed frontostriatal-limbic network, as measured prior to training, predicted the formation of long-term procedural memory, acquired with reward feedback. Similar correlations were not found for either immediate memory nor for memory tested a day after training, although they become stronger over time. The possibility that prediction of long-term memory merely reflected general variability of RSFC within subjects’ brains was ruled out by analyzing two unrelated networks. One composed of regions showing functional connectivity with right intraparietal sulcus (IPS), i.e. the dorsal attention network, and another with left inferior frontal gyrus (IFG), i.e. a frontoparieto-temporal language network. Subject-specific connectivity within these networks showed no significant relationship with any of the memory measures. To further assure the specificity of the frontostriatal-limbic network for predicting reward-based memory formation rather than procedural learning, we trained a control group, who performed the same task with no performance-based reward feedback. Statistically significant correlations were not found between any of the memory measures and RSFC for this group of subjects.

Components of the frontostriatal-limbic network. Extensive evidence establishes a critical role for the ventral striatum, mostly the NAcc, in reward processing. Several reward-related functions such as anticipation (Knutson, Fong, Adams, Varner, & Hommer, 2001b; Kroemer et al., 2014; Schott et al., 2008), encoding of reward prediction error (Abler, Walter, Erk, Kammerer, & Spitzer, 2006; Rodriguez, Aron, & Poldrack, 2006) and evaluation of reward (Liu et al., 2007) have been linked to NAcc. This structure has similarly been shown to be involved in formation of procedural memory (Albouy et al., 2008), and initiation of locomotion (Nicola, 2010). Thus, with respect to reward-based procedural learning the striatum may integrate dopaminergic reward signals with sensory cues via corticostriatal and thalamostriatal afferents and mediate the generation of motor commands via direct and indirect output pathways with the motor cortex (M1) (Wickens, Reynolds, & Hyland, 2003), for instance through a ventral tegmental area (VTA) to M1 projection (Hosp, Pekanovic, Rioult-Pedotti, & Luft, 2011).
Other regions within the frontostriatal-limbic network found here have also been implicated in specific reward-related functions. Activity in ventromedial prefrontal cortex (vmPFC) was found to be associated with formation of a memory for predictable rewards (Bialleck et al., 2011). Orbitofrontal cortex (OFC) is engaged in the encoding of reward value (Elliott, Newman, Longe, & Deakin, 2003) and is also implicated in processing other motivational relevant variables (Elliott, Friston, & Dolan, 2000; Fellows & Farah, 2005). The anterior cingulate cortex has been found to be essential for integrating reward and motor responses (Williams, Bush, Rauch, Cosgrove, & Eskandar, 2004), while structures like the thalamus, amygdala and vmPFC interact during associative learning with reward (Gaffan & Murray, 1990). Other parts of the frontostriatal-limbic network including structures in the medial temporal lobe are known to be crucial for memory formation, and their interactions
with the prefrontal cortex are necessary for the transition into long-term memory (Frankland & Bontempi, 2005; Simons & Spiers, 2003). Amygdala is not only activated by rewarding stimuli (Canli, Sivers, Whitfield, Gotlib, & Gabrieli, 2002), but its activity is also coupled with the striatum during learning (Popescu, Popa, & Paré, 2009) and also interacts with medial temporal lobe systems to coordinate memory formation (Bauer, Paz, & Paré, 2007; Dolcos, LaBar, & Cabeza, 2004). In Figure 1 a network similar to the one found in our study is shown, which is suggested to be involved in memory, learning and reward (Kelley, 2004).

**Reward-based memory formation in frontostriatal-limbic circuits.** The present results link connectivity within a large-scale functional network composed of regions showing functional connectivity with NAcc with formation of long-term reward-based procedural memory. These results confirm the integrative role of the NAcc within the reward network (Camara, Rodriguez-Fornells, Ye, & Münte, 2009; Shohamy & Adcock, 2010). Several subcortical and cortico-subcortical loops may allow reward to mediate memory. For instance, reward-based formation of memory may be achieved via direct projections from VTA to hippocampus, sending disinhibitory projections back via NAcc and globus pallidum (GP) or excitatory projections via prefrontal cortex (PFC) to VTA (Shohamy & Adcock, 2010). Amygdala, PFC and NAcc also receive direct dopaminergic projections from VTA sending disinhibiting projections back to VTA (Düzel et al., 2009).

While correlations of memory and RSFC in our study strengthened over time, functional connectivity in the frontostriatal-limbic network at rest only predicted long-term memory, as assessed 4 weeks after reward-based training. These findings appear to suggest a selective role for this network in formation of lasting reward-based memory formation, but not for more immediate forms of memory and may thus reflect systems consolidation within the related circuits. Whereas synaptic consolidation, that is consolidation, on a cellular, local level, is accomplished within hours, systems consolidation takes weeks and up to months to reorganize the large-scale brain circuits that encode memory (Dudai, 2004). While our findings did not prospectively track reorganization in the frontostriatal-limbic network over time, they document a stronger link between this large-scale network and long-term memory formation, which may reflect the longer time it takes this network to reorganize...
following reward-based learning. Additional work is needed to confirm this hypothesis.

**Possible links with dopaminergic systems.** The recruitment of the ventral striatum by reward-based learning may rely on dopaminergic neurotransmission (Schultz, 2004; Zald et al., 2004). Variation in functional connectivity within the frontostriatal-limbic network as reported here could mirror variability in baseline striatal dopamine synthesis capacity which predicts individual differences in reward-based learning (Cools et al., 2009). Moreover, memory acquisition and consolidation are impaired by dopamine antagonists (Willuhn & Steiner, 2008) and dopamine-release in the striatum is increased while learning a new motor sequence task (Lappin et al., 2009), implying that increased dopamine levels, which can be evoked by reward, positively affect memory formation (Koepp et al., 1998; Lisman & Grace, 2005). Overall, reward-based learning has been suggested to be dependent on a dopamine-driven plasticity (Pessiglione, Seymour, Flandin, Dolan, & Frith, 2006; Samejima, Ueda, Doya, & Kimura, 2005; Willuhn & Steiner, 2008). While our results cannot specifically confirm involvement of dopamine, they complement this framework by linking variation in functional connections in the dopaminergic mesolimbic and mesocortical pathways with inter-individual variability in formation of lasting reward-based procedural memory.

**Distributed Reward Representations in the Brain.** The current results implicate a large-scale frontostriatal-limbic network in long-term memory formation, and are in agreement with recent results on the distributed nature of reward signals throughout the human brain (Lappin et al., 2009; Liu et al., 2011; Vickery, Chun, & Lee, 2011). Primate and other animal models demonstrate that regions within this network are anatomically connected (Haber & Knutson, 2010; Koepp et al., 1998; Lisman & Grace, 2005). For instance projections from limbic areas (Friedman, Aggleton, & Saunders, 2002; Fudge, Kunishio, Walsh, Richard, & Haber, 2002) and the OFC reach the ventral striatum, and similarly from the ventral striatum to pallidum and midbrain (Haber, Kunishio, Mizobuchi, & Lynd-Balta, 1995). In humans, diffusion MRI fiber tracking studies demonstrated structural connectivity between the ventral striatum and the ventromedial frontal cortex and limbic regions such as uncus and amygdala (Johansen-Berg et al., 2008; Lehéricy et al., 2004). Structural connectivity
measured with diffusion MRI can predict system-level properties of functional networks at rest (Honey et al., 2009). Importantly, links between functional connectivity as detected at rest and structural network architecture have been widely reported (Greicius, Supekar, Menon, & Dougherty, 2009; Tomassini et al., 2011), and may potentially explain the results reported here. Namely, stronger functional connectivity of regions with the NAcc, as found here, may facilitate long-term retention of newly acquired skills by maximizing the efficiency of information processing in both local and distant interactions of network components (Sepulcre et al., 2010), conceivably through an underlying variation in structural connectivity (Fields, 2011).
3. ZUSAMMENFASSUNG

3.1 SUMMARY OF RESULTS
Using resting state fMRI and seed-based analysis we identified a large-scale frontostriatal-limbic network, composed of areas implicated in reward processing and memory formation. Two groups of healthy individuals were subsequently trained on a visuomotor procedural learning task receiving reward feedback (n=15) or auditory feedback only (control group, n=15). Memory formation was tested immediately after, one day and one month after training. Functional connectivity within the identified network predicted inter-individual variability of long-term procedural memory in the rewarded group but not in the control group. Two unrelated control networks were analyzed to further test specificity of our results and did not show correlations with any of the behavioral measures. We thus propose that variation in frontostriatal-limbic connectivity may be a source for inter-individual differences in memory formation acquired with reward.

3.2 ZUSAMMENFASSUNG DER ERGEBNISSE
4. LITERATURVERZEICHNIS


RC159.
Robertson, S., Myerson, J., & Hale, S. (2006). Are there age differences in


5. ERKLÄRUNG DES EIGENANTEILS

Die Arbeit wurde in der Klinik und Poliklinik für Neurologie unter der Betreuung von Prof. Dr. med. Christian Gerloff (Doktorvater) und Dr. med. Friedhelm Hummer (Mentor) und an den National Institutes of Health (NIH) unter der Betreuung von Dr. Leonardo Cohen (Institutsleiter National Institute of Neurological Disorders and Stroke) und Dr. Eran Dayan (Mentor) durchgeführt.


Ich übernahm die Durchführung der Pilotstudien, Rekrutierung der Probanden und Datenverwaltung vollständig. Die MRT-Messungen wurden gemeinsam durch mich und Dr. Dayan nach Einarbeitung in die Sicherheitsbestimmungen durchgeführt, während die behavioralen Daten von mir allein erhoben wurden.


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7. Lebenslauf entfällt aus datenschutzrechtlichen Gründen
8. EIDESSTATTLICHE ERKLÄRUNG
Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.
Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: ........................................................................