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Monoamine levels within the orbitofrontal cortex and putamen interact to predict reversal learning performance

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Abstract

Background—The compulsive and inflexible behaviors that are present in many psychiatric disorders, particularly behavioral addictions and obsessive-compulsive disorder, may be due to neurochemical dysfunction within the circuitry that enables goal-directed behaviors. Experimental removal of serotonin or dopamine within the orbitofrontal cortex or dorsal striatum, respectively, impairs flexible responding in a reversal learning test, suggesting that these neurochemical systems exert important modulatory influences on goal-directed behaviors. Nevertheless, the behavioral impairments present in psychiatric disorders are likely due to subtle neurochemical differences, and it remains unknown whether naturally-occurring variation in neurochemical levels associate with individual differences in flexible, reward-directed behaviors.

Methods—The current study assessed the ability of 24 individual juvenile monkeys to acquire, retain and reverse discrimination problems and examined whether monoamine levels in the orbitofrontal cortex, caudate nucleus and putamen could explain variance in behavior.

Results—The interaction between dopamine levels in the putamen and serotonin levels in the orbitofrontal cortex explained 61% of the variance in a measure of behavioral flexibility, but not measures of associative learning or memory. The interaction mirrored that of a hyperbolic function, with reversal learning performance being poorest in either monkeys with relatively low levels of orbitofrontal serotonin and putamen dopamine or in monkeys with relatively high levels of orbitofrontal serotonin and putamen dopamine levels.

Conclusion—These results support the hypothesis that subcortical and cortical neuromodulatory systems interact to guide aspects of goal-directed behavior, providing insight into the neurochemical dysfunction that may underlie the inflexible and compulsive behaviors present in psychiatric disorders.

Keywords

cognitive control; dopamine; serotonin; prefrontal cortex; striatum; individual differences; response inhibition

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Introduction

Adaptive modulation of behavior in response to environmental change is necessary in order for individuals to behave in both a flexible and goal-directed manner. Conversely, the compulsive and rigid behaviors that are present in some individuals with behavioral addictions or obsessive-compulsive disorder may result from impairments in the neural circuitry that normally allows individuals to flexibly update their behaviors. Consistent with this hypothesis, affected individuals exhibit deficits in laboratory tasks of behavioral flexibility (1–3), as well as abnormalities in brain regions that are critical for flexible behavior (4, 5).

Behavioral flexibility can be assessed across species using reversal learning procedures, which index an individual's ability to adaptively modify behavior when previously learned reward associations are altered. The orbitofrontal cortex has been well established as a region critical to flexible, adaptive responding, as lesions to the orbitofrontal cortex impair the extinction of a previously reinforced response (6) and the ability to modify behaviors in a reversal-learning task (7, 8). In addition, efficient updating of behavior during reversal in normal humans is associated with event-related recruitment of the lateral orbitofrontal cortex (9).

The orbitofrontal cortex is connected with both the caudate nucleus and putamen (10) (11), through which it likely acts to alter cognitive and motor behaviors. The striatum has long been recognized as an important brain region for simple forms of goal-directed behaviors (12, 13), and recent evidence has indicated that the dorsal striatum is also involved in reversal learning (14–16). Moreover, functional neuroimaging studies in humans have demonstrated activation within the caudate nucleus and putamen during performance of a reversal learning task (9, 17), suggesting that the ability to adaptively modify behaviors may be governed by an integrative network involving both cortical and subcortical brain nuclei.

Within each of the brain regions implicated in reversal learning, a variety of neurotransmitters can participate in different ways to facilitate behavior. Neurotransmitter depletion studies indicate that within the orbitofrontal cortex, serotonin plays an essential role, while dopamine does not (18). On the other hand, within the dorsal striatum, depletion of dopamine, but not serotonin, impairs reversal learning performance (19), a finding consistent with reported deficits in behavioral flexibility in Parkinson's disease (20).

Although experimental depletion of dopamine and serotonin in these brain regions can alter reversal learning performance, it remains unknown whether naturally-occurring variation in monoamine levels explains individual differences in behavioral flexibility. Because the deficits in behavioral flexibility associated with neuropsychiatric disorders are likely linked to subtle, heritable patterns of neurochemical anomalies, it remains crucial to identify whether normal variation in behavioral abilities are linked with normal variation in neurotransmitter function. One approach for determining the neurochemical basis of cognition is to relate differences in neurochemical measurements with differences in behavior at an individual level (21). We have previously shown that naturally-occurring variation in striatal D₂-like receptor availability is associated with individual differences in reversal-learning performance (22), suggesting that slight differences in neurotransmitter function may contribute to individual variability in behavioral flexibility. Furthermore, the experimental depletion studies were conducted in independent studies and did not examine the possibility that neuromodulatory systems within discrete brain regions functionally interact with each other to influence the neural circuitry that guides behavior.

The current study assessed a cohort of juvenile monkeys for their ability to acquire, retain and reverse novel visual discrimination problems, and their brain monoamine levels were subsequently ascertained. We used these data to test a specific hypothesis: namely, whether the interaction between serotonin levels in the orbitofrontal cortex and dopamine levels in the putamen and caudate nucleus predicted behavioral performance. Specifically, we hypothesized that the interaction between levels of dopamine in the dorsal striatum and serotonin in the orbitofrontal cortex would predict performance during reversal learning, but not the ability to acquire or retain visual discrimination problems.

Methods and Materials

Subjects

24 male juvenile vervet monkeys (*Chlorocebus aethiops sabaues*) born at the UCLA Vervet Research Colony (VRC) were involved in this study. The subjects were recruited from two successive annual birth cohorts (12 from the 2004 cohort and 12 from the 2005 cohort); all subjects were housed in peer groups during the period of testing which began at 2 yrs of age and ended by 3 yrs of age. Subjects had unlimited access to water and received twice daily portions of standard monkey chow (Teklad). Chow was never withheld or reduced to motivate performance of the tasks.

All monkeys were maintained in accordance with the 'Guide for the Care and Use of Laboratory Animals' of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare Publication No. (NIH) 85-23, revised 1996. Research protocols were approved by the UCLA Chancellor's Animal Research Committee.

Discrimination acquisition, retention and reversal learning

Subjects were assessed for their ability to acquire, retain and reverse visual discrimination problems, using procedures similar to those previously described (22, 23). Testing was conducted 6–7 days per week. Monkeys were trained to enter into a metal tunnel which was fitted with a modified Wisconsin General Testing Apparatus. This apparatus was equipped with an opaque screen that could be raised or lowered to present three equally spaced opaque boxes to the monkey. These boxes were fitted hinged lids, that allowed for small pieces of food reward (i.e. a piece of banana, apple or grape) to be concealed within, and inserts on the lid where unique visual stimuli could be displayed (clip art).

Testing sessions began when the opaque screen was raised to present the three boxes (each fitted with a unique visual stimulus) to the monkey. One of the three visual stimuli was uniformly associated with food reward, while the others were not. Monkeys were only allowed to open one box per trial. A trial ended after a correct choice (opening the box containing the food reward), an incorrect choice (opening an empty box), or an omission (no response for 30 s) occurred. The position of the visual stimuli was pseudo-randomly varied across trials. Subjects received up to 30 trials per day.

Initially, subjects were trained to acquire four consecutive discrimination problems, each involving three novel visual stimuli. Subjects were required to learn which one of the three stimuli was associated with food reward by achieving a performance criterion (8 correct choices within 10 consecutive trials). If the performance criterion was not achieved in 30 trials, the session was terminated and the monkey was returned to the social enclosure. The same discrimination problem was presented the following day(s) until the performance criterion was met.

After completing the four discrimination-acquisition problems, monkeys were then trained to acquire, retain and reverse novel discrimination problems. The first phase of each discrimination problem was an acquisition phase which was identical to the acquisition-only training the monkeys had previously received. Once the same performance criterion had been met, the session was terminated and the monkey returned to the social enclosure.

One day after reaching criterion for acquisition, the ability of subjects to remember the stimulus-reward association from the day prior was assessed in a retention phase, where the stimulus-reward contingencies remained unchanged relative to the previous training day. This phase persisted until subjects met the performance criterion of 4 correct responses in 5 consecutive trials. Immediately after reaching the criterion in the retention phase, the reversal phase of the discrimination problem began; there were no environmental events that cued this transition, other than a change in reinforcement contingencies. In the reversal phase, the stimulus that was previously rewarded was no longer rewarded, and one of the two previously unrewarded stimuli was now rewarded. The reversal phase persisted until monkeys achieved criterion (8 correct choices in 10 consecutive trials) or until 30 trials had been completed, whichever occurred first. If monkeys did not reach the performance criterion within 30 trials, the reversal phase continued the following day(s) until the performance criterion was met. Monkeys were assessed on their ability to acquire, retain and reverse three novel discrimination problems. Due to inclement weather that prevented testing, one reversal session was not completed by four different monkeys, and these data were excluded from the analysis.

The primary dependent measures were the total number of trials required to reach criterion and the number of correct responses made in the acquisition, retention and reversal phases. For the reversal phase, the total number of responses directed at the previously rewarded stimulus (a perseverative response) and the never rewarded stimulus (a neutral response) were also recorded. The proportion of each response type (correct, perseverative, or neutral) was determined by dividing the total number of each response by the number of trials required to reach criterion for each phase.

Quantification of monoamine levels

Approximately 2–3 months after completing these behavioral assessments, brain tissue was collected at necropsy, using methods previously described (23, 24). Tissue homogenates were assayed using high pressure liquid chromatography similar to procedures described elsewhere (24) and protein content in homogenates was quantified using the Lowry method (25).

Tissue was acquired from a wide range of brain regions. However, we restricted our analyses here to monoamine levels in the orbitofrontal cortex, putamen and caudate nucleus collected from regions identified in Figure 1. Based on previous evidence (11), tissue acquired from the caudate nucleus and putamen were within the terminal fields of the orbitofrontal and dorsal anterior cingulate cortex. Although behavioral data was collected in 24 monkeys, brain tissue was only collected from 20, as 4 subjects were transferred to another behavioral protocol.

Statistical analyses

All statistical analyses were conducted using SPSS 15.0. The reliability of the behavioral measurements was determined using Cronbach's α , a coefficient of reliability. Linear regressions using cohort (2004 vs. 2005) as an independent variable were then conducted on all measurements prior to any further analysis to remove extraneous variance that was attributable to cohort. These residuals were then centered for further statistical analysis. To

examine the relationship between monoamine levels and behavior measurements, multiple linear regression was conducted with individual monoamine levels and their interaction term(s) as the independent variable(s) and the behavioral measurement(s) as the dependent variable using a forced entry model:

$$\text{Behavior} = \beta_0 + \beta_1(\text{putamen (or caudate) dopamine levels}) + \beta_2(\text{orbitofrontal serotonin levels}) + \beta_3(\text{putamen (or caudate) dopamine levels} * \text{orbitofrontal serotonin levels}) + \epsilon$$

Results

Acquisition, Retention and Reversal Learning

Reliability of the number of trials required to reach criterion across the acquisition and reversal sessions was moderately high (acquisition: Cronbach's $\alpha = 0.72$, reversal: Cronbach's $\alpha = 0.62$). However, contrary to our previous observations, reliability of the number of trials required to reach criterion across the three retention sessions was surprisingly low (Cronbach's $\alpha = 0.33$). The subjects required fewer trials to reach criterion in the acquisition phase (56.2 $\pm$ 28.6) compared to the reversal phase (78.2 $\pm$ 38.7) ($t(22) = -4.93$; $p < 0.001$), indicating that they found the reversal of the stimulus-reward contingencies more difficult than their initial acquisition. Descriptive statistics for the error type in the reversal phase revealed that the probability of making a response to the initially reinforced stimulus was significantly greater than the probability of making a response to the never-rewarded stimulus ($t(23) = 15.58$; $p < 0.001$), showing that the increase in the number of trials required to reach criterion during reversal was driven by an increased probability of making a perseverative response, rather than neutral responses.

Monoamine Levels

The average levels of dopamine and standard error, normalized to protein content, in the putamen and caudate nucleus were 101 $\pm$ 13.2 ng/mg and 110 $\pm$ 5.58 ng/mg, respectively. The average level of serotonin and standard error within the orbitofrontal cortex, normalized to protein content, was 1.94 $\pm$ 0.29 ng/mg.

Neurochemical Correlates of Behavior

Multiple linear regression was used to examine the relationship between serotonin and dopamine levels in the orbitofrontal cortex and putamen, respectively, and the behavioral measures collected. First, the relationship between orbitofrontal serotonin levels, putamen dopamine levels and their interaction was examined with respect to the average number of trials required to reach criterion in the acquisition, retention and reversal phases. Neither levels of dopamine in the putamen, serotonin in the orbitofrontal cortex nor the interaction of the two predicted a significant amount of variance in either acquisition or retention performance (acquisition: $F_{3,14} = 1.97$, $p = 0.16$; retention: $F_{3,11} = 0.079$, $p = 0.97$) (Figure 2A and 2B). Moreover, the main effects of orbitofrontal serotonin and putamen dopamine levels did not predict a significant proportion of variance in the number of trials required to reach criterion in the reversal phase ($F_{2,15} = 0.74$; $p = 0.49$). However, as hypothesized, the regression model including the interaction term was significant (R^2 change $F_{1,14} = 18.82$; $p = 0.001$), accounting for 61% of the variance in the average number of trials required to reach criterion in the reversal phase ($F_{3,11} = 7.36$; $p = 0.003$). In this model, the regression coefficients for orbitofrontal serotonin and putamen dopamine levels remained non-significant (all t 's < 1.5), while the regression coefficient for the interaction was significant ($t(11) = 4.34$; $p = 0.001$), explaining 52% more of the variance than did the main effects of dopamine and serotonin levels. The interaction regression coefficient was positive, such that as levels of dopamine in the putamen increased by one standardized unit, the slope of the relationship between orbitofrontal serotonin and number of trials required to reach criterion

during reversal learning increased by a factor of 0.74. Monkeys with the lowest dopamine and serotonin levels required the greatest number of trials to reach criterion in the reversal. Amongst monkeys with low dopamine levels in the putamen (i.e. one standard deviation below the mean), relatively higher levels of orbitofrontal cortical serotonin were associated with fewer trials required to reach criterion (better performance). However, high putamen dopamine levels (i.e. one standard deviation above the mean) were associated with a positive relationship between serotonin and reversal phase trials: for monkeys with the highest putamen dopamine levels, low serotonin was associated with good reversal learning performance (Figure 2C). Therefore, the interaction between orbitofrontal serotonin and putamen dopamine levels on reversal performance mirrored that of a hyperbolic function, with reversal learning performance being poorest in either monkeys with relatively low levels of orbitofrontal serotonin and putamen dopamine or in monkeys with relatively high levels of orbitofrontal serotonin and putamen dopamine levels.

Next, we sought to determine the neurochemical and anatomical specificity of the relationships mentioned above with reversal performance. To do this, levels of serotonin in the putamen, dopamine in the orbitofrontal cortex and the interaction of the two were regressed against the average number of trials required to reach criterion in the reversal phase. This model did not account for a significant proportion of variance in reversal performance ($F_{3,14}=0.997$; $p=0.42$) (Figure 3A), providing evidence for the selective neurochemical involvement of dopamine in the putamen and serotonin in the orbitofrontal cortex with reversal learning.

Finally, levels of dopamine in the caudate nucleus, serotonin in the orbitofrontal cortex and their interaction were regressed against reversal learning performance. Contrary to our hypothesis, the regression model failed to account for a significant proportion of variance in reversal learning performance ($F_{3,14}=0.34$; $p=0.80$) (Figure 3B).

Discussion

The results of the current study demonstrate that individual differences in monoaminergic systems within distinct brain nuclei interact to predict flexibility of behavior during a reversal learning task. By contrast, these neurochemical measures did not predict basic associative learning or memory (acquisition or retention) performance. Importantly, the relationship between serotonin in the orbitofrontal cortex, dopamine in the putamen and reversal learning was highly specific because a variant model, which included dopamine in the orbitofrontal cortex and serotonin in the putamen as predictors, did not explain a significant amount of variance in reversal performance. Finally, the finding that levels of dopamine in the putamen, and not in a region of anatomical proximity (the caudate nucleus), modified the relationship between orbitofrontal serotonin and reversal learning performance, implicates anatomical specificity of the observed relationships.

Neurochemical interactions within discrete brain regions predict reversal learning performance

Previous studies have found that depletion of serotonin in the orbitofrontal cortex or dopamine in the dorsal striatum can cause reversal learning deficits (18, 19, 26). In these studies, large and systematic reductions in local neurotransmitter levels were produced. However, recently individual differences in methylphenidate-induced dopamine release were reported to correlate with methylphenidate-induced changes in reversal learning performance, indicating that dopaminergic differences, an order of magnitude smaller than depletion studies, within the striatum may directly influence reversal learning performance (27). Building upon these results, the current study provides evidence that orbitofrontal serotonin levels and putamen dopamine levels functionally interact, explaining 61% of the

variance in individual reversal learning performance. The interaction followed a hyperbolic function: in monkeys with low putamen dopamine levels, poor reversal learning was associated with relatively low orbitofrontal cortex serotonin; however, as dopamine levels in the putamen *increased*, the relationship between serotonin and reversal learning reversed, with low levels of serotonin predicting relatively better reversal learning performance. The selective relationship between levels of orbitofrontal serotonin and putamen dopamine on reversal learning performance are likely linked to the increases in perseverative responding that occur during the reversal phase of the task, suggesting that a functional circuit between these monoamine systems may underlie the ability of individuals to inhibit habitual, pre-potent responses.

The current study did not detect a significant interaction between dopamine levels in the caudate nucleus, orbitofrontal serotonin and reversal learning performance, which apparently contrasts with the effects of depletion of dopamine in the dorsomedial striatum on reversal learning (19, 26). Neuroimaging studies in humans have found increased activation during reversal learning in both the putamen (17) and in the caudate (9). Moreover, individual differences in reversal learning performance in monkeys has been associated with PET-based measurements of dopamine D₂-like receptors in both the caudate nucleus and putamen (22), indicating that dopaminergic signaling in both striatal regions is important for flexible, goal-directed behaviors. Because the prefrontal cortex broadly innervates the striatum (11, 28), dopamine within the caudate nucleus may interact with serotonin tone in other prefrontal regions, not investigated here, that are involved in goal-directed behaviors, such as the anterior cingulate cortex (29), and these cortical-striatal circuits work in parallel to encode discrete types of information that together guide goal-directed behavior. Of additional note, our neurochemical assessments were made in a specific OFC region (area 47), though past lesion studies examining effects on reversal learning have involved other or additional OFC regions. Given the potential functional heterogeneity of the OFC, it is possible that the pattern of results we report here are specific to area 47, with different results found (for example) in more ventromedial OFC regions often implicated in reversal performance (6, 30, 31). Systematic investigation into the neurochemical mechanisms within the various OFC subregions is needed and may provide insight into how these neurochemical systems interact with discrete striatal compartments to guide behavior.

Potential links between putamen dopamine and orbitofrontal serotonin

Although the mechanistic nature of the interaction between orbitofrontal serotonin and putamen dopamine systems on reversal learning is unknown, there is evidence to support the idea that striatal dopamine signaling, working through D₂-like receptors, mediates evoked prefrontal serotonin release. Specifically, pharmacological blockade of striatal D₂-like receptors significantly attenuates tail-pinch evoked increases in prefrontal and striatal serotonin efflux (32). Therefore, dopaminergic stimulation of D₂-like receptors within the striatum may directly influence serotonin release within the orbitofrontal cortex, potentially explaining the interaction between these two neurochemical systems on tasks of behavioral flexibility.

Conversely, prefrontal serotonin system may exert a top-down influence over striatal dopamine release. Pharmacological activation of serotonin 2C receptors within the prefrontal cortex potentiates cocaine-induced striatal dopamine release (33), implicating a facilitatory pathway between prefrontal serotonin and striatal dopamine. Based on these data, serotonin within the orbitofrontal cortex and dopamine within the putamen may coalesce at the network level, directly influencing one another, explaining the phenomena reported here. However, the direction of the interaction between dopamine tone in the

striatum and serotonin levels in the orbitofrontal cortex on reversal learning performance remains unknown.

Implications for psychiatric disorders

Neurochemical dysfunction is linked with many of the behavioral impairments that are present in individuals diagnosed with addictions. For example, striatal dopaminergic dysfunction in substance dependent individuals has been found to associate with self-report measures of impulsivity, levels of self-reported craving for drugs, and cognitive abilities (34). Notably, dopaminergic alterations within the striatum that occur in response to chronic drug exposure have been found to correlate with drug-induced changes in reversal learning performance (23). Variation in central serotonin systems also associates with impulsiveness, and pharmacological manipulation of cortical serotonin transmission signaling alters reversal learning performance (35, 36).

The results of the current study indicate that levels of serotonin in the orbitofrontal interact with dopaminergic tone in the putamen to influence reversal learning performance. Therefore, in different individuals, the neurochemical etiology of reversal learning deficits may be slightly different, despite the revealed behavioral deficit being identical, and correspondingly, the pharmacological treatments targeted to improve behavioral flexibility would therefore differ. These data support the notion that pharmacological treatment strategies may achieve greater efficacy if they take into account, and are targeted at remediating, an individual's particular set of regionally-specific neurotransmitter system abnormalities, each of which may exhibit complex cross-system interactions that moderate both the behavior itself and an individual's response to pharmacological intervention.

These data also support the notion that, while experimental manipulations that produce extreme changes in a neurochemical system (e.g., depletion) can reveal the necessary influences of individual transmitters on behavioral processes, additional methods may be required in order to reveal the subtle interactions between neurotransmitters that emerge at a network level, each of which may be both complex and multifaceted. Further studies of the relationship between individual differences in brain neurotransmitter phenotypes and behavior may help to decompose the role for distinct and interacting neuromodulatory systems in aspects of cognition.

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Figure 1.

A depiction of the brain regions collected for quantification of monoamine levels. The shaded area in Figure 1A (predominately Brodmann's area 47) represents the area of the orbitofrontal cortex extracted (Bregma 13.50mm; Interaural 35.40mm). Tissue punches from the caudate nucleus and putamen (represented by the grey circles) were collected from tissue slices similar to that presented in Figure 1B (Bregma 2.70mm; Interaural 24.60mm). Figures were obtained from the Rhesus Monkey Brain in stereotaxic coordinates (37).

Figure 2.

A three-dimensional plot of the relationship between standardized levels of dopamine in the putamen, standardized levels of serotonin in the orbitofrontal cortex and standardized behavioral performance measures in the acquisition (Figure 2A), retention (Figure 2B) and reversal (Figure 2C) phase. Values for individual monkeys are presented in closed circles. The transparent plane overlaid on the individual data points represents how the interaction between levels of dopamine in the putamen and serotonin in the orbitofrontal cortex predicts the number of trials required to reach criterion in each of the phases of the task. Relatively high trials to reach criterion is indicative of poorer performance in each stage.

Figure 3.

A three-dimensional plot of the relationship between prefrontal and striatal neurochemical levels on reversal learning performance. Values for individual monkeys are presented in close circles. The transparent plane overlaid on the individual data points represents how the interaction between levels of monoamines predict the number of trials required to reach criterion in the reversal phase. Figure 3A presents the relationship between standardized levels of serotonin in the putamen, dopamine in the orbitofrontal cortex and reversal learning performance. Figure 3B presents the relationship between standardized levels of dopamine in the caudate nucleus, serotonin in the orbitofrontal cortex and reversal learning

performance. Relatively high trials to reach criterion is indicative of poorer performance in each stage.