Dopaminergic contribution to cognitive sequence learning

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Summary Evidence suggests that dopaminergic mechanisms in the basal ganglia are important in feedback-guided habit learning. To test hypothesis, we assessed cognitive sequence learning in 120 healthy volunteers and measured plasma levels of homovanillic acid [HVA] (a metabolite of dopamine), 5-hydroxyindoleacetic acid [5-HIAA] (a metabolite of serotonin), and 3-methoxy-4-hydroxypheylglycol [MHPG] (a metabolite of norepinephrine). Results revealed a significant negative relationship between errors in the feedback-guided training phase of the sequence learning task and the plasma HVA level. The HVA level accounted for 10.5% of variance of performance. Participant who had lower HVA level than the median value of the whole sample committed more errors during the training phase compared with participants who had higher HVA plasma level than the median value. A similar phenomenon was not observed for the context-dependent phase of the task and for 5-HIAA and MHPG. These results suggest that dopamine plays a special role in feedback-guided cognitive sequence learning.

Keywords: Dopamine, HVA, feedback, sequence learning, basal ganglia

Introduction

Beyond its classic role in the regulation of motor activity, the basal ganglia play an important role in the learning of habits and skills, such as simple associations between stimuli and responses (Yin and Knowlton, 2006). In patients with Parkinson's disease (PD), in which cellular death in the substantia nigra pars compacta leads to the depletion of dopamine in the basal ganglia, Shohamy et al. (2005) found impaired learning of sequential ("chaining") associations.

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During the "chaining" task, each link in a sequence of stimuli leading to reward is trained step-by-step using feedback after each decision, until the complete sequence is learned. In the first phase of this task, the screen showed a room (room 1) with three doors (A, X, Y), each bearing a colored card; the participant was required to choose one of these doors. A correct response (door A) led to a treasure chest (reward), while an incorrect response (X or Y) led to a brick wall. Once this A→reward association was learned, participants were presented with another room (room 2) with three new colored doors (B, W, Z). An incorrect response (W or Z) led to a brick wall, while a correct response (door B) led to room 1, where subjects would again choose the correct door (A) to reach the reward. Once this new association (B→A→reward) was learned, a new room was added to the sequence, until eventually the participant learned a full sequence: D→C→B→A→reward. Patients with PD who never received medications or who were tested off their normal dopaminergic medication performed more poorly on this task than patients with PD who received L-dopa substitution (Shohamy et al., 2005; Nagy et al., 2007), which suggests that L-dopa ameliorates sequential association learning deficits. Frank et al. (2004) proposed that in unmedicated PD patients low level of dopamine in the basal ganglia is not sufficient for reward during positive feedback, whereas in PD patients receiving L-dopa substitution, dopamine "overshoots" disrupt learning about the absence of reward during negative feedback (see also Shohamy et al., 2006). These mechanisms were

proven in healthy participants receiving dopamine receptor agonists and antagonists (Frank and O'reilly, 2006).

Although dopaminergic deficiency is the core feature of PD (Hornykiewicz, 2006), the dopaminergic system is not selectively affected in this disease. Evidence from post-mortem studies, neuroimaging, and animal models suggests that the serotonin and the norepinephrine systems are also impaired (Gesi et al., 2000; Brooks and Piccini, 2006; Scholtissen et al., 2006). Serotonin and norepinephrine may also be important in basal ganglia-dependent learning (Tisch et al., 2004), and hence it can not be excluded that these neurotransmitters may contribute to abnormal cognitive functions in PD and in other neuropsychiatric disorders. Therefore, to further elucidate the specific role of dopamine in sequence learning, we measured the metabolite of dopamine, serotonin, and norepinephrine in the plasma of healthy volunteers, and attempted to find correlations between these neurochemical markers and performance on the "chaining" sequence learning task.

Methods

Participants

Volunteers were 125 healthy people (70 males, 55 females) who were recruited from the community using newspaper advertisements and via acquaintance networks. Exclusion criteria were history of neurological or psychiatric disorders, psychoactive substance dependence, and any other medical condition that can affect central nervous system functions (cardiac, renal, hepatic, metabolic, and hormonal illnesses). All participants were non-smokers and did not take any medication. The Mini-International Neuropsychiatric Interview was used to exclude psychopathology (Sheehan et al., 1998). The mean age was 38.2 years (SD = 9.2). The mean years of education was 13.2 years (SD = 4.1). The mean socioeconomic status, as revealed by the Hollingshead Four-Factor Index, was 36.8 (SD = 22.3) (Cirino et al., 2002). General intellectual abilities were determined with the revised Wechsler Adult Intelligence Scale (WAIS-R) (Wechsler, 1981). All participants gave written informed consent. The study was done in accordance with the Declaration of Helsinki.

The "chaining" task

The task was run on a Macintosh computer, and programmed in the Super-Card language. On each trial of the experiment, the animated character (nicknamed "Kilroy") appears in a room with three colored doors (Fig. 1). The rooms have a uniform white background, and are drawn using perspective lines, with three black doors appearing on the far wall. The doors appear about 2″ high, and the colored cards are each 1″ high by 0.5″ wide, and outlined in white for visual clarity. The animated figure (Kilroy) appears about 2″ tall. For each subject, the colored doors in each of six rooms are selected from a set of 18 unique colors, so that the same three colors appear each time Kilroy enters a particular room, but no color appears in more than one room during training. Thus, for example, room A might have red, green, and purple doors; room B might have yellow, blue, and brown doors; and so on. Spatial layout of these three colors on the doors (left, center, right) is randomized on each trial, so that the correct answer (left, center, right) varies across trials in a room; only the location of the color card indicated which is the correct response. Colors could be easily discriminated.
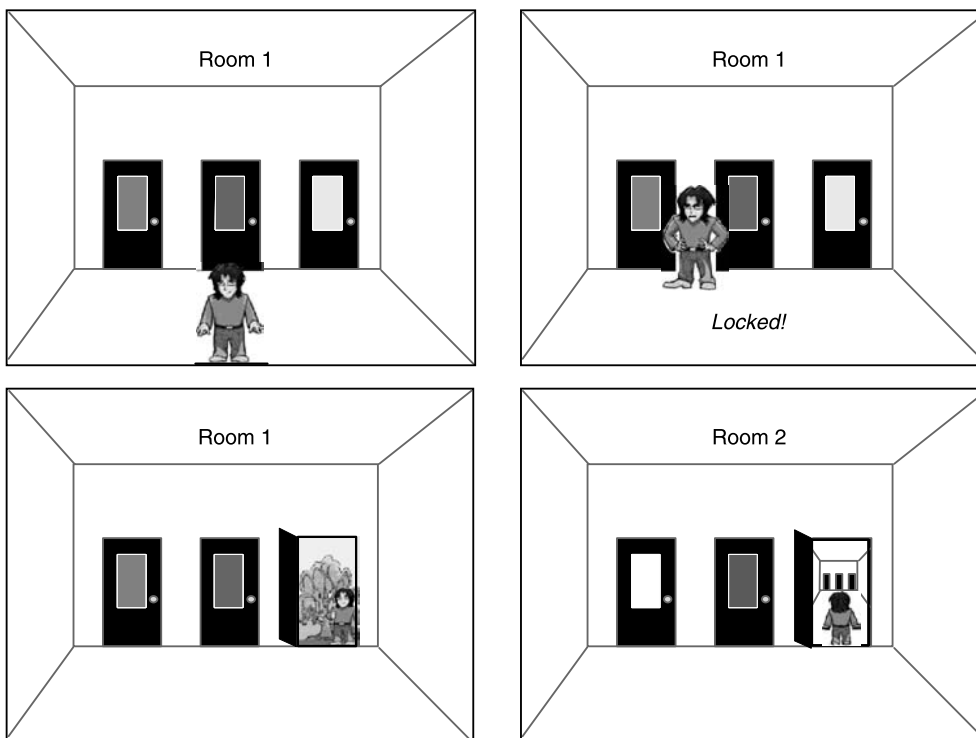

Fig. 1. Display of the cognitive sequence learning ("chaining") task. Upper row: The participants chose the closed door in room 1. Lower row, left panel: The participant chose the open door in room 1. Lower row, right panel: In room 2, the participant chose the open door, and entered into room 1

In each room, the participant uses the computer mouse to move the cursor to click on one of the doors. When the participant selects a door, a few additional drawings of Kilroy appear to approximate a rough animation showing Kilroy turning, walking to the door, and trying to open it. If the participant's choice is incorrect, the door is "locked" and Kilroy cannot open it; he puts his hands on his hips and makes a disappointed face, and the word "Locked!" appears on the bottom of the screen. Kilroy then moves back to the center of the room, and awaits the subject's next choice. If the subject's choice is correct, Kilroy opens the door and steps through. If this room was at the end of the chain, Kilroy reaches the outside, where he turns and gives a thumbs-up sign; if the room was at an earlier stage of the chain, Kilroy steps through into the next room and, once there, waits for further instructions (Fig. 1). In either case (correct or incorrect response), the outcome appears on the screen for 1 sec; there is then a 0.33 sec interval before Kilroy appears at the bottom of the screen again, ready for new instructions. There is no limit on response times.

A trial consists of a full sequence of rooms until Kilroy reaches the outside. The length of this sequence increases from one to four rooms over the course of training. A trial is scored as correct if the subject chooses the correct door on the first opportunity for every room in the chain; however, a subject may make one or more errors on a trial by choosing an incorrect door one or more times before choosing the correct door, in each of one or more rooms in the chain. This means that a subject could make more than one error per trial. Each sequence learning trial continues until the subject completes four consecutive correct trials or to a maximum of 15 trials. If a participant fails to reach criterion within the maximum number of trials for any phase, training of the sequence is terminated, and the subject is taken directly to the last (retraining) phase of the task.

The participant is seated in a quiet testing room at a comfortable viewing distance from the screen. The following instructions appear: "Welcome to the experiment. In this experiment, you will see a character named Kilroy who is trying to get out of the house. Each room in the house has three doors, and each door has a colored card on it. On each trial, two of the doors are locked, and one door is unlocked. In each room, click on the color card of the door that you think is unlocked. If you are correct, Kilroy will get outside. Good luck!" The test then consisted of the following parts:

1. Practice. The Practice Room appears, with three colored doors, and Kilroy in his "waiting-for-instructions" position at the front bottom of the screen. If the participant chooses the correct door, Kilroy makes it outside and the trial is concluded. The practice phase continues until the subject makes four consecutive correct trials (i.e., chooses the correct door on the first response in each of four trials).

2. Sequence training. At this point, new instructions appear: "You've successfully finished practice! Now Kilroy will be put in some new rooms. Again, in each room, two doors are locked and one door is unlocked. Each time, click on the door that you think is unlocked. Sometimes, Kilroy will have to go through more than one room to reach the outside. Good luck!"

   Kilroy now appears in his "waiting-for-instructions" position in Room 1. This phase is identical to the Practice phase, except that three new colored cards are used. Here, subjects have to learn to open the correct door (A). Once this is learned, phase 2 begins, in which Kilroy appears in Room 2, which contains three new colored cards; here, choice of the correct door (B) leads Kilroy to Room 1, where a correct answer leads him outside. Once this is learned, subjects work through phase 3 (door C in Room 3 leads to Room 2 and so on) and phase 4 (door D in Room 4 leads to Room 3 and so on) until, by the end of phase 4, subjects should be choosing the correct door in each room: D→C→B→A→reward.

3. Probe phase. Next comes a probe phase, unsignaled to the subject. At the start of a trial, Kilroy appears in Room 4. Correct responses will, as usual, allow him to progress through the sequence of rooms and reach the outside. Now, however, the colored cards are switched. In each room, one of the three cards is always the correct answer in that room, at that point in the sequence; one of the cards is always a choice that was correct in

a different room; the third card (distracter) is a choice that was never correct in any room. Thus, in Room 2, Kilroy might be presented with a choice between card B, card A, and card X. Card B is the correct choice, and should be chosen by a subject who had learned the chain: i.e., what choice to make at each step in the sequence. But a subject who had merely learned non-sequential stimulus-response associations might choose A, since that is a stimulus that had been directly associated with reward in the past. The probe phase contains six trials, each trial consisting of a trip through the usual four rooms.

4. Retraining phase. Finally comes a retraining phase, in which participants are required to learn a new room with three new colored cards, one of which leads directly to the outside. The purpose of this phase is to determine whether any learning deficits observed on the sequence learning or probe phase could be due to fatigue effects or other nonassociative factors.

Plasma levels of monoamine metabolites

Participants sat in a comfortable chair. Blood levels were collected before cognitive testing between 9 and 10 a.m. in heparinized vacutainer tubes. Plasma levels of metabolites were measured using the coulochem electrode array system (Neurochem, ESA, Inc., MA, USA) (Siuciak et al., 1992). Measurements included the levels of homovanillic acid [HVA] (a metabolite of dopamine), 5-hydroxyindoleacetic acid [5-HIAA] (a metabolite of serotonin), and 3-methoxy-4-hydroxypheylglycol [MHPG] (a metabolite of norepinephrine).

Results

Correlation and linear regression analysis

Five participants did not reach the criterion in the "chaining" task, and therefore they were not able to complete the probe phase. These participants were excluded from the analysis.

The mean plasma levels of metabolites were as follows: HVA: 7.1 ng/ml (SD = 2.5), 5-HIAA: 1.5 ng/ml (SD = 0.9),
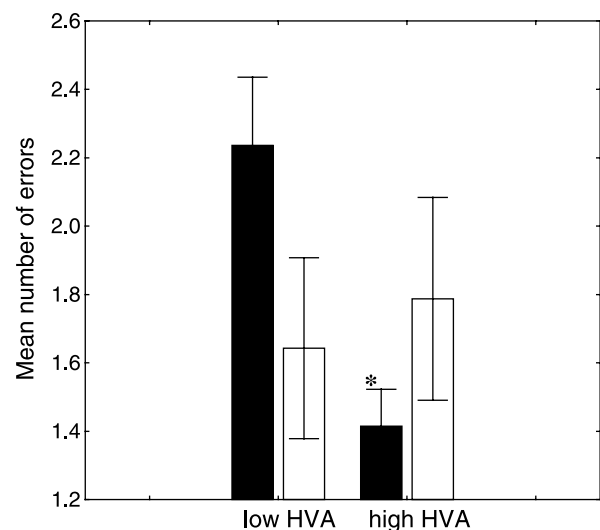

Fig. 2. Mean number of errors in the training phase (stimulus-reward learning) and in the probe phase (sequence change); ■ training, □ probe. Error bars indicate 95% confidence intervals. $^*p = 0.002$

MHPG: 3.8 ng/ml (SD = 1.2). There was a significant nega-
tive relationship between the mean number of errors in the
training phase of the "chaining" task and the HVA level
($R = -0.36$, $p < 0.05$). In contrast, the HVA level did not
correlate with the number of errors in the probe phase of
the "chaining" task ($R = 0.01$) (Fig. 2). 5-HIAA and MHPG
levels did not correlate with the "chaining" task measures
($R < 0.1$).

Linear regression analysis revealed that the HVA level
accounted for 10.5% of variance of training phase errors
($F(1,118) = 13.9$, $p < 0.001$). In the case of 5-HIAA and
MHPG, this value was less than 1% ($p > 0.5$).

Median split analysis

A median-split analysis was also performed. Participant
who had lower HVA plasma level than the median value
of the whole sample committed more errors during the
training phase of the "chaining" task compared with par-
ticipants who had higher HVA plasma level than the median
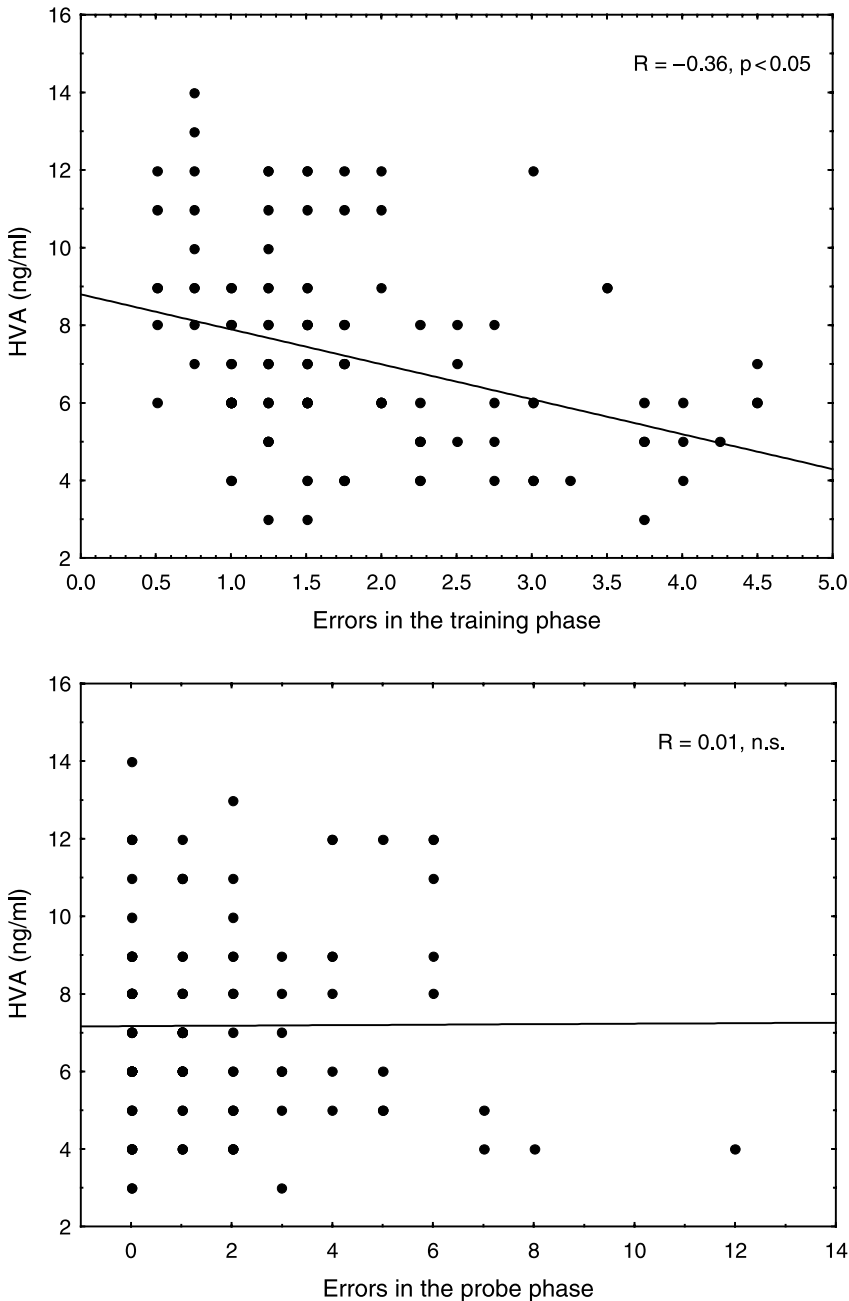

Fig. 3. The relationship between the mean number of errors in the training and probe phase of the "chaining" task and plasma HVA levels

value $(t(118) = -3.12, p = 0.002)$. This difference remained significant when age, gender, education, socioeconomic status, and IQ were included in an analysis of covariance $(F > 8, p < 0.05)$. In contrast, the median-split analysis did not indicate differences between participants with low and high HVA in the probe phase $(p = 0.72)$ (Fig. 3). As expected from the correlation analysis, median-split analyses for 5-HIAA and MHPG did not indicate differences between participants with low and high level of metabolites $(p > 0.5)$. There was no significant difference in IQ between participants with low HVA (mean: 105.6 (SD = 10.8)) and high HVA (mean: 104.9 (SD = 11.3)) $(p > 0.5)$.

To further elucidate the relationship between HVA and sequence learning, we analyzed the number of errors in each step of the chain of associations (room 1-room 4 in the training phase). An analysis of linear trend revealed that the number of errors linearly increased as a function "chaining" associations in participants with low HVA $(F(1,118) = 30.23, p < 0.001)$. This relationship was less pronounced in participants with high HVA $(F(1,118) = 4.47, p = 0.04)$, and the interaction for linear trend between participant with low and high HVA approached the level of significance $(F(1,118) = 3.21, p = 0.07)$ (Fig. 4). Participants with low HVA committed significantly more errors at the third association (phase 3) $(t(118) = 3.0, p = 0.002)$.

Finally, participants with low and high HVA did not differ in the retraining phase (mean number of errors: 1.1 (SD = 0.8) and 1.2 (SD = 0.9), respectively $(p > 0.5)$).
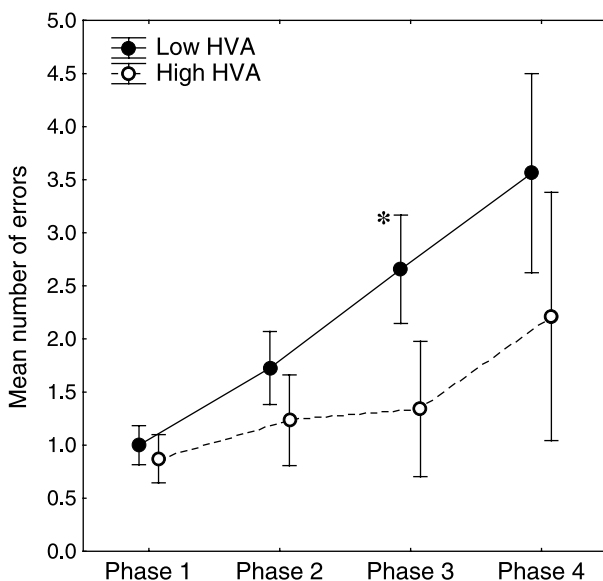

Fig. 4. Mean number of errors from the four phases (four rooms) of the training procedure. Error bars indicate 95% confidence intervals. $^{*}p = 0.002$

Discussion

Our results indicate that sequence learning is specifically related to dopaminergic functions, which is consistent with previous data from patients with PD (Shohamy et al., 2005; Nagy et al., 2007). We found that healthy participants with lower HVA levels, a peripheral indicator of dopaminergic metabolism, committed more errors during the feedback-guided training phase of the "chaining" task. This relationship was not observed in the case of 5-HIAA and MHPG, which are peripheral markers of serotonin and norepinephrine metabolism, respectively.

The finding that participants with low HVA committed more errors on the training phase of the "chaining" task can not be explained by a simple fatigue effect, because these participants showed a similar performance to that of the participants with high HVA in the probe phase and in the retraining phase of the task.

The main limitation of our study was that we used a peripheral marker of monoamine metabolism, which is influenced by multiple factors in addition to central nervous system metabolism. Despite this fact, data indicate that there is a reliable relationship between peripheral metabolites and neurotransmitter levels in the brain (Amin et al., 1992). For example, Verhoeff et al. (2003) found that catecholamine depletion, achieved by the administration of alpha-methyl-para-tyrosine, resulted in increased dopamine receptor binding in the basal ganglia (decreased dopamine level), as measured with positron emission tomography, and in decreased plasma level of HVA and HMPG. Nevertheless, further studies are warranted that use direct measurements in the brain. Furthermore, more extensive neuropsychological assessment is necessary in order to explore the specificity of the relationship between HVA levels and sequence learning.

It is particularly interesting that the HVA level selectively correlated with the number of error in the training phase of the "chaining" task, but not with the performance in the probe phase. After the learning of the chain of associations, in the probe phase, the colors of the incorrect doors were switched such that in each room, in addition to the correct door of that room, there also appeared a door which was the correct door elsewhere in the sequence. For example, in room 2, the subject might be presented with a choice between door B (correct), A (incorrect at this point in the sequence), and X (never correct). The aim of the probe phase was to ascertain that participants learned the correct door in its correct place in the sequence. Recent data indicate that the training and the probe phases of the "chaining" task are neuropsychologically dissociable. In

contrast to patients with PD, patients with medial temporal lobe damage can efficiently complete the training phase and can learn the sequence of associations. These patients, however, display many errors during the probe phase, which is not seen in patients with PD (Nagy et al., 2007). The current neurochemical data support this dissociation, because dopamine metabolism was related only to the training phase of the "chaining" task.

Besides data from PD and from our current study, other evidence also suggests that the dopaminergic system plays an important role in feedback-guided learning, which is an essential component of the "chaining" task. Aron et al. (2004) found that feedback during a cognitive task activated striatal and frontal regions, which receive input from midbrain dopaminergic neurons. Midbrain activity was significantly different for negative versus positive feedback, which is consistent with coding of the "prediction error". In another study, activation in ventral striatum (nucleus accumbens), which is richly innervated by dopaminergic pathways, increased with prediction error for negative feedback during a classification learning task (Rodriguez et al., 2006). These functional neuroimaging data, together with result from PD and from our neurochemical study, support the role of dopaminergic mechanisms in feedback-guided skill and habit learning. These data may be relevant for clinical studies that aim to investigate the role of dopaminergic mechanisms in various neuropsychiatric disorders accompanied by learning impairments.

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