Title: Human cognitive flexibility depends on dopamine D2 receptor signaling

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Supplementary materials and methods

Blood samples for prolactin

Secretion of the hormone prolactin is inhibited by dopamine D2 receptor stimulation in a dose dependent manner. In order to measure the level of prolactin in blood plasma and thereby ascertain the effects of the drugs, we drew blood twice during each of the sessions. After centrifugation, the sample concentrations were labeled with a participant code, study day number, state monitoring number, date and time of blood sampling and stored. Plasma prolactin levels were determined by an electrochemiluminescence immunoassay on a Modular E170 Analyzer (Roche Diagnostics) by Prof Fred Sweep and Rob van den Berg at the Laboratory for Endocrinology of the UMC Nijmegen.

Neuropsychological assessment

Working memory capacity was measured using the Dutch version of the listening span task (Salthouse et al. 1991). Subjects listened to sets of two to seven sentences while answering written questions about the content of each sentence. They then turned the page and wrote down the last word of each sentence. There are three trials at each level and the span represents the maximum number of last words that were remembered correctly on at least two out of three trials (for more details, see (Salthouse et al. 1991)). In this case, recall of final words was scored irrespective of recall-order.
**Supplementary results**

**Table S1** Effects of drugs on prolactin levels

Blood samples were used to determine prolactin values before drug intake and approximately 2.25 hours after bromocriptine intake and 2.75 hours after sulpiride intake (see Methods). All drug-induced changes in prolactin values were significantly different from that during the placebo session. Moreover, we observed no correlations between drug-induced prolactin levels and drug-induced switching performance (all \( p > 0.7 \))

| Drug                        | Mean (s.e.d) difference from placebo (df= 13) |
|-----------------------------|---------------------------------------------|
|                            | Mean (s.e.d.) difference from placebo (df = 45) |
| Bromocriptine              | -130.9**                                 |
| Sulpiride & Bromocriptine  | -99.6*                                   |
| Sulpiride                  | -99.6*                                   |

\( *= p < 0.002, ** = p < 0.001 \)

s.e.d. = standard error of the difference between drug and placebo
Sulpiride & bromocriptine versus sulpiride: \( t (13) = -0.27, p = 0.79 \)

**Learning effect**

This paradigm was designed to measure set switching and effects of incentive motivation by monetary reward. To assess whether the observed drug effects on set switching reflect a form of learning, we analyzed drug effects as a function of time. Specifically, we binned the data in eight successive bins of 20 trials each, and looked at the interaction between SWITCHING, DRUG and TIME. We found no learning effects across the two DATI genotype groups \([\text{SWITCHING} \times \text{DRUG} \times \text{TIME}}: \ F (7,40) < 1\], in the 10R homozygotes \([\text{SWITCHING} \times \text{DRUG} \times \text{TIME}}: \ F (7,19) < 1\], or in the 9R carriers \([\text{SWITCHING} \times \text{DRUG} \times \text{TIME}}: \ F (7,14) = 1.74, p > 0.1\].
Table S2a Mean response times and error rates on reward and switching for the DAT1

9R carriers ($n = 21$)

|                      | High reward | Low reward |
|----------------------|-------------|------------|
|                      | Repeat      | Switch     | Repeat | Switch     |
| Placebo              |             |            |        |            |
| Errors (%)           |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 8.27        | 12.21      | 9.29   | 16.28      |
|                     | (1.45)      | (1.52)     | (1.58) | (2.06)     |
| RT (ms)              |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 344.17      | 348.6      | 350.78 | 358.97     |
| Bromocriptine        |             |            |        |            |
| Errors (%)           |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 7.52        | 12.25      | 11.22  | 15.93      |
|                     | (1.5)       | (1.61)     | (1.96) | (1.69)     |
| RT (ms)              |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 361.3       | 356        | 360.21 | 368.97     |
| s.e.m. = standard error of the mean |

Significant effects:

1. Main effect of reward: $F(1,20) = 19, p < 0.001$; main effect of switching: $F(1,20) = 32.3, p < 0.001$
2. switching: $F(1,20) = 40.7, p < 0.001$; reward: $F(1,20) = 5.1, p < 0.04$
3. switching: $F(1,20) = 7.4, p < 0.02$; reward: $F(1,20) = 13.1, p < 0.002$; reward: $F(1,20) = 14.9, p < 0.001$

Table S2b Mean response times and error rates on reward and switching for the DAT1

10R homozygotes ($n = 27$)

|                      | High reward | Low reward |
|----------------------|-------------|------------|
|                      | Repeat      | Switch     | Repeat | Switch     |
| Placebo              |             |            |        |            |
| Errors (%)           |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 8           | 11.8       | 8.21   | 14.1       |
|                     | (1.20)      | (1.38)     | (1.23) | (1.74)     |
| RT (ms)              |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 320.89      | 325.36     | 327.2  | 329.88     |
| Bromocriptine        |             |            |        |            |
| Errors (%)           |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 9.95        | 11.12      | 11.71  | 13.30      |
|                     | (1.46)      | (1.42)     | (1.53) | (1.83)     |
| RT (ms)              |             |            |        |            |
| s.e.m.               |             |            |        |            |
|                     | 319.36      | 322.97     | 330.93 | 328.75     |
| s.e.m. = standard error of the mean |

Significant effects:

1. Main effect of switching: $F(1,26) = 19.9, p < 0.001$; main effect of reward: $F(1,26) = 4.9, p < 0.04$
2. drug*switching: $F(1,26) = 5.4, p < 0.03$; 1 main effect of switching: $F(1,26) = 25.5, p < 0.001$; 2 main effect of reward: $F(1,26) = 6.9, p < 0.02$; main effect of switching: $F(1,26) = 4.4, p < 0.05$; 1 main effect of reward: $F(1,26) = 13.7, p < 0.002$; 2 main effect of reward: $F(1,26) = 15.1, p < 0.001$
Table S2c Mean response times and error rates on reward and switching for the subgroup of 10R homozygotes who received sulpiride (pre)-treatment \((n = 14)\)

|               | High reward |          | Low reward |          |
|---------------|-------------|----------|------------|----------|
|               | Repeat      | Switch   | Repeat     | Switch   |
| **Placebo**   | **Errors (%)** | **s.e.m.** | **Errors (%)** | **s.e.m.** |
|               | 9.66        | 15.68    | 9.59       | 17.41    |
|               | (1.58)      | (1.74)   | (1.82)     | (2.37)   |
| **RT (ms)**   | 319.14      | 320.42   | 323.25     | 327.7    |
|               | (10.17)     | (11.24)  | (10.4)     | (12.03)  |
| **Bromocriptine** | **Errors (%)** | **s.e.m.** | **Errors (%)** | **s.e.m.** |
|               | 12.09       | 13.32    | 13.39      | 16.06    |
|               | (2.11)      | (1.63)   | (2.16)     | (2.32)   |
| **RT (ms)**   | 305.36      | 310.98   | 316.62     | 317.95   |
|               | (8.39)      | (9.29)   | (10.32)    | (10.58)  |
| **Sulpiride** | **Errors (%)** | **s.e.m.** | **Errors (%)** | **s.e.m.** |
|               | 9.29        | 13.14    | 13.37      | 18.73    |
|               | (1.76)      | (1.87)   | (2.53)     | (2.64)   |
| **RT (ms)**   | 323.67      | 329.18   | 326.08     | 335.54   |
|               | (12.73)     | (14.88)  | (12.47)    | (15)     |
| **Sulpiride - bromocriptine** | **Errors (%)** | **s.e.m.** | **Errors (%)** | **s.e.m.** |
|               | 13.69       | 17.21    | 15.39      | 22.19    |
|               | (2.61)      | (2.82)   | (2.91)     | (3.04)   |
| **RT (ms)**   | 310.61      | 312.35   | 316.71     | 315.77   |
|               | (11.39)     | (10.47)  | (11.98)    | (12.04)  |

s.e.m. = standard error of the mean

Significant effects:

- *Main effect of switching: \(F(1,13) = 22.1, p < 0.001\), drug*switching: \(F(1,13) = 5.6, p < 0.04\); ¹ main effect of switching \(F(1,13) = 24.4, p < 0.001\); ² Main effect of reward \(F(1,13) = 7.8, p < 0.02\); ³ main effect of switching: \(F(1,13) = 5.1, p < 0.05\); main effect of reward: \(F(1,13) = 19.7, p < 0.001\); ⁴ main effect switching \(F(1,13) = 9.6, p < 0.009\); *Main effect of reward \(F(1,13) = 7.6, p < 0.02\)
Figure S1. Dopamine-dependent motivation-cognition interaction

Shown here is the switch cost (switch – repeat) in terms of response times for the two genotype groups. In contrast to the 10R homozygotes ($n = 27$), the 9R carriers ($n = 21$; with relatively higher levels of striatal dopamine) exhibited decreased switch costs under high relative to low reward [REWARD*SWITCHING*DAT1: $F(1,46) = 5.3, p = 0.026$]. Bars represent the standard error of the difference between switch and repeat trials. We did not find these effects in the error rates [REWARD*SWITCHING*DAT1: $F(1,46) < 1$].
Figure S2 a-c. Shown are mean and individual switch costs (switch - repeat) in terms of error rates, from the a) the placebo compared with the bromocriptine session, b) the placebo compared with the sulpiride session, and c) the placebo compared with the bromocriptine after sulpiride pre-treatment session.
### Table S3a Neuropsychological assessment for two DAT1 genotype groups

| DAT1 genotype | 9R Carriers | 10R Homozygotes |
|---------------|------------|---------------|
| n             | 21         | 27            |
| Age (mean ± s.d.)<sup>a</sup> | 21.4 ± 1.9 | 21.7 ± 2.2 |
| DART<sup>*</sup>-score (mean ± s.d.)<sup>a</sup> | 104.1 ± 8.8 | 104.1 ± 10.2 |
| Females: n (%)<sup>c</sup> | 12 (57.1%) | 12 (44.4%) |
| BIS-11 total (mean ± s.d.)<sup>b</sup> | 64.5 ± 10.4 | 64.3 ± 7.5 |
| BDI (mean ± s.d.)<sup>a</sup> | 2.5 ± 2.3 | 2.3 ± 2.7 |
| Listening span (mean ± s.d.)<sup>a</sup> | 5.5 ± 1.2 | 5.4 ± 1.3 |
| STAI (mean ± s.d.)<sup>a</sup> | 32.1 ± 5.7 | 29.9 ± 6.5 |

<sup>a</sup> F(1,46) < 1.2, p > 0.2;  
<sup>b</sup> F(1,43) < 1, p > 0.9;  
<sup>c</sup> χ²(1) < 1, p > 0.3

<sup>*</sup> DART = Dutch Adult Reading Test, verbal IQ measure.

The two genotype groups did not differ in terms of age, IQ, gender, Barratt Impulsiveness Scale (BIS-11), Beck Depression Inventory (BDI), listening span and State-Trait Anxiety Inventory (STAI).

### Table S3b Demographics in low and high listening span groups

| Listening Span | Low | High |
|---------------|-----|------|
| n             | 23  | 25   |
| Age (mean ± s.d.)<sup>a</sup> | 21.5 ± 1.8 | 21.6 ± 2.3 |
| DAT1 10R genotype: n (%)<sup>c</sup> | 11 (47.8%) | 16 (64%) |
| DART<sup>*</sup>-score (mean ± s.d.)<sup>a</sup> | 104.8 ± 9.9 | 103.4 ± 9.4 |
| Females: n (%)<sup>c</sup> | 12 (52.2%) | 12 (48%) |
| BIS-11 total (mean ± s.d.)<sup>b</sup> | 65.6 ± 9.8 | 63.2 ± 7.7 |
| BDI (mean ± s.d.)<sup>a</sup> | 2.6 ± 2.4 | 2.2 ± 2.6 |
| STAI (mean ± s.d.)<sup>a</sup> | 30.8 ± 5.3 | 30.8 ± 7 |
| Listening span score | ≤ 5  | > 5  |

<sup>a</sup> F(1,46) < 1;  
<sup>b</sup> F(1,43) < 1;  
<sup>c</sup> χ²(1) < 1.3, p > 0.3

<sup>*</sup> DART = Dutch Adult Reading Test, verbal IQ measure.

The two listening span groups did not differ in terms of age, DAT1 genotype, IQ, gender, Barratt Impulsiveness Scale (BIS-11), Beck Depression Inventory (BDI), and State-Trait Anxiety Inventory (STAI).
Supplementary discussion

There was no effect of bromocriptine as a function of reward (see main Results). At first sight, this might seem surprising given current literature about dopamine’s role in reward processing. However, bromocriptine has particularly high affinity for the dopamine D2 receptor (Deleu et al. 2002). Accordingly, it remains possible that dopamine D2 receptor signaling is not critical for the effects of motivation in the present task. This hypothesis is in keeping with current theorizing implicating primarily the dopamine D1 receptor in reward processing (Frank 2005).

In order to stress subjects to respond as fast as possible, the response deadline was very strict, possibly causing a floor effect. This might have hampered the improvement of performance in terms of response times on bromocriptine vs. placebo, explaining the absence of any drug-effects on response times.

Supplementary references

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