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**Supplemental Information**

# Dissociable Effects of Dopamine and Serotonin on Reversal Learning

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# Supplemental Information

## Supplemental Data

Demographic Information

Demographic information of the complete sample (Table S1), related to 'Experimental procedures - subjects'. The sample predominantly consisted of young, Caucasian, right-handed subjects with a high level of education. Education levels were divided into high (bachelor student level or higher) and low (primary school or secondary school up to GCSE level).

**Table S1. Demographic information.** Mean ± standard deviation.

| **Gender** | | **Age** | **Handedness** | | **Race** | | **Education** | |
|---|---|---|---|---|---|---|---|---|
| female | n = 489 | 26.3 yr | right | 88.2% | Caucasian | 91.2% | high | 93.3% |
| | (60.4%) | ±11.1 yr | left | 3.8% | Asian | 1.7% | low | 1.1% |
| male | n = 321 | | ambidextrous | 0.4% | other | 1.7% | unknown | 5.6% |
| | (39.6%) | | unknown | 7.5% | unknown | 4.8% | | |

Exclusions

Three subjects (genotypes S'/ S'+10R/10R; S'/L'+9R/10R; L'/L'+9R/9R) were excluded from the final analysis, because they chose the same stimulus on every trial throughout the task, which indicated that they probably did not understand / engage with the task. This resulted in the final sample of 682 subjects.

Covariate independence of results

We confirmed that the effects reported in the main article did not depend on the in- or exclusion of the covariates of no interest, by repeating the statistical analyses reported in the main article, but with the exclusion of these covariates (Table S2). Briefly, like when covariates are included (main article), lose-shift is affected by *SERT* genotype, perseverative errros are affected by *DAT1* genotype as well as an interaction of *DAT1* x acquisiton error rate, and chance errors are partly explained by acquisition error rate.

**Table S2. Re-analysis of main results with exclusion of covariates, Related to Figure 2.**

| | | **F-statistic** | **p-value** | **effect size** |
|---|---|---|---|---|
| **Main effect of error type (rmANOVA)** | *SERT* | F(3.6, 1336) = 2.71 | .035* | $\eta^2$=0.008 |
| | *DAT1* | F(3.6,1336) = 2.76 | .032* | $\eta^2$=0.008 |
| **Lose-shift (uniANOVA)** | *SERT* | F(2,668) = 5.34 | .005** | $\eta^2$=0.016 |
| | *DAT1* | F(2,668) = 0.04 | .9 | |
| **Win-stay (uniANOVA)** | *SERT* | F(2,668) = 1.17 | .3 | |
| | *DAT1* | F(2,668) = 0.81 | .4 | |
| **Perseverative errors** | *SERT* | t(674)=1.11 | .27 | |
| | *DAT1* | t(674)=2.18 | .033* | β=0.082 |
| | acq. correct | t(674)=0.54 | .57 | |
| | *SERT* x acq. correct | t(674)=0.13 | .9 | |
| | *DAT1* x acq. correct | t(674)=2.78 | .006** | β=0.106 |
| **Chance errors** | *SERT* | t(674)=1.12 | .26 | |
| | *DAT1* | t(674)=.021 | .98 | |
| | acq. correct | t(674)=13.6 | <.001*** | β=0.47 |
| | *SERT* x acq. correct | t(674)=0.43 | .67 | |
| | *DAT1 x* acq. correct | t(674)=1.32 | .19 | |

Baseline learning measures.

To establish that subjects successfully learned the task, we conducted two supplementary analyses. First, we investigated whether acquisition and reversal error rates were above chance, and second whether there was evidence that subjects integrated information from past choices and observed outcomes into their current choices. Specifically, we divided subjects into subjects who passed versus failed a formal learning criterion of eight consecutive correct responses that is commonly used for this task (Chamberlain et al., 2006). This criterion requires the subject to ignore at least two instances of misleading punishing feedback on correct responses,

or misleading reward on incorrect responses. Given that this is a very strict criterion, we assessed whether there was evidence in both groups that they successfully learned the task using two additional measures.

First, we used a repeated-measures ANOVA on the proportion of correct responses to confirm that subjects performed significantly better than chance across both groups and stages of the tasks ($F(1,680) = 1494$, $p<.001$; Figure S1A). Post hoc t-tests confirmed that both subjects who pass and fail perform above chance in all phases (pass, both acquisition and reversal: $t(277)> 7$, $p<0.001$; fail: $t(403)> 8$, $p<0.001$).

Next, we used a regression analysis to estimate on a subject-by-subject basis whether subjects integrated information from more than one trial in the past. Specifically, we performed a logistic regression analysis of win-stay/lose-shift behavior going three trials back, separately for subjects who passed or failed the learning criterion (see below). The dependent variable was a binomial vector with [1] vs [0] for the A vs B stimuli. For 'win-stay' regressors all previous loss trials were encoded as [0]. If the previous trial was a win on A, it was encoded as [1], and if the previous trial was a win on B, it was encoded as [-1]. For 'lose-shift' regressor all previous win trials were encoded as [0]. If the previous trial was a loss on A, it was encoded as [-1], and if the previous trial was a loss on B, it was encoded as [1]. Both the win-stay and lose-shift regressors were included for four timelags, representing outcomes on 1-4 trials back. A constant regressor was also included. The estimated beta-values were subsequently analyzed using a repeated-measures GLM to assess effects of integration for win-stay and lose-shift across all lags, with post-hoc t-tests for the individual lags to assess significance for each of the lags.

This logistic regression showed that across win-stay and lose-shift both subjects who fail and subjects who pass the learning criterion integrate outcomes for at least three trials back, with decreasing weights for trials further back in time (trial t-1, t-2, t-3, all: $F(1,680) > 10$, $p < .001$, $\eta^2> 0.020$; Figure S1B).

Despite the evidence for learning in terms of win-staying/lose-shifting, described above, only 41% of subjects passed the formal learning criterion of eight consecutive correct trials. Thus, it should be noted that failure to attain the learning criterion should not be taken as evidence against successful learning. Nevertheless, there was an interaction between the win-stay/ lose-shift behavior and learning criterion attainment ($F(1, 1596.6) = 65.7$, $p < .001$). This interaction was driven by the 'pass' group being more likely to show win-stay behavior ($F(1, 577) = 53.9$, $p < .001$), and

the ‘fail’ group being more likely to show lose-shift behavior ($F(1, 1151) = 9.1$, $p = .003$). As such, learning criterion attainment captured a large amount of variance in our dependent measure of interest (win-staying/lose-shifting). For this reason, we included learning criterion attainment as a factor in our primary analysis of interest, rendering the analysis more sensitive to detecting the subtle genotype effects. Importantly, there was no interaction for either win-stay or lose-shift with learning criterion attainment and genotype (for both polymorphisms: $F(20,661)<2.5$, $p>0.1$). This showed that the genotype effects were independent of overall performance on the task in terms of learning criterion attainment (see also next section).

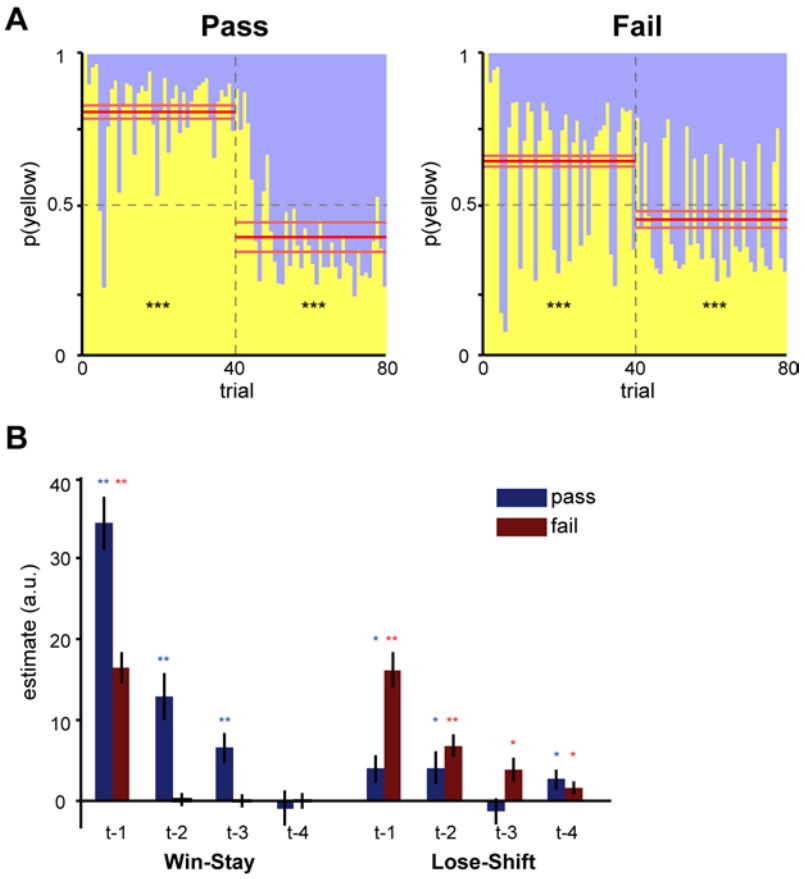


**Figure S1. Baseline learning measures, Related to Figure 2. A) Learning curves.** Average proportion of choices for the initially correct stimulus (here assumed to be yellow) vs. the stimulus that is correct during reversal (here blue), plotted for the groups that passed vs. failed the learning criterion. The solid red lines indicated the mean across the session, and pink lines indicate the 95% confidence intervals, confirming that across all trials, both groups performed above chance in both phases of the task. **B) Win-stay / lose-shift regression** for outcomes up to 4 trials into the past. Across win and loss trials, both groups integrate information from at least 3 trials in the past. The ‘pass‘ group (n = 276) showed relatively more win-stay and less lose-shift than the ‘fail’ group (n = 406). Mean ± SEM. *p < 0.05. **p < 0.01. ***p < 0.005.

Genotype differences on baseline learning measures

To ascertain the specificity of the effects of genotype on perseveration and lose-shift as reported in the main text, we assessed any differences in acquisition error rate or learning criterion attainment as a function of *DAT1* or *SERT* genotype. Using a regression analysis, we confirmed that there was no effect of *DAT1* or *SERT* genotype on the acquisition error rate (summed $R^2$=.001, F(3,675)=.13, p=.95; table S3). Importantly, this means that there were no global differences in terms of reinforcement/choice history at the start of the reversal. There was also no difference in terms of the proportion of subjects who passed the learning criterion (*DAT1* ($\chi^2(2) = 1.50$, $p > 0.1$), *SERT*: ($\chi^2(2) = 0.04$, $p > 0.1$). Given the somewhat arbitrary nature of this learning criterion, we also assessed any genotype effects on learning criteria ranging from 6-10 consecutive correct responses, none of which were significant (p>0.1 for all criteria). Finally, given that we included the learning criterion as a factor in the win-stay / lose-shift regressions, we also ascertained that there was no [genotype x learning criterion attainment x win-stay /lose-shift] (for both polymorphisms: F(20,661)<2.5, p>0.1). These analyses showed that the genotype effects were independent of overall performance on the task in terms of learning criterion attainment and acquisition error rates.

| | ***DAT1*** | | | ***SERT*** | | |
|---|---|---|---|---|---|---|
| **Genotype** | 9R9R | 9R10R | 10R10R | S'S' | S'L' | L'L' |
| **Pass rate (%)** | 40.4 | 37.7 | 42.5 | 40.6 | 41.2 | 40.2 |
| **Acq. error rate (%)** | 27.9 ±13.1 | 29.6±11.9 | 28.6±12.1 | 28.8±12.7 | 29.1±12.5 | 28.5±10.6 |

**Table S3. Acquisition performance, Related to Figure 2.** There was no difference on the percentage of subjects passing the learning criterion of 8 consecutive correct responses, or on the percentage of errors during acquisition, as a function of either polymorphism (see text for statistics). Mean **±** standard deviation.

*SERT* effects on model parameters

We established that the best-fitting parameters did not vary with *SERT* genotype as described in the main text, displayed in figure S2. There was no effect of *SERT* on the perseverative error rate (Fig. S2C, t(671) = 0.10, p=.9), or chance error rate (t(671) = 0.80, p=.42). In addition, there was no change in effect of the choice history on perseveration as a function of *SERT* genotype (Fig. S2D, t(671)=0.13, p=0.9). There were no significant effects on any of the three parameters (Figure S2B, **β**: U = 42147, z = -0.6 p = .5; **φ**: U = 40911, z = -1.2, p = .24; **ρ**: U = 42214, z = -.6, p = .6).

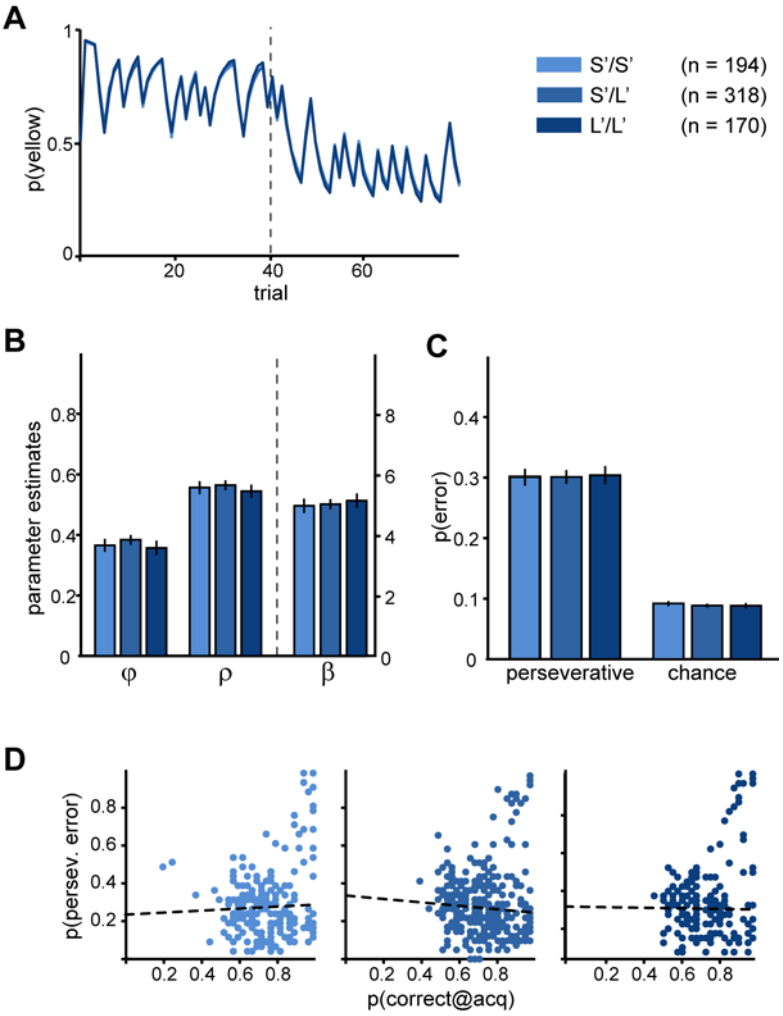


**Figure S2. EWA model simulation, Related to Figure 3.** The choices simulated by the model using the estimated parameters do not vary with *SERT* genotype. **A)** The trial-by-trial estimated probability of chosing the initially correct stimulus, averaged within each *SERT* genotype. There is no difference during acquisition or reversal. **B)** There are no differences in any of the estimated parameters. **C)** There is no effect of the *SERT* polymorphism on perseverative or chance error rate. **D)** There is no interaction between *SERT* genotype, perseveration and choice history. Mean ± SEM.

**Supplemental Experimental Procedures**

Instructions to subjects

The following instructions appeared (in Dutch) on the screen prior to the start of the experiment: *"On the screen two colored patterns are presented: one yellow, one blue. One of these colors is correct more often than the other one and the computer will tell you whether your choice was correct or incorrect. Choose the color which tends to be correct more often. You have to find out by trial and error which color that is. On certain moments the rule can change, i.e. the other color is now correct more often. Then, switch your response to that color. This can happen one or more times during the task"*

Genotyping

Molecular genetic analyses were carried out in a CCKL-certified laboratory at the department of Human Genetics of the Radboud University Nijmegen Medical Centre. DNA was extracted from saliva using the Oragene kits (DNA Genotek Inc., Kanata, Ontario, Canada). In total, 810 participants were genotyped for the variants in the dopamine transporter gene (*SLC6A3/ DAT1*) and the serotonin transporter gene (*SLC6A4/SERT/5HTT*). 5% duplicate DNA samples were included in all genotyping procedures to investigate random genotyping error, which were required to be 100% consistent. In addition, 4% blanks were included, which were required to be negative.

Genotyping of *SLC6A3/DAT1*

Genotyping of the 40 base pair variable number of tandem repeats (VNTR) polymorphism in the 3' untranslated region (UTR) of the *SLC6A3/DAT1* gene encoding the DAT1 was done as described previously.

Genomic DNA (30 ng) was amplified with 0.33 µM of forward primer (NED-5'-TGTGGTGTAGGGACGGCCTGAGAG-3') and reverse primer (5'-CTTCCTGGAGGTCACGGCTCAAGG-3') in 1x AmpliTaq Gold® 360 Mastermix (Applied Biosytems, Nieuwerkerk a/d Ijssel, The Netherlands). The cycling conditions for the PCR started with 10 min at 92°C, followed by 35 cycles of 30 sec at 95°C, 30 sec at the optimized annealing temperature of 64°C, and 1 min 72°C, then followed by an extra 7 min 72°C. The amplifications were performed in a PTC-200 Multicycler (MJ- Research via Biozym, Landgraaf, The Netherlands). Subsequent determination of the length of the alleles was performed by direct analysis on an automated capillary sequencer (ABI3730, Applied Biosystems) using standard conditions.

For *DAT1*, two alleles of interest were analyzed: the common 10R allele and the rarer 9R allele. 470 subjects were homozygous for the common 10R allele, 53 subjects were homozygous for the rarer 9R allele, and 267 subjects were heterozygous; 15 participants had one rarer VNTR in addition to the 9R or 10R alleles (3R (n=1), 6R (n=1), 8R (n=2), or 11R (n=11) repeats); for 5 participants the genotypes could not be determined. Using standard $\chi^2$ tests, no deviations from Hardy-Weinberg Equilibrium (HWE) were observed for either the 10R allele ($\chi^2(1) = 2.67$, $p > 0.05$) or the 9R allele ($\chi^2(1) = 3.21$, $p > 0.05$).

Genotyping of SLC6A4/SERT/5-HTT / rs25531

Genotyping of the 5HTTLPR polymorphism was performed by simple sequence length analysis. PCR was on 50 ng genomic DNA using 0.5 µM fluorescently labeled forward primer (FAM-5'-GGCGTTGCCGCTCTGAATGC-3') and reverse primer (5'-GAGGGACTGAGCTGGACAACCAC-3'), 0.25 mM dNTPs, 1x PCR optimization buffer A (30 mM Tris-HCl pH 8.5, 7.5mM $(NH_4)_2SO_4$, 0.75 mM $MgCl_2$), 10% DMSO and 0.4 U AmpliTaq Gold® DNA Polymerase (Applied Biosystems). The cycling conditions for the polymerase chain reaction started with 12 min at 95°C, followed by 35 cycles of 1 min at 94°C, 1 min at the optimized annealing temperature (57.5°C), and 2 min. 72°C, then followed by an extra 10 min 72°C. Subsequent determination of the length of the alleles was performed by direct analysis on an automated capillary sequencer (ABI3730, Applied Biosystems) using standard conditions.

The SNP present in the 5HTTLPR (rs25531) was genotyped using Taqman analysis (assay ID: Taqman assay: C_25746809_50; Applied Biosystems). Genotyping was carried out in a volume of 10 µl containing 10 ng of genomic DNA, 5 µl of ABgene Mastermix (2x; ABgene Ltd., Hamburg, Germany), 0.125 µl of the Taqman assay and 3.875 µl of $H_2O$. Amplification was performed on a 7500 Fast Real-Time PCR System starting with 15 minutes at 95ºC, followed by 50 cycles of 15 seconds at 95ºC, 1 minute at 60ºC. Genotypes were scored using the algorithm and software supplied by the manufacturer (Applied Biosystems). The assay has been validated by digesting the *5HTT* PCR product with *Msp*I (New England Biolabs, Ipswich, USA) and separating the restriction fragments on a 2% agarose gel. This resulted in restriction fragments of 340 bp,130 bp and 60 bp for the $L_A$ allele, fragments of 175 bp, 165 bp, 130 bp and 60 bp for the $L_G$ allele, and fragments of 300 bp, 130 bp and 60 bp for the S allele.

By combining of 5HTTLPR and rs25531 alleles, the following genotypes were derived for the 810 participants: $L_A/L_A = 190$, $L_A/L_G = 53$, $L_A/S = 329$, $L_G/L_G = 2$,

$L_G$/S = 48, S/S = 176; for 12 participants, the genotypes could not be determined. These genotyping results did not divert from HWE for either rs25531 ($\chi^2$ (1) = 0.70, p > 0.05) or 5HTTLPR ($\chi^2$ (1) = 1.84, p > 0.05). For the behavioral analysis we used a bi-allelic model, where the S allele was grouped with the rare $L_G$ allele, given that the G-substitution in the L allele results in reduced expression more similar to the S allele(Hu et al., 2006; Praschak-Rieder et al., 2007). Thus, functionally, we distinguished between the S' homozygotes (S/S, S/$L_G$ and $L_G$/$L_G$), S'/L' heterozygotes (S/$L_A$, $L_G$/$L_A$) and the L' ($L_A$/$L_A$) homozygotes.

The final sample with complete genotype data for both polymorphisms, with relevant genotypes of *DAT1* (9R/9R, 9R/10R, 10R/10R) and complete data for the PRL task consisted of 685 subjects, of which 3 were excluded from the final analysis based on their behavioral performance, as described under 'Exclusions' above.

**References**

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