1   A causal role for estradiol in human reinforcement learning

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Abstract

The sex hormone estrogen is hypothesized to play a key role in human cognition via its interactions with the dopaminergic system. Work in rodents has shown that estrogen's most potent form, estradiol, impacts striatal dopamine functioning predominately via increased D1-receptor signalling and correlational evidence in humans has suggested high estradiol levels alter reward sensitivity. Here, we addressed two fundamental questions: 1) whether estradiol causally alters reward sensitivity in men, and 2) whether this effect of estradiol is moderated by individual variation in polymorphisms of dopaminergic genes. To test this, we performed a double-blind placebo-controlled administration study in which hundred men received either a single dose of estradiol (2 mg) or placebo. We found that estradiol administration increased reward sensitivity, which was moderated by baseline dopamine. This was observed in choice behaviour and increased learning rates. These results confirm a causal role of estradiol in reinforcement learning in men that is moderated by striatal and prefrontal dopaminergic pathways.

Keywords: Estradiol, reward processing, reinforcement learning, DAT1, COMT, Estrogen receptor

Introduction

Learning which actions to select based on whether the outcome of that action is rewarded or not is a fundamental capacity required for adaptive behaviour. One neuromodulator that has long been linked to this capacity, known as reinforcement learning (RL), is dopamine [1]. More recently, an additional biological substrate that has been suggested to influence RL via dopaminergic mechanisms is the steroid hormone estrogen [2].

Estrogens are a class of steroid hormones important for healthy development in mammals, with estradiol being the most prevalent and potent form [3,4]. Previous human studies implicated estradiol in several cognitive processes with mixed findings in terms of its exact role (for reviews see [2,5]). One recent hypothesis has been that estradiol may specifically impact human reward processing by amplifying dopamine signalling via one of its receptors (i.e. the D1 receptor) [2]. For example, human neuroimaging work has revealed that fluctuations in estradiol levels are correlated with increased reward sensitivity, as documented by an increased BOLD response in the midbrain [6–8]. Similarly, rodent literature has shown that manipulation of estradiol levels affect the striatal dopamine system in various ways, with a net increase in overall dopamine signalling predominantly via the D1 receptor [9–14]. Besides the observed role of estradiol in the striatal dopamine system, it has a hypothesised connection to dopamine in the prefrontal cortex as well. Namely, estradiol metabolites decrease the activity of Catechol-O-methyltransferase (COMT), an enzyme responsible for approximately 60 percent of dopamine degradation in the prefrontal cortex and approximately 15 percent in the striatum [2,15]. Correspondingly, one correlational study previously observed that the association between endogenous estradiol levels and working memory performance is moderated by polymorphisms of the COMT gene [16].

Dopamine's role in reward processing and learning has been well studied using RL tasks and has been formalized with the reward prediction error hypothesis [1,17–19]. A canonical approach to investigate the causal role of dopamine in reward processing is to employ a double-blind placebo-controlled administration protocol using dopamine agonists and

69   antagonists, respectively [20–26]. Extending this approach through pharmacogenetics, which is

70   the interaction between administered drugs and genetic variation, has enabled a better

71   understanding of how genetic variation modulates dopamine availability and how the latter

72   influences reward processing and cognition more generally [16,21,24,27,28].

73   This line of work has shown that causal manipulation of dopamine levels in humans

74   affects performance in reinforcement learning [28], and that these effects can depend on

75   individual differences in baseline dopamine levels [20]. Crucially, such individual differences

76   arise from polymorphisms of dopamine-related genes impacting dopamine synthesis capacity

77   and transmission [16,21,26]. For example, the COMT and dopamine transporter (DAT1) gene have

78   polymorphisms that correlate with differences in performance on working memory and

79   reinforcement learning tasks [16,21,26,29,30]. These polymorphisms are the val[158]met polymorphism

80   of COMT (i.e. the Val/Val, Met/Val, and Met/Met genotypes that are each associated with

81   increasingly higher levels of prefrontal dopamine) and VNTR polymorphism of DAT1 (i.e. the

82   9/10 and 10/10 genotypes are associated with high and low striatal dopamine, respectively).

83   Despite abundant evidence from rodent research and work in humans showing the

84   relation between estradiol, dopamine, and human cognition, results so far have been

85   contradictory in terms of estradiol's effects. Namely, it has been shown that high endogenous

86   estradiol levels increased [6,8] as well as decreased [31] performance on a variety of cognitive

87   tasks. Although previous work on humans provided important insights, these were mostly

88   based on correlations (for exceptions see [6,31,32]), small sample sizes (for exception see [32]),

89   and additionally did not explicitly focus on the importance of baseline differences in dopamine

90   (for exceptions see [16,33]). Therefore, the precise role of estradiol in human reward processing

91   remains unclear (for review see [2]).

92   The aim of the present study was to investigate whether estradiol causally affects

93   reward processing in a probabilistic RL task by employing a pharmacogenetic approach (Fig.

94   1A). The task required subjects to choose between two options on each trial in order to

95   maximize their earnings. The probability of reward of both options was determined by two

96   independent random Gaussian walks while the reward size was constant across trials (Fig.

97   1B). A constant reward size allowed us to isolate estradiol's influence on choice behaviour as

98   a function of receiving versus not receiving a reward on each trial. This allowed for a more

99   precise examination how estradiol influences reward processing. We further investigated

100   whether an effect on reward sensitivity was moderated by individuals' baseline dopamine, as

101   indexed through genetic variation in COMT and DAT1. Our main hypothesis was that estradiol

102   administration would increase reward sensitivity which would be observed through increased

103   choice reactivity. We further predicted that an increase in reward sensitivity would be observed

104   in increased Q-learning learning rates, indicative of higher learning. Finally, we predicted that

105   the behavioural and computational effects would uniquely depend on polymorphisms of both

106   COMT and DAT1, as observed in previous work [21,27].

107   To detect differences at the level of individual genetic variants, we used a sample size

108   (N = 100) in line with previous recommendations in the field [2]. Our sample was pre-screened

109   and matched for key physiological characteristics, behavioural and cognitive traits and states

110   that could have impacted RL behaviour (see Supplementary Materials). Moreover, we aimed

111   at providing a more conclusive and precise account of a dopamine-dependent basis of action

112   through excluding several other mechanistic explanations, which have so far been

113   unaddressed. These included polymorphisms of androgen and estrogen receptors, together

114   with a polymorphism influencing the enzyme aromatase that is responsible for the conversion

115   of androgens to estradiol. These mechanisms are important because previous work has

116   shown that administering estradiol also increases free circulating androgen levels, which are

117   known to be converted to estradiol through aromatase [34] (see also Supplementary Materials).

118   In brief, we have found that estradiol administration increased reward sensitivity as

119   compared to placebo administration. This was observed in choice behaviour and increased

120   learning rates. Furthermore, we observed that the interaction between estradiol administration

121   and dopamine-related genes predicted choice, in line with predictions from previous work

122   reviewed here. Finally, we have observed several effects related to staying and switching

123   behaviour that depended not only on striatal but also prefrontal baseline dopamine levels.

124   Taken together, the described effects are consistent with the hypothesis that estradiol acts by

125   amplifying dopamine signalling via the D1 receptor and extend this by showing that the effects

126   of estradiol are moderated by differences in prefrontal dopaminergic functioning as well.

127

Results

129   Both treatment groups (estradiol and placebo) were matched on several key characteristics.

130   These included age, height, visceral, and abdominal fat, BMI, and individual traits and states

131   that can impact RL behaviour, including working memory, self-reported impulsivity,

132   behavioural inhibition and approach, and mood. As a manipulation check of our administration

133   protocol, estradiol concentrations were significantly elevated in subjects who had received

134   estradiol compared to placebo after ($W = 1545$, 95% CI [0.03, 1.87], $p < .05$), but not before

135   administration (baseline: $W = 1498$, 95% CI [-0.05, 1.03], $p = .09$) and subjects' beliefs about

136   whether they had received estradiol or placebo did not correlate with the actual received drug

137   ($r = 0.02$, $p = .82$; for further details on group characteristics, matching, and manipulation

138   checks see Supplementary Materials).

139   First, we investigated our hypothesis that estradiol administration would alter reward

140   sensitivity, which we expected to observe through a systematic difference in choice behaviour

141   across trials compared to placebo. We quantified this systematic difference by computing the

142   cumulative difference in the probability of choosing option A across trials in both groups. This

143   cumulative difference was then compared to a null distribution demonstrating what would be

144   expected by chance (see Methods and materials). Similarly, we looked at the percentage of

145   trials on which estradiol caused a significant difference in the chosen option compared to

146   placebo. Moreover, we looked at whether these differences in choice behaviour also reflected

147   improved task performance. Secondly, we tested our hypothesis that the effect of estradiol

148   administration on choice behaviour would interact with genetic variation of COMT and DAT1.

149   This was followed by a more detailed examination of whether these interactive effects would

150   be observed in the amount of switching and staying behaviour, and choice autocorrelation

151   throughout the task. Finally, we formalized these differences in behaviour within a

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152 reinforcement learning framework that allowed us to exclude the possibility that choice

153 differences were due to more stochastic responding, but instead were due to higher learning

154 rates, indicative of higher weighing of more recent relative to old task-relevant information.
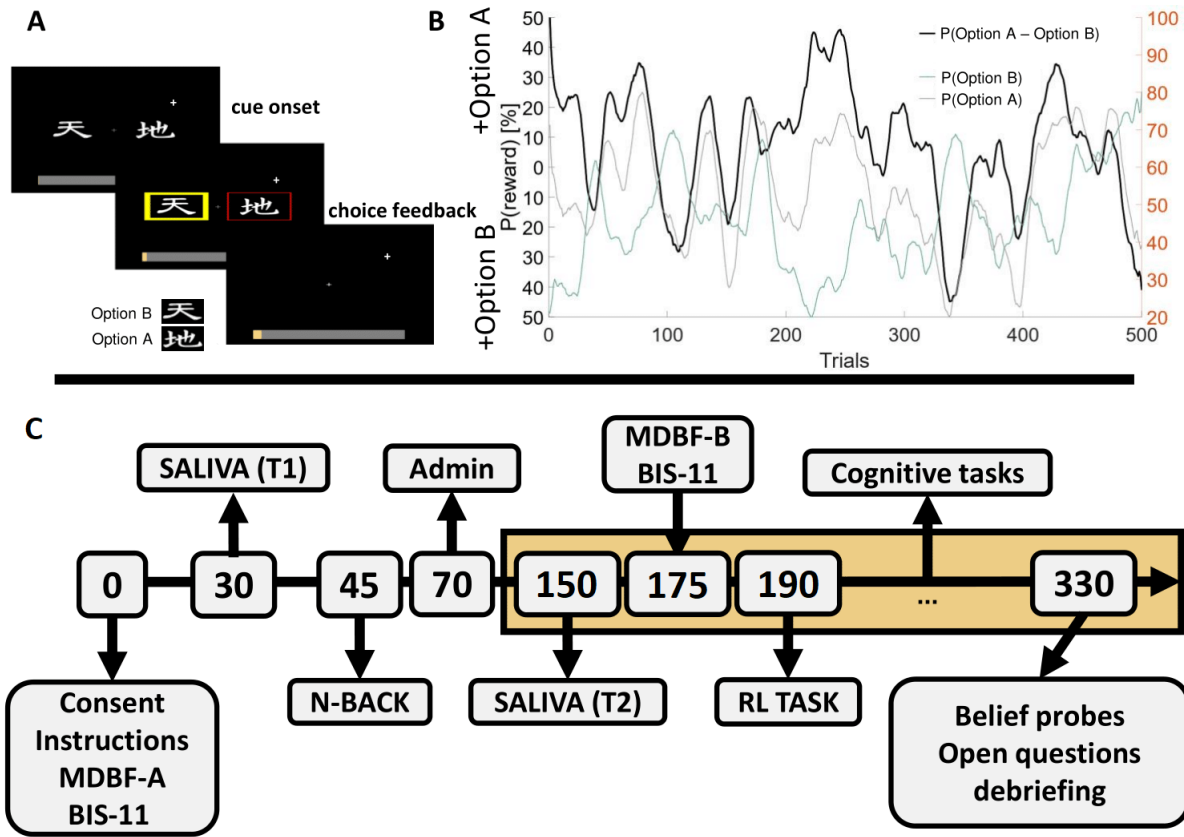

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Fig. 1 A) Outline of a trial of the RL task. Each trial started by the presentation of two options (henceforth option A and option B). Subjects were required to choose one of these options. After they made a choice, subjects were presented with feedback, with the chosen option indicated by a thicker frame and the not chosen option by a thinner frame. A yellow frame indicated the rewarded option, whereas a red frame indicated the unrewarded option. Importantly, both options A and B could yield a reward or no reward on the same trial. **B)** The probability of reward upon choice for each option (green and gray lines), which were determined by two independent random Gaussian walks, with the probability shown in percent on the right y-axis in orange. The black line shows the relative probability of reward for one option over the other, which corresponds to the difference in reward probability for option A and option B. On trials where the black line is reaching the top half of the y-axis, option A was more rewarding, and vice versa. **C)** The timeline of the test session. Values in brackets denote minutes from the onset of the test session. We first collected consent and questionnaire data, which was followed by a baseline saliva sample (T1) and the N-BACK task. After administration of estradiol or placebo, subjects were required to rest for two hours before we collected the second saliva sample (T2) and assessed subjects' mood and impulsivity via questionnaires. The RL task began 120 minutes post-administration. This was followed by three other cognitive tasks that are not the focus of the current paper. At the end of the test session, we probed subjects' beliefs about the drug, the experiment, and debriefed them.

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Estradiol administration alters choice reactivity

175   Our first hypothesis was that estradiol administration would increase reward sensitivity. By

176   reward sensitivity, we refer to a systematic difference in the chosen option across trials

177   between the estradiol and the placebo group. Since reward sizes were constant, the only

178   difference across trials was whether a reward was received or not following choice. An effect

179   of estradiol on reward sensitivity would therefore be observed if the difference between the

180   option that each group chose on average across trials would be higher than would be expected

181   by chance. We investigated whether such a difference in choice behaviour exists in two

182   complementary ways. We first computed the probability for each group to select option A vs.

183   option B across trials, subtracted the two group traces from each other (Fig. 2A) and plotted

184   the cumulative choice difference across trials (Fig. 2B).

185   Under the hypothesis that estradiol systematically influenced choice behaviour, the

186   cumulative difference in the expected chosen option should have exceeded the one obtained

187   from a null distribution. Specifically, in the null distribution choice behaviour was decoupled

188   from the actual treatment (i.e. estradiol vs. placebo) and revealed what degree of cumulative

189   choice difference would be expected by chance or random assignment of treatment (see

190   Methods and materials). Indeed, we observed that the cumulative difference in the expected

191   chosen option between the estradiol and placebo group started to exceed the 100th percentile

192   of a null distribution (Fig. 2B) ($M_{last\ trial}$ = 53.48 %, $z_{last\ trial}$ = 8.44, $p < .001$, threshold value for

193   99.9[th] percentile of null distribution: 46.20 %). This cumulative choice difference between the

194   estradiol and placebo group remained significant when we collapsed it across time (Fig. 2C),

195   which is demonstrated as the mean and the standard error of the mean remaining above the

196   99.9[th] percentile threshold of a null distribution ($M = 25.72 \pm 0.69\%$, $z = 5.80$, $p < .001$, threshold

197   value for 99.9[th] percentile of null distribution: M = 21.02 %) (see Methods and materials). Both

198   results showed that estradiol administration (vs. placebo) led to systematic differences in

199   subjects' choice.

200   Secondly, we tested the percentage of trials on which there was a statistically

201   significant difference between the groups in choice behaviour. To test this, we performed a

202   two-sample proportion z-test on each trial, where we statistically compared the proportion of

203   subjects choosing option A between both groups. We observed that estradiol administration

204   (vs. placebo) led to a statistically significant difference in the proportion of subjects choosing

205   option A vs. option B on 7.6 % of trials (black dots in Fig. 2A). In other words, estradiol

206   administration caused subjects to choose a different option on 7.6 % of trials as compared to

207   placebo. We performed family-wise error control similarly to above (see Methods and

208   materials). For this, we decoupled the responses from the treatment and tested whether this

209   percentage would have been obtained in a null distribution with random allocation of groups.

210   This comparison showed that the change in how groups responded to the rewarding options

211   on 7.6 % of trials exceeded the threshold value of a null distribution ($z = 5.37$, $p < .001$,

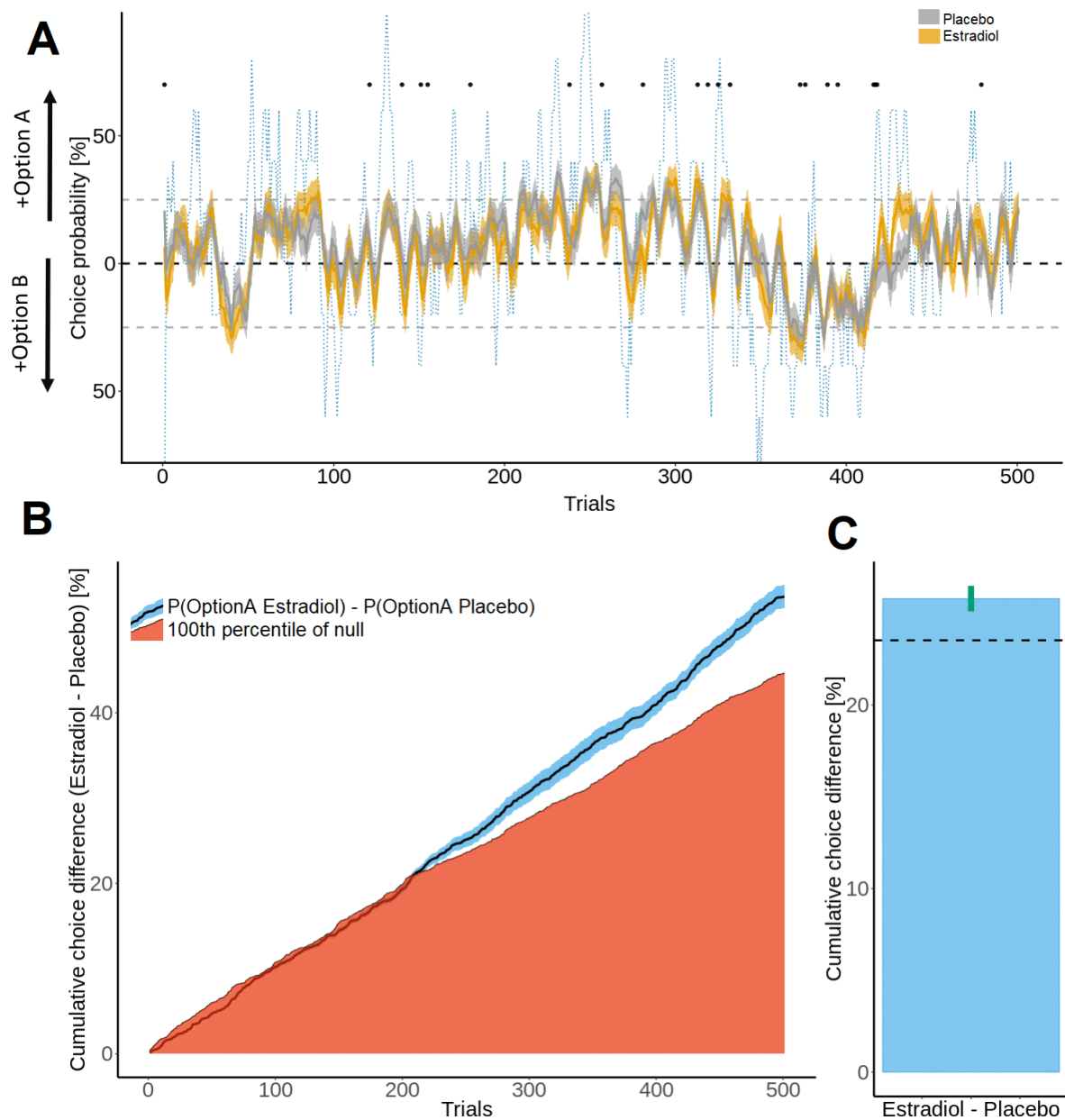212   threshold value for 99.9^{th} percentile of null distribution: 6.4 %).

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Fig. 2 A) Relative choice probability for choosing option A (top of y-axis) vs. choosing option B (bottom of y-axis) for the estradiol (orange) and placebo (gray) group. Solid thick lines represent trial mean, shaded areas around the thick lines denote standard errors of the mean. The blue dotted line denotes the relative reward probability which was computed from the probability of option A (top of y-axis) minus probability of option B (bottom of y-axis). Horizontal gray dotted lines represent where subjects were on average 25% more likely to select option A (upper line) or option B (lower line). All time-series traces were smoothed with a 5-trial moving average for visual purposes. The black dots indicate trials where there was a statistically significant difference ($p < .05$) between the estradiol and placebo group. The number of significant trials was compared to a null distribution (see Methods and materials). **B**) Cumulative choice difference between the estradiol and placebo group over trials compared to a 100th percentile null distribution. The thick black line is the difference between the orange and gray lines presented in figure **A**, and the blue shaded area is the corresponding difference between the standard errors in **A**. The dark orange area denotes the space in which differences are not significant. Conversely, separation between the lines indicate statistical significance. **C)** Mean cumulative choice difference between the estradiol and placebo group collapsed across trials. The dashed line represents the mean cumulative choice difference of the 100th percentile of the null distribution. Error bars indicate standard error of the mean.

231

DAT1 genotype marginally moderates the effects of estradiol on accuracy

233 Following the observed systematic choice difference between both groups, we investigated

234 whether this was reflected in group differences in accuracy (i.e. whether the estradiol group

235 chose the option with higher probability of reward compared to the placebo group). In a

236 comparison of choice accuracy (Fig. 3A), we observed that subjects with exogenously

237 elevated estradiol were not more accurate compared to subjects with placebo ($M_{Estradiol}$ = 57.30

238 ±6.91, $M_{Placebo}$ = 56.80 ±7.09, $t_{(97.94)}$ = 0.36, *95% CI* [-3.28, 2.28], *p* = .72, *d* = 0.07), and

239 responded equally fast ($M_{Estradiol}$ = 0.61 sec ±0.11, $M_{Placebo}$ = 0.62 *sec* ±0.09, $t_{(95.55)}$ = 0.46, *p*

240 = .65, *d* = 0.09).

241 However, based on previous work that showed interactive effects between cognitive

242 performance and dopamine-related genes [20,35], we had hypothesized that the effect of

243 estradiol on accuracy may depend on individual differences in baseline striatal dopamine

244 (indexed with DAT1 polymorphism: 9/10 and 10/10 genotypes are associated with high and

245 low striatal dopamine, respectively). Similarly, we predicted that the effects of estradiol may

246 depend on differences in prefrontal dopamine (indexed with the COMT polymorphism, as

247 Met/Met, Met/Val, and Val/Val genotypes are associated with high, medium, and low prefrontal

248 dopamine, respectively). A general linear model revealed a trend towards an interaction

249 between drug administration and DAT1 genotype on accuracy ($F_{(1, 69)}$ = 3.69, *p* = .06, Ω^2 =

250 0.03, Fig. 3B), while controlling for covariates (see Methods and materials). Following up this

251 trend, pairwise comparisons revealed that estradiol administration increased accuracy in

252 subjects with the 9/10 genotype (i.e. high striatal dopamine levels; *M* = 60.00 ±5.36) compared

253 to those with a 10/10 genotype (i.e. low striatal dopamine levels; 10/10 DAT1, *M* = 56.00 ±6.51;

254 $t_{(39.60)}$ = 2.14, *95% CI* [0.21, 7.63], *p* = .04, *d* = 0.61), but not for the placebo group (9/10

255 genotype: *M* = 57.21 ±6.60; 10/10 genotype: *M* = 56.75 ±6.34; $t_{(31.02)}$ = 0.22, *95% CI* [-3.74,

256 4.66], *p* = .82, *d* = 0.06). Subjects with the 9/10 genotype in the estradiol group were not more

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257 accurate compared to subjects with the 9/10 genotype in the placebo group ($t_{(29.04)}$ = 1.33, *95%*

258 *CI* [-1.48, 7.00], *p* = .19, *d* = 0.45) nor when comparing the groups with the 10/10 genotype

259 $t_{(40.22)}$ = 1.82, *95% CI* [-0.36, 6.80], *p* = .08, *d* = 0.61).

260   Repeating the same analysis for the COMT genotype revealed no interaction between

261 drug administration and COMT on accuracy ($F_{(2, 79)}$ = 1.76, *p* = .18, Ω^2 = 0.02, Fig. 3C).

262   In sum, estradiol administration increased reward sensitivity. We observed this in terms

263 of a cumulative difference in the expected chosen option between the estradiol and the

264 placebo group, both across trials and collapsed across trials. Furthermore, on a subset of trials

265 we found a significant difference in the proportion of subjects from the estradiol group

266 compared to placebo group who chose option A. This systematic difference in how subjects

267 responded throughout the task was not reflected in increased accuracy across both groups.

268 However, in line with our hypothesis we found a significant interaction between genetic

269 variation of DAT1 polymorphism and drug administration, such that in the estradiol group

270 subjects with a 9/10 DAT1 genotype showed an improved accuracy (by trend) relative to those

271 with a 10/10 DAT1 genotype, with no such difference in the placebo group. No such interaction

272 was observed for the COMT polymorphism, indicating that estradiol mainly acted on striatal

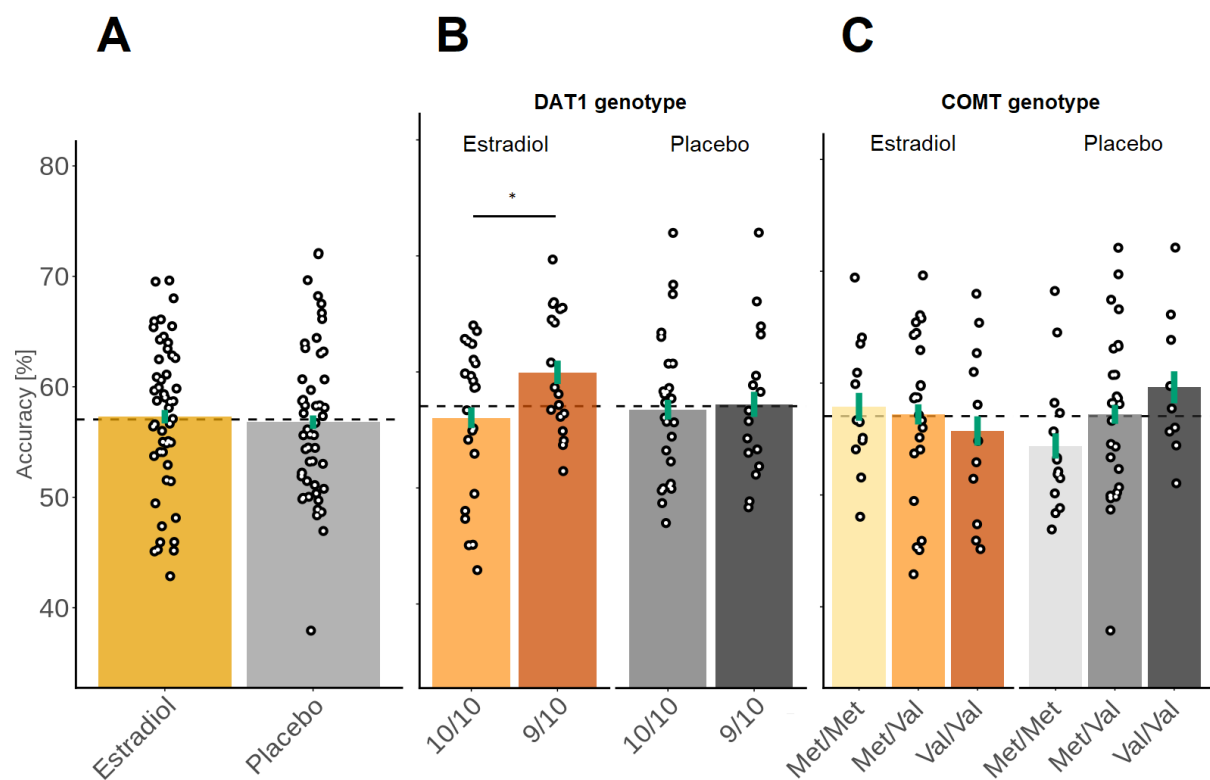273 rather than prefrontal dopamine signaling in terms of its effects on task accuracy.

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Fig. 3 A) Mean accuracy split according to drug administration. **B**) Mean accuracy split according to drug administration and DAT1 polymorphism. **C**) Mean accuracy split according to drug administration and COMT polymorphism. Green error bars are standard errors of the mean. Dots represent individual subjects. The horizontal black dotted line represents grand mean performance collapsed across groups to show the relative change for individual subgroups. * $p < .05$.

The effect of estradiol administration on choice behaviour is moderated by polymorphisms of both COMT and DAT1

To directly test whether the effect of estradiol administration on choice behaviour is moderated by polymorphisms of dopamine-related genes (e.g. COMT, DAT1), and whether individual variability in these effects may be a contributing factor to the observed effects, we used generalized linear mixed models. We tested whether the interaction between drug, polymorphism (COMT or DAT1), and trial are a significant predictor of choice behaviour (i.e. reward sensitivity).

We predicted a significant interaction due to the observed differences in cumulative choice behaviour described above. Based on the inverted U-shape dopamine hypothesis [35], we predicted that estradiol administration would upregulate reward sensitivity in subjects with

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294   low prefrontal dopaminergic activity (i.e. Val/Val) but would not, or would even impair it, in

295   those with high prefrontal dopaminergic activity (i.e. Met/Met). The model predicted that

296   exogenously elevated estradiol in subjects with a Met/Val (β = 0.20 ± 0.04, *95% CI* [0.11, 0.28],

297   *z* = 4.56, *p* < .001) and Val/Val genotype (β = 0.37 ± 0.06, *95% CI* [0.26, 0.48], *z* = 6.99, *p* <

298   .001) were more likely to select option A as trials progressed (Fig. 1B, see also Fig. S2 and

299   Fig. S7. Supplementary Materials) – which was the more rewarding option throughout the task

300   (percent trials rewarded: $M_{optionA}$ = 53.70%, $M_{optionB}$ = 42.91%).

301   Similarly, we predicted that estradiol should indirectly increase striatal dopamine

302   levels, leading to higher reward prediction errors. Based on this, we expected that subjects

303   with the 9/10 genotype (i.e. high striatal dopamine) would select the more rewarding option

304   (i.e. higher value option) more often and less so for subjects with the 10/10 genotype (i.e. low

305   striatal dopamine). This was supported by model predictions showing that that subjects with

306   the 10/10 genotype with placebo (β = -0.12 ± 0.04, *95% CI* [-0.04, -0.20], *z* = -3.03, *p* < .01)

307   were the most likely to select the lower valued option A throughout task progression, while

308   estradiol administration dampened this slope in subjects with the same 10/10 genotype (see

309   Fig. S7. Supplementary Materials). Results from both generalized linear mixed effects models

310   showed that once individual variation was considered, the effect of estradiol administration on

311   choice behaviour across trials was moderated by striatal (DAT1) and prefrontal (COMT)

312   polymorphisms (see Fig. S7. Supplementary Materials for model predictions).

313

Increased reward sensitivity is observed in increased learning rates

315   Given our observation that estradiol increased reward sensitivity and our hypothesis that the

316   mechanistic explanation for a cumulative choice difference in this task may underlie increased

317   striatal and prefrontal dopamine levels, we predicted that estradiol would enhance the learning

318   of reward probabilities. In a RL framework this would be reflected in increased learning rates.

319   The learning rates represent latent variables dictating one's weighing of recent in comparison

320   to older information. To test this, we estimated the learning rate by fitting several Q-learning

321   models (see Methods and materials). To test this, we estimated learning rates by fitting several

322   Q-learning models (see Methods and materials). The best model (model 2, leave one out

323   information criterion (LOOIC) = 60179, Fig. 4A) included separate learning rates for each

324   option, a temperature parameter, and an irreducible noise parameter. The model predicted

325   choice behaviour above chance ($t_{(99)}$ = 13.95, 9$5\%$ CI [0.64, 0.68], p < .001, Fig. 4B, see also

326   Fig. S8. Supplementary Materials) and did not perform better for either group ($M_{Estradiol}$ = 66.26

327   % ±10.77, $M_{Placebo}$ = 64.90 % ±11.85; $t_{(97.115)}$ = 0.76, 95% CI [-0.03, 0.06], p = .45).

328   Our main hypothesis was that if estradiol increases available striatal and prefrontal

329   dopamine concentrations [2,5], then the behavioural differences in choice over time (Fig. 2A)

330   would be captured in the learning rates. We have found that estradiol administration increased

331   the learning rate for both options compared to placebo ($\alpha_{optionB}$: $M_{Estradiol}$ = 0.27 ±0.16, $M_{Placebo}$

332   = 0.17 ±0.13, $t_{(85.36)}$ = 4.47, 95% CI [0.08, 0.21], p < .001, d = 0.9; $\alpha_{optionA}$: $M_{Estradiol}$ = 0.26 ±0.19,

333    $M_{Placebo}$ = = 0.12 ±0.13, $t_{(92.13)}$ = 3.42, 95% CI [0.04, 0.16], p < .001, d = 0.69, Fig. 4C). We

334   expected that estradiol would affect both learning rates in the same direction due to their

335   intrinsic correlation arising from the fact that both capture the same behaviour (r = 0.84, p

336   <.001). However, contrary to our expectations, the observed main effect of estradiol was not

337   moderated by either polymorphisms of DAT1 or COMT (COMT: $\alpha_{OptionB}$: $F_{(2, 81)}$ = 0.37, p = .69;

338    $\alpha_{OptionA}$: $F_{(2, 72)}$ = 0.29, p = .75; DAT1: $\alpha_{OptionB}$: $F_{(1, 71)}$ = 0.02, p = .89, $\alpha_{OptionA}$: $F_{(1, 71)}$ = 0.03, p =
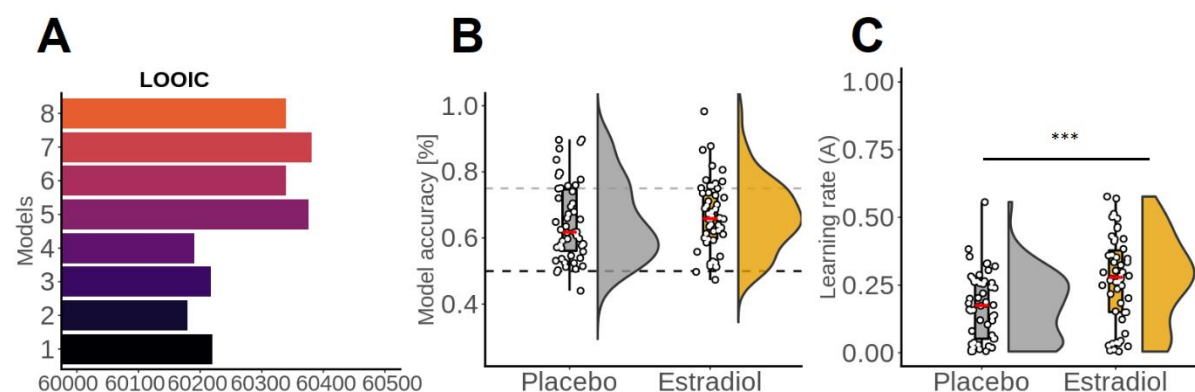
339   .86).

340

341   **Fig. 4 A)** Leave one out information criterion (LOOIC) value for all employed models. Lower LOOIC
342   indicates better model fit – model two was selected as the best model. **B)** The overall model accuracy
343   collapsed over time obtained from the posterior predictive density (see Supplementary Materials) shown
344   for both groups separately. Individual dots represent subjects. The red bar represents the median, the
345   box plot represents the 75% middle most data points, with the whiskers representing 1.95*IQR. **C)**
346   Learning rates by drug treatment. The estradiol group (in orange) had higher learning rates compared
347   to the placebo group (in gray).

348

349   In sum, the estradiol group had higher learning rates compared to the placebo group

350   but we observed no moderation of the polymorphisms of both COMT and DAT1 on the model

351   parameters.

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353   Altered reward sensitivity is driven by differences in the number of stay-switch

354   decisions and moderated by COMT and DAT1 genotype.

355   Finally, to more precisely understand the observed difference in choice behaviour between

356   treatment groups and dopamine-related genes, we tested whether this difference could be

357   attributed to differences in staying and switching behaviour, commonly studied in this field [26,36].

358   Based on our expectation that estradiol would increase striatal dopamine levels, and

359   through that increase reward prediction errors, we predicted that estradiol administration

360   would enhance staying behaviour moderated by DAT1, but not by COMT polymorphism. As a

361   measure of staying, we computed how many trials subjects chose the same option on average

362   if they were previously rewarded for that option (see Fig. 5). Overall, estradiol administration

363   did not increase the number of stay choices ($M = 1.70 \pm 0.03$) compared to placebo ($M = 1.65$

364   ± 0.04; $t_{(97.91)} = 1.07$, *95% CI* [-0.15, 0.04], $p = .29$, $d = 0.21$).

365   However, estradiol administration in 9/10 DAT1 genotype subjects, who were more

366   accurate compared to subjects who received estradiol and had the 10/10 genotype, also chose

367   the same option on more trials on average after being rewarded for their choice ($M = 1.79 \pm$

368   0.18; Fig. 5B). This was observed compared to subjects with placebo who had the 9/10

369   genotype ($M = 1.63 \pm 0.22$; $t_{(29.05)} = 2.33$, *95% CI* [0.02, 0.3], $p = .03$, $d = 0.41$; see also

370   Supplementary Materials Fig. S6), and compared to subjects who had the 10/10 genotype

371   (placebo: $t_{(41.61)}$ = 2.22, *95% CI* [0.01, 0.27], *p* = .03, *d* = 0.41; estradiol: ($t_{(38..86)}$ = 2.49, *95% CI*

372   [0.03, 0.27], *p* = .02, *d* = 0.64). In other words, the increase in accuracy by exogenously

373   elevated estradiol in individuals with a 9/10 genotype was reflected in increased staying with

374   options for which they were previously rewarded. This is consistent with previous work

375   showing increased striatal prediction errors following dopamine precursor administration [28].

376   Furthermore, because estradiol administration likely results in increased prefrontal

377   dopamine levels through downregulating COMT enzyme activity, we predicted that the

378   interaction between estradiol administration and COMT polymorphism would be predictive of

379   switching behaviour [26]. As a measure of switching, we assessed the number of times the

380   option chosen on trial *t* was different from the one chosen at trial *t + 1* (i.e. a switch),

381   irrespective of the choice outcome on trial *t* (see Fig. 5). Estradiol administration did not

382   significantly influence switch decisions (*M* = 162.12 ±56.31) compared to placebo (*M* = 168.82

383   ±68.13; $t_{(94.64)}$ = 0.54, *95% CI* [-18.12, 31.51], *p* = .59, *d* = 0.11). However, we observed a

384   significant interaction of estradiol administration by COMT genotype ($F_{(2, 80)}$ = 3.22, *p* = .05, Ω^2

385   = 0.04, Fig. 5A). The interaction showed that subjects with placebo and a Val/Val genotype

386   (i.e. low prefrontal dopamine availability) switched less often (*β* = -84.07±33.69, *p* = .02)

387   compared to all other groups. As predicted by the inverted U-shaped relationship between

388   prefrontal dopamine levels and behaviour [35], Val/Val placebo subjects (Val/Val: *M* = 132.33

389   ±61.40) switched less compared to Met/Met placebo subjects (i.e. associated with high

390   prefrontal dopamine availability; Met/Met: *M* = 204.27 ±53.52, $t_{(15.10)}$ = 2.91, *95% CI* [19.25,

391   124.54], *p* = .01, *d* = 1.46). For the estradiol group, this difference was not present (Val/Val: *M*

392   = 151.09 ±70.85; Met/Met: *M* = 178.5 ±55.34; $t_{(18.96)}$ = 1.03, *95% CI* [-28.28, 83.10], *p* = .32, *d*

393   = 0.44). That is, estradiol administration attenuated naturally occurring differences in switching

394   behaviour found in subjects with the Met/Met and Val/Val genotypes that are associated with

395   high and low prefrontal dopamine levels, respectively.

396   Crucially, the effects reported for accuracy, staying, and switching were not explained

397   by other mechanistic explanations (i.e. other genetic polymorphisms assessed here), such as

16

398   those related to androgen receptor functioning, androgen to estrogen conversion, or estrogen

399   receptor functioning (see Supplementary Materials), indicating that the observed results are

400   moderated specifically by dopamine-related genes. Furthermore, in Supplementary Materials

401   we further show that the observed differences in staying and switching can be also

402   characterised as differences in choice autocorrelation, and choice autocorrelation as a

403   function of previous reward. In brief, the estradiol group overall exhibited less choice

404   autocorrelation compared to the placebo group, showing that previous responses had a

405   weakened effect on future choices, with these differences being more pronounced based on
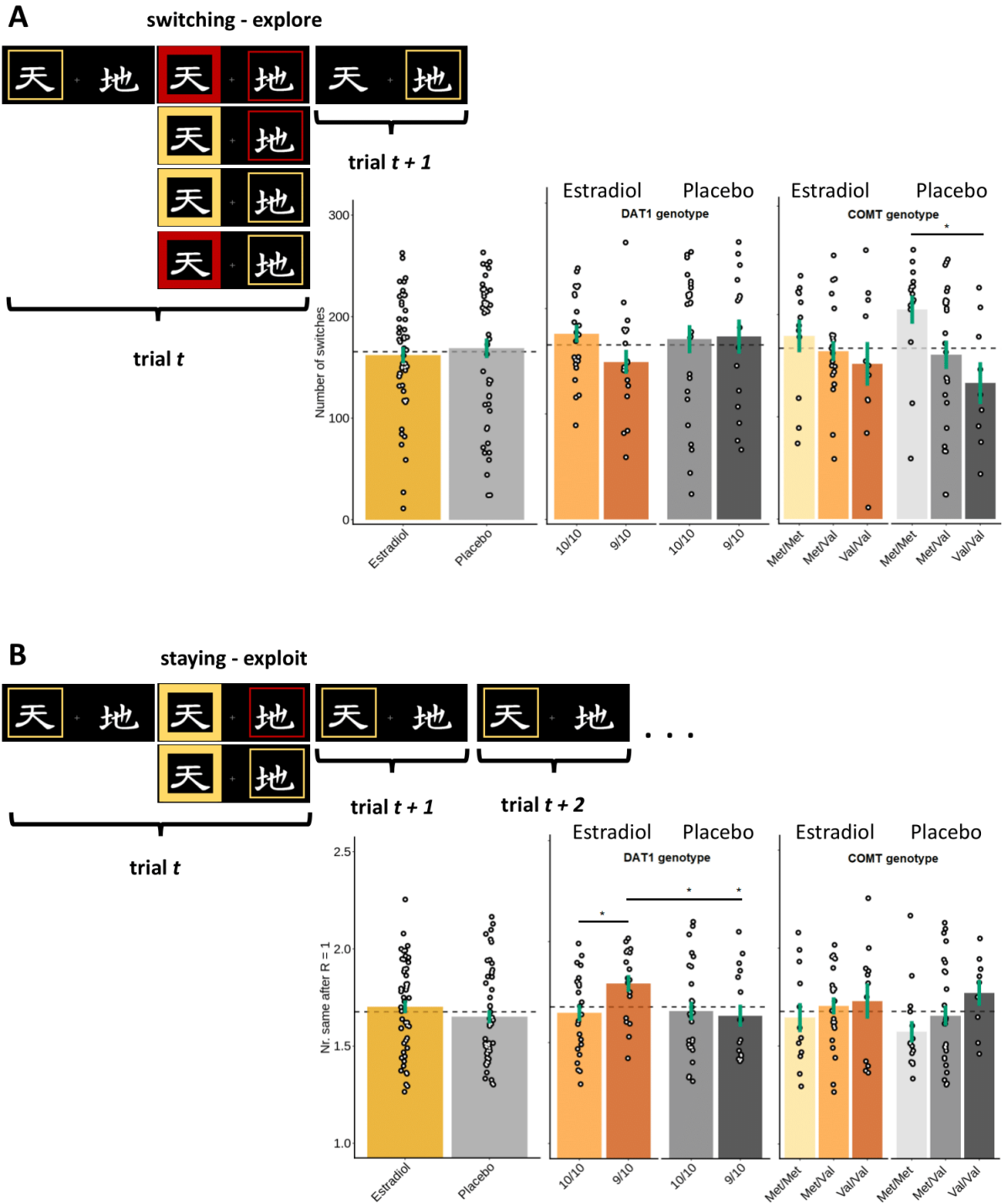
406   DAT and COMT polymorphisms.

Fig. 5 Stay and switch behaviour split according to drug administration and the COMT and DAT1 polymorphisms. **A)** Switching behaviour, measured as the total number of trials on which subjects chose different options on trial t and trial $t + 1$, independent of the choice outcome on trial t. **B)** Staying behaviour, measured as the average number of trials the same option was selected when that choice was previously rewarded. In both plots, each dot represents a subject, the green error bar represents standard error of the mean.

18

Discussion

In this study we examined the causal effects of estradiol on reward processing in human males. A body of previous rodent causal and human correlational work has suggested a role of estradiol in cognition, and reward processing more specifically, via dopamine-related mechanisms [5,7,8,10,12,14,16,37]. However, it remained an open question whether the effect of estradiol administration would be observable in choice behaviour of healthy young men and whether this would be moderated by individual variation in DAT1 and COMT. By employing a pharmacogenetic approach with a probabilistic RL task, we have shown that exogenously elevated estradiol altered various aspects ofchoice behaviour related to reward processing. Moreover, we have shown that effects related to accuracy, staying, and switching were moderated by striatal (DAT1) and prefrontal (COMT) dopamine-related genes, but not by other candidate genes that we tested.

Firstly, we confirmed the hypothesis that estradiol administration increases reward sensitivity in healthy young men as observed through increased choice reactivity. More specifically, we found that the cumulative difference in the expected chosen option between both groups was higher than what would be expected by chance. This was the case when we compared choice behaviour across trials and when we collapsed across trials. When we further quantified the difference by looking at the percentage of trials on which the estradiol group choose a different option compared to the placebo group, we observed they chose differently on a statistically significant subset of trials that was above chance.

In addition to these analyses, we aimed to account for individual variability in the strength of a potential effect on choice behaviour [38,39]. Using two separate generalized linear mixed models, we observed that both models predicted choice, showing that the effect of estradiol on choice behaviour over time was moderated by baseline striatal (DAT1 – first model) and prefrontal dopamine levels (COMT – second model). Overall, these results replicate previous correlational[7,8] neuroimaging work and preliminary evidence from a

19

443   pharmacological study on a small sample of women at menopause (N = 13) showing changes

444   in BOLD signal due to reward-related information in conditions of high vs. low estradiol

445   conditions[6]. Furthermore, our results show, for the first time, that causal exogenous alteration

446   of estradiol leads to differences in choice behaviour on a reinforcement learning task via

447   striatal and prefrontal modulation.

448   However, these differences in choice behaviour alone would not yet clearly establish

449   whether estradiol acted by amplifying dopamine D1 receptor signalling[2]. To test this more

450   directly, we investigated whether the observed choice differences resulted in differences in

451   accuracy on our task, as expected by previous work using dopamine precursor administration

452   [28]. We also tested whether this would be moderated by the DAT1 polymorphism [27,33]. This

453   revealed a trending interaction (p = .06) between estradiol administration and the DAT1

454   polymorphism on accuracy. Specifically, a pairwise comparison showed that estradiol

455   administration significantly increased accuracy in subjects with the 9/10 genotype (i.e. high

456   striatal dopamine), but only compared to subjects with the 10/10 genotype. This effect had a

457   medium effect size (Cohen's d = 0.61).

458   While the observed effect on accuracy is in line with our hypothesis and predictions of

459   estradiol amplifying striatal dopamine D1 receptor signalling[2], our findings should be

460   considered as preliminary and warrant replication in future pharmacogenetic administration

461   studies using larger samples per cell. Of note is that previous research, in which striatal

462   dopamine levels were increased exogenously, showed a deterioration in accuracy in their

463   experimental group with the 9/10 genotype, but an improvement in those with the 10/10

464   genotype [27]. One possible explanation for this contrast with our results is that our administered

465   estradiol dosage most likely acted akin to a "low dosage" of a dopamine precursor. Namely,

466   dopamine precursor administration has been previously shown to impact behaviour in a dose-

467   dependent manner [40,41]. This interpretation is also supported by a recent administration study

468   on a small sample of women (N = 34) where 12 mg of estradiol (i.e. 6 times our dose)

469   decreased working memory performance[31], which was interpreted as an overstimulation of

470 dopaminergic transmission. Similar support comes from a study on hippocampal activity in
471 women following dose-dependent estradiol administration[32].

472   Overall, our finding of a subtle effect on accuracy following estradiol administration
473 when including the genetic DAT1 polymorphism converges with previous work. That is,
474 previous work has interpreted diverging results in the direction of increased and decreased
475 performance in high estradiol conditions due to different baseline dopamine levels [8,37,42,43]. Our
476 results provide empirical evidence for these previous claims by showing that the effect of
477 estradiol on reward processing is better understood when taking baseline dopamine levels
478 into account. Furthermore, they show that investigating whether and how chronic estradiol
479 administration alters reward processing in humans, dependent on one's genotype, may yield
480 important and novel insights for both basic science as well as clinical practice.

481   To better understand what drove the effect on accuracy and choice reactivity, we
482 computed metrics of switching and staying behaviour that are commonly investigated in such
483 tasks [26,36] and performed choice autocorrelation analyses. Both metrics showed that within the
484 estradiol group, the subtle difference in accuracy between the DAT1 polymorphisms was
485 reflected by increased staying behaviour. Namely, subjects with a 9/10 genotype chose the
486 same option on more trials, on average, if they were previously rewarded for that choice,
487 compared to the other subgroups. In addition to finding a weak effect through mechanisms of
488 striatal dopamine, we have observed that the interaction between drug administration and
489 COMT predicted switching behaviour. Specifically, estradiol administration attenuated
490 naturally occurring differences in switching observed in the placebo group. While Val/Val
491 placebo subjects switched least and significantly less compared to Met/Met placebo subjects,
492 this difference disappeared in the estradiol group. The switching and staying effects depending
493 on both the COMT and DAT1 polymorphisms were also supported by analyses of choice
494 autocorrelation, and revealed a comparable pattern to the one described, but also enabled us
495 to better understand the effect of choices several trials ago on the current choice.

496   Finding an effect on switching that is moderated by individual variation in COMT

497   provides, for the first time, evidence for the hypothesis that estradiol has a causal role in frontal

498   dopamine-mediaton[16]. This likely happens due to the inhibition of COMT activity through

499   estradiol metabolites that leads to increased dopamine availability [5,13]. This means that

500   estradiol does not interact only with striatal dopamine levels but also with frontal dopamine

501   levels. Our effect establishes a set of causal findings for future work to replicate and build

502   upon.

503   Finally, because we predicted that increased reward sensitivity would occur due to

504   larger striatal reward prediction errors because of estradiol administration, we hypothesised

505   that this would be reflected in increased learning rates, as compared to placebo. This

506   demonstrates that estradiol increased the weight of new information relative to old information.

507   These effects are consistent with predictions by previous imaging work who found increased

508   reward sensitivity in high estradiol conditions[6]. This is furthermore supported by decreased

509   choice autocorrelation in subjects who were administered with estradiol compared to placebo.

510   However, the effect of estradiol on learning rates was not moderated by the COMT or DAT1

511   polymorphism, in contrast to our predictions. Similarly to our interpretation for accuracy and

512   staying behaviour reported below, it is likely that our sample size was not sufficiently large to

513   detect difference at the polymorphism subgroup level. To the best of our knowledge, this is

514   the first examination of behavioural differences through computational modelling as a function

515   of estradiol administration and polymorphisms of dopamine genes in men. The results provide

516   grounding for future work that may benefit by incorporating a computational approach to

517   elucidate the observable behavioural changes following estradiol administration. Framing

518   behavioural effects through a computational framework would allow future work to compare

519   their findings with our work and findings about other hormones, e.g. testosterone [44,45].

520   Through the behavioural and genetic measures we collected, we were also able to

521   exclude a substantial number of other candidate mechanisms that could have driven some of

522   the effects we observed. These include androgen receptor functioning (polyglutamine (CAG)

523 and polyglycine (GGN) repeats), differences in androgen to estrogen conversion (CYP 19A1)

524 or estrogen receptor functioning (ERα, ERβ), which have been previously unaddressed (see

525 Supplementary Materials). Furthermore, we were able to exclude confounding measures that

526 are known to influence estradiol metabolism upon administration such as changes in self-

527 reported mood and attention due to drug administration, individual differences in

528 impulsiveness, behavioural approach and inhibition, working memory performance assessed

529 via an N-back task, salivary cortisol levels, and differences in body measurements (weight,

530 height, BMI, abdominal and visceral fat) (see Supplementary Materials).

531 The current study also encountered some limitations. Based on these, we provide

532 recommendations for future work employing pharmacogenetics with estradiol.

533 The first is related to increasing sample size. While our sample size was approximately

534 twice as large compared to most previous work [8,16,31,33,37,43,46,47], for one exception see [32]) and

535 in line with suggestions for the field [2], we suspect that we were underpowered to detect all

536 effects of interest at COMT and DAT1 polymorphism level. The reason for this is that we

537 observed several "trend-level" p-values ($p < 0.1$) for which we had strong theoretical

538 predictions. Specifically, this refers to not finding a clear interaction between estradiol

539 administration with both DAT1 and COMT on accuracy [27,33]. In addition, we would have

540 predicted an interaction between estradiol administration with DAT1 on staying, because of

541 increased accuracy. The interaction with COMT on switching behaviour similarly needs

542 replication. Moreover, because previous administration studies did not compute behavioural

543 effect sizes that could have served as a basis for our current work, except of the general

544 recommendation in [2], it was difficult to estimate the minimal viable sample size. Due to general

545 power issues in this field of research, larger sample sizes are required and starting to be used

546 also in other psychoneuroendocrinological work [44,45].

547 The second recommendation relates to the type of reinforcement learning task used.

548 For future research we would suggest using a reversal learning task [20,48] with parametrically

549 changing reward probability contingencies. Based on our findings, we predict that such a task

550 could elucidate more clearly the effect of estradiol on behaviour. Namely, the trials where we

551 observed the clearest effect (e.g. trials around 400) is where the largest probability reversals

552 happened. If our prediction is true, it should also more clearly show improved learning and

553 accuracy compared to the Gaussian random walks employed here. An alternative idea for

554 future work is to use the two-step task [49] which would enable to further disentangle both model-

555 free and model-based behaviour and reveal how variation in COMT and DAT1 moderates the

556 influence of administration. We would predict estradiol to have similar effects as found by other

557 work using dopamine precursors where administration increased model-based learning [50].

558 Our third recommendation is related to dose-dependent effects of estradiol

559 administration. In [31], the authors concluded they may have elicited overstimulation (12 mg) of

560 dopaminergic transmission, while our results (2 mg) show similarity to a low dose of a

561 dopamine precursor due to contrasting results with [27]. An extension through a dose-dependent

562 investigation of choice behaviour would show whether this is true for reward processing

563 similarly to dose-dependent observations in [40,41] and further contribute to the understanding of

564 estradiol in relation to the inverted U-shape hypothesis [35].

565 The final recommendation is to include additional genotypes that may moderate the

566 influence of estradiol on behaviour (e.g. the Taq1A variant in the dopamine D2 receptor gene).

567 This would enable to better disentangle the contribution of different dopamine-related genes

568 [21,24]. Alternatively, neurochemical positron emission tomography as in [20] with estradiol

569 administration would provide a better understanding at the level of receptor binding and show

570 to which degree these effects relate to dopaminergic circuitry in prefrontal and striatal regions.

571 In conclusion, we have shown that estradiol causally influences choice behaviour by

572 altering reward processing. The observed effects were specifically moderated by frontal

573 (COMT) and striatal (DAT) dopamine-related genes but not estrogen and androgen-related

574 genes (CAG, GGN, CYP 19A1, ERα, ERβ). Our results converge with experimental evidence

575 from rodent work that showed amplified striatal dopamine D1 signalling in high estradiol

576 conditions. Moreover, they confirm the prediction that estradiol has a role in frontal dopamine

577 signalling through the COMT polymorphism [5,13,16]. Finally, our behavioural results were

578 supported by computational modelling showing that estradiol causally increased learning

579 rates, supporting the hypothesis that increased reward prediction errors may have driven the

580 increased reward sensitivity

581   In sum, our study shows the importance of using more complex research designs that

582 are supported by causal work from animal models and correlational human studies. Combining

583 predictions from both and augmenting the hypotheses with pharmacogenetics allows us to

584 elucidate the interactions between hormones, neurotransmitter systems, and cognition, both

585 on a mechanistic, behavioural, and computational level. Such an approach has important

586 implications for a better understanding of the biology and neuroscience of human cognition

587 that is moderated by genes in both health and disorder.

588

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Conflict of interests

595 The authors declare no potential conflicts of interest with respect to the research, authorship,

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598 Healthcare regarding clinical research using PET/MR. He is a shareholder of BM Health

599 GmbH since 2019.

600

25

Methods and materials

Subjects

One hundred healthy young males between 19 and 34 years (M_{age} = 24.86, SD = 3.53) participated in the study. We only included men in this study as the employed administration procedure was previously validated on a sample of health young men. Therefore, these results are only representative for the male population and need replication in women as well. All subjects had a body mass index (BMI) between 19.3 and 31.5 (M = 24.45, SD = 2.86). We screened potential subjects for the presence or a history of psychiatric disorders, self-reported weight and height, concurrent involvement in other studies with pharmacological agents, and presence of a chronic physical injury that might have prevented them from participation in a longer experiment. The short version of e-MINI [51] was used to screen and exclude those who had a non-diagnosed, disclosed, or a diagnosed psychiatric disorder. The screening procedure and the sample size estimate were based on previous work for which we obtained pharmacokinetic data for a single 2 mg estradiol dose in topical form [34]. Subjects were recruited through social media, web portals, and flyers on university premises. All subjects provided written informed consent and were financially compensated for the completion of the experiment (50€) and received an additional maximum bonus of 40€ (range 7€ – 30€) based on their performance in the all the tasks. The procedure described was performed in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of the University of Vienna (1918/2015).

Measurement Instruments

Questionnaires

We used a battery of questionnaires to assess self-reported mood (German Multidimensial Mood State Questionnaire; [52], individuals' impulsiveness (Barratt Impulsiveness Scale, BIS-

26

626   11; [53]), and reward responsiveness (BIS/BAS; [54]), to test for changes after estradiol

627   administration and ensure there were no interindividual differences between both groups, as

628   previously both BIS/BAS and BIS-11 scores have been found to correlate with reward learning

629   [55–58] (see Supplementary Materials). In addition, we probed subjects' beliefs and confidence

630   about estradiol (e.g. whether they believed they received estradiol or a placebo, how certain

631   they were of this answer, and whether they noticed any changes). This was done to later

632   regress out the potential contribution of beliefs arising, for example, from subjects researching

633   potential side effects of the hormone prior the experiment. Namely, individuals' beliefs about

634   having received the hormone and beliefs about the effects of the hormone on their

635   performance have previously shown to modulate behaviour independent of whether subjects

636   had received the hormone [59].

637

638   **Hormone concentrations**

639   We collected hormone samples via passive drool and stored them at -30 degrees Celsius.

640   Saliva samples were analyzed for estrone and estradiol using gas chromatography tandem

641   mass spectrometry (GC-MS/MS) and hydrocortisone including testosterone with liquid

642   chromatography tandem mass spectrometry (LCMS/MS) (see Supplementary Materials for

643   details of procedure).

644

645   **Genotyping**

646   We collected DNA using sterile cotton buccal swabs (Sarstedt AG, Germany) and extracted it

647   by applying the QIAamp DNA Mini kit (Qiagen, Germany). Repeat length polymorphisms

648   (AR(CAG), AR(GGN), DAT1(VNTR), ERα(TA) and ERβ(CA)) were investigated by PCR with

649   fluorescent-dye-labeled primers and capillary electrophoresis. The single base primer

650   extension (SBE) method also known as minisequencing was applied for the typing of single

651   nucleotide polymorphism (SNP) variants (Val158Met) in the COMT gene (see Supplementary

652   Materials for details of procedure).

653

654   **Experimental Tasks**

655   For each task, we gave subjects paper instructions including control questions to check

656   whether all subjects understood the instructions. All tasks except for the N-BACK task were

657   monetarily incentivised.

658   **Working memory capacity:** We assessed working memory capacity using an adapted

659   version of the standard N-BACK task [16]. In our version we added a 1-BACK condition, creating

660   four conditions in total (i.e. a 0-BACK, 1-BACK, 2- BACK, 3-BACK). One condition block had

661   20 trials which included 20% target, 65% nontarget, and 15% lure trials. Subjects were

662   presented with a sequence of letters one-by-one. For each letter, they had to decide if the current

663   letter was the same as the one presented *N* trials ago by pressing "R", in case it was not the

664   same they had to press "O". For example, in the 3-back condition, the letter sequence "*A B D A*

665   *A*" would require subjects to press "R" only to the second occurrence of A, as this was the same

666   letter as the one 3 trials ago. The last A in this example sequence is defined as a lure trial, while

667   the other letters were nontarget trials. Lure trials were present only in the 2-BACK and 3-BACK

668   conditions as in [16], and while lure trials were added to keep the task consistent with their

669   implementation, we did not further analyse them separately as they were not relevant for our

670   question. In total, there were four blocks per condition. Each block was announced by an

671   instruction lasting for 2 sec (Fig. 1A), a fixation cross (1 sec) and a sequence of 20 trials. Each

672   trial was presented for 1 sec with a 1 sec feedback phase and a 1 sec inter-stimulus interval.

673   After every 20 trials, subjects had a 3 sec resting period, before the next block was announced.

674   A lack of response to any cue was considered a miss.

675   **Reinforcement Learning:** We employed a probabilistic reinforcement learning task [19] to

676   investigate differences in choice behaviour based on the hypothesized altered reward

28

677 processing. The task consisted of 500 trials, with a 10 second pause after the first 250 trials.

678 Prior to this, subjects performed 10 practice trials with two initial options, which were changed

679 before the main trials. We did this to avoid carry-over effects from practice to the main task.

680 Throughout the task subjects were exposed to the same set of two options with independently

681 varying reward probabilities. We informed subjects that it was possible that both options could

682 be correct (i.e. rewarding) or incorrect (i.e. non-rewarding) on any given trial, as the reward

683 probability of one option was independent of the other and vice versa. As shown in Fig. 1,

684 each trial included three stages: (1) a cue onset stage (5 sec) where subjects had to decide

685 between the two options and press the corresponding key. If they did not respond within that

686 time frame, they would see a warning message indicating they should respond and try to be

687 faster next time; (2) a choice feedback stage (1 s) where subjects received information about

688 both the chosen (thick frame) and unchosen (thin frame) option (yellow - correct, red - wrong);

689 and (3) an inter-trial interval ($M = 1.5$ s, jittered between 0.9 to 2.1 s). Each correct choice was

690 rewarded with 5 eurocents and added to their cumulative balance. To amplify the association

691 between their performance and earnings, subjects saw a yellow bar filling up incrementally

692 with each correct response. Each time the bar was completely filled, a 1 € coin was presented

693 next to the bar indicating they had gained 1 € to their cumulative balance.

694

695 **Procedure**

696 We asked potential candidates to fill out an online survey with screening questions probing for

697 exclusion criteria described in *Subjects*. Following this, we screened them for the general

698 exclusion criteria. We invited suitable candidates to two separate test sessions. They were

699 scheduled to occur with a maximal difference of one week to prevent major changes in weight

700 and/or other bodily measures.

701   The first session always took place at 4.00 pm. We first provided subjects general

702 information about the study procedure, after which subjects provided written informed consent

703 and filled out a battery of questionnaires. Moreover, we assessed their height, weight,

704 abdominal, and visceral fat. These metrics were included as they could impact estradiol

705 metabolization, and therefore, we included them as nuisance regressors in our linear models

706 [60,61]. Twenty minutes after arrival, subjects provided a saliva sample. At the end of the session,

707 we obtained a small amount of blood from the finger on a Micro FTA card and a buccal swab

708 for genotyping.

709   On the second test day (see timeline, Fig. 1, bottom panel) we gave subjects general

710 instructions and information regarding the day. After subjects provided informed consent, they

711 filled out a mood (MDBF-A scale) and impulsiveness (BIS-11) questionnaire. We obtained a

712 first saliva sample (T1, 20 minutes after arrival) to assess baseline hormone concentrations.

713 This was followed by the N-BACK task which we used to assess their baseline working

714 memory performance. Following the N-BACK, subjects applied a topical transparent gel on

715 their chest and shoulders that either contained 2 mg of estradiol (Divigel, Orion Pharma AG,

716 Zug Switzerland) or a placebo. They were randomly assigned estradiol or placebo in a double-

717 blind manner. A male experimenter was present to ensure that the subjects applied the gel

718 correctly. After gel application, we waited for two hours to allow estradiol levels to peak based

719 on our previously established procedure [34]. During this time subjects could read magazines

720 available in the room or books they brought with them. Fifteen minutes prior to the behavioural

721 testing, we required them to fill out a second mood (MDBF-B scale) and impulsiveness (BIS-

722 11) questionnaire followed by a second saliva sample (T2).

723   The behavioural testing commenced two hours after administration of the drug. The

724 first task was the probabilistic reinforcement learning task which contained a block of practice

725 trials to familiarise subjects with the task setup. After they completed the reinforcement

726 learning task, three other decision-making tasks that were not the focus of this publication

727 followed. After the behavioural testing, we probed subjects' beliefs about the treatment and

728 the tasks. At the end of the study, each participant was paid in accordance to their

729 performance.

730

Analysis of behaviour

Statistical analysis of behaviour

For the reinforcement learning task, we first looked at the cumulative difference in response proportions between the estradiol and placebo group. That is, we first computed the relative response probability for each group. This value tells us what percentage of subjects from the estradiol/placebo group chose one of the two options (e.g. option A, Fig. 2A). For the relative response probability, we also computed the corresponding standard errors of the mean which gave us a group-level probability and confidence estimate for choosing, e.g. option A, on each trial. We then subtracted the mean and both the lower and upper bound of the standard error of the mean between both groups for each trial. This gave us a difference in the expected chosen option for each trial that reflected how strong the groups differed in the probability of choosing, e.g. option A. Because we were interested in the absolute difference (i.e. we were not interested in the sign of the difference), we took the absolute value on a per trial basis and computed the cumulative choice difference from this which is presented in Fig. 2B.

To quantify statistical significance for this metric, on each trial we shuffled the responses of subjects and therefore decoupled labels from responses to build a null distribution that would tell us what kind of difference would be expected by chance. By shuffling responses on each trial, we took a more conservative approach to a permutation test when compared to shuffling responses within and across trials as it preserves systematic variance across trials in terms of subjects' choice. We then generated a null distribution of 2000 iterations where for each iteration we computed the cumulative choice difference between two random groups that would be expected by chance. From these cumulative difference traces, we took the 100th percentile of the null distribution for each trial (null distribution in Fig. 2B). This value shows the maximum possible cumulative value that would have been expected by chance (i.e. by two random groups). Therefore, values that exceed this null distribution cannot

756   be attributable due to chance. Namely, if estradiol administration would not have impacted

757   choice behaviour systematically, then cumulatively the difference between the actual estradiol

758   and placebo group would not surpass the threshold of the null distribution.

759   We also computed this metric by averaging across trials. This gave us a measure of

760   the average percentage in choice difference that was cumulative across trials. That is, on

761   average, how strongly estradiol influenced choice difference. As above, we also did the same

762   to the corresponding null distribution to observe whether the obtained empirical percentage

763   exceeded the null distribution showing us what would have been expected by chance.

764   Similarly, we employed two-sample proportion z-tests which tests for whether the

765   proportion of successes from one group is statistically different from the proportion of

766   successes in the other group. These tests were not performed on the relative response

767   probabilities but on the raw responses. That is, we tested whether the number of subjects who

768   chose option A in one group was statistically significantly different from the other group. We

769   repeated this test on every trial to determine on what percentage of trials there was a

770   statistically significant difference between both groups.

771   As a measure of family-wise error control and to ensure that the values we observed

772   were not due to chance, but due to estradiol administration, we shuffled the responses from

773   subjects for each trial 2000 times and thereby decoupled responses from the labels. This

774   yielded a null distribution that showed on what percentage of trials we could expect to find a

775   statistically significant difference between two random groups with intact response variance

776   across trials. By intact response variance we mean that on some trials, both groups were more

777   likely to select one or the other option. Therefore, if we had also shuffled across trials and

778   subjects, it would have been possible to invoke a larger number of false positives in our null

779   distribution (i.e. lower percentages of trials with a statistically significant difference between

780   both random groups). In short, for each permutation test we obtained a percentage reflecting

781   the number of trials with a statistically significant difference in response proportions between

782   two random groups that would have been obtained by chance.

783   For all cases where we computed a null distribution, we computed z-scores as

784   measures of standardized effect size, as in [62]. We obtained a z-score by subtracting from the

785   quantity of interest the mean of the null distribution and dividing it by the standard deviation of

786   the null distribution. From this, we were able to use the Fisher-z-transformation to determine

787   statistical significance.

788   Next, we computed accuracy, defined as the proportion of responses where the option

789   with higher probability of reward was chosen. We collapsed this value across time (Fig. 2C).

790   We computed two additional metrics. The first metric was a measure of switching behaviour;

791   the number of trials where the chosen option on trial t and the one chosen at $t + 1$ were

792   different. The second metric quantified how many trials on average would subjects stay with

793   the same option on subsequent trials if they were rewarded for the same option on trial t. We

794   used this metric as a measure of staying behaviour.

795   Accuracy, reaction times, switching, and staying were statistically evaluated with

796   general linear models where the first model always included a predictor for drug administration

797   (estradiol, placebo). For all models we subsequently included interaction terms for the

798   polymorphisms of genes of interest. Unless explicitly mentioned in the main result section, all

799   reported linear models regressed out z-scored nuissance regressors. These included cortisol

800   levels following administration, beliefs about the drug (see Belief Probes), and body

801   measurement characteristics (weight, BMI, abdominal and visceral fat). Weight and BMI were

802   summed together to generate a composite score [63] because of their high intrinsic correlation

803   ($r = 0.89$). (See also Supplementary Materials: Selecting linear models). General linear models

804   for accuracy also included z-scored reaction times to control for accuracy-speed trade-offs.

805   In addition, we analysed choice autocorrelation (see Supplementary Materials: Impact

806   of previous choice on current choice). In brief, for each participant we computed the relative

807   contribution of choices made from $t - 1$ to $t - 7$ trials back (lags) on current choice. The

808   obtained regression weights indicated how strong the relative influence of individual trials on

809   the current choice was. We performed this both for choice as a function of previous choice

810   (pure choice autocorrelation) and choice as a function of previous rewarded choice (choice

811   autocorrelation as a function of reward). We then performed independent samples Welch *t-*

812   tests on individual lags to assess statistical significance.

813   To control for the variance of random effects such as subjects themselves, we used

814   generalized linear mixed effects models that do not require data aggregation [39,64]. In two

815   separate sets of analyses, we investigated whether treatment group (estradiol, placebo)

816   interacted with the $val^{158}met$ polymorphism of the COMT gene or with the VNTR polymorphism

817   of the DAT1 gene across trials. We fitted separate models, as the sample size per smallest

818   cell was too small otherwise (Table S6, Supplementary Materials). We ran these models using

819   R (version 3.6.0 R Development Core Team, 2019), with the lme4 package [64]. Our simplest

820   model included only an intercept and a random effects structure which included subject-level

821   intercepts. We used a likelihood ratio test to determine whether including group as a fixed

822   factor improved the model fit. From there we fitted separate models for the VNTR

823   polymorphism of the DAT1 gene and the $val^{158}met$ polymorphism of the COMT gene. In both

824   cases, the starting model had a fixed effect interaction between group (estradiol, placebo) and

825   gene (either COMT or DAT1) and subject-level intercepts as random effects. From this model

826   we incrementally increased the complexity of our model until the most complex one. The most

827   complex model was identical for both the VNTR and $val^{158}met$ polymorphism. The model

828   included a three-way interaction between group (estradiol, placebo), gene (COMT or DAT1)

829   and time (trial number). This was our main measure of interest and the one for which we

830   hypothesized effects – that estradiol administration would differentially influence choice as the

831   task progressed, depending on subjects' genotype. The random effect structure for this model

832   included random intercepts for each subject. All models were estimated using the "nloptwrap"

833   optimizer. Models without convergence or singularity warnings were then compared with

834   likelihood ratio tests. We used BIC [65] to pick the winning model but also inspected their AIC

835   [66] and deviance scores for converging information. Below we report the two winning models;

836   both models were identical, except for the polymorphism:

837
$$y = choice \sim group * COMT * trial + (1|subject)$$
1

838 In the case of DAT1, the winning model was:

839
$$y = choice \sim group * DAT1 * trial + (1|subject)$$
2

840

841 **Computational modelling**

842 A canonical approach to estimate subjects' learning is afforded by reinforcement learning. To

843 test if subjects in the estradiol group would behave differently compared to the placebo group,

844 because of increased striatal prediction errors, we formalized behaviour within a reinforcement

845 learning framework and fitted several Q-learning models [67] with softmax choice rules:

846 Q-learning model (equation 3):

847
$$Q_{t+1}^A = Q_t^A + \alpha(R_t^A - Q_t^A)$$
(3)

848 Softmax choice rule (equation 4):

849
$$p = \frac{1}{1 + e^{\tau(Q_t^A - Q_t^B)}}$$
(4)

850 Where, t is time, A is option A, Q is subjective value, α is the learning rate, R is the obtained

851 reward, and τ is the temperature parameter. Equations 3 and 4 represent our first model

852 (model 1). In Q-learning, the basic idea is that agents learn subjective values for actions in

853 their environment. Subjective values are learned and updated through a value function

854 (Equation 3) following feedback after each action. A teaching signal known as the learning

855 rate-weighted prediction error dictates how strongly the subjective value will be updated on

856 each action. The prediction error corresponds to the difference between the obtained and

857 expected reward (i.e. the subjective value prior to making the new choice). Within this process,

858 the learning rate dictates how heavily new information will be weighted in proportion to

859 previous information about the option, and therefore how strongly the subjective value will

860 change from its current estimate. The softmax equation then yields the probability of selecting

35

861 an action given the learning rate and the temperature parameter, which reflects stochasticity

862 of choice behaviour.

863 By employing computational modelling of this sort, we were able to obtain parameter

864 estimates that quantify the difference in subjects' behaviour which we predicted. Our main

865 hypothesis was that estradiol would increase reward sensitivity which should be captured by

866 the learning rate, but not influence choice stochasticity across trials.

867 To obtain a more precise account of the effect of estradiol on reward processing, we

868 extended the basic Q-learning model in several ways, as described below.

869 The first extension (model 2, equation 5a and 5b) allowed for separate learning rates for Q_A

870 and Q_B, because subjects were able to track the outcome of both the chosen and unchosen

871 option.

872 $$Q_{t+1}^A \ = \ Q_t^A + \alpha_A(R_t^A \ - \ Q_t^A) \tag{5a}$$

873

874 $$Q_{t+1}^B \ = \ Q_t^B + \alpha_B(R_t^B \ - \ Q_t^B) \tag{5b}$$

875 Furthermore, due to reward stochasticity of our n-armed bandit implementation (obtained by

876 a Gaussian random walk – Fig. 1B), we added an additional parameter ξ, representing

877 irreducible noise [68] in our perceptual model (model 3, equation 6):

878 $$p \ = \ \frac{1}{1 \ + \ e^{\tau(Q_t^A \ - \ Q_t^B)}}(1-\xi) + \frac{\xi}{2} \tag{6}$$

879 Finally, we added a perseverance parameter λ [69] to the response model (model 4, equation

880 7):

881 $$p \ = \ \frac{1}{1 \ + \ e^{\tau(Q_t^A \ - \ Q_t^B + \lambda C)}}(1-\xi) + \frac{\xi}{2} \tag{7}$$

882 Where C = 1, if the same cue was chosen on trial *n* and trial *n+1*, and C = -1 if the converse

883 was true. In summary, our full model space had separate learning rates for two separate

884   options, a choice stochasticity, and irreducible noise parameter. All other models were

885   reduced cases of this model and all possible combinations of the described free parameters

886   therefore yielded eight models in total for which we estimated parameters. The model fitting

887   was performed using JAGS and the rjags (v 4.9) package in R (v 3.6.0). Each model was run

888   with 5000 samples each with 1000 burn-in samples on three chains. Priors over parameters

889   and hyperparameters were set to default as described in [70]. We computed the leave one out

890   information criterion using the loo package [71] and used this metric to compare the models.

891   Furthermore, we performed Bayesian model comparison by computing the (protected)

892   exceedance probability [72] using the VBA toolbox [73] to determine the best model and compare

893   its congruency with the LOOIC measure. Finally, we extracted the posterior predictive density

894   for each participant as a measure of predictive power of the best model. This was then

895   compared to the actual behaviour as a measure of static (accuracy collapsed across time) and

896   dynamic (accuracy at each trial across subjects) predictive accuracy.

897

References

899   1.   Schultz, W., Dayan, P. & Montague, P. R. A neural substrate of prediction and reward.

900   *Science (80-.).* **275**, 1593–1599 (1997).

901   2.   Diekhof, E. K. Estradiol and the reward system in humans. *Curr. Opin. Behav. Sci.* **23**,

902   58–64 (2018).

903   3.   Saldanha, C. J., Remage-Healey, L. & Schlinger, B. A. Synaptocrine signaling:

904   Steroid synthesis and action at the synapse. *Endocr. Rev.* **32**, 532–549 (2011).

905   4.   Luine, V. N. Estradiol and cognitive function: Past, present and future. *Horm. Behav.*

906   **66**, 602–618 (2014).

907   5.   Colzato, L. S. & Hommel, B. Effects of estrogen on higher-order cognitive functions in

908   unstressed human females may depend on individual variation in dopamine baseline

909   levels. *Front. Neurosci.* **8**, 65 (2014).

910   6.   Thomas, J., Météreau, E., Déchaud, H., Pugeat, M. & Dreher, J. Hormonal treatment

911   increases the response of the reward system at the menopause transition : A

912   counterbalanced randomized placebo-controlled fMRI study.

913   *Psychoneuroendocrinology* **50**, 167–180 (2014).

914   7.   Dreher, J. *et al.* Menstrual cycle phase modulates reward-related neural function in

915   women. *PNAS* **104**, 2465–2470 (2007).

916   8.   Diekhof, E. K. & Ratnayake, M. Menstrual cycle phase modulates reward sensitivity

917   and performance monitoring in young women: Preliminary fMRI evidence.

918   *Neuropsychologia* **84**, 70–80 (2016).

919   9.   Lévesque, D. & Di Paolo, T. Rapid conversion of high into low striatal D2-dopamine

920   receptor agonist binding states after an acute physiological dose of 17β-estradiol.

921   *Neurosci. Lett.* **88**, 113–118 (1988).

922   10.   Becker, J. B. Gender Differences in Dopaminergic Function in Striatum and Nucleus

923   Accumbens. *Pharmacol. Biochem. Behav.* **64**, 803–812 (1999).

924   11.   Becker, J. B. Direct effect of 17β-estradiol on striatum: Sex differences in dopamine

925   release. *Synapse* **5**, 157–164 (1990).

926   12.   Pasqualini, C., Olivier, V., Guibert, B., Frain, O. & Leviel, V. Acute Stimulatory Effect

927   of Estradiol on Striatal Dopamine Synthesis. *J. Neurochem.* **65**, 1651–1657 (1995).

928   13.   Ball, P., Knuppen, R., Haupt, M. & Breuer, H. Interactions Between Estrogens and

929   Cateehol Amines III. Studies on the Methylation of Catechol Estrogens, Catechol

930   Amines and other Catechols by the Catechol-O-Methyltransferase of Human Liver. *J.*

931   *Clin. Endocrinol. Metab.* **34**, 736–746 (1972).

932   14.   Yoest, K. E., Quigley, J. A. & Becker, J. B. Rapid effects of ovarian hormones in

933   dorsal striatum and nucleus accumbens. *Horm. Behav.* **104**, 119–129 (2018).

934   15.   Männistö, P. T. & Kaakkola, S. Catechol-O-methyltransferase (COMT): Biochemistry,

935   molecular biology, pharmacology, and clinical efficacy of the new selective COMT

936   inhibitors. *Pharmacol. Rev.* **51**, 593–628 (1999).

937   16.   Jacobs, E. & D'Esposito, M. Estrogen Shapes Dopamine-Dependent Cognitive

938   Processes: Implications for Women's Health. *J. Neurosci.* **31**, 5286–5293 (2011).

939   17.   Schultz, W., Stauffer, R. W. & Lak, A. The phasic dopamine signal maturing: from

940   reward via behavioural activation to formal economic utility. *Curr. Opin. Neurobiol.* **43**,

941   139–148 (2017).

942   18.   Glimcher, P. W. Understanding dopamine and reinforcement learning: the dopamine

943   reward prediction error hypothesis. *PNAS* **108**, 15647–54 (2011).

944   19.   Frank, M. J., Seeberger, L. C. & O'Reilly, R. C. By carrot or by stick: Cognitive

945   reinforcement learning in Parkinsonism. *Science (80-.).* **306**, 1940–1943 (2004).

946   20.   Cools, R. *et al.* Striatal Dopamine Predicts Outcome-Specific Reversal Learning and

947   Its Sensitivity to Dopaminergic Drug Administration. *J. Neurosci.* **29**, 1538–1543

948   (2009).

949   21.   den Ouden, H. E. M. *et al.* Dissociable Effects of Dopamine and Serotonin on

950   Reversal Learning. *Neuron* **80**, 1090–1100 (2013).

951   22.   Jocham, G., Klein, T. A. & Ullsperger, M. Dopamine-Mediated Reinforcement

952   Learning Signals in the Striatum and Ventromedial Prefrontal Cortex Underlie Value-

953   Based Choices. *J. Neurosci.* **31**, 1606–1613 (2011).

954   23.   Jocham, G., Klein, T. a & Ullsperger, M. Differential Modulation of Reinforcement

955   Learning by D2 Dopamine and NMDA Glutamate Receptor Antagonism. *J. Neurosci.*

956   **34**, 13151–13162 (2014).

957   24.   Eisenegger, C. *et al.* Role of dopamine D2 receptors in human reinforcement learning.

958   *Neuropsychopharmacology* **39**, 2366–2375 (2014).

959 25. Swart, J. C. *et al.* Catecholaminergic challenge uncovers distinct Pavlovian and

960   instrumental mechanisms of motivated (in)action. *Elife* **6**, 1–36 (2017).

961 26. Frank, M. J. *et al.* Genetic triple dissociation reveals multiple roles for dopamine in

962   reinforcement learning. *PNAS* **104**, 16311–16316 (2007).

963 27. Eisenegger, C. *et al.* DAT1 Polymorphism Determines L-DOPA Effects on Learning

964   about Others ' Prosociality. *PLoS One* **8**, e67820 (2013).

965 28. Pessiglione, M., Seymour, B., Flandin, G., Dolan, R. J. & Frith, C. D. Dopamine-

966   dependent prediction errors underpin reward-seeking behaviour in humans. *Nature*

967   **442**, 1042–1045 (2006).

968 29. Doll, B. B., Bath, K. G., Daw, N. D. & Frank, M. J. Variability in dopamine genes

969   dissociates model-based and model-free reinforcement learning. *J. Neurosci.* **36**,

970   1211–1222 (2016).

971 30. Nemmi, F. *et al.* Interaction between striatal volume and DAT1 polymorphism predicts

972   working memory development during adolescence. *Dev. Cogn. Neurosci.* **30**, 191–

973   199 (2018).

974 31. Sommer, T. *et al.* Effects of the experimental administration of oral estrogen on

975   prefrontal functions in healthy young women. *Psychopharmacology (Berl).* **235**, 3465–

976   3477 (2018).

977 32. Bayer, J., Gläscher, J., Finsterbusch, J., Schulte, L. H. & Sommer, T. Linear and

978   inverted U-shaped dose-response functions describe estrogen effects on

979   hippocampal activity in young women. *Nat. Commun.* **9**, 1–12 (2018).

980 33. Jakob, K., Ehrentreich, H., Holtfrerich, S. K. C., Reimers, L. & Diekhof, E. K. DAT1-

981   genotype and menstrual cycle, but not hormonal contraception, modulate

982   reinforcement learning: Preliminary evidence. *Front. Endocrinol. (Lausanne).* **9**, 60

983   (2018).

984   34.   Eisenegger, C., von Eckardstein, A., Fehr, E. & von Eckardstein, S. Pharmacokinetics

985   of testosterone and estradiol gel preparations in healthy young men.

986   *Psychoneuroendocrinology* **38**, 171–178 (2013).

987   35.   Cools, R. & D'Esposito, M. Inverted-U-shaped dopamine actions on human working

988   memory and cognitive control. *Biol. Psychiatry* **69**, 113–125 (2011).

989   36.   Daw, N. D., Doherty, J. P. O., Dayan, P., Seymour, B. & Dolan, R. J. Cortical

990   substrates for exploratory decisions in humans. *Nature* **441**, 876–879 (2006).

991   37.   Reimers, L., Büchel, C. & Diekhof, E. K. How to be patient. The ability to wait for a

992   reward depends on menstrual cycle phase and feedback-related activity. *Front.*

993   *Neurosci.* **8**, 401 (2014).

994   38.   Baayen, R. H., Davidson, D. J. & Bates, D. M. Mixed-effects modeling with crossed

995   random effects for subjects and items. *J. Mem. Lang.* **59**, 390–412 (2008).

996   39.   Bolker, B. M. *et al.* Generalized linear mixed models: a practical guide for ecology and

997   evolution. *Trends in Ecology and Evolution* vol. 24 127–135 (2009).

998   40.   Chowdhury, R. *et al.* Dopamine restores reward prediction errors in old age. *Nat.*

999   *Neurosci.* **16**, 648–653 (2013).

1000 41.   Chowdhury, R., Guitart-Masip, M., Bunzeck, N., Dolan, R. J. & Düzel, E. Dopamine

1001   modulates episodic memory persistence in old age. *J. Neurosci.* **32**, 14193–14204

1002   (2012).

1003 42.   Smith, C. T., Sierra, Y., Oppler, S. H. & Boettiger, C. A. Ovarian Cycle Effects on

1004   Immediate Reward Selection Bias in Humans: A Role for Estradiol. *J. Neurosci.* **34**,

1005   5468–5476 (2014).

1006 43.   Diekhof, E. K. Be quick about it. Endogenous estradiol level, menstrual cycle phase

1007   and trait impulsiveness predict impulsive choice in the context of reward acquisition.

1008   *Horm. Behav.* **74**, 186–193 (2015).

44. Geniole, S. N. *et al.* Using a Psychopharmacogenetic Approach To Identify the Pathways Through Which—and the People for Whom—Testosterone Promotes Aggression. *Psychol. Sci.* **30**, 481–494 (2019).

45. Losecaat Vermeer, A. B. *et al.* Exogenous testosterone increases status-seeking motivation in men with unstable low social status. *Psychoneuroendocrinology* **113**, (2020).

46. Colzato, L. S., Hertsig, G. & Wildenberg, van den Hommel, B. Estrogen modulates inhibitory control in healthy human females: evidence from the stop-signal paradigm. *Neuroscience* **167**, 709–715 (2010).

47. Colzato, L. S., Pratt, J. & Hommel, B. Estrogen modulates inhibition of return in healthy human females. *Neuropsychologia* **50**, 98–103 (2012).

48. Schaaf, M. E. Van Der, Fallon, S. J., Huurne, N., Buitelaar, J. & Cools, R. Working Memory Capacity Predicts Effects of Methylphenidate on Reversal Learning. *Neuropsychopharmacology* **38**, 2011–2018 (2013).

49. Daw, N. D., Gershman, S. J., Seymour, B., Dayan, P. & Dolan, R. J. Model-based influences on humans' choices and striatal prediction errors. *Neuron* **69**, 1204–1215 (2011).

50. Wunderlich, K., Smittenaar, P. & Dolan, R. J. Dopamine Enhances Model-Based over Model-Free Choice Behavior. *Neuron* **75**, 418–424 (2012).

51. Sheehan, D. V. *et al.* The validity of the Mini International Neuropsychiatric Interview (MINI) according to the SCID-P and its reliability. *Eur. Psychiatry* **12**, 232–241 (1997).

52. Steyer, R., Schwenkmezger, P., Notz, P. & Eid, M. Testtheoretische Analysen des Mehrdimensionalen Befindlichkeitsfragebogen. *Diagnostica* **40**, 320–328 (1994).

53. Patton, J. H., Stanford, M. S. & Barratt, E. S. Factor structure of the barratt impulsiveness scale. *J. Clin. Psychol.* **51**, 768–774 (1995).

54. Carver, Charles, S. & White, Teri, L. Behavioral Inhibition, Behavioral Activation, and Affective Responses to Impending Reward and Punishment: The BIS/BAS Scales. *J. Pers. Soc. Psychol.* **67**, 319–333 (1994).

55. Kim, S. H., Yoon, H. S., Kim, H. & Hamann, S. Individual differences in sensitivity to reward and punishment and neural activity during reward and avoidance learning. *Soc. Cogn. Affect. Neurosci.* **10**, 1219–1227 (2014).

56. Sali, A. W., Anderson, B. A. & Yantis, S. Reinforcement learning modulates the stability of cognitive control settings for object selection. *Front. Integr. Neurosci.* **7**, 95 (2013).

57. Unger, K., Heintz, S. & Kray, J. Punishment sensitivity modulates the processing of negative feedback but not error-induced learning. *Front. Hum. Neurosci.* **6**, 186 (2012).

58. Lighthall, N. R., Gorlick, M. A., Schoeke, A., Frank, M. J. & Mather, M. Stress Modulates Reinforcement Learning in Younger and Older Adults. *Psychol Aging* **28**, 35–46 (2013).

59. Eisenegger, C., Naef, M., Snozzi, R., Heinrichs, M. & Fehr, E. Prejudice and truth about the effect of testosterone on human bargaining behaviour. *Nature* **463**, 356–359 (2010).

60. Fishman, J., Boyar, R. M. & Hellman, L. Influence of body weight on estradiol metabolism in young women. *J. Clin. Endocrinol. Metab.* **41**, 989–991 (1975).

61. Schneider, J. *et al.* Effects of Obesity on Estradiol Metabolism: Decreased Formation of Nonuterotropic Metabolites. *J. Clin. Endocrinol. Metab.* **56**, 973–978 (1983).

62. Maidenbaum, S., Miller, J., Stein, J. M. & Jacobs, J. Grid-like hexadirectional modulation of human entorhinal theta oscillations. *PNAS* **115**, 10798–10803 (2018).

63. Aeberli, I., Molinari, L. & Zimmermann, M. B. A composite score combining waist

1059   circumference and body mass index more accurately predicts body fat percentage in

1060   6- to 13-year-old children. *Eur. J. Nutr.* **52**, 247–253 (2013).

1061   64.   Bates, D., Mächler, M., Bolker, B. M. & Walker, S. C. Fitting linear mixed-effects

1062   models using lme4. *J. Stat. Softw.* **67**, (2015).

1063   65.   Schwarz, G. Estimating the Dimension of a Model. *Ann. Stat.* **6**, 461–464 (1978).

1064   66.   Akaike, H. A New Look at the Statistical Model Identification. *IEEE Trans. Automat.*

1065   *Contr.* **19**, 716–723 (1974).

1066   67.   Watkins, C. J. C. H. & Dayan, P. Q-learning. *Mach. Learn.* **8**, 279–292 (1992).

1067   68.   de Boer, L. *et al.* Dorsal striatal dopamine D1 receptor availability predicts an

1068   instrumental bias in action learning. *PNAS* **116**, 261–270 (2019).

1069   69.   Rutledge, R. B. *et al.* Dopaminergic Drugs Modulate Learning Rates and

1070   Perseveration in Parkinson ' s Patients in a Dynamic Foraging Task. *J. Neurosci.* **29**,

1071   15104–15114 (2009).

1072   70.   Ahn, W.-Y., Haines, N. & Zhang, L. Revealing Neurocomputational Mechanisms of

1073   Reinforcement Learning and Decision-Making With the hBayesDM Package. *Comput.*

1074   *Psychiatry* **1**, 24–57 (2017).

1075   71.   Vehtari, A., Gelman, A. & Gabry, J. Practical Bayesian model evaluation using leave-

1076   one-out cross-validation and WAIC. *Stat. Comput.* **27**, 1413–1432 (2017).

1077   72.   Rigoux, L., Stephan, K. E., Friston, K. J. & Daunizeau, J. Bayesian model selection for

1078   group studies - Revisited. *Neuroimage* **84**, 971–985 (2014).

1079   73.   Daunizeau, J., Adam, V. & Rigoux, L. VBA: A Probabilistic Treatment of Nonlinear

1080   Models for Neurobiological and Behavioural Data. *PLoS Comput. Biol.* **10**, (2014).

1081   74.   Longcope, C., Kato, T. & Horton, R. Conversion of blood androgens to estrogens in

1082   normal adult men and women. *J. Clin. Invest.* **48**, 2191–2201 (1969).

1083 75. Yaffe, K. *et al.* Androgen receptor CAG repeat polymorphism is associated with

1084   cognitive function in older men. *Biol. Psychiatry* **54**, 943–946 (2003).

1085 76. Beyenburg, S. *et al.* Androgen receptor mRNA expression in the human

1086   hippocampus. *Neurosci. Lett.* **294**, 25–28 (2000).

1087 77. Kovacs, D. *et al.* The androgen receptor gene polyglycine repeat polymorphism is

1088   associated with memory performance in healthy Chinese individuals.

1089   *Psychoneuroendocrinology* **34**, 947–952 (2009).

1090 78. Comings, D. E., Chen, C., Wu, S. & Muhleman, D. Association of the androgen

1091   receptor gene (AR) with ADHD and conduct disorder. *Neuroreport* **10**, 1589–1592

1092   (1999).

1093 79. Gillies, G. E. & McArthur, S. Estrogen actions in the brain and the basis for differential

1094   action in men and women: A case for sex-specific medicines. *Pharmacol. Rev.* **62**,

1095   155–198 (2010).

1096 80. Bayer, J. *et al.* Estrogen and the male hippocampus: Genetic variation in the

1097   aromatase gene predicting serum estrogen is associated with hippocampal gray

1098   matter volume in men. *Hippocampus* **23**, 117–121 (2013).

1099 81. Kravitz, H. M., Meyer, P. M., Seeman, T. E., Greendale, G. A. & Sowers, M. F. R.

1100   Cognitive Functioning and Sex Steroid Hormone Gene Polymorphisms in Women at

1101   Midlife. *Am. J. Med.* **119**, 94–102 (2006).

1102 82. Ma, S. L. *et al.* Polymorphisms of the estrogen receptor (ESR1) gene and the risk of

1103   Alzheimer's disease in a southern Chinese community. *Int. Psychogeriatrics* **21**, 977–

1104   986 (2009).

1105 83. Almey, A., Milner, T. A. & Brake, W. G. Estrogen receptors in the central nervous

1106   system and their implication for dopamine-dependent cognition in females. *Horm.*

1107   *Behav.* **74**, 125–138 (2015).

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Supplementary Materials:

A causal role for estradiol in human reinforcement learning

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Methods

Genotyping

DNA extraction and quantification

Buccal swabs were collected using sterile cotton swabs (Sarstedt AG, Germany). DNA was extracted from swabs using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) and eluted in a final volume of 50 µL of QIAamp buffer AE (Qiagen). Human nuclear DNA was quantified using the Applied Biosystems (AB) 7500 real-time PCR instrument (Thermo Fisher Scientific, Waltham, MA) and the Quantifiler Human Plus quantification Kit (AB) following manufacturer's recommendations.

Typing of repeat length polymorphisms

Genomic DNA fragments that contain polymorphic repeat sequences were amplified in two separate reactions: i.e. a multiplex PCR (simultaneously targeting AR(CAG)n, DAT1 VNTR, ERα(TA)n and ERβ(CA)n) and a singleplex PCR (targeting solely AR(GGN)n), respectively.

The multiplex PCR was performed using 5 ng template DNA in a reaction mix (total volume of 25 µL) consisting of 1 × GeneAmp PCR buffer (AB), 0.25 mM each dNTP, 2.5 units AmpliTaq Gold polymerase (AB) and target specific primers (AR(CAG), DAT1, ERα and Erβ; including 5'-fluorescent-dye-labeled forward primers; details provided in

1134   Table 1). The following protocol was applied using the Veriti 96-well thermal cycler (AB):

1135   35 cycles at 95 °C for 30 seconds, 55 °C for 1 minute, and 72 °C for 1 minute. Before

1136   the first cycle, an initial denaturation (95 °C for 5 minutes) was included, and the last

1137   cycle was followed by a final extension step at 72 °C for 45 minutes.

1138   The singleplex PCR was conducted using 5 ng template DNA in a reaction mix

1139   (total volume of 20 µL) containing target specific primers (AR(GGN)n, details provided

1140   in Table 1)), 0.5 µL Phire Hot Start II DNA polymerase (Thermo Fisher) in 1 × Phire

1141   reaction buffer (Thermo Fisher). Amplification was carried out on the Veriti thermal

1142   cycler (AB) and included an initial denaturation step at 98 °C for 30 seconds, followed

1143   by 33 cycles of 10 seconds at 98 °C, 30 seconds at 60 °C and 30 seconds at 72 °C.

1144   The last cycle was followed by a final extension at 72 °C for 10 minutes.

1145   Aliquots of PCR products were diluted with Hi-Di formamide (AB), mixed with

1146   internal lane standard LIZ 600 v.2 (AB) and separated on the ABI 3500 Genetic

1147   Analyzer applying standard conditions. The number of repeats predicted by the

1148   GeneMapper ID-X software (AB) was in full agreement to the actual repeats determined

1149   by direct sequencing of PCR products using the BigDye Terminator Sequencing Kit

1150   v3.1 (AB) in selected DNA samples.

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Table SI. Panel of loci and primer sets used for the typing of repeat length polymorphisms

Marker	Location[b]	Primer sequence 5'-3' [c]	Dye	Orientation	Conc. (nM)[d]
AR(GGN)n[a]	chrX:67546447-67546603(+)	CCTGGCACACTCTCTTCACA	VIC	forward	625
		*GTTTCT*GGCCGAGTGTAGCCGTAG		reverse	
AR(CAG)n	chrX:67545237-67545434(+)	CGCGAAGTGATCCAGAACC	6-FAM	forward	200
		*GTTTCT*AGAACCATCCTCACCCTGCT		reverse	
DAT1 VNTR	chr5:1393559-1394008(-)	TGTGGTGTAGGGAACGGCCTGAGA	6-FAM	forward	400
		TGTTGGTCTGCAGGCTGCCTGCAT		reverse	
ERα(TA)n	chr6:151806472-151806594(+)	AACTATCCAAGATTATAGACGCATGA	NED	forward	600
		*GTTTCT*AACATGCACACGCACATACA		reverse	
Erβ(CA)n	chr14:64253529-64253650(-)	GTGCTGCGAGCAGAGATA	PET	forward	800
		*GTTTCT*AATGAGTGGGCCTCCCTTAG		reverse	

[a] AR(GGN)n primers only used in singleplex PCR; all other primers combined in a multiplex PCR
[b] Chromosome number and genomic location of targeted sequence (orientation provided in brackets) according to UCSC version hg38 (http://genome.ucsc.edu/)
[c] The non-specific primer tail is underlined in Italics
[d] The final primer concentrations in the reaction mix

Typing of the COMT Val158Met polymorphism

SNaPshot minisequencing was applied for the typing of Val158Met variants in the COMT gene. Therefore, a 177 bp fragment of genomic DNA harbouring the causative single nucleotide polymorphism (SNP rs4680) in its centre was amplified by PCR. The reaction mix comprised 5 ng template DNA, 1 × GeneAmp PCR buffer (AB), 0.25 mM each dNTP, 2.5 units AmpliTaq Gold polymerase (AB) and target specific primers (details provided in Table 2) in a total reaction volume of 25 µL. Thermal cycling was performed applying the Veriti cycler (AB) and conditions as follows: 95 °C for 5 min; 35 cycles of 95 °C for 15 seconds, 59 °C for 30 seconds and 72 °C for 1 minute; final extension at 72 °C for 5 minutes.

1179 **Table S2. Primer set used for PCR of the COMT fragment**

Marker	Location[a]	Primer sequence 5'-3' [b]	Orientation	Conc. (nM)[c]
COMT	chr22:19963623-19963799(+)	GGGCCTACTGTGGCTACTCA	forward	400
		GCCCTTTTTCCAGGTCTGA	reverse	

1180
1181 [a] Chromosome number and genomic location of targeted sequence (orientation provided in brackets) according to UCSC version hg38 (http://genome.ucsc.edu/).

1182

1183 PCR products were purified from excess primers and dNTPs by ExoSAP-IT (Thermo

1184 Fisher) treatment following manufacturer's recommendations. Minisequencing was

1185 conducted on a Veriti thermal cycler (AB) in a total volume of 10 µL containing 3 µL of

1186 purified PCR product, 5 µL SNaPshot Multiplex Ready Reaction mix (Thermo Fisher)

1187 and 2 µL minisequencing primer (2 µM; details see Table 3). The cycling conditions (25

1188 cycles) were as follows: denaturation at 96 °C for 10 seconds, annealing at 50 °C for 5

1189 seconds and extension at 60 °C for 30 seconds.

1190

1191 **Table S3. Minisequencing primer information**

SNP sequence variation	Location[a]	Primer sequence 5'-3' [b]	Orientation
G>A	chr22:19963728-19963747	*(GATC)₄* GGATGGTGGATTTCGCTGGC	forward

1192
1193
1194
1195
1196 [a] Chromosome number and genomic location of primer binding site (orientation provided in brackets) according to UCSC version hg38 (http://genome.ucsc.edu/). The targeted SNP rs4680 is located at position chr22:19963748.
[b] The non-specific primer tail is underlined in Italics

1197 ExoSAP-IT treatment was again applied for the clean-up of the minisequencing

1198 reaction. 5 µl of purified minisequencing reaction product was then mixed with 9.3 µL

1199 Hi-Di formamide (AB) and 0.2 µL of GeneScan-LIZ 120 internal size standard (AB).

1200 After a denaturing step for 5 min at 98 °C followed by cooling to 4 °C the fragments

1201 were separated on an ABI PRISM 310 Genetic Analyzer (AB) with POP4 polymer and

1202 analysed with GeneMapper v3.2 software. Calling of SNP variants based on

1203 minisequencing was in full agreement to results from direct sequencing of PCR

1204 products in selected DNA samples.

1205

1206 **Hormone concentrations**

1207 Quantification of estrone and estradiol in saliva samples was performed with

1208 derivatization using pentafluorobenzoyl chloride (PFBCl) and the addition of the

1209 isotopically labeled internal standards estrone-d_4 and estradiol-d_5. Organic saliva was

1210 reacted with 1.0 mL 1% PFBCl and 0.1 mL pyridine at 60°C for 30 min. The

1211 derivatization agents were evaporated, the sample was reconstituted with 0.5 mL

1212 NaHCO3 and extracted with 1 mL n-hexane. The organic phase was substituted with

1213 0.2 mL dodecane and subjected to optimized GC-MS/MS analysis using an Agilent

1214 7890 GC with Agilent DB-17ht 15 m x 0.25 mm x 0.15 µm capillary column connected

1215 to an Agilent 7010 tandem mass spectrometer operated in MRM mode using negative

1216 chemical ionization at 150°C with methane as a reaction gas (40%, 2 mL/min). Method

1217 validation was performed using ion transition m/z 464 -> 400 as a quantifier for estrone

1218 and m/z 660 -> 596 for estradiol, whereas a LLOQ of 1.92 fg o.c. and 1.94 fg was

1219 obtained, respectively.

1220 Quantification of hydrocortisone and testosterone in saliva samples was

1221 performed using liquid chromatography tandem mass spectrometry (LCMS/MS), with

1222 an Agilent 6460 with electrospray ionization in positive mode coupled to a 1290 UHPLC

1223 system. Collision energy was optimized for specific MRM transitions of Hydrocortisone

1224 (363.2/121.1 m/z; 363.2/91.1 m/z), Testosterone (289.2/109.1; 289.2/97.1 m/z), 2,3,4-

1225 13C3-Hydrocortisone (366.2/124 m/z) and 2,3,4-13C3-Testosterone (292.2/100 m/z).

1226 Agilent Poroshell 120 EC-C18 was used for chromatographic separation under

1227 reversed phase conditions. The internal standard preparation and internal standard

1228 mixture was prepared containing 2,3,4-13C3-Hydrocortisone; 2,3,4-13C3-

1229 Testosterone, 2,4,16,16,17-d5-17b-Estradiol and concentration of 5ng/mL each.

51

1230   Samples were prepared by adding 100 µl internal standards (5 ng/mL) to 500µl plasma

1231   or saliva and the steroids were extracted using 4 mL MTBE. After 10 min. overhead

1232   shacking, the samples were centrifuged for 5 min. at 3000 rpm and the top MTBE layer

1233   was transferred to a test tube. MTBE was evaporated using a centrivap concentrator at

1234   40°C (Labconco). The residual sample was then re-dissolved in methanol and analyzed

1235   by LC-MS/MS.

1236

1237   **Questionnaires**

1238   ***Mood*:** To control for a potential confound of mood, tiredness, or alertness from the

1239   treatment affecting subjects' performance [24], we assessed participants' self-reported

1240   mood before and after administration of the treatment, using the German Multidimensial

1241   Mood State Questionnaire ("Der Mehrdimensionale Befindlichskeitfragebogen - MDBF)

1242   [52] Both versions of this questionnaire (A and B) contain 12 items with a 5-level Likert

1243   scale and three subscales that test for different continuums of mood (Good-Bad [α_{pre} =

1244   .81, α_{post} = .77], Awake-Tired [α_{pre} = .84, α_{post} = .87], Calm-Nervous [α_{pre} = .73, α_{post} =

1245   .75]).

1246   ***Impulsiveness*:** We used the Barratt Impulsiveness Scale (BIS-11; [53] to measure

1247   participants' impulsiveness as [43] observed that variations in estradiol levels differentially

1248   affected women with low trait as opposed to high trait impulsiveness. BIS-11 is a widely

1249   used measure for impulsiveness with 30 items describing common behaviour and

1250   preferences related to (non)impulsiveness which individuals have to rate on a 4-point

1251   scale (1 - rarely/never, almost always/always - 4). The General Impulsiveness (α_{pre} =

1252   .71, α_{post} = .75) factor together with its three second-order factors (Motor Impulsiveness

1253   (α_{pre} = .47, α_{post} = .54) Nonplanning Impulsiveness (α_{pre} = .6, α_{post} = .63), Attentional

1254   Impulsiveness (α_{pre} = .49, α_{post} = .52) are reported.

1255   ***Behavioural inhibition and activation*:** we measured the trait behavioural activation

1256   and inhibition with the Behavioural inhibiton/Behavioural Activation Scales (BIS/BAS;

1257   [54]. The BAS scale is a 24-item questionnaire answered on a four-level scale (1- very

1258   true for me, 4 - very false for me). It is subdivided into Drive (α= .74), Fun Seeking (α=

1259   .67), and Reward Responsiveness (α= .6) while the BIS scale (α= .77) is

1260   unidimensional. Drive is thought to measure the persistent pursuit of goals (e.g. "I go

1261   out of my way to get the things I want"), Fun Seeking: the desire for new rewards and

1262   willingness to approach events that would be potentially rewarding (e.g. "I crave

1263   excitement and new sensations"), while Reward Responsiveness focuses on positive

1264   responses that would occur if a reward is anticipated (e.g. "When I am doing well at

1265   something I love to keep doing it"). Finally, the BIS scale measures sensitivity to

1266   negative events (e.g. "Criticism or scolding hurts me quite a bit").

1267

1268   **Belief probes**

1269   In addition, we probed participants' beliefs and confidence about estradiol (e.g. whether

1270   they believed they received estradiol or a placebo, how certain they were of this answer,

1271   and whether they noticed any changes). This was done to later regress out the potential

1272   contribution of beliefs arising, for example, from participants researching potential side

1273   effects of the hormone prior the experiment. Namely, individuals' beliefs about having

1274   received the hormone and beliefs about the effects of the hormone on their performance

1275   have previously shown to modulate behaviour independent of whether participants had

1276   received the hormone [59].

1277

1278   **Matching of both groups**

1279   We compared both treatment groups for age and other bodily characteristics (i.e. BMI,

1280   height, weight, visceral, and abdominal fat) and potential differences in self-reported

1281   mood (MDBF), impulsiveness (BIS-11) and reward responsiveness (BIS/BAS) (see

1282   Questionnaires, Table S4 and S5). We used two-tailed independent samples Welch t-

1283   tests, or Wilcoxon signed-rank test if assumptions of normality were not met, to test

1284   whether the groups matched on all variables. To test for mood differences after

1285   administration between the treatment groups, we performed an ANCOVA for each of

1286   the three subscales of the MDBF questionnaire where we controlled for baseline mood

1287   scores. Two-way ANOVAs were further performed on the individual subscales of the

1288   BIS-11 questionnaire to investigate whether there was an interaction between the group

1289   (estradiol, placebo) and session (pre, post) on impulsiveness.

1290   To compare working memory capacity assessed by the N-BACK task, we

1291   analyzed target accuracy, reaction times, and d-prime. We analyzed this with an

1292   ANOVA containing the between-subject variable group (estradiol, placebo) and within-

1293   subject variable for condition together with an interaction term for group and condition.

1294

Results

1296   **Matching of both groups**

1297   In the first part of the supplementary results, Table S4 and S5 show that our random

1298   assignment was successful as the groups did not differ in any of the measured

1299   parameters before (Table S4) administration and as a function of administration (Table

1300   S5). However, we did observe the expected change in estradiol metabolite

1301   concentrations in the estradiol group, outlined below.

1302

1303   **Hormone concentrations**

1304   We observed a statistically significant post-administration difference between both

1305   groups in log-transformed estradiol concentrations (W = 1545, 95% CI [0.03, 1.87], p <

1306   .05) with the estradiol group having higher estradiol metabolite concentration following

1307   administration (estradiol: Mdn = 41.77 ±531.54), placebo: Mdn = 5.55 ±230.23) but not

54

1308   before (estradiol: *Mdn* = 3.38 ±230.97), placebo: Mdn = 1.89 ±21.92) compared to the

1309   placebo group (*W* = 1498, 95% CI [-0.05, 1.03], *p* = .09). We report the median for the

1310   values above because even after log-transforming the metabolite concentrations, they

1311   were not distributed normally. Because of this a mean would not have been a good

1312   measure of central tendency. Importantly, because we have observed high

1313   interindividual variance in estradiol concentrations prior to administration, we have

1314   reason to believe the obtained metabolite concentrations were contaminated during the

1315   handling of the samples following our data collection. Namely, in previous work such

1316   baseline variation was not observed despite an identical procedure and dosage with

1317   the main difference being that serum levels of estradiol were measured there [34]. Log-

1318   transformed estrone and cortisol concentrations after administration were also

1319   examined showing no differences between both groups. Estrone: (experimental: *Mdn* =

1320   8.79 ±4226.69), control: *Mdn* = 5.80 ±161.99) (*W* = 1427, 95% CI [-0.17, 1.05], *p* = .16),

1321   cortisol: (experimental: *Mdn* = 0.77 ±0.94), control: Mdn = 0.73 ±1.15) (*W* = 1207, 95%

1322   CI [-0.31, 0.27], *p* = .90).

1323

1324   **Bodily measures and behavioural characteristics**

1325   As outlined in Table S4, both the estradiol and placebo group were also matched for

1326   their weight, height, BMI, visceral, abdominal fat, and individual sub scales of the

1327   BIS/BAS questionnaire (Drive, Reward, Fun-Seeking, Behavioural Inhibition). Similarly,

1328   separate one-way ANOVAs revealed no interaction for the four subscales of BIS-11

1329   (Table S5) (General: $F_{(1, 195)}$ = 0.01, *p* = 0.91, Attentional: $F_{(1, 195)}$ = 0.04, *p* = .85, Motor:

1330 $F_{(1, 195)}$ = 0.59, *p* = .45, nonplanning: $F_{(1, 195)}$ = 0.08, *p* = .78).

1331   Furthermore, we ensured that both the estradiol and placebo group did not differ

1332   in pre-existing differences in working memory (Figure S2A, S2B, S2C) in addition to

1333   testing whether administration influenced mood (Figure S2D). By doing so we were able

1334   to exclude differences in working memory and mood leading to the observed results

1335   [27,48]. Separate ANCOVAs for the three subscales (Alertness, Mood, Calmness) of the

1336   MDBF revealed no differences in post-administration (Post) scores between the

1337   estradiol and placebo group when controlling for baseline scores (Pre) as a covariate

1338   (Mood: $F_{(1,\ 96)}$ = 0.30, p = 0.58, Ω^2 = 0.08; Alertness: $F_{(1,\ 96)}$ = 1.35, p = .25, Ω^2 = 0.01;

1339   Calmness: $F_{(1,\ 96)}$ = 1.34, p = .25, Ω^2 = 0.01). Similarly, we observed no interaction

1340   between group membership and post-administration score (Mood: $F_{(1,\ 96)}$ = 0.06, p = .81,

1341 Ω^2 = 0.01; Alertness: $F_{(1,\ 96)}$ = 1.88, p = .17, Ω^2 = 0.01; Calmness: $F_{(1,\ 96)}$ = 1.55, p

1342   = .22, Ω^2 = 0.01).

1343

1344   **Table S4: Descriptive statistics by treatment (Estradiol, Placebo).**

	Group				
	Estradiol	**Placebo**	***n***	**statistic [95% CI]**	***p***
Age (years)	25.12 (3.63)	24.6 (3.44)	100	1381 [-0.99, 1.49][1]	0.99
BMI	24.54 (2.65)	24.35 (3.08)	99	1286 [-0.99, 1.99][1]	0.99
Height (cm)	181.90 (6.88)	180.40 (5.95)	99	1.16 [-1.07, 4.07]	0.94
Weight (kg)	81.09 (9.66)	79.48 (11.44)	99	0.76 [-2.61, 5.83]	0.99
Visc. Fat (%)	6.20 (2.48)	6.06 (2.90)	98	1248 [-0.99, 1.00][1]	0.99
Abd. Fat (%)	20.66 (5.97)	19.74 (6.32)	98	0.74 [-1.55, 3.38]	0.99
BIS/BAS					
BIS	17.54 (2.80)	18.40 (3.68)	100	-1.32 [-1.86, -0.25]	0.88
Drive	11.72 (1.77)	12.12 (2.32)	100	1074.5	0.91
Reward	11.62 (1.99)	12.46 (2.04)	100	921[1]	0.18
Fun Seeking	15.88 (2.00)	16.08 (2.17)	100	-0.48 [-1.03, 0.63]	0.99

1345 Note: Values in cells denote M, parentheses denote SD. The superscript 1 denotes the Mann-Whitney-
1346 Wilcoxon W value. For the remaining group comparisons, two-tailed independent samples Welch *t*-tests
1347 were employed. In cases where *n* is not equal to *N* = 100, data was not recorded for that particular variable.
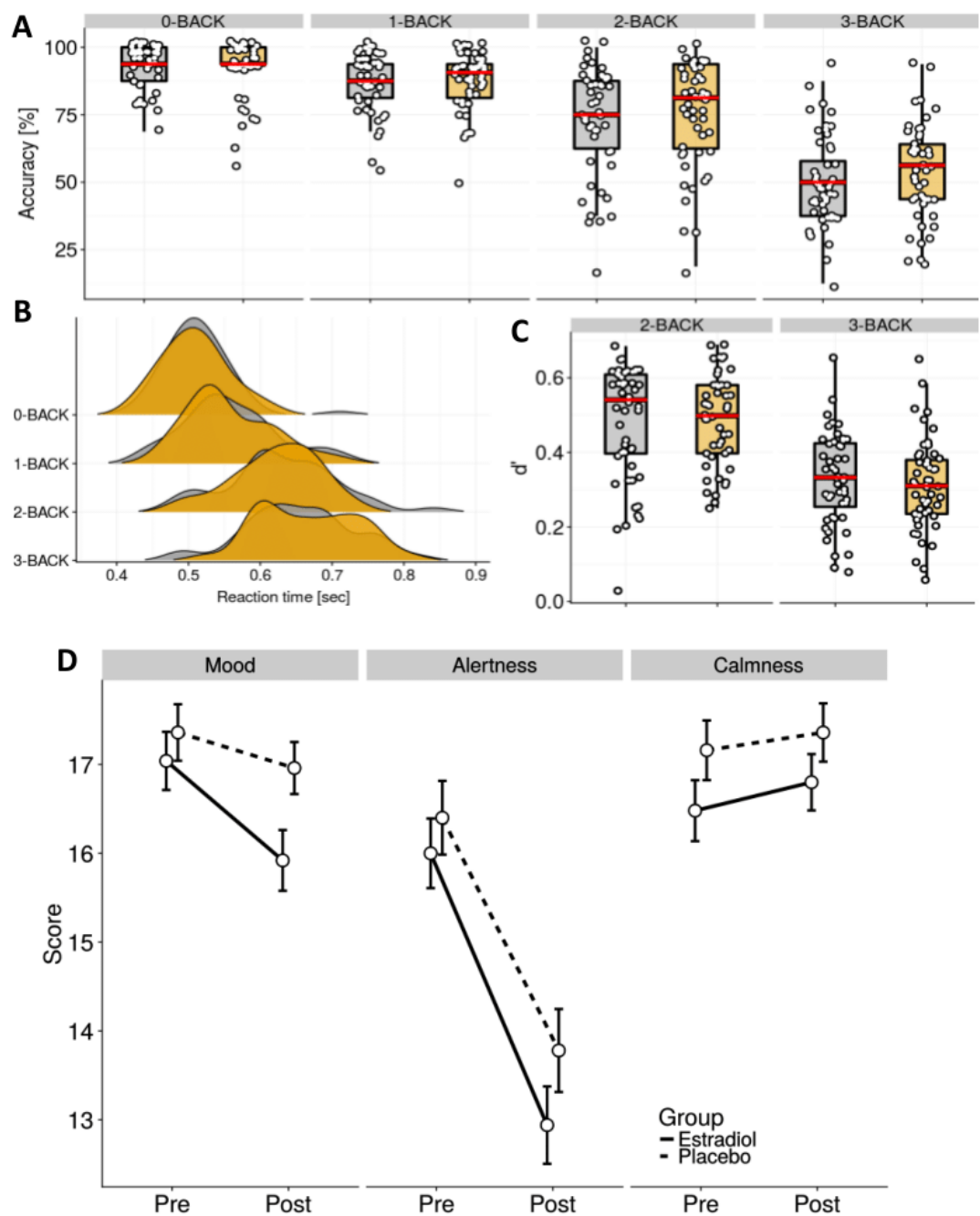1348 p-values are Bonferroni corrected.
1349

Figure S1. A) Accuracy for individual conditions. The red bar represents the median, the box plot represents the 75% middle most data points, with the whiskers representing 1.95*IQR. Orange depicts the estradiol and gray the placebo group. That is, they represent the division of subjects according to whether they would subsequently be allocated to the estradiol or placebo group. This color convention is used throughout all figures. **B**) shows density plots for reaction time data for individual conditions. **C**) shows d' in the most difficult two conditions (2-BACK, 3-BACK) as there were no false alarms in the 0-BACK and 1-BACK, thus accuracy is reduced to d'. **D**) Average scores prior and post administration for the three subscales of the MDBF.

1362   Furthermore, our working memory (N-BACK) task revealed a comparable

1363   picture for accuracy (Figure S2A), reaction times (Figure S2B), and d-prime (Figure

1364   S2C). That is, there was no statistically significant difference between the estradiol and

1365   placebo group in accuracy, average reaction times, and d-prime. We did observe an

1366   expected drop in performance in terms of decreased accuracy (0-BACK: 92.94 ±9.34,

1367   1-BACK: 88.06 ±10.78, 2-BACK: 74.25 ±19.38, 3-BACK: 51.56 ±17.37), and d-prime

1368   (2-BACK: 0.48 ±0.14, 3-BACK: 0.32 ±0.12), and increased reaction times (0-BACK:

1369   0.51 ±0.05, 1-BACK: 0.56 ±0.06, 2-BACK: 0.63 ±0.07, 3-BACK: 0.66 ±0.07) as the

1370   condition became more difficult (i.e. went from 0-BACK to 3-BACK). Separate linear

1371   models were used to compute to check for main effects of drug ($F_{(1, 196)}$ = 2.01, p

1372   = .16, Ω^2 = 0.00) and an interactive effect of drug and condition on d-prime ($F_{(1, 196)}$ =

1373   0.82, p = .37, Ω^2 = 0.00). As mentioned above, we also did this for accuracy (main

1374   effect of drug: $F_{(1, 392)}$ = 1.07, p = .30, Ω^2 = 0.00; drug*condition interaction: $F_{(3, 392)}$ =

1375   2.30, p = .08, Ω^2 = 0.00), and reaction times (main effect: $F_{(1, 347)}$ = 1.31, p = .25, Ω^2 =

1376   0.00; drug*condition interaction: $F_{(1, 347)}$ = 0.99, p = .39, Ω^2 = 0.00).

1377

1378   **Table S5. Descriptive statistics of MDBF and BIS-11 subscales.**

		Pre-administration		Post-administration	
	N	**Estradiol**	**Placebo**	**Estradiol**	**Placebo**
MDBF					
Mood	100	17.04 (2.31)	17.36 (2.25)	15.92 (2.41)	16.96 (2.07)
Alertness	100	16.00 (2.77)	16.40 (2.93)	12.94 (3.08)	13.78 (3.30)
Calmness	100	16.48 (2.43)	17.16 (2.38)	16.80 (2.24)	17.36 (2.32)
BIS-11					
General	100	57.86 (7.68)	59.28 (7.45)	59.96 (8.51)	60.46 (7.86)
Motor	100	20.76 (3.00)	21.78 (3.42)	22.02 (3.42)	22.42 (3.65)
Attention	100	14.34 (3.37)	14.14 (2.17)	15.10 (3.30)	14.64 (2.68)
Nonplanning	100	22.76 (3.61)	23.36 (4.29)	22.84 (4.05)	23.40 (4.21)

1379   Note: values in cells denote *M*, values in parentheses denote *SD*.

1380

1381   In summary, both groups were matched on working memory and post-

1382   administration mood scores. They were additionally matched for age, height, visceral

1383   and abdominal fat, BMI, BIS-BAS, and impulsivity (BIS-11). The estradiol group had

1384   higher estradiol concentrations after but not before administration compared to the

1385   placebo group. Importantly, there was no correlation between subjects' belief about

1386   whether they had received estradiol or placebo and actually receiving estradiol ($r = 0.02$,

1387 $p = .82$), the certainty of that belief and actually receiving estradiol ($r = 0.02$, $p = .82$),

1388   or between the reported observed changes and actually receiving estradiol ($r = -0.08$,

1389 $p = .42$). This shows that our double-blind procedure worked and that our placebo gel

1390   preparation was indistinguishable from the actual drug. Overall, the described results

1391   show that our administration procedure was successful and both groups were matched

1392   on key traits that could have potentially impacted the observed behaviour. This allowed

1393   us to constrain the number of possible alternative explanations of our main results.

1394

1395   **Table S6. Frequencies of individual polymorphisms of DAT and COMT genes.**

Polymorphism	Group	N
9/10	Estradiol	18
9/10	Placebo	16
10/10	Estradiol	21
10/10	Placebo	26
Val/Val	Estradiol	11
Val/Val	Placebo	9
Met/Val	Estradiol	23
Met/Val	Placebo	26
Met/Met	Estradiol	12
Met/Met	Placebo	15

1396   Note: the split according to both COMT and DAT does not sum to 100 because for a few subjects it was
1397   not possible to determine their polymorphism.
1398

1399   **Reinforcement learning task**

1400   **Selecting linear models**

1401   For all general linear models assessing interactions described in our results, we started

1402   with the simplest model which included our interaction of interest (either drug*COMT or

1403   drug*DAT) and regressed out the belief of having received the drug. We considered

59

1404   this belief as a nuisance regressor because of our previous work showing the impact of

1405   beliefs about a hormone on subsequent behaviour [59]. Additional nuisance regressors

1406   included bodily measures known to impact estradiol metabolism which we collected:

1407   weight, BMI, abdominal and visceral fat [60,61] and post-administration cortisol levels [58].

1408   All linear models were compared with BIC and AIC. Unless stated otherwise in the main

1409   text, for all reported results the winning model regressed out cortisol levels following

1410   administration, beliefs about having received the drug, the certainty of that belief and

1411   whether they had observed any changes in themselves, a composite score of weight

1412   and BMI (main text), visceral, and abdominal fat. For general linear models involving

1413   accuracy, we also regressed out reaction times to control for accuracy-speed trade-

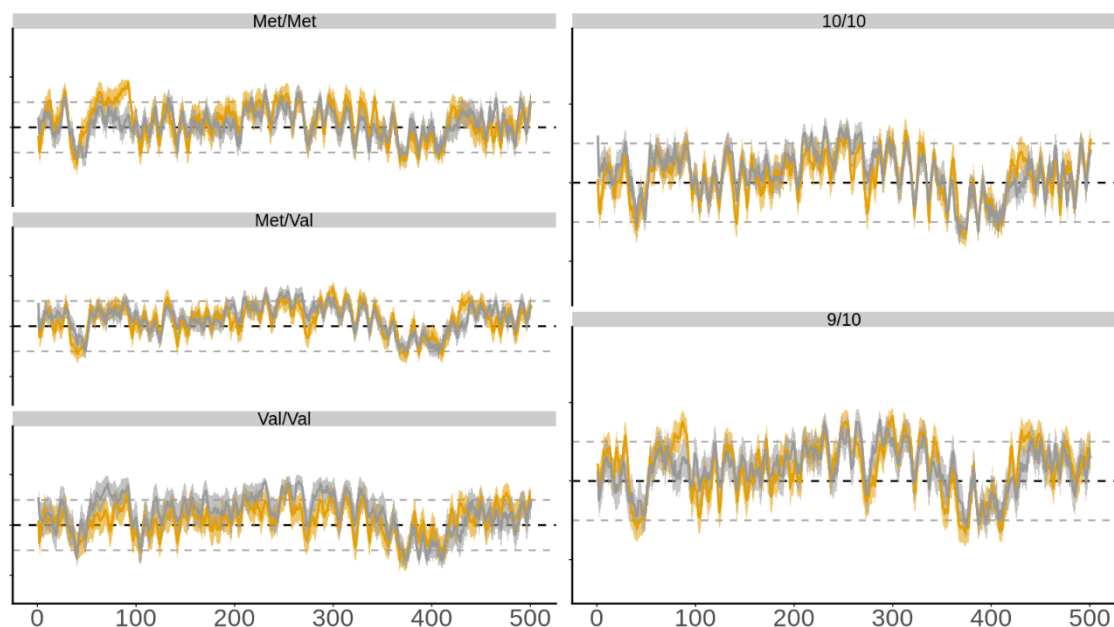1414   offs. All nuisance regressors were z-scored.

1415

1416

1417 **Figure S2.** Relative choice probability for choosing option A (top of y-axis) vs. choosing stimulus 1 (bottom
1418 of y-axis) for the placebo (gray) and estradiol (orange) group split according to both polymorphisms
1419 assessed in the main text: COMT (left panel), DAT (right panel) across trials (1-500). Thick lines represent
1420 trial means, shaded areas denote standard error of the means. The blue line in the background denotes
1421 the empirical relative reward probability which was computed from the probability of stimulus two being
1422 rewarding (top of y-axis) - stimulus one being rewarding (bottom of y-axis). Gray dotted lines represent
1423 where participants were on average 25% more likely to select option A (upper line) or stimulus 1 (lower
1424 line). All time-series traces are smoothed with a 5-trial moving average for visual purposes.

1425

1426   Figure S2 reveals a differential effect of estradiol administration on choice

1427   behaviour that depends on polymorphisms of both COMT and DAT. In the case of the

1428   COMT polymorphism this is most clearly visible in the lower left panel. The panel shows

1429   that placebo Val/Val subjects exhibited a clear tendency towards stimulus two until trial

1430   ~370. After this, they did not reverse back towards choosing it more often despite

1431   stimulus two being more rewarding from trial ~420 onwards. This is in contrast with

1432   results for subjects with other polymorphisms of COMT and results when subjects were

1433   split according to the DAT1 polymorphism. Estradiol Met/Met subjects exhibited choice

1434   behaviour more aligned with the reward probability distribution in the beginning at trial

1435   ~80 compared to subjects from the placebo group with the same polymorphism. When

1436   we then split subjects according DAT1 polymorphism, the estradiol 9/10 subjects can

1437   similarly be seen following the reward probability distribution more closely compared to

1438   the placebo 9/10.

1439

1440   **Model prediction for switching behaviour**
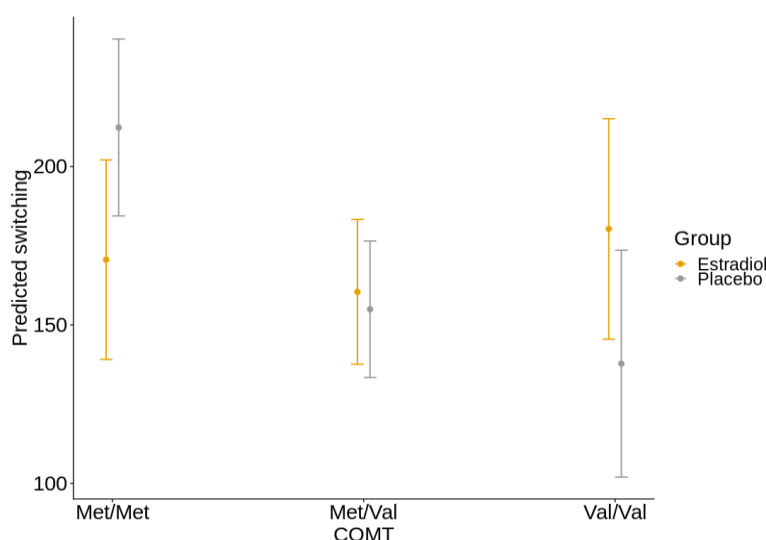

1441

1442   **Figure S3.** General linear model prediction for switching behaviour (i.e. a change in chosen stimulus on
1443   trial $t + 1$ from trial t, independent of choice outcome on trial t). Estradiol administration dampened naturally
1444   occurring differences in switching behaviour when subjects were split according to the COMT
1445   polymorphism, i.e. whether subjects would switch the stimulus they chose on trial t compared to trial $t + 1$
1446   irrespective of choice outcome on trial t. Figure S3 shows that our linear model made comparable
1447   predictions about this switching behaviour for all three polymorphisms in the estradiol group. In contrast, in
1448   the placebo group it predicted a clear linear decrease in switching from the Met/Met genotype (i.e. high
1449   prefrontal dopamine) towards the Val/Val genotype (i.e. low prefrontal dopamine).
1450

The role of CYP 19A1, ERα, ERβ, CAG, and GGN

Because the results we report in the main text and the supplementary materials have other mechanistic explanations and/or could have been moderated through other candidate mechanisms, we further analyzed these mechanisms together by providing theoretical motivation for these analyses. We analyzed the candidate mechanisms for both accuracy and reported switching behaviour. Here, we first briefly outline their importance and then summarize the observed results.

It is known that androgens are converted to estrogen [74]. This means that the increase in estrogen levels arises from the conversion process and the administration more directly. Furthermore, variation in the length of two functional polymorphisms (CAG – polyglutamine, and GGN – polyglycine) are known to modulate the functioning of the androgen receptor gene [75]. This is important for two reasons. The first is that our procedure has previously shown to increase circulating testosterone levels which could have raised estradiol levels whilst being moderated by subjects' androgen receptor characteristics [34]. Following from this, previous work has shown that brain regions important for memory and learning contain androgen receptors [76]. Therefore, it could be possible that interindividual differences in both functional polymorphisms could have moderated our observed results due to interindividual variability. For example, greater CAG repeat length has previously been associated with lower scores in different cognitive tests in older men [75]. Similarly, there has been an association between GGN repeats and immediate and delayed logical memory recall as a function of GGN repeat length found in women [77]. Furthermore, longer repeats of both the CAG and GGN polymorphism have been previously associated with different disorders including attentional deficit and hyperactivity disorder, conduct disorder, and oppositional defiant disorder [78]. All described results show a correlation between interindividual variability in androgen receptor functioning and cognitive performance, giving rise to the CAG and GGN polymorphisms being potential candidate mechanisms moderating the observed effect of estradiol on accuracy and switching behaviour. Repeat polymorphism of two

1479   most studied functional polymorphisms in the androgen receptor gene - CAG and GGN

1480   - were therefore examined.

1481   Throughout the conversion process from androgens to estrogens, the CYP19A1

1482   gene encodes instructions for aromatase – the enzyme converting androgens to

1483   estrogens [79]. The single nucleotide polymorphisms (SNPs) associated with the

1484   CYP19A1 gene regulate the metabolism of androgens and mediate brain estrogen

1485   activity. Two specific SNPs (rS700518, rs936306) have been previously shown to have

1486   a role in cognitive functioning in humans. For example, men with the homozygous AA

1487   allele have been shown to have higher estradiol serum levels and greater bilateral

1488   posterior hippocampal gray matter volume compared to those homozygous with the GG

1489   allele [80]. While other work has shown a differential impact of homozygous CC alleles

1490   versus homozygous TT alleles on episodic memory recall in women [81]. Given that our

1491   procedure has previously shown to increase circulating testosterone levels and that

1492   polymorphisms of the CYP19A1 gene are known to have a role in cognitive functioning,

1493   we aimed to exclude the possibility of that driving our observed effects and analyzed

1494   both single nucleotide polymorphisms of the CYP19A1 gene.

1495   Once androgens are converted to estrogens, estrogen action is mediated

1496   through the known estrogen receptors (ERα, ERβ). Both receptors are widely

1497   distributed throughout the brain in regions important for cognitive functioning. So far, it

1498   has been shown that ERα is responsible for most of estrogen-related activation. For

1499   example, it has been shown that SNPs of ERα are related to Alzheimer's disease and

1500   are associated with the likelihood of developing cognitive impairment [82]. We have,

1501   therefore, focussed on two particular SNPs of ERα: rs9340799, rs2234693. In contrast,

1502   little is known of a potential impact of ERβ. As an exploratory measure, we have

1503   included repeats of this receptor in our analysis as well.

1504   Of the described candidates (CAG, GGN, CYP 19A1, ERα, ERβ), no test

1505   revealed any effect of interest. There was no interaction between group membership

1506   (i.e. estradiol or placebo) and either the SNPs of ERα: rs9340799 ($F_{(2, 84)} = 0.66$, $p =$

63

1507   .52), rs2234693 ($F_{(2, 84)}$ = 0.63, p = .53) in relation to accuracy. Furthermore, the same

1508   was true for the interaction between CAG repeats and group membership ($F_{(1, 87)}$ = 0.45,

1509 p = .51), GGN repeats and group membership ($F_{(1, 87)}$ = 1.31, p = .26), and SNPs of the

1510   CYP19A1 gene and group membership (rs700518 $F_{(2, 84)}$ = 1.84, p = .15, rs936306 $F_{(2,}$

1511 $_{84)}$ = 0.34, p = .72). In a final examination, we also looked at the repeats of ERβ to

1512   determine whether this could have driven any of the observed effects. However, this

1513   was not the case for either recorded variant of ERβ (ERβ1: $F_{(1, 87)}$ = 0.02, p = .89, ERβ2:

1514 $F_{(1, 87)}$ = 0.00, p = .96).

1515   Identical results were obtained for switching behaviour. While we observed a

1516   statistically significant interaction between estradiol administration and the COMT

1517   polymorphism, this was not true for any of the other mechanistic explanations. That is,

1518   no model showed an interaction between group membership and either of the SNPs of

1519   ERα: rs9340799 ($F_{(2, 84)}$ = 2.90, p = .06), rs2234693 ($F_{(2, 84)}$ = 2.88, p = .06), CAG repeats

1520   ($F_{(1, 87)}$ = 0.10, p = .76), GGN repeats $F_{(1, 87)}$ = 1.32, p = .25), and SNPs of the CYP19A1

1521   gene (rs700518 $F_{(2, 84)}$ = 1.81, p = .17, rs936306 $F_{(2, 84)}$ = 1.08, p = .35) in relation to

1522   switching behaviour. As in the case of accuracy, we also looked at the repeats of ERβ.

1523   Again, there was no statistically significant contribution to switching behaviour from this

1524   predictor for either recorded variant of ERβ (ERβ1: $F_{(1, 87)}$ = 3.05, p = .08; ERβ2: $F_{(1, 87)}$

1525   = 0.96, p = .33).

1526   We finally repeated the set of analyses for staying behaviour with no effects

1527   found. SNPs of ERα: rs9340799 ($F_{(2, 84)}$ = 1.69, p = .19), rs2234693 ($F_{(2, 84)}$ = 1.79, p =

1528   .17), CAG repeats ($F_{(1, 87)}$ = 0.38, p = .54), GGN repeats $F_{(1, 87)}$ = 0.30, p = .59), SNPs

1529   of the CYP19A1 gene (rs700518 $F_{(2, 84)}$ = 1.27, p = .29, rs936306 $F_{(2, 84)}$ = 0.59, p = .55),

1530   and variant of ERβ (ERβ1: $F_{(1, 87)}$ = 1.35, p = .25; ERβ2: $F_{(1, 87)}$ = 0.86, p = .36).

1531   In brief, we have shown that the effects did not depend on overall androgen

1532   receptor functioning assessed by investigating the repeat length of two different

1533   functional polymorphisms (CAG and GGN). Both polymorphisms were investigated due

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1534   to the known conversion process of androgens to estrogen which could have

1535   moderated these results [74,80]. We excluded that interindividual variability in the

1536   conversion process itself would predict the observed effects, by investigating two

1537   polymorphisms of the CYP19A1 gene which plays a key role in converting androgens

1538   to estrogens [80,81]. Finally, we excluded the possibility that following the conversion

1539   process, the observed effects were a consequence of polymorphisms (ERα) or repeats

1540   (ERβ) of known estrogen receptors, given that both are widely distributed throughout

1541   the brain, especially in regions of importance for reward processing [83]. All of the

1542   described candidates revealed no effect for either accuracy or switching behaviour that

1543   are reported above.

1544

1545   **Impact of previous choice on current choice**

1546   Since we observed a difference in group choice behaviour in Figure S2 in the main

1547   results, and that the estradiol and placebo group systematically chose differently on

1548   7.5% of the trials, we ran separate logistic regressions to compute whether this would

1549   also be observed in how past choices would affect the current choice. We predicted

1550   there would be a difference between the estradiol and placebo group in pure choice

1551   autocorrelation (i.e. if I choose option A on trial t, is it more likely I will choose it again

1552   on trial $t + 1$) and reward-related autocorrelation (i.e. if I choose option A on trial t and

1553   it is rewarded, is it more likely I will choose it again on trial $t + 1$). We further predicted

1554   that splitting these two groups according to the DAT1 and COMT polymorphism would

1555   show differences depending on the polymorphism.

1556   Information about subjects' choices n trials ago was varied from 1 trial to 7 trials

1557   ago and used as a regressor to predict current choice. Therefore, in the design matrix

1558   we had information about their choice from 7 trials to 1 trial ago. The value 1 meant

1559   they repeated their choice, while 0 meant they did not. We first split participants

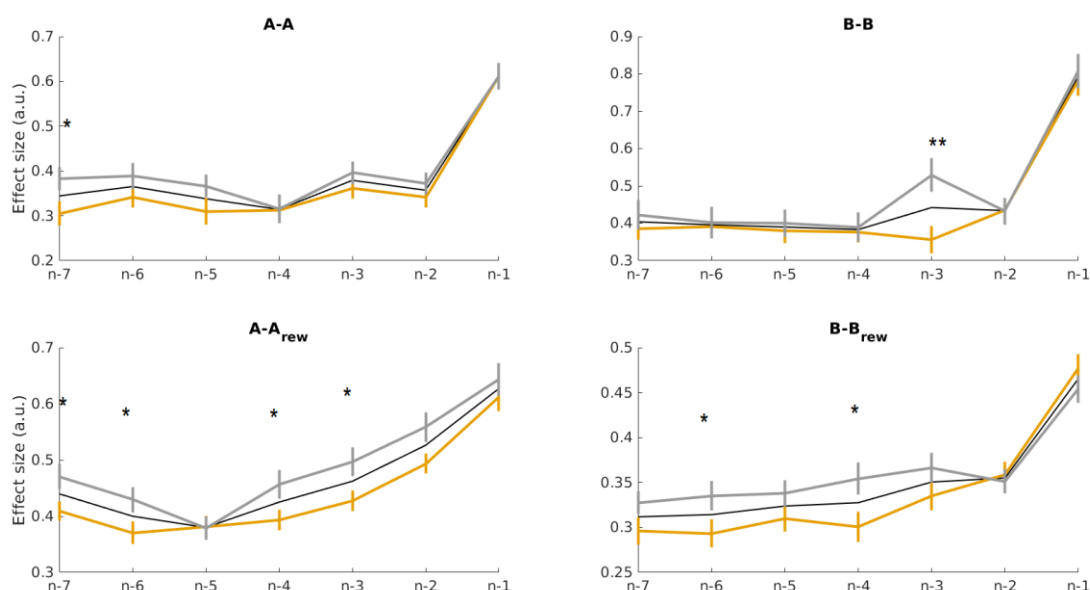1560   according to the estradiol and placebo group (Figure S4).

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Figure S4. The top panels show pure choice autocorrelation: Choosing A if A was chosen previously or choosing B if B was chosen previously. Bottom panels show reward-related choice autocorrelation: Choosing A if A was previously rewarded or choosing B if B was previously rewarded. The lines show the averaged beta coefficient from the regression. Error bars are standard errors of the mean. Orange line depicts the estradiol group, gray lines depict the placebo group. * $p < .05$, ** $p < .01$.

Contrary to our prediction, the top panel in Figure S4 does not reveal a systematic difference in choice autocorrelation between the estradiol and placebo group. One notable exception is the contribution of the choices made three trials ago where the placebo group was more likely to consider those choices compared to the estradiol group ($p < .01$). However, the bottom panel reveals that the estradiol group had lower reward-related autocorrelation for both options. That is, if they were rewarded for a choice several trials ago, they were less likely to persevere with that choice compared to the placebo group. This is consistent with Figure 2A where the estradiol group followed the reward probability distribution better compared to the placebo group. Figure S4 reveals why that may have been the case; they were less likely to persevere due to information received several trials ago, but not the one that just occurred $t - 1$ trials ago.

We then further split the same participants according to the COMT (Figure S5) DAT (Figure S6) polymorphisms. We see that the autocorrelation difference for choosing option B three trials ago reported in Figure S4 was driven by the group with the Val/Met genotype specifically. In contrast, the difference between the estradiol and placebo group in terms of reward-related choice autocorrelation was driven by the placebo group with the Val/Val genotype (i.e. low prefrontal dopamine), as seen in the third column. Only in the Val/Val comparison was there a systematic difference between the estradiol and placebo subgroup. This difference disappeared in the other COMT polymorphisms and was also only true for option A. Conversely, in column four a difference between the estradiol and placebo group only became observable in subjects with the Met/Met genotype (i.e. high prefrontal dopamine).
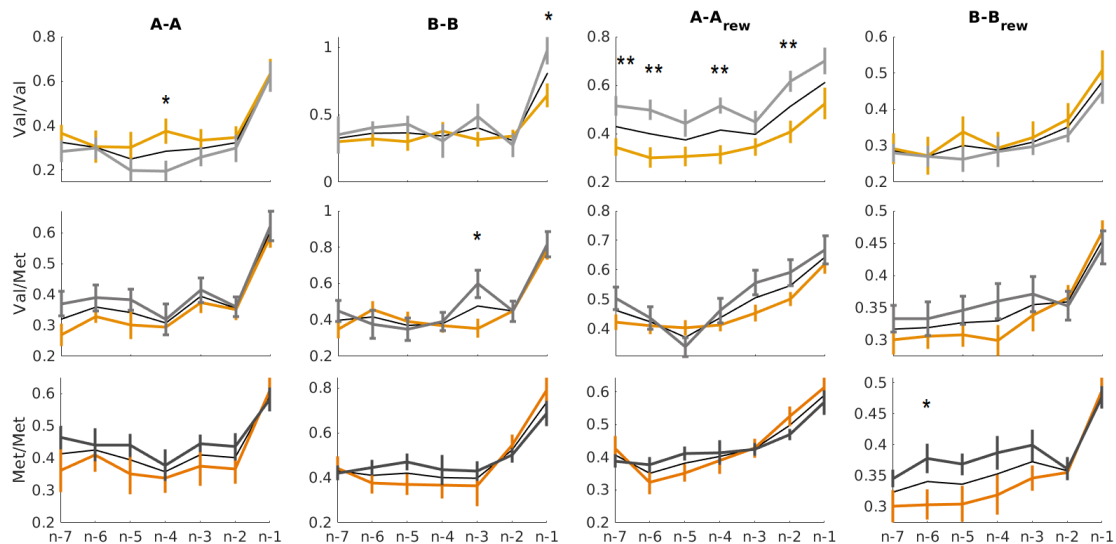

Figure S5. Individual columns show the same as individual panels in Figure S4. Here, they are additionally split according to the COMT polymorphism. The lines show the averaged beta coefficient from the regression. Error bars are standard errors of the mean. Orange line depicts the estradiol group, gray lines depict the placebo group. $*p < .05$, $**p < .01$.

The final split was according to the DAT1 polymorphism. This did not reveal clearly interpretable systematic differences apart from the autocorrelation difference for option B between the estradiol and placebo group being driven by subjects with the 10/10 genotype (i.e. low striatal dopamine) as opposed to subjects with the 9/10

1607   genotype. Similarly, estradiol 10/10 genotype subjects also exhibited lower reward-

1608   related autocorrelation compared to the placebo 10/10 genotype subjects. However,

1609   this was also present in the 9/10 subjects for both stimuli, indicative of them being more

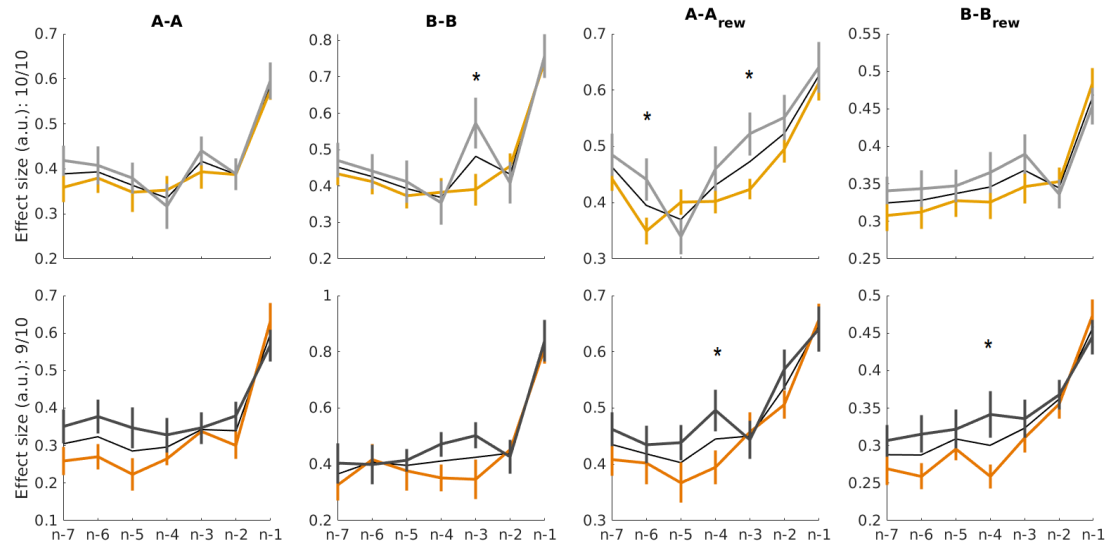1610   likely to stick with identical choices after being rewarded.

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1613
1614   **Figure S6.** Individual columns show the same as individual panels in Figure S4. Here, they are split
1615   according to the VNTR polymorphism of the DAT gene. * $p < .05$, ** $p < .01$.
1616

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1618   **Generalized linear mixed effects model predictions for choice**

1619   Figure S7 reveals strong interactive effects for both the DAT polymorphism with drug

1620   over time on choice (A) and the COMT polymorphism (B) with the same model

1621   structure. We did not include models that would combine both genotypes as they would

1622   have given rise to an insufficient size per smallest cell (Table S6).
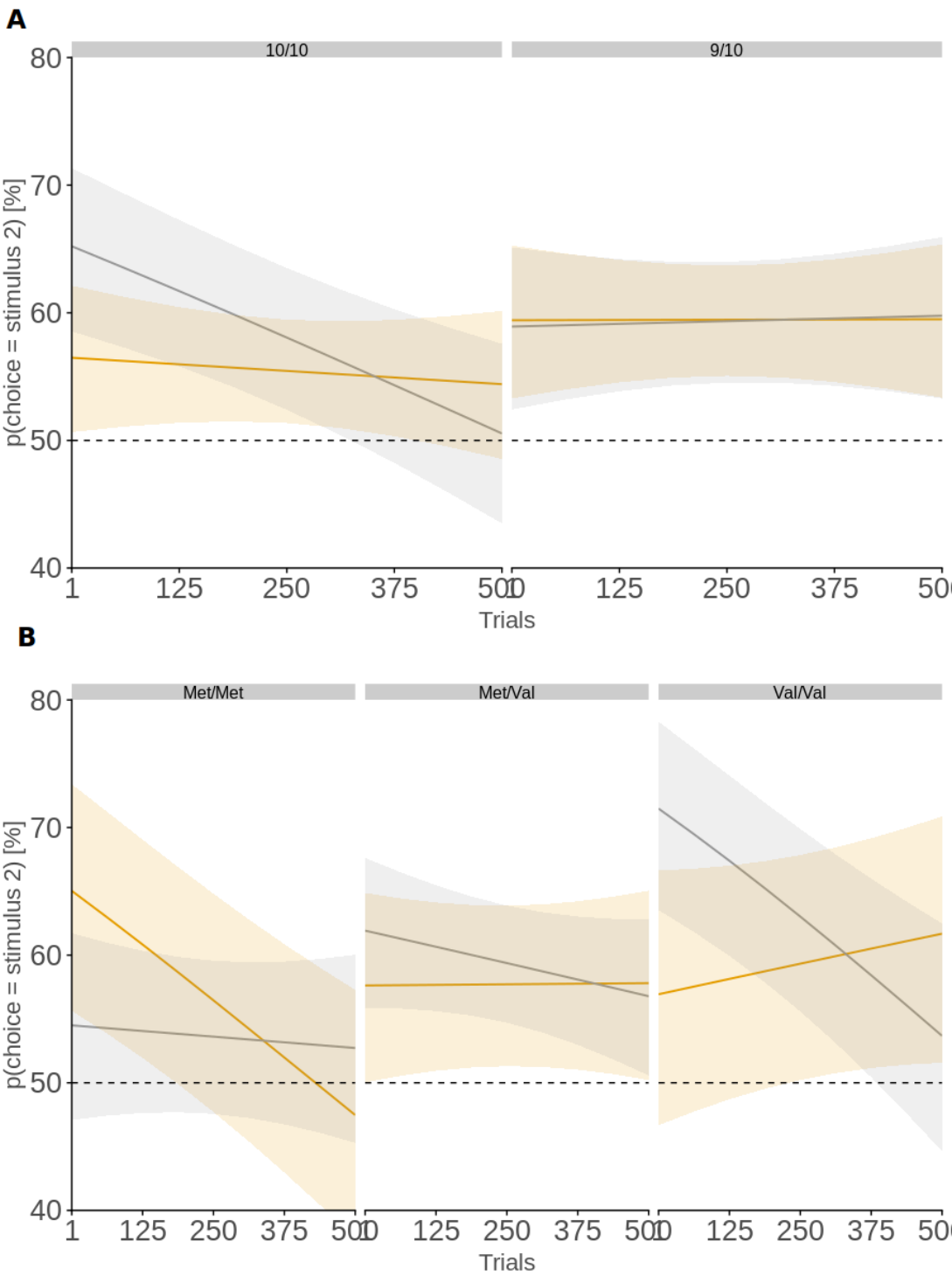
1623

Figure S7. Predictions from winning models of the generalized linear mixed effects models for **A**) the interaction between drug, DAT, and trial on choice, and **B**) the interaction between drug, COMT, and trial on choice.

Formal model comparison

In addition to computing the leave-one-out information criterion to perform model comparison [71] we similarly computed the exceedance probability of the winning model using the VBA toolbox [73]. This value showed a strong preference for the winning model

1633 *P*(model two) = 98%. Furthermore, we computed protected exceedance probability [72]

1634 as an extension which, while yielding an expected decrease in the winning model

1635 probability, still favoured model two over other competing models (*P*(model two) =

1636 12.5%). The likely decrease was due to the reinforcement learning task not being

1637 optimized to detect behavioural differences between the models tested. However, in all

1638 reported models, the latent variable of interest, i.e. the learning rate, remained

1639 unaltered. We would therefore expect the increase in learning rates to be present if we

1640 were to select the learning rates from models that best fit individual subjects.

1641

1642 **Validating model**

1643 We further tested the model validity and predictions by computing posterior predictive

1644 densities, i.e. what predictions does the model make on a trial by trial basis for subjects

1645 with the parameters such as those that were extracted from our participants. Posterior

1646 predictive densities showed no difference in a fit between both the estradiol and placebo

1647 group and approximated the empirical reward probability distribution (Figure S8A). To

1648 quantify this, we then compared model predictions from posterior predictive densities

1649 with actual participant behaviour to assess model accuracy collapsed across time

1650 (Figure 4B) showing it performed above chance and equally well for both groups. We

1651 further compared accuracy on each trial across participants to ensure that there were

1652 no unexpected drops in accuracy. This did not happen as the model (Figure S8C) had

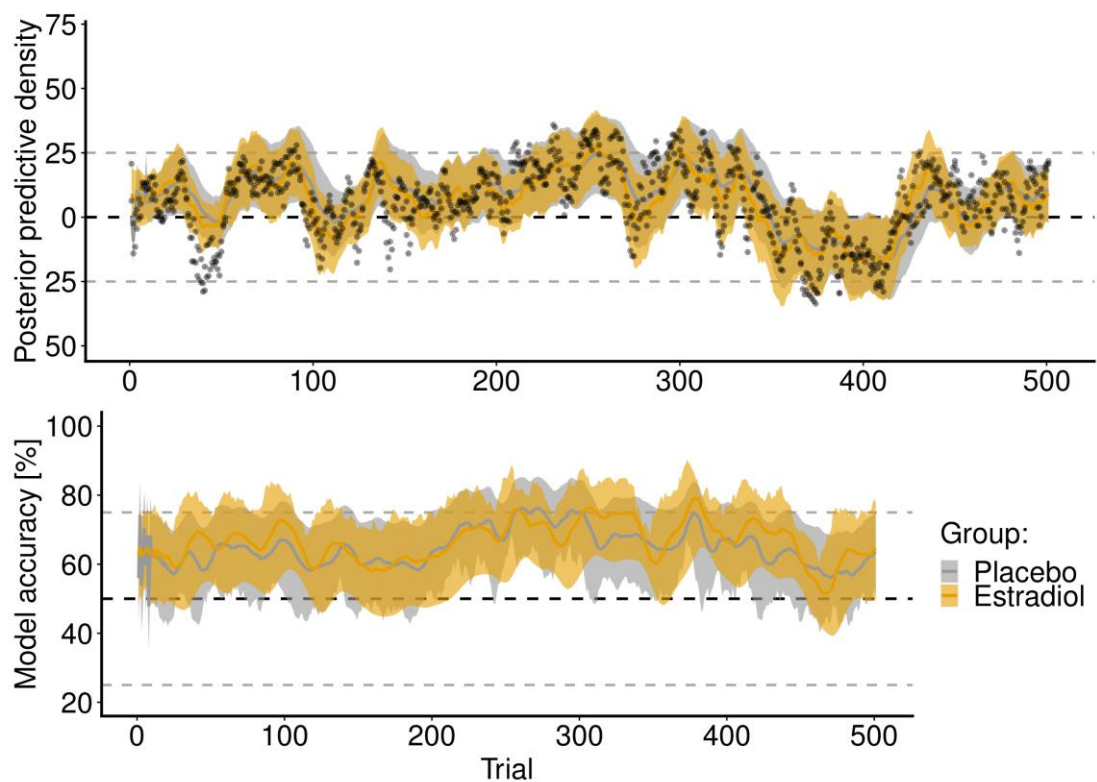1653 no discernible drops in performance.

1654

Figure S8. A) Posterior predictive density computed for both groups with overlaid average responses for both groups across trials **B**) Accuracy for both groups obtained from the posterior predictive density for both groups separately.