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Variations in the *GRIN2B* gene are associated with risky decision-making

Vanessa Ness [a,*,1], Larissa Arning [b,1], Hanna E. Niesert [b], Maik C. Stüttgen [a], Jörg T. Epplen [a], Christian Beste [b]

[a] Institute of Cognitive Neuroscience, Department of Biopsychology, Ruhr-University Bochum, Universitätsstr. 150, D-44780 Bochum, Germany
[b] Department of Human Genetics, Medical Faculty, Ruhr-University Bochum, Germany

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ABSTRACT

The dopaminergic system is known to modulate decision-making. As N-methyl-D-aspartate (NMDA) receptors strongly influence dopaminergic function, it is conceivable that the glutamatergic system is also involved in decision-making. We examined whether polymorphisms in the N-methyl-D-aspartate receptor 2B subunit gene (*GRIN2B*) influence decision-making using the Iowa Gambling Task (IGT). In total, 245 ($n = 245$, 127 female) healthy German students were included in the analysis. Two synonymous SNPs in exon 13, rs1806191 (H1178H) and rs1806201 (T888T) showed the strongest association with aspects of IGT performance. Females with a CC allele in rs1806201 made less use both of a win-stay strategy and demonstrated more exploratory behaviour during task execution. For rs1806191, we found a strong additive effect in usage of a win-stay strategy. This, partly sex-dependent, correlation of the win-stay/lose-shift behaviour with *GRIN2B* genotypes suggests that healthy individuals with certain *GRIN2B* variations respond differently to ambiguous conditions, possibly by altered perception of wins and losses. These findings underline the necessity to integrate the glutamatergic system when examining decision-making processes.

1. Introduction

Every day, humans face situations requiring the simultaneous consideration of numerous factors relevant for adaptive decision-making, *i.e.* the selection of appropriate responses to obtain natural rewards. Thus, optimal decision-making depends on the learning of associations between stimuli, actions and outcomes (reward or punishment) and on the use of this knowledge to adapt flexibly (Morton and Avanzo, 2011).

Reward processing mainly depends on mesocorticolimbic dopaminergic systems that comprise dopamine (DA) neurons in the ventral tegmental area (VTA) and their projections to nucleus accumbens (NAc), amygdala, prefrontal cortex (PFC), amongst other forebrain regions (Schultz, 1998; Wise and Rompre, 1989). Additionally, reward-related behaviour emerges from the dynamic activity of entire neural networks. For example, integration of glutamatergic and dopaminergic afferents by the NAc plays an important role in incentive motivation, goal-directed behaviours, and learning processes related to reinforcing properties of natural rewards and addictive drugs (Goto and Grace, 2008; Hyman et al., 2006; Kelley and Berridge, 2002; Wolf et al., 2004). The role of neurotransmitters, such as dopamine and serotonin, in decision-making has been demonstrated many times by studies of pathological conditions (Nussbaum et al., 2010; Rogers, 2011). Parkinson's disease (PD) treatment that increases dopamine levels has for example been related to significant alterations in decision-making in some patients, which is expressed as increased likelihood of developing pathological gambling and impulsive behaviours (Antonini and Cilia, 2009; Cools et al., 2003; Weintraub et al., 2009). Also, decision-making impairments in schizophrenia, a disease characterised by aberrant dopaminergic, serotonergic and glutamatergic systems, have been reported (Strauss et al., 2011; Waltz et al., 2007). Here, it is suggested that schizophrenics display some of the same characteristics as pathological gamblers (Borras and Huguelet, 2007). In the same manner, both pathologically related and induced neurotransmitter alterations impact on neural mechanisms underlying decision-making also genetically determined differences in transmitter homeostasis or expression may play a role. Indeed, variations in the serotonin transporter length polymorphic region (5-HTTLPR), the catechol-*O*-methyltransferase (COMT) and brain-derived neurotrophic factor (BDNF) have been identified as likely contributors to differences in decision-making aspects in healthy subjects using the Iowa Gambling Task (IGT, Homberg et al., 2008; Roussos et al., 2008; Van den Bos et al., 2009; Kang et al., 2010).

* Corresponding author. Tel.: +49 234 32 24082; fax: +49 234 321 4377.
E-mail addresses: vanessa.ness@rub.de (V. Ness), larissa.arning@rub.de (L. Arning), hannah.e.niesert@rub.de (H.E. Niesert), maik.stuettgen@rub.de (M.C. Stüttgen), joerg.t.epplen@rub.de (J. T. Epplen), christian.beste@rub.de (C. Beste).
[1] Both authors contributed equally to the manuscript.

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The IGT has been designed to mimic real-life decision-making in a laboratory situation and is one of the most frequently used tasks to assess decisions under uncertainty. Also, the IGT is a comparatively complex task mostly used to examine deficits in decision-making in patients with brain damage (Bechara et al., 1994, 1999; Brand et al., 2007). Furthermore, performance on the IGT is confounded by interrelations between cognitive processes, exacerbating the identification of specific processes responsible for observed deficits (Busemeyer and Stout, 2002). Participants performing the IGT are asked to successively draw at will cards from one of four decks. Unknown to the subjects, each deck is associated with a certain range of gains and losses. The participants' task is to maximise their total gain by learning which of the four decks are the most profitable. Optimal performance on the IGT depends on an intact prefrontal cortex (Bechara et al., 1997). Since glutamate is an important neurotransmitter in the cortex it is a likely candidate to influence decision-making on the IGT. Additionally, glutamate modulates dopamine activity in the in the VTA, and therefore presents a clear pathway to impact reward-related effects of dopamine on decision-making (Wolf et al., 2004). Yet, associations of the glutamatergic transmitter system with IGT performance have not been investigated so far.

In order to elucidate the relationship between glutamate and decision-making, we recruited healthy men and women with the goal of characterising relationships between sequence variations in a gene coding for the N-methyl-D-aspartate receptor (NMDAR) channel subtype NR2B (*GRIN2B*) and IGT performance. NR2B appears to be involved in mediating neural plasticity leading to drug dependence (Schumann et al., 2009), and NR2B-containing NMDARs in the NAc and the dorsal hippocampus play a significant role in mediating the reinstatement of rewarding responses to morphine (Ma et al., 2007). Single-nucleotide polymorphisms (SNPs) in *GRIN2B* have been implicated in phenotypes associated with reward-related and impulsive behaviour, such as alcohol-related traits (Wernicke et al., 2003), smoking initiation (Vink et al., 2009), obsessive-compulsive disorder (OCD; Arnold et al., 2004, 2009), impulse control and addictive behaviours (*i.e.* pathological gambling, compulsive shopping) in PD (Lee et al., 2009) and attention deficit/hyperactivity disorder (ADHD; Dorval et al., 2007). In addition, the pathology of common disorders, such as OCD (Endrass et al., 2010), PD (Beste et al., 2009a, 2009b; Willemssen et al., 2009), Huntington's disease (HD, Beste et al., 2010) and ADHD (Shiels and Hawk, 2010) is frequently characterised by abnormal performance monitoring, a cognitive mechanism important for successful decision-making (Walton et al., 2004). Given the role of *GRIN2B* variations in phenotypes related to reward-seeking behaviour, we hypothesised that *GRIN2B* variations might also be involved in the process of decision-making in complex and conflicting situations, as measured in the widely used IGT. In total, we examined 16 SNPs within *GRIN2B* in a sample of 245 healthy German students and analysed the data with respect to different IGT outcome variables in regard to risky decision-making.

2. Method

2.1. Participants

245 physically and mentally healthy (127 female and 118 male), genetically unrelated participants of Caucasian ancestry (211 of German ancestry, 34 of other Caucasian ancestry) took part in this study. Ancestry was established by questioning participants about the ancestry of all grandparents. Participants had no history of neurological or psychiatric diseases as assessed by means of a psychiatric screening. The mean age was 23.33 (range 19–30, SD = 2.81) and mean education was 14.57 years (range 10–16 yrs, SD = 1.07). The mean alcohol consumption was 2.31 units (SD = 2.44) per week. None of the included participants reported current, clinically significant depressive symptoms according to the BDI (German Version; Beck and Steer, 1993; $M = 4.41$, SD = 3.69) or other psychiatric diseases. Also, the sexes were comparably distributed within the different SNPs (f). Additionally, the genotype groups of all polymorphisms did not differ with respect to their age, education, and BDI scores (Table 1). Participation was rewarded with 2 credit points, or 20€ payment. All participants were naive to the hypotheses. The study was conducted and approved by the ethics committee of the medical faculty of the Ruhr-University Bochum, Germany. All procedures were carried out with adequate understanding and written consent of the participants.

2.2. Genotyping

For non-invasive sampling, exfoliated cells were brushed from the oral mucosa of the participants. DNA isolation was performed with QIAamp DNA mini Kit (Qiagen GmbH, Hilden, Germany). SNP genotyping was conducted by polymerase chain reaction (PCR) and differential enzymatic analysis with the PCR restriction fragment length polymorphism method. Initially, the frequently studied C2664T variation in exon 13 (rs1806201) was genotyped. In order to evaluate the genetic variability of additional parts of *GRIN2B*, fifteen additional SNPs were selected for fine-mapping analysis spanning 10 kb of the 3′ part of GRIN2B. SNPs were primarily selected on the basis of their frequency (MAF >0.1) from dbSNP (www.ncbi.nlm.nih.gov/projects/SNP/): rs1806213 (IVS10), rs3026164 (IVS10), rs1806195 (IVS10), rs1806194 (IVS10), rs16909222 (IVS11), rs10772692 (IVS11), rs4764011 (IVS11), rs3026160 (Ex12), rs3026159 (IVS12), rs1806205 (IVS12), rs1806191 (Ex13), rs1805247 (Ex13), rs890 (3UTR), rs1805503 (3UTR) and rs1805476 (3UTR). Further details of methodology and primer sequences are available upon request.

2.3. Iowa Gambling Task

The IGT was presented using the program PEBL (Version 0.09; Mueller, 2009). The procedure is identical to the one applied by Bechara et al. (1994), and only visual feedback was provided. At the beginning of the IGT the participants were endowed with a loan of $2000 in computer money. They were instructed to maximise the money by choosing cards (one at a time) from four decks (A–D) displayed on the computer screen. The participants chose a deck by pressing keys 1–4 of the

Table 1
Demografic Variables.

SNP	*N*	AGE					SEX					BDI					EDUCATION				
		M	SD	χ^2	df	*p*	♂	♀	χ^2	df	*p*	*M*	SD	χ^2	df	*p*	*M*	SD	χ^2	df	*p*
rs1805476	125	23.53	2.89	4.19	11	0.973	56	69	1.68	1	0.217	4.28	4.05	0.97	1	0.449	14.60	1.12	2.45	3	0.540
rs890	141	23.35	2.89	10.06	11	0.498	65	76	2.94	1	0.093	4.28	4.05	3.79	1	0.089	14.60	1.13	2.11	4	0.963
rs1805247	239	23.31	2.79	1.90	11	0.999	112	127	0.003	1	1.000	4.41	3.70	0.63	1	1.000	14.56	1.07	2.42	4	0.679
rs1806191	117	23.37	2.79	11.29	11	0.423	50	67	3.01	1	0.095	4.37	3.72	2.60	1	0.207	14.67	1.02	2.63	4	0.753
rs1806201	245	23.33	2.80	8.09	11	0.718	118	127	0.41	1	0.524	4.41	3.69	1.05	1	0.395	14.57	1.06	4.53	4	0.356
rs1806205	240	23.33	2.80	7.35	11	0.782	115	125	0.27	1	0.608	4.47	3.69	0.79	1	0.408	14.56	1.07	3.35	4	0.555
rs3026159	230	23.33	2.75	8.10	11	0.718	115	115	0.57	1	0.546	4.44	3.71	1.99	1	0.196	14.57	1.07	2.69	4	0.641
rs3026160	242	23.34	2.81	13.53	11	0.259	117	125	0.22	1	0.653	4.38	3.69	0.002	1	1.000	14.56	1.06	4.70	4	0.321
rs4764011	114	23.45	2.95	5.62	11	0.913	47	67	1.25	1	0.329	4.28	3.91	1.86	1	0.246	14.64	1.02	3.36	4	0.564
rs10772692	126	23.28	2.89	9.24	11	0.626	56	70	1.74	1	0.210	4.59	2.89	2.87	1	0.126	14.51	3.85	0.92	3	1.000
rs16909222	229	23.44	2.82	10.82	11	0.466	112	117	0.92	1	0.357	4.50	3.73	0.56	1	0.533	14.60	1.04	2.91	4	0.600
rs1806194	131	23.16	2.78	14.66	11	0.193	64	67	3.38	1	0.750	3.97	3.60	3.14	1	0.110	14.57	1.02	6.42	4	0.160
rs1806195	124	23.45	2.84	5.75	11	0.905	64	60	2.57	1	0.150	4.33	3.99	0.76	1	0.476	14.66	1.03	2.87	4	0.725
rs3026164	242	23.34	2.79	7.68	11	0.755	116	126	0.97	1	0.378	4.45	3.68	0.001	1	1.000	14.56	1.06	7.18	4	0.130
rs1806213	235	23.40	2.82	8.59	11	0.671	117	118	0.57	1	0.474	4.43	3.72	0.000	1	1.000	14.57	1.08	7.35	4	0.124

typewriter keys (1 = A, 2 = B, 3 = C, 4 = D). The participants were informed that each time they would receive monetary gains or losses. Unknown to the subjects, cards of decks A and B are associated with higher monetary gains (both $100) but also higher penalties (monetary losses, ranging from $150 to $350 for deck A and $1250 for deck B) than decks C & D, which feature both lower gains (both $50) and lower penalties (deck C: $25-$75, deck D: $250). After selecting a card the monetary outcomes are displayed on the screen. The IGT consists of 100 trials. The probabilities of receiving a penalty are adjusted such that decks A & B have a negative expected value and C & D have a positive expected value. Accordingly, the optimal strategy to maximise the net gain is the exclusive choice of decks C & D. The participants were not given information about how long the task will go on. The following performance indices were calculated: (a) IGT Net Score, calculated as the difference between the number of good and bad deck choices ([C + D]−[A + B]), (b) relative frequency of win-stay trials (frequency of choosing the same deck as the previous trial after a positive outcome, divided by the total number of trials with a positive outcome), (c) relative frequency of lose-switch trials (frequency of switching to another deck after a negative outcome, divided by the total number of trials with a negative outcome), (d) an index of exploratory behaviour, calculated as the minimum number of cards taken from any of the four decks, and (e) the number of choices from card decks with high variability in the amount of losses that could be encountered (decks A&C).

2.4. Statistical analyses

For each of the five dependent variables, we investigated the effects of sex and *GRIN2B* genotypes using two-way analysis of variance (ANOVA), complemented with η^2 as effect size index. The analyses were performed assuming a co-dominant or a dominant effect for each polymorphism. In the dominant model, both, the heterozygous and the rarely observed homozygous variation were combined (rs1805247, rs1806201, rs1806205, rs3026159, rs3026160, rs16909222, rs3026164, rs1806213). In the co-dominant model, all three genotypes were analysed (rs1805476, rs890, rs1806191, rs4764011, rs10772692, rs1806194, rs1806195). SNP rs1805503 was excluded from the analysis due to its low allele frequency (see allele frequencies in Table 2). All statistical analyses were conducted in MATLAB 7.8.0 (The Mathworks, Natick, MA). Hardy–Weinberg equilibrium (HWE) was tested for each SNP. The strength of linkage disequilibrium (LD) between pairs of SNPs was measured as D' by using HAPLOVIEW [http://www.broad.mit.edu/mpg/haploview/]. PHASE (2.1) was utilised to estimate haplotype frequencies (Stephens and Scheet, 2005).

3. Results

Bivariate correlational analyses across the whole sample revealed that all performance variables were correlated (Fig. 1). In order to elucidate which behavioural strategies lead to overall performance on the IGT, we carried out a multiple regression analysis with Net Score as the criterion variable and sex as well as a number of process-variables as predictors: fraction of win-stay trials, fraction of lose-shift trials, the exploration index and the frequency of card choices from the two high-variance decks. Entering these variables into a multiple regression analysis resulted in a highly significant multiple correlation ($R^2 = 0.39$, $p < 10^{-23}$). The strongest predictor variables turned out to be the number of choices from high-variance decks ($\beta = 0.44$, $p < 10^{-13}$, semipartial $r = 0.355$) and the exploration index ($\beta = -0.49$, $p < 10^{-10}$, semipartial $r = -0.403$). In addition, the relative frequency of lose-shift trials made a significant but somewhat smaller contribution ($\beta = -0.13$, $p = 0.031$, semipartial $r = -0.36$), while the relative fraction of win-stay trials and sex failed to do so.

Table 2
General information on genotyped SNPs (MAF: minor allele frequency).

dbSNP ID	*GRIN2B* gene region	Alleles	MAF
rs1806213	IVS10	T > G	0.08
rs3026164	IVS10	T > G	0.08
rs1806195	IVS10	G > T	0.48
rs1806194	IVS10	T > C	0.36
rs16909222	IVS11	A > G	0.13
rs10772692	IVS11	C > A	0.47
rs4764011	IVS11	A > G	0.45
rs3026160	Ex12	G > A	0.13
rs3026159	IVS12	A > G	0.13
rs1806205	IVS12	G > C	0.29
rs1806201	Ex13	C > T	0.26
rs1806191	Ex13	A > G	0.49
rs1805247	Ex13	A > G	0.09
rs890	3UTR	T > G	0.47
rs1805503	3UTR	T > G	0.02
rs1805476	3UTR	G > T	0.37

In view of these results, we further analysed whether the significant predictor variables are associated with *GRIN2B* genotypes. We first analysed the C2664T variation in exon 13 (rs1806201) since the effect of this polymorphism has already been found to be associated significantly in patients with alcoholism, Alzheimer's disease, schizophrenia, tardive dyskinesia, PD and HD (Chiu et al., 2003; Kim et al., 2006; Liou et al., 2007; Arning et al., 2007; Wu et al., 2010). We found a significant interaction between sex and genotype for the fraction of win-stay trials ($F(1,244) = 7.9$, $p = 0.005$, $\eta^2 = 0.03$) in the absence of any main effects (both F's < 1.6, p's > 0.2; see Figs. 2a and 5). Post-hoc t-tests revealed differences between genotypes in females ($t(125) = 2.8$, $p = 0.005$) but not males ($t(116) = 1.1$, $p > 0.25$). No effect was observed for the fraction of lose-shift trials (all F's < 1.5, p's > 0.2), but we found a significant interaction for sex and genotype for exploratory behaviour ($F(1,244) = 9.3$, $p = 0.003$, $\eta^2 = 0.04$; see Figs. 2b and 5) in the absence of any main effects (both F's < 1.6, p's > 0.2). Again, post-hoc t-tests detected significant differences between genotypes in females ($t(125) = 3$, $p < 0.003$) but not males ($t(116) = 1.3$, $p > 0.15$; see Fig. 5 for overview over results for all SNPs). Net Score, the most "classic" IGT-parameter, as well as the number of choices from high-variance decks, was not significantly associated with either sex or genotype (all F's < 2.2, p's > 0.1). These results were largely unaltered when the session was split up into an early (trials 1–40) and a late part (41–100).

The direct functional significance of the synonymous rs1806201 polymorphism is unknown, and previously reported results on the effect of this SNP have been inconsistent. Therefore, we chose to narrow the region of interest, which, via LD, could explain the association initially detected by rs1806201. We analysed 15 additional SNPs in the 3′ part of *GRIN2B*. One of these SNPs could not be analysed due to insufficient number of cases (see methods). Out of the remaining 14 SNPs, nine showed statistically significant associations with IGT process-variables.

The strongest effect in terms of effect size and p-value was observed for rs1806191. Here, we found a main effect of genotype for the fraction of win-stay trials ($F(2,233) = 7.6$, $p = 0.001$, $\eta^2 = 0.06$) in the absence of differences between the sexes ($F = 0.24$, $p = 0.625$; Figs. 3 and 5). Inspection of the results revealed that individuals carrying the GG genotype followed a win-stay strategy most often, while individuals with the AA genotype showed the least fractions of win-stay trials, with heterozygous subjects intermediate. No other significant associations of rs1806191 genotypes with any other process-variable were observed.

Additional significant main and interaction effects of *GRIN2B* genotypes are given in Fig. 5 (for further information view supplementary data). To single out a further example, there are significant main effects for rs3026164 (see Fig. 4) and rs1806213 genotypes for the fraction of lose-shift trials (F's > 9.4, p's = 0.002, $\eta^2 = 0.04$) and sex (all F's > 6.4, p's < 0.012, $\eta^2 = 0.03$; Fig. 6). Examining LD among the *GRIN2B* variations revealed, that the variations rs1805476 (3UTR), rs890 (3UTR), rs1805247 (Ex13), rs1806191 (Ex13), rs1806205 (IVS12), rs3026159 (IVS12), rs3026160 (Ex12), rs4764011 (IVS11), rs10772692 (IVS11), rs16909222 (IVS11), rs1806194 (IVS10) and rs1806195 (IVS10) were in high LD in the cohort ($D' = 1.0$, $r^2 = 0.65$–0.95). In 5′ direction the LD breaks down, and a block of very strong LD ($D' = 1.0$, $r^2 \geq 0.97$) is observed for rs3026164 (IVS10) and rs1806213 (IVS10, Fig. 6). Yet, LD-based haplotype analysis did not reveal a haplotype that explained the association substantially better than any individual SNP.

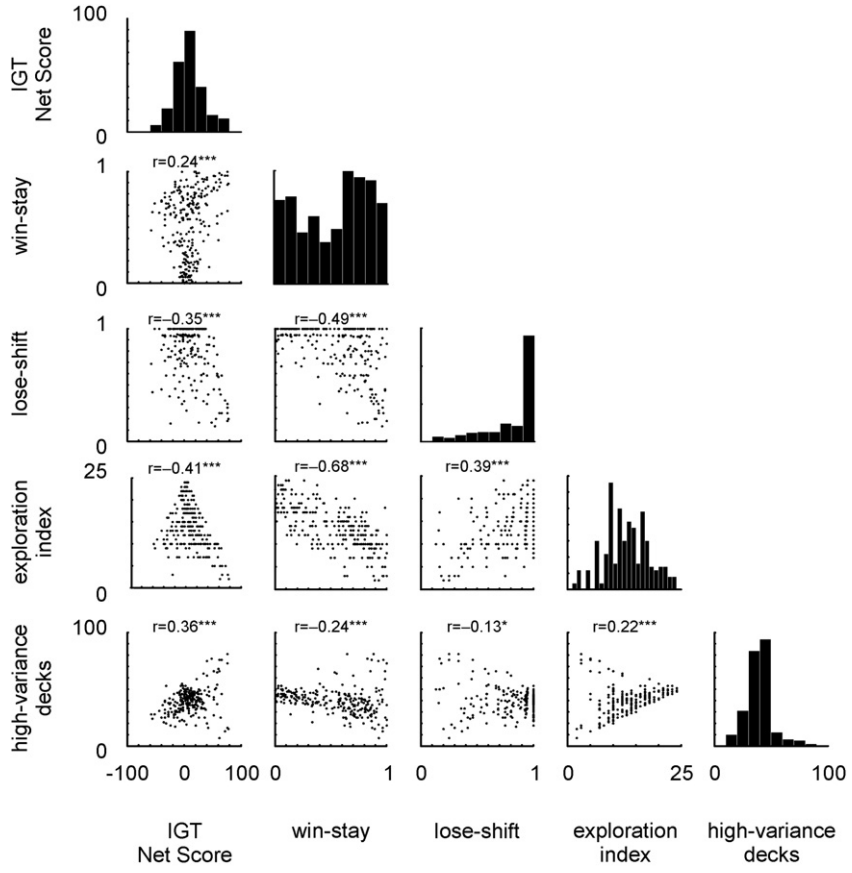

Fig. 1. Scatterplot matrix of dependent variables analyzed in this study with Pearson correlation coefficients for each pair. Key: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$. Histograms represent binned frequency distributions of variables.

4. Discussion

The current study focussed on the relevance of *GRIN2B* polymorphisms for risky decision-making. The analysis was inspired by the fact that the integration of glutamatergic and dopaminergic afferents plays an important role in processes related with decision-making, *e.g.* goal-directed behaviours and learning processes related to reinforcing properties of reward (Goto and Grace, 2008; Hyman et al., 2006; Kelley and Berridge, 2002; Walton et al., 2004; Wolf et al., 2004). Additionally, the glutamatergic system, like the dopaminergic system, has known influences on cognitive processes (Villmann and Becker, 2007). Our analyses revealed that the most uniform GRIN2B association was observed for the fraction of win-stay trials. Here, two synonymous SNPs in exon 13, rs1806191 (H1178H) and rs1806201 (T888T), which are in high LD with each other, showed the strongest association. For rs1806191 we found the strongest main effect of genotypes for usage of a win-stay strategy, independent of sex, with the number of G alleles significantly correlating with higher fractions of win-stay trials (Fig. 3). For rs1806201 we detected

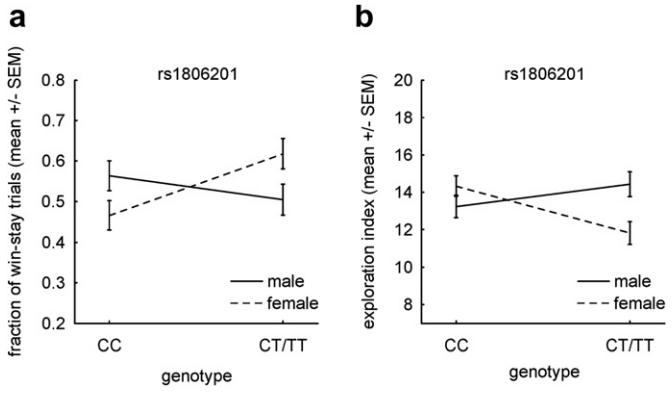

Fig. 2. (a) Interaction of sex and rs1806201 genotypes for following a win-stay strategy. (b) Interaction of sex and s1806201 genotypes for the exploration index, *i.e.* the minimum number of cards taken from any of the four available decks.

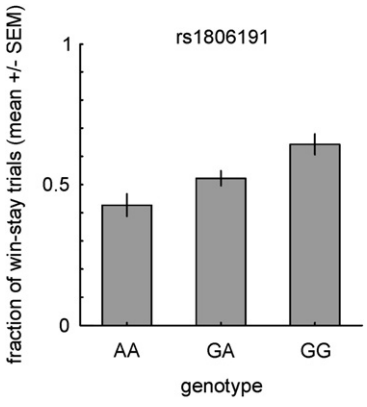

Fig. 3. Main effect of rs1806191 genotypes on fraction of win-stay trials.

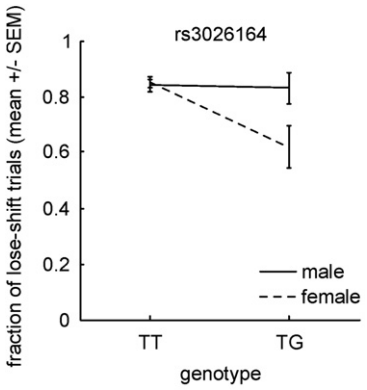

Fig. 4. Interaction of sex and rs3026164 genotypes for following a lose-shift strategy.

a significant interaction between sex and genotypes. Females carrying at least one rare T allele significantly more often used a win-stay strategy more often compared to females homozygous for the wild-type C allele (Fig. 2a). Female CC carriers also showed a higher minimum number of cards taken from any deck, thus indicating more exploratory behaviour in this subgroup of females (Fig. 2b). In contrast, men showed no differences in any of the variables examined in this study. Furthermore, significant main effects for two polymorphisms in intron 10 (rs3026164 and rs1806213) and sex could be observed for the fraction of lose-shift trials. The two SNPs are highly linked with each other but show only low correlation with the remaining markers in 3′ direction (Fig. 6).

Since even the advantageous decks render occasional loss, it is crucial that over time the participants suppress their tendency to respond to moderate losses at the advantageous decks and instead learn to recognise this as the more successful long-term alternative despite the occasional losses. The partly sex-dependent correlation of the win-stay/lose-shift behaviour with *GRIN2B* genotypes in our cohort thus suggests that healthy individuals with certain *GRIN2B* sequence variation(s) respond differently to ambiguous conditions, possibly by altered perception of wins and losses.

Glutamate participates in the transition from reward learning to repetitive behaviours in substance and behavioural addictions and the level of glutamate within the NAc seems to mediate reward-

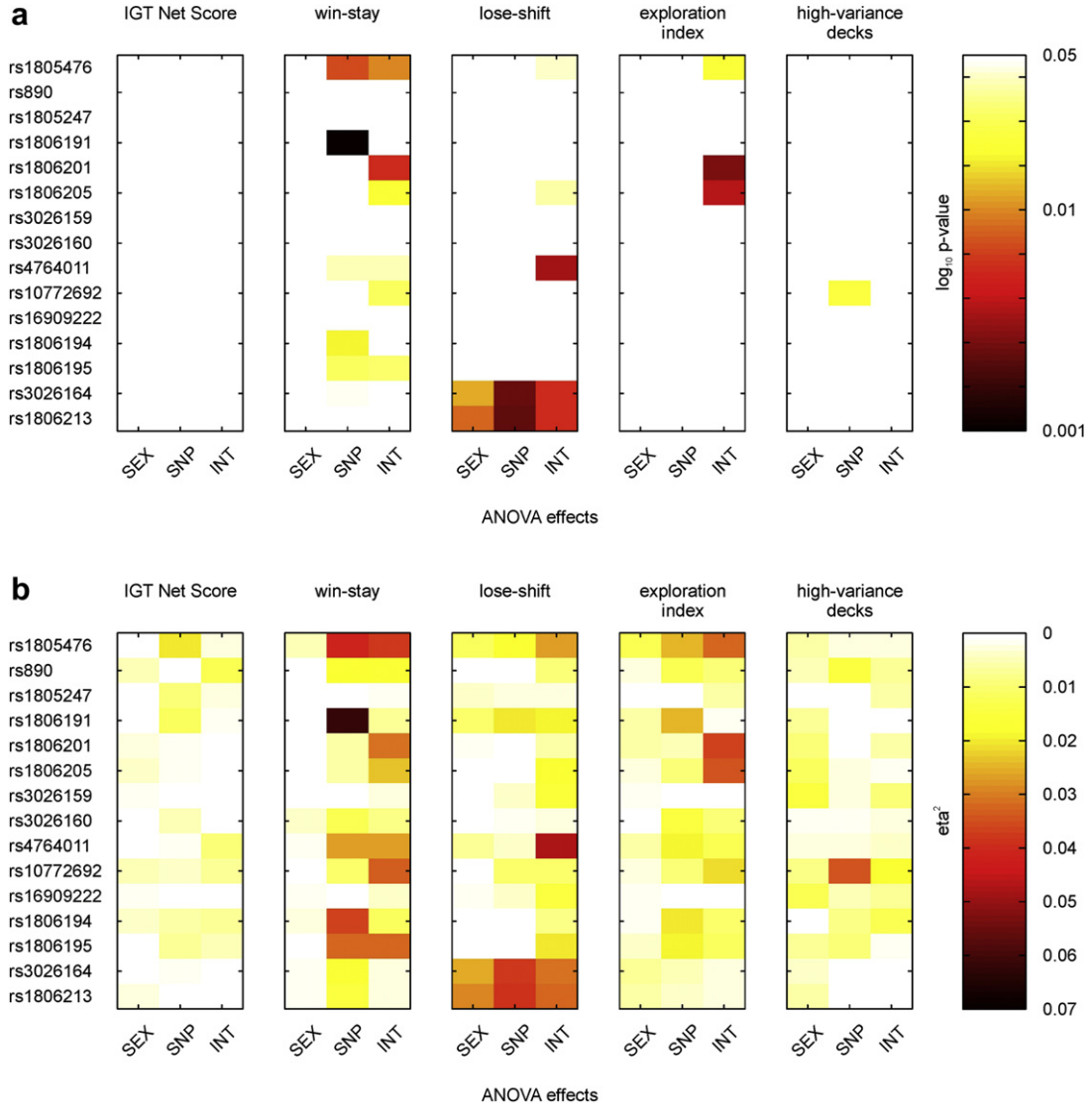

Fig. 5. (a) Inversely scaled heat map of significant effects, separately for all SNPs (rows), dependent variables (panels), main effects of sex and genotype and their interaction (columns). Darker colours represent smaller log(p); see colour bar. (b) Inversely scaled heat mat of effect size (eta squared). Conventions as in (a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

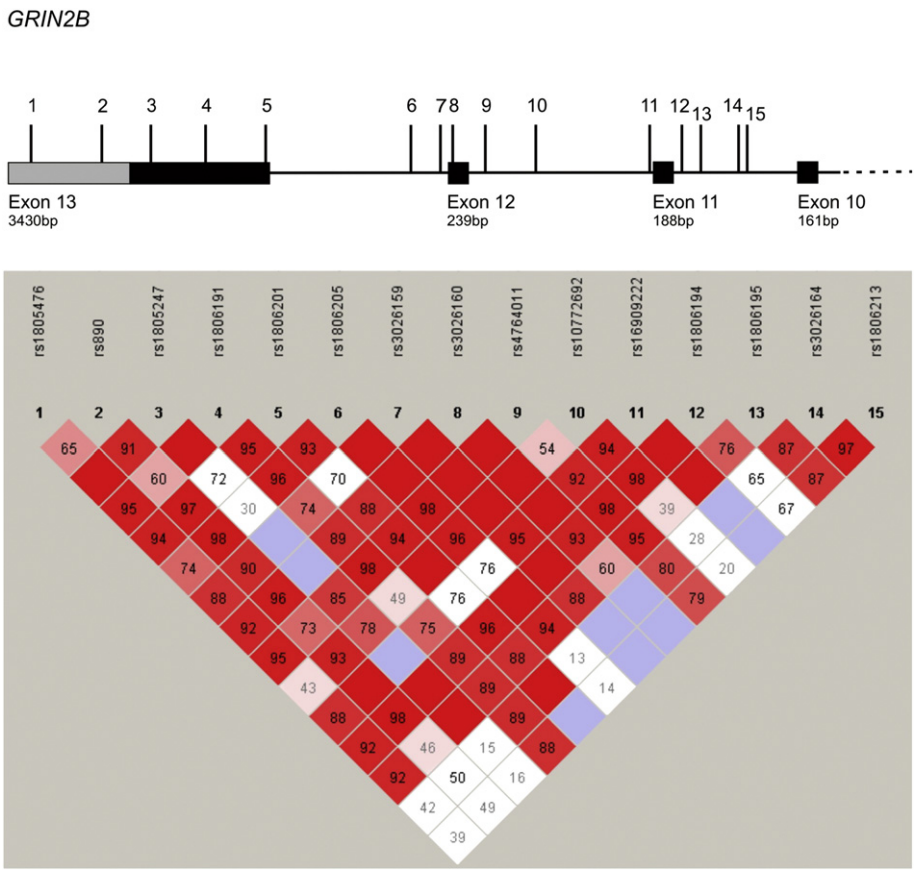

Fig. 6. Graphical representation of single-nucleotide polymorphisms in relation to the exon-intron structure and Haploview LD structure of the 3′ part of GRIN2B. Haploview plot showing pairwise LD (D′ values) for 15 SNPs based on genotypes of 245 individuals of the study. Each square plots the level of LD between a pair of SNPs; comparisons between neighbouring SNPs lie along the first line under the names of the SNPs. Colour scheme is based on D′ and LOD score values: white, D′ < 1 and LOD < 2; blue, D′ = 1 and LOD < 2; shades of pink/red, D′ < 1 and LOD ≥ 2; bright red, D′ = 1 and LOD ≥ 2. Numbers in squares are D′ values (values of 1.0 are not shown). The numbered vertical lines indicate positions of the SNP in the gene. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

seeking behaviour (Brewer and Potenza, 2008; McFarland et al., 2003). Unfortunately, none of the SNPs associated has been validated functionally. Yet, synonymous SNPs like rs1806191 and rs1806201 might be causal through influencing messenger RNA splicing, stability, and structure as well as changing the rate of protein folding (Hunt et al., 2009). All these changes can have a significant effect on the function of proteins, and slight differences in the NMDAR channel subtype NR2B could explain the repeatedly described associations with *GRIN2B* variations and correlated phenotypes associated with reward-related and impulsive behaviour (Wernicke et al., 2003; Vink et al., 2009; Arnold et al., 2004, 2009, Lee et al., 2009; Dorval et al., 2007).

Yet, the study is limited by the fact that the sample size, while relatively large for a neuropsychological experiment, was somewhat small for standard genetic analyses and included only healthy participants. In this group, the differences in IGT outcome are expected to be small, since the IGT is a method initially developed for investigating basic decision-making deficits of individuals with neuropsychological disorders. Therefore, the results should be viewed as hypothesis generating that warrant replication. Nonetheless, we believe that the present results encourage further investigation of glutamatergic influences on decision making. Also, the lack of effects for the IGT Net Score, combined with moderately strong effects for performance variables not included in standard studies employing the IGT, render it worthwhile to include these indices in future studies to complement IGT Net Score, especially since we observed genotype differences for these novel measures.

It might be criticized that we did not corrected for multiple comparisons, but our measures show modest to strong intercorrelations (see Fig. 1). Therefore, a Bonferroni correction does not seem adequate (*e.g.* Perneger, 1998). Additionally, the inclusion of 15 SNPs, which again are strongly intercorrelated, prohibits the application of a simple Bonferroni correction. Therefore, we adhere to the recommendations of Perneger and prefer to report the original *p*-values and effect sizes for an unbiased assessment of our findings.

In summary, our data suggests that variations in *GRIN2B*, may contribute to risky decision-making with respect to reward-related NMDAR mechanisms. The present data show that the glutamatergic system should be considered when examining decision-making processes.

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Appendix. Supplementary material

Supplementary material related to this article can be found online at doi:10.1016/j.neuropharm.2011.06.023.

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