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Cognitive and emotional processing in high novelty seeking associated with the L-DRD4 genotype

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ABSTRACT

The personality trait of novelty seeking (NS) has been associated with the long variant of the dopamine D4 receptor (L-DRD4) VNTR polymorphism. This is the first study to examine the influence of L-DRD4 polymorphism on some of the cognitive (i.e. decision making) and emotional underpinnings of the NS phenotype. One hundred and eighteen healthy males grouped in a L-DRD4 ($n = 24$) and a S-DRD4 ($n = 94$) group, completed multimodal assessment for personality, planning for problem solving and decision making. Two age-matched L-DRD4 and S-DRD4 sub-samples ($n = 17$ each) entered and completed emotional processing using startle modulation by affective pictures. ANOVAs showed that L-DRD4 individuals had higher NS, made more risky choices and won less money in the decision making task, but had intact planning for problem solving. They also had reduced startle reactivity and late startle modulation by both pleasant and unpleasant pictures. Early, attentional startle modulation by the affective pictures was intact. NS correlated negatively with startle reactivity and performance in the emotional decision task. These results suggest that the L-DRD4 polymorphism is associated with high NS and risk taking, underreactivity to unconditioned aversive stimuli, constricted emotional responses but preserved attentional processing of emotional stimuli and efficient problem solving. These results extend animal evidence on DRD4-mediated control of decision making and emotional processing to humans. The proposed role of the NS phenotype in human evolution and in disorders of impulsivity is discussed under the light of the present findings.

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1. Introduction

The dopamine D4 receptor in the human brain is highly distributed in prefrontal and limbic regions such as the amygdala and hippocampus (Meador-Woodruff et al., 1996). The 48 bp repeat in the portion of the dopamine D4 receptor (DRD4) gene coding for the third intracytoplasmic loop is a polymorphism in exon III, which varies between 2 and 11 copies with the 4-repeat being the most common in Caucasians (Vallone, Picetti, & Borrelli, 2000). There is a long (L-DRD4; 6–8 repeats) and a short polymorphism group (S-DRD4; 2–5 repeats). Studies of G protein coupling (Asghari et al., 1994), cyclic AMP synthesis (Asghari et al., 1995), in vitro expression (Schoots & Van Tol, 2003) and chaperone-induced folding (Van Craenenbroeck et al., 2005) provide increasingly solid evidence that the shorter exon III repeats code for a more efficient gene at the level both of transcription, translation and second messenger generation compared to the long repeat (Ebstein, 2006). Haplotype data indicate that the L-DRD4 and particularly the seven repeats (7R) allele, originated as a rare mutational event that increased to high frequency in human populations by positive selection (Ding et al., 2002), possibly due to its association with behavioral traits which facilitated human migration 40,000–50,000 years ago (Chen, Burton, Greenberger, & Dmitrieva, 1999; Ding et al., 2002) when the 7R allele is estimated to have emerged (Wang et al., 2004).

The less efficient L-DRD4 variant has been associated with the personality trait of novelty seeking (NS) (Benjamin et al., 1996; Ebstein et al., 1996), although evidence is inconsistent (Paterson, Sunohara, & Kennedy, 1999; Savitz & Ramesar, 2004) and meta-analyses do not support a strong relationship (Munafo, Yalcin, Willis-Owen, & Flint, 2008). NS relates to the tendency towards exploratory behavior and intense excitement in response to novel stimuli and is a complex personality trait, likely to be underpinned by many genes, each with a relatively small effect. The biological basis of NS and its association with DRD4 is not completely understood (Oak, Oldenhof, & Van Tol, 2000) and it is therefore important to determine the NS phenotype more accurately. To this effect, perhaps a more relevant question is whether the L-DRD4 is associated with aspects of cognitive and emotional processing likely to be more or less efficient in individuals with high NS. The cognitive and emo-

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tional underpinnings of NS are potentially easier to map onto neural systems and this would allow better understanding of the biological basis of NS.

NS is defined as a heritable tendency to respond strongly to novelty and cues for reward or relief from punishment, which leads to exploratory activity in pursuit of rewards as well as active avoidance of monotony and punishment (Cloninger, Svrakic, & Przybeck, 1993). This definition is cross-validated by the association between NS and the less efficient L-DRD4 since (a) the latter is associated with increased reward-related ventral striatum reactivity and self-reported impulsivity in humans (Forbes et al., 2009), (b) DRD4 knockout mice are supersensitive to alcohol and cocaine (Rubinstein et al., 1997) and exhibit reductions in anxious behavioral responses to novel environments (Dulawa, Grandy, Low, Paulus, & Geyer, 1999) and (c) pharmacological DRD4 blockade led to an increase in responding on the lever producing an aversive conditioned stimulus in a rat decision making paradigm indicating diminished ability of the conditioned punisher to reduce behavioral responding (Killcross, Everitt, & Robins, 1997).

Based on the above, it is possible that NS can be operationally construed as behavior relatively uninhibited by fear due to reduced fear-processing such that deficient emotional information processing about the potential consequences of "risky" actions, lead to a greater incidence of these behaviors. This definition predicts that compared to S-DRD4 individuals, L-DRD4 individuals (who presumably have high NS levels) should present with (a) deficient emotional decision making and (b) reduced responses to negative emotion. We tested the first prediction with the Iowa Gambling Task, a reliable probe of emotional decision making (Bechara, Damasio, & Damasio, 2000; Bechara, Damasio, Damasio, & Anderson, 1994; Pecchinenda, Dretsch, & Chapman, 2006). Choices in this simulated gambling task are made under conditions of uncertainty. Subjects must make discriminative instrumental choices that minimize the presentation of secondary negative reinforcing stimuli (i.e. cards associated with monetary loss). This type of decision making is motivated by reward, punishment and the uncertainty of outcomes and has been regarded as a type of emotional decision making. Its premises lie in the "somatic marker" hypothesis (Damasio, 1996) which proposes that the body states evoked by the experience of reward or punishment signal the potential occurrence of an outcome, and these emotional signals guide behavior and help bias the choices made in the gambling task, in a manner that is advantageous to the organism in the long-term (Bechara et al., 2000). We also used a cognitive decision making task as a control, to rule out deficiencies in decision making per se. We tested the second prediction in a subsample of our subjects, using the paradigm of startle modulation by affective pictures (Lang, Bradley, & Cuthbert, 1990), which allowed us to measure directly their emotional responses.

2. Materials and methods

2.1. Subjects

We restricted the sample to men to avoid additional, gender-related variability in the gambling task (Bolla, Eldreth, Matochik, & Cadet, 2004; Overman et al., 2004; Reavis & Overman, 2001) and in affective picture processing (Bradley, Codispoti, Sabatinelli, & Lang, 2001). One hundred and thirty unrelated Greek/Caucasian healthy males aged 18–35 years (mean ± SD, 26.0 ± 4.2), mostly university students, were recruited. All participants underwent IQ testing with the Raven's progressive matrices, psychiatric and physical assessment including a urine toxicology screening. Exclusion criteria were left-handedness, personal history of head trauma, medical and neurological conditions, use of prescribed and recreational drugs and personal or family history of DSM-IV axis I disorders. One hundred and eighteen subjects entered the study. The study was approved by the Ethics Committee of the University of Crete. After complete description of the study to the subjects, written informed consent was obtained.

2.2. Genotyping

Blood DNA was extracted using the Flexigene DNA kit (Qiagen, Valencia, CA). DRD4 exon III genotypes were determined with polymerase chain reaction, using forward, 5′-CTCATGCTGCTGCTCTACTG-3′ and reverse, 5′-CAGTGTAGATGACGGGGTTG-3′. PCR amplification was carried out in a final reaction volume of 25 μl containing 100–125 ng of genomic DNA, 75 mM Tris–HCl, 20 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 200 mM dNTPs mix, 10 pmol of each primer, 1 M Betaine and 0.5 U Taq Polymerase (Fermentas, Ontario, Canada). The cycling conditions were (i) initial denaturation at 95 °C for 3 min, (ii) 35 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 45 s and extension at 72 °C for 90 s followed by (iii) a final extension at 72 °C for 10 min. PCR products were separated by 12% polyacrylamide gel electrophoresis and visualized following ethidium bromide staining. For confirmation, sequence analysis of 10 samples was carried out in Applied Biosystems 3100 Genetic analyzer. Based on previous studies and molecular work suggesting that the 7R allele is distinct from the 2- to 6-repeat alleles (Ding et al., 2002), genotypes were classified into two groups according to the presence or absence of the 7R allele. Of the 118 individuals studied, 24 had at least one copy of the 7R allele, corresponding to 20.3% of the sample.

2.3. Personality questionnaires

All subjects were administered the Tridimentional Personality Questionnaire (TPQ), which evaluates four personality dimensions of temperament – novelty seeking, harm avoidance (HA) and reward dependence (RD) which are hypothesized to be based on distinct neurochemical and genetic substrates (Cloninger et al., 1993).

2.4. Cognitive assessment

2.4.1. Iowa Gambling Task (IGT) (Bechara et al., 1994)

Participants were instructed to select one card at a time from four decks (A, B, C, D) displayed on the screen in order to win "pretend" money. Unknown to the subjects, decks A and B were associated with high monetary rewards but also high penalties (monetary loses) while decks C and D had lower rewards but also lower penalties. The win or loss associated with the selection of a card appeared visually on the screen. Across 100 trials, more choices from the decks C and D lead to a net gain while choosing from the other two decks resulted in greater loss. Dividing card selections into 5 blocks of 20 allowed us to determine the rate of learning over the course of the task. Scores were (a) total numbers of cards selected from advantageous decks C and D minus total numbers of cards selected from "risky" decks A and B, with a higher score indicating superior performance (b) total money won (c) overall learning defined as the difference between block 5 and block 1 in the number of advantageous minus disadvantageous card selections.

2.4.2. Stockings of Cambridge (SoC) (Owen, Downes, Sahakian, Polkey, & Robbins, 1990)

Subjects were asked to rearrange in the minimum possible number of moves, "balls" presented in "socks" in the lower half of the screen such that their positions match a target arrangement in the upper half. The test presents the subject with easy 2- and 3-move and harder 4- and 5-move problems. Subjects are asked to plan the complete sequence of moves required to solve the problem prior to their first move. Initial thinking time (ITT) prior to execution of the first move, subsequent thinking time (STT) for the execution of all subsequent moves, and problems solved in minimum moves are recorded. Poor performance in this test translates into shorter ITT (less time planning), and/or longer STT (more time executing the solution) with less perfect solutions.

2.5. Affective startle modulation

This was performed in a separate session, on all 24 L-DRD4 subjects however, because of documented age effects on startle (Ellwanger, Geyer, & Braff, 2003) and startle reactivity to unpleasant pictures (Smith, Hillman, & Duley, 2005) we included only 24 S-DRD4 individuals matched 1:1 for age ($F_{age} = 0$, $p = 1$). All subjects had a hearing threshold greater than 40-dB at 1 kHz. Startle testing and scoring was blind to subjects' genotype. Our equipment, set up and standard protocol regarding caffeine and nicotine consumption have been described in detail previously (Bitsios, Giakoumaki, & Frangou, 2005). Subjects rated their mood and feelings using 10-cm visual analogue scales (VAS) before and after startle testing. Ratings for valence and arousal of the International Affective Picture System (IAPS) pictures were obtained post-testing with the Self-Assessment Manikin (SAM) (Lang, 1980). The session started with a 4-min acclimation period. All subjects were then presented with 54 pictures [18 pleasant (babies, family and love scenes), 18 unpleasant (mutilated bodies, angry faces and snake attacks), 18 neutral (household objects and mushrooms)], each for 6 s, taken from the IAPS.[2] Of these, 36 pictures (12/valence type)

[2] IAPS numbers for neutral pictures used in the experiment are: 2200, 5500, 5510, 7000, 7002, 7009, 7010, 7020, 7040, 7050, 7060, 7080, 7090, 7100, 7150, 7170, 7175, 7500; for the pleasant pictures: 1650, 2040, 2050, 2057, 2080, 2150, 2160, 4650,

were accompanied by an acoustic probe (50 ms, 104-dB white noise over 70-dB background noise running throughout). Of the 12 probes presented during each affective category, 4 probes were presented at 300 ms after picture onset, 4 at 3000 ms and 4 probes at 4500 ms after picture onset. Six pictures of each valence type were not accompanied with a startle probe to increase unpredictability of startle stimuli. Unpredictability was also increased by another 15 startle probes occurring randomly during inter-picture intervals (6 s blank screens). EMG of the orbicularis oculi was recorded from the left eye. Trial exclusion and scoring criteria were identical to those used in previous studies (Kaviani et al., 2004). Subjects' exclusion criteria from further analysis were negligible startle responses (mean amplitude < 10 mV) and/or more than two discarded trials per trial type. Seven subjects from the L-DRD4 group were excluded based on these criteria, leaving 17 subjects from each group for analysis. Basal EMG activity and startle amplitude were examined. Basal EMG activity was defined as the mean EMG activity to the three different probe onsets, each one averaged across their presentations in each valence. Startle amplitude responses were averaged across the same probe onsets/valence type.

2.6. Statistical analysis

Analyses of variance (ANOVAs) or the non-parametric Mann–Whitney test as appropriate and χ^2-tests were performed to examine genotype differences in demographic, personality and cognitive variables. More detailed analyses of the IGT and SoC data included 2 × 5 (group-by-block) and 2 × 4 (group-by-difficulty level) ANOVAs respectively. Startle measures were analyzed with 2 × 3 × 3 (group-by-valence-by probe onset) ANOVA. Post–pre-testing (Δ)VAS mood ratings were compared using one-way ANOVAs. Picture ratings for valence and arousal were analyzed with 2 × 3 (group-by picture type) ANOVA. Effect sizes (partial η^2) are reported for significant results.

3. Results

Ninety-four and twenty-four subjects were classified as S-DRD4 and L-DRD4 respectively, a distribution consistent with Hardy–Weinberg expectations ($\chi^2 = 1.75$, df = 2, $P = 0.42$).

3.1. Personality and cognitive testing

Table 1 shows that NS was higher and RD and HA were lower in the L-DRD4 group and that there were no group effects on demographic variables or SoC performance. The ANOVAs for ITT and STT, revealed the expected significant main effect of difficulty level (ITT: $F(3,345) = 30.1$, $p < 0.001$, $\eta^2 = 0.207$; STT: $F(3,345) = 7.1$, $p < 0.001$, $\eta^2 = 0.06$) but not group or interaction (all F values < 1). Table 1 shows that in the IGT task, the L-DRD4 individuals selected more cards from the "risky" decks A and B, won less money and their overall learning was worse compared to the S-DRD4 individuals. Fig. 1 shows the progressive switch toward more advantageous choices in the two groups. The ANOVA revealed significant main effects of genotype group [$F(1,116) = 11.8$; $p < 0.001$; $\eta^2 = 0.092$] and block [$F(4,464) = 15.3$; $p < 0.001$; $\eta^2 = 0.117$] and a significant interaction [$F(4,464) = 5.5$; $p < 0.003$; $\eta^2 = 0.045$]. Inclusion of smoking status as a between-subject factor showed no main effect of smoking or interactions involving smoking status (all F values < 1). NS correlated negatively with CD-AB difference score in the L-DRD4 group ($r = -0.519$, df: 24, $p = 0.009$).

3.2. Startle testing – subjective ratings

The group means for ΔVAS mood and affective picture ratings are shown in Table 2. There were no group differences in any of the ΔVAS mood ratings [$F_{\text{alertness}}(1,35) = 2.0$, $p > 0.17$; $F_{\text{anxiety}}(1,35) = 1.8$, $p > 0.19$; $F_{\text{discontentment}}(1,35) = 1.8$, $p > 0.19$). Picture ratings showed the expected main effects of valence [$F(2,68) = 175.3$; $p < 0.001$)] and arousal [$F(2,68) = 99.3$; $p < 0.001$)] but not group or interaction (Fs < 1). Significant linear trends in the case of valence ratings [$F(1,34) = 362.7$; $p < 0.001$] confirmed the categorization of pictures

4660, 7330, 8030, 8080, 8502, 8540 and for females 4490, 4520, 4530, 4550 while for males 4002, 4180, 4210, 4232; for the unpleasant pictures: 1030, 1111, 1270, 2120, 3051, 3062, 3063, 3064, 3100, 3102, 3140, 3150, 3210, 6242, 6570, 9050, 9405, 9810.

Table 1
Demographic, personality and cognitive performance characteristics for each DRD4 VNTR genotype group (mean ± S.D.).

	S-DRD4 ($n = 94$)	L-DRD4 ($n = 24$)	F or U or x^2	p
Age (years)[a]	25.9 ± 4.0	26.3 ± 4.9	1070	>0.8
Estimated IQ	113.7 ± 10.2	114.2 ± 11.0	<1	>0.9
Education (years)[a]	16.9 ± 2.4	17.6 ± 2.7	901	>0.2
Smokers/non-smokers[b]	43/51	11/13	0.0	>0.9
Smokers: cigarettes/day	16.2 ± 6.7	20.1 ± 9.9	2.4	>0.1
TPQ				
Novelty seeking[a]	10.03 ± 3.6	12.2 ± 3.9	515.0	<0.001
Reward dependence	9.1 ± 2.6	8.1 ± 3	5.1	<0.03
Harm avoidance	8.5 ± 5.1	6.4 ± 4.6	3.2	<0.08
IGT				
Total CD – AB	16.4 ± 25.8	−2.4 ± 15.4	11.7	<0.001
Money won	1630.8 ± 1206	977.1 ± 716.7	6.4	<0.012
Overall learning[a]	9.7 ± 11.1	2.4 ± 7.9	701.5	<0.004
SoC				
Mean ITT (ms)[a]	8787.1 ± 6260	9189.9 ± 4316	999.0	>0.3
Mean STT (ms)[a]	809.6 ± 1046.6	1393.8 ± 2295	961.0	>0.2
Problems solved[a]	9.7 ± 1.9	9.7 ± 1.7	1078	>0.7

TPQ: Tridimensional personality questionnaire; IGT: Iowa Gambling Task; Total CD – AB refers to total numbers of cards selected from advantageous decks C and D minus the total numbers of cards selected from ("risky") decks A and B; Overall learning refers to the difference between block 5 and block 1 in the number of advantageous minus disadvantageous card selections; SoC: Stockings of Cambridge; ITT: initial thinking time; STT: subsequent thinking time.

[a] For this measure, the overall distribution of the score differed from normality, and the equivalent non-parametric Mann–Whitney procedure was applied.

[b] Chi square comparison.

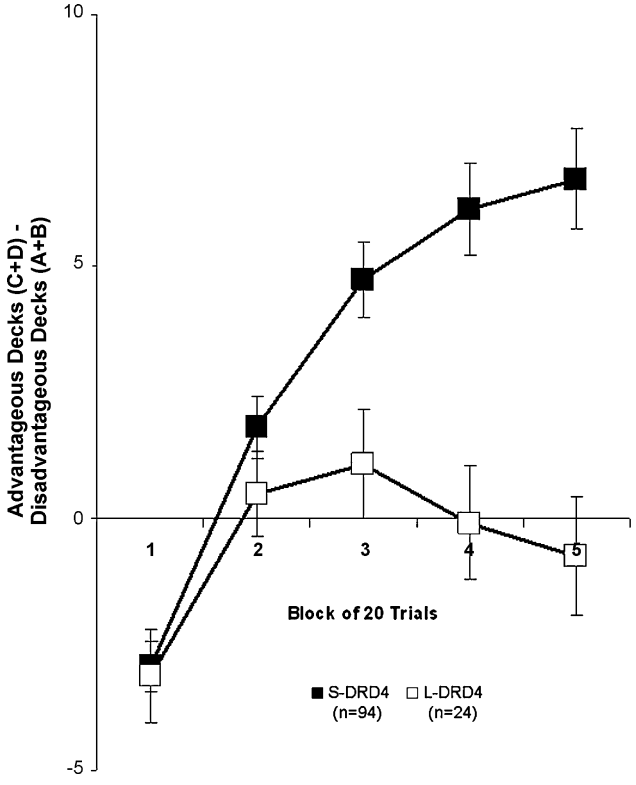

Fig. 1. Numbers of cards selected from advantageous decks C and D minus the numbers of cards selected from the "risky" decks A and B (CD – AB) per block of 20 trials, for the two genotype groups in the IGT test. Squares represent group means and bars represent SEM.

Table 2
VAS mood and affective picture ratings for the two genotype groups (mean ± S.D.).

	S-DRD4 (n = 19)	L-DRD4 (n = 17)	Entire group
Mood ratings post–pre-test differences			
Anxiety	0.28 ± 2.4	−0.6 ± 1.1	−0.13 ± 1.9
Discontentment	−0.06 ± 0.8	−0.8 ± 2.1	−0.4 ± 1.6
Alertness	−0.05 ± 0.6	0.2 ± 0.4	0.05 ± 0.5
Affective picture ratings: valence			
Pleasant	6.4 ± 1.0	6.4 ± 0.9	6.4 ± 0.87
Neutral	3.6 ± 1.3	3.3 ± 1.3	3.55 ± 1.3
Unpleasant	2.4 ± 0.8	2.2 ± 1.1	2.35 ± 0.97
Affective picture ratings: arousal			
Pleasant	4.5 ± 1.5	4.9 ± 1.5	4.6 ± 1.5
Neutral	2.0 ± 1.1	2.2 ± 1.0	2.1 ± 1.0
Unpleasant	5.0 ± 1.5	5.6 ± 1.1	5.3 ± 1.4

as pleasant, neutral and unpleasant and significant quadratic trends in the case of arousal ratings [$F(1,34) = 270.0$; $p < 0.001$] confirmed that both pleasant and unpleasant pictures were more arousing than neutral ones, as expected.

3.3. Affective startle modulation

The 3 × 3 × 2 (valence-by-probe onset-by-group) ANOVA of the baseline EMG activity data revealed no significant effects (all p values > 0.122). Fig. 2 shows the startle amplitude for the three affective valences and three different probe onsets in the two genotype groups. A 3 × 3 × 2 (valence-by-probe onset-by-group) ANOVA showed significant main effects of group [$F(1,34) = 20.6$; $p < 0.001$; $\eta^2 = 0.377$], valence [$F(2,68) = 12.01$; $p < 0.001$; $\eta^2 = 0.261$], and probe onset [$F(2,68) = 31.7$; $p < 0.001$; $\eta^2 = 0.483$]. There were also significant valence-by-group [$F(2,68) = 5.04$; $p = 0.009$; $\eta^2 = 0.129$], probe onset-by-group [$F(2,68) = 12.6$; $p < 0.001$; $\eta^2 = 0.271$] and a significant three-way interaction [$F(4,136) = 2.7$; $p = 0.05$; $\eta^2 = 0.074$)].

Separate follow up ANOVAs in each genotype group showed that the valence and probe onset main effects were much more robust in the S-DRD4 group (η^2 range: 0.224–0.666, all p values < 0.001) compared to the L-DRD4 group (η^2 range: 0.08–0.53, all p values < 0.037). The valence-by-probe interaction was significant in the S-DRD4 group [$F(4,72) = 5.2$; $p < 0.001$; $\eta^2 = 0.224$] but critically, this interaction was not significant in the L-DRD4 group [$F(4,64) = 1.4$; $p > 0.2$]. Follow up of the significant valence-by-probe interaction in the S-DRD4 group with separate ANOVAs for each probe, showed significant effect of valence with the late probe (4500 ms) only [$F(2,36) = 10.9$; $p < 0.001$; $\eta^2 = 0.377$]. Bonferoni-corrected paired sample t-tests showed that startle was significantly increased in the unpleasant compared to neutral ($p < 0.014$) and pleasant pictures ($p < 0.001$) and it was significantly attenuated in the pleasant compared to neutral pictures ($p < 0.012$). Similar post-hoc analyses for the L-DRD4 group showed that late-probe startle differed only between pleasant and unpleasant ($p = 0.007$) pictures (all other comparisons $p > 0.06$).

Mean startle across the blank screens and across all affective trials was lower in the L-DRD4 group (Cohen's d for independent non-equal groups: 1.46 and 1.56 respectively). Exploratory Pearson's correlations revealed inverse relationships between NS and mean startle across all affective ($r = -0.583$, df: 17; $p < 0.01$), pleasant ($r = -0.659$, df: 17; $p < 0.004$) or blank screens ($r = -0.732$, df: 17; $p < 0.001$) in the L-DRD4 but not in the S-DRD4 group (all p values > 0.9).

4. Discussion

4.1. Decision making

This is the first study to demonstrate that high NS related to the L-DRD4 VNTR is associated with risky and potentially harmful decision making. Compared to the S-DRD4 individuals, the L-DRD4 subjects had higher picks from the risky A and B decks and the number of their risky decisions correlated highly with NS. As a result of their disadvantageous choices, they lost more money and it was evident that they failed to learn to modify their behavior based on its consequences. Importantly, L-DRD4 individuals had no difficulty in planning ahead and executing the solutions in the cognitive decision making task, reaching the same amount of perfect solutions as S-DRD4 participants.

It is interesting that the L-DRD4 VNTR differentiates between two tests of planning ability, one of which involves planning based on emotional processing of incentive information for decision making. Such dissociation indicates that L-DRD4 status may not compromise problem solving in cognitive decision making but may be associated with impaired performance when emotional/motivational feedback is required for decision making. It is possible that this is because the prefrontal neural systems underlying these two tests may be different. Indeed, functional

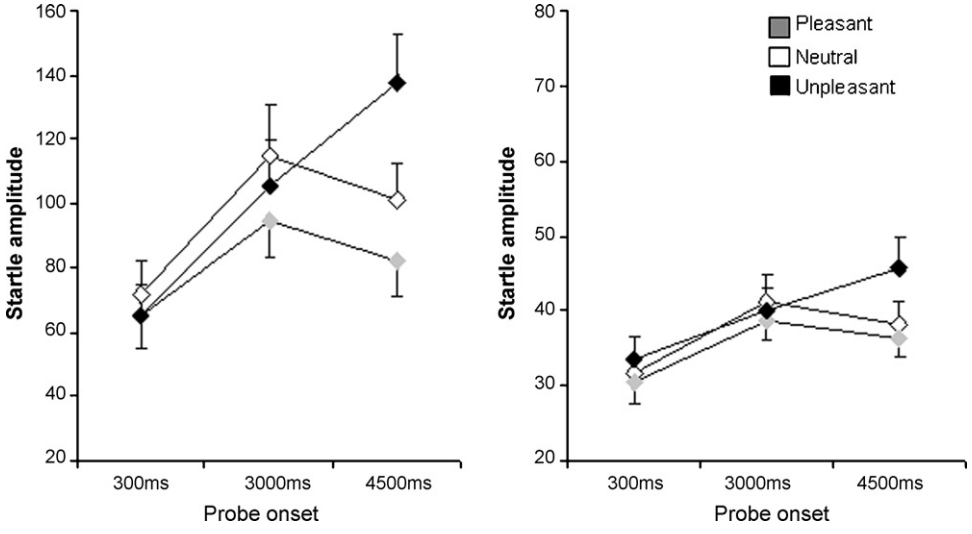

Fig. 2. Startle amplitude in digital units for the three affective valences and three different probe onsets in the S-DRD4 (left panel) and the L-DRD4 (right panel) genotype groups. Please note the difference in scale. Squares represent group means and bars represent SEM.

neuroimaging studies have shown that the SoC depends more on the DLPFC (Owen et al., 1990) while the IGT depends more on the ventrolateral PFC (VLPFC) and orbitofrontal cortex (OFC) (Bechara, Damasio, Tranel, & Anderson, 1998). Our findings are in accordance with the fronto-limbic distribution of DRD4 and the connectivity of the OFC and medial PFC regions with the limbic areas (Meador-Woodruff et al., 1996). In the rat, intra PFC infusion of DRD4 antagonists produced exploratory behaviors and anxiolytic effects (Shah, Sjovold, & Treit, 2004) and abolished the acquisition of olfactory fear conditioning (Laviolette, Lipski, & Grace, 2005). In animals therefore, impaired decision making and increased responses to novelty have been attributed to a reduction in the control which fear-related stimuli exert over the suppression of behavior, a process controlled by DRD4 in the PFC (Floresco & Magyar, 2006). The use of the affective startle modulation paradigm allowed us to examine these possibilities.

4.2. Affective startle modulation

We replicated the well-documented linear increase in startle amplitude across the affective picture categories, as evidenced by the significant and linear effect of valence. Startle responses to early-onset (300 ms) probes were attenuated compared to late-onset probes as evidenced by the significant and linear probe onset effect, replicating previous findings (Bradley, Cuthbert, & Lang, 1993). This early-onset startle attenuation during affective picture viewing is an example of prepulse inhibition, whereby picture onset serves as a prepulse; early-onset startle attenuation has been attributed to the recruitment of non-volitional attentional processes that serve to protect the processing of the (pictorial) prepulse stimulus from the disruptive startle stimulus (Bradley et al., 1993). Although the above effects were weaker in the L-DRD4 group, they were nevertheless present in both groups suggesting preserved attentional processing of and preserved response pattern to affective stimuli in L-DRD4 individuals.

The most important findings were (A) general reduction in startle reactivity in L-DRD4 individuals with a 37.7% of total variance attributable to DRD4 genotype. It seems that startle reactivity is tonically attenuated in L-DRD4 subjects and that this attenuation is greater with higher NS scores, as evidenced by the correlations. Given that startle is an unconditioned reflex response to loud aversive stimuli, our results suggest robust under-responsivity to unconditioned aversive stimuli in L-DRD4 subjects (B) reduced late-onset affective startle modulation in L-DRD4 compared to S-DRD4 individuals as evidenced by the significant three-way interaction, suggesting anomalies in later-onset processes that subserve responses to the valence properties of affective and motivational stimuli. Our results argue for bidirectional reductions in emotional reactivity in L-DRD4 individuals (constricted affect) in agreement with reports of L-DRD4 status on subjective emotional reactivity (Oniszczenko & Dragan, 2005). It is therefore possible that L-DRD4 individuals may appear phenotypically not only "fearless" but also "anhedonic". Interestingly, reduced startle reactivity and late-onset startle attenuation by pleasant pictures have been previously reported in subjects with low HA and in depressive/anhedonic states (Corr, Kumari, Wilson, Checkley, & Gray, 1997; Kaviani et al., 2004), while reduced startle modulation by unpleasant pictures has been reported in psychopathy (Patrick, Bradley, & Lang, 1993).

The groups were highly homogeneous with no state-dependent mood differences and both rated the affective pictures identically. The lack of correspondence between self-reports and objective (startle) measures of emotional reactivity has been observed before in depressed and psychopathic patients (Kaviani et al., 2004). It is possible that startle, an automatic reflex response, is a more accurate and sensitive measure of mood, independent of the slower language-based appraisal processes (Lang, Davis, & Ohman, 2000).

4.3. General discussion

Although our findings were obtained in an ethnically and demographically highly homogeneous cohort of healthy male subjects, the risk of demographic or genetic inhomogeneity was not entirely removed, since we did not control for population stratification or linkage disequilibrium. Also, the startle data were obtained in relatively small samples. Given these limitations, the present data should better be treated as preliminary and they certainly need replication in larger studies utilizing prospective genotyping to follow-up.

This is a first attempt to "decompose" the high NS associated with the L-DRD4 polymorphism, in its cognitive and emotional underpinnings. High NS related to the L-DRD4 VNTR is associated with risky decision making when emotional/motivational feedback is required, while planning and decision making for problem solving is intact. Our data suggest that NS and risky decision making in the L-DRD4 group may be the result of attenuated processing of (a) negative stimuli leading to indifference to the potentially harmful consequences of behavior (b) pleasurable stimuli leading to overcompensation by engagement in risky actions and novel environments. It would be interesting for future fMRI studies to test subjects characterized for their DRD4 status for their performance in decision making tasks and affective picture viewing, and for PET studies to examine DA release in relevant limbic regions (e.g. striatum, amygdala, OFC).

NS has the potential for negative consequences as well as rewards. It has been suggested that the high NS associated with the L-DRD4 VNTR would have great evolutionary importance contributing to major human migratory expansions in the past (Chen et al., 1999; Wang et al., 2004). Indeed, it is conceivable that risk taking with efficient problem solving, under-reactivity to unconditioned aversive stimuli and low emotional reactivity in the face of preserved attentional processing of emotional stimuli may have been advantageous phenotypic characteristics fostering migration and expansion. Low emotional reactivity is associated with high emotional endurance (Oniszczenko & Dragan, 2005) which can afford physical, emotional and mental resilience in the face of adversity in perilous environments. The disadvantageous decision making in L-DRD4 high NS individuals does not necessarily result in dysfunctional behavior, since all our subjects were normal healthy volunteers, with no history or presence of psychiatric illness. It may even be that L-DRD4 genotype may be protective against stress, anxiety and depression by moving attention away from emotional adversity, as an analogue to the psychological term of "denial". More research is required into the role of the L-DRD4 VNTR in executive functions and early information processing as well as in acquisition and extinction of fear and appetitive conditioning.

On the other hand, such implicit disadvantageous decision making in the context of critical or chronically stressful life situations may lead to a vicious cycle of adverse outcome, stress, and further disadvantageous decisions, especially if L-DRD4 subjects are stress-intolerant due to gene–environment or gene–gene (e.g. L-DRD4 by 5-HTTLPR) interactions (Lakatos et al., 2000, 2003). Such a mechanism may be at least one route to disorders of affect and impulsivity in L-DRD4 subjects and indeed, susceptibility for extreme phenotypes with dysregulated affect and impulsivity such as ADHD (Swanson et al., 2007), substance abuse (Kotler et al., 1997) and pathological gambling (Comings et al., 1999), has been reported for L-DRD4 individuals. Studies with patient populations and high risk individuals are required to determine the relationship of such L-DRD4-driven high NS, risky decisions, reduced physiological response to unconditioned aversive stimuli and constricted emotional responses, to risk for these disorders but also for depression and bipolar disorder (Lopez Leon et al., 2005; Serretti & Mandelli, 2008) with which they are highly comorbid.

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