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ORIGINAL INVESTIGATION

Multilevel impact of the dopamine system on the emotion-potentiated startle reflex

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Abstract
Rationale/objectives The pathogenetic mechanism of emotion-related disorders such as anxiety disorders is considered to be complex with an interaction of genetic, biochemical, and environmental factors. Particular evidence has accumulated for alterations in the dopaminergic system—partly conferred by catechol-*O*-methyltransferase (*COMT*) gene variation—and for distorted emotional processing to constitute risk factors for anxiety and anxiety-related disorders.
Methods Applying a multilevel approach, we analyzed the main and interactive effects of the functional *COMT* val158met polymorphism and L-dopa (single-dose 50 mg levodopa and 12.5 mg carbidopa; double-blind, placebo-controlled design) on the emotion-potentiated (unpleasant, neutral, and pleasant IAPS pictures) startle response as an intermediate phenotype of anxiety in a sample of 100 healthy probands (f=52, m=48).
Results The *COMT* 158val allele was associated with an increased startle potentiation by unpleasant stimuli as compared with neutral stimuli irrespective of L-dopa or placebo intervention. *COMT* 158met/met genotype carriers, while displaying no difference in startle magnitude in response to unpleasant or neutral pictures in the placebo condition, showed startle potentiation by unpleasant pictures under L-dopa administration only.
Conclusions The present proof-of-concept study provides preliminary support for a complex, multilevel impact of the dopaminergic system on the emotion-potentiated startle reflex suggesting increased phasic dopamine transmission driven by the more active *COMT* 158val allele and/or a single dose of L-dopa to predispose to maladaptive emotional processing and thereby potentially also to anxiety-related psychopathological states.

Keywords COMT · Catechol-*O*-methyltransferase · Val158met · L-dopa · Genetic

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Introduction

Beside the GABA/glutamate, the serotonin, and the noradrenaline systems, there is accumulating evidence for the dopamine system to influence the pathogenesis of anxiety and anxiety-related disorders on a genetic and a biochemical/neurotransmitter level (cf. Durant et al. 2010).

On a genetic level, the catechol-*O*-methyltransferase (COMT) enzyme, crucially involved in the inactivation of monaminergic neurotransmitters, particularly dopamine and

norepinephrine, has been suggested as one of the most promising candidates in the pathogenesis of anxiety and anxiety disorders. A single nucleotide polymorphism (472G/A) in the *COMT* gene on chromosome 22q11.2 (Winqvist et al. 1992), causes an amino acid change from valine to methionine at position 158 (val158met), with the val allele (472G) conferring an at least 40 % higher COMT activity (Chen et al. 2004; Lachman et al. 1996). The more active *COMT* 158val allele, while decreasing overall dopamine levels in the prefrontal cortex and tonic dopamine levels subcortically, has been shown to increase phasic dopamine transmission and to enhance paleocortical processes such as arousal mediated by an orbital and ventrolateral frontal cortex/amygdala-ventral striatum network, whereas the *COMT* 158met allele, while increasing overall dopamine in the frontal cortex and tonic dopamine levels subcortically, has been suggested to decrease phasic dopamine release in subcortical regions (Bilder et al. 2004). The more active val allele has been reported to be associated with panic disorder (Domschke et al. 2004, 2007; Hamilton et al. 2002; Lonsdorf et al. 2010; Rothe et al. 2006), phobic anxiety (McGrath et al. 2004) and dimensional anxiety-related traits such as neuroticism (Hettema et al. 2008), and harm avoidance (Kim et al. 2006). In contrast, there are also reports demonstrating association of the less active met allele with anxiety-related phenotypes (Eley et al. 2003; Enoch et al. 2003; Olsson et al. 2005; Stein et al. 2005; Woo et al. 2004) or indicating no influence of *COMT* val158met on anxiety disorders or related phenotypes (Ohara et al. 1998; Samochowiec et al. 2004; Wray et al. 2008). Accordingly, imaging genetic studies in healthy probands as well as in patients with panic disorder have suggested amygdala activation during emotional processing to be driven by *COMT* gene variation with, however, diverging support for the direction of the allelic association (val allele: Domschke et al. 2008, 2012a; met allele: Drabant et al. 2006; Smolka et al. 2005).

Also on a biochemical level, the dopamine system has been proposed to crucially modulate anxiety-related mood states. However, corresponding to the equivocal findings regarding the association of *COMT* gene variation with anxiety-related phenotypes as reported above, results regarding the role of dopamine neurotransmission in anxiety are mixed: In animal models, differential anxiety-related effects of dopamine agonist injections have been reported dependent on the site of action with an anxiogenic effect in the limbic system but the opposite effect in the ventral tegmental area (Bartoszyk 1998; Hood et al. 2010; Talalaenko et al. 1994). In humans, L-dopa, as the natural amino acid precursor of the central neurotransmitter dopamine and amphetamine-induced dopamine release have been reported to induce anxiety (Bailer et al. 2012; Murphy 1973), and, despite of their severe motor side effects, dopamine receptor 2 (DRD2) antagonists such as fluspirilene have previously been used in the treatment of generalized anxiety disorder and anxiety states in general (Heinrich and Lehmann 1992; Wurthmann et al. 1995). In social phobia, both dopamine agonist (pramipexole) and antagonist (sulpiride) treatment was associated with significant increases in anxiety, with SSRI treatment reducing anxiety only in the dopamine agonist-induced anxiety states (Hood et al. 2010). Significantly elevated erythrocyte COMT activity has been reported in patients with anxiety states (Shulman et al. 1978), and COMT inhibitors are effectively used in the treatment of anxiety symptoms in Parkinson's disease (Richard et al. 1996).

In order to further elucidate the role of dopamine in anxiety in a multilevel model integrating genetic (*COMT* val158met) and biochemical (L-dopa) factors, in the present proof-of-concept study, we investigated the role of a phasic increase in dopamine levels by a single-dose administration of L-dopa on the background of *COMT* val158met genotype-driven phasic/tonic dopamine transmission in modulating the emotion-potentiated startle reflex as a neurobiologically founded measure of emotional reactivity and an intermediate phenotype of anxiety and anxiety disorders (Grillon and Baas 2003; Vaidyanathan et al. 2009). We hypothesized that an increase in phasic dopamine levels as conferred by a single dose of L-dopa and/or—according to the hypothesis by Bilder et al. (2004)—the *COMT* 158val allele would lead to increased startle magnitudes in response to unpleasant stimuli. Alternatively, given a previously reported association of increased startle responses to unpleasant stimuli with the *COMT* 158met allele (Montag et al. 2008)—possibly driving elevated tonic dopamine levels (Bilder et al. 2004)—L-dopa and the 158met allele might interactively increase startle magnitudes in the unpleasant condition. The presently expected results are hoped to inform the ongoing and highly equivocal discussion on the role of phasic vs. tonic dopamine in the pathogenesis of anxiety.

Methods

Sample

A sample of 115 (male=55, female=60; mean age, 24.57 years; SD, 4.82) unrelated healthy subjects was consecutively recruited at the Departments of Psychiatry, Universities of Muenster and Wuerzburg, Germany, between 2009 and 2012. In order to minimize the risk of ethnic stratification, Caucasian (German) descent was ascertained by Caucasian background of both parents. Current or prior diagnosis of DSM-IV axis I disorders was excluded using the Mini-International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al. 1998). Additionally, anxiety sensitivity (AS), the general tendency to fear anxiety-related symptoms, was recorded by the German version of the Anxiety Sensitivity

Index (ASI; Alpers and Pauli 2001). To exclude any neurological or other somatic disorders, subjects underwent a physical and neurological examination in a screening session 1 week before the experiment, where additionally heart activity (electrocardiogram) and basic blood parameters were checked. Further exclusion criteria comprised L-dopa or lactose intolerance, high and frequent caffeine consumption (more than three cups of coffee per day), illegal drug consumption (assessed by a urine drug screening for amphetamine, barbiturates, benzodiazepines, cocaine, ecstasy, methamphetamine, methadone, opiates, tricyclic antidepressants, tetrahydrocannabinol), alcohol consumption of more than 140 g/week (equivalent to about 15–20 UK units of alcohol), daily smoking of more than 20 cigarettes a day, daily use of any medication (except for hormonal contraception), pregnancy or breast feeding, less than a high school education, age under 18 and over 50 years, and left handedness. Subjects were asked not to smoke, consume alcohol (assessed by a breath alcohol test), or take any medication for at least 24 h prior to the investigation. All female probands had to be using a reliable method of hormonal contraception (either oral contraceptives or hormonal intrauterine devices). The protocol was approved by the ethics committees of the Universities of Muenster and Wuerzburg, Germany, and written informed consent was obtained from all subjects during the screening session.

Genotyping

Subjects were genotyped for the functional val158met polymorphism in the *COMT* gene according to published protocols (Domschke et al. 2008). According to previous findings, subjects were pre-stratified into valine allele carriers (val/val and val/met) and met/met homozygotes (Domschke et al. 2008; Lonsdorf et al. 2010).

L-dopa intervention

The study utilized a one-session, double-blind, placebo-controlled between-subject design. All experimental sessions were conducted from 08:15 to 12:00 a.m. After a negative drug and pregnancy urine test, all electrodes were fixed and checked for impedances below 5 kΩ. L-dopa intervention was performed by a single oral administration of Nacom® (50 mg levodopa and 12.5 mg carbidopa, MSD, Haar). Nacom® was administered in white opaque gelatin capsules, placebo capsules contained mannitol and aerosil (99.5:0.5) according to the German Drug Law (Arzneimittelgesetz (AMG)). At about 09:00 a.m.—after the first self-report anxiety measurement—subjects were given a placebo or L-dopa capsule with a glass of water.

Emotionally relevant stimuli

Twenty-four emotionally threatening unpleasant images taken from the International Affective Picture System (IAPS; Lang et al. 2005) were selected as anxiety-relevant emotional cues along with 24 neutral and 24 pleasant IAPS pictures.[1] Ninety-five percent of all pictures were exactly the same for both genders, while different erotic pictures were chosen for men and women to ensure comparable valence and arousal levels (see footnote).

Objective outcome measure: emotion-potentiated startle paradigm

At the assumed maximum plasma level of L-dopa according to information provided by the manufacturer (60 min after administration), the emotion-potentiated startle experiment was started at 10:00 a.m. In order to get subjects used to the startle stimulus (50 ms of 95 dB white noise with an instantaneous rise-time presented via Bose® Around-Ear Headphones) and to prevent outlier startle responses during the critical trials, eight startle stimuli at random intervals of 1 to 12 s were presented. The startle experiment per se consisted of three blocks of 24 unpleasant, neutral or pleasant IAPS pictures, respectively, as described above and 3-min breaks between the blocks. An experimental block contained eight pictures of each of the three categories in random order with the constraint that no two of the same type (unpleasant, neutral, or pleasant) were presented successively. During the experiment in a dimly lit room, subjects sat in a recliner which was separated by a room divider from the experimenter. Visual stimuli were presented for 8 seconds (intertrial interval (ITI): mean=21 s; range=16.5–25.5 s) on a 19 in. LCD computer screen approximately 1 m away from the subject. Startle probes were administered 2.5, 4, or 5.5 s after picture onset during picture presentation and 10 as well as 12 s after picture offset during the ITI. In each block, as well as in the overall experiment, 75 % of all trials contained startle probes during picture presentation (evenly distributed across each picture category), 12.5 % of all trials contained startle probes during the ITI, and 12.5 % of the trials did not contain any startle probe.

Electromyogram (EMG) activity of the M. orbicularis oculi, which is responsible for eyelid closure, was measured as a

[1] Unpleasant IAPS pictures: 3000, 3053, 3170, 3102, 9410, 3080, 6313, 3120, 3130, 3071, 3100, 3010, 3060, 3064, 3140, 3110, 3150, 9300, 2800, 3030, 6540, 9252, 9250, and 9040; neutral IAPS pictures: 2200, 2880, 5510, 5531, 7002, 7004, 7006, 7009, 7010, 7025, 7034, 7050, 7080, 7090, 7100, 7130, 7175, 7185, 7217, 7224, 7950, 2215, 5535, and 7031; pleasant IAPS pictures: 1710, 2091, 2160, 2216, 2340, 2345, 4608, 4626, 4641, 8120, 5623, 5831, 5833, 8041, 8370, 8200, 8210, 8461, 8496, and 5814; for men: 4220, 4290, 4607, and 4680; and for women: 4550, 4658, 4687, and 5631.

commonly used variable for startle reaction recordings in humans (Blumenthal et al. 2005). For this purpose, two transparent pediatric 13-mm electrodes were placed under the left eye. The reference electrode was placed on the forehead, 2 cm beneath the hairline, and the ground electrode was placed on the processus mastoideus behind the left ear (sintered Ag/AgCl electrodes). EMG activity was recorded by V-Amp 16 (Brain Products GmbH, Gilching, Germany), a 16 channel DC amplifier system using the BrainVision Recorder Software (V-Amp Edition 1.10, Brain Products GmbH, Gilching, Germany). Sampling rate was 1000 Hz, and an online notch filter of 50 Hz was applied. Stimuli were presented using the software package Presentation (v13.0, Neurobehavioral Systems, Albany, CA). BrainVision Analyzer 2 (Brain Products GmbH, Gilching, Germany) was used as offline analyzing software, with which the signals were rectified, filtered (low cutoff, 28 Hz; high cutoff, 500 Hz; notch, 50 Hz) and smoothed (using a time constant of 50 ms). Startle magnitude was quantified as the difference between the highest peak 21 to 200 ms after and the average during 50 ms before startle probe presentation. Startle data were checked for zero responses and artifacts in each subject. Startle reactions with no detectable responses (<5 μV) were scored as zero. Artifacts were defined as spontaneous eye-blinks during baseline or within 20 ms after startle probe onset and scored as missing values. Subjects were excluded from data analysis when having too many zero responses (more than 2.5 standard deviations above mean number of zero responses) or less than three valid startle responses in one picture category. All startle magnitudes were T transformed within subjects (to the overall mean including zero responses) in order to assure comparability of the data (Blumenthal et al. 2005; Muhlberger et al. 2008; Pauli et al. 2010). Response probability data for the three-picture categories (unpleasant, neutral, pleasant) classified by intervention, gender, and genotype were calculated according to Blumenthal et al. (2005) by "the total number of detected responses divided by the total number of eliciting stimuli presented (after adjusting for trials contaminated by artifact)."

After the experiment, electrodes were removed, and subjects were given the possibility to clean their faces from the electrode paste and have a short break. Finally, each subject had to rate all pictures in a free-viewing condition by valence (1=highly pleasant, 9=highly unpleasant) and arousal (1=exited, 9=calm) using Self-Assessment-Manikin (SAM) scales (Lang et al. 2005). At about 12:00 a.m., subjects were discharged by a physician and paid an allowance of 100 €; ca. 72 h after discharge, subjects were contacted by telephone to ask for possible side effects as a safety measure.

Subjective outcome measures: VAS and POMS

Subjects were asked to rate their anxiety level by placing a mark on a visual analog scale (VAS) consisting of a 100 mm horizontal line labeled "not at all anxious" (score of 0) and "extremely anxious" (score of 100). Additionally, the 35-item German version of the Profile of Mood States (POMS) was administered, on which subjects report their current mood on a 7-point scale from "not at all" (score of 0) to "extremely" (score of 6) (Biehl et al. 1986; McNair et al. 1992). Four categories have been distinguished within the German version of the POMS: (1) "depression-anxiety," ((2) "fatigue," (3) "vigor," and (4) "hostility" (Albani et al. 2005). In the present study, the subscale "depression-anxiety" was used as a subjective measure of anxiety. VAS and POMS measurements were taken at three points in the course of the study: (1) before the respective intervention (L-dopa/placebo), (2) at the anticipated maximum L-dopa plasma level according to the manufacturer (60 min), and (3) after emotion-potentiated startle.

Statistical analysis

Sample characteristics were evaluated by χ^2 tests for genotype (*COMT* val/val and val/met vs. met/met genotypes) and gender with intervention (L-dopa versus placebo) as between-subject factor as well as by one-way analyses of variance (ANOVAs) for age and anxiety sensitivity with intervention, genotype and gender as between-subject factors.

VAS and POMS ratings were analyzed with ANOVAs for repeated measures with genotype and intervention (L-dopa vs. placebo) as between-subject factors and measurement time (before substance intake, 1 h after substance intake and after the startle experiment) as within-subject factor. Baseline startle response (assessed in the ITIs) was analyzed by ANOVA for repeated measures with "block" (the 12 ITI startle responses were divided into 4 blocks (T1 to T4) each being the mean of three consecutive startle responses) as a within-subject factor and genotype and intervention as between-subject factors. Picture ratings, which were conducted at the end of the startle experiment, and picture viewing times—defined as times between picture onset and consecutive ratings—were analyzed using ANOVA for repeated measures with genotype and intervention as between-subject factors and picture category (unpleasant, neutral, pleasant) as within-subject factor. Pairwise comparisons of picture valence or time of measurement were assessed by using post-hoc *t* tests.

According to a priori hypotheses, the main multilevel analysis of emotion-potentiated startle response was performed by using ANOVA for repeated measures with genotype and intervention as between-subject factors and picture category (unpleasant, neutral, pleasant) as within-subject factor. Picture categories were further analyzed by post-hoc univariate ANOVAs.

A further explorative analysis was performed by using gender as an additional independent variable in an ANOVA for repeated measures with genotype, intervention, and gender as between-subject factors and picture category (unpleasant, neutral, pleasant) as within-subject factor.

Alpha level was set at 5 % using Greenhouse-Geisser corrections where appropriate.

Results

Sample characteristics

Fifteen subjects of initially 115 recruited subjects showed too many zero startle responses or less than three valid startle responses in one-picture category (see "Methods") and were therefore excluded from further analyses.

The remaining sample of 100 subjects was equally distributed regarding genotype (*COMT* val/val and val/met vs. met/met genotypes) and gender across intervention groups (L-dopa vs. placebo; both $\chi^2(1)<.38$, $p>.54$; see Table 1).

Factorial ANOVA revealed that neither genotype nor intervention condition groups differed in age (all F (1, 92)<1.14, $p>.28$), but there was a statistical trend for males being slightly older than female probands (men: mean age= 25.4 years, women: mean age=23.6 years; F (1, 92)<3.47, $p=.07$). For AS, factorial ANOVA revealed no effects of genotype, intervention condition or gender groups (F (1, 88)<1.89, $p>.17$)[2] (see Table 1). Mean anxiety sensitivity was 13.4 (SD, 6.5; range, 1–44; median, 13), which is lower than the expected mean AS of about 18 in a nonclinical population (Peterson and Reiss 1992).

Picture ratings and viewing times

Valence ratings corresponded to a priori categories (F (2, 192)= 4617.01, $p<.001$; linear trend, F (1, 96)=5839.06, $p<.001$; pleasant>neutral>unpleasant: all t (99)>|40.94|, $p<.001$). There were no direct or interaction effects of genotype or intervention on valence ratings (all F (2, 192)<0.32, $p>.63$).

Arousal ratings were affected by picture valence (F (2, 192)= 708.06, $p<.001$), with highest ratings for unpleasant pictures, followed by relatively high ratings for pleasant pictures and low ratings for neutral pictures (all t (99)>|12.90|, $p<.001$). There were no direct or interaction effects of genotype or intervention on arousal ratings (all F (2, 192)<0.61, $p>.54$).

Analysis of picture viewing times revealed no significant main effect of picture category, intervention, or genotype, nor additional interactions of genotype or intervention (all F (2, 192)<1.09, $p>.32$).

Response probability, habituation, and baseline startle response

Response probability data as classified by intervention, genotype, and gender were distributed as follows: (1) unpleasant pictures: placebo, 79 %; L-dopa, 82 %; *COMT* met/met, 77 %; *COMT* val/val and val/met, 82 %; males, 76 %; females, 84 %; (2) neutral pictures: placebo, 74 %; L-dopa, 78 %; *COMT* met/met, 76 %; COMT val/val and val/met, 76 %; males, 71 %; females, 80 %; and (3) pleasant pictures: placebo, 73 %; L-dopa, 81 %; *COMT* met/met, 73 %; COMT val/val and val/met, 79 %; males, 72 %; females, 81 %.

Analysis of the startle response during the intertrial interval as a measurement of habituation revealed a significant effect of "block" on startle magnitude irrespective of intervention group (F (3, 231)=40.49, $p<.001$; linear trend, F (1, 77)= 108.80, $p<.001$). Mean baseline startle magnitudes declined across the four blocks (all t (80)>2.04, $p<.05$).[3] There was no genotype or intervention effect or a genotype x intervention interaction effect on baseline startle response times (all F (3, 231)<1.02, $p>.38$).

Startle modulation: influence of picture category

ANOVA revealed a significant main effect of picture category on startle response across both intervention groups (F (2, 192)= 28.32, $p<.001$), which was due to increasing startle magnitudes from pleasant to neutral to unpleasant pictures (linear trend, F (1, 96)=55.43, $p<.001$; each t (99)>3.47, $p<.002$).

Genotype effects on startle modulation

There was no significant main effect of genotype on mean startle magnitudes across both intervention groups (F (1, 96)= 1.76; $p=.19$), and no significant interaction effect of genotype (*COMT* val/val and val/met vs. met/met genotypes) and picture category could be discerned (F (2, 192)=1.24, $p=.29$).

Effects of intervention (L-dopa vs. placebo) on startle modulation

There was no significant main effect of intervention (L-dopa vs. placebo) on mean startle magnitudes (F (1, 96)=0.88; $p=.35$). However, a significant interaction effect of intervention (L-dopa vs. placebo) and picture category was observed (F (2, 192)= 4.25, $p=.02$). Post-hoc separate analyses for each intervention group revealed that in the L-dopa group startle magnitudes differed in all picture categories (unpleasant>neutral>pleasant; all t (48)>2.21, $p<.04$). In the placebo group, no difference in startle magnitude between unpleasant and neutral pictures could be discerned (t (50)=1.12, $p=.27$), while startle magnitudes differed significantly between unpleasant and pleasant as well as between neutral and pleasant pictures (unpleasant>pleasant and neutral>pleasant; both t (50)>3.11, $p<.004$; see Fig. 1).

[2] Four additional subjects had to be excluded from this analysis because of missing results of the Anxiety Sensitivity Index.

[3] Nineteen additional subjects had to be excluded from this analysis because of consecutive zero responses leading to a missing mean in one of the four blocks (see "Methods").

Table 1 Sample characteristics

Intervention	COMT val158met	Gender										Total				
		Men					Women[a]									
		N=16	*N*=10	*N*=14	*N*=8	*N*=48	*N*=15	*N*=10	*N*=18	*N*=9	*N*=52	*N*=31	*N*=20	*N*=32	*N*=17	*N*=100
Placebo	val/val, val/met	Age=25.4 (8.05) ASI=13.9 (6.91)					Age=23.7 (3.35) ASI=13.8 (6.33)					Age=24.6 (6.19) ASI=13.9 (6.50)				
	met/met		Age=25.6 (4.91) ASI=11.6 (4.20)					Age=23.2 (2.15) ASI=9.7 (4.37)					Age=24.4 (5.77) ASI=10.7 (4.28)			
Verum (L-dopa)	val/val, val/met			Age=26.0 (3.94) ASI=13.0 (7.43)					Age=24.3 (2.27) ASI=15.4 (8.94)					Age=25.0 (3.17) ASI=14.3 (8.25)		
	met/met				Age=24.0 (3.07) ASI=13.3 (3.54)					Age=22.3 (1.87) ASI=15.0 (5.32)					Age=23.11 (2.57) ASI=14.2 (4.52)	
Total						Age=25.4 (6.23) ASI=13.0 (5.99)					Age=23.6 (2.58) ASI=13.7 (7.03)					Age=24.4 (4.76) ASI=13.4 (6.53)

Standard deviation in parentheses; genotype distribution: *COMT* met/met=37, *COMT* val/val=49, *COMT* val/met=14

ASI anxiety sensitivity index

[a] *N*=13 in follicular phase, *N*=35 in luteal phase (missing data for *N*=4 female probands)

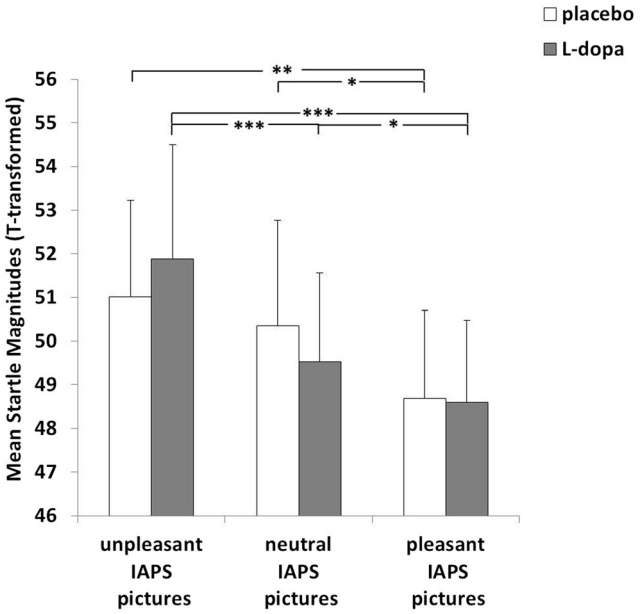

Fig. 1 Interaction of intervention (L-dopa vs. placebo) and picture category. A significant interaction effect of intervention (L-dopa vs. placebo) and picture category was observed (F (2, 192)=4.25, p=.02); error bars symbolize standard deviations; *p≤.05; **p≤.01; ***p≤.001—significance level

Genotype×intervention effects on startle modulation

A significant interaction between two-group genotype (*COMT* val/val and val/met vs. met/met), intervention and picture category was discerned (F (2, 192)=4.18, p=.02; see Fig. 2), which could also be shown for the three-group genotype design (*COMT* val/val vs. val/met vs. met/met) (F (2, 188)=3.49, p=.01), but not for the recessive genotype design when assuming the *COMT* val allele to be the risk allele (*COMT* val/val vs. val/met and met/met) (F (2, 192)=0.22, p=.79). Post-hoc separate analyses for each genotype group (*COMT* val/val and val/met vs. met/met) revealed that val allele carriers did not display a significant interaction of picture category and intervention (F (2, 122)=0.12, p=.89; see Fig. 2). However, met/met homozygotes showed a significant interaction of picture category and intervention (F (2, 70)= 7.95, p=.001): under placebo, no difference in startle magnitudes was discerned between unpleasant and neutral pictures (t (19)=−1.11, p=.28), but between unpleasant and pleasant as well as neutral and pleasant pictures (unpleasant> pleasant and neutral>pleasant; both t (19)>3.24, p<.005), while in response to L-dopa, there was a significant difference in startle magnitudes between unpleasant and neutral as well as unpleasant and pleasant pictures (unpleasant>neutral and unpleasant>pleasant; both t (19)>3.82, p<.002) and no difference between neutral and pleasant pictures (t (16)=1.10, p=.29) (see Fig. 2).

Explorative analysis of gender effects on startle modulation

When using gender as additional independent variable in the analysis of startle modulation, no significant main effect of gender on mean startle magnitudes was observed (F (1, 92)= 0.32; p=.57). The above mentioned significant effects of picture category (F (2, 184)=27.74, p<.001; unpleasant> neutral>pleasant: all t (99)>3.47, p≤.001), picture category x intervention (F (2, 184)=4.06, p<.02) and picture category x genotype x intervention (F (2, 184)=4.18, p<.02) remained significant, when adding gender into the overall ANOVA.

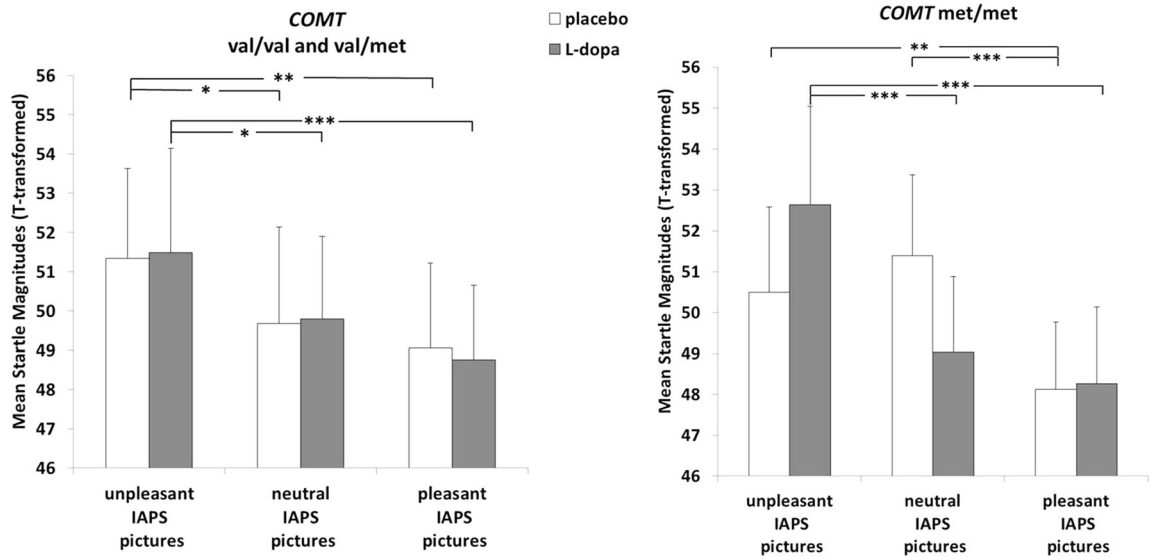

Fig. 2 Multifactorial startle modulation by genotype (*COMT* val158met), intervention (L-dopa vs. placebo) and picture category. A significant interaction between genotype, intervention and picture category was discerned (F (2, 192)=4.18, p=.02); *error bars* symbolize standard deviations; *p≤.05; **p≤.01; ***p≤.001—significance level (significant post-hoc t tests)

Subjective measures of anxiety

A significant main effect of measurement time on VAS anxiety ratings was observed (F (2, 192)=4.04, p=.04), with significantly decreasing anxiety levels from point 1 (before capsule intake) to point 2 (1 h after capsule intake; t (99)=4.52, p<.001) and significantly increasing anxiety levels from points 2 to 3 (after the startle experiment; t (99)=−2.21, p=.03). However, there were no significant effects of genotype, challenge condition or genotype x challenge condition on VAS ratings (all F (2, 192)<2.65, p>.09).

Analysis of the POMS subscale "depression-anxiety" revealed no significant main effect of measurement time (F (2, 192)=1.88, p<.17) and no significant effects of genotype, challenge condition, or genotype×challenge condition (all F (2, 192)<.87, p>.40).

Discussion

In the present pilot study, genetic factors (*COMT* val158met genotype), biochemical factors (L-dopa intervention) and anxiety-related emotional stimuli were for the first time observed to interactively influence the startle reflex as a potential psychophysiological parameter of anxiety: In *COMT* 158val allele carriers, startle potentiation by unpleasant emotional stimuli was higher as compared with neutral stimuli in both the L-dopa and the placebo condition. *COMT* 158met/met genotype carriers, however, while displaying no difference between negative and neutral stimuli in the placebo condition, showed startle potentiation by unpleasant pictures under L-dopa administration only. Across the entire sample, we observed an overall drug effect with L-dopa conferring an increased startle potentiation by unpleasant emotional stimuli, which was, however, mainly explained by the response of *COMT* 158met/met homozygotes. Thus, subjects either receiving L-dopa or carrying the *COMT* 158val allele as single anxiety risk-increasing factors reacted to unpleasant stimuli in an aroused manner as reflected by increased startle magnitudes under negative emotional stimulation, which has previously been linked to fearfulness and behavioral inhibition as dimensional phenotypes of anxiety disorders and therefore has been suggested as a promising intermediate phenotype of anxiety (Cook et al. 1992; Grillon and Baas 2003; Hamm et al. 1997; Hawk and Kowmas 2003; Koch 1999; Vaidyanathan et al. 2009). In contrast, the *COMT* 158met allele seemed to be rather associated with defensive responding deficits and thereby to potentially confer some resilience to anxiety or anxiety disorders, which might be reversed by phasic increases in dopamine levels as modeled by a single-dose L-dopa intervention.

How could the present results be interpreted against the background of current dopaminergic hypotheses of emotion processing and anxiety, respectively?

The more active *COMT* 158val allele—previously found to be associated with panic disorder and other anxiety-related phenotypes (Domschke et al. 2004, 2007; Hamilton et al. 2002; Hettema et al. 2008; Kim et al. 2006; Lonsdorf et al. 2009; McGrath et al. 2004; Rothe et al. 2006)—while decreasing overall dopamine levels in the prefrontal cortex and tonic dopamine levels subcortically, has been shown to increase phasic dopamine transmission potentially via D2 transmission and to enhance paleocortical processes such as arousal mediated by an orbital and ventrolateral frontal cortex/amygdala-ventral striatum network (Bilder et al. 2004). Given this val allele-driven increased phasic dopamine transmission, the *COMT* 158val allele might confer startle potentiation towards unpleasant stimuli in general and therefore potentially an enhanced risk for anxiety, with a single dose of L-dopa as administered in the present study not further increasing startle potentiation possibly due to a ceiling effect. The *COMT* 158met allele on the other hand, while increasing overall dopamine in the prefrontal cortex and tonic dopamine levels subcortically, has been suggested to decrease phasic dopamine release in subcortical regions (Bilder et al. 2004) and might thus exert a "protective" effect regarding unpleasant, anxiety-related emotional processing as presently reflected by a blunted startle response to unpleasant pictures not different from neutral pictures. On the background of a decreased phasic dopamine release in subcortical regions driven by the met allele, acute administration of L-dopa might in turn increase subcortical responsiveness towards unpleasant stimuli and thereby potentially anxiety-related arousal as presently mirrored by increased startle responses to unpleasant pictures.

However, as the phasic/tonic hypothesis of *COMT*-driven dopamine tone has not unequivocally been supported by newer data and there is accumulating evidence for the *COMT* val/val genotype to rather confer a lower dopamine tone leading to, e.g., a compensatory upregulation of dopamine D1 receptors in cortical and limbic areas (Slifstein et al. 2008), the role of dopamine in the etiology of anxiety with so far divergent evidence for both a dopamine deficiency and increased dopamine neurotransmission to possibly increase anxiety states remains to be further elucidated. This particularly, as—besides support for the *COMT* val allele to confer higher anxiety states and related intermediate phenotypes (see above)—there is also compelling evidence for the *COMT* met allele to drive higher responsivity to unpleasant pictures in imaging genetic studies (see Drabant et al. 2006; Heinz and Smolka 2006; Rasch et al. 2010; Smolka et al. 2005) and as in the present study *COMT* 158met/met homozygotes showed a relatively high startle response to neutral pictures, which could be interpreted as reflecting anxiety, such that neutral stimuli are perceived similarly to negative ones as described in social

anxiety disorder (Yoon and Zinbarg 2007; Yoon and Zinbarg 2008). Also, the present results are in contrast to a recent study by Corr and Kumari (2013), who found a reduced startle response to unpleasant pictures under D-amphetamine, and to a study by Montag et al. (2008), who reported increased startle reflexes in the unpleasant condition of an acoustic affect-modulated startle paradigm to be conferred by the less active *COMT* met allele. Two other studies failed to discern any influence of *COMT* gene variation on the emotional modulation of the startle reflex in healthy probands (Armbruster et al. 2011; Pauli et al. 2010). These studies, however, differ from the present one in several aspects: As discussed by the authors, the effect of D-amphetamine in the study by Corr and Kumari (2013) might have been conveyed not exclusively by dopamine but also by serotonin and norepinephrine release. Montag et al. (2008) investigated a purely female sample controlled for hormonal status, while in the present study, female as well as male probands were included. Since an estrogenic response element in the *COMT* gene promoter region might render COMT expression particularly dependent on estrogen levels (Xie et al. 1999) and estrogen levels have been shown to influence emotional processing as well as the startle reflex (Amin et al. 2006; Epperson et al. 2007), this might account for differing startle responses across studies. Also, in the study by Montag et al. (2008) in addition to the *COMT* Val158Met polymorphism probands were pre-stratified for the *DRD2/ANKK1* Taq IA variant, which could have influenced their results in an epistatic way. Furthermore, probands in the two studies failing to discern an effect of *COMT* gene variation on affect-modulated startle response, probands were significantly older (61.13+2.57 years, Armbruster et al. 2011; m: 35.16+10.29 years, f: 35.00+10.18 years, Pauli et al. 2010) 61.13±2.57 years, than probands investigated in the study by Montag et al. (22.11+3.29 years, Montag et al. 2008) and the present one (26.42±6.11 years). This might have influenced the respective results, as an age-related decrease in emotional recognition and processing has been observed (Ruffman et al. 2008).

A major limitation of the present study is the modest sample size, which is within the range of some comparable previous studies applying the startle reflex paradigm or measures of neuronal activation as an intermediate phenotype approach (Corr and Kumari 2013; Domschke et al. 2012b; Giakoumaki et al. 2008; Mattay et al. 2003; Pauli et al. 2010) but small for a placebo-controlled pharmacological challenge study particularly when analyzing the impact of one genetic variant on several dependent variables. If a more stringent, conservative statistical threshold was applied, most results would not survive (all $p \geq 0.01$). Along these lines, the lack of a replication sample applying a comparable design decreases the potential significance of the present results. However, in an attempt to test the replicability and thus the robustness of the present results, in a sample of $N=50$ probands generated via random split a statistical trend supporting our key finding of a three-way interaction "two-group genotype (*COMT* val/val and val/met vs. met/met)×intervention×picture category" was observed ($F(2, 92)=3.09$, $p=.055$). Furthermore, the inclusion of only a single dose of the challenge drug is to be considered an important limitation. On a neuropsychological level, the present sample appears to be "super normal" with regard to anxiety (i.e., lower anxiety sensitivity scores than expected from a healthy population) potentially due to the present sampling strategy selective for relatively young and mainly highly educated participants (i.e., students), which limits the generalizability and the theoretical transfer of the results to developmental or even clinical models of anxiety disorders (cf. Baumann Klauke et al. 2013). Also, in the present study female as well as male probands were included, which—given accumulating evidence for the *COMT* val158met polymorphisms conferring sexual dimorphism in anxiety and affective phenotypes (e.g., Domschke et al. 2004, 2007, 2012a; Harrison and Tunbridge 2008)—might have influenced the present results. However, no significant influence of gender on the present results has been identified statistically. Finally, not having controlled for a possibly confounding effect of life events is a limitation of the present study, since it has been reported that the impact of *COMT* gene variation on the emotion-potentiated startle reflex depends on the number of negative life events experienced by the probands (Klauke et al. 2012).

In summary, the present pilot study for the first time probing a multilevel model of dopaminergic influences on the emotion-potentiated startle response provides preliminary support for the more active *COMT* 158val allele to confer an increased startle potentiation in response to unpleasant pictures as compared with neutral pictures, while the less active *COMT* 158met allele seemed to be associated with defensive responding deficits, which was reversed by a single-dose L-dopa intervention. Thus, increased phasic dopamine transmission potentially driven by the more active *COMT* 158val allele and/or L-dopa is suggested to predispose to maladaptive emotional processing and thereby possibly also towards anxiety-related psychopathological states.

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References

Albani C, Blaser G, Geyer M, Schmutzer G, Brähler E, Bailer H, Grulke N (2005) The German short version of "Profile of Mood States" (POMS): psychometric evaluation in a representative sample. Psychother Psychosom Med Psychol 55(7):324–30

Alpers GW, Pauli P (2001) Angstsensitivitäts-Index. Julius-Maximilians-Universität, Würzburg

Amin Z, Epperson CN, Constable RT, Canli T (2006) Effects of estrogen variation on neural correlates of emotional response inhibition. Neuroimage 32:457–464

Armbruster D, Mueller A, Strobel A, Lesch KP, Kirschbaum C, Brocke B (2011) Variation in genes involved in dopamine clearance influence the startle response in older adults. J Neural Transm 118:1281–1292

Bailer UF, Narendran R, Frankle WG, Himes ML, Duvvuri V, Mathis CA, Kaye WH (2012) Amphetamine induced dopamine release increases anxiety in individuals recovered from anorexia nervosa. Int J Eat Disord 45:263–271

Bartoszyk GD (1998) Anxiolytic effects of dopamine receptor ligands: I. Involvement of dopamine autoreceptors. Life Sci 62:649–663

Baumann Klauke B, Weber H, Domschke K, Zwanzger P, Pauli P, Deckert J, Reif A (2013) The interaction of early life experiences with COMT val158met affects anxiety sensitivity. Genes Brain Behav 12:821–829

Biehl B, Dangel S, Reiser A (1986) Profile of Mood States. In: Collegium Internationale Psychiatriae (CIPS) (ed) Beltz Test, Weinheim,

Bilder RM, Volavka J, Lachman HM, Grace AA (2004) The catechol-*O*-methyltransferase polymorphism: relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes. Neuropsychopharmacology 29:1943–1961

Blumenthal TD, Cuthbert BN, Filion DL, Hackley S, Van BA (2005) Committee report: guidelines for human startle eyeblink electromyographic studies. Psychophysiology 42:1–15

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-*O*-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821

Cook EW III, Davis TL, Hawk LW, Spence EL, Gautier CH (1992) Fearfulness and startle potentiation during aversive visual stimuli. Psychophysiology 29:633–645

Corr PJ, Kumari V (2013) Effect of D-amphetamine on emotion-potentiated startle in healthy humans: implications for psychopathy and antisocial behaviour. Psychopharmacology (Berlin) 225:373–379

Domschke K, Baune BT, Havlik L, Stuhrmann A, Suslow T, Kugel H, Zwanzger P, Grotegerd D, Sehlmeyer C, Arolt V, Dannlowski U (2012a) Catechol-*O*-methyltransferase gene variation: impact on amygdala response to aversive stimuli. Neuroimage 60:2222–2229

Domschke K, Deckert J, O'donovan MC, Glatt SJ (2007) Meta-analysis of COMT val158met in panic disorder: ethnic heterogeneity and gender specificity. Am J Med Genet B Neuropsychiatr Genet 144B: 667–673

Domschke K, Freitag CM, Kuhlenbaumer G, Schirmacher A, Sand P, Nyhuis P, Jacob C, Fritze J, Franke P, Rietschel M, Garritsen HS, Fimmers R, Nothen MM, Lesch KP, Stogbauer F, Deckert J (2004) Association of the functional V158M catechol-*O*-methyl-transferase polymorphism with panic disorder in women. Int J Neuropsychopharmacol 7:183–188

Domschke K, Gajewska A, Winter B, Herrmann MJ, Warrings B, Muhlberger A, Wosnitza K, Glotzbach E, Conzelmann A, Dlugos A, Fobker M, Jacob C, Arolt V, Reif A, Pauli P, Zwanzger P, Deckert J (2012b) ADORA2A gene variation, caffeine, and emotional processing: a multi-level interaction on startle reflex. Neuropsychopharmacology 37:759–769

Domschke K, Ohrmann P, Braun M, Suslow T, Bauer J, Hohoff C, Kersting A, Engelien A, Arolt V, Heindel W, Deckert J, Kugel H (2008) Influence of the catechol-*O*-methyltransferase val158met genotype on amygdala and prefrontal cortex emotional processing in panic disorder. Psychiatry Res 163:13–20

Drabant EM, Hariri AR, Meyer-Lindenberg A, Munoz KE, Mattay VS, Kolachana BS, Egan MF, Weinberger DR (2006) Catechol *O*-methyltransferase val158met genotype and neural mechanisms related to affective arousal and regulation. Arch Gen Psychiatry 63:1396–1406

Durant C, Christmas D, Nutt D (2010) The pharmacology of anxiety. Curr Top Behav Neurosci 2:303–330

Eley TC, Tahir E, Angleitner A, Harriss K, McClay J, Plomin R, Riemann R, Spinath F, Craig I (2003) Association analysis of MAOA and COMT with neuroticism assessed by peers. Am J Med Genet B Neuropsychiatr Genet 120B:90–96

Enoch MA, Xu K, Ferro E, Harris CR, Goldman D (2003) Genetic origins of anxiety in women: a role for a functional catechol-*O*-methyltransferase polymorphism. Psychiatr Genet 13:33–41

Epperson CN, Pittman B, Czarkowski KA, Stiklus S, Krystal JH, Grillon C (2007) Luteal-phase accentuation of acoustic startle response in women with premenstrual dysphoric disorder. Neuropsychopharmacology 32:2190–2198

Giakoumaki SG, Roussos P, Bitsios P (2008) Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val158Met polymorphism. Neuropsychopharmacology 33:3058–3068

Grillon C, Baas J (2003) A review of the modulation of the startle reflex by affective states and its application in psychiatry. Clin Neurophysiol 114:1557–1579

Hamilton SP, Slager SL, Heiman GA, Deng Z, Haghighi F, Klein DF, Hodge SE, Weissman MM, Fyer AJ, Knowles JA (2002) Evidence for a susceptibility locus for panic disorder near the catechol-*O*-methyltransferase gene on chromosome 22. Biol Psychiatry 51:591–601

Hamm AO, Cuthbert BN, Globisch J, Vaitl D (1997) Fear and the startle reflex: blink modulation and autonomic response patterns in animal and mutilation fearful subjects. Psychophysiology 34:97–107

Harrison PJ, Tunbridge EM (2008) Catechol-*O*-methyltransferase (COMT): a gene contributing to sex differences in brain function, and to sexual dimorphism in the predisposition to psychiatric disorders. Neuropsychopharmacology 33:3037–3045

Hawk LW Jr, Kowmas AD (2003) Affective modulation and prepulse inhibition of startle among undergraduates high and low in behavioral inhibition and approach. Psychophysiology 40:131–138

Heinrich K, Lehmann E (1992) Low dose neuroleptanxiolysis in anxiety states. Prog Neuropsychopharmacol Biol Psychiatry 16:135–143

Heinz A, Smolka MN (2006) The effects of catechol *O*-methyltransferase genotype on brain activation elicited by affective stimuli and cognitive tasks. Rev Neurosci 17:359–367

Hettema JM, An SS, Bukszar J, van den Oord EJ, Neale MC, Kendler KS, Chen X (2008) Catechol-*O*-methyltransferase contributes to genetic susceptibility shared among anxiety spectrum phenotypes. Biol Psychiatry 64:302–310

Hood SD, Potokar JP, Davies SJ, Hince DA, Morris K, Seddon KM, Nutt DJ, Argyropoulos SV (2010) Dopaminergic challenges in social anxiety disorder: evidence for dopamine D3 desensitisation following successful treatment with serotonergic antidepressants. J Psychopharmacol 24:709–716

Kim SJ, Kim YS, Kim SY, Lee HS, Kim CH (2006) An association study of catechol-*O*-methyltransferase and monoamine oxidase A polymorphisms and personality traits in Koreans. Neurosci Lett 401: 154–158

Klauke B, Winter B, Gajewska A, Zwanzger P, Reif A, Herrmann MJ, Dlugos A, Warrings B, Jacob C, Muhlberger A, Arolt V, Pauli P, Deckert J, Domschke K (2012) Affect-modulated startle: interactive influence of catechol-*O*-methyltransferase Val158Met genotype and childhood trauma. PLoS ONE 7:e39709

Koch M (1999) The neurobiology of startle. Prog Neurobiol 59:107–128

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM (1996) Human catechol-*O*-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250

Lang PJ, Bradley MM, Cuthbert BN (2005) International Affective Picture System (IAPS). University of Florida, Gainesville

Lonsdorf TB, Ruck C, Bergstrom J, Andersson G, Ohman A, Lindefors N, Schalling M (2010) The COMTval158met polymorphism is associated with symptom relief during exposure-based cognitive-behavioral treatment in panic disorder. BMC Psychiatry 10:99

Lonsdorf TB, Weike AI, Nikamo P, Schalling M, Hamm AO, Ohman A (2009) Genetic gating of human fear learning and extinction: possible implications for gene-environment interaction in anxiety disorder. Psychol Sci 20:198–206

Mattay VS, Goldberg TE, Fera F, Hariri AR, Tessitore A, Egan MF, Kolachana B, Callicott JH, Weinberger DR (2003) Catechol *O*-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proc Natl Acad Sci U S A 100: 6186–6191

McGrath M, Kawachi I, Ascherio A, Colditz GA, Hunter DJ, De V (2004) Association between catechol-*O*-methyltransferase and phobic anxiety. Am J Psychiatry 161:1703–1705

McNair D, Lorr M, Droppleman L (1992) Profile of mood states manual (rev.). Educational and Industrial Testing Service, San Diego

Montag C, Buckholtz JW, Hartmann P, Merz M, Burk C, Hennig J, Reuter M (2008) COMT genetic variation affects fear processing: psychophysiological evidence. Behav Neurosci 122:901–909

Muhlberger A, Wieser MJ, Pauli P (2008) Darkness-enhanced startle responses in ecologically valid environments: a virtual tunnel driving experiment. Biol Psychol 77:47–52

Murphy DL (1973) Mental effects of L-dopa. Annu Rev Med 24:209–216

Ohara K, Nagai M, Suzuki Y, Ochiai M, Ohara K (1998) No association between anxiety disorders and catechol-*O*-methyltransferase polymorphism. Psychiatry Res 80:145–148

Olsson CA, Anney RJ, Lotfi-Miri M, Byrnes GB, Williamson R, Patton GC (2005) Association between the COMT Val158Met polymorphism and propensity to anxiety in an Australian population-based longitudinal study of adolescent health. Psychiatr Genet 15:109–115

Pauli P, Conzelmann A, Mucha RF, Weyers P, Baehne CG, Fallgatter AJ, Jacob CP, Lesch KP (2010) Affect-modulated startle reflex and dopamine D4 receptor gene variation. Psychophysiology 47:25–33

Peterson RA, Reiss S (1992) Anxiety sensitivity index manual (2nd ed.). Worthington, OH: International Diagnostic Systems

Rasch B, Spalek K, Buholzer S, Luechinger R, Boesiger P, de Quervain DJ, Papassotiropoulos A (2010) Aversive stimuli lead to differential amygdala activation and connectivity patterns depending on catechol-*O*-methyltransferase Val158Met genotype. Neuroimage 52:1712–1719

Richard IH, Schiffer RB, Kurlan R (1996) Anxiety and Parkinson's disease. J Neuropsychiatry Clin Neurosci 8:383–392

Rothe C, Koszycki D, Bradwejn J, King N, Deluca V, Tharmalingam S, Macciardi F, Deckert J, Kennedy JL (2006) Association of the Val158Met catechol *O*-methyltransferase genetic polymorphism with panic disorder. Neuropsychopharmacology 31:2237–2242

Ruffman T, Henry JD, Livingstone V, Phillips LH (2008) A meta-analytic review of emotion recognition and aging: implications for neuropsychological models of aging. Neurosci Biobehav Rev 32:863–881

Samochowiec J, Hajduk A, Samochowiec A, Horodnicki J, Stepien G, Grzywacz A, Kucharska-Mazur J (2004) Association studies of MAO-A, COMT, and 5-HTT genes polymorphisms in patients with anxiety disorders of the phobic spectrum. Psychiatry Res 128:21–26

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC (1998) The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 20:22–33

Shulman R, Griffiths J, Diewold P (1978) Catechol-*O*-methyl transferase activity in patients with depressive illness and anxiety states. Br J Psychiatry 132:133–138

Slifstein M, Kolachana B, Simpson EH, Tabares P, Cheng B, Duvall M, Frankle WG, Weinberger DR, Laruelle M, Abi-Dargham A (2008) COMT genotype predicts cortical-limbic D1 receptor availability measured with [11C] NNC112 and PET. Mol Psychiatry 13:821–827

Smolka MN, Schumann G, Wrase J, Grusser SM, Flor H, Mann K, Braus DF, Goldman D, Buchel C, Heinz A (2005) Catechol-*O*-methyltransferase val158met genotype affects processing of emotional stimuli in the amygdala and prefrontal cortex. J Neurosci 25:836–842

Stein MB, Fallin MD, Schork NJ, Gelernter J (2005) COMT polymorphisms and anxiety-related personality traits. Neuropsychopharmacology 30: 2092–2102

Talalaenko AN, Abramets IA, Stakhovskii Y, Shekhovtsov AA, Chernikov AV, Shevchenko SL (1994) The role of dopaminergic mechanisms on the brain in various models of anxious states. Neurosci Behav Physiol 24:284–288

Vaidyanathan U, Patrick CJ, Cuthbert BN (2009) Linking dimensional models of internalizing psychopathology to neurobiological systems: affect-modulated startle as an indicator of fear and distress disorders and affiliated traits. Psychol Bull 135:909–942

Winqvist R, Lundstrom K, Salminen M, Laatikainen M, Ulmanen I (1992) The human catechol-*O*-methyltransferase (COMT) gene maps to band q11.2 of chromosome 22 and shows a frequent RFLP with BglI. Cytogenet Cell Genet 59:253–257

Woo JM, Yoon KS, Choi YH, Oh KS, Lee YS, Yu BH (2004) The association between panic disorder and the L/L genotype of catechol-*O*-methyltransferase. J Psychiatr Res 38:365–370

Wray NR, James MR, Dumenil T, Handoko HY, Lind PA, Montgomery GW, Martin NG (2008) Association study of candidate variants of COMT with neuroticism, anxiety and depression. Am J Med Genet B Neuropsychiatr Genet 147B:1314–1318

Wurthmann C, Klieser E, Lehmann E, Pester U (1995) Test therapy in the treatment of generalized anxiety disorders with low dose fluspirilene. Prog Neuropsychopharmacol Biol Psychiatry 19: 1049–1060

Xie T, Ho SL, Ramsden D (1999) Characterization and implications of estrogenic down-regulation of human catechol-*O*-methyltransferase gene transcription. Mol Pharmacol 56:31–38

Yoon KL, Zinbarg RE (2007) Threat is in the eye of the beholder: social anxiety and the interpretation of ambiguous facial expressions. Behav Res Ther 45:839–847

Yoon KL, Zinbarg RE (2008) Interpreting neutral faces as threatening is a default mode for socially anxious individuals. J Abnorm Psychol 117:680–685