Dopamine β-Hydroxylase Gene (*DβH*) -1021C→T Influences Self-Reported Paranoia during Cocaine Self-Administration

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Background: Variation in the gene for *dopamine* β-*hydroxylase (DβH)* has been reported to associate with cocaine-induced paranoia as assessed by retrospective self-report. This association has yet to be tested prospectively.

Methods: Visual analog scale (VAS) ratings of paranoia were obtained in 31 cocaine users during three cocaine self-administration sessions (8, 16, and 32 mg/70kg). Pharmacogenetic interactions between cocaine and a putative functional polymorphism in *DβH* (-1021C→T) were assessed.

Results: VAS self-ratings showed significant or trend-level interactions of genotype and time during each session ($p = .004$, .09 and .003, respectively) with TT homozygotes endorsing greater paranoia over time than either CT or CC individuals. Interactions were significant at all doses in African Americans ($n = 19$; $p = .02$, .04 and .05). No other demographic or experimental variable distinguished genotypic groups.

Conclusions: Results indicate that individuals homozygous for the 'very low-activity' T allele at *DβH* -1021C→T show an increased propensity to paranoia over time during cocaine self-administration.

Key Words: Cocaine, dependence, dopamine β-hydroxylase, genetics, paranoia, psychosis, self-administration

Paranoia is commonly endorsed (50–80%) in cocaine users (Bartlett *et al.* 1997; Brady *et al.* 1991; Cubells *et al.* 2005; Kalayasiri *et al.* 2006a; Rosse *et al.* 1994; Satel *et al.* 1991) and is well-evidenced during laboratory cocaine administration (Angrist 1990; Kalayasiri *et al.* 2006b; Mooney *et al.* 2006; Muntaner *et al.* 1989; Pearlson *et al.* 1989; Sherer *et al.* 1988). Vulnerability to the trait is variable (Kalayasiri *et al.* 2006b), with studies pointing to the importance of both environmental (Brady *et al.* 1991; Cubells *et al.* 2005; Kalayasiri *et al.* 2006a) and genetic factors (Cubells *et al.* 2000; Gelernter *et al.* 1994, 2005). A prior retrospective report by Cubells *et al.* (2000) noted an association between a dopamine β-hydroxylase (*DβH*) haplotype associated with low plasma *DβH* activity and cocaine-induced paranoia.

Our group has developed human laboratory methods that validly and reliably assess paranoia in response to self-administered cocaine (Kalayasiri *et al.* 2006b; Lynch *et al.* 2006; Sughondhabirom *et al.* 2005). In the current study, we used these methods and a putative functional polymorphism in *DβH* (-1021C→T) (Zabetian *et al.* 2001) to test prospectively whether variation in *DβH* influences "in vivo" measures of paranoia.

Methods and Materials

Participants were 31 nontreatment-seeking volunteers, who were dependent on cocaine and actively using (smoked or intravenous) as confirmed by unstructured interview and urine toxicology testing, respectively (Table 1 for demographics). Individuals with a comorbid psychiatric and/or primary psychotic disorder (DSM-IV); alcohol, sedative-hypnotic, opiate dependence, or significant medical illness were excluded. All subjects were medication free. Subjects participated in a cocaine safety-eligibility session prior to study participation (Sughondhabirom *et al.* 2005). Written informed consent was obtained, as approved by the Yale Human Investigations Committee.

Subjects participated in a "binge" paradigm of intravenous cocaine self-administration under a fixed-ratio 1:5-min timeout schedule as previously described (Lynch *et al.* 2006; Sughondhabirom *et al.* 2005). Subjects had two hours of self-regulated, bolus cocaine infusions (8, 16, and 32 mg/70 kg body weight; one dose condition per day), preceded and followed by 30-min baseline and 60-min washout periods, respectively. Except for the first five subjects (Sughondhabirom *et al.* 2005), who received an escalating dose regimen, session order was randomized and double-blind. Self-administration and subjective effects (but not genotype) data have appeared in prior validation and reliability studies of the paradigm (Lynch *et al.* 2006; Sughondhabirom *et al.* 2005) and paranoia phenotype (Kalayasiri *et al.* 2006b).

Genotyping at *DβH* -1021C→T (rs1611115) was performed using polymerase chain reaction (PCR)- Restriction Fragment Length Polymorphism (RFLP) methods as reported previously (Zabetian *et al.* 2003). Gels were independently double scored. Of the 31 subjects, the final 13 were prospectively genotyped to enrich for rarer CT and TT individuals. In all instances, experimental sessions were conducted blind to genotype and independent of information regarding history of cocaine-induced paranoia (i.e., retrospective report data was not solicited).

Cocaine-induced subjective effects ("paranoid"; "high"; "want cocaine") were assessed at 5-min intervals by visual analog scale self-ratings [VAS; 0 (not at all), 10 (most ever)] on a touch-screen laptop computer. Cocaine responses, infusions, inter-infusion interval, session intake, and vital signs (heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP)) were also recorded.

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Received June 12, 2006; revised August 16, 2006; accepted August 17, 2006.

0006-3223/07/$32.00
doi:10.1016/j.biopsych.2006.08.012

Table 1. Demographic, Cocaine use, and Self-Administration Data According to *DBH*-1021C→T Genotype[a]

	Total (n = 31)				AAs (n = 19)			
	TT (n = 5)	CT (n = 11)	CC (n = 15)	p-Value	TT (n = 4)	CT (n = 5)	CC (n = 10)	p-Value
Age	41 ± 4	38 ± 7	38 ± 6	.48	40 ± 4	37 ± 6	39 ± 6	.64
Gender	3 M, 2 F	9 M, 2 F	9 M, 6 F	.46	3 M, 1 F	4 M, 1 F	5 M, 5 F	.45
Race	1 EA, 4 AA	6 EA, 5 AA	5 EA, 10 AA	.35	—	—	—	—
Age of First Cocaine Use (years)	20 ± 6	20 ± 4	19 ± 3	.86	20 ± 7	21 ± 6	19 ± 3	.73
Duration of Cocaine Use (years)	21 ± 8	19 ± 5	18 ± 6	.63	21 ± 9	15 ± 4	20 ± 6	.34
Dollars Spent for Cocaine per Day	154 ± 136	135 ± 139	138 ± 98	.85	168 ± 153	99 ± 44	122 ± 44	.83
Days per Week of Cocaine Use	5.6 ± 1.9	5.6 ± 1.6	5.7 ± 1.9	1.00	6.3 ± 1.5	6.0 ± 1.7	6.1 ± 1.5	.98
Baseline "Paranoia" (VAS score)	.0 ± .0	.5 ± 1.0	.3 ± .5	.31	.0 ± .0	.7 ± 1.1	.1 ± .2	.32
Responses (i.e., button presses)	45 ± 74 Median = 19	68 ± 169 Median = 7	56 ± 180 Median = 8	.77	51 ± 84 Median = 12.5	131 ± 249 Median = 7	9 ± 6 Median = 7.5	.26
Infusions	8.8 ± 4.4	7.5 ± 2.7	7.4 ± 3.1	.68	8.0 ± 4.6	8.0 ± 2.0	6.6 ± 2.9	.63
Inter-infusion Interval (minutes)	20.3 ± 14.2	20.7 ± 18.4	20.1 ± 9.5	.72	22.8 ± 15.1	14.5 ± 3.6	22.0 ± 9.4	.38
Cocaine Intake (mg)								
8 mg session	106 ± 31	86 ± 34	93 ± 31	.52	102 ± 35	99 ± 20	85 ± 28	.49
16 mg session	150 ± 68	156 ± 65	155 ± 68	.99	144 ± 76	192 ± 38	125 ± 52	.11
32 mg session	282 ± 140	239 ± 85	237 ± 100	.68	256 ± 148	256 ± 64	211 ± 93	.48

M, male; F, female; EA, European American; AA, African American; VAS, visual analog scale.
[a]For 32 mg/70 kg session, unless otherwise noted.

Normally distributed data were analyzed using repeated-measure analysis of variance (ANOVA; Huynh-Feldt corrected), or one-way ANOVA. In the absence of normality, variables were transformed (log or reciprocal) or, in the absence suitably normalizing transformation, analyzed by Kruskal Wallis or non-parametric methods for repeated-measure data (the latter in 30 min time bins given their computational intensity) (Brunner *et al.* 2002; Kalayasiri *et al.* 2006b). Categorical data were analyzed by two-tailed χ^2 or Fisher's exact tests. Analyses were performed using SPSS 11.0 for Mac OS X (SPSS Inc., Chicago, Illinois) or SAS Version 9.12 (SAS Institute, Cary, North Carolina).

Results

Categorical comparisons of genotypic groups according to the presence (VAS ≥ 2.0) or absence (VAS ≤ 1.5) of cocaine-induced paranoia were not significant (χ^2 = 1.2, df = 2, p = .6; see Kalayasiri *et al.* 2006b for threshold criteria). As shown previously (Kalayasiri *et al.* 2006b), VAS self-ratings of paranoia were non-normally distributed. They were, therefore, subjected to analysis by the nonparametric ANOVA-Type Statistic (ATS) for repeated measures (Brunner *et al.* 2002). No main effect of genotype was observed (8 mg: ATS_2 = .04, p = .95; 16 mg: ATS_2 = .3, p = .75; 32 mg: ATS_2 = .7, p = .48). However, significant main effects of time were present (8 mg: ATS_{13} = 5.6, p = .0002; 16 mg: ATS_{13} = 7.0, p < .0001; 32 mg: ATS_{13} = 14.2, p < .0001), as were statistically significant (8 mg: ATS_{26} = 3.0, p = .004; 32 mg: ATS_{26} = 2.8, p = .003) or trend-level (16 mg: ATS_{26} = 1.7, p = .09) interactions of genotype and time. Self-reported paranoia (peak 30-min bin) was higher in TT homozygotes compared to CT and CC individuals at all doses, including 8 mg (1.8 ± 1.7 vs. 1.0 ± 1.6 vs. .5 ± .8), 16 mg (2.2 ± 2.1 vs. 1.2 ± 1.8 vs. 1.0 ± 1.2), and 32 mg (3.9 ± 3.8 vs. 1.4 ± 2.0 vs. 1.7 ± 1.7) (Figure 1). To control for population stratification, identical analyses were performed in African Americans (AA; n = 19, 4 TT, 5 CT, and 10 CC). Analyses in European Americans were precluded by the solitary TT in that group. Significant genotype x time interactions were present at all doses in AAs (8 mg: ATS_{26} = 2.5, p = .02; 16 mg: ATS_{26} = 2.1, p = .04; 32 mg: ATS_{26} = 2.1, p = .05; peak 30 min bin, 8 mg: 2.3 ± 1.5 vs. 1.4 ± 2.0 vs. .7 ± 1.0; 16 mg: 2.8 ± 2.0 vs. 1.8 ± 2.0 vs. 1.0 ± 1.3; 32 mg: 4.9 ± 3.7 vs. 2.5 ± 2.4 vs. 1.9 ± 1.9).

Though significant main effects of time (range, p = .0001-.05) were observed for other subjective effects ("high", "want cocaine") and vital sign measures (SBP, DBP, HR), no significant main effects of genotype or interactions were found, except for HR (8 mg: genotype x time; p = .05). Genotypic groups did not differ with respect to other demographic, cocaine use, and self-administration measures (Table 1), either in the combined or the AA samples.

Discussion

Our data demonstrate a significant interaction between *DβH* -1021C→T genotype and cocaine self-administration with respect to self-reported paranoia, with a "very low-activity" *DβH* genotype (TT) conferring an increased vulnerability to cocaine-induced paranoia over time. This finding was replicated across

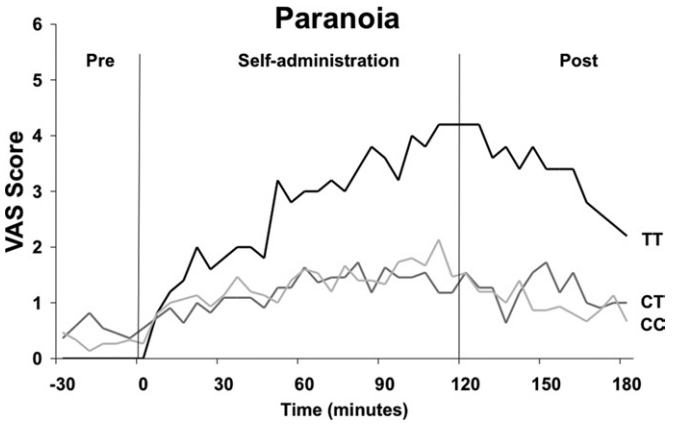

Figure 1. Time-activity curves of visual analog scale (VAS) self-ratings of paranoia are depicted according to dopamine β-hydroxylase (*DβH*) -1021C→T genotype (n = 5 TT, 11 CT, and 15 CC; 32 mg/70kg cocaine self-administration session). A significant interaction of genotype and time was observed (ATS_{26} = 2.8, p = .003). ATS, ANOVA (analysis of variance)-type statistic.