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Original article

Quantitative-trait loci analysis of cocaine-related behaviours and neurochemistry

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We recently conducted a dose–response study of the effects of cocaine on several activity measures in the panel of BxD/Ty recombinant inbred mice. Animals were tested in an automated activity chamber over 2 days with i.p. saline on day 1 and i.p. cocaine on day 2, at one of four doses, 5, 15, 30 or 45 mg kg^{-1}. The monitor recorded total distance traveled, nosepokes in a holeboard, repeated movements and time spent by an individual in proximity to the centre of the apparatus. Dose–response curves for locomotor activation, i.e. the difference between cocaine and saline scores, showed that for all strains tested, scores increased 5–30 mg kg^{-1}. With few exceptions, locomotor activity at 45 mg kg^{-1} was not significantly higher than that at 30 mg kg^{-1}. Repeated movement scores showed patterns similar to locomotor activity and nosepokes tended to be progressively inhibited by increasing doses of cocaine. Recombinant inbred strain mean distributions for all behaviours and at all doses exhibited continuous, rather than discrete variation, thus providing evidence of multiple-gene effects on cocaine-related behaviours. Quantitative trait loci (QTL) analysis pointed to several chromosomal locations associated with variations in cocaine-related behaviours and some are either identical or close to QTL reported by others. In separate groups of animals, densities of dopamine D_1, and D_2 receptors and dopamine uptake transporters were measured in the medial prefrontal cortex, caudate-putamen, nucleus accumbens and ventral midbrain. In all areas, all measures showed distributions consistent with polygenic influence and were associated with QTL. Of particular interest was our finding of a large segment on chromosome 15, which is related to dopamine receptor densities and cocaine-related behaviours. Pharmacogenetics 9:607–617 © 1999 Lippincott Williams & Wilkins

Keywords: cocaine, behaviour, genetic, dopamine, recombinant inbred, mice, quantitative trait loci

Introduction

The misuse of cocaine in North America has developed into a major public health concern over the past 25 years. During that time, and despite large increments in our knowledge about basic biological mechanisms of cocaine, problems associated with its use appear not to have abated. Basic researchers have elucidated neurobiological mechanisms to describe and explain cocaine actions; however, one very important area concerning vulnerability to cocaine addiction has received relatively little attention. Among humans, very little is known about individual differences in susceptibility to cocaine addiction, much less indeed than about individual differences in such vulnerability to alcohol. This is probably because of strict laws governing cocaine possession and use, thereby forcing such to be covert. Alternatively, during the past 10 years in human research on use and misuse of a legally available drug, alcohol, genetic-based, individual differences in vulnerability to several types of alcohol-related problems have been shown in humans (Cloninger, 1987). Furthermore, intensive

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study of alcohol-related phenotypes has been conducted in genetically defined laboratory animals, most notably in selected lines and inbred strains of rodents (McClearn, 1991). While little is known about genetic-based, individual differences related to cocaine in humans, a growing body of literature has revealed a picture of genetic involvement with cocaine phenotypes in laboratory animals which is similar to that seen for alcohol. Morse *et al.* (1995) provide a recent review of this literature. Specifically, most cocaine-related behavioural phenotypes show large, genetically based differences among lines and strains of rodents, and, similar to outcomes with alcohol, most of these appear to be influenced by multiple genes (Tolliver *et al.*, 1994; Miner & Marley, 1995; Phillips *et al.*, 1998).

Another point to consider in cocaine research is that it is probably unreasonable to assert, despite what some consider to be a well-understood mechanism of action of cocaine, that one behavioural or neurochemical phenotype can capture the important characteristics of initial response, compulsive use, or addiction. Thus, based on our findings and those of others, we argue that the overall picture of cocaine sensitivity and susceptibility to be best presented and understood from the perspective of multiple measures. For example, Costall *et al.* (1989) demonstrated that in addition to effects that make cocaine attractive, cocaine may also be anxiogenic. More recently, we demonstrated coadministration of ethanol to reverse cocaine's inhibition of exploration and increased stereotypy, while having little or no effect on increases in locomotion (Cook *et al.*, 1998). This last behaviour has been proposed to be an index of the abuse liability for such agents (Wise, 1987). An individual's tendency to self-administer, maintain use and develop addiction to a drug involves many dynamic processes indeed and one of these is likely to be the initial effect of the drug including its summed attractive and aversive effects. Of course, the relative weights of these effects are very likely to be influenced by both individual genetic makeup and experiential history.

The use of genetically defined animals such as selected lines and inbred strains is an accepted approach to model behavioural effects of drugs and their neurochemical correlates (Crabbe & Belknap, 1992). Recombinant inbred (RI) mouse strains are particularly valuable for pharmacogenetic research (DeFries *et al.*, 1989; Gora-Maslak *et al.*, 1991; Rodriguez *et al.*, 1994, 1995). One such panel of RI mouse strains is the panel of 26 RI strains derived from the well-known C57BL/6 J and DBA/2 J mouse strains by Taylor (1989), namely, BXD/Ty. These strains have been characterized genetically on more than 1000 markers, including anynomous (RFLP, microsatellite repeats, etc.) and known genes. Each marker thus has a characteristic strain distribution pattern. Using simple correlational analysis, one can determine which markers are associated with specific phenotypes, i.e. quantitative trait loci (QTL) analysis. As argued by others (Belknap *et al.*, 1996), QTL markers identified in RI strains are subject to problems involving both alpha and beta errors (false positives and misses). As such, all QTLs identified in RI strains of rodents should be considered as provisional and subject to independent verification.

We report behavioural effects of four doses of cocaine, related regional dopamine receptor data and QTL related to behaviour, to neurobiology, and intersecting QTL. The behaviours measured were locomotor activity, holeboard exploration, repeated movements and time spent in the centre of an open-rield activity monitor. Neurochemical measures included densities of dopamine D_1 and D_2 receptors and the dopamine uptake transporter (DAUT) in the medial prefrontal cortex, caudate-putamen and ventral midbrain.

Materials and methods

Study population

Male and female BXD RI mice, 60–80 days of age were obtained from our production colony consisting of 26 strains at Penn State University. All animals were housed in unisex littermate groups of 2–4, maintained on laboratory chow, *ad libitum*, with temperature and humidity maintained at 21 °C and 40%, respectively. Bedding consisted of hardwood chips and the light–dark cycle was on a 12 h rotation with onset at 06.00 h and lights off at 18.00 h. Testing was done in groups of approximately 50, distributed across different strains and in overlapping batches to maximize efficiency. For behavioural testing, the number of animals per strain, sex and dose ranged from 4 to 10. Thus for each dose, there was a minimum of eight animals tested. For the neurochemical measures, tissues were pooled from three to five animals per strain and sex and each estimate of B_{max} for the receptors was based on three replicate experiments from the pooled tissues.

Cocaine

Cocaine as the HCl salt was prepared fresh each day. The drug was dissolved in sterile saline at a concentration of 1.5 mg ml^{-1}. Sterile saline (0.9% w/v) was used as control vehicle. The doses used were 5, 15, 30 and 45 mg kg^{-1}. Larger doses were not used because of the probable convulsive effects of cocaine beyond 50 mg kg^{-1} (Miner & Marley, 1995a).

Apparatus

All testing was conducted using automated activity monitors (Omnitech, Inc., Columbus, OH, USA). Construction of the monitors was of transparent

Plexiglas® and dimensions were 40 × 40 × 30 cm high. Each monitor was housed in a sound-attenuated chamber and a fan provided masking noise and ventilation.

Testing procedure

All animals were tested between 08.00 h and 12.00 h over two consecutive days. On the first day, each animal was weighed, received an ip injection of sterile saline solution (0.01 ml kg^{-1}) and placed into the activity monitor for a 30 min period. Cocaine treatment, via ip injection, followed weighing on the second day and as on the day before, the animal was placed into the activity monitor, this time for 15 min. The animals were tested in groups of approximately 50 over 1 week each for each group. There were four activity monitors and we were able to test 6–7 groups of four animals on each day for 2 days each (Monday to Tuesday, Wednesday to Thursday with Friday for data management). This test order has been adopted by our research team based on our earlier observation that cocaine treatment in a novel environment may attenuate cocaine-induced locomotor activity (Jones *et al.*, 1993). The shorter period for cocaine testing was used because of the relatively short half-life for cocaine in mice and because of possible differences in clearance rates among the strains (Womer *et al.*, 1994; Azar. *et al.*, 1998). We thus wanted to capture the *initial* effects of cocaine, i.e. when brain cocaine concentrations would be highest.

Activity measures

Locomotor activity, measured as total distance traveled, nosepokes, repeated movements and time spent in the centre of the apparatus were sampled every 5 min. The longer test period on day 1 was chosen to assess the rate at which the animals would adapt to a novel environment, a characteristic which has been shown to be relevant to amphetamine-related behaviours (Piazza *et al.*, 1989). The use of the Omnitech apparatus for measuring repeated movements and centre time has been validated previously using direct observational techniques (Sanberg *et al.*, 1987; Fitzgerald *et al.*, 1988). These researchers have shown that repeated movements produced by amphetamine and scored by the apparatus correlate highly with observationally scored stereotypy. Nonetheless, we prefer to use the term, repeated movements, because we believe that this more accurately reflects what is actually scored by the machine.

Dopamine neurochemistry

Separate groups of animals that were not treated with cocaine or otherwise tested were sacrificed at 60–70 days of age, their brains quickly removed and dissected on an ice-cold aluminium block into medial prefrontal cortex, caudate-putamen, nucleus accumbens and ventral midbrain as described by Jones *et al.* (1996). Receptor binding studies were conducted in the tissue homogenates for dopamine D_1 and D_2 receptors and the dopamine uptake transporter (DAUT). The ligands used were [^{3}H]-SCH23390, [^{125}I]-epidepride and [^{3}H]-GBR12935, and the concentrations used were saturating, thus yielding estimates of maximum binding capacities (Erwin *et al.*, 1997). The data obtained thus, were B_{MAX} values for each ligand in each region, based on two to three replicate experiments using tissue pooled from three to five animals of like strain and sex. Between 16 and 18 strains were examined.

Statistical analyses

Behavioural data were quantified as the difference between the cocaine score for the first 15 min minus the saline score obtained for the first 15 min. Extreme outlying datapoints (± 3 SD from the group mean) were removed from the dataset. In order to assess strain and sex effects on the difference scores, a two-between subjects variables analysis of variance (ANOVA) was employed. We used the SAS General Linear Model (GLM) for unweighted means solutions (SAS Institute, Inc., 1989) which accommodates unequal cell sizes when those differences are not reflected in the population. Estimation of genetic contribution (i.e. for strain main effect) to variance was made using estimated omega squared (Myers & Well, 1995). We also performed analysis of covariance, using saline scores (which were significantly correlated with the cocaine scores) as the covariate. This was done for QTL analysis in an attempt to remove pre-extant subject characteristics that could confound the effects of the drug. Where examination of difference and adjusted means revealed close agreement, QTL for difference scores are presented. In a few cases as seen below, adjusted means revealed some different QTL.

In order to develop a metric that would reflect the overall potential of cocaine to increase locomotor activity among the strains, we performed linear regression, distance difference scores versus log dose (5, 15 and 30 mg kg^{-1}). The highest dose was not used because some strains were showing a trend towards decreased locomotion at this dose). The slopes of difference scores on log dose were then used as the unit of measurement. We developed this method based on our earlier work with ethanol (Erwin *et al.*, 1993).

QTL correlations were calculated using point-biserial correlation between the marker (0 as C57-like and 1 as DBA-like) and the continuously distributed behavioural or neurochemical phenotype. For our behavioural and neurochemical measures, we adopted the criterion for QTL at a LOD (logarithm likelihood ratio for linkage) score of 3. For comparison purposes however, we listed

QTL in agreement between our and the other three studies which met our criterion of $P < 0.01$ for the maximum likelihood statistic generated by Mapmanager QT. We believed that this was justified because of the independence of the experiments.

Results

COCAINE-RELATED BEHAVIOURS

Effects of cocaine on total distance

The analysis of variance revealed significant ($P < 0.001$) between-strain effects at all dose levels; all strains evinced increased locomotion, compared to saline. Each *F*-ratio was based on 22 and 407–398 degrees of freedom and as such significance values are relatively uninformative. More useful information may be delivered by statistics such as r^2 that report effect size in terms of factor contribution to total variance. Estimated ω^2 is one such statistic and as such revealed that strain accounted for 14% and 16% of the total variance in this measures at 5 and 15 mg kg^{-1} and 32% and 20% of total variance at 30 and 45 mg kg^{-1}, respectively. At 5 mg kg^{-1}, we also observed a significant effect of sex ($P < 0.01$, 1% of variance explained) with females tending to be more highly activated than males.

Table 1. QTL identified for cocaine-related behaviour

Behaviour	*Marker*	*Chr*	*Dist (cM)*	*LOD*	r	n
Total distance						
At 5 mg kg^{-1} (M)	D15mit3	15	39.6	3.7	0.77	19
At 15mg kg^{-1} (F)	afp	5	50.0	3.1	–0.72	19
	D5Mit7	5	50.0	3.1	–0.74	19
At 45 mg kg (C)	D5Mit10	5	54.0	3.1	–0.73	18
Nosepokes (a)						
At 5 mg kg^{-1} (M)	D9Ncvs45	9	70.0	3.2	0.76	17
	D11Ncvs60	11	74.0	3.1	0.45	20
At 15 mg kg^{-1} (C)	D3Ncvs49	3	~70	3.7	–0.72	23
At 30 mg kg^{-1}	D12Ncvs31	12	6.0	3.4	0.72	21
Nosepokes						
At 15 mg kg^{-1} (c)	D1Ncvs12		74.5	4.8	–0.81	18
At 30 mg kg^{-1} (C)	D2Ncvs54	2	Syntenic	3.4	0.71	22
	D2Mit17	2	69.0	3.2	0.73	19
	D2Byu3	2	69.0	3.3	0.69	23
Repeated movements (a)						
At mg kg^{-1} (F)	D5Mit7		50.0	3.3	–0.74	19
Repeated movements						
At mg kg^{-1} (C)	adfp	4	38.9	3.7	0.74	21
(C)	D6Rik55	6	26.5	3.3	0.60	22
At 30 mg kg^{-1} (C)	D15Ncvs29	15	51.0	3	0.69	21
(M)	acrg	1	52.3	3.3	0.74	19
At 45 mg kg^{-1} (C)	D6Rik55	6	26.5	3.7	0.73	22
	D1Mit7	1	41.0	3.1	0.67	23
	inha	1	41.6	3.1	0.67	23
	Sac	19	83.0	3.3	0.44	22
(F)	D9Mit4	9	29.0	3.8	–0.52	18
	Drd2	9	28.0	3	–0.48	21
Centre time (a)						
At 30 mg kg^{-1} (F)	D5Ncvs60	5	59.0	3.2	0.69	22
At 45 mg kg^{-1} (C)	pmv22	11	8.0	3.8	–0.72	23
	D11Ncvs73	11	4.0	3.3	–0.71	22
	D11Mit2	11	2.0	3.8	–0.72	18
	glns-ps1	11	11.0	4.2	–0.75	23
Slope						
Combined	D9Ncvs47	9	42.0	3	–0.75	21

[a]Indicates ANOCOVAR adjusted scores. F, female; M, male; C, sexes combined.

Effects of cocaine on nosepokes

Overall, cocaine tended to inhibit this form of exploratory behaviour. The results indicated significant between-strain effects at all cocaine dose levels ($P < 0.001$ at all doses except for 15 mg kg^{-1} which was $P < 0.03$), and proportion of variance explained at 5, 15, 30 and 45 mg kg^{-1} at 15%, 4%, 13% and 13%, respectively. ANOVA also indicated significant effects of sex on nosepokes at 45 mg kg^{-1} ($P < 0.001$, with 2% of variance explained. Females tended to show a greater effect of cocaine on reduction of nosepokes than did the males.

Effects of cocaine on repeated movements

Cocaine increased repeated movements at all doses with its peak effect observed at 30 mg kg^{-1}. As with the previous measures, significant between-strain effects were detected at all dose levels ($P < 0.001$). Proportion of variance explained at each dose was 10%, 15%, 19% and 23% for 5, 15 30 and 45 mg kg^{-1}, respectively. ANOVA also revealed a significant effect of sex ($P < 0.05$, with 1% of variance explained) on repeated movements at 15 mg kg^{-1} only with females tending to show greater effects of cocaine than males.

Effects of cocaine on centre time

In general, cocaine tended to reduce the time spent in the centre of the arena. Estimated ω^2 at each dose were 0.10, 0.16, 0.18 and 0.15 at 5, 15, 30 and 45 mg kg^{-1}, respectively ($P < 0.001$ at all doses). No significant sex effects were detected for centre time at any dose.

Dose–response activation scores

This measure was calculated by regressing distance traveled in cm on log dose of cocaine. A test of normality using the Kolmogorov–Smirnov Goodness of Fit Test supported our hypothesis that the distribution did not differ significantly from normal.

The Pennsylvania State University Cooperative Data Registry

Investigators interested in the raw means and other measures on which this paper is based have access through the Penn State Cooperative Data Registry (Plomin *et al.*, 1991; Blizard *et al.*, 1995). Interested investigators should contact the first author.

QTL identified for cocaine-related behaviours

Table 1 presents QTL, including LOD scores and the point-biserial correlation between the QTL and the behaviour. For total distance, QTL were detected for females at 15 and 45 mg kg^{-1} on chromosome 5 and for males at 5 mg kg^{-1} on chromosome 5. For males, we identified a QTL for slope on chromosome 9. QTL for nosepokes at 30 mg kg^{-1} were detected for males and females on chromosome 2, and for males chromosomes 12 and 9 at 30 and 5 mg kg^{-1}, respectively. QTL for repeated movements for sexes combined at 5 and 45 mg kg^{-1} were detected on chromosome 4 and 1, respectively, and for males at 30 mg kg^{1}, on chromosome 1.

DOPAMINE NEUROCHEMISTRY

Distributions of strain means for dopamine D_1, D_2 and the dopamine uptake transporter are illustrated in Figs 1–3, respectively. As the figures indicate, the caudate-putamen had the highest densities of all receptors, followed by the nucleus accumbens, frontal cortex and ventral midbrain. The distributions also are consistent with polygenic influence on densities. We did not observe significant differences between the sexes overall, therefore the data were collapsed across sex.

QTL identified for dopamine measures

QTL identified for regional dopamine receptor densities by type are presented in Table 2. In the frontal cortex, we identified two, sex-specific loci. For the D_2 receptor, we identified a QTL on chromosome 5 in males; for the dopamine uptake transporter, a location on chromosome 1 for females. For sexes combined, we identified three loci on chromosome 7.

In the nucleus accumbens, we identified loci for sexes combined, one for the D_1 receptor on chromosome 16 and one for the D_2 receptor on chromosome 15. For males, we identified QTL on chromosomes 12 and 15, with the latter showing at least five significant markers

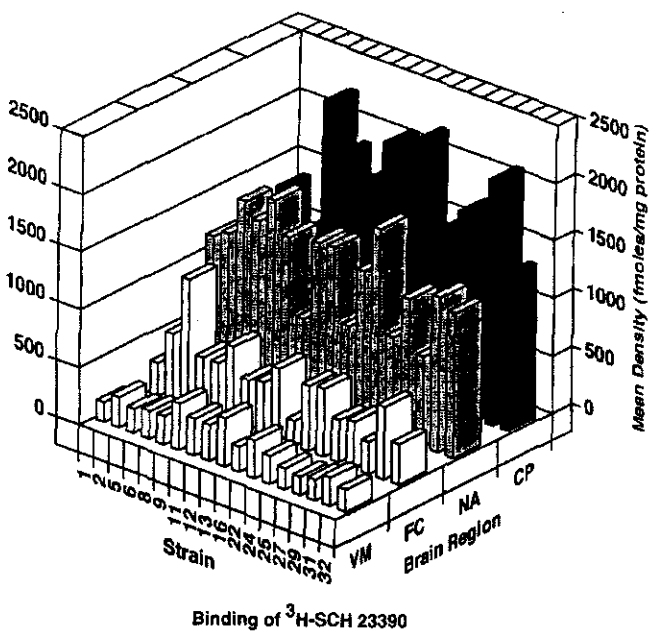

Fig. 1. Dopamine D_1 receptor densities by region in the panel of BXD/Ty recombinant inbred mouse strains. See text for specific details. VM, ventral midbrain; FC, medial prefrontal cortex; NA, nucleus accumbens; CP, caudate-putamen.

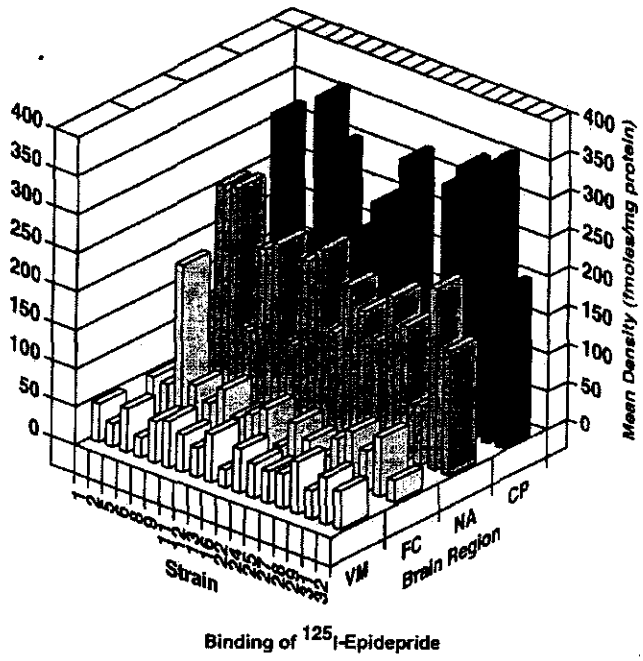

Fig. 2. Dopamine D_2 receptor densities by region in the panel of BXD/Ty recombinant inbred mouse strains. See text for specific details. VM, ventral midbrain; FC, medial prefrontal cortex; NA, nucleus accumbens; CP, caudate-putamen.

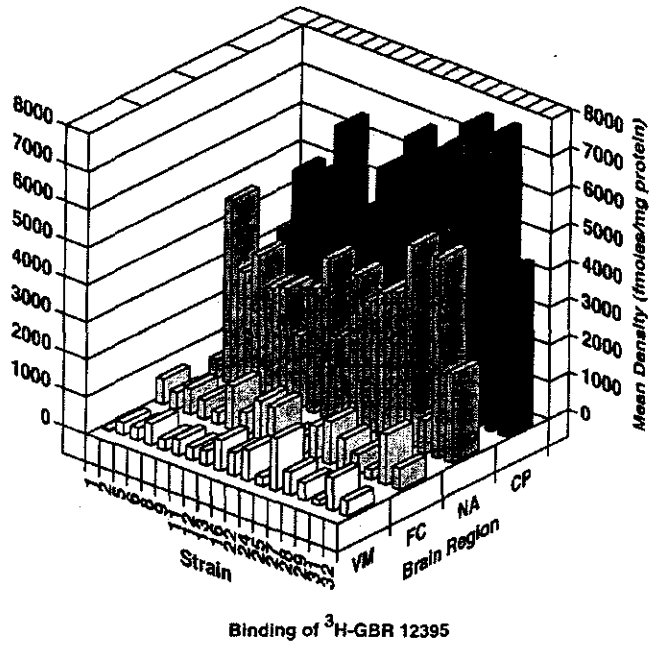

Fig. 3. Dopamine uptake transporter densities by region in the panel of BXD/Ty recombinant inbred mouse strains. See text for specific details. VM, ventral midbrain; FC, medial prefrontal cortex; NA, nucleus accumbens; CP, caudate-putamen.

between 43 and 48 cM. One of the markers, atf4, was the same marker on chromosome 15 for sexes combined.

In the caudate-putamen, for sexes combined, we observed QTL on chromosomes 12 for the D_1 receptor, chromosome 15 for the D_2 receptor and chromosomes 11 and 15 for the dopamine uptake transporter, the latter QTL being in the same area as for the D_2 receptor. We also observed sex-specific QTL in the caudate-putamen, but only for females. For the D_1 receptor, we identified QTL on chromosome 5, 12 and 15, for the D_2 receptor, we located QTL on chromosomes 9 and 15, the latter showing nearly complete overlap with the region identified for the D_1 receptor. The area on chromosome 15 identified for the sexes combined for the D_2 receptor is also in the same region. In the ventral midbrain, we located one locus on chromosome 15 for sexes combined, in the same area (46 cM) as for several the other receptors as noted above. We identified also a QTL, specific for females, on chromosome 9 for the D_2 receptor.

QTL in common for behaviours and dopamine

Table 3 presents QTL that we identified as likely being in common between cocaine-related behaviours and the neurochemical measures. We identified common QTL on chromosomes 1, 5, 12 and 15. On chromosome 1, D1Ncvs68 (69.9 cM) correlated with dopamine uptake transporter density and in the same area, we identified D1Ncvs12 (74.5 cM) as a QTL for nosepokes at 15 mg kg^{-1}. Both markers were identified in females only. On chromosome 5, D5Mit10 (54.0 cM) was correlated with D_2 receptor densities in the frontal cortex (males) and distance travelled at 45 mg kg^{-1} (sexes combined). On chromosome 12, D12Mit1 (2.0 cM) was correlated with D_1 receptor densities in the caudate-putamen (sexes combined) and D12Ncvs31 (6.0 cM) was correlated with nosepokes at 30 mg kg^{-1} (males). Chromosome 15 provided the richest source for common QTL. An area spanning approximately 40–55 cM, correlated with D_1 and D_2 receptor densities in the caudate-putamen (sexes combined, females) was also correlated with repeated movements at 30 mg kg^{-1} (sexes combined) and with distance travelled at 5 mg kg^{-1} (males). It should be recalled, that there was also a significant correlation between D_1 receptor densities in the caudate-putamen (sexes combined) and repeated movements (sexes combined), $r = 0.55$, $P < 0.05$.

Discussion

The findings we have presented extend our knowledge about genetic influences on cocaine-related behaviours in several important ways. First, this is the first study in

Table 2. QTL for regional dopamine receptors

Receptor	*Marker*	*Chr*	*Dist*	*LOD*	r	n
Medical prefrontal cortex						
D_2 (M)	D5Mit10	5	54.0	3.1	–0.80	14
DAUT (F)	D1Ncvs68	1	69.9	3.3	–0.88	10
DAUT (C)	D7Ncvs41	7	45.0	3.2	0.75	15
	D7Ncvs42	7	45.0	3.2	0.76	17
	mod2r	7	46.2	3.5	0.76	18
Nucleus accumbens						
D_1 (C)	D16Byu216	16	69.1	3.1	–0.78	15
D_2 (M)	D12Byu212	12	59.0	3.4	0.80	15
	D15Ncvs20	15	Syntenic	3.4	0.78	17
	rangap1	15	43	4.7	0.83	18
	pgdfb	15	46.8	4.7	0.83	18
	cyp2d9	15	47.2	3.5	0.81	15
	D15Mit2	15	46.9	3.6	0.81	15
	atf4	15	43.3	4.0	0.82	16
D_2 (C)	atf4	15	43.3	3.1	0.77	16
Caudate-putamen						
D_1 (C)	D12Mit1	12	2.0	3.1	0.80	14
D_1 (F)	D5Ncvs55	5	7.0	3.5	–0.79	16
	D12Ncvs47	12	25.0	4.0	0.81	17
	eif4e	12	25.0	4.8	0.84	16
	D15Ncvs21	15	53.3	3.1	0.76	16
	D15Ncvs29	15	51.0	3.9	0.84	16
	D15Ncvs23	15	51.0	4.8	0.85	17
	D15Ncvs24	15	51.0	4.8	0.85	17
D_2 (C)	rangap1	15	43.0	3.2	0.74	18
	pdgfb	15	46.8	3.2	0.74	18
D_2 (F)	D9Rik67	9	61.0	3.6		
	D15Ncvs29	15	51.0	4.0	0.84	15
	D15Ncvs22	15	51.0	3.5	0.82	14
	D15Ncvs24	15	51.0	3.8	0.83	15
	D15Rik36	15	Syntenic	4.2		
DAUT (C)	D11Ncvs44	11	25.0	3.3	–0.78	17
	D11Mit34	11	46.0	3.6	–0.78	18
	D15Mit1	15	46.3	3.1	0.74	18
	D15Ncvs29	15	51.0	3.2	0.76	17
DAUT (F)	atf4	15	43.3	5.1	0.89	15
Ventral midbrain						
D_2 (C)	D15Mit1	15	46.3	3.1	0.74	18
D_2 (F)	cyp2d9	15	47.2	3.4	0.84	13

F, female; M, male; C, sexes combined.

which cocaine behaviours across a wide range of doses have been related to dopamine neurochemistry. Second, the monitoring of multiple measures simultaneously provides a more comprehensive view of cocaine effects than would be available had only one measure been used. Third, to the best of our knowledge, this is the first report showing increased genetic influence with increasing doses of cocaine. This implies, of course that at different doses of cocaine, the role of genetic makeup changes to reflect either different genes coming into play or a changing effect of those genes already operative at other doses. Fourth, we report QTL that are both in common to both sexes and those that are sex-specific to both cocaine-related behaviours and dopamine receptors.

All of the behaviours measured showed distribution patterns consistent with polygenic influence. Genetic correlations within behaviours across dose were high between adjacent doses and tended to decrease as distance between doses increased. With the exception of

Table 3. QTL in common for dopamine receptors and cocaine behaviours

Marker	*Dopamine receptor*	*Behaviour*	*Marker*
Chromosome 1			
D1Ncvs68	DAUT in FC (F)	NP15 (F)	D1Ncvs12
Chromosome 5			
D5Mit10	D_2 in FC (M)	TD45 (C)	D5Mit10
Chromosome 12			
D12Mit1	D_1 in CP (C)	NP30 (M)	D12Ncvs31
Chromosome 15			
Several at 40–55 cM	D_1, D_2 in CP (F,C)	RM30 (C) TD5	Several at 40–55 cM

TD, total distance (dose of cocaine); NP, nosepokes (dose of cocaine); RM, repeated movements (dose of cocaine); FC, medial prefrontal cortex; CP, caudate-putamen; M, male, F, female, C, sexes combined.

locomotor activity and repeated movements and locomotor activity and centre time, our behavioural measures were largely orthogonal. QTL identified for the behaviours also reflected this orthogonality with most of the QTL for locomotion located on chromosomes 5, 9 and 15, nosepokes on chromosomes 2, 3, 9, 11 and 12, repeated movements on chromosomes 1, 4, 5, 6, 9, 15 and 19, and centre time on chromosomes 5 and 11.

How our results fit with those obtained by Tolliver *et al.* 1994, Miner and Marley *et al.* 1995b and Phillips *et al.* 1998 are presented in Table 4. The protocol of Phillips *et al.* (1998) most closely matched ours and not unexpectedly, produced more matching QTL to our study than did the others. Markers in common for cocaine-related locomotor activity between ours and the Phillips study exist on chromosomes 3, 4, 5, 12, 13 and 15. The name of Miner and Marley's (1995b) chromosome 4 marker, Nhe1, has been replaced by *Slc9a1*, a sodium/hydrogen exchanger. This marker is in common with our results at 5 mg kg^{-1} and is located at 64.6 cM. The Phillips study located a marker (also in common with our study), *Iapls3–10*, located at 54.4 cM; this may indicate an area overlapping the three studies. Possible candidate genes in this region include *Grik3* (glu k3 receptor), *Il14* (Interleuken 14) and *Oprd1* (Opioid delta 1 receptor). On chromosome 5, towards the telomere,

Table 4. Comparisons of QTL among previous studies with the present study

Miner and Marley (1995a,b)	*Tolliver* et al. *(1994)*	*Phillips* et al. *(1998)*
Chromosome 3		
		D3Ncvs26 5,10 (5,45)
Chromosome 4		
Nhel 10 (5)		Iapls3-10 5,10 (5,)
Chromosome 5		
		Nfc2u 40 (45)
Chromosome 9		
	d 32 (30)	
	D9Mit8 32 (30)	
Chromosome 11		
	D11Mit2 32 (30)	
Chromosome 12		
		Cbg 10 (15)
Chromosome 13		
		D13Ncvs48 5 (5)
Chromosome 15		
		D15Ncvs26 10 (15)
Chromosome 17		
D17Ncvs7 10 (5)		

The behaviour for locomotor activity and cocaine doses for the three investigative teams are indicated immediately to the right of the marker. Doses for comparison from this study are in parentheses. Comparisons from our study are same sex. Miner and Marley and Tolliver *et al.* used males, Phillips *et al.* used females.

Nfc2u was identified by the Phillips group and replicated by us. Possible neurologically relevant candidate genes may include *Ache* (acetylcholine esterase), *GnB2* (guanine nucleotide binding protein) and *Nos1* (nitric oxide synthase – neuronal). On chromosome 9, we identified two markers in common with that reported by the Tolliver group. The markers, *d* (38 cM) and D9Mit8 (40 cM), are in the region containing *Nedd4* (neural precursor cell expressed, developmentally down-regulated gene 4) and *Tcf4* (transcription factor 12) as possible candidate genes. The dopamine D_2 receptor gene at 28 cM is also within the 95% confidence interval and is discussed below. Also in common with the Tolliver study, we observed one marker on chromosome 11, D11Mit2 (2 cM). Possible candidate genes include *Ddc* (DOPA decarboxylase), and *Camk2b* (calmodulin kinase II, beta subunit). On chromosome 12 the Phillips and our studies identified *Cbg* (corticosteroid binding globulin) at 51 cM. In addition to *Cbg* identified as a possible candidate gene, *Ckb* (creatine kinase, brain) might be included. On chromosome 13, D13Ncvs48 (71 cM) is in common between the Phillips and our studies and possible candidate genes include *crhbp* (corticotropin releasing hormone binding protein) and *Htr1a* (serotonin receptor, 1a). The marker, D15Ncvs26, at 38.2 cM and observed by the Phillips group and us is quite near to a large area on chromosome 15 that in our study is associated with densities of dopamine D_1 and D_2 receptors, cocaine-related locomotor activity and repeated movements. This area is discussed in greater detail below. On chromosome 17, Miner and Marley and we identified a marker, D17Ncvs7 at 31 cM. In the region are the genes, *Ckb-rs2* (creatine kinase, brain, related sequence 2) and *Efna5* (ephrin A5).

Others have conducted QTL analysis of psychostimulant actions in the BXD RI panel. Alexander and his colleagues (1996) observed QTL for both phencyclidine and amphetamine and Grisel *et al.* (1997) revealed QTL for methamphetamine-induced hyperlocomotion in the home cage. As concerns the Alexander study, we observed one common QTL for locomotor activity, i.e, for phencyclidine and cocaine, D15Ncvs26, which at 40 cM on chromosome 15 is in the large group of QTL discussed below. One QTL which we identified in common with the Grisel study for amphetamine home cage activity was D1Mit1 on chromosome 1 at 8.7 cM. This locus was associated with cocaine-induced hyperlocomotion at 5 and 15 mg kg^{-1}. Possible candidate genes include *Oprk1* (opioid receptor, kappa 1), Grip1, glucocorticoid receptor interacting protein 1 and Kcnb2 (potassium voltage gated channel, *Shab*-related subfamily, member 2). Also in common with methamphetamine activity in the Grisel study was D15Mit7 at 14.5 cM. This marker also correlated with cocaine-induced hyperlocomotion at 5, 15 and 30 mg kg^{-1}. For this marker one possible candidate gene is Trhr (thyrotropin releasing hormone receptor).

A recent study from the laboratory of RJ Hitzemann (Kanes *et al.*, 1996), reported QTL associated with haloperidol-induced catalepsy in male mice from the BXD/Ty RI panel. Haloperidol is a potent D_2 receptor antagonist and thus it would be of interest to know whether the QTL identified for this drug coincide with QTL identified for densities of D_2 receptors in our study. The Kanes *et al.* (1996) study identified QTL on chromomes 2, 4, 6, 9, 15 and 16. Interestingly, one of the markers on chromosome 9, *Drd2* which they identified for haloperidol-induced catalepsy, we also identified for cocaine-increased repetitive movements. Furthermore, the markers on chromosome 15 (between 41 and 50 cM) and on chromosome 16 (*COMT*) match markers which we found to be associated with D_2 receptors in the caudate-putamen and nucleus accumbens. The markers that they identified on chromosome 15 provide additional evidence for the importance of one or more genes on that chromosome and the marker on chromosome 16 is *COMT*, catechol-*O*-methyl transferase, a reasonable candidate gene which could affect the density of dopamine receptors. More recently, Hitzemann (1998) proposed that at least for the D_2 receptor, differences in densities, i.e., gene expression, could be reasonably expected to be under the control of genes other than (not to exclude in addition to) the structural gene (*Drd2*). This author indicated among others, loci on chromosome 4 near *Tyrp1* and on chromosome 9, distal to the D_2 structural gene (*Drd2*), and near the 5HT1b gene (*Htr1b*). The 5HT1b gene is located at 46 cM and our marker found for females, D9Rik67, is located at 61 cM, within the confidence interval (approximately 20 cM) for such markers identified in RI strains and F_2 hybrid crosses.

With two exceptions, and in line with Hitzmann's (1998) assertion, none of the markers that we found to be associated with densities of dopamine receptors or the transporter was located on chromosomes containing the structural genes for any of the dopamine receptors or the transporter. The first exception noted is our finding of a marker at 61 cM on chromosome 9 associated with D_2 receptor densities for females in the caudate-putamen. This is somewhat distal to the *Drd2* gene and may be related to a marker, *d* (currently *Myo5a*), found in the vicinity for haloperidol-induced catalepsy (Hitzemann, 1998). Recall that this marker was identified also by us and by Tolliver for cocaine-related locomotion and is in the general vicinity of markers located by Phillips also for cocaine-related locomotion. The second exception noted was chromosome 16 which has both the genes for catechol-*O*-methyl transferase (approximately 10 cM) and the structural gene for the dopamine D_3 receptor (23.3 cM). Thus, we may have identified chromosomal areas containing

putative genes that alter the expression of the receptors, either directly or indirectly (or affected their availability for detection in our assay method).

The increase in genetic influence on cocaine-related behaviours at increasing doses is a new observation and its exact meaning is not clear. The increase could reflect either an increased activity of genes already affected by the drug or a recruitment of new genes. The answer to the problem may have to await confirmation of candidate genes and subsequent experimental manipulations.

The involvement of the large area on chromosome 15 with cocaine-related behaviours and neurochemistry warrants special discussion. It appears from our work and from others as discussed above that there is a large segment of chromosome 15 that is associated with cocaine and phencyclidine actions and dopamine D_1 and D_2 receptor densities in the caudate-putamen and nucleus accumbens. The area spans approximately 30–60 cM and numerous markers from different sources show significant correlations and LOD scores. A paucity of tissue prevented us from also examining possible structural differences (K_d) in receptors among the BXD RI strains. The numerous markers for each receptor subtype in this area would make chromosome 15 a target for intensive study. Possible candidate genes might include the peripheral type benzodiazepine receptor, *Bzrp*, *Cacnb* (calcium channel beta3 subunit), *Gpt1* (glutamic pyruvic transaminase 1, soluble) and *Scn8a* (sodium channel, voltage-gated, type VIII, alpha polypeptide). Recently, Bucan *et al.* (1993) mapped roughly the same area from mouse chromosome 15 onto human chromosome 22. This is particularly exciting, since most of the genes found in the mouse in this area are also found in the human genome, and may point to genes affecting sensitivity to drugs misused by humans.

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