Drug and Alcohol Dependence Reports 2 (2022) 100012

Contents lists available at ScienceDirect

Drug and Alcohol Dependence Reports

journal homepage: www.elsevier.com/locate/dadr

Genetic association of nucleus accumbens 5-hydroxyindoleacetic acid level and alcohol preference drinking in a quasi-congenic male mice: Potential modulation by *Grm7* gene polymorphism

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ARTICLE INFO

Keywords:
Addiction
Alcohol
Genetics
Serotonin
5-HIAA
Grm7
mGluR7

ABSTRACT

Objective: To test the hypothesis that predisposition to high alcohol drinking behavior is genetically associated with hypoactive serotonergic function in the Nucleus Accumbens (NAc).
Method: Alcohol avoiding C5A3 and alcohol preferring I5B25A mice of the Quasi-congenic Recombinant QTL Introgression (RQI) mouse strains were subjected to in vivo microdialysis in the NAc. Neurotransmitter and metabolite contents were analyzed by HPLC and samples were collected in three phases: Baseline, Control, and Alcohol. Samples were collected with 20 min intervals.
Results: Between-strain differences restricted to small chromosome segments significantly affected both alcohol preference drinking and NAc 5-HIAA levels [$F_{1,13}$ = 5.569 p=.035 (General Linear Model Repeated Measures ANOVA and Tests of Between-Subjects Effects)]. Whole genome biallelic DNA marker genotyping allowed the identification of 16 differential microsatellite markers associated with low 5-HIAA levels and excessive alcohol drinking. Chromosome 6 markers were linked to *Grm7* (51.19 centimorgan), a reported candidate gene for modulation of addiction. The results are consistent with earlier reports of association of low 5-HIAA and high alcohol consumption in rats and primates, including Homo sapiens.
Conclusion: Low NAc 5-HIAA and high alcohol consumption are genetically associated in a quasi-congenic mouse model carrying variants of the *Grm7* gene. We propose that constitutional polymorphism in *Grm7* may modulate CRF neuron activity via altered mGluR7 expression thus targeting CRF pathways to substance use circuits. This raises the possibility of modulation of DRN 5-HT neurons leading to hypo- or hyper-serotonergic condition in NAc and higher or lower alcohol preference drinking.

1. Introduction

Inheritance of complex traits, like alcohol preference drinking, is polygenic but their genetic architecture is not well known. At the turn of the millennium mutagenesis was hoped to allow discovery of genes involved in complex traits, however, the utility of mutagenesis was criticized that it may not be an efficient way of producing appropriate models of naturally occurring genetic variants (such as human genetic disorders) (Vadasz, 2000). Indeed, N-ethyl-N-nitrosourea (ENU)-induced nucleotide changes are known to exhibit a strong bias towards particular lesions (Bronstein et al., 1992), and possibilities remain that this property should restrict the general applicability of ENU (Takahasi et al., 2007). Approaches utilizing animal models with natural gene variants may offer useful insights.

Natural allelic variation affects mesencephalic (MES) tyrosine hydroxylase (TH) activity (TH/MES), which can be traced back to differences in the number of TH positive neurons residing in the dopaminergic A9, A10 cell groups (Ross et al., 1976). Because the mesotelencephalic dopaminergic system plays pivotal roles in motor behavior, reward system, attention, etc., we initiated a genetic project to select for high or low TH/MES. Replicated mouse lines were developed by recurrent backcrossing to a mouse strain (C57BL/6By) with concomitant selection for high or low TH/MES. Using the method of Recombinant QTL Introgression (RQI) QTLs from the CXBI RI strain were transferred to two incipient " low TH/MES" lines (B6.I-α and B6.I-β), and QTLs from the BALB/cJ

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[1] Development of animal models was supported by NIH NINDS NS19788, experiments were supported by The National Institute on Alcohol Abuse and Alcoholism R01 AA11031, and DoD DAMD 17-00-1-0578.

https://doi.org/10.1016/j.dadr.2021.100012
Received 9 September 2021; Received in revised form 11 November 2021; Accepted 29 November 2021

donor strain were transferred to two incipient high "TH/MES" lines (B6.C-α and B6.C-β) (Vadasz et al., 1998) . After completing the construction of four RQI strain sets mice were phenotyped for alcohol preference drinking and the whole genome was genotyped for microsatellite markers. QTL mapping identified six QTLs located on chromosomes 6 and 12 (Vadasz et al., 2007a). One of them (*Eac2)* was further investigated and we identified *Grm7* (metabotropic glutamate receptor subtype 7) as the first mammalian gene accounting for alcohol preference (Vadasz et al., 2007b). Here we further investigate the alcohol preferring (I5B25A) and the alcohol avoiding (C5A3) quasi-congenic RQI strains. After five cycles the genetic background of each QTL-Introgression line is about 97% identical with that of the background strain, i.e., the remaining 3% genetic difference is responsible for the phenotypic differences. Here, we test the hypothesis that the genetic differences between the two RQI strains affect accumbal serotonergic function in addition to the previously established differences in alcohol consumption (Vadasz et al., 2007a, 2007b).

2. Methods

2.1. Animals

The experiments involved 17 89±1 (mean±SE) day-old male mice of the C5A3 and I5B25A quasi-congenic strains (n = 6–8). In the construction of RQI strains B6 (C57BL/6ByJ) served as background strain; C (BALB/cJ, donor strain), and I (CXBI/By) served as donor strains as described (Vadasz et al., 1982, Vadasz et al., 2007a) . C5A3 is of B6.C introgression type in which BALB/cJ donor segments are distributed on B6 background; I5B25A is of B6.I introgression type in which CXBI donor segments are distributed on B6 background. We use abbreviated RQI strain names as C5A3 (B6.Cb_5i_7-α3/Vad) and I5B25A (B6.Ib_5i_7-β25A/Vad). The care and use of animals met the standards and recommendations of the IACUC of the Nathan S. Kline Institute for Psychiatric Research in accordance with US Department of Agriculture and US Public Health Service guidelines.

2.1.1. Recombinant QTL introgression (RQI)

RQI is a method for the genetic analysis of complex quantitative traits by combining short-term phenotypic selection-introgression to reach quasi-congenic condition, recombination and inbreeding (Vadasz et al., 1998). Use of RQI allows transfer of segregated heritable increaser or decreaser factors which control the phenotype, and preserve them in homozygous recombinant form in numerous quasi-congenic (near-isogenic) highly inbred strains. For phenotype a mesotelencephalic dopamine-system related trait was chosen because of its critically important roles in the control of motor activity, motivation, emotion, addiction, and learning. QTLs that are responsible for the continuous variation of mesencephalic tyrosine hydroxylase (TH/MES) activity, an index trait for midbrain dopamine neuron number (Ross et al., 1976), were introgressed onto B6 background strain from BALB/cJ and CXBI donor strains. CXBI is a recombinant inbred strain carrying B6 and BALB/cBy genes . Two types of F2s (B6XC and B6XI), were produced and in each type replicate lines (α and β) were created by equal division of each F2 litter. In each of the four lines, at least 45 F2 males were tested for the phenotype, and 15 were selected for the first backcross to B6 females. Then, at least 45 backcross$_1$ (b_1i_0) male offspring were tested, and 15 males were selected and intercrossed with non-littermate females, resulting in b_1i_1 generation. The QTL transfer was carried out in two directions by backcross-intercross cycles with concomitant selection for the extreme high and low expressions of TH/MES activity in replicates, resulting in four QTL introgression lines. In these lines, the top and bottom one-third of each generation was selected. These steps were repeated for five (b_5i_7 series) cycles. The QTL introgression phase was followed by initiation of brother sister (bxs) mating for at least 30 generations in closed lines. Inbred RQI strains of the b_5i_7 series carry an estimated <3% of the donor genome on the background B6 genome.

2.2. Surgery and MD (microdialysis)

Implantation of guide cannula was performed in a stereotaxic apparatus with ketamine (100 mg/bwkg i.p.) + xylazine (10 mg/bwkg i.p.) anesthesia. The head position was adjusted so that bregma and lambda were aligned at the same height. The guide cannula (CMA/7 guide; CMA Microdialysis, North Chelmsford, MA, USA) was implanted vertically into the accumbens shell (anterior, 1.3 mm; lateral, 0.7 mm; vertical, −5.2 mm from bregma), according to the atlas of Franklin and Paxinos (Franklin and Paxinos, 1997), then fixed on the skull with dental cement as described previously (Hungund et al., 2003). Animals were let to recover for 5–6 days before inserting the probe for dialysis. Mice were housed in a transparent Plexiglas hemisphere, closed with a top hemisphere, with food and water available. The probe (CMA/7, O.D. 0.24 mm; 2 mm cuprofen membrane; CMA Microdialysis) was inserted in the guide cannula and was left overnight with artificial cerebrospinal fluid (expressed in mM: NaCl 147, KCl 2.7, $CaCl_2$ 1.2, $MgC1_2$ 0.85; CMA/Microdialysis AB, Stockholm, Sweden) pumped through the dialysis probe at a constant flow rate of 0.5μL/min. The flow rate was increased to 1μL/min on the day of the experiment and we waited for 2 h before starting collecting the samples. We put 1μL 0.1 M PCA in each microcentrifuge tube and collected the samples in it in every 20 min. Sample collection was scheduled as follows. Baseline samples were collected at −80 min, −60 min, −40 min, −20 min, 0 min. Saline samples were collected at 20 min, 40 min, 60 min. Physiological saline (0.9%) was administered immediately after collecting last baseline sample (0 min). Alcohol samples were collected at 80 min, 100 min, 120 min, 140 min, 160 min, 180 min. Alcohol (1.5 g/kg alcohol in saline, i.p.) was injected immediately after collecting the last saline sample (60 min). Amphetamine sulfate (3 mg/kg, i.p.) was administered immediately after collecting the last alcohol sample (180 min). Data from amphetamine samples of 200 min, 220 min, 240 min were not included in the analysis. Crude dialysate samples (15μL) were injected directly into an HPLC apparatus (C18 UniJet Microbore column, Bioanalytical Systems, West Lafayette, IN, USA) coupled to an electrochemical detector (Bioanalytical Systems). Mobile Phase Constituents (for 1 L) were Na-citrate ($C_6H_5Na_3O_7$ $2H_2O$, 14.3 g), Na-phosphate (NaH_2PO_4, 3.0 g), Diethylamine ($C_4H_{11}N$ HCl, 1.09 g), 1-Octanesulfonic acid (OSA, $C_8H_{17}NaO_3S$ H_2O, 475 mg), Na-EDTA ($Na_2C_{10}H_{14}O_8N_2$ $2H_2O$, 10 mg), Acetonitrile (CH_3CN, 16.4 mL), N,N-Dimethylacetamide ($CH_3CON(CH_3)_2$, 21.1 ml). The mobile phase was pumped at a flow rate of 0.1 mL/min.

Brains were fixed and 50-μm-thick sections cut with a vibratome and stained with cresyl violet to confirm the accuracy of the probe placements. Only data from animals with correct probe placements were included in the final analysis.

2.3. Statistical analysis

Data were analyzed using General Linear Model (GLM) Repeated Measures (RM) ANOVA as implemented in the IBM SPSS Statistics 24 package. To analyze NAc metabolite levels across time RM ANOVA was used applying Mauchly's test of sphericity and Levene's test of equality of error variance. Within-subject factor measure was neurotransmitter or metabolite level (pg/15μL), between-subject factor was strain. GLM RM ANOVA was followed up by independent-samples t-test with two tails. Data of one subject (mouse #147 of the IB25A strain) for all sampling intervals were lost and were not used for analysis. An outlier HVA measure (IB25A, mouse i.d.#158, baseline sample #2) was replaced by group mean=807.96. Statistical significance of strain and treatment effects were tested at alpha=0.05.

3. Results

3.1. Genetic sources of differences

Results of genotyping of 396 microsatellite markers covering 19 chromosomes confirmed biallelic variation between C5A3 and I5B25A quasi-congenic strains . Briefly, after the recurrent backcrossing to C57BL/6By background strain in the process of quasi-congenic strain construction each strain differed from the common background strain in about 3%, and from each other in about 7% of the markers, suggesting that this difference can be responsible for the observed neurochemical differences in NAc MD.

The biallelic nature of the RQI strains allowed the identification of differential chromosome regions associated with both low accumbal 5-HIAA and high alcohol drinking. Based on the RQI construction strategy, the X and Y sex chromosomes can be considered as background- and donor-type, respectively. The tested strains were highly inbred, accordingly we detected only homozygous C57BL/6By-type (BB) or BALB/cJ-type (CC) markers, but no heterozygotes.

Testing the autosomal chromosomes of the C5A3 and I5B25A strains we found 24 differential markers on 7 chromosomes out of 324 reliably genotyped markers on 19 chromosomes (using a genotype database at www.rqigenetics.org/RQI.gbase), indicating a small genetic distance (23/324=0.070 ratio) between the two RQI strains. This can be compared with the biallelic mapping and genotyping of 673 SNPs in 55 of the most commonly used mouse strains, which resulted in 374.5 fixed alleles that differ between a given pair of strains by complete-linkage hierarchical clustering (Tsang et al., 2005) corresponding to a larger genetic distance (374.5/673=0.556). Using the notation CC=homozygous BALB/c-type; BB=homozygous C57BL/6By-type alleles, and strain order: C5A3 followed by I5B25A we found the following marker genotypes:

Chr.1 D1Mit167 CC, BB; **Chr.5** D5Mit145 CC, BB; D5Mit48 CC, BB; D5Mit331 CC, BB; D5Mit223 CC, BB; D5Mit286 CC, BB; **Chr.6** D6Mit19 CC, BB; D6Mit228 CC, BB; D6Mit230 CC, BB; D6Mit105 CC, BB (49.71 cM); D6Mit327 CC, BB (49.99 cM); D6Mit287 CC, BB (52.14 cM); **Chr.7** D7Mit14 CC, BB**;** D7Mit223 CC, BB; D7Mit259 CC, BB; **Chr.8** D8Mit155 BB, CC; **Chr.12** D12Mit60 BB, CC; D12Mit46 BB, CC; **Chr.13** D13Mit236 BB, CC; D13Mit55 BB, CC; D13Mit158 BB, CC; D13Mit17 BB, CC; D13Mit115 BB, CC.

These genetic differences may modulate the observed neurotransmitter and metabolite levels in the NAc and may contribute to the reported significant strain difference in alcohol preference drinking (Vadasz et al., 2007a). Indeed, the differential chr. 6 region harbors *Grm7* (chr6: 51.19 cM) which has been reported to affect both alcohol and cocaine related behaviors (Gyetvai et al., 2011; Vadasz and Gyetvai, 2020). However, the MD neurochemical phenotypes have not been subjected to QTL mapping in the RQI system, and specific gene variant effect on a biochemical pathway leading to neurotransmitter and metabolite level modulation in the NAc has not been identified.

3.2. In vivo NAc neurotransmitter and metabolite levels

3.2.2. Factors time and strain as sources of differences in MD samples

For each subject 13 MD samples (−60 through 180 min) were analyzed. For establishing baseline levels the first four 20-minute intervals were used taking samples at −60, −40, −20, and 0 min. The *saline* phase comprised three intervals (sampled at 20, 40, and 60 min), while *alcohol* effects were detected in six intervals of the alcohol phase sampled at 80, 100, 120, 140, 160, and 180 min. In the statistical analysis full factorial RM model with Type III sum of squares was applied with RM contrasts as “simple (first)“ and “difference” for factors time and strain, respectively. In all analyses the first baseline sample, #1 at-80 min, was excluded because it was an outlier among the baseline intervals reflecting instability due to first interaction with the animal and the brain tissue surrounding the microdialysis guide cannula. Amphetamine effects were detected in three intervals sampled at 200, 220, and 240 min.

Table 1.
Strain differences in NAc DA concentration* of in vivo microdialysis samples in alcohol avoiding (C5A3) and preferring (I5B25A) quasi-congenic mice .

Sampling (min)		Mean	Std. Deviation	N
−60	C5A3	3.791	2.685	7
	I5B25A	2.200	1.059	7
−40	C5A3	3.835	2.483	7
	I5B25A	2.342	1.365	7
−20	C5A3	3.310	2.365	7
	I5B25A	2.327	1.081	7
0	C5A3	3.437	1.857	7
	I5B25A	2.057	0.997	7
20	C5A3	4.230	2.147	7
	I5B25A	1.951	0.922	7
40	C5A3	3.349	2.210	7
	I5B25A	2.064	1.049	7
60	C5A3	3.164	2.213	7
	I5B25A	2.218	1.208	7
80	C5A3	4.369	2.899	7
	I5B25A	3.200	1.986	7
100	C5A3	2.724	1.932	7
	I5B25A	2.505	1.528	7
120	C5A3	2.736	2.096	7
	I5B25A	2.535	1.585	7
140	C5A3	2.908	2.085	7
	I5B25A	3.011	1.872	7
160	C5A3	3.782	3.395	7
	I5B25A	1.918	1.346	7
180	C5A3	3.420	2.811	7
	I5B25A	2.035	1.022	7
200	C5A3	5.188	4.881	7
	I5B25A	4.682	2.710	7
220	C5A3	8.987	6.724	7
	I5B25A	11.246	7.736	7
240	C5A3	9.738	7.290	7
	I5B25A	8.972	5.997	7

* pg/15uL.

3.2.2.1. Dopamine levels. Data were subjected to GLM RM ANOVA where 13 MD samples (interval −60 min to 180 min) were used as levels of within-subject factor (Table 1). Between-subjects factor was strain (C5A3 $n = 7$, I5B25A $n = 7$). Mauchly’s Test of Sphericity ($W = 0.000$; $p<.001$) rejected the null hypothesis that the error covariance matrix of the orthonormalized transformed dependent variables is proportional to an identity matrix. Because sphericity could not be assumed, Greenhouse-Geisser (GG) correction (Epsilon GG=0.368) was applied. After correction, the within-subjects (WS) effects of time ($p=.142$) or time*strain interaction ($p=.079$) were not significant. Also, tests of Between-Subjects (BS) effects showed no significant effects ($p=.378$).

3.1.2.2. DOPAC levels. In GLM RM ANOVA between-subjects factor was strain (C5A3 $n = 8$, I5B25A $n = 7$; Table 2). Mauchly’s Test of Sphericity ($W = 0.000$; $p<.001$) rejected the null hypothesis, Greenhouse-Geisser (GG) correction (Epsilon GG=0.298) was applied. After correction, the WS effect of time was significant ($F_{3.573} = 7.521$ $p=.002$). The time factor in tests of WS contrasts was significant source of increase from baseline level (at-60 min) vs levels at 120 min ($F_{1,13} = 3.682$ $p<.047$) and 140 min ($F_{1,13} = 4.730$ $p<.049$) in the alcohol phase. In tests of WS contrasts time*strain interaction effects were not significant ($p>.05$).

The results suggest that NAc DOPAC levels were at increased levels after alcohol exposure when compared to the baseline level at the-60 min interval without reaching statistically significant strain differences.

3.2.2.3. HVA levels. GLM RM ANOVA with 13 sampling intervals were used as levels of WS factor, between-subjects factor was strain (C5A3 $n = 8$, I5B25A $n = 7$; Table 3). Mauchly’s Test of Sphericity rejected the null hypothesis, and Greenhouse-Geisser (GG) correction (Epsilon

Table 2
Strain differences in NAc DOPAC concentration* of in vivo microdialysis samples in alcohol avoiding (C5A3) and preferring (I5B25A) quasi-congenic mice .

Sampling (min)	strain	Mean	Std. Deviation	N
−60	C5A3	778.200	389.346	8
	I5B25A	688.783	301.971	7
−40	C5A3	754.838	392.619	8
	I5B25A	666.283	309.061	7
−20	C5A3	746.688	382.134	8
	I5B25A	655.133	320.468	7
0	C5A3	750.000	365.958	8
	I5B25A	658.400	339.192	7
20	C5A3	754.925	352.470	8
	I5B25A	671.767	317.496	7
40	C5A3	780.838	375.998	8
	I5B25A	666.200	297.855	7
60	C5A3	741.500	370.641	8
	I5B25A	696.783	336.160	7
80	C5A3	825.813	415.529	8
	I5B25A	742.733	364.272	7
100	C5A3	811.400	356.123	8
	I5B25A	808.633	361.569	7
120	C5A3	797.163	297.296	8
	I5B25A	837.817	375.121	7
140	C5A3	823.063	300.103	8
	I5B25A	826.750	355.929	7
160	C5A3	849.775	325.708	8
	I5B25A	775.367	328.447	7
180	C5A3	804.650	310.982	8
	I5B25A	714.867	311.020	7
200	C5A3	706.314	295.444	8
	I5B25A	635.617	313.721	7
220	C5A3	376.614	113.439	8
	I5B25A	290.550	82.878	7
240	C5A3	235.200	49.096	8
	I5B25A	202.083	37.419	7

* pg/15uL.

Table 3
Strain differences in NAc HVA concentration* of in vivo microdialysis samples in alcohol avoiding (C5A3) and preferring (I5B25A) quasi-congenic mice .

Sampling (min)	strain	Mean	Std. Deviation	N
−60	C5A3	970.188	367.914	8
	I5B25A	807.966	197.679	7
−40	C5A3	941.638	364.016	8
	I5B25A	830.414	198.066	7
−20	C5A3	953.400	350.460	8
	I5B25A	826.671	217.839	7
0	C5A3	953.488	341.361	8
	I5B25A	823.886	207.996	7
20	C5A3	974.363	328.129	8
	I5B25A	838.557	234.934	7
40	C5A3	1014.063	351.057	8
	I5B25A	864.757	217.713	7
60	C5A3	994.700	331.003	8
	I5B25A	913.210	260.137	7
80	C5A3	1026.450	330.930	8
	I5B25A	919.871	271.920	7
100	C5A3	1014.400	364.870	8
	I5B25A	946.000	248.722	7
120	C5A3	1012.013	285.417	8
	I5B25A	997.014	268.497	7
140	C5A3	1017.838	280.401	8
	I5B25A	1001.557	219.480	7
160	C5A3	1079.038	299.284	8
	I5B25A	991.657	194.072	7
180	C5A3	1063.163	276.674	8
	I5B25A	974.214	181.220	7
200	C5A3	925.350	264.105	8
	I5B25A	850.200	131.634	7
220	C5A3	799.067	172.885	8
	I5B25A	730.517	126.504	7
240	C5A3	657.717	76.875	8
	I5B25A	599.783	67.824	7

* pg/15uL.

GG=0.290) was applied. After GG correction the within-subjects effects of time was significant ($F_{3.482}$ = 6.507 p=.001). The time factor in tests of WS contrasts was a significant source of increase from baseline level (at-60 min) vs levels at 120 min ($F_{1,13}$ = 9.066 p<.010), 140 min ($F_{1,13}$ = 12.059 p<.004), 160 min ($F_{1,13}$ = 17.837 p<.001), 180 min ($F_{1,13}$ = 14.309 p<.002) while time*strain interaction in tests of WS contrasts was not significant. Tests of Between-Subjects (BS) effects showed no significant effects ($F_{1,13}$ = 0.477 p=.502). The results suggest that NAc HVA levels were significantly higher at 140–180 min when compared to the baseline level (at the −60 min interval) without reaching statistically significant strain differences.

3.2.2.4. 5-HIAA levels. As above, GLM RM ANOVA with 13 sampling intervals (−60 min to 180 min) were used as levels of WS factor. Between-subjects factor was strain (C5A3 n = 8, I5B25A n = 7; Table 4). Greenhouse-Geisser (GG) correction (Epsilon GG=0.351) was applied because Mauchly's Test of Sphericity rejected the null hypothesis. After GG correction the within-subjects effects of time were not significant ($F_{4.211}$ = 2.295 p=.068). Also, WS time*strain interaction was not a significant source of 5-HIAA level variation (p=.477). Tests of WS contrasts for time or time*strain interaction showed no significant change from the baseline sample at-60 min. Tests of Between-Subjects Effects showed significant strain effect $F_{1,13}$ = 5.569 p=.035 (see Fig. 1).

4. Discussion

We examined the interactions between genetic factors and alcohol administration in the NAc applying our in vivo microdialysis procedure developed for mice (Hungund et al., 2003). For determining the genetic factors we took advantage of an earlier large-scale QTL mapping study which identified two extreme RQI mouse strains: The alcohol avoiding C5A3, and the alcohol preferring I5B25A strains with reported alcohol preference drinking (g/kg/day) M = 2.57 SD=2.71 (n = 40) and M = 10.87 SD=2.94 (n = 40), respectively (Vadasz et al., 2007a). The unique characteristic of the QTL Introgression lines is that their genome is estimated to be ~97% identical with the background C57BL/6By strain. Chromosome linked strain differences have been identified by microsatellite DNA marker polymorphism therefore the association of differential neurochemical and behavioral phenotypes point to potentially common genetic determinants. The approximately 93% genomic similarity of the C5A3 and I5B25A strains suggests that no phenotypical differences are expected except those which are controlled by functional gene variants which are located on the small differential chromosome segments representing the estimated 7% of the genome.

4.1. Variations of monoamines and their metabolites

Within the constraints of the applied MD/HPLC method and the relatively small sample size, levels of DA and its metabolites did not show significant within subject time*strain interaction or between strain effects giving no indication of association with the ethanol consumption differences between C5A3 and I5B25A. In similar experiments, studying the extracellular levels of monoamines in the nucleus accumbens of the alcohol-preferring AA and alcohol-avoiding ANA rats with in vivo microdialysis, alcohol administration significantly increased the extracellular levels of dopamine, DOPAC, and HVA. However, no difference between the AA and ANA rats in the extent or time course of stimulation of dopamine release was found (Kiianmaa et al., 1983).

In the C5A3 and I5B25A mice alcohol administration did not induce significant response in 5-HIAA levels and the within-subjects effects of time and within-subjects time*strain interaction were not significant

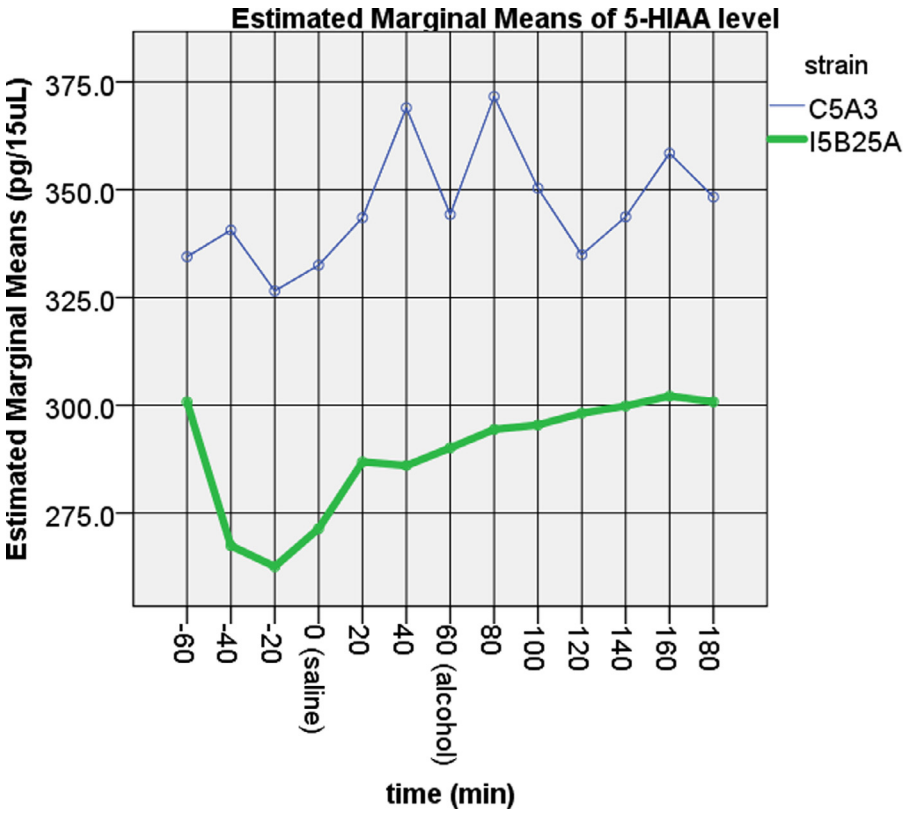

Fig. 1.. Genetic differences in NAc 5-HIAA levels determined by in vivo microdialysis. GLM RM ANOVA with13 sampling intervals (−60 min to 180 min) were used as levels of within-subject factor. Between-subjects factor was strain (C5A3 n = 8, I5B25A n = 7). Mauchly's Test of Sphericity rejected the null hypothesis, Greenhouse-Geisser (GG) correction (Epsilon GG=0.351) was applied. After GG correction the within-subjects effects of time ($F_{4.211}$ = 2.295 p=.068) and WS time*strain interaction (p=.477) were not significant sources of 5-HIAA level variation. Tests of WS contrasts for time or time*strain interaction showed no significant change from baseline (−60 min sample). Tests of Between-Subjects Effects showed significant effects $F_{1,13}$ = 5.569 p=.035).

Table 4
Strain differences in NAc 5-HIAA concentration* of in vivo microdialysis samples in alcohol avoiding (C5A3) and preferring (I5B25A) quasi-congenic mice .

Sampling (min)	strain	Mean	Std. Deviation	N
−60	C5A3	334.457	44.197	8
	I5B25A	300.780	16.436	7
−40	C5A3	340.600	74.870	8
	I5B25A	267.400	38.979	7
−20	C5A3	326.513	64.948	8
	I5B25A	262.557	48.102	7
0	C5A3	332.500	36.716	8
	I5B25A	271.371	48.185	7
20	C5A3	343.488	48.855	8
	I5B25A	286.843	64.520	7
40	C5A3	368.988	57.133	8
	I5B25A	286.000	67.566	7
60	C5A3	344.238	53.164	8
	I5B25A	290.100	60.960	7
80	C5A3	371.613	56.956	8
	I5B25A	294.371	57.563	7
100	C5A3	350.338	48.695	8
	I5B25A	295.414	57.864	7
120	C5A3	334.913	46.787	8
	I5B25A	298.157	58.044	7
140	C5A3	343.713	49.245	8
	I5B25A	299.829	56.463	7
160	C5A3	358.438	64.861	8
	I5B25A	302.114	56.803	7
180	C5A3	348.288	54.475	8
	I5B25A	300.786	52.153	7
200	C5A3	341.300	49.344	8
	I5B25A	288.000	50.926	7
220	C5A3	302.275	46.096	8
	I5B25A	266.057	54.220	7
240	C5A3	299.171	47.911	8
	I5B25A	261.083	35.245	7

* pg/15uL.

sources of 5-HIAA level variation (p=.477), and no significant changes from baseline sample levels were detected in tests of contrasts. However, 5-HIAA levels in the alcohol preferring I5B25A strain were consistently lower in dialysate samples in the course of the baseline, saline control, and alcohol phases (p=.035). The marked differences between C5A3 and I5B25A in alcohol consumption and in NAc 5-HIAA levels seem to be constitutional, the latter could be explained in terms of differences in serotonin function in the nucleus accumbens, suggesting genetic association of the two phenotypes.

Our results are consistent with numerous studies on laboratory rodents namely in the *HAD/LAD* rats (Gongwer et al., 1989), the 5-HT deficient Fawn-Hooded rats which display a preference towards ethanol intake (Rezvani et al., 1990), the alcohol-preferring P and alcohol-non-preferring NP rats (Zhou et al., 1994; Zhou et al., 1994), and the Sardinian alcohol-preferring (sP) and Sardinian alcohol-non-preferring (sNP) rats (Devoto et al., 1998).

Studies on mice are somewhat contradicting. Daszuta and Portalier found a higher number of 5-HT neurons in the most caudal part (B6) of nucleus raphe dorsalis of BALB/c compared to C57BL (Daszuta and Portalier, 1985). Siesser et al., concluded that Tph2 genotype determines brain serotonin synthesis but not tissue content in C57BL/6 and BALB/c congenic mice (Siesser et al., 1985). Primates readily consume alcohol solution for its reinforcing effects. Cloninger proposed a psychobiological model of alcoholism (Type II) in its original formulation as male-limited, and characterized by impaired impulse control resulting in unrestrained alcohol consumption (Cloninger, 1987). Cloninger attributed impulse-mediated alcoholism (Type II) primarily to CNS serotonin deficit. Linnoila's investigations showed that men with low CSF 5-HIAA concentrations frequently exhibit behavioral problems that may be indicative of impaired impulse control and excessive alcohol consumption (Linnoila et al., 1994). Considering the evolutionary underpinnings of excessive alcohol consumption and integrating behavioral and neuroendocrine data from captive and semi-free-ranging rhesus macaques, Gerald and Higley hypothesized that benefits derived from

impulsive and aggressive behaviors in some contexts might contribute indirectly to the maintenance of traits involved in excessive alcohol intake (Gerald and Higley, 2002). Extensive studies on both humans and rhesus macaques showed relationships between excessive alcohol consumption and serotonergic function, as measured by concentrations of 5-HIAA in the cerebrospinal fluid (CSF). Rhesus monkeys with low CSF 5-HIAA concentrations also exhibited deficits in impulse control and consumed large amounts of alcohol similarly to individuals characterized by Type II-like deficits (Higley and Bennett, 1999).

As in other vertebrates, alcohol response and consumption in primates is a complex trait affected by both environmental and genetic factors, and their interactions during development. A review of studies from the National Institutes of Health Animal Center (NIHAC) (Schwandt et al., 2010) confirmed that alcohol response and alcohol consumption are influenced by life history variables such as age, sex, and adverse early experience in the form of peer-rearing. Furthermore, genetic variants that alter functioning of the serotonin, endogenous opioid, and corticotropin releasing hormone systems were shown to influence both physiological and behavioral outcomes, in some cases interacting with early experience to indicate gene by environment interactions.

Candidate gene studies identified a common polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) which altered in vitro gene transcription (Lesch et al., 1996), in vitro transporter activity (Stoltenberg et al., 2002), and in vivo serotonin transporter density (Heinz et al., 2001). A variant in the μ-opioid receptor gene (OPRM1) has also been identified. The single nucleotide polymorphism (SNP A118G) in the gene has been associated with increased subjective euphoria and stimulation following intravenous alcohol administration. It has been suggested that the increased alcohol-induced positive reinforcement that is mediated by the OPRM1 A118G polymorphism could be a heritable factor that increases susceptibility to both initiation and maintenance of alcohol seeking behavior (Ray and Hutchison, 2004). The results of investigation of the OPRM1 C77G polymorphism in rhesus macaques indicated that males carrying the G allele displayed increased alcohol-induced stimulation (Barr et al., 2007). The evidence linking 5-HTTLPR variation with level of response to alcohol in humans has been mixed (cf. Schwandt et al., 2010). Longitudinal studies on alcohol consumption of 156 rhesus macaques demonstrated that, as in humans, there are additive genetic factors that contribute to variation in alcohol consumption in rhesus macaques (19.8% of the total variance, (Lorenz et al., 2006)).

4.1.1. *Limitations of position based search for candidate genes*

Mouse strains of different origin usually carry variants at about 50% of their genes, therefore chance association of independently controlled phenotypes is relatively high (Tsang et al., 2005). The two quasi-congenic strains carry variants at about 7% of their genes and one of the differential chromosome segments harbors *Grm7* gene variants, which have been reported to modulate alcohol drinking (Vadasz et al., 2007b). The smaller genomic difference between the quasi-congenic strains significantly reduces chance association of phenotypes in comparison with experiments on commonly used strains, however, potential contribution of other unknown and known genetic factors residing in this 7% needs further investigation. As to alcohol preference drinking, it is possible that one of the modulatory genes is *Grm7* (chromosome 6: 51.19 cM) because there are closely positioned differential markers on chr6 (D6Mit327 49.99 cM, D6Mit105 49.71 cM, D6Mit 287 52.14 cM).

4.2. *The* Grm7 *pleiotropy hypothesis*

The main result, genetic association between low NAc 5-HIAA and high alcohol preference drinking, raises the questions of identity of underlying genes and neural mechanisms. As discussed above, a few genes have been reported as modulators of alcohol related behaviors while the genetic architecture of alcohol drinking is not well known. Here we propose that variation in cis-regulated *Grm7* may be one of the involved genes and as a pleiotropic gene it may modulate both NAc 5-HIAA and high alcohol preference drinking based on:

(1) work showing a link between stress and addiction via corticotropin-releasing factor (CRF) modulation of the DRN 5-HT system demonstrates that CRF acts at CRF1 receptors to inhibit the DRN 5-HT system via GABA release leading to decreased 5-HT release contributing to impulsivity and substance abuse initiation. With increasing CRF concentration the inhibitory effects are lost (cf. Valentino et al., 2010)).
(2) reports indicating that mGlu7 presynaptic inhibitory heteroreceptor (produced via expression of *Grm7* mRNA) may modulate the increase in stress hormones induced by group-III mGluR agonists [(Mitsukawa et al., 2006) for mechanistic models see also (Johnson et al., 2001) and (Tasker et al., 1998)].
(3) our reports demonstrating that *Grm7* is cis-regulated and its variant gene expressing lower levels of mRNA in various brain regions can predispose to higher alcohol preference drinking (Vadasz et al., 2007a,Vadasz et al., 2007b, Gyetvai et al., 2011).

We propose that genetic variation in *Grm7* mRNA abundance can affect mGluR7 expression and function in brain stress-circuitries: *Grm7* gene variants lead to quantitative differences in mGlu7 receptor field density and the activation of variant fields can drive different levels of disinhibition of CRF-containing hypothalamic paraventricular nucleus (PVN) neurons which target dorsal raphe nuclei (DRN), among others. For example, alcohol avoiding C5A3 mice carrying the C-type *Grm7* gene variant would show a higher level of disinhibition of PVN CRF neurons and higher concentration of CRF in DRN. At this higher CRF concentration range the inhibitory effects are lost leading to uninhibited release of 5-HT in NAc and constitutionally higher levels of in vivo 5-HIAA.

Authors contribution

Csaba Vadasz conceived and designed the project. Beatrix Gyetvai contributed to genotyping, and husbandry. Csaba Vadasz and Beatrix Gyetvai analyzed the data, interpreted the results and wrote the manuscript.

Funding

This work including the construction of animal models was supported by The National Institute of Neurological Disorders and Stroke NS19788, The National Institute on Alcohol Abuse and Alcoholism R01 AA11031, and United States Department of Defense (US Army Medical Research and Materiel Command DAMD 17-00-1-0578).

Disclosures

We are not aware of any funding, affiliations, memberships, or other factors that might be perceived as affecting the objectivity of this work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are grateful to Katalin Kékesi (HPLC measurements), Attila Kaszás (microdialysis), Melinda Oros (genotyping), Réka Linn (genotyping database quality control) and lab members, colleagues including Drs. Péter Kabai, György Kóbor, Mariko Saito, Ágota Ádam, István Szakáll, the late István Sziráki who contributed to the construction of the RQI strain system, and to Rui Mao, Ray Wang, and János Piturca for excellent technical work in the maintenance of strains.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dadr.2021.100012.

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