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A genetic determinant of the striatal dopamine response to alcohol in men

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Abstract

Excessive alcohol use, a major cause of morbidity and mortality, is less well understood than other addictive disorders. Dopamine release in ventral striatum is a common element of drug reward, but alcohol has an unusually complex pharmacology, and humans vary greatly in their alcohol responses. This variation is related to genetic susceptibility for alcoholism, which contributes more than half of alcoholism risk. Here, we report that a functional *OPRM1* A118G polymorphism is a major determinant of striatal dopamine responses to alcohol. Social drinkers recruited based on *OPRM1* genotype were challenged in separate sessions with alcohol and placebo under pharmacokinetically controlled conditions, and examined for striatal dopamine release using positron emission tomography and [^{11}C]-raclopride displacement. A striatal dopamine response to alcohol was restricted to carriers of the minor 118G allele. To directly establish the causal role of *OPRM1* A118G variation, we generated two humanized mouse lines, carrying the respective human sequence variant. Brain microdialysis showed a four-fold greater peak dopamine response to an alcohol challenge in h/mOPRM1-118GG than in h/mOPRM1-118AA mice. *OPRM1* A118G variation is a genetic determinant of dopamine responses to alcohol, a mechanism by which it likely modulates alcohol reward.

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CONFLICT OF INTEREST

None of the authors has any competing interest, financial or otherwise.

Keywords

alcohol; dopamine; opioids; reward; polymorphism; positron emission tomography

INTRODUCTION

Alcohol use is a major cause of disability worldwide 1, but available treatments have limited efficacy 2. Development of novel, mechanism-based pharmacotherapies will require an improved understanding of the neurobiology that underlies addictive properties of alcohol 3. Compared to other addictive drugs, alcohol has a complex pharmacology. Sedative, ataxic and anxiolytic alcohol effects are primarily mediated through GABA- and glutamate signaling. In contrast, rewarding properties of alcohol such as euphoria and psychomotor stimulation are thought to involve endogenous opioids and mesolimbic dopamine (DA). In response to alcohol, μ-opioid receptor (*OPRM1*) activation in the ventral tegmental area (VTA) suppresses the activity of inhibitory GABA-ergic interneurons, resulting in disinhibition of DA-neurons and DA release from their terminals in the ventral striatum 4. Accordingly, OPRM1 blockade is a treatment for alcohol dependence 3.

Humans vary substantially in their alcohol responses, and this variability is related to genetic susceptibility for alcohol use disorders, which accounts for more than half the disease risk in this condition 5,6. Striatal DA-release is a common element of drug reward 7, and alcohol-induced DA release has been shown both in rodents 8,9 and humans 10. There is, however, marked individual variation in alcohol-induced behavioral responses thought to be related to DA activation, such as psychomotor stimulation. Functional variation in opioid genes may contribute to this variation by modulating alcohol-induced DA release. A functional *OPRM1* 118G variant 11 confers enhanced subjective alcohol responses 12, and a functionally equivalent rhesus macaque 77G variant 13 confers enhanced alcohol-induced psychomotor stimulation 14. Despite this, it remains controversial whether the *OPRM1* A118G polymorphism is a risk factor for alcohol use disorders 15.

Here, we asked whether *OPRM1* A118G modulates striatal DA release in response to alcohol. We first carried out a human positron emission tomography (PET) study using displacement of the DA-D_2 ligand [^{11}C]-raclopride, an indirect measure of endogenous DA release 16, and found that 118G carriers had a markedly more vigorous striatal DA response to alcohol compared to subjects homozygous for the major 118A allele. In parallel, we isolated the influence of *OPRM1* A118G variation by generating humanized mice, carrying the human exon 1 of the *OPRM1* gene, either as the major 118A allele, or with the 118G SNP introduced through site directed mutagenesis. Direct microdialysis measures of the response to a rewarding dose of alcohol showed a four-fold higher peak DA response to the alcohol challenge.

MATERIALS AND METHODS

Additional details for all methods are provided in Supplementary Information.

PET-study

Subjects were healthy male social drinkers, consuming less than 20 standard drinks per week. They were non-smokers who had never smoked, or had quit at least a year prior to enrolling in the study. Because the ventral striatum shrinks an average of 3.6 to 3.7% per decade 17, the age range was restricted to 21–45 years in order to reduce age-related variability. Furthermore, because rhesus macaque *OPRM1* genotype predominantly modulates psychomotor responses to alcohol in males 14, and because recent human data indicate only borderline detectable striatal DA release in response to alcohol in females 18, the study population was restricted to males. Subject demographics are provided in table S1. Allele frequencies of *OPRM1* 118G in Caucasian populations are around 15% 19, and 118G homozygotes are rare. Subjects were recruited into two approximately equally sized groups: 1) individuals homozygous for the major 118A allele (118AA genotype; n=16) of the OPRM1 polymorphism; 2) individuals carrying one or two copies of the variant 118G allele (118AG or 118GG genotype; because no subject with the rare 118GG genotype was in fact found, this group is hereafter called 118AG; n=12) of the *OPRM1* polymorphism. Genotyping was on DNA from whole blood. SNP rs1799971 (A/G) was genotyped using the assay-on-demand (assay ID: C_8950074_1) from Applied Biosystems (Foster City, CA, USA). The alleles were discriminated by post-PCR plate read on ABI PRISM® 7900HT Sequence Detector System (Applied Biosystems).

Because blood alcohol concentrations (BACs) after oral alcohol administration are highly variable 20, we used a physiologically based pharmacokinetic model, and intravenous (i.v.) alcohol infusion to achieve highly standardized brain alcohol exposure 21 successfully used in prior studies 20,22–27. Subjects underwent three infusion sessions, on separate days, separated by approximately 1 week.

A baseline session was carried out outside the PET scanner to ensure that subjects tolerated the alcohol infusion without experiencing nausea or marked sedation, and allow them to habituate to the procedure. All subjects received i.v. alcohol that raised BACs from 0 to 80 mg/dl within 15 min. This is a peak BAC commonly achieved by social drinkers, although the infusion procedure produced this level at a faster rate than is typically achieved with oral alcohol use. The exposure profile was chosen based on prior findings showing that it robustly activates the ventral striatum in a manner that can be detected by functional brain imaging 27. Subjective response to alcohol was measured using a modified Drug Effects Questionnaire [DEQ; 28] obtained before the beginning of the infusion and every 7.5 min minutes during the infusion. Breath alcohol concentrations (BrAC) were monitored every 7.5 min during the infusion, and every 30 min thereafter. Blood samples for BACs were collected before starting the infusion, and then at 15, 30 and 45 min.

In the second and third sessions, subjects received infusions of alcohol or placebo in counter-balanced order while undergoing a PET scan with [^{11}C]-raclopride (GE Advance Tomograph, Waukesha, WI). An 8-min transmission scan was first acquired with 2 rotating rod sources for the purpose of attenuation correction. Next, a 10 mCi dose of [^{15}O]-water was administered over 1 min through an IV line to assess regional perfusion and co-register the PET image with a brain MRI obtained from the subject during a separate visit.

Following this, the infusion (6% v/v ethanol or normal saline) was started. At 15 min after the start of the infusion, a 12 mCi dose of [^{11}C]-raclopride was administered over 1 min. Dynamic PET scans began with the [^{11}C]-raclopride injection and continued for 90 min. A schematic of the PET sessions is shown in Figure 1D.

PET Analysis was based on a Region of Interest (ROI) methodology. A structural MR scan was co-registered with the subject's PET scans. ROIs were drawn in the primary areas of post-synaptic dopamine release, namely the anterior ventral striatum, posterior ventral striatum, caudate and putamen (figure S1). The anterior ventral striatum was defined as striatum anterior and inferior to the anterior commisure. The posterior ventral striatum was defined as striatum posterior and inferior to the anterior commisure. The PET data from the placebo session were used as a measure of baseline raclopride binding potential, using the simplified reference tissue model 29. Percent change in binding potential (%ΔBP) following alcohol was calculated as ($BP_{placebo}$ - $BP_{alcohol}$)/($BP_{placebo}$). Reduction in raclopride binding is attributed to competition with dopamine endogenously released by the alcohol challenge, and the percent change in binding potential has been shown to be proportional to the magnitude of dopamine release 16.

PET data were examined for homogeneity of variance and analyzed using general linear models (GLM, Statistica 6.0, StatSoft, Tulsa, OK). For the %ΔBP, a repeated measures analysis of covariance (ANCOVA) was performed, with genotype and testing sequence as between subjects variables, region of interest as the within subjects variable, and drinking frequency (measured by the Lifetime Drinking History questionnaire 30) as the covariate. Subjective responses to alcohol were analyzed using repeated measures ANOVA with genotype and testing sequence as between subjects variables and time point as the within subjects variable. *Post hoc* comparisons were conducted using Newman-Kuel tests. Effect sizes were calculated using partial eta2 from Statistica, and Cohen's f calculated using GPower 3.1.0 (Franz Faul, Universität Kiel, Germany).

Humanized mouse lines

At the *OPRM1* locus, linkage disequilibrium (LD) exists between *OPRM1* A118G and other markers 31. We therefore generated two humanized mouse lines, where the *OPRM1* exon 1 was replaced by the corresponding human sequence. One of the lines, h/mOPRM-118AA, is homozygous for the major human 118A allele. For the other (h/mOPRM1–118GG), site directed mutagenesis was used to introduce a G in position 118. Thus, the lines are genetically identical, with the exception of the A → G substitution (Figure 2A–C). The lines are on a C57BL/6 background, where robust voluntary alcohol consumption is observed 32.

Generation of the h/mOPRM1–118AA and h/mOPRM1–118GG lines is detailed in Supplementary Information. Insertion of sequence corresponding to human *OPRM1* exon 1 is shown in Fig. 2C. Determination of the respective h/mOPRM1–118 genotype was carried out using the PCR method described for the human PET study. First generation h/mOPRM1–118AA and GG mice obtained from a cross of the founder lines were assessed with regard to overall health and behavior using an established testing battery 33. Table S2 shows that there were no genotype differences, with the exception of some excessive running in GG subjects upon introduction into the novel environment, and a modest but

statistically significant increase in locomotion in h/m118AA males compared to h/m118GG males. Male mice aged 13–16 weeks were used for the binding, signaling experiments, and male mice aged 24 – 32 weeks were used for the microdialysis experiments.

Receptor binding studies

Membrane binding was assessed in preparations from CHO-K1 cells transfected with OPRM1 receptor cDNA and incubated with 2nM [^{3}H]-D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin (DAMGO; 37Ci/mmol; NEN, Boston, MA) and nonradioactive naloxone, morphine, and β-endorphin (β-End) ranging from 0.01nM to 1000 nM. The mixture was incubated at room temperature, filtered through Whatman GF/B filters (FP-100, Brandel), washed with ice-cold Tris-HCl, and assayed for radioactivity by liquid scintillation. Data were analyzed by nonlinear analysis using the Prism computer program (GraphPad Software, Inc.).

Autoradiographic binding was studied on 20 μm coronal cryosections as described 34, using 5 nM [^{3}H]DAMGO (48.9 Ci/mmol, NEN). Non-specific binding was determined from adjacent sections in the presence of the radioligand plus 10 μM naloxone. Radiolabeled, dried tissue sections and [^{3}H]microscales (Amersham) were apposed to tritium-sensitive screens (Fujifilm imaging plates), and images were obtained using a Fujifilm BAS 5000 PhosphorImager.

Agonist-induced GTP[γ -^{35}S] activity was evaluated as described 35 on 20 μm coronal sections, by incubating GTP[γ-^{35}S] (0.04 nM) with 3 μM DAMGO in assay buffer at 25°C for 2 hr. Agonist studies were also performed in the presence of the antagonist naloxone to verify the receptor specificity of G-protein activation. Basal activity was assessed in each experiment with GDP in the absence of agonist, and nonspecific binding was evaluated in the presence of 10 μM unlabeled GTP[γS] without GDP. Slides were rinsed, dried and exposed to tritium-sensitive screens (Fujifilm imaging plates).

Images were analysed using MCID Image Analysis Software (Imaging Research Inc., UK). Regions of interest were defined by anatomical landmarks 36. Agonist-stimulated activity in brain sections was calculated by subtracting the optical density in basal sections (incubated with GDP alone) from that of agonist-stimulated sections; results were expressed as percentage of basal activity.

Electrophysiology

Enzymatically dissociated trigeminal ganglion neurons were used. Ca2+ currents were recorded using whole-cell patch-clamping as described 37. Currents were acquired with the Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA), filtered, and cell membrane capacitance and pipette series resistance were electronically compensated. The concentration-response relationships were determined by sequential application of the respective agonist in increasing concentrations. Two to three different concentrations were used with each cell in order to minimize desensitization. The results were pooled and the concentration-response curves were fit to the Hill equation. Data and statistical analyses were performed with Igor Pro (Lake Oswego, OR) and Prism 4.0 (GraphPad Software, Inc.) software packages, respectively.

Microdialysis

Under isofluorane anaesthesia, mice were implanted with microdialysis probes as previously described 38, aimed at the nucleus accumbens (NAc), and secured to the skull using a skull screw and cement. Histologically verified placement within the NAc for each subject is shown in Figure 3A. During a 12h postsurgical recovery period, probes were slowly perfused with artificial cerebrospinal fluid. The perfusion flow rate was then increased to 0.6μL/min for 2h to reach an interstitial equilibrium before sample collection. Dialysate fractions were subsequently collected, during the dark cycle, at 10min intervals during a 60min baseline period and, after an injection of EtOH (2g/kg, ip), during a 120min post-injection period. Samples were analyzed for DA and 5-HT using high-performance liquid chromatography coupled with electrochemical detection (HPLC-ECD).

After confirmation by ANOVA of no group differences in baseline DA or 5-HT concentrations, data were converted to percent change from the average baseline concentration obtained prior to pretreatment. Within- and between-groups analyses were performed by two-way ANOVA with repeated measures over time, with separate analyses performed for the DA and 5-HT data.

RESULTS

Human PET-study

BACs at the end of the alcohol infusion during the PET session were, on average, within 1 mg/dl of the target level, confirming the high level of accuracy and precision of the method. There were no differences between BACs achieved in the AA and AG groups (ANOVA: $F[1, 24]=0.287$, $p = 0.597$). Baseline binding potential (BP) for [^{11}C]-raclopride, as measured on the saline infusion session, also did not differ between genotypes (AA: 3535.8±107.8; AG: 3722.7±124.5 nCi/cc, mean±SEM; $F[1,26]=1.3$, $p=0.27$), making pre-existing differences in D2 receptor densities unlikely.

Across the four striatal subregions of interest (anterior ventral striatum, posterior ventral striatum, caudate and Putamen, figure S1), the change in BP (ΔBP) attributable to the alcohol challenge [$(BP_{placebo} - BP_{alcohol})/(BP_{placebo})$] differed markedly as a function of genotype, with AG subjects consistently showing signs of greater DA-release than AA individuals (main genotype effect: $F[1,22]=9.3$, $p=0.006$). Genotype accounted for 29.7% of the variance in response, measured as partial eta2, translating into an effect size of Cohen's $f=0.65$, i.e. between “moderate” – “large”. There was also a significant genotype x region interaction ($F[3,66]=3.8$, $p=0.01$); accordingly, *post-hoc* tests showed that the main genotype effect was predominantly driven by significant differences in the anterior and posterior ventral striatum, regions most closely associated with drug reward (Figure 1A–C). The highest response to alcohol was observed in the anterior ventral striatum of the AG group, where ΔBP was approximately 9%. This is more than half of the response seen with amphetamine 16, a drug that potently enhances synaptic DA by acting on the DA transporter. This relative potency is in agreement with direct measures obtained by brain microdialysis in experimental animals 9,16. In contrast, AA subjects did not show an alcohol-induced displacement of the radioligand in any region. Inclusion of the BAC at 45

min as a covariate in the statistical analysis of the %ΔBP data did not influence the main effect of genotype (F[1,19]=9.7, p=0.0057). The BAC at 45 min *per se* showed a trend for a significant effect on the %ΔBP (F[1,19]=3.86, p=0.064).

Similar to other imaging genetic studies 39,40, subjective ratings showed higher variability than the biological measure. The only effect detected was a genotype x time interaction, with AG subjects reporting lower levels of intoxication than AA subjects at the later time points, suggestive of faster acute tolerance development (see Supplementary Materials and Figure S2 E).

Characterization of humanized receptors and mouse lines

When expressed in CHO cells, both humanized receptors showed binding of the classical OPRM1 selective ligand [^{3}H]-DAMGO, displaced by the endogenous OPRM1 agonist β-End with expected low nanomolar IC_{50}, and with no difference between genotypes (F[4,46]=1.3, p=0.3; Fig. 2D). Signaling, assessed as modulation of Ca^{2+} currents in acutely isolated superior trigeminal ganglion neurons, was also normal in response to both DAMGO and β-End, once again without any genotype differences (DAMGO: F[4,12]= 1.8, p=0.2; β-End: F[4,12]=1.2, p=0.4; Fig 2E–F). Finally, [^{3}H]-DAMGO binding on mouse brain sections did not show genotype differences in receptor densities across a number of brain regions examined, including ventral and dorsal striatum and the ventral tegmental area (VTA; Figure S5).

DA responses to alcohol in humanized mouse lines

Microdialysis probes placement in ventral striatum / Nucleus Accumbens is shown in Figure 3A. Baseline DA levels did not differ between genotypes (F[1,22]= 6.9×10^{-6}, n.s.) and were 2.42±0.4 nM for h/mOPRM1–118AA and 2.43±0.6 nM for h/mOPRM1–118GG mice, respectively (n=12/group). Alcohol injection (2g/kg, a dose that robustly induces conditioned place preference in C57BL/6 mice) increased dialysate DA in both lines (main effect: F[6,132]=12.4; p<0.0001), However, response differed markedly between genotypes (main genotype effect: F[1,22]=7.2; p<0.05; genotype x time interaction: F[6,132]= 12.4; p<0.005; Figure 3B). Peak DA increase was nearly 4-fold greater in GG mice than in AA animals. The genotype effect was specific for DA release induced by alcohol; although alcohol also significantly increased 5-HT (main effect: F[6,132]=15.6; p<0.0001), this was not affected by genotype (main genotype effect: F[1,22]=3.6; n.s; genotype x time interaction: F[6,132]=0.92; n.s.; Figure 3C).

DISCUSSION

Taken together, our data strongly support a causal role of the *OPRM1* 118G allele to confer a more vigorous DA response to alcohol in the ventral striatum. These findings may be related to observations of differential subjective alcohol effects as a function of *OPRM1* A118G genotype in humans 12, and of markedly increased psychomotor responses to alcohol in rhesus males carrying the functionally equivalent 77G variant 14. Interestingly, we also found that subjective feelings of intoxication over time differed as a function of genotype. Carriers of the 118G allele showed signs of rapid acute tolerance, similar to that

previously described in men at genetic risk for alcoholism 41. The use of a reverse-translational approach allowed us to effectively isolate the influence of the human 118G variant from that of other polymorphisms with which it might be in LD.

Excessive activation of brain reward systems by alcohol in *OPRM1* 118G carriers might be expected to confer susceptibility for alcohol use disorders. Although we in fact found evidence in support of this notion in an ethnically homogenous Caucasian sample 42, others have not. One study found that other polymorphisms within the same haplotype block, but not A118G, were associated with diagnoses of substance dependence 31, while a meta-analysis of all available data for A118G was negative 43. This apparent discrepancy closely parallels recent findings with the 5-HT transporter gene promoter length polymorphism (5-HTTLPR). A robust role of 5-HTTLPR variation has been found using an imaging based endophenotype that is closely related to negative affect 39,44, and these findings are paralleled by experimental data in a non-human primate model 45,46. Nevertheless, findings from 5-HTTLPR association studies in depression have not held up on meta-analysis 47,48. The reasons for these discrepancies are presently unknown, much debated, and critical to resolve. One important possibility is that current diagnostic categories pool genetically heterogeneous phenocopies, as has specifically been proposed in depression 49. If so, the use of biological endophenotypes, such as in the present study, can potentially aid the dissection of these populations into genetically and pathophysiologically more homogenous categories.

Irrespectively of its possible role as a susceptibility factor, excessive reward-related DA-response to alcohol in carriers of *OPRM1* 118G might be of pharmacogenetic importance in treatment of excessive alcohol use. Indeed, two studies have found that the presence of the 118G allele was associated with a therapeutic response to the opioid antagonist naltrexone 50,51. Although one study failed to find such an effect 52, recent non-human primate data provide strong evidence for this notion under closely controlled experimental conditions 53. Our data are consistent with a selective role of endogenous opioids for alcohol reward in *OPRM1* 118G carriers, and provide a possible biological mechanism for the preferential naltrexone response in this population. However, the dissociation between objective measures of alcohol-induced DA-release and subjective reports of alcohol effects may argue against a simplistic view of alcohol-induced DA-release as an immediate mediator of drug reward. Instead, these data may be more compatible with a view of ventral striatal DA-activation as a signal predictive of alcohol reward, and perhaps involved in learning processes important for addiction 54. Of note, the level of alcohol-induce DA-activation in our study was overall lower than previously reported 10. A difference between the two studies is that alcohol administration was oral in the prior report, while we used intravenous administration. This may indicate that striatal DA response to alcohol is only in part driven by direct pharmacological actions of alcohol, while an additional component of the activation is evoked by smell or taste cues.

Our data demonstrate that the 118G allele, which encodes an amino acid substitution in the N-terminal extracellular loop of the receptor, confers a higher striatal DA-activation in response to alcohol regardless of whether it occurs in the context of other markers within its haplotype block or not. However, the molecular mechanism that mediates functional

consequences of the 118G variant remains controversial. Initially described as a gain-of-function mutation due to increased affinity for β-End 11, *OPRM1* 118G has since been proposed to instead confer decreased expression in several systems, including human post-mortem brain tissue 55, and a mouse model where a mutation was introduced in a position corresponding to the human A118G marker 56. The latter model differs from that used in our study, in that it introduced the N → D substitution in the context of a murine N-terminal receptor protein sequence. In contrast, we generated mice expressing humanized receptors throughout this region by replacing all of exon 1, the main region of divergence between the two species (Figure 2A). Using this model, our present findings show that a key functional phenotype associated with the 118G variant, alcohol-induced striatal DA-release, is replicated across species in the absence of effects on receptor affinity, binding density, or signaling. A remaining possibility is altered oligomerization, a mechanism that is critical for opioid receptor desensitization, endocytosis, and re-insertion 57. *OPRM1* A118G modifies a glycosylation site in the N-terminal extracellular loop of the receptor, and could thereby influence receptor dimerization and trafficking.

Functionally equivalent *OPRM1* variants have evolved independently in two primate lineages, the rhesus macaque 13 and man 11. In both species, minor allele carriers are more sensitive to disruption of social attachment bonds 58,59. In rhesus, this is accompanied by bold - exploratory traits, while lower conscientiousness has been observed in human 118G carriers 13,58,60. These traits may have been advantageous in the evolutionary history of the respective species, and recent studies suggest that the 118G allele has been under positive selection 61. In contrast, the ability of these variants to modulate DA responses to alcohol is likely a coincidental evolutionary byproduct.

Identifying genetic sources of individual variation in complex behavioral phenotypes has proven challenging. Our findings support the notion that use of biological intermediate phenotypes and translational approaches can contribute important data to facilitate this difficult endeavor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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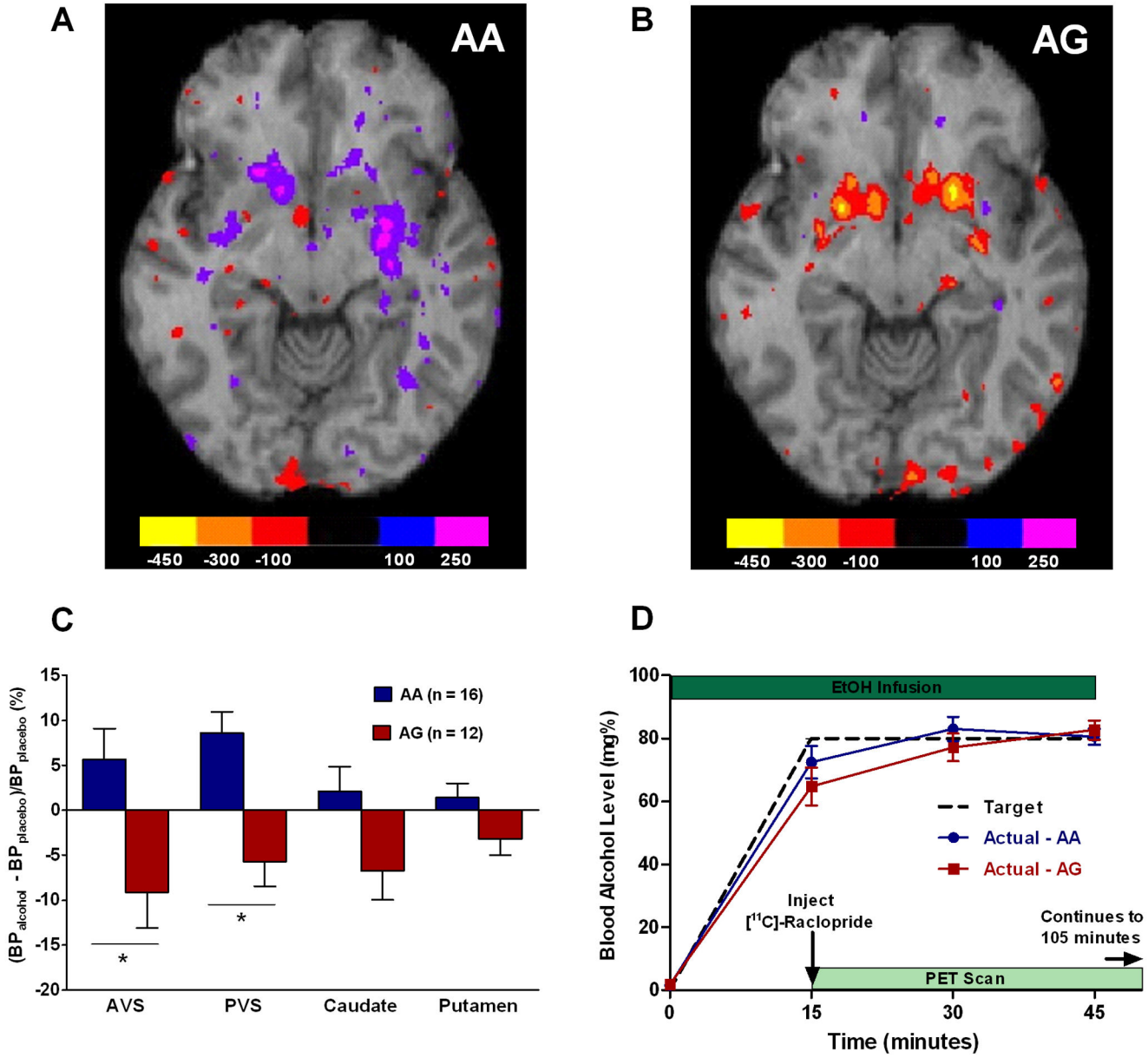

Fig. 1.

Human PET study. Axial view of group maps showing change of [^{11}C]-raclopride binding potential (ΔBP; nCi/cc) between placebo and alcohol sessions in **(A)** AA individuals and **(B)** AG individuals. Color bars indicate corresponding ΔBP values. Reduction in raclopride binding is attributed to competition with dopamine released by the alcohol challenge; thus, a negative ΔBP indicates an increase in endogenous dopamine release. **(C)** Relative change in binding potential (%ΔBP) for [^{11}C]-raclopride between alcohol and placebo sessions in four striatal regions of interest. Data are least square means (±SEM). Main genotype effect: p=0.006; *p<0.05 on *post hoc* tests within individual regions. AVS = anterior ventral striatum, PVS = posterior ventral striatum. **(D)** Schematic of PET sessions, and blood

alcohol concentration profiles over time during the alcohol session (mean±SEM). There was no significant difference between genotypes ($F[1,24]=0.51$, $p=0.48$).

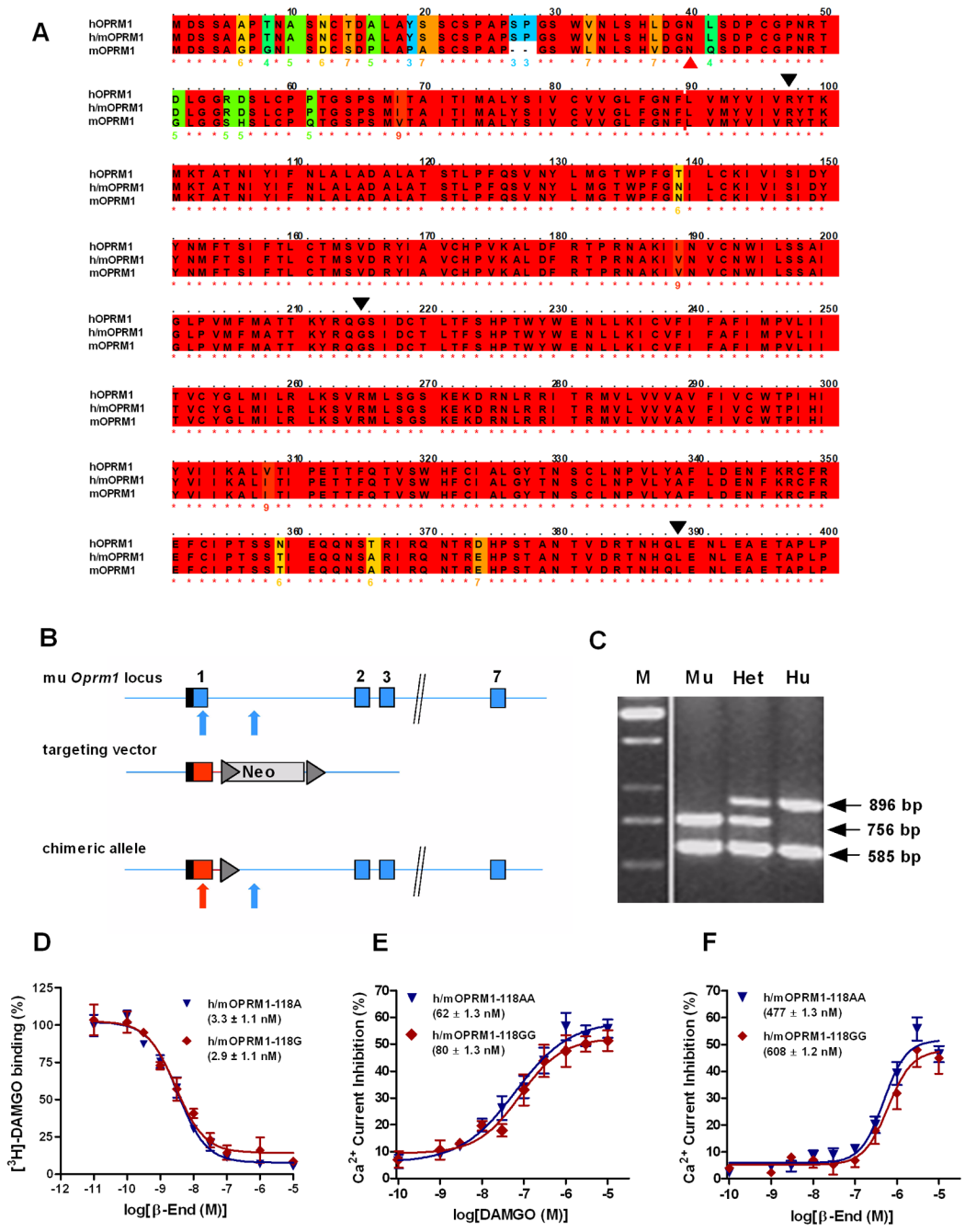

Fig. 2.

Targeting strategy and functional confirmation for humanized *OPRM1* mouse lines. **(A)** Alignment of human (hOPRM1), mouse (mOPRM1) and humanized (h/mOPRM1) protein sequences. Black triangles indicate exon boundaries. Red triangle points to site of the N40D substitution, corresponding to A118G allelic variation. Color-coding of background indicates degree of conservation, with red indicating full human – mouse homology, and blue non-conservative substitution. As can be seen, replacement of mouse exon 1 with the corresponding human sequence yields a receptor that is highly homologous to the human

protein. **(B)** Exon 1 of the murine *OPRM1* locus including additional 400 bp of intron 1 was replaced by the corresponding human sequence, containing either A or G in position 118. Blue and red colors represent mouse and human sequences, respectively. Boxes: respective exons of the *OPRM1* coding region. Hatched region: 5'-UTR. Triangle: frt site for flox recombinase. Neo: neomycin selection marker. Arrows point to position of the human and mouse specific PCR primers. **(C)** PCR based identification of the human (Hu), mouse (Mu) and heterozygote (Het) genotypes. M: Marker lane. **(D)** [^{3}H]-DAMGO binding to respective humanized receptor transiently expressed in CHO cells. Data are means (±SEM) of triplicates, and mean (±SEM) IC_{50} values for the respective genotypes are indicated. Non-linear curve-fitting established that no significant genotypes differences were present **(E – F)** Concentration-response curves for DAMGO and β-endorphin (β-End) in trigeminal ganglion (TG) neurons isolated from h/m*OPRM1* 118AA or 118GG mice. Data points represent the mean (±SEM) of the agonist-mediated Ca^{2+} current inhibition. Mean EC_{50} values (±SEM) for the respective genotypes are indicated. Similar to the binding experiment, no genotype differences were found.

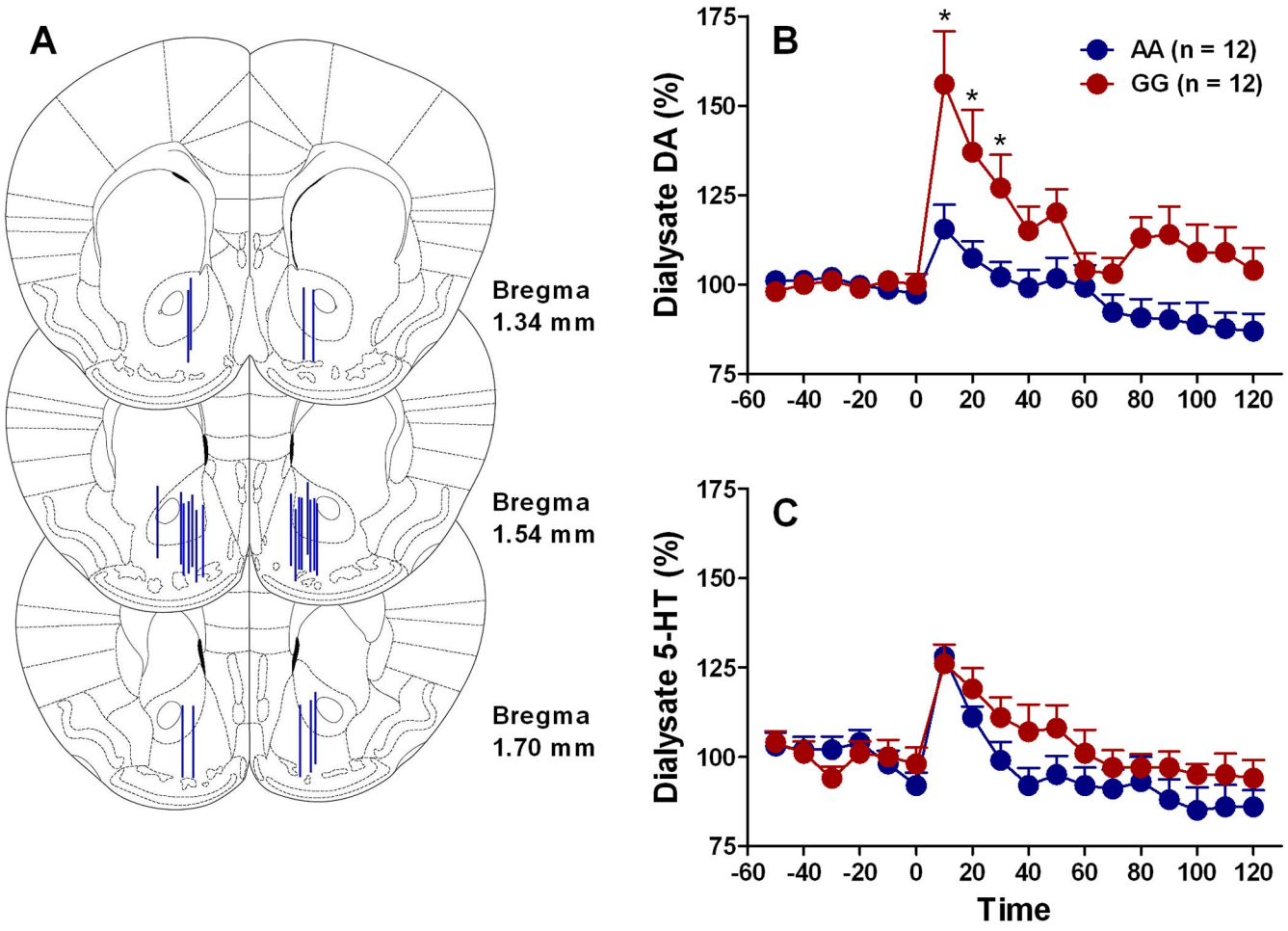

Fig. 3.

Striatal DA response to alcohol (2g/kg) in the humanized mouse lines, determined by brain microdialysis. **(A)** Individual probe placements in ventral striatum / Nucleus Accumbens. **(B)** Dialysate DA levels in h/mOPRM1-118AA and h/mOPRM1-118GG mice. After confirmation of no group differences in baseline concentrations, the data were converted to the percent change from the average baseline concentration obtained prior to pretreatment. Alcohol was given at time point 0. Differential response was indicated by a genotype x time interaction ($p<0.005$); differences at individual data points as determined by *post hoc* test are indicated by *$p<0.05$ **(C)** In contrast to the DA response, the 5-HT response to the alcohol dose did not differ between genotypes.