ORIGINAL ARTICLE

Association of a Functional Polymorphism in the μ-Opioid Receptor Gene With Alcohol Response and Consumption in Male Rhesus Macaques

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Context: Innate differences in opioid neurotransmission are hypothesized to influence abuse liability of alcohol. In humans, a variant of the μ-opioid receptor gene (*OPRM1A118G*) increases receptor affinity, alcohol-induced euphoria, and risk for alcohol use disorders.

Objective: To determine whether a variant in the μ-opioid receptor gene (*OPRM1C77G*) that increases affinity of the receptor is associated with alcohol response and consumption in macaques.

Design: Young adult rhesus macaques (*Macaca mulatta*) were intravenously administered 2.0 to 2.1 g of ethanol per kilogram of body weight and assessed for alcohol response. Animals were later given simultaneous access to an aspartame-sweetened 8.4% (vol/vol) ethanol solution and a vehicle for 1 hour per day, 5 days a week, for a period of 6 weeks. Animals (N=82) were genotyped for the *OPRM1C77G* polymorphism; the effects of the genotype on alcohol response and consumption were determined by analysis of variance, with sex included as a nominal independent variable.

Main Outcome Measures: Alcohol response (ataxia, stimulation, and sedation), average alcohol consumption, the percentage of days during which an animal consumed alcohol at a level sufficient to produce intoxication (≥0.67 g of alcohol per kilogram of body weight), and alcohol preference (calculated as 100 × {alcoholic solution/[alcoholic solution + nonalcoholic solution]}).

Results: Increased alcohol-induced stimulation was observed among male macaques carrying the *OPRM1C77G* allele. *OPRM1C77G* allele carriers consumed more ethanol and exhibited increased ethanol preference. Male carriers of the *OPRM1C77G* allele exhibited higher alcohol preference and consumption, and drank to intoxication more frequently than did *C/C* males.

Conclusions: These findings demonstrate that the rhesus macaques' equivalent of the *OPRM1A118G* variant is associated with increased alcohol response, consumption, and preference. Our results reveal effects of the *OPRM1C77G* genotype to be male-restricted or more marked among male macaques. This is of interest, given the fact that early-onset type II alcoholism is more common among men and that, among addicted individuals, men are more responsive to μ-opioid receptor blockade.

Arch Gen Psychiatry. 2007;64:369-376

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Endogenous opioid peptides mediate natural rewards as well as ethanol-induced positive reinforcement (ie, psychomotor stimulation and euphoria).[1-3] The exact mechanism for these actions remains to be determined but may involve both dopamine-dependent and dopamine-independent mechanisms. In experimental animals, activation of μ-opioid receptors in the ventral tegmental area activates dopamine neurons by releasing them from tonic inhibition by γ-aminobutyric acid interneurons[4,5]; alcohol activates dopamine release in the ventral tegmental area in a manner sensitive to blockade of μ receptors.[6] In addition, direct opioid inputs to the nucleus accumbens are capable of producing psychomotor stimulation in a dopamine-independent manner,[7,8] likely contributing to positive reinforcement of alcohol actions.

As predicted by the mechanisms outlined, blockade of opioid signaling results in suppression of alcohol consumption in several animal models with alcohol dependence.[9] Among the 3 cloned opioid receptors, the μ subtype is likely key to opioid-mediated ethanol reinforcement. This interpretation is consistent with the lack of ethanol self-administration in the null mutants of the μ-opioid receptor gene (*Oprm1*).[10,11] Blockade of μ-opioid receptors decreases ethanol drinking in nonselected Wistar rats.[12,13] μ-Antagonism also appears to be effective in suppressing the pharmacologically significant elevations in

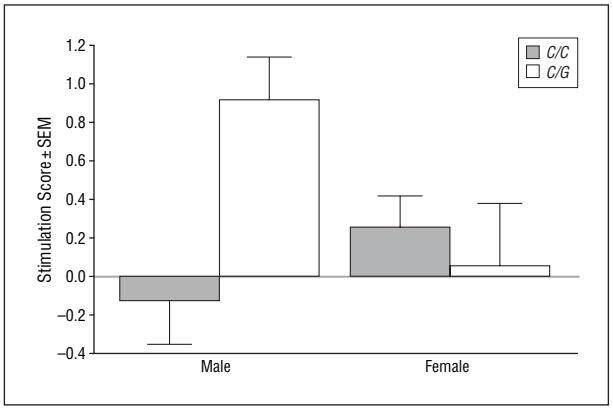

Figure 1. Effect of the *OPRM1C77G* genotype and sex on alcohol-induced stimulation in rhesus macaques. Values are the results from factor analysis performed on behavior observed following intravenous infusion of 2.0 to 2.2 g of ethanol per kilogram of body weight. Sample size: female *C/C*, n=38; male *C/C*, n=16; female *C/G*, n=14; male *C/G*, n=5.

ethanol consumption that are demonstrated in animal models of alcoholism based on genetic selection. This reduction appears to be unrelated to effects on ethanol palatability and, therefore, presumably reflects direct attenuation of alcohol's reinforcing actions.[14-16] Supporting a role of endogenous opioids in addiction vulnerability, altered function of endogenous opioid systems has been demonstrated in several ethanol-preferring lines.[17,18]

Numerous studies have demonstrated an efficacy of the opioid receptor antagonist naltrexone in clinical treatment of alcohol dependence.[19-21] Despite 1 large negative trial,[22] meta-analyses support naltrexone efficacy on several drinking variables.[23,24] Family history of alcohol dependence has been reported as a predictor of therapeutic response to naltrexone.[25,26] Together these findings point to an intriguing possibility that the modest overall effect size of naltrexone in treatment of alcohol dependence reflects heterogeneity in patient responses and may be considerably improved in appropriately selected patient populations.

Data demonstrating that family history of alcoholism is a predictor of naltrexone response are converging with recent findings that suggest that the role of opioid signaling in ethanol reward, as well as alcoholism treatment response, is linked to genetic factors. For instance, laboratory drinking studies have shown that subjects with a genetic risk for alcoholism experience increased ethanol-induced euphoria, which is selectively sensitive to naltrexone. Subjects at low genetic risk have a diminished euphorigenic ethanol response, and, consequently, naltrexone is shown to have an effect in blocking ethanol-induced euphoria in this group.[27] Several polymorphisms exist in the human μ-opioid receptor gene (*OPRM1*). One of these, *A118G*, is a functional nonsynonymous single nucleotide polymorphism (*ASN40ASP*); in vitro studies have demonstrated that this polymorphism confers a 3-fold increase in the affinity of the μ-opioid receptor for β-endorphin. The functional importance of this variant in vivo is supported by the data indicating that carriers of the *OPRM1118G* allele have significantly elevated pain thresholds and also experience increased euphoria (subjective high) following consumption of alcohol.[28,29] The latter leads to the prediction that *OPRM1118G* carriers may be more susceptible to developing alcohol dependence. Although results are mixed,[30,31] several studies have linked the *OPRM1118G* allele to alcohol dependence.[32,33] More importantly, recent data suggest that, among alcohol-dependent individuals, carrying the *OPRM1118G* allele predicts a therapeutic response to naltrexone.[34] This observation potentially links the previously established role of family history in alcoholism vulnerability, enhanced alcohol-induced euphoria, and naltrexone response. Specifically, it may be that increased positive reinforcement response to alcohol mediated by the *OPRM1118G* allele of *OPRM1* could be a heritable factor that increases susceptibility to both initiation and maintenance of alcohol dependence. Because of this, reversal of enhanced alcohol reinforcement by naltrexone might provide a particular therapeutic benefit in this group.

The role of the *OPRM1A118G* single nucleotide polymorphism cannot be addressed experimentally in rodents, but rhesus macaques (*Macaca mulatta*) may offer an opportunity to do so. In rhesus macaques, there is a single nucleotide polymorphism (*OPRM1C77G*) that causes an amino acid change (*ARG26PRO*) in the N-terminal arm of the μ-opioid receptor, conferring a 3.5-fold increase in the affinity for β-endorphin.[35] We therefore wanted to address the hypothesis emerging from the human studies that predicts that the *OPRM1C77G* polymorphism would be associated with enhanced alcohol stimulation and, therefore, consumption, in rhesus macaques. It has previously been reported that early life stress in the form of peer rearing leads to elevated alcohol consumption in rhesus macaques[36] and that rearing history and sex interact with a functional variant in the serotonin transporter–linked polymorphic region (*5-HTTLPR*) to modulate alcohol intake.[37] Therefore, sex and rearing history were included in the analyses to evaluate whether they modified potential effects of the *OPRM1C77G* genotype. As the *OPRM1A118G* polymorphism and naltrexone treatment influence the frequency of alcohol consumption, we also wanted to determine whether carriers of the *OPRM1C77G* allele more frequently consumed alcohol to intoxication. Finally, endogenous opioids are known to influence feeding behavior and, in particular, increase preference for sweet solutions.[38,39] To exclude the potential confound of altered ethanol palatability, we determined preference for the 8.4% (vol/vol) alcohol solution over the sweetened vehicle.

METHODS

SUBJECTS

Study animals were 82 young adult rhesus macaques obtained from 6 birth-year cohorts, ranging in age from 2.8 to 4.5 years (mean±SD, 3.8±0.4 years) at the initiation of the alcohol self-administration study. The sample size for each dependent measure, broken down by sex and genotype, is indicated in each respective figure (**Figures 1**, **2**, **3**, and **4**). Animals were socially housed in sex-limited, age-matched groups and were tested within their respective social groups in runs at the National In-

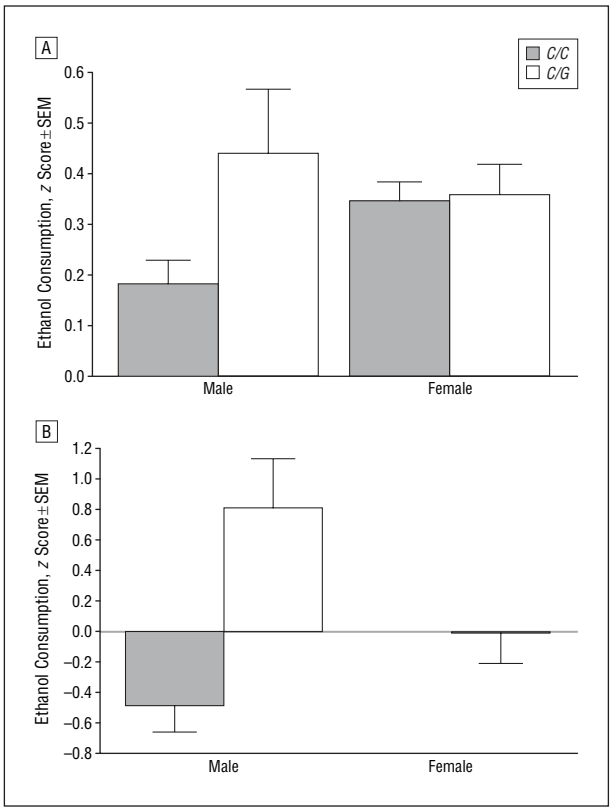

Figure 2. Effect of the *OPRM1C77G* genotype and sex on voluntary alcohol consumption in rhesus macaques. Values reflect the average amount of ethanol consumed in an hour of testing (grams per kilogram of body weight), both prior to (A) and following (B) statistical correcting for the testing cohort. Sample size: female *C/C*, n=42; male *C/C*, n=15; female *C/G*, n=17; male *C/G*, n=8.

stitutes of Health Animal Center. The animals' weights ranged from 4.1 to 9.3 kg (mean±SD, 6.2±0.15 kg). Protocols for the use of experimental animals were approved by the Institutional Animal Care and Use Committee of the National Institute on Alcohol Abuse and Alcoholism.

ALCOHOL ADMINISTRATION

To assess ethanol sensitivity, each animal was removed from its home cage and restrained on a flat surface while ethanol (16.8% [vol/vol], United States Pharmacopeia) was infused into the saphenous vein (males, 2.1 g/kg of body weight; females, 2.0 g/kg of body weight) at a constant rate for 15 minutes. The rationale for administering a higher dose to males is that, as in humans, rhesus males have less body fat than females and have been shown to require more alcohol per kilogram of body weight to produce identical blood alcohol concentrations.[40,41] Doses were based on pilot data showing that with a dose of 1.0 g of alcohol per kilogram of body weight, a small number of monkeys showed no evidence of intoxication, and with a dose of 3.0 g of alcohol per kilogram of body weight, some animals became unconscious. Pilot data also supported our theory that females required a lower dose than males did, with the differential dosing producing identical blood ethanol contents (BECs). Each animal went through 2 separate alcohol infusion and behavioral testing sessions so that averages could be taken. At 5, 10, and 60 minutes following initiation of the infusion, blood samples were obtained from the femoral vein for assessment of BEC; BECs were quantified enzymatically using a commercial kit (Sigma-Aldrich Corp, St Louis, Mo).

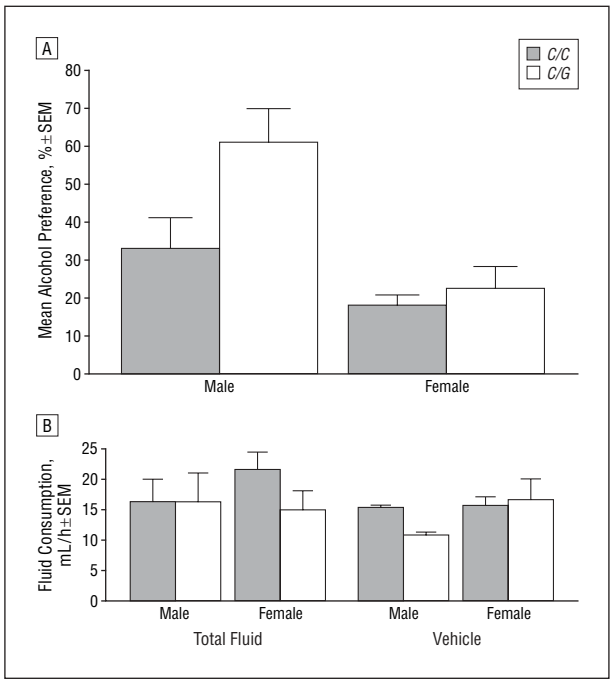

Figure 3. Effect of the *OPRM1C77G* genotype and sex on alcohol preference in rhesus macaques. A, Mean alcohol preference calculated as 100 × [alcoholic solution/(alcoholic solution + nonalcoholic vehicle)]. B, Total fluid consumption and vehicle consumption. Sample size: female *C/C*, n=18; male *C/C*, n=12; female *C/G*, n=9; male *C/G*, N=7.

ASSESSMENT OF ETHANOL SENSITIVITY

Following the intravenous ethanol infusions, animals were placed in a testing room for 30 minutes. Because there was a potential for severely ataxic animals to injure themselves, the floor was cushioned with 30 to 45 cm of wood shavings. Each subject's general motor behavior was scored for 30 minutes by experienced investigators (blind to each subject's genotype) who observed the subject through a 1-way viewing window. Scored behaviors included locomotion, passivity, stumbling, falling, hitting a wall, swaying, unsuccessful jumping, and successful jumping. Locomotion and passivity were recorded as seconds in duration, while the remaining behaviors were recorded as frequencies. Descriptions of the objectively defined behaviors are as follows:

Locomotion: any directed movement across the substrate, either vertical or horizontal.

Passivity: absence of directed movement.

Stumbling: when the animal loses balance and appears to trip over its own feet or lose its footing while moving.

Falling: any time the animal involuntarily drops from a higher to a lower area of the substrate, either while moving or while stationary; or when the animal loses balance and involuntarily topples over and drops to the floor or shelf (depending on where the animal is located), either while moving or while stationary.

Hitting a wall: when the animal's body hits the sides of the Plexiglas box while attempting to escape or the inside wall of the animal room while moving or while stationary.

Swaying: each time the animal's body veers in any direction (forward, back, left, or right) out of a controlled, upright posture, either while passive or while moving.

Unsuccessful jumping: when an animal falls down or misses its target when attempting a leap either vertically or horizontally across the substrate. This behavior is mutually exclusive with *falling*.

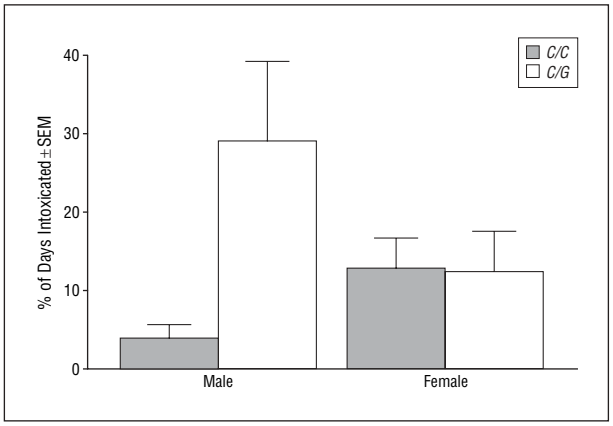

Figure 4. Effect of the *OPRM1C77G* genotype and sex on the number of days during which rhesus macaques consumed sufficient quantities of alcohol to produce signs of intoxication (0.67 g/kg of body weight). Sample size: female *C/C*, n=42; male *C/C*, n=15; female *C/G*, n=17; male *C/G*, n=8.

Successful jumping: when an animal leaps either vertically or horizontally across the substrate.

ALCOHOL CONSUMPTION

After a period of at least 1 month, animals were allowed to freely consume an aspartame-sweetened 8.4% (vol/vol) alcohol solution for 1 hour per day, 5 days a week in their home cages.[42] This standardized method consisted of 3 phases:

1. Spout training. The animals were trained for 1 hour per day during a 1-week period to drink from nipple-like spouts dispensing aspartame-sweetened water. This phase lasted 5 days, during which all animals consumed more than 50 mL of the vehicle.

2. Initial alcohol exposure. This phase was designed to ensure that all animals experienced the pharmacological effects of alcohol before beginning the experimental phase of the study. To begin this phase, the color of the sweetened vehicle was changed, and alcohol was added to the vehicle to produce an 8.4% (vol/vol) alcohol solution. During the initial alcohol exposure phase, animals were given free access to the alcohol solution for 1 hour per day. Each of the animals included in this phase of the study fulfilled a pre-established criterion of consuming more than 0.67 g of the ethanol solution per kilogram of body weight per hour (a dose shown to produce signs of intoxication in rhesus macaques) on 2 or more occasions. Once all animals met the criterion, both nonalcoholic and 8.4% (vol/vol) alcoholic aspartame-sweetened solutions were available (in addition to normal drinking water) for 1 hour per day. No special methods, such as deprivation of food or water, were used to induce ethanol consumption; animals established stable consumption patterns within 2 weeks.

3. Experimental period. During the 6-week experimental phase, alcohol and the vehicle were dispensed 5 days a week (Monday-Friday) from 1 PM to 2 PM while the animals were in their home cage environment. Animals were fitted with a collar that was implanted with an identifier chip so that consumed volumes could be recorded for each individual. Although the vehicle was available for all cohorts tested, levels of consumption of the vehicle were recorded for only 2 of the testing cohorts.

GENOTYPING

Using standard extraction methods, DNA was isolated from whole blood, which was collected from the femoral vein under ketamine anesthesia (15 mg/kg of body weight, administered intramuscularly). Genotyping was performed using the procedure modified from the method reported by Miller et al.[35] A portion of *OPRM1* exon 1 was amplified from 25 ng of genomic DNA by flanking oligonucleotide primers museekr (5′-TCAGTACCATGGACAGCAGCGCTGTCCCCACGAA-3′) and museekr1 (5′-GTCGGACAGGTTGCCATCTAAGTG-3′) in 15-µL reactions using AmpliTaq Gold (Applied Biosystems, Foster City, Calif) and 2.5 mmol of magnesium chloride per liter, according to the manufacturers' instructions (Invitrogen, Carlsbad, Calif). Amplifications were performed on a PerkinElmer 9700 thermocycler (PerkinElmer, Wellesley, Mass) with 1 cycle at 96°C, followed by 30 cycles at 94°C for 15 seconds, 56°C for 15 seconds, and 72°C for 30 seconds, and a final 3-minute extension at 72°C. Restriction digest by *Fnu*4HI (New England Biolabs, Beverly, Mass) was then performed using 0.5 µL of polymerase chain reaction product in a total volume of 20 µL for 2 hours at 37°C. Samples were separated by electrophoresis on 10% polyacrylamide gels, and the *OPRM177C* and *OPRM177G* alleles were identified by direct visualization following ethidium bromide staining.[35]

DATA ANALYSIS

For the behaviors scored during alcohol intoxication, factor analysis was performed to yield alcohol response factors to be used as dependent variables in analysis of variance (ANOVA). Scores for each behavior were expressed as the mean frequency or duration of the behavior for the 2 testing periods. Factor analysis was performed using principal components extraction and varimax rotation.

To determine whether the *OPRM1C77G* genotype influenced alcohol response, we performed ANOVA using rotated orthogonal factor scores as dependent variables. Analysis of variance was also performed to assess the effects of the *OPRM1C77G* genotype on alcohol consumption and preference. Three-way ANOVA was conducted initially, with genotype, sex, and rearing included as independent variables. The rearing conditions have been described in detail in previous publications.[37,43,44] Because rearing history did not yield a main effect or interact with sex or genotype on any of the dependent variables in our study, it was dropped from the analysis, and 2-way ANOVA with genotype and sex as independent variables was ultimately performed. The influences of these variables on alcohol consumption, the number of days during which an animal consumed alcohol at a level above 0.67 g/kg of body weight per hour, and alcohol preference were determined. Post hoc comparisons were performed using the Newman-Keuls test.

Previous studies in our laboratory have demonstrated that alcohol consumption varies among testing cohorts. It is thought that this occurs as a result of specific social group dynamics or external disturbances that globally influence alcohol intake (eg, inclement weather, infectious disease, and procedures or social conflicts occurring in adjacent runs). Because ethanol consumption (grams per kilogram of body weight per hour) differed significantly among the testing cohorts used in this study, even after controlling for sex differences, a z score for alcohol consumption that controlled for cohort was generated to test the effects of *OPRM1C77G* on ethanol intake. To determine alcohol preference in the 2 birth-year cohorts in which vehicle consumption was recorded, average volumes of alcoholic and nonalcoholic (sweetened vehicle) solutions consumed during the course of the drinking study were calculated as 100 × [alcoholic solution/(alcoholic solution + nonalcoholic solution)].

The average identity by descent for the animals included in the study was 1.45%. This indicates that 2 randomly selected

macaques would share only 1.45% of their genes by descent (approximately equivalent to a degree of relationship that is observed between second cousins once removed and third cousins). This demonstrates that most pairs of individuals have a low degree of relationship, approximating the relationship observed in some human study populations. Because the identity by descent was sufficiently low, standard statistical procedures were applied for testing the association of *OPRM1C77G* with ethanol consumption.[43]

The frequency of the *OPRM177G* allele was 18%, and genotype frequencies did not deviate from the Hardy-Weinberg equilibrium (χ^2=0.23; *P*=.89). Because preliminary analyses demonstrated no difference in outcomes between *G/G* and *C/G* animals, these were collapsed into an *OPRM177G* allele carrier group for all the analyses performed. There were no *OPRM1C77G* frequency differences among the cohorts tested, nor were there any frequency differences according to sex or dominance rank, or between Chinese- and Indian-derived rhesus macaques (n=8 and n=74, respectively). The Kolmogorov-Smirnov test demonstrated that alcohol consumption data did not deviate from normality. All analyses were performed using StatView Statistical software (StatView, Cary, NC). The criterion for significance was set at *P*≤.05.

RESULTS

ALCOHOL RESPONSE

Factor analysis of alcohol sensitivity measures yielded 3 factors—disinhibition, stimulation, and ataxia—which together accounted for 74% of the variance (**Table**). There were no main effects of the *OPRM1C77G* genotype or of sex on stimulation factor scores ($F_{1,69}$=2.35, *P*=.13, and $F_{1,69}$=1.37, *P*=.25, respectively); however, there was a significant interaction between the 2 ($F_{1,69}$=5.25; *P*=.02) (Figure 1). Post hoc testing demonstrated that this was attributable to male *OPRM177G* carriers exhibiting significantly higher stimulation scores than males homozygous for the *OPRM177C* allele (*P*<.05). There were no main effects of sex or of the *OPRM1C77G* genotype, nor were there any interactions between the 2 for disinhibition or ataxia scores (data not shown). Because of the potential confound of variation in BECs or age to influence alcohol response, analyses were repeated including only 3- and 4-year-old animals with 10-minute BECs between 0.2% and 0.3%, but results remained the same ($F_{1,37}$=4.1; *P*<.05; data not shown).

To rule out a pharmacokinetic effect of the *OPRM1C77G* polymorphism, repeated measures of ANOVA were also performed with sex and genotype as independent and BEC as the dependent variable. Follow-up ANOVA was performed at each time point, with body weight included as a continuous independent variable. There were no effects of *OPRM1C77G* nor were there any interactive effects of *OPRM1C77G* and sex on BECs (Figure 1).

ALCOHOL CONSUMPTION

There was a main effect of the *OPRM1C77G* genotype on alcohol consumption ($F_{1,78}$=4.62; *P*<.04), with carriers of the *OPRM177G* allele consuming more ethanol than *C/C* animals (Figure 2A). In addition, there was an interactive effect between genotype and sex ($F_{1,78}$=4.0; *P*≤.05). Post hoc comparison demonstrated that male carriers of the *OPRM177G* allele consumed more ethanol than *C/C* males (*P*<.05) (Figure 2A). When using *z* scores to statistically control for cohort effects, these effects remained and were strengthened. We observed a main effect of the *OPRM1* gene variation ($F_{1,78}$=7.2; *P*=.009) but not of sex ($F_{1,78}$=0.49; *P*=.48), in addition to an interactive effect between the *OPRM1* genotype and sex ($F_{1,78}$=7.4; *P*=.008). Post hoc comparisons demonstrated that male carriers of the *OPRM1G* allele consumed more alcohol than did all other groups of the study (*P*<.05) (Figure 2B).

Table. Results of Factor Analysis Performed on Behaviors Scored Following Alcohol Administration

Factor	Eigenvalue	Behavior	Factor Loading
Disinhibition	3.855	Successful jumping	0.774
		Unsuccessful jumping	0.928
		Swaying	0.411
		Falling	0.542
Stimulation	1.655	Locomotion	0.977
		Passivity	–0.979
Ataxia	0.870	Stumbling	0.858
		Swaying	0.754
		Falling	0.656
		Hitting a wall	0.456

ALCOHOL PREFERENCE

There was a main effect of *OPRM1C77G* on alcohol preference ($F_{1,42}$= 9.6; *P*<.008). Carriers of the *OPRM177G* allele exhibited increased alcohol preference compared with *OPRM177C* homozygotes (*P*<.05) (Figure 3). There was also a main effect of sex with a higher level of alcohol preference in males than in females ($F_{1,42}$=17.4; *P*=.008). Finally, the *OPRM1* genotype and sex interacted to influence alcohol preference ($F_{1,42}$= 4.2; *P*=.05). Post hoc comparisons demonstrated the effect of *OPRM1C77G* to be present among male but not female rhesus macaques; males that were carriers of the *OPRM177G* allele exhibited higher alcohol preference than did *C/C* males (*P*<.01) (Figure 3).

DAYS INTOXICATED

There was a main effect of genotype on the number of study days during which an animal consumed an amount of ethanol that exceeded 0.67 g/kg of body weight per hour ($F_{1,78}$=4.4; *P*<.04) but not of sex ($F_{1,78}$=0.012; *P*=.92) (Figure 4). *OPRM177G* allele carriers consumed alcohol to intoxication more frequently than did *OPRM177C* homozygotes. Similar to the other dependent variables considered, there was an interactive effect between *OPRM1C77G* and sex ($F_{1,78}$=4.2; *P*<.05), and post hoc comparison demonstrated that, among males, those carrying the *OPRM177G* allele consumed doses of alcohol sufficient to produce intoxication more frequently than did those homozygous for the *OPRM177C* allele (*P*<.05) (Figure 4).

COMMENT

The human *OPRM1118G* allele confers increased affinity of the receptor for β-endorphin and has, therefore, been referred to as a gain-of-function polymorphism.[45] Although recent findings of decreased allelic transcription efficiency of this variant suggest the possibility of a more complex picture,[46] human in vivo findings would seem to support the original proposal, because the *OPRM1118G* variant has been associated with elevated pain thresholds,[28] increases in both euphorigenic response to alcohol and susceptibility to alcohol use disorders in some populations,[29,33] and a higher probability of treatment response to the opioid antagonist naltrexone.[34,47] However, human data are mostly limited to correlative studies. Available results from alcohol-challenge experiments are obtained at relatively low BECs because of ethical constraints. Furthermore, in human studies, complex conditioned effects may interact with direct pharmacological actions of alcohol to determine psychostimulant responses. Nonhuman primates therefore offer an attractive model system to evaluate the functional role of the *OPRM1* genetic variation for alcohol responses and alcohol preference.

We found that an *OPRM1* variant in rhesus macaques that has similar functional effects in vitro to the human *118G* allele increased ethanol-induced psychomotor stimulation, a commonly used marker of euphorigenic and positively reinforced alcohol actions.[48] This effect was behaviorally selective, in that ethanol-induced ataxia and disinhibition were not affected by genotype. The effect was only observed in males carrying the *OPRM177G* allele, and, congruent with the alcohol-challenge data, our alcohol consumption and preference data support the notion that positively reinforcing effects of alcohol are more pronounced in male *OPRM177G* carriers. Male carriers of the *OPRM177G* allele consumed more alcohol under free-choice limited-access conditions, consumed it more frequently, and exhibited increased preference for the alcohol solution over the sweetened vehicle.

In humans, it was originally reported that attenuated, rather than elevated, alcohol responses are characteristic of family history–positive subjects,[49] in addition to being associated with increased risk for later developing alcoholism.[50] Subsequent work in family history–positive subjects, however, demonstrated that attenuated responses are primarily seen during late stages, while increased stimulation is observed among the same subjects during the early phases of alcohol exposure.[51] Based on these findings, a differentiator model has been proposed, according to which, family history–positive individuals are motivated to drink because they exhibit both greater initial responses and greater acute tolerance to alcohol[52]; our data appear to be in agreement with this model. Modulation of alcohol reinforcement by genetic variation at the *OPRM1* locus in humans may relate to μ-opioid receptor control of hypothalamic-pituitary-adrenal axis responses. Suppression of alcohol self-administration and craving by naltrexone is accompanied by activation of the hypothalamic-pituitary-adrenal axis,[53] and the human *OPRM1118G* variant confers an elevated adrenocorticotropic hormone response to naltrexone.[54] We and others have found that the *OPRM177G* allele is associated with differences in hypothalamic-pituitary-adrenal axis output in rhesus macaques under a variety of testing conditions,[36] including following alcohol challenge (C.S.B., unpublished data, 2006).

In our study, macaques were given limited access to alcohol in social groups. Alcohol intake under these conditions is lower than in animals tested in isolation or in continuous access paradigms. This is in part because of competition for access to alcohol dispensers but (presumably) also because of the social pressures that come with being housed in an enclosure containing a troop of animals and the possible repercussions of becoming intoxicated in that setting. The fact that we did not observe an effect of rearing history on alcohol consumption in this data set may also be partially attributed to the application of these testing conditions; peer-reared monkeys are not only lower in social rank, potentially minimizing their access to the alcohol dispensers, but also tend to be more stress reactive and anxious.[37,43]

On average, animals tested in our social groups consumed only 0.3 g of alcohol per kilogram of body weight per hour. However, male carriers of the *OPRM177G* allele exhibited average levels of consumption that were almost twice that amount. This rate of alcohol consumption, though still considerably lower than that seen in alcohol-dependent animals that have had access to alcohol for years,[55] is pharmacologically active, as shown by its ability to produce signs of intoxication in rhesus macaques in our colony (J.D.H., unpublished data, 1998). Consumption at this level is, therefore, likely to occur at least in part because of the reinforcing pharmacological properties of alcohol, rather than because of its taste or caloric content alone. This is further supported by the observation that, opposite to the consumption of alcohol, intake of the sweetened vehicle solution was somewhat diminished in male carriers of the *OPRM177G* allele. Moreover, these subjects consumed alcohol to intoxication on almost 30% of testing days, whereas animals in the other groups did so on only 8% of testing days. This parallels data indicating that the human *OPRM1118G* allele is associated with an increase in the number of days during which alcohol-dependent subjects consume alcohol[34] and that, conversely, blockade of μ-opioid receptors with naltrexone results in a decreased frequency of heavy alcohol consumption.[23]

Although the observed effects of the *OPRM177G* allele on alcohol intake appears to be attributable to potentiated alcohol reinforcement, it is noteworthy that altered nociception may potentially contribute to the drinking phenotype. Carriers of the human *OPRM1118G* variant are more resistant to pain.[28] If the same is true in the macaque, it might render *OPRM177G* carriers less avoidant of aggressive encounters in a social setting and thus more likely to gain and retain access to the alcohol dispensers during social drinking sessions. Potentially related to this point, the *OPRM177G* allele is also associated with increased alcohol-induced aggression in our colony (C.S.B., unpublished data, 2005) and has been associated with aggression in another colony of rhesus ma-

caques, even in the absence of alcohol exposure.[35] It remains to be established whether the observed increased aggression, increased alcohol intake, and altered pain sensitivity are interrelated. However, neither altered nociception nor generally altered motivation for consummatory behavior are in our opinion likely to directly account for the increased alcohol consumption in *OPRM177G* carriers observed in our study, because, contrary to the alcoholic fluid intake, consumption of the sweetened vehicle was, if anything, decreased.

The male-restricted influence of the *OPRM177G* variant is of particular interest. Alcohol dependence is clinically and genetically heterogeneous. A distinct form characterized by a family history of alcoholism, an early onset of alcohol problems, and a psychostimulant-like response to alcohol is predominantly found in men.[56] The relevance of this heterogeneity has recently been validated by the demonstration of differential treatment response to the 5-HT_3 antagonist ondansetron, which has beneficial effects exclusively in early-onset subjects.[57] In a suggestive parallel, a growing body of evidence suggests that the role of opioid transmission for alcohol-related phenotypes is sex-restricted both in rodents and humans. Studies in mice have shown a sexual dichotomy with regard to the influence of *Oprm1* knockout on alcohol consumption[11]; alcohol-dependent human subjects who are either men or who are carriers of the *OPRM1118G* allele are more responsive to μ-opioid receptor blockade.[34,47] Our findings are in agreement with these observations. A possible conclusion is that in one population of men, in particular those with an early onset of alcohol problems and a positive family history, alcohol intake is more likely to be driven by the positively reinforcing effects of alcohol (reward craving), while in late-onset men and a majority of women, alcohol intake may be more often affected by negative reinforcement of alcohol (relief craving).[58]

In this study, we employed function-guided association with the coding nonsynonymous single nucleotide polymorphism (*OPRM1C77G*) rather than identifying and genotyping additional markers in the *OPRM1* locus and applying a haplotype-based approach in relating behavior to genetic variation. This decision was based on 2 considerations. First, the *OPRM1C77G* marker has been demonstrated to be functional and alter OPRM1 binding.[35] This prompts specific testable hypotheses about its consequences at a systems level, which merit evaluation. Second, the number of animals tested under uniform conditions and available for sequence analysis gave us insufficient power for a haplotype-based approach. Future analyses may therefore reveal additional coding variants or other functionally important variants in noncoding regions that are in linkage disequilibrium with the *OPRM1C77G* polymorphism and may contribute to the observed phenotype.

Although the pathway to alcoholism is influenced by many factors, repeated exposures to alcohol constitute a minimum requirement for the pathogenesis of this condition. We have found that a variant of the μ-opioid receptor gene (*OPRM1C77G*) is associated with increased sensitivity to the psychomotor stimulant properties of alcohol and influences early patterns of alcohol consumption in rhesus macaques. Male carriers of the variant allele exhibit increased alcohol preference and consume alcohol to intoxication more frequently. Our data support and extend emerging human literature that suggest that genetic variability at the *OPRM1* locus modulates alcohol reinforcement and preference, that these effects are moderated by sex, and that μ-opioid transmission primarily influences susceptibility for alcohol use and treatment responses in males.

Submitted for Publication: April 4, 2006; final revision received July 14, 2006; accepted July 14, 2006.
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Financial Disclosure: None reported.
Funding/Support: Supported by the National Institute of Child Health & Human Development and National Institute on Alcohol Abuse and Alcoholism Intramural Research Programs.
Previous Presentation: This paper was presented as a poster at the Research Society on Alcoholism meeting in Santa Barbara, Calif, on June 26, 2005.
Acknowledgment: The authors would like to acknowledge Amelia Chapelle, BA, Sue Higley, BA, Kelli Chisholm, BA, Courtney Shannon, BA, Ruth Woodward, DVM, Michelle Keawphalouk, BS, and Clarissa Parker, BA, for their support of this research.

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