

Overexpression of dopamine D2 receptors reduces alcohol self-administration

Panayotis K. Thanos,* Nora D. Volkow,* Paul Freimuth,† Hiroyuki Umegaki,‡ Hiroyuki Ikari,‡ George Roth,§ Donald K. Ingram§ and Robert Hitzemann¶

Departments of *Medicine and †Biology, Brookhaven National Laboratory, Upton, New York, USA

‡Department of Geriatrics, University of Nagoya School of Medicine, Aichi, Japan

§Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, USA

¶Department of Behavioral Neuroscience, Oregon Health Sciences University, Portland, Oregon, USA

Abstract

The mechanism(s) underlying predisposition to alcohol abuse are poorly understood but may involve brain dopamine system(s). Here we used an adenoviral vector to deliver the dopamine D2 receptor (DRD2) gene into the nucleus accumbens of rats, previously trained to self-administer alcohol, and to assess if DRD2 levels regulated alcohol preference and intake. We show that increases in DRD2 (52%) were associated with marked reductions in alcohol preference (43%), and alcohol intake (64%) of ethanol

preferring rats, which recovered as the DRD2, returned to baseline levels. In addition, this DRD2 overexpression similarly produced significant reductions in ethanol non-preferring rats, in both alcohol preference (16%) and alcohol intake (75%). This is the first evidence that overexpression of DRD2 reduces alcohol intake and suggests that high levels of DRD2 may be protective against alcohol abuse.

Keywords: adenovirus, alcoholism, animal model, drinking preference, gene therapy, nucleus accumbens.

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Alcoholism is a devastating disease that affects over 11 million individuals in the USA (Williams *et al.* 1987). Epidemiological studies have documented an important role of genetics in predisposition to alcoholism as evidenced by the higher concordance for alcoholism in identical twins than in fraternal twins and by the fourfold increased risk for alcoholism in children of alcoholics than in the general population (Cotton 1979; Goodwin 1979; Cloninger 1987; Schuckit and Gold 1988). The biological mechanisms underlying the predisposition to alcoholism are poorly understood. A target neurotransmitter for alcoholism is dopamine (DA) since it is believed to underlie the reinforcing effects of drugs of abuse including those of alcohol (Koob *et al.* 1987; Wise and Bozarth 1987; Di Chiara *et al.* 1992; Weiss 2000). It has been proposed that DA is one of the neurotransmitters that modulate the predisposition to alcohol abuse (Blum *et al.* 1996; George *et al.* 1995; Di Chiara *et al.* 1996; Repo *et al.* 1999; Li 2000). It is the projections of the DA cells to the nucleus accumbens (NAc) that have been implicated in the reinforcing effects of alcohol (Koob *et al.* 1987); where alcohol dose-dependently increases dopamine concentration (Le and Kiianmaa 1988; Weiss *et al.* 1993).

Chronic alcoholism has been shown to produce significant changes in dopamine D2 receptors (DRD2) concentrations (Tajuddin and Druse 1996). More importantly, of the dopamine receptor subtypes, the (DRD2) appear to be involved in transmitting the dopamine mediated reinforcing effects of alcohol (Stefanini *et al.* 1992; McBride *et al.* 1993b; Nowak *et al.* 2000). This was evidenced, by the reduced reinforcing effects of alcohol in DRD2 knockout mice (Phillips *et al.* 1998). Studies in human subjects also implicate DRD2 in alcoholism (Volkow *et al.* 1996a; Guardia *et al.* 2000). Alcoholics have reduced levels of DRD2 in brain (Volkow *et al.* 1996b) and epidemiological studies, though not always consistent (Cook *et al.* 1996), show a higher frequency of the DRD2 A1 *Taq* allele (Blum *et al.* 1990; Noble *et al.* 1991; Parsian *et al.* 1991; Persico *et al.*

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Address correspondence and reprint requests to Panayotis K. Thanos, Department of Medicine, Building 490, Brookhaven National Laboratory, Upton, NY 11973-5000, USA. E-mail: thanos@bnl.gov

Abbreviations used: DA, dopamine; DRD2, dopamine D2 receptor; NAc, nucleus accumbens; OD, optic density; P, ethanol-preferring rats; PET, positron emission tomography.

1996; Eriksson *et al.* 2000), which is an allele associated with low DRD2 density (Noble *et al.* 1991). Because the higher frequency of the A1 *Taq* allele as well as the reduction in brain DRD2 has also been documented with other drugs of abuse, it has been hypothesized that low levels of DRD2 predispose subjects to use drugs or alcohol as a means to compensate for the decrease in the activation of the associated reward circuits (Phillips *et al.* 1998). Though this hypothesis could be perceived as being in apparent conflict with the results from animal studies showing that removal of DRD2 results in a decrease in alcohol consumption (Phillips *et al.* 1998; Myers and Robinson 1999), one can not assume that the behavioral effects of absence of DRD2 receptors can be extrapolated to the behavioral consequences of variability in DRD2 receptor levels. Nor is it possible to predict on the basis of the data from the DRD2 knockout mice, the dose effect relationship between the levels of DRD2 and the effects on the behavior.

The purpose of this study was to investigate if we could modulate alcohol intake by varying the levels of DRD2 in the NAc, which is the brain region associated with the reinforcing effects of drugs of abuse (Pontieri *et al.* 1996). Brain levels of DRD2 in rats were modified using a replication-deficient adenoviral vector containing the rat cDNA insert for DRD2 (AdCMV.DRD2), which was injected into the NAc. Gene transfer via adenoviral vector is an effective strategy that can be utilized to introduce particular genes into tissue and provides a high specificity targeting and delivery (Crystal 1992; Suhr and Gage 1993). The effectiveness of the present vector for intracerebral transfer of DRD2 as well as the expression of functional DRD2 effects has been previously well established (Ikari *et al.* 1995, 1999; Umegaki *et al.* 1997; Ingram *et al.* 1998; Ogawa *et al.* 2000). Specifically, injection of the DRD2 adenoviral vector in the rat brain has been shown to increase the expression of functional DRD2 and that *in vitro* autoradiography visualizes this overexpression. More recently, we demonstrated that positron emission tomography (PET) was able to image the overexpression of DRD2 induced by adenoviral-mediated gene transfer into the rat brain (Ogawa *et al.* 2000). In addition, this overexpression of DRD2 by adenoviral vector was not strain or species specific and could be visualized by both PET and autoradiography (Ogawa *et al.* 2000). Ethanol intake was assessed using the popular sucrose-fading procedure (Samson 1986; Tolliver *et al.* 1988; Samson *et al.* 1989) and analyzed in terms of overall ethanol preference in a two-bottle choice paradigm. This technique has been widely used to train animals to drink ethanol and preference ratio is reflective of the CNS pharmacological effects of ethanol rather than attributed to taste as a factor (Samson *et al.* 1996).

Our working hypothesis was that DRD2 overexpression induced by vector administration would alter the reinforcing effects of alcohol and change ethanol intake.

Materials and methods

All studies were conducted in accordance with the guidelines established by the National Institutes of Health in *The Guide for Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of SUNY at Stony Brook School of Medicine. Two parallel experiments were performed; one to evaluate DRD2 levels and the other to evaluate alcohol self-administration at different time-points after DRD2 vector injection into the NAc.

D2 vector assessment

This experiment was done in 15 male adult Sprague–Dawley rats (350–450 g) and examined the efficacy of the AdCMV.DRD2 vector on the DRD2 levels of the NAc.

Microsurgery

Rats were anesthetized with an equal mixture of ketamine xylazine (100 mg/kg, 10 mg/kg) and placed in a Kopf stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Rats were implanted unilaterally with a 22-gauge guide cannula (Plastics One, Roanoke, VA, USA) into the NAc according to coordinates (+1.2 A, +1.4 L, –6.6 V) in the rat brain atlas of Paxinos and Watson (1986). The guide cannula was secured to the skull with four small stainless steel screws and dental cement. The rats were then allowed 1 week to recover the surgery before being microinjected with the DRD2 vector (as previously described in Umegaki *et al.* 1997). Briefly, the fragment containing the entire DRD2 coding region was cloned into the shuttle vector, pCMV.SV2 and the resulting plasmid was cotransfected into 293 cells and purified. A 26-gauge 5 μ L Hamilton microsyringe connected to a 28-gauge internal cannula was used to microinject 2 μ L over 10 min of the adenoviral vector containing the cDNA for the DRD2 (10^{10} pfu/mL). Subsequently, the rats were divided into five groups of three. Rats depending on their group were killed at 4, 6, 8, or 10 days postvector injection. Another group of rats received a second vector injection on day 10, and killed 3 days later (10 + 3 days). Their brains were rapidly removed and frozen in isopentane and stored at –80°C. Next, 20- μ m thick coronal sections were cut on a cryostat. Sections were then kept at –80°C until [³H]raclopride DRD2 autoradiography was conducted.

Autoradiography and image analysis

Autoradiography was performed as previously described (Ikari *et al.* 1995). Briefly, sections were preincubated for 1 h at room temperature (24°C) in 0.1 M Tris buffer. Sections were then incubated in buffer containing 5 nM [³H]raclopride (New England Nuclear, Boston, MA, USA) for 1 h at room temperature. Next, the sections were rinsed twice with cold Tris buffer (5°C) for 5 min each, rinsed in ice-cold distilled water and allowed to air dry for 24 h before being exposed under 3H Hyperfilm for 3 weeks. Optic density of brain regions was measured using a computer-based densitometer, image analyzer (MCID-M1, Imaging Research Inc., St. Catherines, Ontario, Canada). The NAc was outlined and the optic density (OD) recorded. Five consecutive sections containing the NAc were used for the autoradiography analyses of D2 receptor binding and were then averaged for each animal. Concurrently, DRD2 binding levels were assessed in the striatum of the same sections as a control. DRD2 specific binding levels were expressed as the percent difference between the vector-injected side and the contralateral

control side. Nonspecific binding (cerebellum) was subtracted from the total binding to yield specific binding. It is well established that the cerebellar subtraction technique (Hitzemann *et al.* 1991; Qian *et al.* 1992; Kanes *et al.* 1993) accounts for about 90–95% of the total binding, and has considerably less variability than other techniques since the target tissue (NAc) and the cerebellum are on the same glass slide. In addition, the cerebellum subtraction technique is particularly useful when a one has a relatively small region of interest and requires less isotope.

Behavioral assessment

Self-administration was monitored using the two-bottle choice preference test, which is a model that captures aspects of voluntary alcohol consumption in humans (Samson *et al.* 1989). This experiment was done in 15 adult Sprague–Dawley rats and examined the effect of the AdCMV.DRD2 vector on a continuous access two-bottle choice-drinking paradigm using the standard sucrose-fading technique (Samson 1986; Tolliver *et al.* 1988; Samson *et al.* 1989).

(i) Following a 1-week adaptation period to the home cage environment, rats ranging in weight between 391 and 483 g (mean = 431 g) were given a two-bottle preference test for 5 days. Animals had continuous access to ethanol (5% v/v) and tap water in their home cages from two 150 mL Kimax drinking bottles. Every day at the same time (9:00 AM) fluid intake as well as the animal's body weight was recorded. The position of the bottles was reversed daily to prevent a position habit. The amount of ethanol consumed divided by total fluid consumed $\times 100$ was used as a measure of ethanol preference.

(ii) After this initial preference test animals were then initiated to self-administer ethanol using the *sucrose-fading procedure*. This procedure has been widely used and previously described in detail (Samson 1986; Tolliver *et al.* 1988; Samson *et al.* 1989). Briefly, animals are first presented with a choice between tap water and a 20% (w/v) sucrose solution for 2 days. Then the sucrose concentration was reduced to 10% (w/v) for 2 days. Over a series of days, ethanol was added to the sucrose solution in graded amounts, with the sucrose concentration being simultaneously reduced. More specifically the sequence of fluid presentations were five sessions with: 10% sucrose–1% ethanol (all ethanol solutions are v/v), 10% sucrose–2% ethanol, 10% sucrose–4% ethanol, 10% sucrose–7% ethanol, 7% sucrose–7% ethanol, 5% sucrose–7% ethanol, 4% sucrose–7% ethanol, 10 sessions with 2% sucrose–7% ethanol, 10 sessions with 1% sucrose–7% ethanol, and finally 10 sessions with 7% ethanol. At the end of this procedure rats were assigned to an alcohol preferring group if they showed greater than a 60% preference to ethanol (7% v/v) versus water. In contrast, rats were assigned to an alcohol non-preferring group if they showed greater than a 60% preference to water versus ethanol (7% v/v).

(iii) *Preoperative drinking-baseline*. Following the sucrose-fading technique both groups of rats were given 7 days of ethanol preference assessment between water and ethanol (7% v/v). This phase was the baseline drinking preference prior to surgery.

(iv) *Postoperative drinking-baseline*. As previously described in experiment I, all animals were stereotaxically implanted with an injection cannula into the NAc. All animals were allowed 1-week recovery from surgery at which time they had free access to both food and water. Following this recovery period, all animals were given 7 days of the same ethanol preference assessment between

water and ethanol (7% v/v) and this data was compared with the preoperative data.

(v) *Vector treatment drinking*. Rats were treated (on day 0) with a microinjection of the control replication-deficient adenovirus vector expressing nothing (AdCMV.Null) vector into the NAc as previously described in experiment (i) and then returned to the two-bottle ethanol preference assessment for 7 days. Subsequently on day 8, all animals were similarly microinjected with the AdCMV.DRD2 vector into the NAc and then returned to the two-bottle ethanol preference assessment between water and ethanol (7% v/v). This assessment continued until day 28. At day 28, half the animals in each group were re-injected with the AdCMV.DRD2 vector, for a second time; and the other half with the control AdCMV.Null vector. All animals were again monitored for ethanol preference for an additional 4 days.

Results

D2 vector assessment

Histological examination of the area of injection, as previously described did not reveal any unusual neuropathology or significant signs of inflammation associated with sites infected with AdCMV.DRD2 and those receiving control treatments. In addition, as demonstrated in our previous studies (Ikari *et al.* 1995, 1999; Umegaki *et al.* 1997; Ingram *et al.* 1998; Ogawa *et al.* 2000) rats receiving the DRD2 vector exhibited an increase in the DRD2 binding only at the injection site (NAc). This binding extended across several serial sections and no [3 H] raclopride binding was detected in contralateral sides. Figure 1 illustrates the NAc microinjection sites in these animals and an example of DRD2 binding in the NAc.

Autoradiography assessment of DRD2 in the NAc after AdCMV.DRD2 microinjection revealed a localized increase of DRD2 levels in animals examined 4, 6, 8 or 10 days following vector treatment (Fig. 2). Specifically, a one-way ANOVA comparing NAc DRD2 levels in different groups of rats revealed a significant difference [(4, 3.99) $p < 0.05$]. Subsequently, *post hoc t*-test comparisons between DRD2 levels on the vector-injected side versus the contralateral control side revealed the following increases in DRD2: day 4, 52.1% ($T_{\text{obs}} = 12.28$, $*p < 0.001$); day 6, 37.4% ($T_{\text{obs}} = 6.60$, $*p < 0.001$); day 8, 25.2% ($T_{\text{obs}} = 3.7$, $*p < 0.01$); day 10, 8.3% ($T_{\text{obs}} = 1.28$, ns), respectively. Rats treated a second time with a vector injection at day 10 showed an increase in DRD2 levels at day 13 of 25.6% ($T_{\text{obs}} = 4.37$, $*p < 0.01$). Furthermore, as a control we examined DRD2 levels in the caudate putamen of the same sections. A one-way ANOVA revealed no significant differences between DRD2 levels on the vector-injected side versus the contralateral control side [(4, 0.36) $p > 0.05$].

Behavioral assessment

Drinking preference was assessed as the amount of ethanol consumed divided by total fluid consumed $\times 100$. In the initial preference test it was found that all animals showed

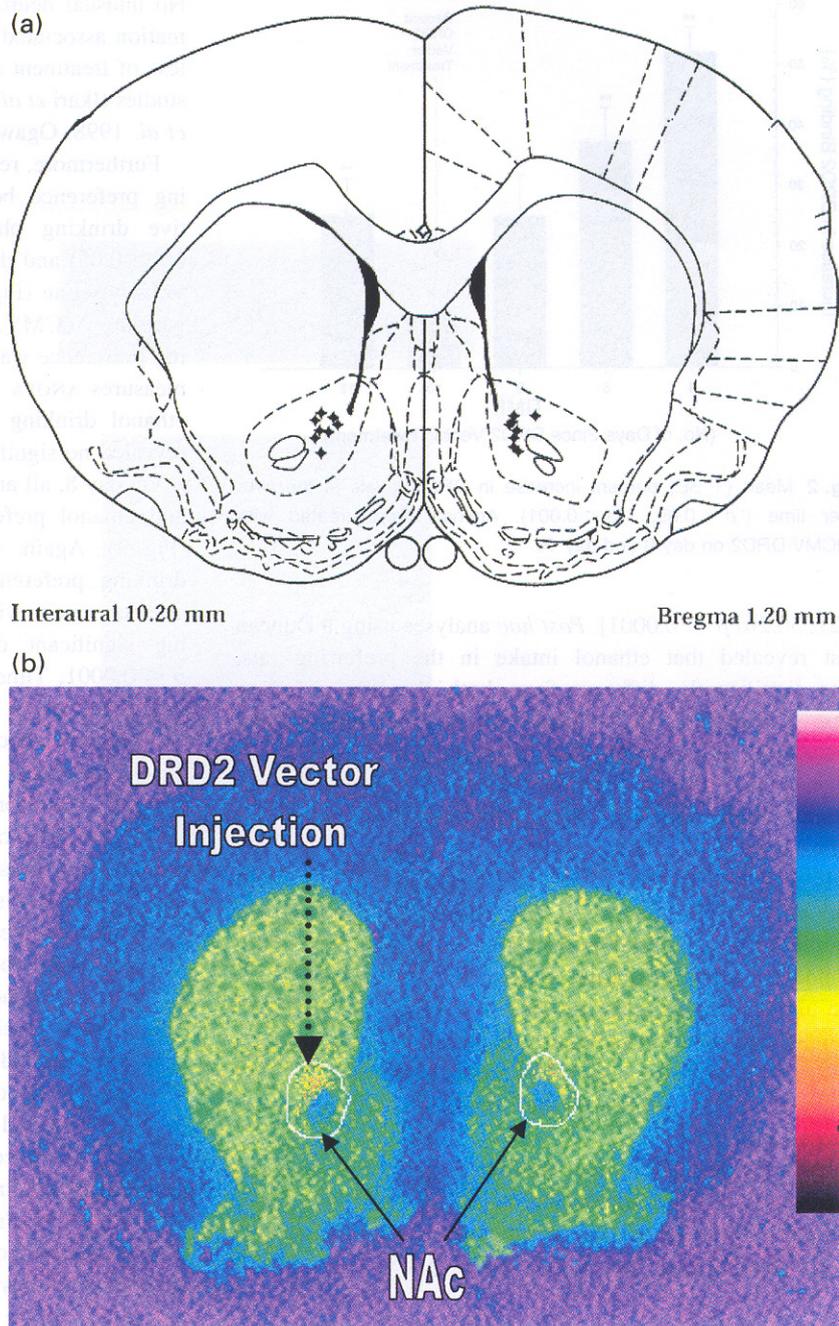


Fig. 1 (a) A coronal section of the rat brain illustrating the NAc and the location of injection sites (adopted from Paxinos and Watson 1986). (b) Qualitative assessment of DRD2 binding 4 days after treatment with the DRD2 vector into the left NAc. Autoradiography of a rat coronal brain section treated with AdCMV.DRD2. Intense concentration of DRD2 demonstrates binding [^3H]raclopride in the NAc near the injection site (see arrow).

little ethanol intake and followed water as it alternated positions each day. The mean intakes were expressed as milliliters and grams of ethanol/kg of body weight/day (Table 1).

Assessment of each animal's ethanol drinking preference following the sucrose-fading procedure (previously described) resulted in six rats in the alcohol preferring group [$> 60\%$ preference of ethanol (7% v/v) versus water], and nine rats in the alcohol non-preferring group [$> 60\%$ preference of water versus ethanol (7% v/v)]. The mean

weight of the animals increased by the end of the sucrose-fading technique to 522 g (range 442–611). Furthermore, it was found that the non-preferring rats showed little ethanol intake and followed water as it alternated positions each day. In contrast, preferring rats increased their ethanol intake (Table 1). A series of one-way ANOVA tests revealed significant differences in ethanol intake before and after sucrose fading. Specifically, significant differences were found in ethanol intake [(27, 43.09) $p < 0.0001$], water intake [(27, 17.86) $p < 0.0001$], and g/kg/day ethanol intake

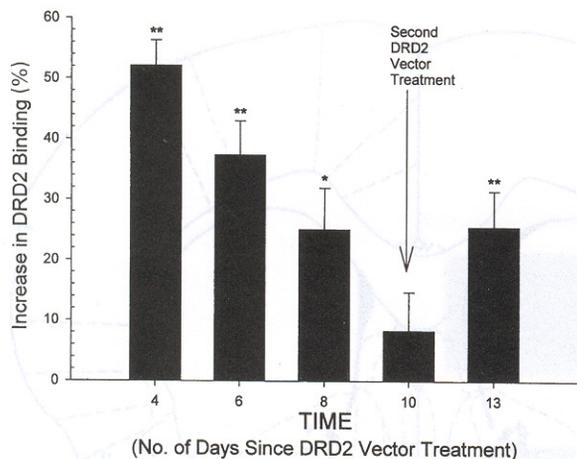


Fig. 2 Mean (+ SE) percent increase in DRD2 levels of the NAC over time (* $p < 0.05$; ** $p < 0.001$). Animals were treated with AdCMV.DRD2 on day 0 and day 10.

[(27, 50.26) $p < 0.0001$]. *Post hoc* analyses using a Duncan test revealed that ethanol intake in the preferring rats, was significantly different from both the non-preferring rats, $p < 0.0001$; and ethanol intake prior to training; $p < 0.0001$ (before; see Table 1). In contrast, the same type of *post hoc* analysis between the non-preferring rats and ethanol intake prior to training, revealed no significant differences.

In addition, given a mean total fluid intake of 37.1 mL and 40.4 mL for preferring and non-preferring rats, this resulted in a mean fluid intake of 7.1 mL and 7.7 mL/100 g of body weight. Calculation of the same intake measures for the initial preference test resulted in 7.9 mL/100 g of body weight, and is not significantly different between the two test periods. Thus on a per weight basis fluid intake had not changed but the amount of fluid ingested daily as ethanol was altered.

Cannula placement was confirmed as in the previous experiment through histological examination as previously described. One animal was not included in the behavioral assessment because the cannula placement was too dorsal.

Table 1 Twenty-four hour ethanol intakes in a two-bottle choice condition measured before and after sucrose-fading technique

	Ethanol (mL)	Ethanol (g/kg)	Water (mL)
Before training	6.2 (5.1)	0.57 (0.48)	28.1 (6.2)
After training			
Non-preferring rats	24.0 (8.4)**	2.53 (0.28)**	13.1 (5.9)*
Preferring rats	8.11 (1.9)	0.85 (0.13)	32.3 (6.8)

Values are means with standard deviations in parentheses. * $p > 0.05$; ** $p > 0.001$.

No unusual neuropathology or significant signs of inflammation associated with injection sites was observed regardless of treatment and this was consistent with our previous studies (Ikari *et al.* 1995, 1999; Umegaki *et al.* 1997; Ingram *et al.* 1998; Ogawa *et al.* 2000).

Furthermore, repeated measures ANOVA comparing drinking preference between the preoperative and postoperative drinking phases revealed no statistical difference ($p > 0.05$) and this data was pooled together and referred to as baseline (Fig. 3). Subsequently, animals were treated with the AdCMV.Null (vehicle) vector on day 0 and drinking preference was recorded for 7 days (Fig. 3). A repeated measures ANOVA comparing baseline ethanol drinking and ethanol drinking after treatment with the control vector revealed no significant difference ($p > 0.05$; Fig. 3).

On day 8, all animals were treated with the DRD2 vector and ethanol preference drinking monitored until day 28 (Fig. 3). Again, a repeated measures ANOVA comparing drinking preference in both groups of rats across time after treatment with the DRD2 vector revealed the following significant differences: group effect (12, 45.414), $p < 0.0001$; Time effect (21, 13.668), $p < 0.0001$ and the interaction group \times time effect (21, 3.761) $p < 0.0001$.

Post hoc t-test comparisons were then performed between baseline drinking preference and drinking at different times after DRD2 vector treatment and revealed several significant differences illustrated in Fig. 3 by an asterisk ($p < 0.05$). At day 12, 4 days after DRD2 vector treatment (time when peak DRD2 levels were observed in experiment 1), ethanol preference was decreased in the preferring rats from 70% to 27% and in the non-preferring rats from 20% to 4% (Fig. 3). Ethanol preference in the preferring rats returned to pretreatment levels at day 16, but preference in the non-preferring rats did not return to pretreatment levels until day 25 (Fig. 3). At day 28 half the animals in each group received a second DRD2 vector treatment, which decreased ethanol preference at day 32 to the same extent as it had done during the first treatment (Fig. 3). In contrast, the other half of the animals received a vehicle (null vector) treatment at day 28, and showed no significant effect on their ethanol preference on day 32 (Fig. 3).

Ethanol intake (g/kg/day) was also examined in both groups of animals (Fig. 4). A repeated measures ANOVA comparing ethanol intake in both groups of rats across time after treatment with the DRD2 vector revealed the following significant differences: group effect (12, 263.05), $p < 0.0001$; time effect (21, 17.77), $p < 0.0001$ and the interaction group \times time effect (21, 4.54) $p < 0.0001$. *Post hoc t*-test comparisons were then performed on ethanol intake between baseline and at different times after DRD2 vector treatment and revealed several significant differences illustrated in Fig. 4 by an asterisk ($p < 0.05$). At day 12, four days after DRD2 vector treatment (time when peak DRD2 levels were observed in experiment i), ethanol intake

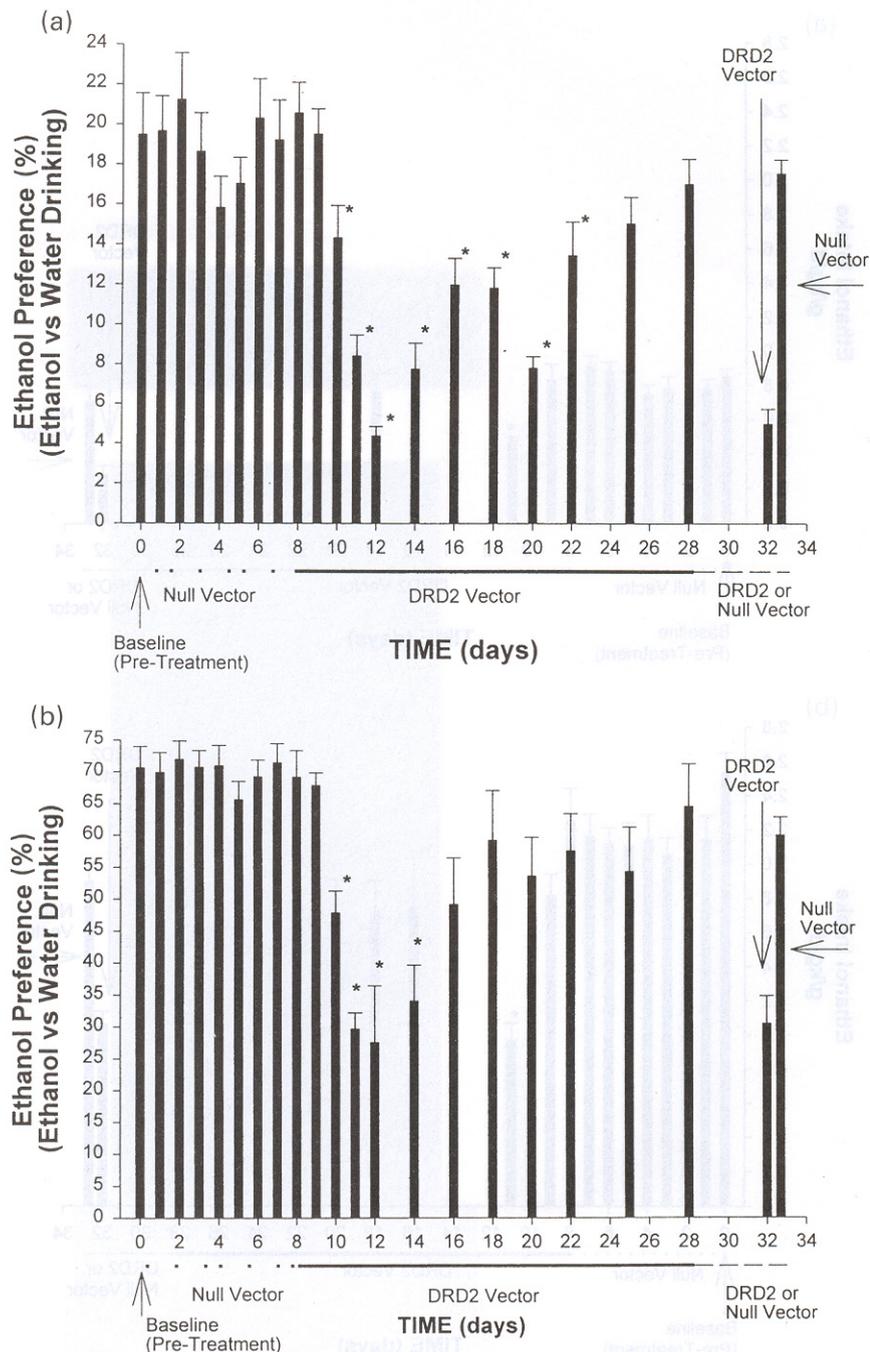


Fig. 3 Mean (+ SE) percent ethanol preference over time in (a) preferring and (b) non-preferring rats (* $p < 0.05$). Both groups of animals were treated at two time points with the DRD2 and vehicle (null) vectors.

was decreased in the preferring rats from 2.2 to 0.8 g/kg/day and in the non-preferring rats from 0.8 to 0.2 g/kg/day (Fig. 4). Ethanol intake returned to baseline levels 8 days later and a second challenge with either the vehicle or DRD2 vector at day 28 revealed similar effects on ethanol intake.

Overall, it should be noted, there was no significant decrease in total fluid intake after treatment with the vector, but rather a decrease in ethanol preference (drinking from the ethanol bottle versus the water bottle) and ethanol intake. No visible decrease in locomotor activity was

observed in animals following vector treatment. In addition, rats did not show any signs of malaise or weight loss due to treatment with the vector and this was consistent with previous studies (Ikari *et al.* 1995, 1999; Umegaki *et al.* 1997; Ingram *et al.* 1998).

Discussion

In this study the role of DRD2 in alcohol abuse was examined by observing the effects of manipulating the

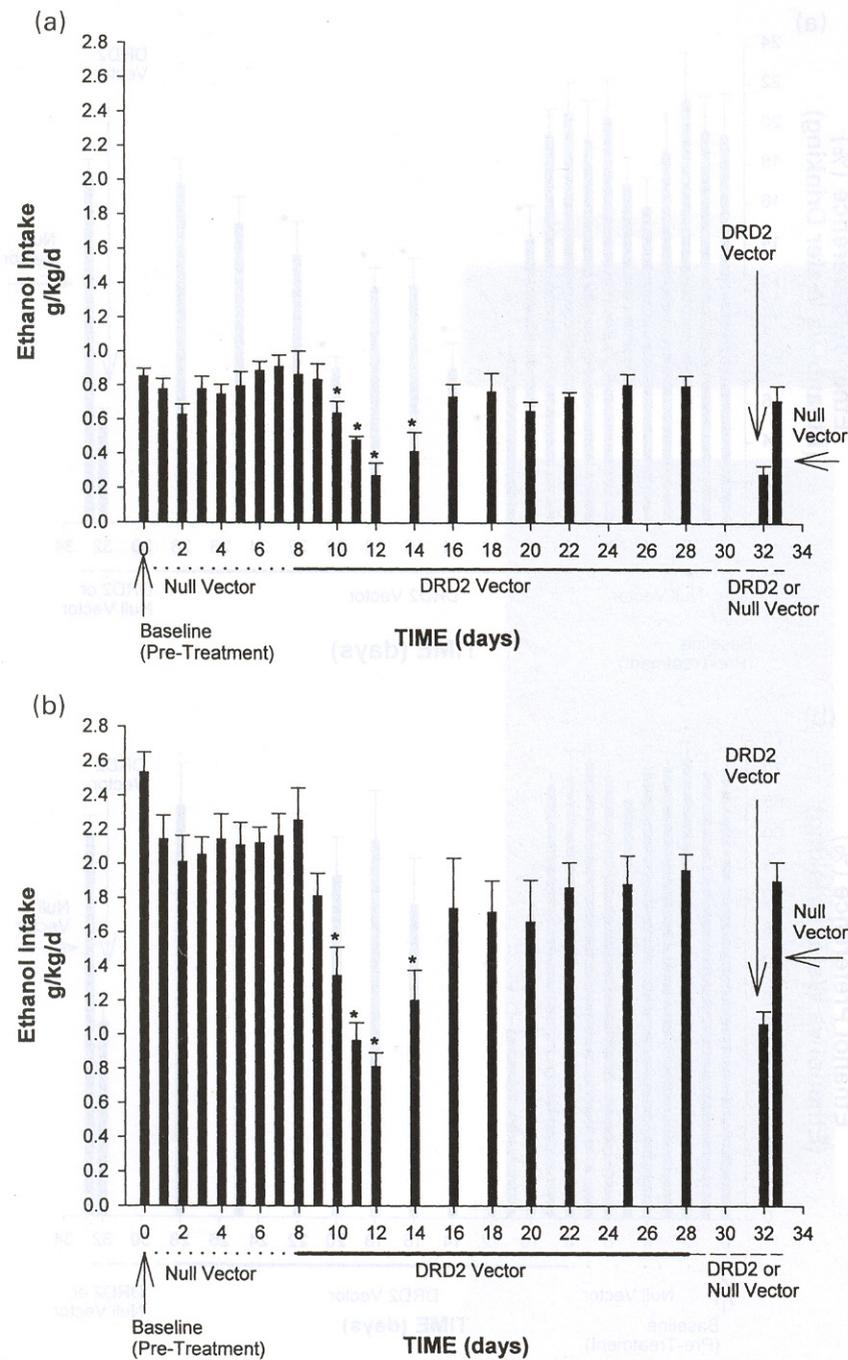


Fig. 4 Mean (+ SE) ethanol intake (g/kg/day) over time in (a) preferring and (b) non-preferring rats (* $p < 0.05$). Both groups of animals were treated at two time points with the DRD2 and vehicle (null) vectors.

levels of the DRD2 gene in the NAC. This was accomplished by means of an adenovirus engineered to express the DRD2 gene in infected cells. In the current study we observed that rats microinjected with the DRD2 vector into the NAC produced a significant (52%) overexpression of DRD2 binding levels. This DRD2 overexpression was visualized at day 4 after microinjection of the DRD2 vector and returned to baseline at day 8. Therefore the DRD2 binding overexpression induced by the adenoviral vector in the brain was transient and returned to baseline after about a week. When

treated with the vector a second time DRD2 levels increased to significant levels (3 days later). This transient DRD2 binding overexpression was consistent with our previous studies (Ikari *et al.* 1995, 1999; Umegaki *et al.* 1997; Ingram *et al.* 1998; Ogawa *et al.* 2000). Interestingly, Fig. 2 shows a smaller response to a second treatment with the vector. One potential explanation could be that there is sensitization to the second vector that would result in the same behavioral effects for a lower DRD2 expression level. However, further studies are required to assess if tolerance or sensitization

develop to the DRD2 overexpression. It is possible that the DRD2 overexpression can result in constitutive activity producing a decrease in drinking, although this has not yet been demonstrated *in vivo*. Future studies will examine this by microinjecting a D2 gene that encodes for a DRD2 that is constitutively active.

In addition, the current study demonstrated that DRD2 gene transfer into the NAc regulated alcohol preference and intake. Alcohol Preferring rats treated with intra-accumbens DRD2 vector infusion decreased their ethanol preference by 43% and returned to baseline levels by day 8. When these animals were treated a second time with the vector a similar decline in preference was seen 4 days later. In addition, ethanol intake in the same animals decreased by 64% before returning to baseline levels 8 days later.

Similarly, alcohol non-preferring rats treated with the DRD2 vector displayed significant reductions in both ethanol preference (16%) and intake (75%). Interestingly, in this group, ethanol preference returned back to baseline levels after 14 days, whereas ethanol intake returned to baseline levels after 8 days. It remains uncertain as to why this effect on preference seems to be more prolonged in the alcohol non-preferring rats. One potential explanation is that the DRD2 levels that are required for drinking to return to baseline in preferring and non-preferring rats may be different and thus while for preferring rats drinking may return at a higher level than in the non-preferring rats for whom relatively low levels may still be able to interfere with the drinking. One other explanation could be that the return of DRD2 levels to baseline after vector injection may differ between alcohol preferring and non-preferring rats. This is something that we will be examining with PET. In addition, another possibility could be that the reduction in drinking is a secondary effect from DRD2 stimulation then the return to baseline could follow a different temporal course than the return of DRD2 to baseline and this could differ between preferring and non-preferring rats. Although the binding data and preference behavior are positively correlated they are not a one to one relationship. In fact, we know from the human data that while in normal controls DRD2 levels differ between those liking the drug versus those that do not; the variability can not be explained solely on the differences in DRD2. This indicates that there are other variables that modulate these responses. As a result of this the correlation is not one to one. In fact, it is also possible that the relative role that the DRD2 has on drinking behavior may differ between preferring and non-preferring rats; so in the preferring rats, DRD2 may play a greater role in regulating drinking behavior than in non-preferring rats. The present findings pertain to alcohol predisposition in that one can interpret the high DRD2 levels as being protective against alcohol administration. Conversely, one can hypothesize that low DRD2 levels with a consequent decrease in DA stimulation may favor alcohol-self administration. This

hypothesis is supported by studies showing that the selectively bred ethanol-preferring rats (P) have moderate to low DRD2 levels (Kanes *et al.* 1993; McBride *et al.* 1993a) and are generally considered to be strains with low DA brain activity (McBride *et al.* 1991; Ng and George 1994b; Hitzemann *et al.* 1995; Zhou *et al.* 1995). Moreover, systemic administration of DA agonists in these two strains markedly reduces ethanol intake (Russell *et al.* 1996; Ng and George 1994a) and microinjection of a D2 antagonist into the NAc of the P rat increases ethanol intake (Levy *et al.* 1991), which is compatible with the notion that the levels of DA stimulation via DRD2 modulate ethanol intake.

In this study, we observed a decrease in alcohol intake, whether the animal exhibited high or low ethanol consumption. In the preferring rats DRD2 up-regulation decreased ethanol intake to levels similar to those of Non-preferring rats whereas in the latter, receptor up-regulation almost completely abolished ethanol intake. This indicates that DRD2 modulate alcohol intake irrespective of the baseline level of consumption.

These results have therapeutic implications for they suggest that strategies aimed at increasing brain DRD2 levels, which are significantly reduced in alcoholics, could be beneficial in the prevention and treatment of alcoholism. Though the use of vector delivered genes as performed in this study is not clinically appropriate, in the future, as gene therapies become less invasive and more specific, this approach may become feasible. Also, as we learn how D2 receptors are regulated it may be possible to develop interventions that can increase their expression.

The present study provides evidence that high DRD2 levels may have a protective role against alcohol abuse. The expression of DRD2 in the brain, which is modulated by both genetic and environmental factors such as stress (Papp *et al.* 1994), provides a molecular mechanism that can account for the involvement of both genetic as well as environmental factors in the predisposition to alcohol abuse. Information encoded in a cell's genetic material directs the synthesis of a given protein. Subtle variations among genes account for the normal range of inherited differences between individuals in a population. More importantly, major genetic variation may promote an individual's vulnerability to disease including alcohol abuse.

Future studies will examine the role of D1 in a similar self-administration alcohol study using a D1 vector. This will allow further insight into the possibility that the D2 effects described is not due to an imbalance of DA receptor subtypes in the nucleus accumbens. Researchers have also emphasized the effect of alcohol on other neurotransmitters, particularly an interaction of the dopamine and opioid system (Gonzales 1996), as well as the role of neurotrophic factors and the effect of ethanol on levels of selective growth factors (Crews 2000). Future studies will look at the

relationship between these DA receptor subtypes and specific growth factors in alcohol abuse.

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Overexpression of dopamine D2 receptors reduces alcohol self-administration

Panayotis K. Thanos,* Nora D. Volkow,* Paul Freimuth,†
 Hiroyuki Umegaki,‡ Hiroyuki Ikari,‡ George Roth,§ Donald K. Ingram§ and
 Robert Hitzemann¶

Departments of *Medicine and †Biology, Brookhaven National Laboratory, Upton, New York, USA

‡Department of Geriatrics, University of Nagoya School of Medicine, Aichi, Japan

§Gerontology Research Center, National Institute on Aging, Baltimore, Maryland, USA

¶Department of Behavioral Neuroscience, Oregon Health Sciences University, Portland, Oregon, USA

The authors of the above paper, which appeared in *J. Neurochem.* 78, pp. 1094–1103, wish to make a correction to the legends of Figs 3 and 4. The figures and their corrected legends are reproduced below.

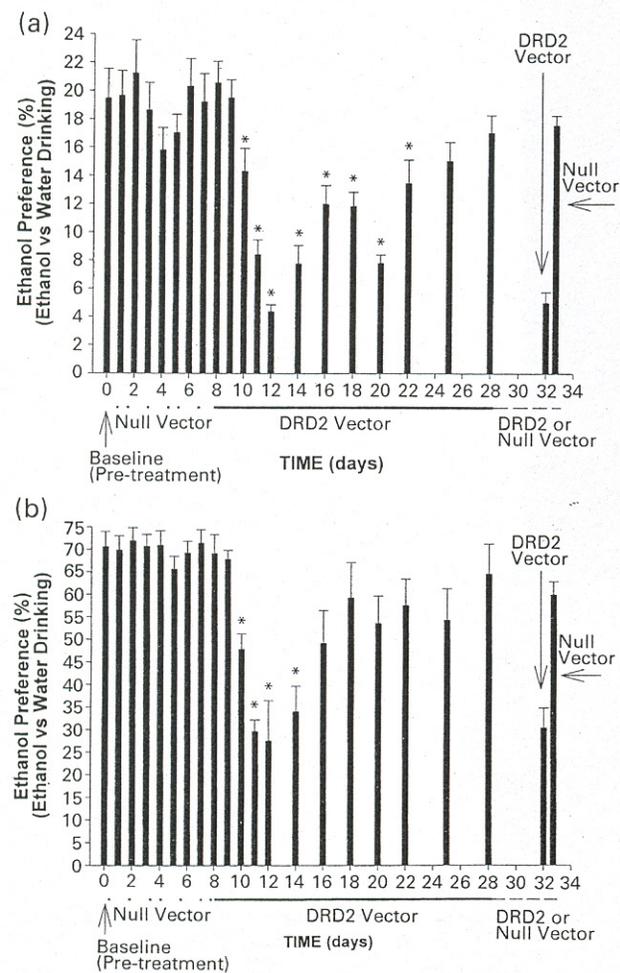


Fig. 3 Mean (+ SE) percentage ethanol preference over time in (a) non-prefering and (b) preferring rats ($*p < 0.05$). Both groups of animals were treated at two time points with the DRD2 and vehicle (null) vectors.

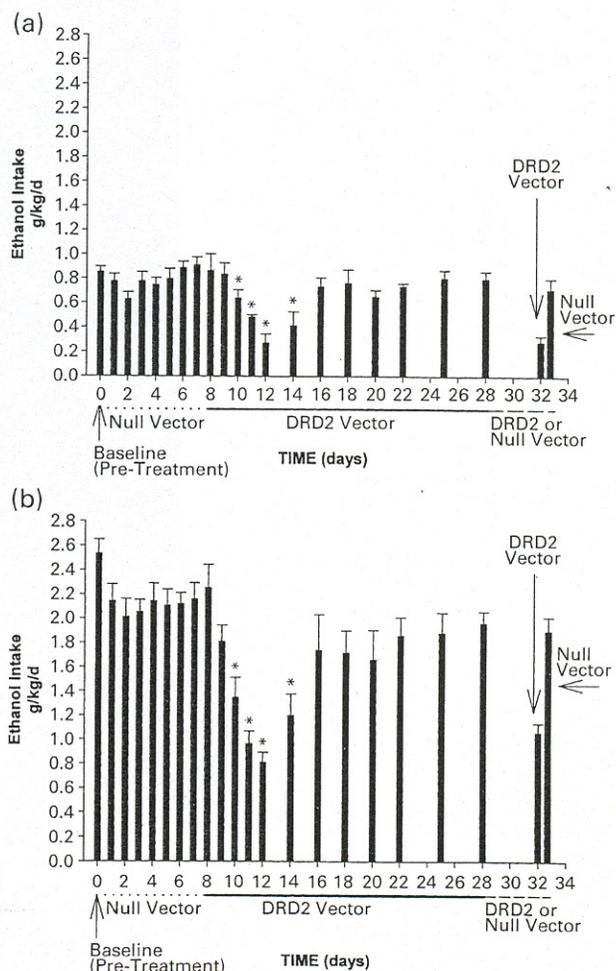


Fig. 4 Mean (+ SE) ethanol intake (g/kg/day) over time in (a) non-prefering and (b) preferring rats ($*p < 0.05$). Both groups of animals were treated at two time points with the DRD2 and vehicle (null) vectors.