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DRD2 Gene Transfer Into the Nucleus Accumbens Core of the Alcohol Preferring and Nonpreferring Rats Attenuates Alcohol Drinking

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Background: Transient overexpression of the dopamine D2 receptor (DRD2) gene in the nucleus accumbens (NAc) using an adenoviral vector has been associated with a significant decrease in alcohol intake in Sprague Dawley rats. This overexpression of DRD2 reduced alcohol consumption in a two-bottle-choice paradigm and supported the view that high levels of DRD2 may be protective against alcohol abuse.

Methods: Using a limited access (1 hr) two-bottle-choice (water versus 10% ethanol) drinking paradigm, we examined the effects of the DRD2 vector in alcohol intake in the genetically inbred alcohol-preferring (P) and -nonpreferring (NP) rats. In addition, micro–positron emission tomography imaging was used at the completion of the study to assess in vivo the chronic (7 weeks) effects of ethanol exposure on DRD2 levels between the two groups.

Results: P rats that were treated with the DRD2 vector (in the NAc) significantly attenuated their alcohol preference (37% decrease) and intake (48% decrease), and these measures returned to pretreatment levels by day 20. A similar pattern of behavior (attenuation of ethanol drinking) was observed in NP rats. Analysis of the [^{11}C]raclopride micro–positron emission tomography data after chronic (7 weeks) exposure to ethanol revealed clear DRD2 binding differences between the P and NP rats. P rats showed 16% lower [^{11}C]raclopride specific binding in striatum than the NP rats.

Conclusions: These findings further support our hypothesis that high levels of DRD2 are causally associated with a reduction in alcohol consumption and may serve as a protective factor against alcoholism. That this effect was seen in P rats, which are predisposed to alcohol intake, suggests that they are protective even in those who are genetically predisposed to high alcohol intake. It is noteworthy that increasing DRD2 significantly decreased alcohol intake but did not abolish it, suggesting that high DRD2 levels may specifically interfere with the administration of large quantities of alcohol. The significantly higher DRD2 concentration in NP than P rats after 7 weeks of ethanol therefore could account for low alcohol intake.

Key Words: Alcoholism Adenovirus Addiction Positron Emission Tomography (PET) Gene Therapy.

ETHANOL STIMULATES BOTH dopamine (DA) neurons and DA release in the rat nucleus accumbens (NAc) (Blanchard et al., 1993; Imperato and Chiara, 1986; Weiss et al., 1993; Wozniak et al., 1990; Yoshimoto et al., 1991). In addition, it has been established that the various DA receptors play a significant role in alcohol and substance abuse (Caine et al., 1999; Di Chiara, 1995; Gonzales, 1996; Munzar and Goldberg, 2000; Volkow et al., 1993). Among the various DA receptor subtypes, the DRD2 receptor has been most frequently associated with the reinforcing effects of alcohol (Hitzemann et al., 2003; McBride et al., 1993; Nowak et al., 2000; Stefanini et al., 1992).

Similarly, chronic alcoholism produced significant changes in DRD2 levels (Guardia et al., 2000; Tajuddin and Druse, 1996; Volkow et al., 1996, 2002). These data have led to the hypothesis that DRD2 deficiency or down-regulation may predispose subjects to drug use as a means of compensating for the decrease in activation of reward circuits activated by these receptors (Blum et al., 1996; Volkow et al., 1996, 2002). Multiple genes and environmental conditions have been implicated in producing low levels of DRD2 receptors in the brain. Low levels of DRD2 in brain have been postulated to lead to a reward deficiency syndrome that predisposes an individual to multiple addictive, impulsive, and compulsive behavioral propensities,

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such as alcohol and drug abuse, glucose binging, pathologic gambling, sex addiction, attention-deficit/hyperactivity disorder, Tourette's syndrome, autism, chronic violence, posttraumatic stress disorder, schizoid/avoidant personality, conduct disorder, and antisocial behavior (Blum et al., 2000; Comings and Blum, 2000; Volkow et al., 1993, 1996, 2001, 2002; Wang et al., 1997).

Imaging studies (Volkow et al., 1999b, 2002) have shown significant correlation between DRD2 density and the reinforcing response to psychostimulants, suggesting that low DRD2 levels may be involved in the predisposition to drug abuse. Supporting this view, several animal studies have reported that DRD2 antagonists enhanced ethanol self-administration in selectively bred alcohol-preferring (P) rats (Dyr et al., 1993; Levy et al., 1991). In addition, it has been demonstrated that there was 20 to 25% lower DRD2 binding in the NAc of P rats when compared with alcohol nonpreferring (NP) rats (McBride et al., 1993), as well as 20% lower DRD2 binding between the Sardinian ethanol-preferring and the Sardinian ethanol nonpreferring rats (Stefanini et al., 1992).

It is well understood that DA projections from the ventral tegmental area (VTA) to the NAc are the most studied system implicated in the control of ethanol self-administration behavior (Kalivas et al., 1993; Koob et al., 1987). Previous studies have examined the subterritories of the NAc into core and shell with regard to alcohol preference (Samson and Hodge, 1996; Zocchi et al., 2003). It has been hypothesized that both are critical in alcohol consumption but that the NAc shell is involved in mediating the excitatory effects of stimuli, the anticipation of reward, and goal-directed behaviors (Corbit et al., 2001; Johnson et al., 1995; Sokolowski et al., 1998; Sokolowski and Salamone, 1998), whereas the NAc core is involved in mediating and encoding the incentive value of the instrumental outcome on the performance of goal-directed actions (Corbit et al., 2001; Samson and Chappell, 2003) and implicated in mechanisms of learning such as the expression of conditioned stimulus and unconditioned stimulus associations (Parkinson et al., 1999).

An adenoviral gene transfer technique was used to deliver the DRD2 gene into the NAc core of adult Sprague Dawley rats that were trained to self-administer alcohol (Thanos et al., 2000, 2001). Results indicated that DRD2 up-regulation in P rats produced marked reductions in alcohol preference and consumption, which returned to baseline as the DRD2 levels returned to their initial values. This was the first evidence that overexpression of DRD2 in the NAc core attenuated alcohol intake and suggested that high levels of DRD2 may be protective against alcohol abuse. More recently, it was reported that DRD2 gene transfer into the NAc core of rats that were trained to self-administer intravenous cocaine significantly attenuated the number of infusions and the number of lever presses associated with cocaine (Thanos et al., 2002a). This effect was significant for several days and was positively correlated with the time frame of peak DRD2 up-regulation. The present study used a similar approach of NAc core DRD2 up-regulation and examined the effects on alcohol consumption in the P and NP rats.

The biochemical and behavioral profile of the P and NP rats (developed by Li and colleagues at the Indiana University Alcohol Research Center) has been described extensively in the literature (Lumeng et al., 1982; Zhou et al., 1995). These rats were used in the current study and received a microinfusion of an adenoviral vector containing the rat D2cDNA insert into the NAc. Using a two-bottle-choice paradigm, ethanol preference and intake were assessed for each rat. We hypothesized that DRD2 gene transfer into the NAc of P rats would produce a significant attenuation in ethanol preference and ethanol consumption.

At the completion of this experiment, rats were imaged in vivo with micro–positron emission tomography (μPET) to assess the chronic effects (7 weeks) of ethanol exposure on DRD2 binding. Previous studies have indicated that quantitative assessment of DRD2 binding with μPET is a reliable, noninvasive technique (Alexoff et al., 2003; Ogawa et al., 2000; Thanos et al., 2002a,b). These data will help us better understand the effects of chronic (7 weeks) acute ethanol self-administration on DRD2 levels. Finally, these results were compared with previous binding data on naïve P and NP rats (McBride et al., 1993).

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 Brookhaven National Laboratory.

Subjects

Thirty male adult rats (350–450 g) were used in this study [15 P and 15 NP]. Rats were housed individually in a room controlled for temperature and humidity as well as a 12-hr light/dark (lights off at 7:00 AM) cycle. Food was provided ad libitum, whereas water and ethanol access were limited and available only during daily 1-hr testing sessions.

Procedures

Behavioral Testing and Microinfusions. The effect of the DRD2 vector was examined using a two-bottle-choice limited access paradigm, which captures aspects of voluntary alcohol consumption in humans. Limiting access to alcohol to a short period daily causes rats to drink alcohol immediately when first made available each day and at a constant amount during each access period. Moreover, ethanol drinking increased by limiting the opportunity to obtain alcohol (Files et al., 1994; Wilson et al., 1997). Briefly, the cage of each rat was fitted with two 150-ml (Kimax) drinking bottles. One bottle contained tap water, and the other contained a 10% (v/v) alcohol solution. The position of the bottles were reversed daily to prevent a position habit. Each morning (9:00 AM), the fluid intake and body weight of each animal were recorded. Each animal was given a 1-hr session daily (to a choice between the two drinking bottles). Behavioral assessment consisted of percentage of ethanol preference and ethanol intake. Ethanol preference was calculated from the ratio volume of ethanol consumed divided by the total fluid consumed (water bottle + ethanol bottle) each day $\times$ 100. Ethanol intake was calculated in grams

per kilogram using the mass of ethanol consumed divided by the rat's body weight.

After a 1-week adaptation period to the home cage environment [containing two bottles (water and 10% v/v ethanol)], rats that ranged in weight between 386 and 477 g (mean, 426 g) were given a second week of daily access and tested for their preference (water versus ethanol). This second week of drinking was considered the preoperative baseline. Next, each animal had a cannula surgically implanted into the NAc and was allowed 1 week of recovery time before being returned to the same two-bottle preference test for 1 week (postoperative baseline).

All animals next were treated with a microinfusion into the NAc of the control, replication-deficient adenovirus null vector (AdCMV.Null), as previously described (Thanos et al., 2001), and then returned to the home cage for two-bottle ethanol preference assessment for another week. Subsequently on day 0, all animals similarly received a microinfusion once into the NAc with the AdCMV.DRD2 vector (Thanos et al., 2001) and then were returned to the home cage for ethanol preference assessment. This final assessment continued for 24 days.

Microinfusion was carried out using an automated syringe pump (Razel, Stamford, CT) and a 26-gauge 5-μl Hamilton microsyringe connected to a 28-gauge internal cannula. Each microinfusion administered 2 μl of vector [adenoviral vector containing the cDNA for the DRD2 receptor (AdCMV.DopD2R; 10^{10} pfu/ml)] over 10 min so as to reduce the risk of procedure-induced lesions.

Surgery. Rats were anesthetized with ketamine and xylazine (100 mg/kg, 10 mg/kg) and placed in a Kopf stereotaxic apparatus. A 22-gauge guide cannula was then implanted unilaterally (Plastics One, Roanoke, VA) into the NAc core [+1.2 mm AP, ±1.4 mm ML, −6.6 mm DV (Paxinos and Watson, 1986)]. Laterality of cannula placement was randomly assigned so that half of the rats received left NAc implants and the other half received implants into the right NAc. The guide cannula was then secured to the skull with four small stainless steel screws and dental cement. The animals were then allowed 1 week to recover.

μPET. Upon completion of the behavioral experiment, 6 P and 6 NP rats were anesthetized intraperitoneally with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic head holder in a prone position on the bed of the μPET R4 scanner (Concorde Microsystems, Knoxville, TN). Animals then received an injection via the tail vein of a mean dose of 3.34 nmol/kg [^{11}C]raclopride (245.5 ± 26.2 μCi for P; 244.8 ± 27.8 μCi for NP; specific activity 1.4–2.2 mCi/nmol and injected volumes of 200 μl). [^{11}C]raclopride binding in the μPET R4 has been previously demonstrated as a reproducible and suitable method in studying in vivo the DRD2 availability in the rodent brain (Alexoff et al., 2003; Thanos et al., 2002b). The μPET R4 scanner has a 12-cm animal port with an image field of view of ~11.5 cm. Total acquisition time was 70 min [(22 frames: 1 (5 sec), 5 (10 sec), 1 (15 sec), 2 (20 sec), 1 (40 sec), 2 (60 sec), 1 (180 sec), 5 (300 sec), 1 (450 sec), 3 (600 sec)] and data were acquired in full three-dimensional mode with maximum axial acceptance angle (±28 degrees). Images were reconstructed using FORE rebinning (Matej et al., 1998) followed by two-dimensional filtered backprojection with a ramp filter cutoff at the Nyquist frequency. Using the rat stereotaxic atlas (Paxinos and Watson, 1986) and the Harderian glands as reference points, the coronal planes of striatum (ST) and cerebellum (CB) were identified in the same manner. Specifically, for each animal, the ST and CB were identified as 6 and 16 slices, respectively, caudal to the Harderian glands (slice thickness was 1.2 mm), which are routinely used as markers in rodent PET studies (Hume et al., 1996; Matej et al., 1998; Paxinos and Watson, 1986; Thanos et al., 2002b).

[^{11}C]raclopride was synthesized according to previously described methods (Farde et al., 1986). [^{11}C]raclopride doses were small fractions of routine syntheses prepared for human subject studies. Specific activity determination was made using mass measurements acquired during radiotracer purification by high-performance liquid chromatography (Novapak C18, Milford, MA), and radioactivity measurements were obtained with a calibrated ion chamber (Capintec, Ramsey, NJ).

μPET Analysis. Regions of interest (ROI) in ST and CB were selected using a rat stereotaxic atlas (Paxinos and Watson, 1986). Specific binding was estimated using the ST/CB ratio, which was calculated for each animal as previously described (Thanos et al., 2002b). The ST is an area rich in DRD2 and can reliably be imaged and analyzed using the μPET R4 (Alexoff et al., 2003; Thanos et al., 2002b). Similarly, the CB is a structure that lacks DRD2 and is commonly used as a reference structure in PET studies by using a ratio of ST to CB DRD2 binding (Alexoff et al., 2003; Thanos et al., 2002b). Briefly, the left and right ST ROI were averaged and divided by CB ROI over the 70-min duration of each scan. Time activity data were then used to calculate distribution volume ratios (DVRs), using the Pixel-wise Modeling graphic analysis software (Mikolajczyk et al., 1998). This PET quantitation application is widely used and provides a linear function of receptor availability of PET data that does not require blood sampling (Logan et al., 1996; Mikolajczyk et al., 1998).

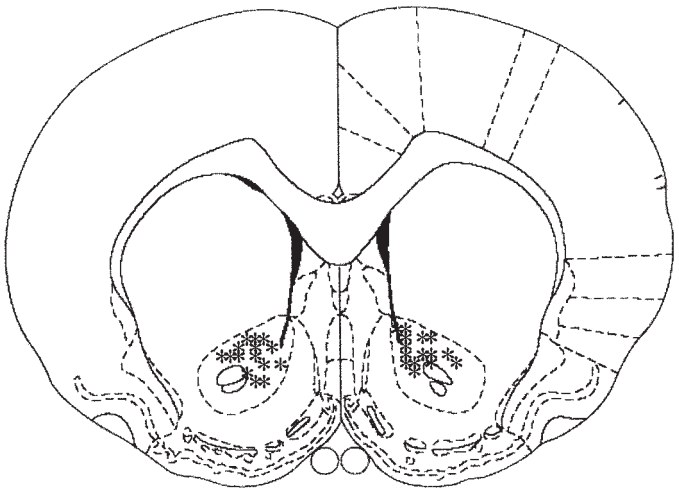

Fig. 1. Coronal section of the rat brain (AP +1.2 mm): the NAc and the location of microinfusion sites (adapted from Paxinos and Watson, 1986).

Histology. After completion of the behavioral and μPET experiments of the study, the brains were harvested from all rats for histological confirmation of the cannula placements. Briefly, each animal was deeply anesthetized with ketamine/xylazine, and the brain was rapidly removed and frozen in an isopentane/dry ice bath and stored in a freezer at −80°C. Next, 20-μm-thick coronal sections were cut on a cryostat (Leica CM3050S; Leica Microsystems, Nussloch, Germany).

Sections were stained with cresyl violet by serial immersion in the following solutions (time given in parentheses): dH_2O (1 min), 1% cresyl violet/0.3% acetic acid (15 min), dH_2O (2 min), dH_2O (2 min), dH_2O (2 min), 0.4% formalin/0.2% acetic acid (15 min), dH_2O (2 min), dH_2O (2 min), 95% ethanol (30 sec), 100% ethanol (30 sec), and xylene (2 min). Slides were then coverslipped with Permount and allowed to air dry before verification of cannula placement in the NAc under light microscopy (Fig. 1).

RESULTS

Rats did not show any signs of malaise or weight loss after treatment with the vector, and this was consistent with previous studies (Ikari et al., 1999, 1995; Ingram et al., 1998; Thanos et al., 2001; Umegaki et al., 1997). No alterations in general behavior or locomotor activity were noted (during observation of the animals).

Ethanol Preference

Baseline drinking behavior in both groups of rats (P and NP) was consistent with previous reports (Zhou et al.,

1995). A one-way, repeated measures ANOVA comparing drinking preference (preoperative baseline and postoperative drinking phases) revealed no statistical difference ($p > 0.05$). Subsequently, animals were treated with the AdCM-V.Null (vehicle) vector, and drinking preference was assessed for 7 days. Similarly, a one-way repeated measures ANOVA comparing baseline ethanol drinking and ethanol drinking after treatment with the control vector revealed no significant difference ($p > 0.05$).

All animals were then treated with the DRD2 vector (day 0), and ethanol drinking was monitored for 24 days. A one-way, repeated measures ANOVA, comparing baseline ethanol preference (day 0) and ethanol preference after DRD2 vector treatment revealed a significant difference in P rats ($F = 9.337$; $df = 24$; $p < 0.001$; Fig. 2A). Subsequently, a Tukey test was used to examine all pairwise multiple comparisons. Comparisons between baseline (day 0) ethanol preference and post-DRD2 vector treatment revealed several significant differences (illustrated in Fig. 2A by an asterisk; $p < 0.05$). Specifically, at day 2 (after DRD2 vector treatment), ethanol preference was decreased in the P rats from 81 to 44% and returned to pretreatment levels by day 20 (Fig. 2A).

Similarly, a one-way repeated measures ANOVA, comparing baseline ethanol preference (day 0) and ethanol preference after DRD2 vector treatment, revealed a significant difference in NP rats ($F = 1.88$, $df = 24$, $p < 0.01$; Fig. 2A). Pairwise multiple comparisons (Tukey test), however, did not yield any significant differences between baseline ethanol preference and post-DRD2 vector drinking.

Ethanol Intake

A one-way repeated measures ANOVA comparing baseline ethanol intake (day 0) in P rats with drinking after treatment with the active vector was significantly decreased ($F = 15.06$, $df = 24$, $p < 0.001$; Fig. 2B). At day 2 (after DRD2 vector treatment), ethanol intake was decreased in the P rats from 2.7 g/kg to ~1.3 g/kg, and intake returned to pretreatment levels by day 20 (Fig. 2B). Pairwise multiple comparisons (Tukey test) between baseline ethanol intake and post-DRD2 vector intake revealed several significant differences illustrated by an asterisk ($p < 0.05$; Fig. 2B).

A similar one-way repeated measures analysis of the data in NP rats baseline ethanol intake (day 0) and intake after treatment with the active vector revealed a statistical difference ($F = 1.687$, $df = 24$, $p < 0.05$; Fig. 2B). Subsequent, pairwise multiple comparisons (Tukey test), however, did not yield any significant differences between baseline ethanol intake and post-RD2 vector intake in NP rats.

Overall, it should be noted that 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. Baseline total fluid (water + 10% ethanol) intake in the 1-hr two-bottle-choice procedure was similar in both the P (27.3 ml) and NP (25.2 ml) rats. Specifically, P rats showed a mean fluid intake of 21.1 ± 1.6 ml of ethanol and 6.2 ± 2.7 ml of water, whereas NP rats showed a mean fluid intake of 5.7 ± 1.8 ml of ethanol and 19.5 ± 5.2 ml of water.

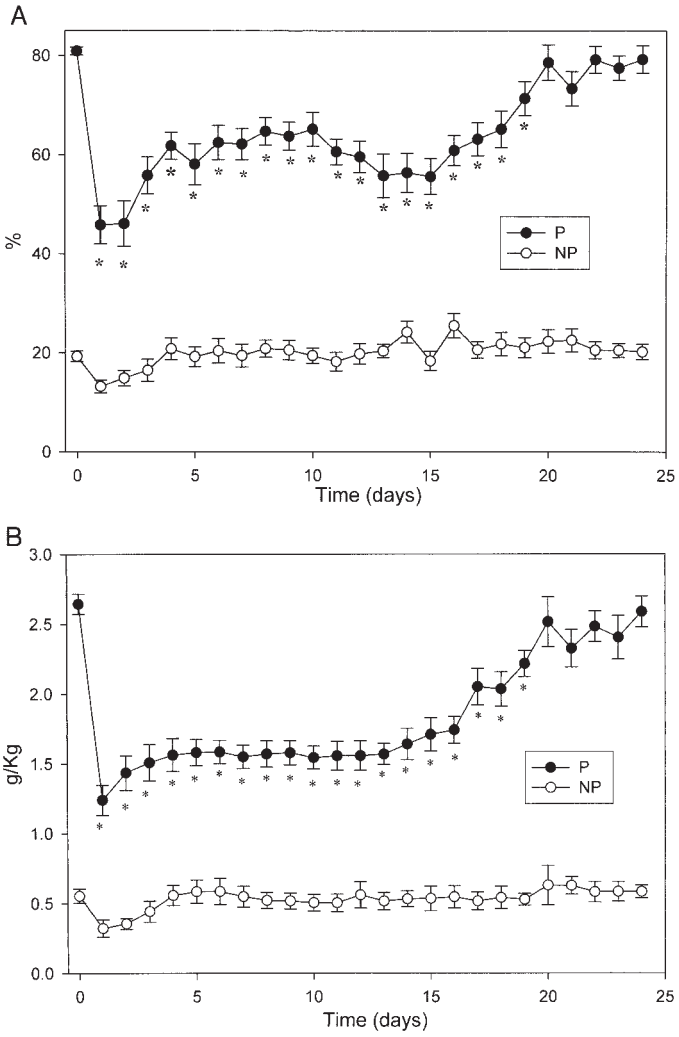

Fig. 2. (A) Mean percentage (± SE) of ethanol preference over time in P and NP rats in a daily 1-hr limited-access session (*$p < 0.05$). Day 0 represents baseline drinking. Animals were treated with the DRD2 vector on day 0. (B) Mean (± SE) ethanol intake (g/kg) over time in P and NP rats in a daily 1-hr limited-access session (*$p < 0.05$). Day 0 represents baseline drinking. Animals were treated with the DRD2 vector on day 0.

μPET

Representative examples of coronal μPET images of the ST after intravenous injection of [^{11}C]raclopride are shown in Fig. 3. A significant difference in [^{11}C]raclopride binding in the ST was observed between the P and NP rats.

Quantitative analysis of the [^{11}C]raclopride μPET consisted of (1) the ST/CB binding ratios over time, (2) [^{11}C]raclopride binding over time, and (3) the DVR. *T* test comparisons between the two strains revealed a statistically significant difference in the ST/CB ratios ($t_{obs} = 3.26$, $df =$

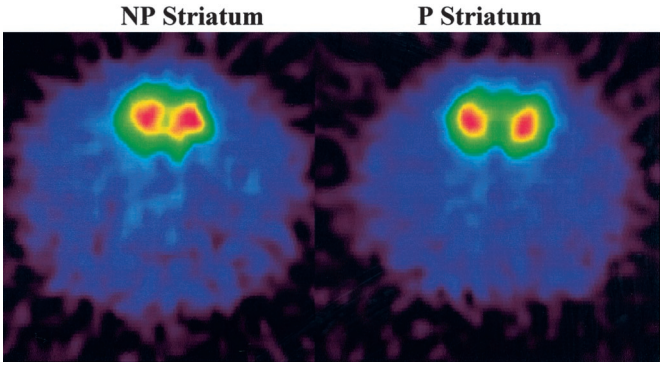

Fig. 3. Coronal images (plane thickness = 1.2 mm) of a P and NP rat brain at the level of the ST obtained using the μPET R4 scanner with [^{11}C]raclopride.

21, $p < 0.001$). Specifically, the mean ratio from time point 0 to 70 min for each strain was 1.84 ± 0.08 and 1.56 ± 0.05 for the NP and P rats, respectively (Fig. 4A). The [^{11}C]raclopride binding kinetics or time activity curve (TAC) is shown for each strain in Fig. 4B. A *t* test comparison of the TAC for the ST between the P and NP rats revealed a significant difference ($t_{obs} = 3.31$, $df = 21$, $p < 0.001$). Finally, the time activity data were then used to calculate DVRs, using the Pixel-wise Modeling graphic analysis software. The DVR was calculated for each strain (NP = 2.66 and P = 2.25) and provided a linear function of the DRD2 receptor availability between P and NP rats as observed with μPET.

Histology

Localization of the area of microinfusion was assessed using a stereotaxic atlas (Paxinos and Watson, 1986) (Fig. 1). Histological examination of the area of microinfusion did not reveal any unusual neuropathology or significant signs of inflammation associated with sites infected with AdCMV.DRD2 or control vector.

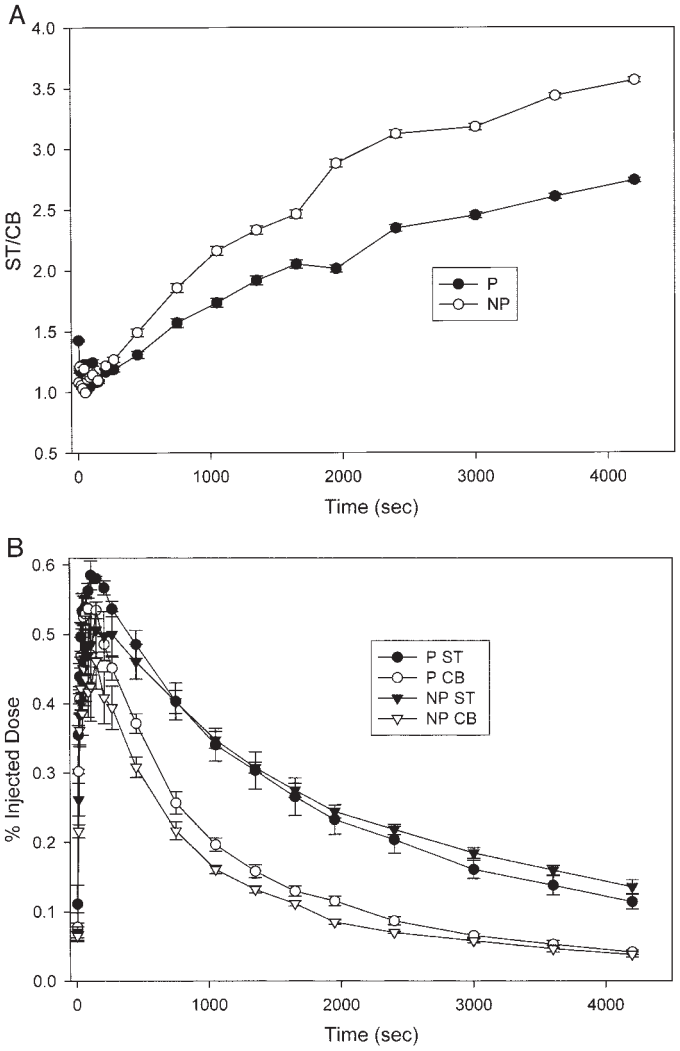

Fig. 4. (A) ST/CB, [^{11}C]raclopride binding ratio (mean ± SE) in P and NP rats. (B) [^{11}C]raclopride TAC in P and NP rats. Mean (± SE) percentage injected dose/ml binding in ST and CB.

DISCUSSION

The present study examined the role of DRD2 gene transfer and selective up-regulation in a rodent ethanol self-administration paradigm. Ethanol intake and preference were significantly reduced in P rats that were treated with the DRD2 vector. Specifically, P rats that were treated with the DRD2 vector showed that ethanol preference was attenuated for a period of 20 days before returning to baseline levels, with a maximum effect seen (37% decrease) within the first few days posttreatment. Similarly, ethanol intake was attenuated after treatment with the DRD2 vector before returning to baseline, in 20 days, with a maximum effect (48% decrease) within the first few days posttreatment. These data further supported our hypothesis that DRD2 levels in the NAc play an important role in ethanol drinking and may be associated with the significant differences in ethanol preference and consumption observed between P and NP rats. NP rats that were treated with the DRD2 vector also showed a significant main effect on ethanol preference and intake; however, these changes were not as large in magnitude or specific over time and could be associated with the already low baseline consumption of NP rats.

The duration of the effect of the DRD2 vector on behavior was consistent with previous studies (Ingram et al., 1998; Thanos et al., 2001; Umegaki et al., 1997). In particular, ethanol preference and intake returned to baseline levels 20 days posttreatment with the DRD2 vector, and this was similar to previous results observed in Sprague Dawley rats (Thanos et al., 2001). This effect on ethanol consumption by the DRD2 vector was observed beyond the 2 weeks reported in previous binding studies. Specifically, previous in vitro autoradiography studies reported the transient nature of DRD2 overexpression induced by the vector in the rat, with DRD2 levels returning to baseline within 2 weeks and peak expression at days 2 to 5 after infusion (Ingram et al., 1998; Thanos et al., 2001; Umegaki et al.,

1997). Therefore, DRD2 vector treatment could not have influenced μPET [^{11}C]raclopride binding at 7 weeks. The difference in DRD2 binding (as observed with μPET) between P and NP rats thus was negatively correlated with ethanol drinking. Specifically, although P rats exhibited greater alcohol consumption, they displayed lower [^{11}C]raclopride binding, and vice versa for the NP rats.

The in vivo effects of chronic ethanol exposure on DRD2 levels in P and NP rats were also examined. μPET analyses revealed significantly lower DRD2 binding in the P rats that were chronically exposed to ethanol compared with NP rats. The ST/CB ratio over time provided a relative μPET comparison of specific (ST) DRD2 binding relative to nonspecific (CB) binding. This popular method of μPET analysis of DRD2 binding is unaffected by dose of the injected radiotracer and revealed that NP rats displayed 15% greater [^{11}C]raclopride binding compared with P rats. Next, the DRD2 binding kinetics (TAC data) were consistent with the ratio data and revealed a consistently and significantly higher ST binding in the NP rats. Similarly, the DVR analysis described DRD2 receptor availability between P and NP rats and showed a highly significant difference between the groups (~16% greater in the NP versus P rats). Comparing the DVR of the P and NP rats with the ST/CB ratio data revealed a high degree of concurrence, and this was consistent with the literature (Logan et al., 1996; Thanos et al., 2002b). Although animals were not repeatedly examined with μPET over time, these results provided evidence that μPET could be used to effectively examine quantitatively and noninvasively DRD2 binding in P and NP rats. Furthermore, these data indicated that chronic alcohol consumption in these animals maintained a similar DRD2 profile as previously observed with autoradiography in naïve P and NP rats (McBride et al., 1993). That is, P rats displayed lower [^{11}C]raclopride binding in comparison with NP rats. These μPET findings were not in agreement with some previous studies that reported an up-regulation of DRD2 in several rodent strains in a variety of forced chronic ethanol administration procedures (Hruska, 1988; Lograno et al., 1993; Tajuddin and Druse, 1996). However, our μPET findings were in accordance with several other rodent studies that have described that chronic ethanol administration produced functional down-regulation or desensitization of DRD2 in several rat strains (Lucchi et al., 1988; Muller et al., 1980; Rommelspacher et al., 1992; Syvalahti et al., 1988), including most recently in P rats (Engleman et al., 2003). In addition, our findings were consistent with the clinical report that alcoholics showed a DRD2 down-regulation compared with controls (Volkow et al., 1996).

The μPET data helped assess the effects of chronic acute ethanol exposure (albeit different doses/group) on DRD2 levels in P and NP rats. These results were comparable to the DRD2 data reported previously in naïve P and NP rats (McBride et al., 1993). Recently, evidence was provided that alcohol drinking by P rats attenuated D2 autoreceptor function in the NAc (Engleman et al., 2003). In the present study, rats were scanned for μPET after ~7 weeks of ethanol exposure and ~4 weeks after treatment with the DRD2 vector. Finally, although the present study provided important insight into the chronic effects of ethanol exposure on DRD2, it is important to point out a limitation in interpreting these results, which is the disparate ethanol dose consumed between the two groups of rats, which is unavoidable in self-administration procedures. Furthermore, interpretation of the μPET data requires caution because these results were compared with DRD2 binding data from naïve P and NP rats in the literature (McBride et al., 1993).

The present results emphasized the important role that DRD2 levels in the NAc may play in the unique ethanol self-administration behavior profile of the P rats. This was in agreement with previous studies that have reported that DRD2 levels were significantly different between naïve P and NP rats. Specifically, DRD2 density was reported to be 20% lower in the olfactory tubercle and NAc of the Sardinian P rats compared with Sardinian NP rats (Stefanini et al., 1992). In a comparable study in P and NP rats, P rats showed 20 to 25% lower [^{3}H]sulpiride binding in the caudate putamen, medial and lateral NAc, and VTA compared with NP rats (McBride et al., 1993). This intrinsic lower DRD2 profile observed in P rats may suggest that some type of adaptive process to the chronic effects of ethanol is present before ethanol exposure.

In evaluating the mechanisms underlying the protective effects of high D2R level in alcohol intake, it is worth noting that increasing D2R produced a marked decrease in alcohol intake but did not abolish it. This suggests that high D2R levels may be specifically interfering with the administration of high concentrations of alcohol. This could explain why NP rats, which have high D2R levels, still consume alcohol, albeit at much lower concentrations. Indeed, we had postulated that there is an optimal level for the activation of reward circuits after which further stimulation becomes aversive to explain why in individuals with high striatal D2R levels a relatively large dose of a stimulant drug (methylphenidate) was perceived as aversive, whereas in individuals with low D2R levels, it was perceived as pleasurable (Volkow et al., 1999a, 2002).

Although the complex interaction within the mesocortical/mesolimbic system that processes the various stimuli that regulate ethanol self-administration is beyond this discussion, the data suggest that DRD2 levels in the NAc core play an important role in ethanol drinking. One possible mechanism influencing self-administration alcohol consumption is a complex network of feedforward and feedback loops within the mesolimbic system between the NAc core, the medial prefrontal cortex (mPFC), and the VTA (Kalivas et al., 1993; Samson and Chappell, 2003; Samson and Hodge, 1996). According to this system organization, alcohol consumption is influenced by environmental stimuli that function as discriminative and conditioned reinforcing

stimuli (processed by the mPFC). The mPFC then sends excitatory input to the NAc core that influences the release of DA (You et al., 1998), as well as having direct actions on the medium spiny output neurons in the NAc core (Kiyatkin and Rebec, 1996). In addition to the environmental stimuli, internal stimuli that reach the mPFC from the hypothalamus, amygdala, and hippocampus interact to determine the degree of glutamate output to the NAc core (Kalivas et al., 1993; Pennartz et al., 1994). This part of the system, therefore, is testing the salience of the external environmental stimuli in conjunction with internal generated stimuli (e.g., degree of deprivation, anxiety). Alcohol is known to increase the extracellular levels of DA (Fadda et al., 1989; Imperato and Chiara, 1986) and will significantly increase DA output to both the NAc and the mPFC (Kalivas et al., 1993). Furthermore, excess DA in the NAc core can extend an ethanol self-administration session well beyond the "normal" limits (Samson et al., 1991). DA release in the NAc provides a feedforward process to maintain the current behavior in relation to the salience of the current multiple-stimulus inputs to the NAc core. However, the release of DA in the mPFC would tend to decrease mPFC output, thus reducing the excitatory facilitation of NAc core and VTA cells in a negative feedback function. The termination of conditioned responding (i.e., to ethanol-related stimuli) is most likely controlled by a shift in the NAc core output to the ventral pallidum and its projections to the thalamus, which send additional feedback projections to the mPFC (Pennartz et al., 1994). Therefore, DRD2 up-regulation in the NAc core (in the present study) potentiated the output signal of these GABAergic medium spiny neurons, which in turn feedback and inhibit the mPFC, which provides control over the onset and offset of a self-administration bout (Samson and Hodge, 1996). In contrast, DRD2 deficiency or down-regulation in the NAc may predispose a subject to drug use as a means of compensating for the decrease in activation of reward circuits activated by these receptors.

The expression of DRD2 in the brain, which has been shown to be modulated by both genetic and environmental factors such as stress (Morgan et al., 2002; Papp et al., 1994), provides a molecular mechanism that can account for the involvement of both genetic and environmental factors in the predisposition to drug and alcohol abuse. Thus, possibilities exist for developing strategies to modulate the expression of DRD2 as a means of attenuating alcohol preference and dependency.

Understanding alcohol preference in rodent models requires the elucidation of the genotypes and phenotypes involved. Previous studies have reported several differences (besides in the mesolimbic dopamine reward system) in biochemistry between the P and NP rats. Specifically, with these selected lines, the data include differences in serotonin, GABA, endogenous opioid, and neuropeptide Y systems (McBride et al., 1990; Thielen et al., 1997). In addition, phenotypic and genotypic mapping studies have identified quantitative trait loci influencing alcohol consumption on chromosomes 3, 4, and 8 in the inbred P/NP rats (Murphy et al., 2002). Therefore, it is imperative to study further the interaction and regulation/modulation of the above neurotransmitters and genes to understand better the mechanism(s) of alcohol abuse.

Finally, it was demonstrated that longitudinal in vivo assessment of vector-mediated DRD2 expression in the rat brain can be accomplished with μPET. This technique also provides future opportunities in studying rodent models of alcoholism as well as evaluating possible new-generation viral vectors that could offer longer duration of action and, hopefully, longer functional effects. Further studies will seek to identify the types and number of cells transduced by the DRD2 vector.

Future studies will examine this DRD2 hypothesis and alcoholism in transgenic mice, as well as develop and evaluate a second-generation DRD2 vector that is capable of providing DRD2 up-regulation beyond the 2 weeks observed with the present vector. Similarly, this approach should be used to examine the role of other receptors (within the D1 and D2 family) on alcoholism.

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REFERENCES

Alexoff D, Vaska P, Marsteller D, Li J, Logan J, Fowler JS, Taintor N, Thanos PK, Volkow ND (2003) Reproducibility of C-11 raclopride binding in the rat brain measured with the microPET R4: effects of scatter correction and tracer-specific activity. J Nucl Med 44:1–8.

Blanchard BA, Steindorf S, Wang S, LeFevre R, Mankes RF, Glick SD (1993) Prenatal ethanol exposure alters ethanol-induced dopamine release in nucleus accumbens and striatum in male and female rats. Alcohol Clin Exp Res 17:974–981.

Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, Lubar JO, Chen TJ, Comings DE (2000) Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. J Psychoactive Drugs (Suppl) 32:i–iv, 1–112.

Blum K, Cull JC, Braverman ER, Commings DE (1996) Reward deficiency syndrome. Am Sci 84:132–145.

Caine SB, Negus SS, Mello NK, Bergman J (1999) Effects of dopamine D(1-like) and D(2-like) agonists in rats that self-administer cocaine. J Pharmacol Exp Ther 291:353–360.

Comings DE, Blum K (2000) Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog Brain Res 126:325–341.

Corbit LH, Muir JL, Balleine BW (2001) The role of the nucleus accumbens in instrumental conditioning: evidence of a functional dissociation between accumbens core and shell. J Neurosci 21:3251–3260.

Di Chiara G (1995) The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 38:95–137.

Dyr W, McBride WJ, Lumeng L, Li TK, Murphy JM (1993) Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. Alcohol 10:207–212.

Engleman EA, McBride WJ, Li TK, Lumeng L, Murphy JM (2003) Ethanol drinking experience attenuates (-)sulpiride-induced increases in extracellular dopamine levels in the nucleus accumbens of alcohol-preferring (P) rats. Alcohol Clin Exp Res 27:424–431.

Fadda F, Mosca E, Colombo G, Gessa GL (1989) Effect of spontaneous ingestion of ethanol on brain dopamine metabolism. Life Sci 44:281–287.

Farde L, Hall H, Ehrin E, Sedvall G (1986) Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. Science 231:258–261.

Files FJ, Lewis RS, Samson HH (1994) Effects of continuous versus limited access to ethanol on ethanol self-administration. Alcohol 11: 523–531.

Gonzales R (1996) In vivo links between neurochemistry and behavioral effects of ethanol. Alcohol Clin Exp Res 20:203A–209A.

Guardia J, Catafau AM, Batlle F, Martin JC, Segura L, Gonzalvo B, Prat G, Carrio I, Casas M (2000) Striatal dopaminergic D(2) receptor density measured by [(123)I]iodobenzamide SPECT in the prediction of treatment outcome of alcohol-dependent patients. Am J Psychiatry 157:127–129.

Hitzemann R, Hitzemann B, Rivera S, Gatley J, Thanos PK (2003) Dopamine D2 receptor binding and the number of dopamine neurons in the BXD recombinant inbred series Genetic relationships to alcohol and other drug associated phenotypes. Alcohol Clin Exp Res 27:1–11.

Hruska RE (1988) Effect of ethanol administration of striatal D1 and D2 dopamine receptors. J Neurochem 50:1929–1933.

Hume SP, Lammertsma AA, Myers R, Rajeswaran S, Bloomfield PM, Ashworth S, Fricker RA, Torres EM, Watson C, Jones T (1996) The potential of high-resolution positron emission tomography to monitor striatal dopaminergic function in rat models of disease. J Neurosci Methods 67:103–112.

Ikari H, Umegaki H, Iguchi A (1999) [Gene therapy to treat Parkinson's disease]. Nippon Ronen Igakkai Zasshi 36:103–109.

Ikari H, Zhang L, Chernak JM, Mastrangeli A, Kato S, Kuo H, Crystal RG, Ingram DK, Roth GS (1995) Adenovirus-mediated gene transfer of dopamine D2 receptor cDNA into rat striatum. Brain Res Mol Brain Res 34:315–320.

Imperato A, Chiara GD (1986) Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 239:219–228.

Ingram DK, Ikari H, Umegaki H, Chernak JM, Roth GS (1998) Application of gene therapy to treat age-related loss of dopamine D2 receptor. Exp Gerontol 33:793–804.

Johnson PI, Goodman JB, Condon R, Stellar JR (1995) Reward shifts and motor responses following microinjections of opiate-specific agonists into either the core or shell of the nucleus accumbens. Psychopharmacology (Berl) 120:195–202.

Kalivas PW, Churchill L, Klitnick MA (1993) Limbic motor circuits and neuropsychiatry, in *The Circuitry Mediating the Translation of Motivational Stimuli Into Adaptive Motor Responses* (Kalivas PW, Barnes CD eds), pp 237–288. CRC Press, Boca Raton, FL.

Kiyatkin EA, Rebec GV (1996) Dopaminergic modulation of glutamate-induced excitations of neurons in the neostriatum and nucleus accumbens of awake, unrestrained rats. J Neurophysiol 75:142–153.

Koob GF, Vaccarino FG, Amalric M, Bloom FE (1987) *Positive Reinforcement Properties of Drugs: Search for Neural Substrates, in Brain Reward Systems and Abuse*. Raven Press, New York.

Levy AD, Murphy JM, McBride WJ, Lumeng L, Li TK (1991) Microinjection of sulpiride into the nucleus accumbens increases ethanol drinking in alcohol-preferring (P) rats. Alcohol Alcohol Suppl 1:417–420.

Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL (1996) Distribution volume ratios without blood sampling from graphical analysis of PET data. J Cereb Blood Flow Metab 16:834–840.

Lograno DE, Matteo F, Trabucchi M, Govoni S, Cagiano R, Lacomba C, Cuomo V (1993) Effects of chronic ethanol intake at a low dose on the rat brain dopaminergic system. Alcohol 10:45–49.

Lucchi L, Moresco RM, Govoni S, Trabucchi M (1988) Effect of chronic ethanol treatment on dopamine receptor subtypes in rat striatum. Brain Res 449:347–351.

Lumeng L, Waller MB, McBride WJ, Li TK (1982) Different sensitivities to ethanol in alcohol-preferring and -nonpreferring rats. Pharmacol Biochem Behav 16:125–130.

Matej SK, Karp JS, Lewitt RM, Becher AJ (1998) Performance of the Fourier rebinning algorithm for PET with large acceptance angles. Phys Med Biol 43:787–795.

McBride WJ, Chernet E, Dyr W, Lumeng L, Li TK (1993) Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats. Alcohol 10:387–390.

McBride WJ, Murphy JM, Lumeng L, Li TK (1990) Serotonin, dopamine and GABA involvement in alcohol drinking of selectively bred rats. Alcohol 7:199–205.

Mikolajczyk K, Szabatin M, Rudnicki P, Grodzki M, Burger C (1998) A JAVA environment for medical image data analysis: initial application for brain PET quantitation. Med Inform (Lond) 23:207–214.

Morgan D, Grant K, Gage H, Mach R, Kaplan J, Prioleau O, Nader S, Buchheimer N, Ehrenkaufer R, Nader M (2002) Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. Nat Neurosci 5:169–174.

Muller P, Britton RS, Seeman P (1980) The effects of long-term ethanol on brain receptors for dopamine, acetylcholine, serotonin and noradrenaline. Eur J Pharmacol 65:31–37.

Munzar P, Goldberg SR (2000) Dopaminergic involvement in the discriminative-stimulus effects of methamphetamine in rats. Psychopharmacology (Berl) 148:209–216.

Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li TK (2002) Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. Behav Genet 32:363–388.

Nowak KL, Ingraham CM, McKinzie DL, McBride WJ, Lumeng L, Li TK, Murphy JM (2000) An assessment of novelty-seeking behavior in alcohol-preferring and nonpreferring rats. Pharmacol Biochem Behav 66:113–121.

Ogawa O, Umegaki H, Ishiwata K, Asai Y, Ikari H, Oda K, Toyama H, Ingram DK, Roth GS, Iguchi A, Senda M (2000) In vivo imaging of adenovirus-mediated over-expression of dopamine D2 receptors in rat striatum by positron emission tomography. Neuroreport 11:743–748.

Papp M, Klimek V, Willner P (1994) Parallel changes in dopamine D2 receptor binding in limbic forebrain associated with chronic mild stress-induced anhedonia and its reversal by imipramine. Psychopharmacology (Berl) 115:441–446.

Parkinson JA, Olmstead MC, Burns LH, Robbins TW, Everitt BJ (1999) Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. J Neurosci 19:2401–2411.

Paxinos G, Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York.

Pennartz CM, Groenewegen HJ, Lopes da Silva FH (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. Prog Neurobiol 42:719–761.

Rommelspacher H, Raeder C, Kaulen P, Bruning G (1992) Adaptive changes of dopamine-D2 receptors in rat brain following ethanol withdrawal: a quantitative autoradiographic investigation. Alcohol 9:355–362.

Samson HH, Chappell A (2003) Dopaminergic involvement in medial prefrontal cortex and core of the nucleus accumbens in the regulation of ethanol self-administration: a dual-site microinjection study in the rat. Physiol Behav 79:581–590.

Samson HH, Hodge CW (1996) Pharmacological effects of ethanol on the nervous system, in *Neurobehavioral Regulation of Ethanol In-take* (Deitrich R, Erwin V eds), pp 203–226. CRC Press, Boca Raton, FL.

Samson HH, Tolliver GA, Haraguchi M, Kalivas PW (1991) Effects of d-amphetamine injected into the nucleus accumbens on ethanol reinforced behavior. Brain Res Bull 27:267–271.

Sokolowski JD, Conlan AN, Salamone JD (1998) A microdialysis study of nucleus accumbens core and shell dopamine during operant responding in the rat. Neuroscience 86:1001–1009.

Sokolowski JD, Salamone JD (1998) The role of accumbens dopamine in lever pressing and response allocation: effects of 6-OHDA injected into core and dorsomedial shell. Pharmacol Biochem Behav 59:557–566.

Stefanini E, Frau M, Garau MG, Garau B, Fadda F, Gessa GL (1992) Alcohol-preferring rats have fewer dopamine D2 receptors in the limbic system. Alcohol Alcohol 27:127–130.

Syvalahti EK, Hietala J, Roytta M, Gronroos J (1988) Decrease in the number of rat brain dopamine and muscarinic receptors after chronic alcohol intake. Pharmacol Toxicol 62:210–212.

Tajuddin NF, Druse MJ (1996) Effects of chronic alcohol consumption and aging on dopamine D2 receptors in Fischer 344 rats. Alcohol Clin Exp Res 20:144–151.

Thanos P, Taintor N, Umegaki H, Ikari H, Roth GS, Ingram DK, Volkow ND (2002a) Dopamine D2 receptor upregulation reduces cocaine self-administration, in *2002 Abstract Viewer/Itinerary Planner*, Program No 119.114. Society for Neuroscience, Washington, DC.

Thanos PK, Taintor NB, Alexoff D, Vaska P, Logan J, Grandy DK, Fang Y, Lee JH, Fowler JS, Volkow ND (2002b) In vivo comparative imaging of dopamine D2 knockout and wild-type mice with (11)C-raclopride and microPET. J Nucl Med 43:1570–1577.

Thanos PK, Volkow ND, Freimuth P, Umegaki H, Ikari H, Roth G, Ingram DK, Hitzemann R (2000) Dopamine D2 Receptor upregulation in the nucleus accumbens of the rat after treatment with a D2 adenovirus vector and its effects on alcohol abuse. Alcohol Clin Exp Res 24:154.A.

Thanos PK, Volkow ND, Freimuth P, Umegaki H, Ikari H, Roth G, Ingram DK, Hitzemann R (2001) Overexpression of dopamine D2 receptors reduces alcohol self-administration. J Neurochem 78:1094–1103.

Thielen RJ, McBride WJ, Chernet E, Lumeng L, Li TK (1997) Regional densities of benzodiazepine sites in the CNS of alcohol-naive P and NP rats. Pharmacol Biochem Behav 57:875–882.

Umegaki H, Chernak JM, Ikari H, Roth GS, Ingram DK (1997) Rotational behavior produced by adenovirus-mediated gene transfer of dopamine D2 receptor into rat striatum. Neuroreport 8:3553–3558.

Volkow N, Wang G, Fowler J, Logan J, Gatley S, Gifford A, Hitzemann R, Ding Y, Pappas N (1999a) Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. Am J Psychiatry 156:1440–1443.

Volkow ND, Chang L, Wang GJ, Fowler JS, Ding YS, Sedler M, Logan J, Franceschi D, Gatley J, Hitzemann R, Gifford A, Wong C, Pappas N (2001) Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. Am J Psychiatry 158:2015–2021.

Volkow ND, Fowler JS, Wang GJ (1999b) Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. J Psychopharmacol 13:337–345.

Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ, Dewey SL, Wolf AP (1993) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse 14:169–177.

Volkow ND, Wang G-J, Fowler JS, Logan J, Hitzemann R, Ding Y-S, Pappas N, Shea C, Kathleen P (1996) Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol Clin Exp Res 20:1594–1598.

Volkow ND, Wang GJ, Fowler JS, Thanos P, Logan J, Gatley SJ, Gifford A, Ding YS, Wong C, Pappas N (2002) Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. Synapse 46:79–82.

Wang GJ, Volkow ND, Fowler JS, Logan J, Abumrad NN, Hitzemann RJ, Pappas NS, Pascani K (1997) Dopamine D2 receptor availability in opiate-dependent subjects before and after naloxone-precipitated withdrawal. Neuropsychopharmacology 16:174–182.

Weiss F, Lorang MT, Bloom FE, Koob GF (1993) Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. J Pharmacol Exp Ther 267:250–258.

Wilson AW, Neill JC, Costall B (1997) Strain differences in ethanol preference and reinforced behaviour: a comparison of two-bottle choice and operant self-administration paradigms. Behav Pharmacol 8:37–46.

Wozniak KM, Pert A, Linnoila M (1990) Antagonism of 5-HT3 receptors attenuates the effects of ethanol on extracellular dopamine. Eur J Pharmacol 187:287–289.

Yoshimoto K, McBride WJ, Lumeng L, Li TK (1991) Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. Alcohol 9:17–22.

You ZB, Tzschentke TM, Brodin E, Wise RA (1998) Electrical stimulation of the prefrontal cortex increases cholecystokinin, glutamate, and dopamine release in the nucleus accumbens: an in vivo microdialysis study in freely moving rats. J Neurosci 18:6492–6500.

Zhou FC, Zhang JK, Lumeng L, Li TK (1995) Mesolimbic dopamine system in alcohol-preferring rats. Alcohol 12:403–412.

Zocchi A, Girlanda E, Varnier G, Sartori I, Zanetti L, Wildish GA, Lennon M, Mugnaini M, Heidbreder CA (2003) Dopamine responsiveness to drugs of abuse: a shell-core investigation in the nucleus accumbens of the mouse. Synapse 50:293–302.