# Central Overexpression of Angiotensin $AT_{1A}$ Receptors Prevents Dopamine $D_2$ Receptor Regulation of Alcohol Consumption in Mice

Rosanna Moore, Elena V. Krstew, Jeppe Kirchhoff, Robin L. Davisson, and Andrew J. Lawrence

**Background:** While angiotensin receptors are found on the soma and terminals of dopaminergic neurons, controversy surrounds the potential role of angiotensin in alcohol consumption.

**Methods:** Using a transgenic mouse with a brain-specific overexpression of angiotensin $AT_{1A}$ receptors (NSE-$AT_{1A}$ mice), we have examined the role of angiotensin in alcohol consumption and alcohol-induced regulation of the dopaminergic system.

**Results:** The functional relevance of the overexpressed $AT_{1A}$ receptors was confirmed by an exaggerated rehydration response following 24-hour dehydration. NSE-$AT_{1A}$ mice showed a high preference for alcohol (similar to wild-type mice); yet, raclopride treatment had no effect on alcohol consumption in NSE-$AT_{1A}$ mice, while significantly reducing consumption in wild-type mice. In contrast, NSE-$AT_{1A}$ mice showed enhanced sensitivity to raclopride compared with wild types in terms of $D_2$ receptor up-regulation within the ventral mesencephalon. In addition, striatal $D_2$ receptors in NSE-$AT_{1A}$ mice were sensitive to up-regulation by chronic alcohol consumption.

**Conclusions:** Collectively, these data imply that while expression of angiotensin $AT_{1A}$ receptors on striatal neurons has no impact upon basal alcohol consumption or preference, $AT_{1A}$ receptors do modulate the sensitivity of dopamine $D_2$ receptors to regulation by alcohol and the ability of a $D_2$ receptor antagonist to reduce consumption.

**Key Words:** Angiotensin, AT1A Receptor, Alcohol, Dopamine, Transgenic Mice.

IT HAS BEEN known for some time that alcohol stimulates the release of renin in humans, which was proposed to be related to increased blood pressure in drinkers (e.g., Puddey et al., 1985). Similarly, in experimental rats, chronic ethanol treatment elevates plasma angiotensin II levels (Wright et al., 1986). Subsequently, a number of studies addressed the potential role of angiotensin signaling in alcohol consumption. Systemic administration of angiotensin [or angiotensin-converting enzyme (ACE) inhibitors] appeared to reduce the voluntary consumption of ethanol in rodents (Grupp et al., 1988, 1992; Lingham et al., 1990; Spinosa et al., 1988). The locus of action for this observation was suggested to be the subfornical organ, which would be accessible to circulating angiotensin II (Grupp et al., 1989b). Furthermore, in certain strains of alcohol-preferring rats, alcohol intake was apparently inversely related to plasma renin activity (Grupp et al., 1989a). The receptor subtype responsible for mediating the reduction in ethanol consumption following angiotensin administration was characterized as being the $AT_1$ receptor (Grupp and Harding, 1994).

While the subfornical organ was suggested to be a likely central target for angiotensin to modulate alcohol consumption (Grupp et al., 1989b), subsequent studies by the same group failed to demonstrate regulation of alcohol consumption by central [intracerebroventricular (icv)] infusion of angiotensin II or angiotensin III (Grupp and Harding, 1995). Furthermore, another experiment suggested that systemic administration of angiotensin II only reduced ethanol consumption if injected immediately before access to ethanol (Kulkosky et al., 1996). In contrast, however, other studies have clearly demonstrated that central administration of angiotensin II in either mice (Weisinger et al., 1999b) or rats (Fitts, 1993; Weisinger et al., 1999a) actually increases free-choice alcohol intake. Consequently, the mechanism(s) for the regulation of ethanol intake by angiotensin is/are somewhat controversial.

*From the Howard Florey Institute, University of Melbourne, Parkville, Vic., Australia (RM, EVK, JK, AJL); the Department of Anatomy & Cell Biology, University of Iowa, Iowa City, Iowa (RLD); and the Centre for Neuroscience, University of Melbourne, Parkville, Victoria, Australia (AJL).*

*Received for publication November 28, 2006; accepted March 3, 2007.*

*AJL is a Senior Research Fellow of the NHMRC, Australia, supported by an NHMRC program grant (236805). RLD is an Established Investigator of the American Heart Association. This work was also supported by grants from the National Institutes of Health to RLD (HL 14388 and HL 63887).*

*Reprint requests: Andrew. J. Lawrence, Brain Injury & Repair Group, Howard Florey Institute, University of Melbourne, Parkville, Victoria, 3010, Australia; Email: andrew.lawrence@hfi.unimelb.edu.au*



**DOI: 10.1111/j.1530-0277.2007.00399.x**

To address the controversy surrounding the role of angiotensin in volitional alcohol consumption, a recent study has utilized transgenic mice with either gain of function or loss of function with respect to the central renin–angiotensin system (RAS) (Maul et al., 2001). In summary, this particular study provided evidence for a direct positive correlation between brain angiotensinogen and alcohol consumption and preference. Thus, mice lacking the angiotensinogen gene (TLM) showed reduced free-choice ethanol consumption over a 4-week period and in parallel, the preference ratio for ethanol was also reduced. Conversely, mice with a brain overexpression of the angiotensinogen gene (TGM123) consumed more ethanol with a higher preference over a 7-week period compared with wild-type littermates. Interestingly, this increased ethanol preference in TGM123 mice was attenuated by treatment with the ACE inhibitor, spirapril, and also by treatment with the dopamine $D_2$ receptor antagonist, fluphenazine (Maul et al., 2001). Transgenic mice [TGR(ASrAOGEN)680] with reduced central angiotensin II show reduced alcohol consumption, an observation that is paralleled in $AT_1$ receptor knockout mice, and [TGR(ASrAOGEN)680] mice also show reduced dopamine levels within the midbrain compared with wild types (Maul et al., 2005). Collectively, these data suggest that elevated angiotensin II in the brain may increase ethanol consumption and preference via an interaction with central dopaminergic systems. It is noteworthy that the presence of angiotensin $AT_1$ receptors in the ventral mesencephalon and striatal complex is well established (Allen et al., 1992).

To extend the study of how central angiotensin may regulate complex behavior patterns, such as motivation to consume ethanol, we have utilized mice that overexpress angiotensin $AT_{1A}$ receptors in a brain (neuron)-specific manner. These mice are hypersensitive to both endogenously released and exogenously administered angiotensin (Lazartigues et al., 2002, 2004), and are therefore a useful model to examine the role of angiotensin in physiological processes. In addition, we sought to characterize dopamine receptor function in these mice, as dopamine receptor ligands can regulate alcohol preference (e.g., Pfeffer and Samson, 1988).

## METHODS

### *Animals*

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986, Australia, under the guidelines of the Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. NSE-$AT_{1A}$ mice and nontransgenic littermates were utilized in this study, the creation and genetic background of which have been described in detail previously by Lazartigues et al. (2002). All mice were genotyped by polymerase chain reaction and $AT_1$ receptor autoradiography, as detailed elsewhere (Lazartigues et al., 2002, 2004).

Age-matched male mice (6–9 weeks old at the beginning of the study) were utilized and housed individually in standard mouse boxes, with cat litter (Breeders Choice$^{TM}$ Nerang, Queensland, Australia) and standard mouse food under a controlled 12-hour light/dark cycle; boxes were cleaned and mice were weighed weekly. During the course of the study, all mice were housed at the Integrative Neuroscience Facility (INF) at the Howard Florey Institute. All mice were acclimatized for a minimum of 7 days before experiments commenced. A total of 52 NSE-$AT_{1A}$ mice and 60 nontransgenic littermates were used in the present study.

### *Drinking Study*

Mice were placed on a continual-access free-choice drinking paradigm, whereby each cage was equipped with 2 drink bottles, positioned to allow equal access to both containers. One bottle was filled with tap water and one bottle was filled with ethanol (5% v/v) solution. The mice were free to choose between tap water and the test solution. The position of the bottles was altered randomly to avoid the development of place preference (Short et al., 2006). The bottles were monitored daily, and the total daily fluid intake (mL/kg/d), consumption of (g/kg/d), and relative preference (volume of test solution consumed as a percentage of total daily fluid intake) were calculated. The standard protocol for alcohol consumption consisted of mice exposed to 5% (v/v) ethanol for at least 14 days, followed by 14 days at 10% (v/v) ethanol, and then 14 days at 15% (v/v) ethanol. Cohorts of mice were finally subjected to a further 14 days at 20% (v/v) ethanol. In some mice ($n = 13$ per genotype), total fluid deprivation (TFD) occurred for a period of 24 hours to quantify rehydration in response to increased endogenous angiotensin.

### *Drug Treatments in Conjunction With Alcohol Consumption*

After standard acquisition of ethanol consumption, mice ($n = 9/10$ per genotype) were injected for 9 consecutive days: the first 3 days with saline [0.1 mL/10 g body weight, intraperitoneal (i.p.)] and the following 6 days with raclopride (3 mg/kg, i.p.) while still under continual access to 15% (v/v) ethanol in a 2-bottle free-choice drinking paradigm. At the end of drug treatment, mice were killed by cervical dislocation, and the brains were removed for autoradiography.

### *Autoradiography*

Alcohol-naïve mice, chronic alcohol-drinking mice (a total of 6 weeks of consumption of ethanol), and raclopride-treated mice (typically $n = 5$ per genotype per group) were killed 24 hours after the last drug treatment and their brains were removed for autoradiography. Tissue levels of the dopamine transporter (DAT) were determined using a previously published protocol (Cowen et al., 2005; McGregor et al., 2003). Sections were preincubated in buffer (0.1 M $NaH_2PO_4$/0.1 M sucrose, pH 7.4) for 30 minutes at room temperature. Sections were then incubated in buffer containing 50 pM [$^{125}$I]RTI-55 (2200Ci/mmol; Perkin Elmer, Waltham, MA) and 100 nM fluoxetine (60 minutes, room temperature). GBR12935 (10 $\mu$M) was used to determine nonspecific binding. Sections were then washed in ice-cold buffer (1×1 minute, 2×20 minutes), rinsed in ice-cold $dH_2O$, and desiccated overnight. Slides were then apposed to a Kodak X-omat AR film in the presence of standard [$^{14}$C]microscales (American Radiolabelled Chemicals, St. Louis, MA) for 7 hours.

Tissue levels of the dopamine $D_2$-like receptor were determined using a standard protocol (Djouma and Lawrence, 2002; Lawrence et al., 1995). [$^{125}$I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using [$^{125}$I]Na (2200Ci/mmol; Amersham International, Chalfont St. Giles, Buckinghamshire, UK) via the chloramine-T iodination technique purified from the iodination reaction mixture by paper chromatography as described previously (Lawrence et al., 1995). Sections were preincubated in buffer (170 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.001% w/v ascorbic acid) for 30 minutes. Sections were then incubated in buffer for 1 hour with 0.3 nM [$^{125}$I]NCQ298. Nonspecific binding was determined using 10 $\mu$M raclopride. Sections were

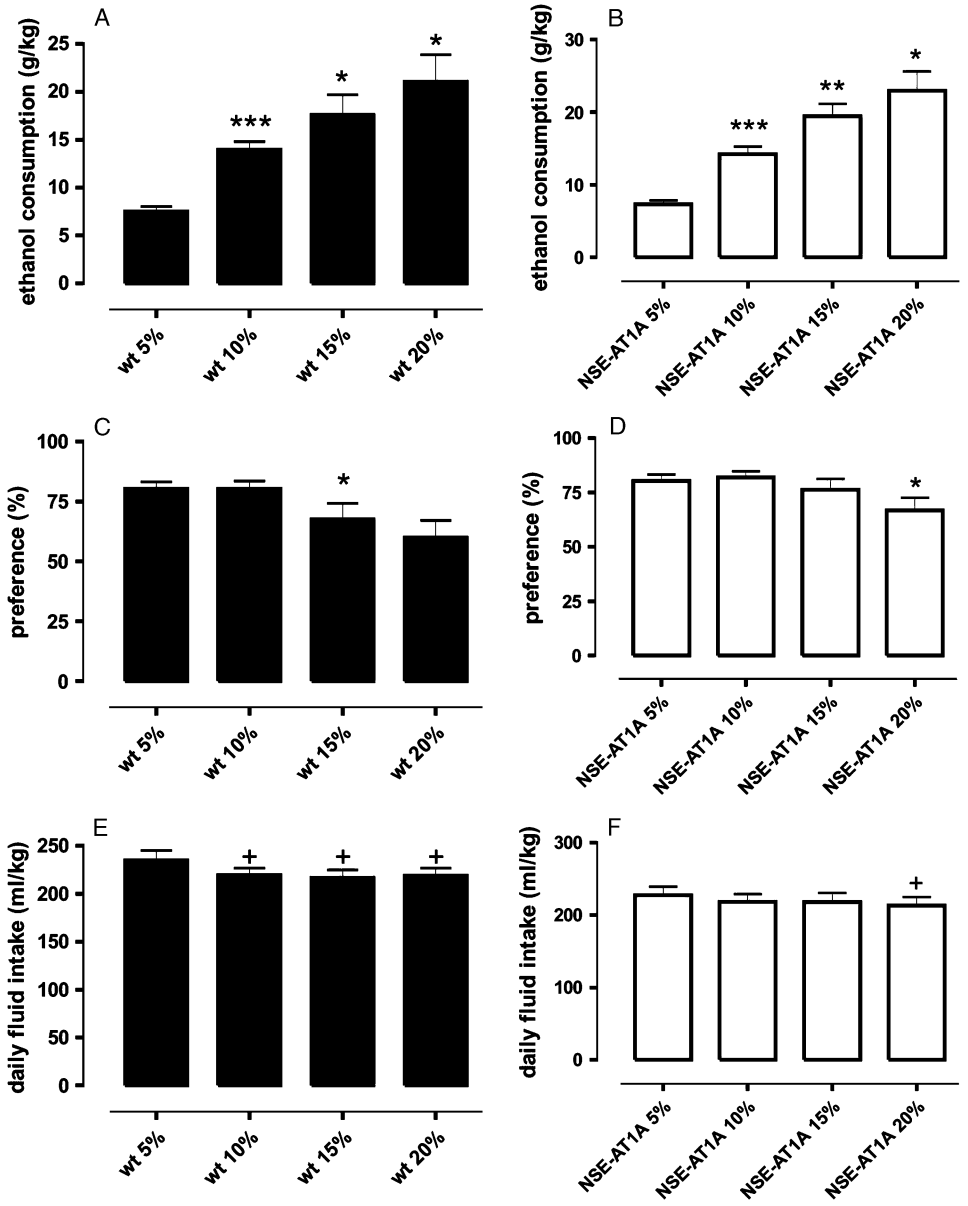


**Fig. 1.** Ethanol consumption and preference in NSE-$AT_{1A}$ and wild-type mice. (**Panels A** and **B**) represent ethanol consumption, (**panels C** and **D**) preference, and (**panels E** and **F**) daily fluid intake. Filled columns represent wild-type mice and open columns NSE-$AT_{1A}$ mice. Data are mean ± SEM ($n = 13$ per genotype). $^{*}p<0.05$, $^{***}p<0.001$ versus previous concentration of ethanol. $^{+}p<0.05$ versus 5% ethanol within groups.

washed in ice-cold buffer (4×2 minutes) and rinsed in ice-cold $dH_2O$ (2×30 seconds). Following overnight desiccation, slides were apposed to film for 3 hours for striatal sections and 23 hours for mesencephalon sections.

Films were developed with a Kodak D-19 developer and analyzed using the SCION imaging system (Scion Corp., Frederick, MD) for densitometry, by comparing the optical densities resulting from the radioactive ligands with that of corresponding standard microscales under constant illumination. Data are expressed as dpm/$mm^2$.

### *Statistical Analysis*

Autoradiographic images were quantified by comparing regions of interest (delineated with a computer mouse) between genotype and treatment groups. Brain nuclei were defined by referring thionin-stained sections with a standard mouse atlas (Franklin and Paxinos, 1997). Graphical representations of data were created using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). Statistical analysis of data was performed using SigmaStat 3.10 (Systat Software Inc., Point Richmond, San Jose, CA). Two-way analysis of variance (ANOVA) was used to compare within and between the groups as appropriate, followed by Student Newman–Keuls post hoc tests for drinking behavior or Holm–Sidak tests for autoradiography. If only 2 groups were being compared, *t*-tests were used to analyze the data. In all cases, $p \leq 0.05$ was considered to be significant.

### *Materials*

Proteinase K was supplied by Boehringer (Mannheim, Germany). Agarose was DNA grade and supplied by Gibco BRL Life Technologies (Mount Waverley, Victoria, Australia). The 1 kb DNA ladder

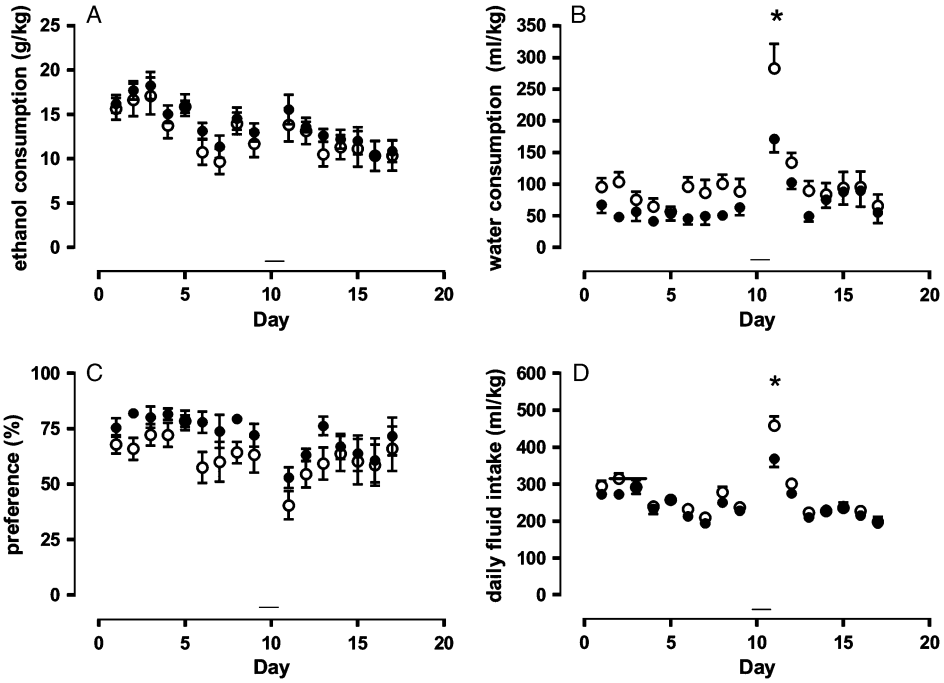


**Fig. 2.** Rehydration responses following 24 h of total fluid deprivation in NSE-$AT_{1A}$ and wild-type mice drinking a choice of ethanol or water. In all cases, filled circles represent wild-type mice and open circles indicate NSE-$AT_{1A}$ mice. The horizontal bar represents the 24-h period of total fluid deprivation. Data are means ± SEM ($n = 13$ per genotype). (**A**) Ethanol consumption. (**B**) Water consumption. (**C**) Ethanol preference. (**D**) Daily fluid intake. $^{*}p < 0.05$ between genotypes.

was from Gibco BRL Life Technologies. All other chemicals and reagents were of laboratory grade from various sources. [$^{125}$I]RTI-55 and [$^{125}$I]-Sar$^{1}$-Ile$^{8}$-Angiotensin II (specific activities both 2000 Ci/mmol) were purchased from Perkin Elmer. [$^{125}$I]Na (2000 Ci/mmol) was purchased from Amersham International. NCQ634 was a gift from Astra Pharmaceuticals (Södertälje, Sweden).

## RESULTS

### *Drinking Patterns*

Figure 1 illustrates the drinking data for both genotypes at 5, 10, 15, and 20% (v/v) ethanol concentrations. No differences were found between the genotypes in ethanol consumption, ethanol preference, or daily fluid intake at any of the tested ethanol concentrations. An increase in ethanol consumption was observed for both of the genotypes as the concentration of ethanol increased, as illustrated (Fig. 1A–1C).

As illustrated in Fig. 2 on the day after a 24-hour period of TFD (rehydration day), an increase in water consumption and daily fluid intake was observed for both genotypes. Rehydration occurred primarily with water for mice that had been chronically drinking ethanol in the 2-bottle free-choice paradigm, and this effect was exaggerated in the NSE-$AT_{1A}$ mice compared with wild types as shown by a significantly greater daily fluid intake ($t_{26} = 2.653$, $p = 0.0134$) and water consumption ($t_{26} = 2.529$, $p = 0.0178$) for the 24-hour period immediately after deprivation (Fig. 2C and 2D).

As illustrated in Fig. 3, in nontransgenic mice raclopride treatment (3 mg/kg, i.p.) caused a significant reduction in ethanol consumption [$F(2, 34) = 2.401$, $p = 0.01$] compared with saline, but this had no effect in NSE-$AT_{1A}$ mice. Thus, in nontransgenic mice, the average alcohol consumption for the 3 days before saline treatment was $17.3 \pm 0.8$ g/kg while consumption in NSE-$AT_{1A}$ mice was $19.6 \pm 0.9$ g/kg. Treatment with vehicle had no effect (nontransgenic mice: $16.5 \pm 0.9$ g/kg; NSE-$AT_{1A}$ mice: $20.4 \pm 0.3$ g/kg), whereas treatment with raclopride reduced ethanol consumption in nontransgenic mice ($13.6 \pm 0.8$ g/kg) but had no effect in NSE-$AT_{1A}$ mice ($20.9 \pm 1.2$ g/kg). A significant main effect on ethanol preference [$F(1, 17) = 3.110$, $p = 0.026$] and ethanol consumption [$F(1, 17) = 2.401$, $p = 0.022$] was evident between the genotypes during raclopride treatment, and there was a significant interaction between genotype×treatment for both ethanol preference [$F(2, 34) = 3.944$, $p = 0.029$] and ethanol consumption [$F(2, 34) = 4.190$, $p = 0.024$]. In contrast, while there was a main effect of treatment on daily fluid intake [$F(2, 34) = 13.59$, $p < 0.01$], there was no main effect of genotype [$F(1, 17) = 0.628$, $p = 0.439$] nor was there an interaction between treatment×genotype [$F(2, 34) = 0.258$, $p = 0.774$].

### *Autoradiography*

$D_2$-like receptor binding was examined using [$^{125}$I]NCQ-298 at the level of the mesencephalon (Fig. 4) and basal ganglia (Fig. 5). In both wild-type and NSE-$AT_{1A}$ mice, dense binding was essentially confined to the substantia nigra pars compacta (SNc) and the VTA, and binding was

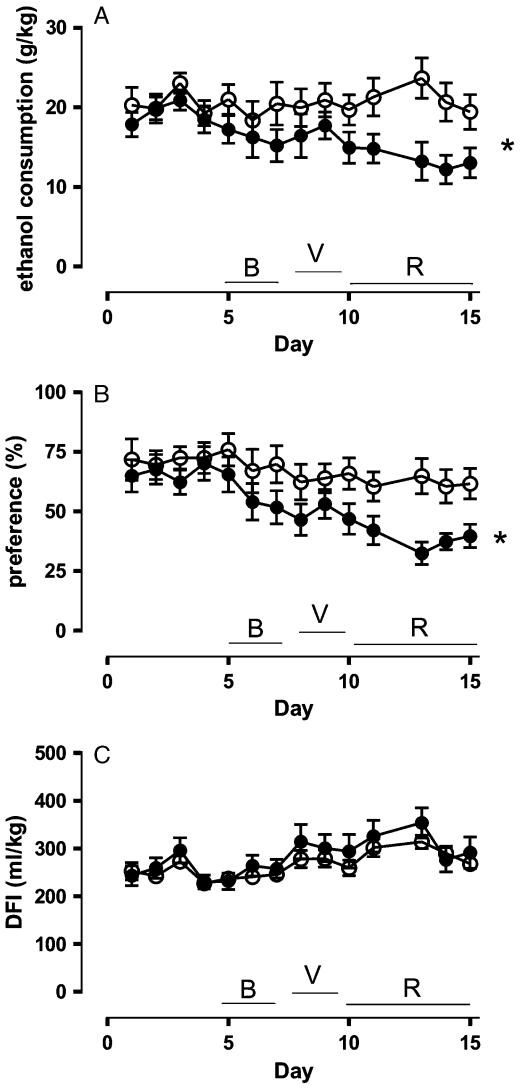


**Fig. 3.** Effect of raclopride (3 mg/kg, intraperitoneal (i.p.) on ethanol consumption (A), preference (B), and daily fluid intake (C). In all cases filled circles represent wildtype mice and open circles represent NSE-$AT_{1A}$ mice. Data are means ± SEM ($n = 9$–10 per genotype). B, baseline; V, vehicle; R, raclopride treatment. *$p < 0.05$, effect of drug treatment.

fully displaced by raclopride. In the SNc (Fig. 4E), there was no effect of genotype on binding in naïve mice. Furthermore, chronic alcohol consumption had no impact on [$^{125}$I]NCQ-298 binding; however, there was a main effect of treatment [$F(2, 182) = 26.996$, $p < 0.001$] with raclopride to increase binding significantly in both the wild-type ($t = 2.584$, $p = 0.011$) and NSE-$AT_{1A}$ mice ($t = 6.06$, $p < 0.001$), and there was an interaction between genotype×treatment [$F(2, 182) = 6.661$, $p = 0.002$]. In the VTA, there was a main effect of raclopride treatment [$F(2, 171) = 39.777$, $p < 0.001$] and an interaction between treatment×genotype [$F(2, 171) = 10.685$, $p < 0.001$], indicating increased binding in both wild-type ($t = 3.969$, $p < 0.001$) and NSE-$AT_{1A}$ mice ($t = 7.793$, $p < 0.001$), again to a greater extent in NSE-$AT_{1A}$ mice compared with wild-type littermates ($t = 3.894$, $p < 0.001$).

In the striatum, [$^{125}$I]NCQ-298 binding was largely confined to the caudate putamen (CPu), nucleus accumbens (NAcc), and the olfactory tubercule (OT). Genotype×treatment interactions were apparent in the dorsolateral [$F(2, 199) = 5.596$, $p = 0.004$], dorsomedial [$F(2, 206) = 7.720$, $p < 0.001$], and ventromedial [$F(2, 220) = 7.319$, $p < 0.001$] quadrants of the CPu. NSE-$AT_{1A}$ alcohol-naïve mice were found to show significantly lower binding compared with alcohol-naïve wild-type mice in the dorsolateral ($t = 3.47$, $p = 0.001$), dorsomedial ($t = 3.856$, $p = 0.001$), and ventromedial CPu quadrants ($t = 3.34$, $p = 0.001$), as well as the NAcc core ($t = 2.006$, $p < 0.05$). In wild-type mice, chronic consumption of ethanol resulted in an up-regulation in $D_2$-like binding only in the ventromedial quadrant of the CPu ($t = 2.21$, $p = 0.05$, Fig. 5F). In contrast, in NSE-$AT_{1A}$ mice, chronic consumption of ethanol resulted in an up-regulation in $D_2$-like binding throughout the CPu, being significant in the dorsolateral ($t = 3.148$, $p = 0.002$), dorsomedial ($t = 3.572$, $p < 0.001$), and ventromedial ($t = 3.188$, $p = 0.002$) CPu quadrants. Accumbal binding of [$^{125}$I]NCQ298 was not affected by ethanol consumption in either genotype. Raclopride treatment elevated $D_2$-like binding in the dorsolateral CPu of wild-type mice ($t = 2.681$, $p = 0.008$, Fig. 5C).

Dopamine transporter binding was examined with [$^{125}$I]RTI-55 at the level of the striatum. Dense DAT binding was essentially confined to the CPu, Nacc, and OT (Fig. 6). Dopamine transporter binding was fully displaced by GBR 12935. A genotype×treatment interaction was apparent in the ventromedial [$F(2, 305) = 3.875$, $p = 0.022$] quadrant of the CPu. For alcohol-naïve mice, binding was significantly lower in NSE-$AT_{1A}$ mice compared with wild-type mice in the dorsomedial ($t = 2.336$, $p = 0.02$), ventromedial ($t = 4.816$, $p < 0.001$), and ventrolateral ($t = 3.614$, $p < 0.001$) CPu. In NSE-$AT_{1A}$ mice, chronic consumption of ethanol resulted in an up-regulation in [$^{125}$I]RTI-55 binding in the ventrolateral ($t = 2.539$, $p = 0.012$) and ventromedial ($t = 3.209$, $p = 0.001$) CPu quadrants. In contrast, in wild-type mice there was no effect of ethanol consumption on [$^{125}$I]RTI-55 binding.

## DISCUSSION

The present study has highlighted that while NSE-$AT_{1A}$ mice are similar to wild-type mice in terms of basal (unstimulated) parameters, if challenged with a relevant physiological or pharmacological manipulation, a clear phenotype is observed, suggestive of altered dopamine $D_2$ receptor function in NSE-$AT_{1A}$ mice. Specifically, while antagonism of dopamine $D_{2/3}$ receptors by raclopride reduced alcohol consumption and preference in wild-type mice, the same drug was without effect on these parameters in NSE-$AT_{1A}$ mice. This finding provides further support for a role of the $AT_{1A}$ receptor in the central regulation of alcohol consumption.

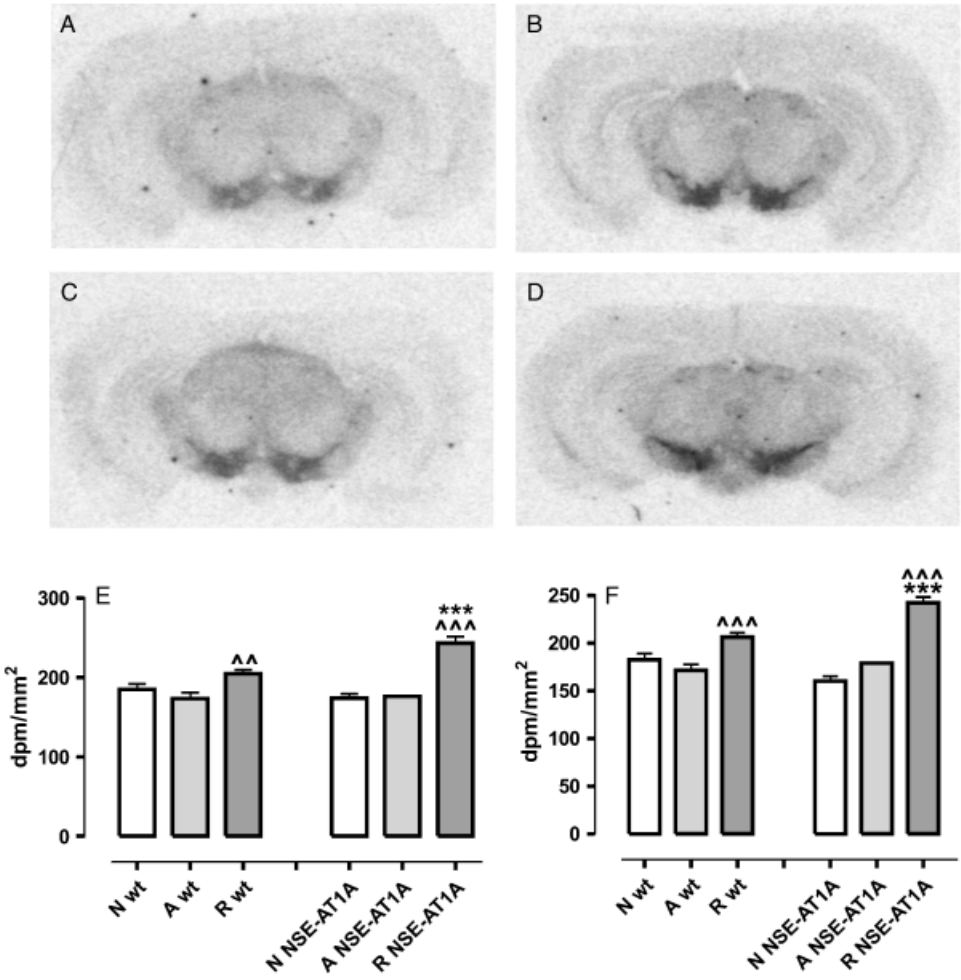


**Fig. 4.** Dopamine $D_2$ receptor binding in the ventral mesencephalon of NSE-$AT_{1A}$ and wild-type mice. Wild-type (WT) mouse alcohol naïve, WT mouse raclopride treated, NSE-$AT_{1A}$ mouse alcohol naïve, NSE-$AT_{1A}$ mouse raclopride treated, quantification of data from substantia nigra pars compacta [N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, intraperitoneal (i.p.)]. Quantification of data from the ventral tegmental area (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.), Data are means ± SEM ($n = 5$–7 per genotype). $^{\wedge\wedge}p < 0.01$,$^{\wedge\wedge\wedge}p < 0.001$ versus naïve mice within groups. $^{***}p < 0.001$ versus wild type within treatment.

The NSE-$AT_{1A}$ mouse line is maintained on a C57/BL6J background, which has a high preference for ethanol (Bachmanov et al., 1996), and this phenotype was observed in both NSE-$AT_{1A}$ and wild-type mice. There was no difference between the genotypes in terms of preference or consumption of ethanol at any of the tested concentrations, suggesting that overexpression of brain $AT_{1A}$ receptors does not alter ethanol-seeking behaviors under these conditions. Total fluid deprivation stimulates peripheral and central angiotensin (Ang II) release (Chen and Morris, 2001; Johnson et al., 2003; Morris et al., 1999), a potent dipsogen (Bagi et al., 2003; Blair-West et al., 1996). In ethanol-experienced mice in the 2-bottle free-choice paradigm, NSE-$AT_{1A}$ mice consumed more water (and total fluid) than wild-type littermates following TFD. This exaggerated response supports the functional relevance of overexpressed $AT_{1A}$ receptors in NSE-$AT_{1A}$ mice (Lazartigues et al., 2002, 2004). Importantly, both wild-type and NSE-$AT_{1A}$ mice that had been previously consuming ethanol with high preference preferentially rehydrated with water as opposed to ethanol. Moreover, during rehydration, ethanol consumption was not different between genotypes.

Recently, there has been renewed interest in a possible link between angiotensin and alcohol consumption (e.g., Cowen and Lawrence, 2006; Crabbe et al., 2006). While preventing the breakdown of angiotensin II to angiotensin III by deletion of aminopeptidase A has no impact upon alcohol consumption or preference (Faber et al., 2006), other studies suggest that the $AT_{1A}$ receptor is implicated in voluntary alcohol intake (e.g., Maul et al., 2005). Interestingly, it appears that angiotensin may act centrally to modulate alcohol consumption via an interaction with dopaminergic pathways (see Maul et al., 2001, 2005). While basal alcohol consumption parameters in NSE-$AT_{1A}$ mice were similar to wild types, we also examined

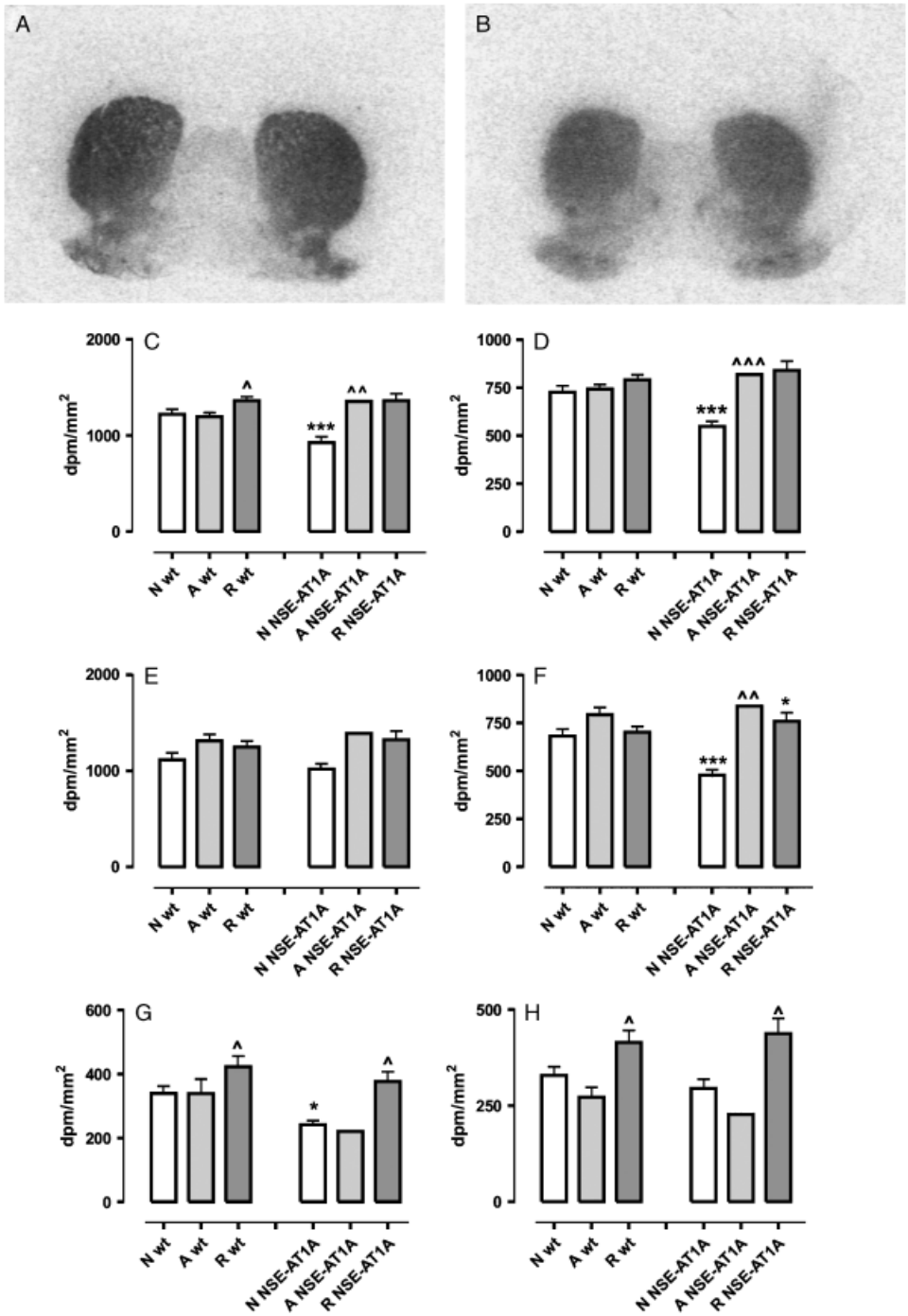


**Fig. 5.** Dopamine $D_2$ receptor binding in the basal ganglia of NSE-$AT_{1A}$ and wild-type mice. (**A**) WT mouse alcohol naïve. (**B**) NSE-$AT_{1A}$ mouse alcohol naïve. (**C**) Quantification of dorsolateral striatum [N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, intraperitoneal (i.p.)]. (**D**) Quantification of dorsomedial striatum (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). (**E**) Quantification of ventrolateral striatum (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). (**F**) Quantification of ventromedial striatum (N, naïve, A, chronic alcohol, R, raclopride, 3 mg/kg, i.p.). (**G**) Quantification of accumbens core (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). (**H**) Quantification of accumbens shell (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). Data are means ± SEM ($n = 5$–7 per genotype). $^{\wedge}p<0.05$, $^{\wedge\wedge}p<0.01$, $^{\wedge\wedge\wedge}p<0.001$ versus naïve mice within groups. $^{*}p<0.05$, $^{***}p<0.001$ versus wild type within treatment.

the possibility of an interaction between angiotensin and dopamine in this context. Raclopride is a $D_2/D_3$ antagonist; however, the striatal density of $D_3$ receptors is low (approximately 1% of $D_2$), and therefore the consequences of raclopride treatment are essentially attributed to $D_2$ receptor antagonism (Hall et al., 1994, 1996). When administered in rats via microinjections into the NAcc, $D_2$ antagonists consistently reduce ethanol drinking

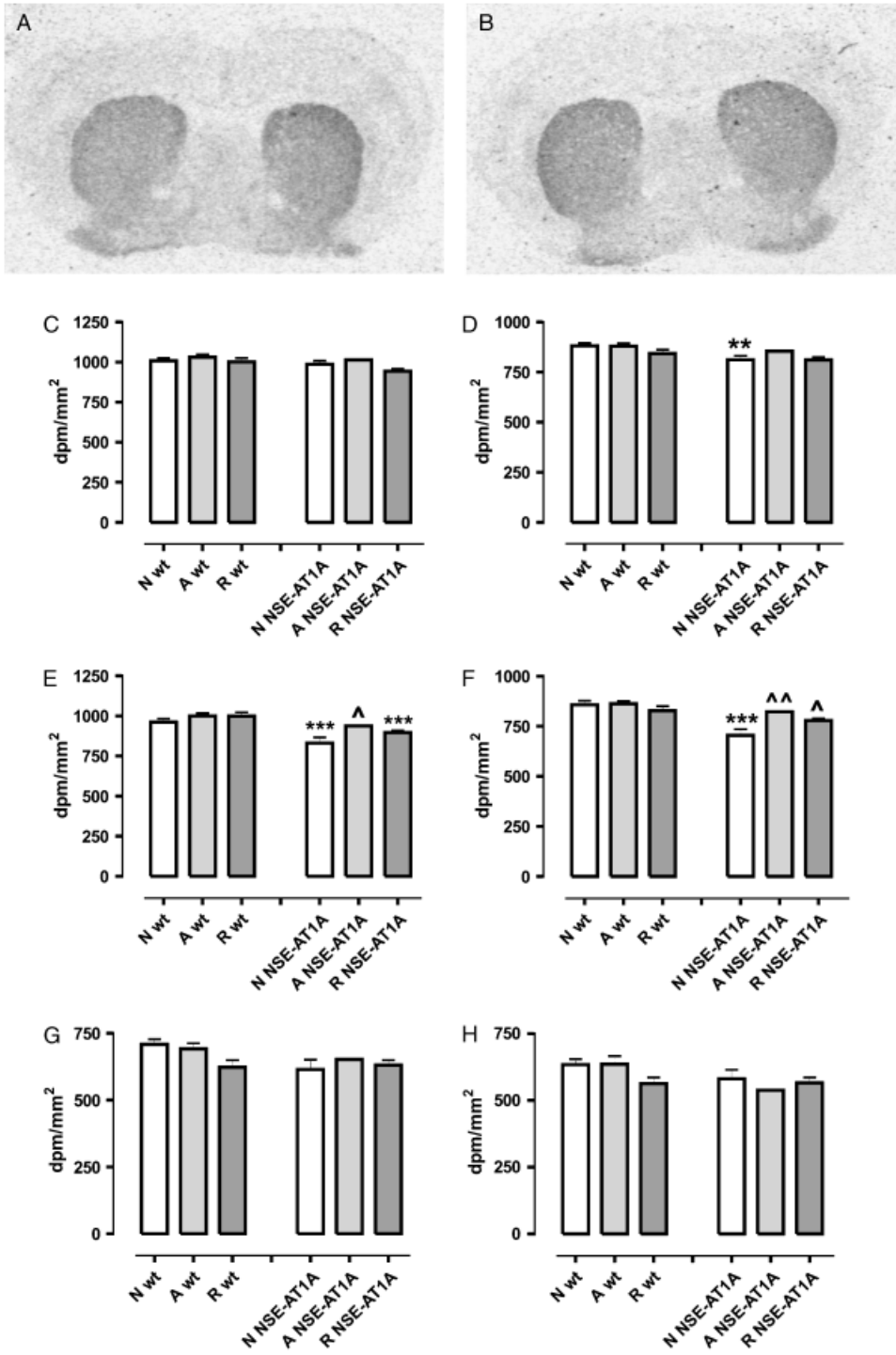


**Fig. 6.** Dopamine transporter binding in the basal ganglia of NSE-$AT_{1A}$ and wild-type mice. (**A**) WT mouse alcohol naïve. (**B**) NSE-$AT_{1A}$ mouse alcohol naïve. (**C**) Quantification of dorsolateral striatum [N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, intraperitoneal (i.p.)]. (**D**) Quantification of dorsomedial striatum (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). (**E**) Quantification of ventrolateral striatum (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). (**F**) Quantification of ventromedial striatum (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). (**G**) Quantification of accumbens core (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). (**H**) Quantification of accumbens shell (N, naïve; A, chronic alcohol; R, raclopride, 3 mg/kg, i.p.). Data are means ± SEM ($n = 5$–7 per genotype). $^{p<0.05}$, $^{^{p<0.01}}$ versus naïve mice within groups. $^{**p<0.01}$, $^{***p<0.001}$ versus wild type within treatment.

(Hodge et al., 1997) and ethanol-seeking behavior (Samson et al., 1993). Following systemic injections, most researchers report a reduction in ethanol seeking (Dyr et al., 1993; Pfeffer and Samson, 1988), although dose is an issue (Levy et al., 1991). In the present study, raclopride injections (i.p.) reduced ethanol preference and consumption in wild-type mice, whereas NSE-$AT_{1A}$ mice were resistant, maintaining pretreatment ethanol consumption

and preference throughout. This finding supports the notion that angiotensin systems can regulate dopamine-mediated behaviors. In terms of alcohol consumption in mice, dopamine $D_2$ receptor function is pertinent. Thus, vector-mediated reintroduction of dopamine $D_2$ receptors into the accumbens of $D_2$ deficient mice caused a transient increase in alcohol consumption and preference (Thanos et al., 2005). Similarly, striatal release of dopamine following systemic alcohol is dramatically attenuated in $D_2$ deficient mice compared with wild types (Job et al., 2006). Consequently, the inability of raclopride to reduce alcohol consumption or preference in NSE-$AT_{1A}$ mice would suggest that overexpression of $AT_{1A}$ receptors is functionally influencing the signaling via dopamine $D_2$ receptors.

Analysis of $D_2$-like binding found a genotype difference in alcohol-naïve mice, with significantly reduced binding in the CPu and NAcc core of NSE-$AT_{1A}$ mice. Whether these differences are due to altered development of the dopaminergic system in transgenic mice is not clear, although regulation of dopamine $D_2$-like receptors in adult mice was also genotype-dependent. In NSE-$AT_{1A}$ mice, ethanol consumption was found to increase $D_2$-like receptor binding in the CPu, a result echoed by an increase in DAT. In contrast, essentially no effect of ethanol consumption was observed for wild-type mice. Studies surrounding the regulation of $D_2$ receptors following ethanol consumption are somewhat controversial, with some researchers reporting that $D_2$ receptors in rats are resistant to modulation following ethanol consumption (Cowen and Lawrence, 2001), and other researchers suggesting ethanol consumption may alter the $B_{max}$ for $D_2$ receptors (Tajuddin and Druse, 1996). Indeed, the present study confirms, with the exception of the ventromedial CPu, a lack of effect of ethanol consumption per se on $D_2$ receptor density in wild-type mice, as previously noted in rats (Cowen and Lawrence, 2001); however, the $D_2$ receptors in NSE-$AT_{1A}$ mice are sensitive to ethanol consumption. This also suggests altered dopaminergic function in the NSE-$AT_{1A}$ mice, possibly in the form of $AT_1$-$D_2$ receptor interactions that are not found in wild-type mice, but that confer sensitivity to ethanol.

Raclopride treatment resulted in an up-regulation of $D_2$-like receptors in the SNc and VTA in both genotypes, with an exaggerated effect in the NSE-$AT_{1A}$ mice. This confirms that raclopride was administered at a pharmacologically relevant dose in both genotypes. While presynaptic $D_2$-like receptors were more sensitive to regulation by raclopride in NSE-$AT_{1A}$ mice, the altered behavioral phenotype observed in NSE-$AT_{1A}$ mice may also be due to the presence of $AT_{1A}$ receptors on $D_2$ receptor positive cells within the basal ganglia, which would not occur in wild-type mice (Allen et al., 1991, 1992). This would provide an explanation for the lack of behavioral response to raclopride, despite the dose changing receptor levels.

In conclusion, the present data indicate that central overexpression of angiotensin $AT_{1A}$ receptors results in a complex phenotype. Thus, neither alcohol preference nor consumption is altered; however, NSE-$AT_{1A}$ mice are resistant to the effects of a dopamine $D_2$ receptor antagonist on alcohol consumption and preference, and yet sensitive to $D_2$ receptor regulation at the biochemical level. Collectively, these data imply that expression of angiotensin $AT_{1A}$ receptors on striatal neurons impacts upon dopamine $D_2$ systems.

## ACKNOWLEDGMENTS

We would like to thank A/Prof. John Drago and Dr. Maarten Van den Buuse for critical appraisal.

## REFERENCES

Allen AM, MacGregor DP, Chai SY, Donnan GA, Kaczmarczyk S, Richardson K, Kalnins R, Ireton J, Mendelsohn FA (1992) Angiotensin II receptor binding associated with nigrostriatal dopaminergic neurons in human basal ganglia. Ann Neurol 32:339–344.

Allen AM, Paxinos G, McKinley MJ, Chai SY, Mendelsohn FA (1991) Localization and characterization of angiotensin II receptor binding sites in the human basal ganglia, thalamus, midbrain pons, and cerebellum. J Comp Neurol 312:291–298.

Bachmanov AA, Tordoff MG, Beauchamp GK (1996) Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. Alcohol Clin Exp Res 20:201–206.

Bagi EE, Fekete E, Lenard L (2003) Angiotensin II and III microinjections into the zona incerta influence drinking behavior. Brain Res 977:199–208.

Blair-West JR, Denton DA, McBurnie MI, Weisinger RS (1996) The dipsogen angiotensin II does not stimulate ethanol intake in mice. Physiol Behav 60:481–487.

Chen Y, Morris M (2001) Differentiation of brain angiotensin type 1a and 1b receptor mRNAs: a specific effect of dehydration. Hypertension 37:692–697.

Cowen MS, Djouma E, Lawrence AJ (2005) The metabotropic glutamate 5 receptor antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine reduces ethanol self-administration in multiple strains of alcohol-preferring rats and regulates olfactory glutamatergic systems. J Pharmacol Exp Ther 315:590–600.

Cowen MS, Lawrence AJ (2001) Alterations in central preproenkephalin mRNA expression after chronic free-choice ethanol consumption by fawn-hooded rats. Alcohol Clin Exp Res 25:1126–1133.

Cowen MS, Lawrence AJ (2006) Alcoholism and neuropeptides: an update. Curr Drug Targets, CNS Neurol Dis 5:233–239.

Crabbe JC, Phillips TJ, Harris RA, Arends MA, Koob GF (2006) Alcohol-related genes: contributions from studies with genetically engineered mice. Addict Biol 11:195–269.

Djouma E, Lawrence AJ (2002) The effect of chronic ethanol consumption and withdrawal on mu-opioid and dopamine $D_1$ and $D_2$ receptor density in Fawn-Hooded rat brain. J Pharmacol Exp Ther 302: 551–559.

Dyr W, McBride WJ, Lumeng L, Li TK, Murphy JM (1993) Effects of $D_1$ and $D_2$ dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. Alcohol 10:207–212.

Faber F, Gembardt F, Sun X, Mizutani S, Siems W-E, Walther T (2006) Lack of angiotensin II conversion to angiotensin III increases water but not alcohol consumption in aminopeptidase A-deficient mice. Regul Pept 136:130–137.

Fitts DA (1993) Angiotensin and captopril increase alcohol intake. Pharmacol Biochem Behav 45:35–43.

Franklin KBJ, Paxinos G (1997) *The Mouse Brain in Stereotaxic Coordinates*. Academic Press Inc., San Diego.

Grupp LA (1992) Effects of angiotensin II and an angiotensin converting enzyme inhibitor on alcohol intake in P, NP rats. Pharmacol Biochem Behav 41:105–108.

Grupp LA, Harding S (1994) The reduction in alcohol drinking by peripherally injected angiotensin II is selectively mediated by the $AT_1$ receptor subtype. Pharmacol Biochem Behav 47:385–392.

Grupp LA, Harding S (1995) Intracerebroventricularly infused angiotensin II or III do not alter voluntary alcohol intake in rats. Pharmacol Biochem Behav 51:593–599.

Grupp LA, Kalant H, Leenen FH (1989a) Alcohol intake is inversely related to plasma renin activity in the genetically selected alcohol-preferring and -nonpreferring lines of rats. Pharmacol Biochem Behav 32:1061–1063.

Grupp LA, Perlanski E, Stewart RB (1988) Angiotensin II-induced suppression of alcohol intake and its reversal by the angiotensin antagonist Sar-1 Thr-8 angiotensin II. Pharmacol Biochem Behav 31:813–816.

Grupp LA, Perlanski E, Stewart RB (1989b) Systemic angiotensin II acts at the subfornical organ to suppress voluntary alcohol consumption. Pharmacol Biochem Behav 34:201–205.

Hall H, Farde L, Halldin C, Hurd YL, Pauli S, Sedvall G (1996) Autoradiographic localization of extrastriatal $D_2$-dopamine receptors in the human brain using [$^{125}$I]epidepride. Synapse 23:115–123.

Hall H, Sedvall G, Magnusson O, Kopp J, Halldin C, Farde L (1994) Distribution of $D_1$- and $D_2$-dopamine receptors, and dopamine and its metabolites in the human brain. Neuropsychopharmacology 11:245–256.

Hodge CW, Samson HH, Chappelle AM (1997) Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. Alcohol Clin Exp Res 21:1083–1091.

Job MO, Ramachandra V, Anders S, Low MJ, Gonzales RA (2006) Reduced basal and ethanol stimulation of striatal extracellular dopamine concentrations in dopamine D2 receptor knockout mice. Synapse 60:158–164.

Johnson RF, Beltz TG, Thunhorst RL, Johnson AK (2003) Investigations on the physiological controls of water and saline intake in C57BL/6 mice. Am J Physiol Regul Integr Comp Physiol 285:R394–403.

Kulkosky PJ, Allison TG, Carr BA (1996) Angiotensin II reduces alcohol intake and choice in water- or food-restricted rats. Alcohol 13:359–363.

Lawrence AJ, Krstew E, Jarrott B (1995) Functional dopamine D2 receptors on rat vagal afferent neurones. Br J Pharmacol 114:1329–1334.

Lazartigues E, Dunlay SM, Loihl AK, Sinnayah P, Lang JA, Espelund JJ, Sigmund CD, Davisson RL (2002) Brain-selective overexpression of angiotensin (AT1) receptors causes enhanced cardiovascular sensitivity in transgenic mice. Circ Res 90:617–624.

Lazartigues E, Lawrence AJ, Lamb FS, Davisson RL (2004) Renovascular hypertension in mice with brain-selective overexpression of AT1a receptors is buffered by increased nitric oxide production in the periphery. Circ Res 95:523–531.

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 1 (suppl): 417–420.

Lingham T, Perlanski E, Grupp LA (1990) Angiotensin converting enzyme inhibitors reduce alcohol consumption: some possible mechanisms and important conditions for its therapeutic use. Alcohol Clin Exp Res 14:92–99.

Maul B, Krause W, Pankow K, Becker M, Gembardt F, Alenina N, Walther T, Bader M, Siems WE (2005) Central angiotensin II controls alcohol consumption via its AT1 receptor. FASEB J. 19:1474–1481.

Maul B, Siems WE, Hoehe MR, Grecksch G, Bader M, Walther T (2001) Alcohol consumption is controlled by angiotensin II. FASEB J 15:1640–1642.

McGregor IS, Clemens KJ, Van der Plasse G, Li KM, Hunt GE, Chen F, Lawrence AJ (2003) Increased anxiety 3 months after brief exposure to MDMA (''Ecstasy'') in rats: association with altered 5-HT transporter and receptor density. Neuropsychopharmacology 28:1472–1484.

Morris M, Li P, Callahan MF, Oliverio MI, Coffman TM, Bosch SM, Diz DI (1999) Neuroendocrine effects of dehydration in mice lacking the angiotensin AT1a receptor. Hypertension 33:482–486.

Pfeffer AO, Samson HH (1988) Haloperidol and apomorphine effects on ethanol reinforcement in free feeding rats. Pharmacol Biochem Behav 29:343–350.

Puddey IB, Vandongen R, Beilin LJ, Rouse IL (1985) Alcohol stimulation of renin release in man: its relation to the hemodynamic, electrolyte, and sympatho-adrenal responses to drinking. J Clin Endocrinol Metab 61:37–42.

Samson HH, Hodge CW, Tolliver GA, Haraguchi M (1993) Effect of dopamine agonists and antagonists on ethanol-reinforced behavior: the involvement of the nucleus accumbens. Brain Res Bull 30:133–141.

Short JL, Ledent C, Drago J, Lawrence AJ (2006) Receptor crosstalk: characterization of mice deficient in dopamine $D_1$ and adenosine $A_{2A}$ receptors. Neuropsychopharmacology 31:525–534.

Spinosa G, Perlanski E, Leenen FH, Stewart RB, Grupp LA (1988) Angiotensin converting enzyme inhibitors: animal experiments suggest a new pharmacological treatment for alcohol abuse in humans. Alcohol Clin Exp Res 12:65–70.

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 PK, Rivera SN, Weaver K, Grandy DK, Rubinstein M, Umegaki Wang GJ, Hitzemann R, Volkow ND (2005) Dopamine D2R DNA transfer in dopamine D2 receptor-deficient mice: effects on ethanol drinking. Life Sci 77:130–139.

Weisinger RS, Blair-West JR, Burns P, Denton DA (1999a) Intracerebroventricular infusion of angiotensin II increases water and ethanol intake in rats. Am J Physiol 277:R162–172.

Weisinger RS, Blair-West JR, Denton DA, McBurnie MI (1999b) Angiotensin II stimulates intake of ethanol in C57BL/6J mice. Physiol Behav 67:369–376.

Wright JW, Morseth SL, Abhold RH, Harding JW (1986) Elevations in plasma angiotensin II with prolonged ethanol treatment in rats. Pharmacol Biochem Behav 24:813–818.