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Regional CNS densities of serotonin$_{1A}$ and dopamine D_2 receptors in periadolescent alcohol-preferring P and alcohol-nonpreferring NP rat pups

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Abstract

The objective of the present study was to use quantitative autoradiography to determine binding densities of serotonin$_{1A}$ (5-HT$_{1A}$) and dopamine (DA) D_2 receptors in alcohol-naive periadolescent P and NP rat pups. P ($n=8$) and NP ($n=7$) rat pups, 25 days of age, from different litters were used. Coronal brain sections were incubated with 2 nM [^{3}H]8-OH-DPAT or 20 nM [^{3}H]sulpiride for 5-HT$_{1A}$ or D_2 binding, respectively. Approximately 15–40% higher densities of [^{3}H]8-OH-DPAT binding were observed in the anterior cortical regions of the periadolescent P rat compared with NP rat pups. Similar differences were also observed in posterior cortical regions with P rats having 25–40% higher [^{3}H]8-OH-DPAT binding than NP rats. [^{3}H]8-OH-DPAT binding was approximately 10–20% higher in posterior hippocampal regions of the P rat pups compared with the NP line. [^{3}H]sulpiride binding was significantly different only in the ventral tegmental area (VTA), where binding was approximately 20% lower in the periadolescent P rats compared with the NP rat pups. Overall, these results are very similar to findings observed in adult alcohol-naive P and NP rats, and suggest that the innate differences in the neural systems implicated in high alcohol drinking behaviors may already be established in the periadolescent animal.

Keywords: Autoradiography; Alcohol-preferring rats; Alcohol-nonpreferring rats; 5-HT$_{1A}$ receptors; D_2 receptors; Periadolescence

1. Introduction

The serotonergic and dopaminergic neurotransmitter systems have been implicated in the neurochemistry of high alcohol drinking behaviors. Rat lines selectively bred for their alcohol preference display innate differences in these two monoamine systems (for review, see McBride and Li, 1998). Adult alcohol-preferring P rats have lower contents of serotonin (5-HT) and dopamine (DA) in several brain regions compared with the alcohol-nonpreferring NP rat line (Murphy et al., 1982, 1987; McBride et al., 1993a,b). Adult P and NP rats have also been shown to have different innate levels of several neurotransmitter receptors (for review, see McBride and Li, 1998). 5-HT$_{1A}$ receptor levels are elevated in several cerebral cortical and hippocampal subregions and are lower in raphe nuclei of adult alcohol-naive P rats compared with NP rats (Wong et al., 1993; McBride et al., 1994). On the other hand, DA D_2 receptor levels have been shown to be lower in certain limbic regions (e.g. nucleus accumbens and ventral tegmental area [VTA]) of the adult P rat compared with NP rats (McBride et al., 1993a,b). These innate differences in adult rat brain 5-HT$_{1A}$ and D_2 receptor levels have been shown to be due to a change in B_{max}, indicating changes in receptor number (Wong et al., 1990; McBride et al., 1993a,b). Consistent with the finding of reduced contents of 5-HT in several CNS regions of the P rat, neuroanatomical studies have demonstrated decreased serotonergic innervation in many of the same CNS regions (Zhou et al., 1994). In addition, the reduced densities of 5-HT$_{1A}$ receptors in the raphe nuclei (McBride et al., 1994) may reflect the reduced numbers of 5-HT neurons in these nuclei (Zhou et al., 1994). Neuro-

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anatomical studies indicate that there may be reduced dopaminergic innervation from the VTA to the nucleus accumbens in the P rat compared with the NP rat (Zhou et al., 1995), a finding consistent with the differences in DA content in the nucleus accumbens of the P rat compared with the NP rat line (Murphy et al., 1987).

A significant area of interest in alcohol research has begun to focus on high alcohol drinking behaviors in young animals. Recently, P rat pups have been shown to rapidly acquire high alcohol drinking behavior by 3 to 4 weeks of age (McKinzie et al., 1998). This would suggest that the neural substrates underlying high alcohol drinking behavior are already present in the preadolescent animal. The current study was undertaken to determine [^{3}H]8-OH-DPAT and [^{3}H]sulpiride binding levels in the CNS of periadolescent alcohol-naive P and NP rats. The hypothesis to be tested is that if the differences in 5-HT$_{1A}$ and D$_2$ receptor densities observed in adulthood between the P and NP rat lines are associated with divergent alcohol drinking, then these differences should also be present at the age of onset of high alcohol drinking.

2. Method

2.1. Animals

Postnatal day 25, ethanol-naïve, P ($n=8$) and NP ($n=7$) rat pups were used. All pups were from the 48th generation and no more than two pups per litter were used. The P and NP lines have been selectively bred from Wistar stock for their ethanol preference and nonpreference and have been well characterized (Li et al., 1993; Lumeng et al., 1977). Animals were triple housed under a 12:12 light cycle in a temperature-controlled colony room for at least 1 week before the experiment, and were handled daily. The animals used in this experiment were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, NIH and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

2.2. Autoradiography procedure

Rats were killed by decapitation, their brains quickly removed and frozen in isopentane over dry ice (-50 °C). Brains were wrapped in foil and stored in airtight freezer bags at -70 °C until sectioned. Coronal serial sections (20 μm) were cut on a Leica cryostat microtome (Leica Microsystems, Deerfield, IL) set at -20 °C. A total of six sections per slide, eight slides per animal were taken for each specific ligand. Adjacent slides were used for the determination of nonspecific binding. The procedures for 5-HT$_{1A}$ binding have been previously described (McBride et al., 1994). For 5-HT$_{1A}$ receptor binding, slides from each brain were incubated in a 0.17-M Tris buffer with 4 mM $CaCl_2$ and 2 nM [^{3}H]8-OH-DPAT (224 Ci/mmol; Amersham Life Science, Arlington Heights, IL) for 60 min at room temperature. For D$_2$ receptor binding, the incubation buffer consisted of 0.17 M Tris, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.001% ascorbate and 20 nM [^{3}H]sulpiride (80 Ci/mmol; Amersham Life Science) for 20 min at room temperature (McBride et al., 1993a,b). Nonspecific binding was determined in the presence of 1 μM 5-HT for 5-HT$_{1A}$ binding or 1 μM haloperidol for D$_2$ binding. Slides were rinsed in radiolabel-free buffer 2×5 min, then were quickly dipped in glass distilled H_2O and dried rapidly with a cold-air dryer. When completely dry (overnight), slides were apposed to sheets of tritium-sensitive Ultrofilm (LKB, Mager Scientific, Dexter, MI) and placed in X-ray cassettes with calibrated high-activity [^{3}H] microscale standards (Amersham). Each cassette contained one set of standards and sections for total and nonspecific binding from the same brain areas of P and NP rats. The cassettes were sealed and stored at 4 °C for 30 days for [^{3}H]8-OH-DPAT binding and 30–90 days for [^{3}H]sulpiride binding.

2.3. Data analysis

Films were processed in Kodak D19 Developer, fixed by standard procedures and air dried. Autoradiograms were digitized and analyzed using NIH IMAGE for Macintosh computers. Brain areas were delineated according to the rat brain atlas of Paxinos and Watson (1986). In most cases, a total of five to six bilateral readings were taken for each area, nuclei or subregion for each animal. Specific binding was determined by subtraction of nonspecific binding from total binding in adjacent sections. The mean value of readings taken in each region or subregion for each individual animal was then grouped by rat line and was analyzed by region or subregion.

Data are presented in units of fmol/mg tissue (means ± S.E.M.). Statistical analysis was performed using either a one-way ANOVA for regions without subregional divisions or a two-way mixed ANOVA (region × line) for areas that had subregions with repeated measures for region and post hoc Neuman–Keuls tests. Due to the high number of multiple comparisons made with the two-way mixed ANOVA, the significance level was adjusted to $P<.01$ for that test. For all other comparisons, a significance level of $P<.05$ was used.

3. Results

3.1. [^{3}H]8-OH-DPAT binding

The binding densities of [^{3}H]8-OH-DPAT in the prefrontal, cingulate, frontal and parietal cortices are displayed graphically in Fig. 1. A one-way ANOVA revealed significant differences in [^{3}H]8-OH-DPAT binding between peri-

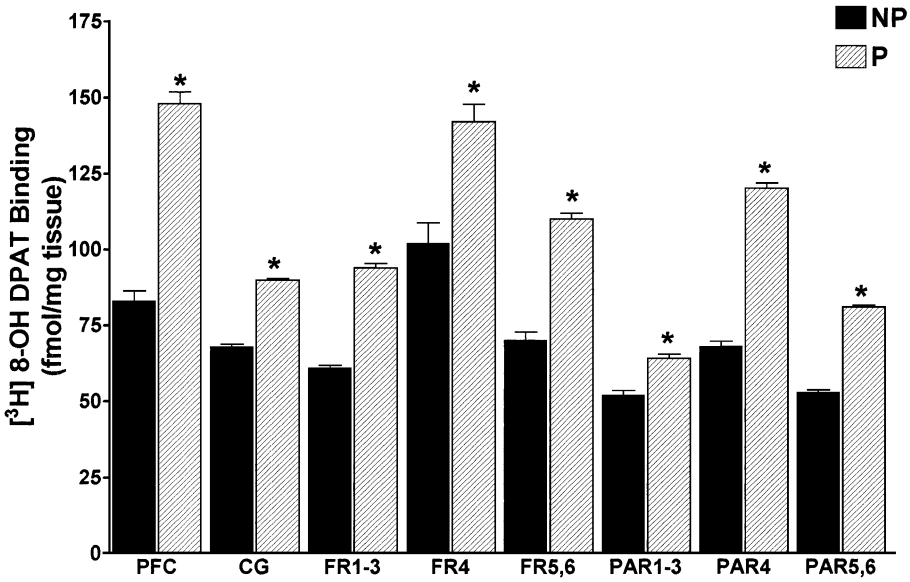

Fig. 1. [^{3}H]8-OH-DPAT binding in subregions of the anterior cerebral cortex of periadolescent P and NP rats. Values are the mean ± S.E.M.; $n = 7$ for NP and $n = 8$ for P rats, * $P < .05$. Abbreviations: PFC, prefrontal cortex; CG, cingulate cortex; FR1–3, FR4, FR5–6, frontal cortex layers 1–3, 4, 5–6; PAR1–3, PAR4, PAR5–6, parietal cortex layers 1–3, 4, 5–6.

adolescent P and NP rats in the prefrontal cortex [$F(1,14) = 178.9$, $P < .0001$] and in the cingulate cortex [$F(1,14) = 524.1$, $P < .0001$], with P rats having greater binding in these areas compared with NP rats. Mixed two-way ANOVAs were used to determine differences between the two rat lines among the layers within the frontal and parietal cortices. In the frontal cortex, there were significant differences in line [$F(1,44) = 134.2$, $P < .0001$] and layer [$F(2,44) = 86.1$, $P < .0001$], but no Line × Layer interaction [$F(2,44) = 3.58$, $P = .0424$]. Post hoc analysis revealed that Layer 4 had the highest level of [^{3}H]8-OH-DPAT binding, followed by Layers 5–6 and lowest levels in Layers 1–3. Post hoc tests also showed that P rats had significantly ($P < .05$) higher levels of [^{3}H]8-OH-DPAT binding than did NP rats. In the parietal cortex, there were significant differences in line [$F(1,44) = 753.1$, $P < .0001$], layer [$F(2,44) = 327.8$, $P < .0001$] and Line × Layer interaction [$F(2,44) = 174.3$, $P < .0001$]. Post hoc analysis revealed that Layer 4 had the highest levels of [^{3}H]8-OH-DPAT binding and that there was no difference in binding levels between Layers 1–3 and 5–6. Post hoc tests also showed that P rats had significantly ($P < .05$) higher [^{3}H]8-OH-DPAT binding in all layers compared with NP rats.

Fig. 2 illustrates [^{3}H]8-OH-DPAT binding in the retrosplenial, occipital, temporal and entorhinal cortices. A one-way ANOVA revealed no significant differences in [^{3}H]8-OH-DPAT binding between periadolescent P and NP rats in the retrosplenial cortex [$F(1,14) = 3.54$, $P = .08$]. A one-way ANOVA revealed significant differences in binding in the entorhinal cortex [$F(1,14) = 497.9$, $P < .0001$], with higher binding levels in the P rats as compared with the NP rats. Mixed two-way ANOVAs were used to determine differences between the two rat lines among the layers within the occipital and temporal cortices. In the occipital cortex, there were significant differences in line [$F(1,29) = 680.4$, $P < .0001$], but not in layer [$F(1,29) = 4.79$, $P = .04$], nor was there a Line × Layer interaction [$F(1,29) = 5.48$, $P = .04$]. In the temporal cortex, there were significant differences in line [$F(1,29) = 218.0$, $P < .0001$], layer [$F(1,29) = 20.0$, $P < .001$] and Line × Layer interaction [$F(1,29) = 24.2$, $P < .0005$]. Post hoc analysis showed that binding was not significantly different between Layers 1–3 and 4–6, but that [^{3}H]8-OH-DPAT binding was significantly higher in the P rats compared with NP rats.

Fig. 3 depicts [^{3}H]8-OH-DPAT binding in the anterior and posterior hippocampus. A mixed two-way ANOVA was used to determine differences between the two rat lines among the different subregions of the anterior and posterior hippocampus. There were significant differences in line [$F(1,119) = 248.0$, $P < .0001$], subregion [$F(7,119) = 1996$, $P < .0001$] and Line × Subregion interaction [$F(7,119) = 26.0$, $P < .0001$]. Post hoc analysis revealed that there were significant differences in the levels of [^{3}H]8-OH-DPAT binding in all regions of the posterior hippocampus compared with the anterior hippocampus. There were no significant differences in binding between the P and NP rats in the anterior hippocampus. In the posterior hippocampus, post hoc tests determined that binding was greatest in the dorsal dentate gyrus and lowest in the CA3 subfield. Significant differences in [^{3}H]8-OH-DPAT binding were found between P and NP rats in all five subregions of the posterior hippocampus.

Table 1 indicates [^{3}H]8-OH-DPAT binding in other selected brain regions that were examined. One-way ANOVAs revealed no significant differences in binding in the caudate–putamen [$F(1,14) = 0.372$, $P = .552$], the

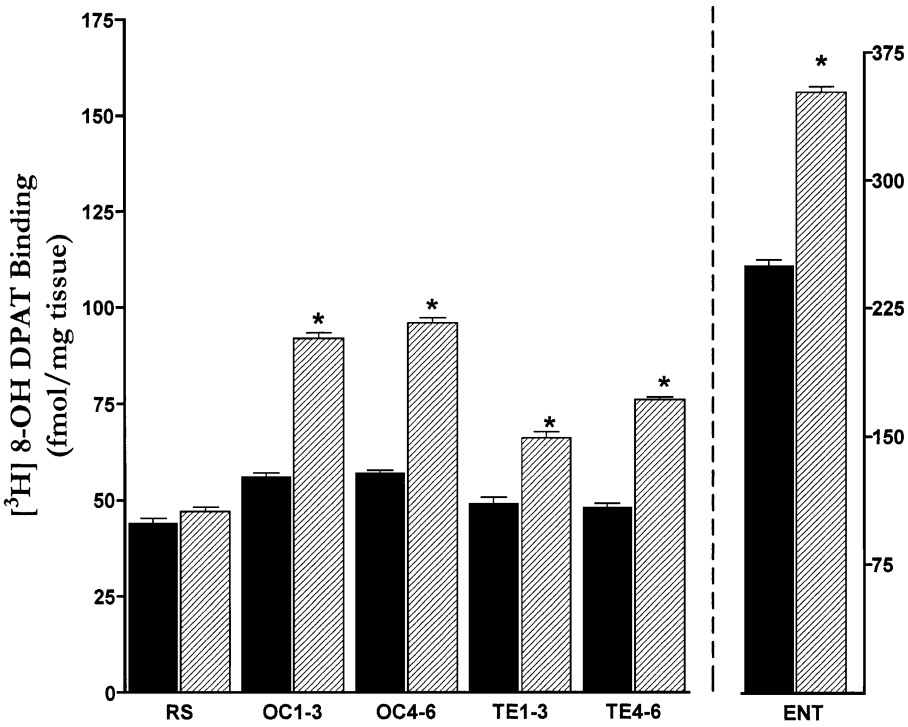

Fig. 2. [^{3}H]8-OH-DPAT binding in subregions of the posterior cerebral cortex of periadolescent P and NP rats. Values are the mean ± S.E.M.; $n = 7$ for NP and $n = 8$ for P rats, * $P < .05$. Abbreviations: RS, retrosplenial cortex; OC1–3, OC4–6, occipital cortex layers 1–3, 4–6; TE1–3, TE4–6, temporal cortex layers 1–3, 4–6; ENT, entorhinal cortex.

nucleus accumbens [$F(1,14) = 3.78$, P=.057], the olfactory tubercles [$F(1,14) = 2.41$, P=.115], the bed nucleus of the stria terminalis [$F(1,13) = 0.015$, P=.903] or the amygdala [$F(1,14) = 0.065$, P=.802]. Mixed two-way ANOVAs were performed on subregions of the septum and hypothalamus. In the septum, there was no significant line difference [$F(1,44) = 0.245$, P=.629], a significant subregion difference [$F(2,44) = 651.35$, $P < .0001$] and no significant Line × Subregion interaction [$F(2,44) = 0.425$, P=.658]. The significant subregion difference was due to the much greater binding found in the lateral intermediate and lateral dorsal nuclei than the medial septum in both lines. In the

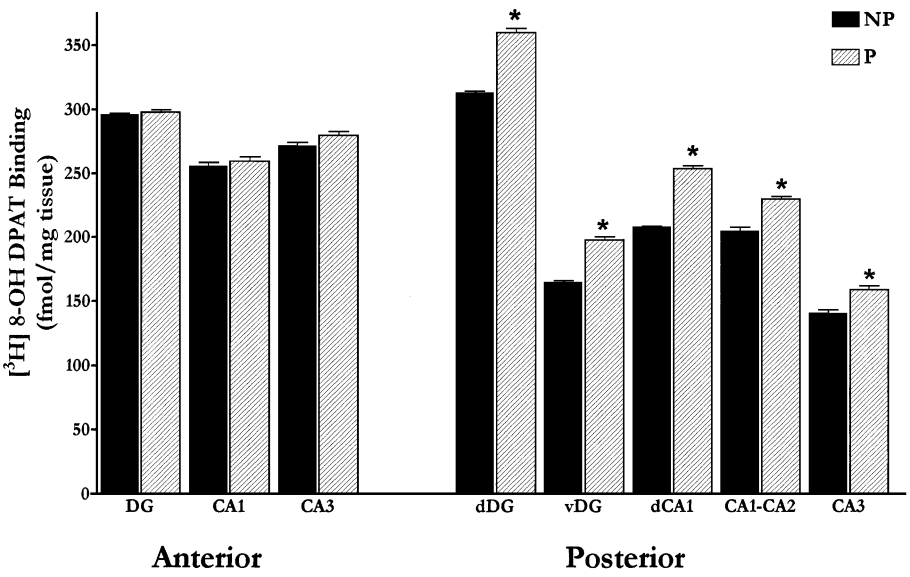

Fig. 3. [^{3}H]8-OH-DPAT binding in subregions of the posterior hippocampus of periadolescent P and NP rats. Values are the mean ± S.E.M.; $n = 7$ for NP and $n = 8$ for P rats, * $P < .01$. Abbreviations: DG, dentate gyrus; CA1 subfield; CA3 subfield; dDG, dorsal dentate gyrus; vDG, ventral dentate gyrus; dCA1, dorsal CA1 subfield.

Table 1
Densities of [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in selected brain regions of postnatal day 25 alcohol-naive P and NP rats (fmol/mg tissue)

Region	NP	P
Caudate–putamen	21.6 ± 0.6	22.1 ± 0.4
Nucleus accumbens	23.5 ± 1.3	24.3 ± 2.2
Olfactory tubercle	49.3 ± 0.9	47.0 ± 1.2
Bed nucleus stria terminalis	68.7 ± 1.6	67.9 ± 1.2
Septal nuclei		
lateral intermediate	333 ± 8	342 ± 7
lateral dorsal	319 ± 3	317 ± 2
medial	137 ± 2	136 ± 2
Hypothalamus		
medial preoptic	71.1 ± 2.9	71.4 ± 1.7
lateral preoptic	51.2 ± 1.5	54.2 ± 1.1
ventromedial	63.9 ± 1.7	62.8 ± 2.0
Amygdala	116 ± 2	117 ± 4

There were no significant differences between P and NP pups in these regions.

hypothalamus, there was no line difference [$F(1,44) = 1.25$, P=.28], a significant subregion difference [$F(2,44) = 140.3$, $P < .0001$], and no Line × Subregion interaction [$F(2,44) = 1.80$, P=.186]. The significant subregion difference was due to the higher binding levels in the medial preoptic area as compared to the lateral preoptic area and the ventromedial nucleus in both lines.

3.2. [^{3}H]sulpiride binding

The binding densities of [^{3}H]sulpiride in selected brain regions of periadolescent P and NP rats are graphically depicted in Fig. 4. All analyses were done with one-way ANOVAs. The VTA was found to be significantly different [$F(1,12) = 5.47$, P=.0383], with P rats having lower binding levels than the NP rats. All other regions were found not to differ. The F and P values are as follows: anterior striatum [$F(1,11) = 3.75$, P=.08]; posterior striatum [$F(1,11) = 0.132$, P=.724]; nucleus accumbens [$F(1,11) = 2.38$, P=.154]; olfactory tubercles [$F(1,11) = 3.49$, P=.09]; substantia nigra pars compacta [$F(1,11) = 2.41$, P=.15]; substantia nigra pars reticulata [$F(1,11) = 2.68$, P=.13].

4. Discussion

The present results indicate that there are significant differences between the periadolescent P and NP rat lines in the binding densities of 5-HT$_{1A}$ and D$_2$ receptors at an age when disparate drinking behaviors are also observed. [^{3}H]8-OH-DPAT binding was higher in most of the cortical regions examined (Figs. 1 and 2) and in the posterior regions of the hippocampus of the P compared to NP rat pups (Fig. 3). All other regions examined were not statistically different between the two lines (Table 1). [^{3}H]sulpiride binding was significantly lower in the VTA of the periadolescent P rats as compared with the NP rat pups (Fig. 4). It should be noted here that although we used [^{3}H]sulpiride to demonstrate DA D$_2$ receptors, it is possible that D$_3$ receptor binding may also be reflected by the concentration of [^{3}H]sulpiride used in this study, particularly in the ACB where D$_2$ and D$_3$ receptors are prevalent (Mansour and Watson, 1994). However, a previous study from our laboratory has demonstrated no significant alter-

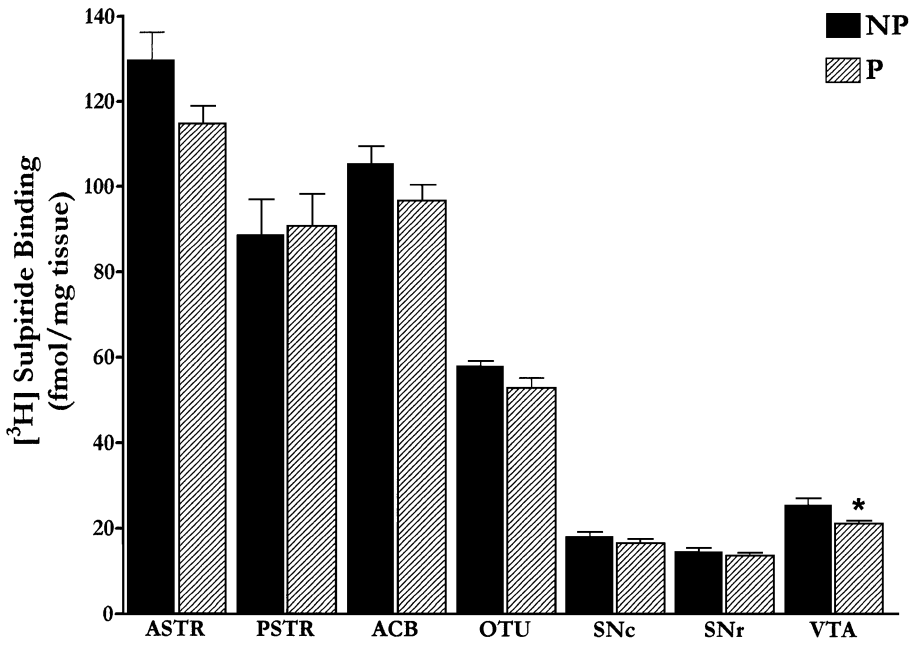

Fig. 4. [^{3}H]sulpiride binding in CNS regions of periadolescent P and NP rats. Values are the mean ± S.E.M.; $n = 7$ for NP and $n = 8$ for P rats, $^*P < .05$. Abbreviations: ASTR, anterior striatum; PSTR, posterior striatum; ACB, nucleus accumbens; OTU, olfactory tubercles; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area.

ations in D_3 receptor binding using [^{3}H]7-OH-DPAT between adult P and NP rats (McBride et al., 1997). In the present study, [^{3}H]sulpiride binding in the VTA almost certainly reflects DA D_2 receptors due to the fact that neither D_3 receptor mRNA nor ligand binding have been found in the VTA of rats (Mansour and Watson, 1994). All other regions examined with [^{3}H]sulpiride were not statistically significant (Fig. 4). Because divergent alcohol drinking behaviors are already exhibited around this age (McKinzie et al., 1998), these results suggest that the innate differences in the 5-HT_{1A} and D_2 receptor systems of the 25-day-old P rat may contribute to the differences in alcohol drinking behaviors between the periadolescent P and NP rats.

The higher densities of 5-HT_{1A} receptors in the cerebral cortical regions and the posterior hippocampus may reflect abnormal development of the 5-HT system, which interferes with the normally programmed reduction in receptors that would usually occur with maturation (Daval et al., 1987; Whitaker-Azmitia, 1991). Furthermore, the higher densities of 5-HT_{1A} receptors in the prefrontal, frontal and cingulate cortical areas, and in the posterior hippocampus of the P than NP rat may be associated with their disparate alcohol drinking behaviors (McBride et al., 1990), and the development (Waller et al., 1983) and persistence (Gatto et al., 1987) of alcohol tolerance observed between these selected lines. The prefrontal, frontal and cingulate cortical areas have been implicated in motivated behaviors and in mediating alcohol drinking behavior (Koob et al., 1998; McBride and Li, 1998). Alcohol self-administration enhances 5-HT release in the nucleus accumbens (Weiss et al., 1996), and injections of ethanol also increase 5-HT release in the frontal cortex (Portas et al., 1994). Therefore, it is possible that ethanol drinking may increase 5-HT neuronal activity in anterior cerebral cortical areas and the released 5-HT may act at 5-HT_{1A} receptors in this region to regulate alcohol drinking. Systemic administration of 8-OH-DPAT has been shown to reduce alcohol drinking in P rats (McBride et al., 1990). This reduction in alcohol intake could occur as a result of 8-OH-DPAT mimicking the actions of ethanol at 5-HT synapses.

The P line of rats develops tolerance more readily than the NP line (Waller et al., 1983), and tolerance once developed persists longer in the P than in the NP line (Gatto et al., 1987). The 5-HT system projecting from the median raphe nucleus has been implicated in the development of tolerance (Le et al., 1981). Because 5-HT innervation of the ventral hippocampus, which makes up the largest proportion of the posterior hippocampus, comes mainly from the median raphe nucleus (Azmitia and Segal, 1978), it is possible that this region plays a role in the development and persistence of tolerance, and the differences in 5-HT_{1A} receptors observed in the posterior hippocampus between the P and NP lines may play a role in the differences observed in the development and persistence of tolerance.

The single difference found in the densities of the D_2 receptors between the P and NP lines in the VTA cell body region but not in the substantia nigra cell body region may indicate that the mesolimbic DA system may be more sensitive to alterations in developmental factors that regulate the expression of the D_2 receptor. However, it is not known what factors may have contributed to this difference in only one cell body region. The lower densities of DA D_2 receptors within the VTA of the P rat likely indicate reduced numbers of D_2 autoreceptors. There is evidence that ethanol self-administration increases the release of DA within the nucleus accumbens of P rats to a greater extent than observed for Wistar rats (Weiss et al., 1993). If the mesolimbic DA system is involved in regulating alcohol drinking, then a reduction in negative feedback regulation could lead to ‘a loss of control’, resulting in higher ethanol intakes. The importance of D_2 autoreceptors within the VTA in regulating alcohol drinking is illustrated by the findings that microinjections of D_2 agonists into the VTA reduced ethanol intake of P rats (Nowak et al., 2000). In addition, another study indicated that overexpression of D_2 receptors within the nucleus accumbens reduced alcohol self-administration (Thanos et al., 2001). These studies point out the importance of D_2 receptors within the mesolimbic region in regulating alcohol intake, and that alterations in the densities of this receptor could have a significant impact on ethanol intake.

Although it is possible to speculate about the possible roles that 5-HT_{1A} and D_2 receptors may have, it is not possible with the present measures to give any definitive interpretation of their roles in alcohol drinking or other behaviors. In addition, interpretation of the data is limited because differences in binding densities do not distinguish between alterations in affinity or number of receptors, nor does the autoradiography procedure delineate functional activity or second messenger coupling of the receptor, which also may be occurring.

5-HT_{1A} receptor binding has been shown to reach adult levels in the cerebral cortex, hippocampus and septum by the third postnatal week (Daval et al., 1987). The current results correlate well with binding studies in adult P and NP rats. 5-HT_{1A} receptor binding with [^{3}H]8-OH-DPAT in adult alcohol-naive P and NP rats was found to be 15–40% higher in most cortical regions examined and 10–15% higher in the posterior regions of the hippocampus of the P rat compared with the NP rat (McBride et al., 1994). Membrane binding studies using [^{3}H]8-OH-DPAT revealed the differences in the cortical regions to be due to a greater number of binding sites in the P line, i.e. change in B_{max} (Wong et al., 1993). Although membrane binding studies have not yet been performed on periadolescent P and NP animals, it would seem likely that the differences in cortical binding found in the periadolescent rats would also be due to a change in the number of receptors.

DA D_2 autoradiography studies in alcohol-naive adult P and NP rats have shown that [^{3}H]sulpiride binding was 20–25% lower in the caudate–putamen, nucleus accumbens and VTA of P rats compared with NP rats (McBride et al., 1993a,b). Although the caudate–putamen (ASTR and PSTR in Fig. 4) and nucleus accumbens were not significantly

different in the current study, there was a trend toward lower binding levels in these regions in the P rat pups. The dopaminergic receptor system is still in a state of dynamic flux at postnatal day 25 in the rat with evidence of receptor overproduction and later pruning in development. Two recent studies suggest this is particularly true of the caudate–putamen and nucleus accumbens, where D_2 receptor levels peaked by postnatal day 28 and decreased significantly by postnatal day 35 and remained at the lower levels through adulthood (Tarazi et al., 1998, 1999). These results may explain why D_2 receptor binding in the present study had not yet achieved the adult-like pattern of the P and NP rat lines. It is also possible that in the nucleus accumbens D_2 receptor expression in cholinergic interneurons (Jongen-Relo et al., 1995) and GABA projection neurons (Lu et al., 1998) may have masked D_2 autoreceptor densities at this stage of development. That stated, the small, but significant, changes in D_2 receptor binding levels in the VTA of the P rat pup may indicate that the VTA plays a role in the alcohol drinking behaviors in the young animal. This will have to be investigated by future studies. In adult membrane binding preparations, [^{3}H]sulpiride binding established that the differences in D_2 binding were due to changes in B_{max}, reflecting differences in receptor number between P and NP rats (McBride et al., 1993a,b). However, the VTA was not specifically looked at in the McBride et al. (1993a,b) study, due to difficulty in isolating the VTA by dissection. Membrane binding studies have not yet been performed in periadolescent rats, therefore it is not clear if the observed difference in the VTA found here would also be due to changes in B_{max}. The differences in 5-HT_{1A} and D_2 receptor binding reported here in the periadolescent animal and previously in the adult P rat suggests that these neurotransmitter systems are fundamentally (i.e. in development) changed as a likely consequence of genetic selection for alcohol preference.

There is abundant evidence to support the role of altered serotonergic and dopaminergic neurotransmitter systems in high alcohol drinking, particularly in the genetically selected rodent lines (for review, see McBride and Li, 1998). In the P and NP rat lines, not only are there differences in the 5-HT_{1A} and D_2 receptor systems discussed above, but also in 5-HT_{1B} (McBride et al., 1997), 5-HT_2 (McBride et al., 1993a,b) and 5-HT_{2C} (Pandey et al., 1996) receptors between P and NP adult rats. D_1 and D_3 DA receptors do not appear to differ between the two rat lines (McBride et al., 1997). In addition to receptor differences, regional contents of 5-HT and DA are altered (Murphy et al., 1982, 1987) as well as innervation (Zhou et al., 1994, 1995) in the adult P animal. The pharmacological manipulation of the two receptor systems has also been shown to alter drinking behavior in the P rats as well as in other high alcohol selected rodent lines (see McBride and Li, 1998 for review). However, the complexity of alcohol drinking certainly involves many other neurotransmitter systems. The opioid, cholinergic, GABAergic and glutaminergic systems have all been implicated, as well. The vast majority of the studies have been done in adult animals, leaving the systems and neurocircuitry at work in the adolescent animal to little more than speculation.

Alcohol abuse among adolescents is a worldwide problem and in humans, a major topic of study (for review, see Bauman and Phongsavan, 1999; Windle and Windle, 1999). Children of alcoholics (COAs) have been perhaps the most extensively studied population and lend many clues to the genetics and risk factors involved in the development of alcoholism (for review, see Enoch and Goldman, 1999; Johnson and Leff, 1999). The paucity of adolescent rodent work is beginning to improve. Although relatively few studies have been done specifically on adolescent P rats, they have thus far demonstrated the usefulness of selected lines in studying the development of high alcohol drinking behaviors, particularly in relation to the human COA literature. Periadolescent and adolescent P rat pups readily acquire high voluntary alcohol drinking by 3–4 weeks of age and consume amounts comparable to adults by 38–41 days of age (McKinzie et al., 1998). COAs and others with a family history of alcoholism often report an early age of heavy drinking onset, either before or during adolescence (Schuckit and Russell, 1983; Morrison et al., 1995). Adolescent ethanol exposure in P rats has been shown to lead to long-lasting changes in the adult P rat. Salimov et al. (1996) found adult P rats that consumed alcohol during adolescence had reduced novelty-induced anxiety in a cross-maze and a lower stress response in a slip-funnel test as compared with alcohol-naive P rats. This study suggests that adolescent alcohol exposure may lead to long-term changes in adulthood. Although adult COAs have been reported to be at higher risk for anxiety and mood disorders over a nonrisk population (Cuijpers et al., 1999) and may have impaired startle reflexes as children (Grillon et al., 1997), several studies have found that alcohol reduces anxiety measures from baseline in young male COAs (Newlin and Thomson, 1990; Grillon et al., 2000). Finally, the current study was undertaken to continue to bridge the adult P work with emerging adolescent studies. We demonstrate here that there are innate differences in the 5-HT_{1A} and D_2 receptor systems in the periadolescent P rat that are similar to those found in the adult P animal. These intrinsic alterations may contribute to the high alcohol drinking behaviors seen in the adolescent P rat, as they appear to in the adult animal.

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