Available online at www.sciencedirect.com

SCIENCE DIRECT

Pharmacology, Biochemistry and Behavior 80 (2005) 229–237

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Dopamine and serotonin content in select brain regions of weanling and adult alcohol drinking rat lines

Wendy N. Strother[a,*], Lawrence Lumeng[b,c,d], Ting-Kai Li[b,c,1], William J. McBride[a,c]

[a]*Department of Psychiatry and Institute of Psychiatric Research, 791 Union Drive, Indianapolis, IN 46202-4887, United States*
[b]*Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202, United States*
[c]*Department of Biochemistry, Indiana University School of Medicine, Indianapolis, IN 46202, United States*
[d]*VA Medical Center, Indianapolis, Indianapolis, IN 46202, United States*

Received 5 February 2004; received in revised form 11 November 2004; accepted 16 November 2004
Available online 8 December 2004

Abstract

The objective of the present study was to examine innate differences in the tissue content of dopamine (DA), serotonin (5-HT) and their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in five brain regions of weanling and adult alcohol-preferring (P), alcohol-nonpreferring (NP), high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) selected rat lines. Adult male and weanling (postnatal day 25) male rats were killed by decapitation and brains were rapidly dissected for the following regions: olfactory tubercles (OTU), nucleus accumbens (ACB), septum (SEP), anterior cerebral cortex (ACTX) and amygdala (AMYG). Tissue extracts were assayed by HPLC with electrochemical detection. Due to significantly higher content levels in the adults, adult and weanling animals were analyzed separately. Significant differences were found in the ACB and OTU between the adult lines in both DA and 5-HT content, with P and HAD rats having lower levels than NP and LAD rats, respectively. Significant differences in DA content between the weanling lines were also found in the OTU, with P and HAD rats having lower DA levels than NP and LAD rats, respectively. These results confirm previous findings of an association between innate low DA content in select limbic regions and high alcohol drinking behavior.

Keywords: P; NP; HAD; LAD; Dopamine; Serotonin; Alcohol drinking

1. Introduction

Studies with lines of rats selectively bred for high or low alcohol intake suggest that differences in the dopamine (DA) and serotonin (5-HT) systems may underlie their disparate alcohol intakes. Findings with the alcohol-preferring (P) and -nonpreferring (NP) lines indicate that the tissue levels of 5-HT and/or its primary metabolite, 5-hydroxyindoleacetic acid (5-HIAA) were lower in several CNS regions of the P line, e.g., cerebral cortex, striatum, nucleus accumbens (ACB), olfactory tubercles (OTU), hippocampus and hypothalamus (McBride et al., 1993b; Murphy et al., 1982, 1987). Studies conducted with the F2 generation of P×NP intercrosses support an association between low contents of 5-HT in the nucleus accumbens and high alcohol preference (McBride et al., 1995). Lower contents of 5-HT and/or 5-HIAA have also been reported in parts of the cerebral cortex, posterior striatum, nucleus accumbens, septum (SEP), hippocampus and hypothalamus of the high-alcohol-drinking (HAD) than low-alcohol-drinking (LAD) rats from replicate line 1 (Gongwer et al., 1989). In addition, an inverse relationship between alcohol intake and brain 5-HT levels has also been found for several inbred strains of mice (Yoshimoto and Komura, 1987). However, similar differences in regional CNS levels of 5-HT and/or 5-

* Corresponding author. Tel.: +317 274 2333; fax: +317 274 1365.
E-mail address: wstrothe@iupui.edu (W.N. Strother).
[1] Present address: NIAAA, The Willco Building, 6000 Executive Boulevard, Suite 400, Bethesda, MD 10892-7003, United States.

0091-3057/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.11.003

HIAA have not been found between the ALKO alcohol-drinking (AA) and the ALKO nonalcohol (ANA) rat lines (Korpi et al., 1988).

The lower contents of 5-HT observed in the CNS of P rats compared to NP rats (Murphy et al., 1982, 1987) appear to be due to reduced 5-HT innervation. Zhou et al. (1991, 1994a) reported that immunoreactive 5-HT fibers were lower in several CNS regions of the P rat line compared to the NP line, e.g., frontal cortex, nucleus accumbens, hippocampus. Furthermore, the lower densities of 5-HT immunostained fibers in the CNS of the P line compared to the NP rat line may be a result of fewer 5-HT neurons in the medial and dorsal raphe of P rats (Zhou et al., 1994b).

Compared with NP rats, P rats also have lower contents of DA and its major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the cerebral cortex, nucleus accumbens and olfactory tubercles (McBride et al., 1993b; Murphy et al., 1982, 1987). In agreement with these results, male rats with high alcohol intakes in the F2 generation from P×NP intercrosses were shown to have a 25% lower content of DA in the nucleus accumbens compared with low alcohol drinkers (McBride et al., 1995). Similar differences were not found in the nigrostriatal DA system (Murphy et al., 1982, 1987). Gongwer et al. (1989) reported lower contents of DA, DOPAC and HVA in the nucleus accumbens and anterior striatum of the HAD compared to the LAD line. In addition, C57BL/6J mice, which demonstrate high alcohol-drinking behavior, have lower contents of DA in the olfactory tubercle and hypothalamus compared to DBA/2J and BALBc mice, which both display low alcohol intakes (George et al., 1995).

The lower contents of DA in the nucleus accumbens and cerebral cortex in P rats (McBride et al., 1993b; Murphy et al., 1982, 1987) may also be a result of reduced innervation. Zhou et al. (1995) reported lower densities of dopaminergic fibers in the cingulate cortex and shell of the nucleus accumbens of the P rat compared to the NP rat. This study also demonstrated that the subpopulation of DA neurons in the ventral tegmental area (VTA) projecting to the nucleus accumbens was decreased in the P rat line compared to the NP line.

The mesolimbic DA system, including its 5-HT innervations, is considered to be involved in regulating alcohol drinking behaviors (Koob et al., 1998; McBride and Li, 1998). The findings to date suggest that differences in DA and 5-HT function may be important factors contributing to the disparate alcohol drinking behaviors of some of the selectively bred rat lines. If the lower DA and 5-HT content differences are associated with high alcohol intakes of the P and HAD lines, then such differences might be expected to be found at the age of onset of high alcohol intakes in these lines. McKinzie et al. (1998) reported that P rats initiated free-choice intake of 10% ethanol as early as postnatal days (PNDs) 22–25. In addition, HAD rats from both replicate lines readily initiated ethanol drinking during adolescence (McKinzie et al., 1996, 1998). Therefore, the present study was undertaken to test the hypothesis that lower contents of DA, 5-HT and/or their major metabolites are associated with high ethanol intakes in P and HAD rat pups at the age of onset of high alcohol drinking behavior. Furthermore, because the VTA dopaminergic system innervates the ACB, OTU, AMYG, SEP and ACTX, and these structures are involved in motivated behaviors, the present study focused on determining the contents of DA and 5-HT in these five regions of weanling and adult P, NP, HAD and LAD rats.

2. Methods

Animals were obtained from the breeding colonies generated and maintained at Indiana University School of Medicine, Indianapolis, Indiana. Adult alcohol naïve P, NP (44th generation; N=10), HAD and LAD (34th generation, replicate line 1; N=10) male rats between 90 and 120 days old were used. Weanling alcohol naïve P, NP (44th generation; N=10, 8), HAD and LAD (30th generation, replicate line 1; N=8, 10) male rats were used on postnatal day 25. Rats were double housed in colony rooms with 12:12 lighting (lights on at 0800) with food and water available ad libitum. 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).

Rats were handled daily for at least 1 week and were killed between the hours of 1300–1500. Rats were decapitated and whole heads were immersed in isopentane (−50 °C) over dry ice for 10 s. Brains were rapidly removed and dissected in a cold box maintained at −20 °C. The brains were dissected according to the procedure previously published (Gongwer et al., 1989) into the following five regions: olfactory tubercles, anterior cortex, nucleus accumbens, septum and amygdala. Brain regions were frozen in isopentane over dry ice and individually wrapped in foil. Tissue was stored at −70 °C until homogenized. Tissue was homogenized in 0.5 M perchloric acid (15 ml/g tissue) over ice with a Dremel Tissue Tearer (Fisher Scientific). Samples were placed on ice for 30 min with vortexing every 10 min. Homogenates were then centrifuged at 2000 rpm at −4 °C for 15 min. Supernatants were placed into microcentrifuge filter tubes (Denville Scientific) and centrifuged at 7000×g for 15 min. Filters were removed and liquid samples were stored at −70 °C until assayed for the contents of DA, DOPAC, HVA, 5-HT and 5-HIAA by HPLC with electrochemical detection according to previously published procedures (Murphy et al., 1982). Due to higher content levels in the adult animals, adult and weanling animals were

analyzed separately. Statistical differences of tissue compounds were determined by two-way repeated-measures ANOVAs (line×compound) for each brain region. The three dopamine compounds (DA, DOPAC and HVA) were grouped, and 5-HT was grouped with 5-HIAA for statistical analysis. Significant interactions between line and compounds were followed up with one-way ANOVAs across line for each compound (DA, DOPAC, HVA, 5-HT, 5HIAA) and unpaired Student's *t*-tests for direct line comparisons.

3. Results

3.1. Adults

The regional brain contents for DA and 5-HT in the adult and weanling rats are shown in Figs. 1 and 2; values for the metabolites of DA and 5-HT for adult P, NP, HAD and LAD rats are shown in Table 1, whereas similar weanling data are shown in Table 2.

3.1.1. Nucleus accumbens

A two-way RM ANOVA by dopaminergic compounds showed a significant effect of compound [$F(2,72)=940.52$; $p<0.0001$], line [$F(3,36)=9.52$; $p<0.0001$] and compound×line interaction [$F(6,72)=2.92$; $p<0.01$] in the adults. Follow-up one-way ANOVAs revealed that DA [$F(1,38)=8.13$; $p<0.01$], DOPAC [$F(1,38)=13.56$; $p=0.001$] and HVA [$F(1,38)=5.212$; $p<0.05$] were all significantly different among the lines. Unpaired Student's *t*-tests found DA and DOPAC to be significantly lower in the P rats compared to NP rats and lower in HAD rats compared to LAD rats (Fig. 1; Table 1). The P line was not different from the HAD line and NP rats were not different from the LAD rats. HVA was found to be significantly lower in the P rats compared to NP rats, but not in HAD rats compared to LAD rats. The P and HAD rat groups were not

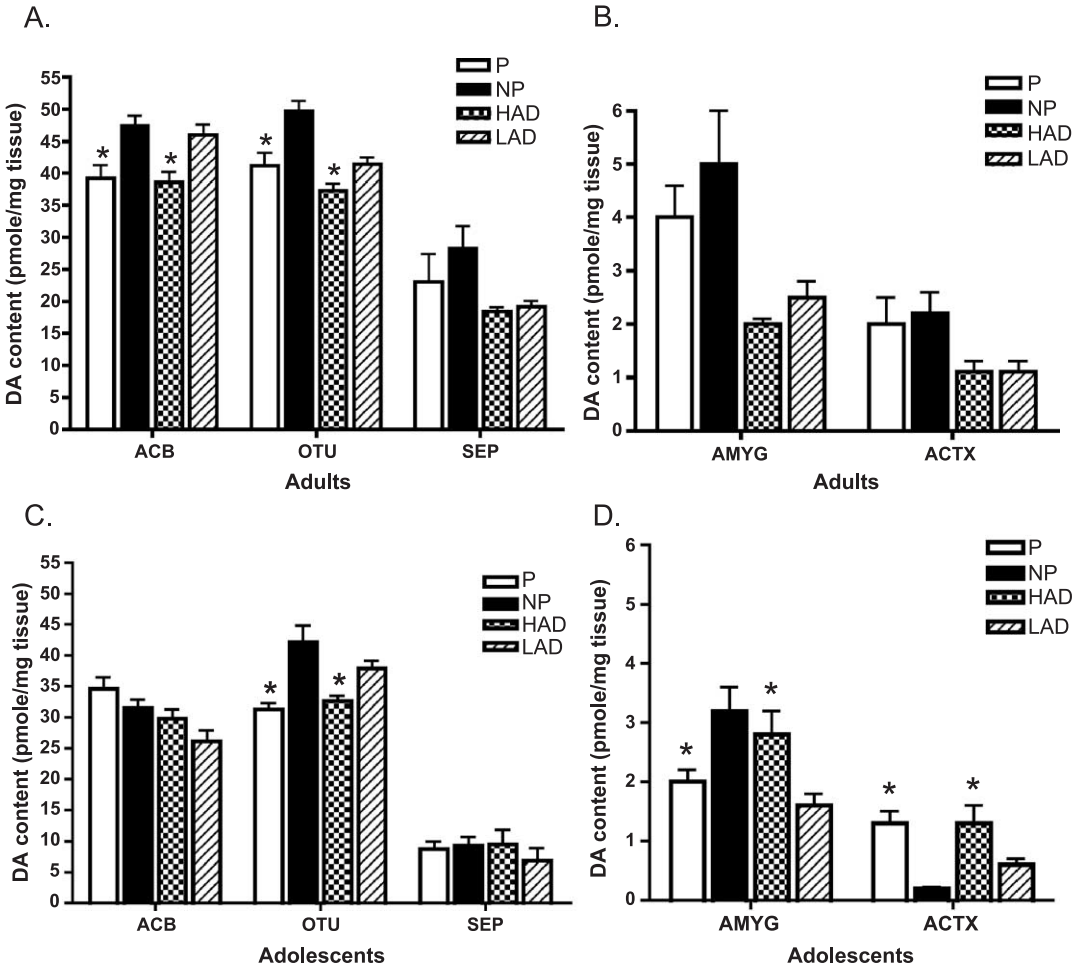

Fig. 1. Dopamine (DA) content (pmol/mg tissue) in the five brain regions between adult (A, B) and periadolescent (C, D) P, NP, HAD and LAD rat lines. (A) Adult P and HAD rats had lower DA content in the nucleus accumbens (ACB) and olfactory tubercles (OTU) compared to the NP and LAD rats, respectively. (B) There were no significant differences in the amygdala (AMYG) nor anterior cortex (ACTX) in the adult rat lines. (C) Similar to the adults, periadolescent P and HAD rats had lower DA content in the OTU compared to the NP and LAD rats, respectively. (D) Unlike the adult rats, periadolescent P and HAD rats had higher DA content in the ACTX compared to the NP and LAD rats, respectively. Data are expressed as means±S.E.M. *Indicates a significant difference ($p\leq0.05$) between P versus NP and between HAD versus LAD.

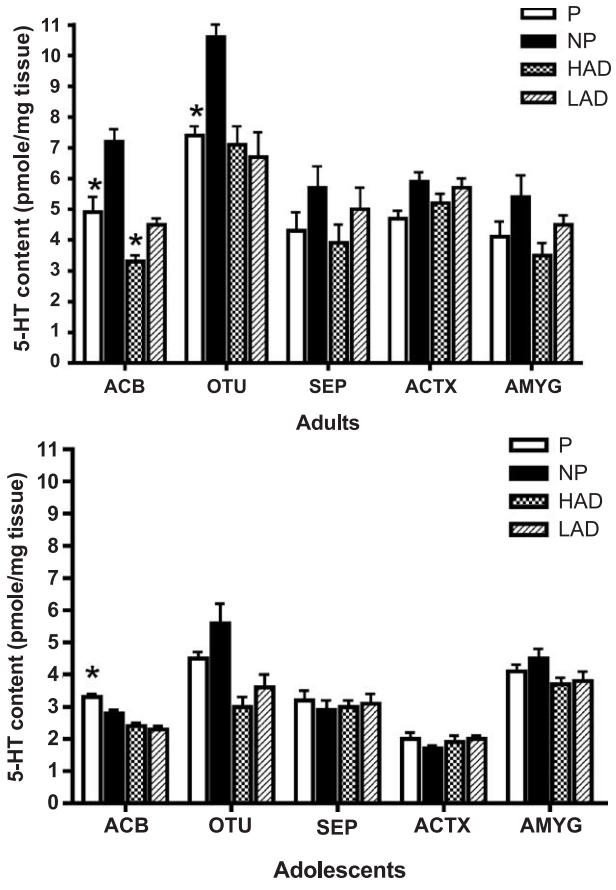

Fig. 2. Serotonin (5-HT) content (pmol/mg tissue) in the five brain regions between adult (Top) and periadolescent (Bottom) P, NP, HAD and LAD rat lines. 5-HT levels were lower in the ACB of adult P and HAD rats compared to NP and LAD rats, respectively, and in the OTU of adult P rats compared to NP rats. Unlike the adult rats, periadolescent P rats had higher 5-HT content in the ACB compared to NP rats. Data are expressed as means±S.E.M. *Indicates a significant difference ($p \leq 0.05$) between P versus NP and between HAD versus LAD.

significantly different in their levels of HVA, but NP rats and LAD rats were significantly different.

A two-way RM ANOVA for the serotonergic compounds found no compound effect [$F(1,36)=2.40$; $p=0.13$] nor compound×line interaction [$F(3,36)=0.817$; $p=0.49$], but a significant effect of line [$F(3,36)=14.85$; $p<0.0001$] in the adults. Because of the significant line difference, one-way ANOVAs were performed by compound across line. Both 5-HT [$F(3,39)=16.24$; $p<0.0001$] and 5-HIAA [$F(3,39)=7.26$; $p=0.001$] were significantly different among the lines. The levels of 5-HT were significantly lower in P rats compared to NP rats and in HAD rats compared to LAD rats (Fig. 2). All groups were significantly different from each other, as well. The significant effect of 5-HIAA was due to higher levels in P rats compared to HAD rats and higher levels in NP rats compared to LAD rats (Table 1).

3.1.2. Olfactory tubercles

Analysis in the olfactory tubercles revealed there was a significant effect of dopaminergic compound [$F(2,54)=579.8$; $p<0.0001$], line [$F(3,27)=11.32$; $p<0.0001$] and compound×line interaction [$F(6,54)=2.914$; $p<0.05$]. One-way ANOVAs by compound found DA [$F(3,39)=9.49$; $p<0.001$] and DOPAC [$F(3,39)=5.21$; $p<0.01$] to be significantly different among the lines. Unpaired Student's t-tests found DA levels to be significantly lower in both the P and HAD rats compared to NP and LAD rats, respectively (Fig. 1). The P and HAD rats did not differ in their DA levels, but NP and LAD levels were significantly different from each other. DOPAC levels were only significantly different in the NP rats compared to the LAD rats.

Two-way RM ANOVA by serotonergic compounds demonstrated a significant effect of compound [$F(1,35)=139.40$; $p<0.0001$], line [$F(3,35)=12.29$; $p<0.0001$] and compound×line interaction [$F(3,35)=5.10$; $p<0.005$] in the adults. One-way ANOVAs by component found both 5-HT [$F(3,39)=10.50$; $p<0.0001$] and 5-HIAA [$F(3,39)=9.06$; $p<0.0001$] to be significantly different among the lines (Fig. 2; Table 1). Post hoc analyses revealed 5-HT was significantly lower in P rats compared with NP rats and NP rats significantly different from LAD rats. 5-HIAA levels were significantly different between P and HAD rats and between NP and LAD rats.

Table 1
Metabolite levels (pmol/mg tissue) in adult P, NP, HAD and LAD rats

	DOPAC	HVA	5-HIAA
Nucleus accumbens			
P	11.2±1.7*	3.5±0.4*	4.9±0.6
NP	19.2±2.0	5.6±0.7	6.4±0.6
HAD	14.5±1.0*	3.4±0.3	3.3±0.3
LAD	19.0±1.0	3.5±0.3	3.7±0.6
Olfactory tubercles			
P	12.8±1.4	2.9±0.3	5.7±0.5
NP	15.3±1.7	3.1±0.2	5.8±0.3
HAD	10.4±1.0	2.5±0.5	3.9±0.2
LAD	9.4±0.7	2.8±0.2	3.6±0.4
Septum			
P	12.5±2.0	1.9±0.3	4.8±0.4
NP	11.3±1.0	2.5±0.2	5.9±0.4
HAD	3.1±0.4	1.7±0.3	3.2±0.5
LAD	3.3±0.3	1.7±0.2	4.0±0.5
Amygdala			
P	1.3±0.2	0.3±0.1	3.9±0.3
NP	1.2±0.1	0.3±0.1	4.4±0.2
HAD	1.1±0.2	0.7±0.2	2.7±0.2
LAD	1.6±0.2	0.5±0.04	3.2±0.2
Anterior cortex			
P	0.7±0.1	0.2±0.02	4.2±0.2
NP	0.8±0.1	0.3±0.03	4.4±0.3
HAD	0.7±0.03	0.3±0.02	2.4±0.2
LAD	0.7±0.03	0.3±0.02	2.4±0.2

Data are means±S.E.M.; n=NP, HAD=8; P, LAD=10. n.d.=not detectable.
* Indicates that the value for P or HAD versus NP or LAD, respectively, was significantly ($p<0.05$) different.

Table 2
Metabolite levels (pmol/mg tissue) in adolescent P, NP, HAD and LAD rats

	DOPAC	HVA	5-HIAA
Nucleus accumbens			
P	6.9±0.4*	4.2±0.1	4.8±0.2
NP	12.2±0.6	4.8±0.2	4.8±0.2
HAD	12.9±1.5*	n.d.	4.0±0.3
LAD	8.5±0.8	n.d.	4.2±0.3
Olfactory tubercles			
P	6.7±0.5*	2.4±0.2	4.7±0.2
NP	9.1±1.0	3.0±0.2	5.0±0.3
HAD	5.5±0.3	n.d.	3.3±0.2
LAD	6.1±0.7	n.d.	3.6±0.4
Septum			
P	2.5±0.3	2.2±0.1	5.2±0.3
NP	3.4±0.6	2.4±0.2	4.9±0.4
HAD	3.0±0.5	0.8±0.1	3.4±0.2
LAD	2.6±0.4	1.0±0.2	3.6±0.2
Amygdala			
P	0.3±0.02*	0.2±0.02*	2.4±0.2
NP	0.6±0.06	0.4±0.05	2.7±0.2
HAD	1.3±0.2	0.9±0.1	3.8±0.2
LAD	1.1±0.1	1.0±0.2	4.3±0.2
Anterior cortex			
P	0.4±0.1	0.8±0.1*	1.7±0.2
NP	0.3±0.03	0.5±0.1	1.6±0.2
HAD	1.2±0.2	n.d.	2.2±0.2*
LAD	1.0±0.1	n.d.	2.7±0.1

Data are means±S.E.M.; n=NP, HAD=8; P, LAD=10. n.d.=not detectable.
* Indicates that the value for P or HAD versus NP or LAD, respectively, was significantly ($p<0.05$) different.

3.1.3. Septum

Across the dopaminergic compounds, there was a significant effect of compound [$F(2,68)=360.2$; $p<0.0001$], line [$F(3,34)=12.97$; $p<0.0001$] and a compound×line interaction [$F(6,68)=6.63$; $p\leq0.0001$] in the adults. One-way ANOVAs by compound found DA [$F(3,39)=7.27$; $p=0.001$] and DOPAC [$F(3,38)=19.89$; $p<0.0001$] significantly different among the lines with P and NP rats having higher DA and DOPAC levels compared with HAD and LAD rats, respectively (Fig. 1; Table 1).

A two-way RM ANOVA for the serotonergic compounds (Fig. 2; Table 1) revealed no significant effect of compound [$F(1,36)=1.533$; $p=0.22$] and no compound×line interaction [$F(3,36)=2.57$; $p=0.07$], but a significant effect of line [$F(3,36)=3.23$; $p=0.03$], which was due to higher values in NP than HAD rats.

3.1.4. Amygdala

A two-way repeated-measures ANOVA across line for the three dopaminergic compounds revealed a significant effect of compound [$F(2,68)=71.88$; $p<0.0001$] and a compound×line interaction [$F(6,68)=6.02$; $p<0.0001$], and a tendency for a significant effect of line [$F(3,34)=2.67$; $p=0.06$] in the adults. One-way ANOVAs by compound found DA [$F(3,39)=5.20$; $p<0.01$] and HVA [$F(3,37)=3.62$; $p<0.05$] to be significantly different among the lines. Unpaired Student's t-tests found DA levels to be significantly higher in the P rats compared to HAD rats as well as higher in the NP rats compared to the LAD rats (Fig. 1). Conversely, HVA levels were found to be significantly lower in the P and NP rats compared to the HAD and LAD rats, respectively (Table 1).

Two-way repeated-measures ANOVA for the two serotonergic compounds (Fig. 2; Table 1) found a significant effect of compound [$F(1,36)=16.64$; $p<0.0001$], line [$F(3,36)=5.83$; $p<0.01$] but no compound×line interaction [$F(3,36)=1.31$; $p=0.286$].

3.1.5. Anterior cerebral cortex (ACTX)

A repeated-measures two-way ANOVA across the lines (Fig.1; Table 1) found a significant effect of dopaminergic compounds [$F(2,50)=28.19$; $p<0.0001$], line [$F(3,25)=76.352$; $p<0.0001$], but no compound×line interaction [$F(6,50)=1.27$; $p=0.29$] in adults. A two-way RM ANOVA across the lines by serotonergic compounds (Fig. 2; Table 1) demonstrated a significant effect of compound [$F(1,32)=267.8$; $p<0.0001$], line [$F(3,32)=5.35$; $p<0.005$] and a significant compound×line interaction [$F(3,32)=31.42$; $p<0.0001$]. One-way ANOVAs for compound found a significant main effect of 5-HIAA [$F(3,36)=18.96$; $p<0.0001$]. Unpaired Student's t-tests revealed 5-HIAA levels were significantly higher in the P and NP rats compared to the HAD and LAD rats, respectively.

3.2. Weanlings

Figs. 1 and 2 and Table 2 illustrate the regional brain contents of DA, 5-HT and their metabolites in weanling P, NP, HAD and LAD rats. Note that HVA levels were below detection in many of the HAD and LAD samples (Table 2).

3.2.1. Nucleus accumbens

A two-way RM ANOVA by dopaminergic compounds showed a significant effect of compound [$F(2,30)=485.07$; $p<0.0001$], and compound×line interaction [$F(2,30)=9.29$; $p<0.001$], but no effect of line [$F(1,15)=0.782$; $p=0.39$] in the weanlings. Follow-up one-way ANOVAs revealed that DA [$F(3,33)=4.68$; $p<0.01$] and DOPAC [$F(3,34)=10.28$; $p<0.0001$] were significantly different among the lines (Fig. 1; Table 2). Unpaired Student's t-tests found DA was significantly higher in the NP rat line compared to the LAD line. DOPAC was found to be significantly lower in the P rats compared to NP rats. The P line was not different from the HAD line and NP rats were not different from the LAD rats.

A two-way RM ANOVA for the serotonergic compounds (Fig. 2; Table 2) found a significant compound effect [$F(1,30)=443.2$; $p\leq0.0001$] and effect of line [$F(3,30)=6.50$; $p<0.01$], but no compound×line interaction [$F(3,30)=1.711$; $p=0.19$]. Because of the significant line

difference, one-way ANOVAs were performed by component across line. Both 5-HT [$F(3,33)=15.35$; $p<0.0001$] and 5-HIAA [$F(3,34)=3.02$; $p<0.05$] were significantly different among the lines. Levels of 5-HT were significantly higher in P rats compared to NP and HAD rats and higher in NP rats compared to LAD rats. The significant effect of 5-HIAA was due to higher levels in P rats compared to HAD rats.

3.2.2. Olfactory tubercles

Analysis in the olfactory tubercles revealed there was a significant effect of dopaminergic compound [$F(2,28)=504.1$; $p<0.0001$], line [$F(1,14)=20.50$; $p<0.0001$] and compound×line interaction [$F(2,28)=11.22$; $p<0.0001$]. One-way ANOVAs by compound found DA [$F(3,32)=10.00$; $p<0.0001$] and DOPAC [$F(3,33)=4.72$; $p<0.01$] to be significantly different among the lines (Fig. 1; Table 2). Unpaired Student's *t*-tests found DA levels to be significantly lower in both the P and HAD rats compared to NP and LAD rats, respectively (Fig. 1). DOPAC levels were also significantly lower in the P rats compared to the NP rats, as well as different between the P and HAD rats and between the NP and LAD rats.

Two-way RM ANOVA by serotonergic components demonstrated no significant effect of compound [$F(1,28)=0.067$; $p=0.80$] or compound×line interaction [$F(3,28)=1.73$; $p=0.18$], but a significant line effect [$F(3,28)=9.89$; $p<0.0001$]. The significant line effect was followed up with one-way ANOVAs by compound, which found both 5-HT [$F(3,31)=7.08$; $p<0.001$] and 5-HIAA [$F(3,33)=6.36$; $p<0.005$] levels to be significantly different among the lines (Fig. 2; Table 2). Post hoc *t*-tests found that 5-HT and 5-HIAA was significantly higher in the P and NP rats compared with HAD and LAD rats, respectively.

3.2.3. Septum

Across the dopaminergic components (Fig. 1; Table 2), there was a significant effect of compound [$F(2,52)=69.68$; $p<0.0001$], but no line [$F(3,26)=0.742$; $p=0.53$] or compound×line interaction [$F(6,52)=0.432$; $p=0.85$]. A two-way RM ANOVA for the serotonergic compounds (Fig. 2; Table 2) revealed a significant effect of compound [$F(1,31)=35.71$; $p<0.0001$], line [$F(3,31)=5.68$; $p<0.005$] and compound×line interaction [$F(3,31)=4.56$; $p<0.01$]. Follow-up one-way ANOVAs found a significant difference in 5-HIAA content [$F(3,34)=10.85$; $p<0.0001$] across lines. This difference was due to higher tissue levels in the P and NP rats compared to the HAD and LAD rats, respectively.

3.2.4. Amygdala

A two-way repeated-measures ANOVA across lines for the three dopaminergic compounds (Fig. 1; Table 2) revealed a significant effect of compound [$F(2,58)=105.07$; $p<0.0001$], line [$F(3,29)=4.243$; $p<0.01$] and a compound×line interaction [$F(6,58)=5.79$; $p<0.0001$] in the weanlings. One-way ANOVAs by compound found that the levels of DA [$F(3,34)=3.72$; $p<0.05$], DOPAC [$F(3,33)=12.94$; $p<0.0001$] and HVA [$F(3,34)=12.31$; $p<0.0001$] were significantly different among the lines. Unpaired Student's *t*-tests found DA levels to be significantly lower in the P rats compared to the NP rats, but not significantly different from the HAD or LAD rat levels. Conversely, DA levels in the HAD rats were significantly higher compared to the LAD rats. Unpaired Student's *t*-tests found DOPAC levels to be significantly lower in the P rats compared to the NP rats and HAD rats as well as lower in the NP rats compared to the LAD rats. HVA levels were lower in the P rats compared to NP rats, but these levels did not differ between the HAD and LAD rats lines. Two-way repeated-measures ANOVA for the two serotonergic compounds (Fig. 2; Table 2) found a significant effect of compound [$F(1,31)=28.26$; $p<0.0001$] and a compound×line interaction [$F(3,31)=18.56$; $p<0.0001$], but no significant effect of line [$F(3,31)=2.63$; $p=0.07$]. The levels of 5-HIAA were significantly [$F(3,34)=19.92$; $p<0.0001$] different among the lines with lower levels in the P and NP rats compared to the HAD and LAD rats, respectively.

3.2.5. Anterior cerebral cortex

A repeated-measures two-way ANOVA across the lines found a significant effect of dopaminergic compounds [$F(2,28)=7.23$; $p<0.005$], line [$F(1,14)=25.67$; $p<0.0001$] and a compound×line interaction [$F(2,28)=11.82$; $p<0.0001$]. Follow-up one-way ANOVAs by compound found DA [$F(3,33)=7.74$; $p<0.001$], DOPAC [$F(3,33)=8.87$; $p<0.0001$] and HVA [$F(1,16)=5.62$; $p=0.03$] levels significantly different among the lines (Fig. 1; Table 2). DA levels were higher in the P and HAD rat lines compared to the NP and LAD rat lines, respectively. DOPAC levels were lower in the P rats compared to the HAD rats, and HVA levels were higher in P rats compared to NP rats.

A two-way RM ANOVA across the lines by serotonergic compounds (Fig. 2; Table 2) demonstrated a significant effect of compound [$F(1,31)=10.14$; $p<0.005$], line [$F(3,31)=2.93$; $p<0.05$] and a significant compound×line interaction [$F(3,31)=19.74$; $p<0.0001$]. One-way ANOVAs for component found a significant main effect of 5-HIAA [$F(3,34)=7.13$; $p<0.001$] due to significantly lower levels in the HAD rats compared to the LAD rats.

4. Discussion

The major findings of this study were (a) lower contents of DA were found in the OTU of the weanling and adult P versus NP line and HAD versus LAD line (Fig. 1); (b) lower contents of DA were found only in the ACB of the adults in both pairs of lines (Fig. 1); (c) in contrast to the adult findings, lower contents of 5-HT were not observed in the ACB of the weanling P versus NP rat and HAD versus the LAD rat (Fig. 2); and (d) significantly lower contents of 5-HT were observed in the OTU of adult P versus NP rats, which were

not found between the HAD and LAD lines, although there was a tendency for this difference to be found between the weanling P and NP rats. These results also expand on previous results of tissue content levels in adult selected rat lines (Murphy et al., 1982, 1987; Gongwer et al., 1989; McBride et al., 1995). It should be noted that much of the previous work involved ethanol exposed animals, while the current study is limited to ethanol naïve rats.

The results support a role for differences in the DA system projecting to the OTU in the divergent alcohol drinking behaviors observed during the weanling and periadolescent time periods between the P and NP and HAD and LAD rats (McKinzie et al., 1996, 1998). On the other hand, the present findings do not support a role for differences in the DA or 5-HT system projecting to the ACB as predictors of divergent alcohol intakes in the weanling P–NP and HAD–LAD line pairs. However, interpretations of differences in tissue contents of DA and 5-HT are very limited. Differences in release and neurotransmission could be present between the lines but would not necessarily be detected with measures of tissue levels. Also, developmental differences between the lines may also hinder detecting differences, because regional changes in the monoamine systems are occurring during this early post-weaning period (Tarazi et al., 1998a,b, 1999), and, consequently, significant differences between the lines may not be detected with measures of tissue contents until later in development.

Lower contents of DA in the ACB and OTU of adult and in the OTU of weanling P versus NP, and HAD versus LAD rats may indicate lower DA innervation, reduced synthesis, increased release and/or increased metabolism. If the lower contents of DA were due to increased metabolism, then higher tissue levels of DOPAC and/or HVA might be expected in the P and HAD lines compared to their low ethanol intake counterparts. In the OTU of weanling P versus NP rats, DOPAC levels were significantly lower in the P line, whereas no significant differences were observed in the OTU of between adult P and NP rats, or between adult HAD and LAD rats (Tables 1 and 2). These results suggest that increased metabolism of DA may not underlie the lower tissue contents of DA found in the OTU and ACB of P and HAD rats compared to NP and LAD rats, respectively.

The OTU is part of the mesolimbic DA system and is heavily innervated by the VTA (Gilad and Reis, 1979; Voorn et al., 1986). Immunohistochemical findings by Zhou et al. (1995) indicated fewer DA fibers projecting from the VTA to the ACB in adult P rats compared to NP rats. Therefore, it is possible that the lower DA contents in the weanling OTU may be a result of fewer DA projections to the OTU from the VTA. In the ACB of adult P compared to NP rats and adult HAD compared to LAD rats, lower contents of DA were observed in the P and HAD rats. However, similar differences were not seen between the lines in the weanling rats. If the difference in content reflects a difference in innervation, these differences suggest that there may be post weanling developmental changes occurring in the VTA DA innervations between P versus NP and HAD versus LAD rats. The relative rate of maturation of this DA projection system may be slightly retarded in the P and HAD lines and/or slightly more developed in the NP and LAD lines.

The differences in maturation of VTA DA projections may be region specific. In the adult, the contents of DA were lower in the ACB and OTU of the P rat compared to the NP rat, and between the HAD and LAD rats. The AMYG, ACTX and SEP also receive DA innervations from the VTA. The finding that lower contents of DA were not observed in these three regions between adult and weanling P versus NP and HAD versus LAD rats suggest that factors within the target regions of the VTA may be involved in programming DA innervation. In contrast, the levels of DA in the ACTX of weanling P and HAD rats were higher than values in NP and LAD rats. However, by adulthood, these differences were no longer apparent (Fig. 1). This was due to a greater increase in the contents of DA in the ACTX of LAD and NP rats than HAD and P rats, suggesting that maturation of DA projections to the ACTX may have been slower in developing in LAD and NP rats compared to HAD and P rats.

The ACB and OTU receive their 5-HT inputs primarily from the dorsal raphe nucleus (DRN), whereas the ACTX, AMYG and SEP receive 5-HT inputs from both the DRN and median raphe nucleus (Azmitia and Segal, 1978; O'Hearn and Molliver, 1984; Vertes, 1991; Vertes and Martin, 1988). In the ACB, lower contents of 5-HT were observed in the adult P and HAD rat lines compared to the NP and LAD rats, respectively (Fig. 2). The lower contents of 5-HT in the ACB and OTU of the adult P and HAD lines were not accompanied by lower levels of 5-HIAA (Table 2). In the ACB of adult P rats, lower 5-HT tissue contents were associated with reduced densities of 5-HT immunoreactive fibers, suggesting reduced 5-HT innervation (Zhou et al., 1991). The reduced number of 5-HT immunoreactive fibers in several regions of P versus NP rat appears to be a result of fewer 5-HT neurons in the DRN and MRN of P rats. However, only the ACB in P versus NP rats and HAD versus LAD rats, and OTU in P compared to NP showed lower 5-HT contents, suggesting that 5-HT projections to the other three regions and OTU of HAD versus LAD rats were not different. The contents of 5-HT in the ACTX, ACB, OTU and SEP were higher in adult than in adolescent rats, suggesting additional post weanling development of the 5-HT system in all four rat lines in these four brain regions. However, similar increases in the contents of 5-HT in the AMYG were not observed between the adult and weanling P, NP, HAD and LAD lines (Fig. 2).

In young and adult P and NP rats, intraperitoneal injection of amphetamine (AMPH) has been shown to increase locomotor activity (LMA) more in NP than in P rats (McKinzie et al., 2002). The LMA-stimulating effects of AMPH may be mediated by the ACB (Kelly et al., 1975;

West et al., 1999). The lower responses to AMPH in adult P rats compared to adult NP rats suggest that the mesolimbic DA system in the adult P rat is operating at a lower level than in adult NP rats. The lower response to AMPH in the periadolescent P compared to the NP rat might also indicate that the OTU and the mesolimbic DA system play roles in the AMPH response.

A previous study indicated that differences in 5-HT_{1A} and DA D_2 receptors, which were evident in adulthood (McBride et al., 1993a, 1994), could also be detected at PND 25 between P and NP rats (Strother et al., 2003). These results suggest that these differences could be related to the differences in ethanol intake between the P and NP lines. These results also indicate that differences in the DA and 5-HT systems are present at the age of alcohol drinking onset (PND 22–25) and that the receptor autoradiography technique offers a more sensitive measure of detecting changes in the DA and 5-HT system than does measuring tissue contents.

The OTU has been postulated, along with the ACB, as being involved in regulating alcohol drinking (McBride et al., 1993a,b). Because of its relatively small size and close proximity to the ACB, it has been difficult to study this region and provide a better understanding of its role in alcohol drinking behavior. However, a limited number of studies suggest that this system can support the reinforcing actions of some drugs of abuse and that the OTU appears to be activated by administration of ethanol, cocaine and morphine (Kornetsky et al., 1991; Quarfordt et al., 1991; Moore et al., 1998; Cowen and Lawrence, 2001). In sP alcohol-preferring rats, ethanol administration significantly increased the content of the dopamine metabolites DOPAC and HVA in the OTU compared to non-preferring sNP rats (Fadda et al., 1991). While ethanol and other drugs of abuse studies suggest a role for the OTU in the adult animal, developmental studies are needed to assess the role of the OTU in drug seeking and reward in the young animal.

Acknowledgements

This work was supported in part by AA07611, AA10721, AA10722 and AA10256.

References

Azmitia EC, Segal M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 1978;179:641–67.

Cowen MS, Lawrence AJ. Alterations in central preproenkephalin mRNA expression after chronic free-choice ethanol consumption by fawn-hooded rats. Alcohol, Clin Exp Res 2001;25:1126–33.

Fadda F, Colombo G, Gessa GL. Genetic sensitivity to effect of ethanol on dopaminergic system in alcohol preferring rats. Alcohol Alcohol, Suppl 1991;1:439–42.

George SR, Fan T, Ng GYK, Jung SY, O'Dowd BF, Naranjo CA. Low endogenous dopamine function in brain predisposes to high alcohol preference and consumption: reversal by increasing synaptic dopamine. J Pharmacol Exp Ther 1995;273:373–9.

Gilad GM, Reis DJ. Collateral sprouting in central mesolimbic dopamine neurons: biochemical and immunocytochemical evidence of changes in the activity and distrubution of tyrosine hydroxylase in terminal fields and in cell bodies of A10 neurons. Brain Res 1979;160:17–26.

Gongwer MA, Murphy JM, McBride WJ, Lumeng L, Li T-K. Regional brain contents of serotonin, dopamine, and their metabolites in the selectively bred high and low alcohol-drinking lines of rats. Alcohol 1989;6:317–20.

Institute of Laboratory Animal resources, Commission on Life Sciences, National research Council T-K, 1996. Guide for the care and use of laboratory animals. Washington, (DC): National Academy Press; 1996.

Kelly PH, Seviour PW, Iversen SD. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 1975;94:507–22.

Koob GF, Roberts AJ, Schulteis G, Parsons LH, Heyser CJ, Hyytia P, et al. Neurocircuitry targets in ethanol reward and dependence. Alcohol, Clin Exp Res 1998;22:3–9.

Kornetsky C, Huston-Lyons D, Porrino LJ. The role of the olfactory tubercle in the effects of cocaine, morphine and brain-stimulation reward. Brain Res 1991;541:75–81.

Korpi ER, Sinclair JD, Kaheinen P, Viitamaa T, Hellevuo K, Kiianmaa K. Brain regional and adrenal monoamine concentrations and behavioral responses to stress in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 1988;5:417–25.

McBride WJ, Li T-K. Animal models of alcoholism: neurobiology of high alcohol-drinking in rodents. Crit Rev Neurobiol 1998;12:339–69.

McBride WJ, Chernet E, Dyr W, Lumeng L, Li T-K. Densities of dopamine D2 receptors are reduced in CNS regions of alcohol-preferring P rats. Alcohol 1993a;10:387–90.

McBride WJ, Murphy JM, Gatto GJ, Levy AD, Yoshimoto K, Lumeng L, et al. CNS mechanisms of alcohol self-administration. Alcohol Alcohol 1993b;2:463–7.

McBride WJ, Guan X-M, Chernet E, Lumeng L, Li T-K. Regional serotonin-1A receptors in the CNS of alcohol-preferring and -non-preferring rats. Pharmacol Biochem Behav 1994;49:7–12.

McBride WJ, Bodart B, Lumeng L, Li T-K. Association between low contents of dopamine and serotonin in the nucleus accumbens and high alcohol preference. Alcohol, Clin Exp Res 1995;19:1420–2.

McKinzie DL, Eha R, Murphy JM, McBride WJ, Lumeng L, Li T-K. Effects of taste aversion training on the acquisition of alcohol drinking in adolescent P and HAD rat lines. Alcohol, Clin Exp Res 1996;20:682–7.

McKinzie DL, Nowak KL, Murphy JM, Li T-K, Lumeng L, McBride WJ. Development of alcohol drinking behavior in rat lines selectively bred for divergent alcohol preference. Alcohol, Clin Exp Res 1998;22:1584–90.

McKinzie DL, McBride WJ, Murphy JM, Lumeng L, Li T-K. Effects of amphetamine on locomotor activity in adult and juvenile alcohol-preferring and -nonpreferring rats. Pharmacol Biochem Behav 2002;71:29–36.

Moore RJ, Vinsant SL, Nader MA, Porrino LJ, Friedman DP. Effect of cocaine self-administration on dopamine D2 receptors in rhesus monkeys. Synapse 1998;30:88–96.

Murphy JM, McBride WJ, Lumeng L, Li T-K. Regional brain levels of monoamines in alcohol-preferring and -nonpreferring lines of rats. Pharmacol Biochem Behav 1982;16:145–9.

Murphy JM, McBride WJ, Lumeng L, Li T-K. Contents of monoamines in forebrain regions of alcohol-preferring (P) and -nonpreferring (NP) lines of rats. Pharmacol Biochem Behav 1987;26:389–92.

O'Hearn E, Molliver ME. Organization of raphe-cortical projections in rat: a quantitative retrograde study. Brain Res Bull 1984;13:709–26.

Quarfordt SD, Kalmus GW, Myers RD. Ethanol drinking following 6-OHDA lesions of nucleus accumbens and tuberculum olfactorium of the rat. Alcohol 1991;8:211–7.

Strother WN, Lumeng L, Li TK, McBride WJ. Regional CNS densities of serotonin 1A and dopamine D2 receptors in periadolescent alcohol-preferring P and alcohol-nonpreferring NP rat pups. Pharmacol Biochem Behav 2003;74:335–42.

Tarazi FI, Tomasini EC, Baldessarini RJ. Postnatal development of dopamine and serotonin transporters in rat caudate–putamen and nucleus accumbens septi. Neurosci Lett 1998a;254:21–4.

Tarazi FI, Tomasini EC, Baldessarini RJ. Postnatal development of dopamine D_4-like receptors in rat forebrain regions: comparison with D_2-like receptors. Dev Brain Res 1998b;110:227–33.

Tarazi FI, Tomasini EC, Baldessarini RJ. Postnatal development of dopamine D1-like receptors in rat cortical and striatolimbic brain regions: an autoradiographic study. Dev Neurosci 1999;21:43–9.

Vertes RP. A PHA-L analysis of ascending projections of the dorsal raphe nucleus in the rat. J Comp Neurol 1991;313:643–68.

Vertes RP, Martin GF. Autoradiographic analysis of ascending projections from the pontine and mesencephalic reticular formation and the median raphe nucleus in the rat. J Comp Neurol 1988;275:511–41.

Voorn P, Jorritsma-Byham B, Van Dijk C, Buijs RM. The dopaminergic innervation of the ventral striatum in the rat: a light- and electron-microscopic study with antibodies against dopamine. J Comp Neurol 1986;251:84–99.

West CHK, Boss-Williams KA, Weiss JM. Motor activation by amphetamine infusion into nucleus accumbens core and shell subregions of rats differentially sensitive to dopaminergic drugs. Behav Brain Res 1999;98:155–65.

Yoshimoto K, Komura S. Re-examination of the relationship between alcohol preference and brain monoamines in inbred strains of mice including senescence-accelerated mice. Pharmacol Biochem Behav 1987;27:317–22.

Zhou F, Bledsoe S, Lumeng L, Li T-K. Immunostained serotonergic fibers are decreased in selected brain regions of alcohol-preferring rats. Alcohol 1991;8:425–31.

Zhou F, Bledsoe S, Lumeng L, Li T-K. Reduced serotonergic immunoreactive fibers in the forebrain of alcohol-preferring rats. Alcohol, Clin Exp Res 1994a;18:571–9.

Zhou F, Pu CF, Lumeng L, Li T-K. Serotonergic neurons in alcohol-preferring rats. Alcohol 1994b;11:397–403.

Zhou F, Zhang JK, Lumeng L, Li T-K. Mesolimbic dopaminergic system in alcohol preferring rats. Alcohol 1995;12:403–12.