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Prolonged Changes in Neurochemistry of Dopamine Neurones After Chronic Ethanol Consumption

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BAILEY, C. P., N. ANDREWS, A. T. MCKNIGHT, J. HUGHES, AND H. J. LITTLE. *Prolonged changes in neurochemistry of dopamine neurones after chronic ethanol consumption*. PHARMACOL BIOCHEM BEHAV **66**(1) 153–161, 2000.—The effects of 3 weeks of chronic ethanol consumption in mice on brain concentrations and turnover of monamine transmitters was examined. The measurements were made at 24 h, 6 days and 2 months after cessation of the ethanol intake to examine changes that might be relevant to relapse drinking. Increases in noradrenaline and dopamine concentrations, and decreases in the ratios of dopamine metabolites to dopamine, were seen in ventral tegmental tissue at 24 h after alcohol consumption. Increased noradrenaline was also evident at the 6-day interval, but no other changes were seen at this time. At the 2-month interval, the ventral tegmentum from ethanol-treated animals showed decreases in metabolite/dopamine ratios. No changes were seen in 5-hydroxytryptamine or its metabolite. In striatal tissue, none of these changes were seen, but at 24 h decreases occurred in the content of dopamine and its metabolites and a decrease in 5-hydroxyindoleacetic acid. The results indicate changes occur in monoamine turnover in the VTA as long as 2 months after cessation of chronic ethanol consumption; such changes may be related to the prolonged nature of alcohol dependence. © 2000 Elsevier Science Inc.

Alcohol Dopamine Noradrenaline 5-HT Chronic ethanol intake Ventral tegmental area

THE major problem in dependence on alcohol is that of relapse drinking. Alcoholics frequently undergo the withdrawal syndrome and remain abstinent for weeks, months, or even years, then return to excess drinking. Such relapse has been reported, for example, in 74% to 90% (27) and 80% (29) of alcoholics during 1 to 2 years follow-up after treatment. Prolonged behavioral changes, including craving, are seen in alcoholics after months or even years of abstinence, but the neuronal basis of this is not understood. The aim of the present work was to investigate changes in neurotransmitters in the ventral tegmental area (VTA) and striatum during the abstinence phase following withdrawal from chronic ethanol consumption.

The present study was designed as a parallel investigation to previous work on prolonged behavioral changes after chronic alcohol intake. We have recently found that chronic alcohol consumption by rodents results in certain prolonged, specific behavioral changes. When amphetamine and cocaine were given by repeated injection at intervals after the acute alcohol withdrawal syndrome had subsided, the development of sensitization to the locomotor activating effects of the psychostimulants was increased (26). The behavior of the rodents was overtly normal in the absence of drug administration. Sensitization has been implicated in drug dependence and relapse, as the phenomenon lasts for many months, possibly permanently, after cessation of drug intake (36). Increased sensitization to amphetamine and cocaine was seen in our behavioral studies even when these drugs were not administered for the first time until 2 months after cessation of the ethanol consumption. We have also found increased locomotor stimulation by nicotine after chronic ethanol consumption when this drug was administered repeatedly after the cessation of acute ethanol withdrawal (46). This study showed, in addition, that mice that had previously consumed alcohol chronically developed a conditioned activity to nicotine that was not seen in the absence of the ethanol intake.

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Considerable evidence implicates the mesolimbic dopamine system in drug dependence and sensitization. Acutely, alcohol causes excitation of dopaminergic neurones in the VTA (17,4), by a direct action on these neurones (5) and, consequently, increases extracellular dopamine in the nucleus accumbens (11). The extracellular dopamine level in the nucleus accumbens is decreased during the acute phase of withdrawal, a pattern similar to that seen during withdrawal from other drugs of dependence, such as morphine and cocaine (38), and the firing rate of mesolimbic dopaminergic neurones is decreased (12). Rodents will self-administer alcohol and also morphine directly into the VTA (16,10), and injection of a nicotine antagonist into the VTA decreased nicotine self-administration by rats (9). There is considerable evidence to indicate the VTA as the site of initiation of psychostimulant sensitization (21,8). Sensitization to amphetamine is produced by local administration into the VTA and prevented by injection of an NMDA receptor antagonist into that area (22). Neuronal changes reported after psychostimulant sensitization include subsensitivity of D2 receptor– and $GABA_B$-receptor–mediated responses, and increased glutamate release and D1 receptor–mediated activation in the VTA, and increased dopamine release and D1 receptor–mediated responses in the nucleus accumbens (33,19,20). There is evidence that such changes occur progressively during the abstinence phase; for example, dopamine autoreceptor subsensitivity in the VTA is transient, but increased accumbal dopamine release is seen later after the cessation of chronic psychostimulant treatment (47). Fewer electrophysiologic studies have been made on nicotine sensitization, but increased accumbal dopamine release has been demonstrated (3).

The current study measured the effects of chronic and acute ethanol treatments on the brain contents of monoamine neurotransmitters and their metabolites in the VTA and striatum of mice. This study used identical strains and ages of mice, and identical chronic ethanol treatments and time intervals from alcohol withdrawal, as the behavioral studies. The treatments were also the same as previous electrophysiologic experiments that demonstrated decreases in spontaneous firing rate of dopaminergic neurones lasting at least 6 days after cessation of ethanol consumption (1,2). The specific intention was to determine whether or not there were changes in contents of monoamines and their metabolites in the mesolimbic system of mice, which may be present up to 2 months after the cessation of chronic ethanol treatment. For comparison, the effects of acute administration of ethanol on the neurotransmitter levels was also studied.

METHOD

Male TO strain mice (Bantin and Kingman, Hull, UK) 25 to 30 g were used in all studies. They were housed in groups of 9, with lights on a 12 h/12 h cycle with the lights on between 0800 to 2000 h. Temperature and relative humidity were 21° to 1°C and 55% to 10% respectively. The sample sizes were 6 to 9 per treatment group. All experimentation was carried out in accordance with the UK Animals Scientific Procedures Act 1986.

Chronic Administration of Ethanol

The mice were made physically dependent on ethanol by a liquid diet (Dyets Inc., Bethlehem, PA, USA) schedule lasting 23 days. The first 3 days they received just a control liquid diet to familiarize them, then diet containing 3.5% ethanol for 2 days, followed by 5% for 9 days then 8% for 9 days. The ethanol intake at the beginning of the treatment was 25 g/kg/day, rising to 28 g/kg/day by the end of the treatment. Control mice were pair fed a control liquid diet that contained no ethanol, isocalorific with the ethanol-containing diet (23). At the end of the diet schedule, the liquid diet was replaced with standard laboratory chow at 0900 h and the animals were left undisturbed until the tissue preparation. Tap water was available ad libitum throughout the treatment and post-treatment periods. We have previously demonstrated that in mice this chronic ethanol treatment schedule causes a withdrawal syndrome, with convulsive responses to gentle handling but not spontaneous convulsions lasting 18 h from the start of withdrawal (2). Following cessation of the alcohol liquid diet treatment, the mice were withdrawn from the alcohol and fed a normal laboratory diet for either 24 h, 6 days, or 2 months, at which time the tissues were prepared for the monamine measurements.

Acute Administration of Ethanol

Mice were given a single intraperitoneal (IP) injection of 3 g/kg ethanol, as a 12% w/v solution in 0.9% saline. Control animals were given equivalent volumes of 0.9% saline. After administration of the ethanol, the mice showed initial increases in locomotor activity followed by signs of incoordination of movement and ataxia.

Tissue Preparation

The tissue samples were taken at 24 h, 6 days, or 2 months after the cessation of chronic liquid diet treatment. For the studies on the acute effects of ethanol, sampling was at 45 min or 24 h after ethanol administration. At the appropriate times, the mice were killed by cervical dislocation. The injections and dissections for each treatment group were alternated between ethanol and control administrations to minimize variations. Dissections were performed on an inverted petri dish resting on dry ice to separate the VTA and striatum (40,18). After dissection, the tissue samples were placed in pre-weighed microcentrifuge tubes, weighed, and immediately frozen on dry ice and then stored at −70°C for not longer than 2 weeks.

Measurements of Monoamine Concentrations and Turnover

For the monoamine measurements, 0.1 M of ice-cold perchloric acid in HPLC grade water was added to the frozen tissue such that 15 μl was added per mg of tissue. This was immediately sonified with a Heat Systems Microson ultrasonic cell disrupter for 1 min and spun at 12,000 g for 15 min at +4°C with a refrigerated centrifuge. The supernatant was removed, passed through a 0.2-μm cellulose filter and stored at −70°. Pellet was stored separately at −70°C. Samples from control and ethanol-treated animals from each treatment group were prepared in the same day, with alternating control and ethanol-treated samples throughout the day.

The concentrations of dopamine, noradrenaline, 5-hydroxytryptamine (5-HT), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindole acetic acid (5-HIAA) in the purified brain samples were assayed by reversed phase high-pressure liquid chromatography using electrochemical detection. Twenty-microliter aliquots were injected onto the HPLC system. The mobile phase consisted of 0.1 M NaH_2PO_4, 0.2 mM octanesulphonic acid, 8% v/v methanol and 0.1 mM disodium EDTA, pH 4.2, and was filtered through a 0.2-μm cellulose filter before use. The output from

the detector was plotted and measured on computer, using Turbochrom 4 (Perkin-Elmer). Elution was performed at 1 ml/min and the working electrode potential was set at 0.8 V.

These measurements were converted to quantitative values by comparison with a standard curve derived from solutions of HPLC grade dopamine, noradrenaline, 5-HT, DOPAC, HVA, and 5-HIAA AT 50, 25 and 5 pg/ml, assayed by HPLC using the same methodology. All the samples from each treatment group were assayed on the same day, using the same standard curve. Samples were assayed in a random order throughout the day, and at least 5 separate samples were used to make up the standard curve.

Protein Content of Tissue Samples

The quantities of protein in the pellets, taken as the quantity of protein in each brain sample, were assayed using Peterson's modification of Lowry's method (Lowry et al., 1951; Peterson, 1977). The pellets were dissolved by addition of 1 ml of Lowry Reagent solution (consisting of 0.1% copper sulphate, 0.2% potassium tartrate, 10% sodium carbonate) and allowed to stand at room temperature for 20 min. Then 0.5 ml 2N Folin & Ciocalteu's Phenol Reagent was added with rapid and immediate mixing and allowed to stand for 30 min. The absorbance at 750 nm was measured using a Pharmacia Biotech Ultraspec 3000 spectrophotometer. The protein levels were quantified by comparisons with a calibration curve created from data using solutions of bovine serum albumin at 50, 100, 200, 300, and 400 mg/ml.

Data Analysis

Brain contents of neurotransmitters and metabolites measured are expressed as nanogram of compound per milligram

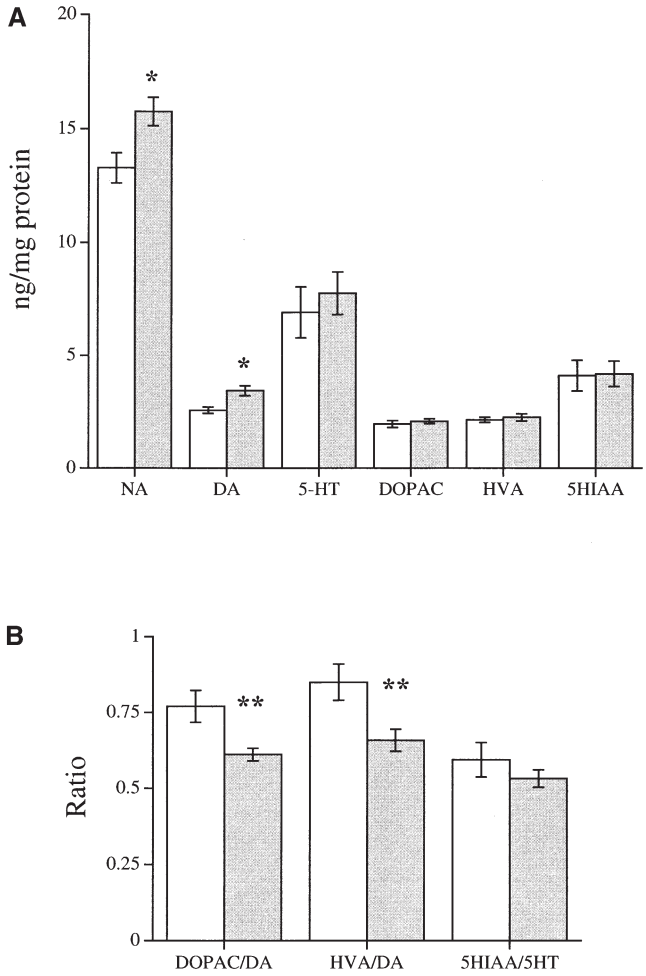

FIG. 1. Concentrations of monamines and metabolites (A) and ratios of monamines to metabolites (B) in ventral tegmental tissue at 1 day after cessation of chronic ethanol consumption. Open columns are the control results; shaded columns the results after the chronic ethanol intake. DA = dopamine; NA = noradrenaline; 5-HT = 5-hydroxytryptamine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; 5-HIAA = 5-hydroxyindole acetic acid. $*p < 0.05$, $**p < 0.01$, for comparison between controls and ethanol treatment.

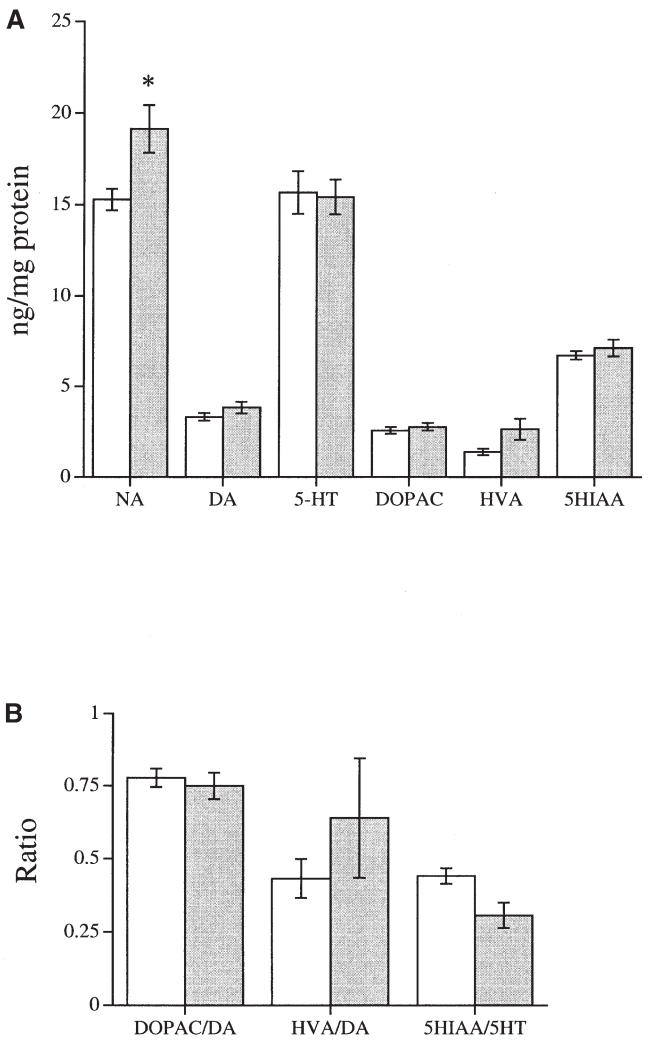

FIG. 2. Concentrations of monamines and metabolites (A) and ratios of monamines to metabolites (B) in ventral tegmental tissue at 6 days after cessation of chronic ethanol consumption. Open columns are the control results; shaded columns the results after the chronic ethanol intake. Abbreviations as in Fig. 1. $*p < 0.05$ for comparison between controls and ethanol treatment.

of sample tissue protein. Ratios of the brain content per milligram of sample protein of DOPAC/dopamine, HVA/dopamine, and 5-HIAA/5-HT were also calculated. All data are given as means and standard errors of the mean. The statistical test used was a 2-tailed, unpaired Student's *t*-test.

RESULTS

Chronic Ethanol Administration

Ventral tegmental area. The measurements of brain contents and turnover for the VTA are illustrated in Figs. 1 to 3. There were significant increases in noradrenaline and dopamine concentrations 1 day after cessation of the liquid diet treatment ($P < 0.05$) and decreases in DOPAC/dopamine and HVA/dopamine ratios ($P < 0.01$, $P < 0.05$, respectively) in tissues from ethanol-treated mice, compared with control values (Fig. 1B).

Fig. 2A shows that the only significant change seen in the VTA 6 days after chronic ethanol treatment was an increase in noradrenaline content ($p < 0.05$) after the chronic ethanol consumption.

At 2 months after cessation of the ethanol intake, there was a significant ($p < 0.001$) decrease in DOPAC/dopamine turnover (Fig. 3B). Although there was a decrease in the mean value of the HVA/dopamine ratios after the ethanol consumption, the difference just failed to reach significance ($p = 0.06$).

Table 1 summarizes the changes seen in the ventral tegmental area following chronic ethanol consumption.

Striatum. Figs. 4, 5, and 6 illustrate the striatal brain content after withdrawal from the chronic ethanol liquid diet treatment. The only time interval at which differences between tissues from control and ethanol-treated animals were seen was 24 h after the cessation of ethanol consumption (Fig. 4A) At this point, there were significant decreases in dopamine, DOPAC, 5-HIAA, and HVA contents (all $P < 0.005$, except DOPAC $P < 0.01$) in tissues from ethanol-treated animals. there were no significant changes in turnover and no significant changes at either of the later time intervals of 6 days and 2 months after cessation of chronic ethanol intake.

Table 1 summarizes the changes seen in the striatal area following chronic ethanol consumption.

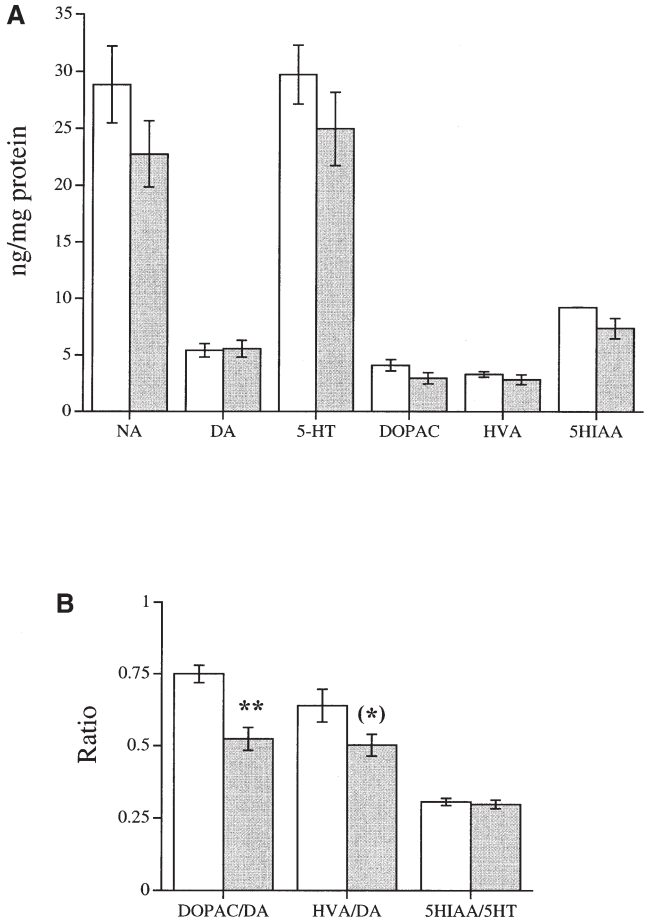

FIG. 3. Concentrations of monamines and metabolites (A) and ratios of monamines to metabolites (B) in ventral tegmental tissue 2 months after cessation of chronic ethanol consumption. Open columns are the control results; shaded columns the results after the chronic ethanol intake. Abbreviations as in Fig. 1. *$p < 0.05$ for comparison between controls and ethanol treatment. (*)$p = 0.06$ for comparison between controls and ethanol treatment.

Single Dose of Ethanol

Ventral tegmental area. Table 2 shows that, in the VTA, 45 min after administration of 3 g/kg ethanol IP, there was a significant decrease in dopamine content ($p < 0.01$) and significant increases in the turnover of DOPAC/dopamine and HVA/dopamine ($p < 0.0001$) compared with control values. At the 24-h interval, these alterations were no longer seen.

Striatum. From Table 2 it can be seen that the only significant change in brain content in the striatum following the acute dose of ethanol was an increase in the concentration of DOPAC at 45 min after injection ($p < 0.05$).

Table 3 summarizes the changes seen in the ventral tegmental and striatal areas after the acute injections of ethanol.

DISCUSSION

The present study is the first to show changes in monoamine content at these long time intervals from cessation of chronic ethanol treatment. All the time-points of investigation were after cessation of the behavioral symptoms of ethanol withdrawal hyperexcitability, which lasts up to 18 h after ethanol withdrawal (2). The results demonstrate that acute and chronic ethanol administration produce differential effects in monoamine content in the VTA and striatum of mice, with changes seen in the VTA even at 2 months after cessation of chronic ethanol treatment.

At 24 h after cessation of chronic ethanol treatment there were significant increases in noradrenaline and dopamine levels and decreases in DOPAC/DA and HVA/DA ratios in the VTA, suggesting increases in noradrenaline and dopamine synthesis and decreased dopamine turnover. There were no changes in dopamine content, release or neurotransmitter/metabolite ratios in the VTA, 6 days after cessation of chronic ethanol treatment, but there was a highly significant decrease in the DOPAC/DA ratio at the 2-month interval. Although the HVA/DA ratio did not quite show significance ($p = 0.06$) at the latter time, the mean value was much lower in ethanol-treated animals. These results suggest that dopamine turnover in the VTA may be decreased 2 months after ethanol withdrawal, which would have important implications for the continuation of craving and propensity to relapse

TABLE 1
SUMMARY OF EFFECTS OF CHRONIC ETHANOL CONSUMPTION

	NA	DA	5-HT	DOPAC	HVA	5-HIAA	DOPAC/DA	HVA/DA	5-HIAA/5-HT
VTA 1 day	↑	↑	—	—	—	—	↓	↓	—
VTA 6 days	↑	—	—	—	—	—	—	—	—
VTA 2 months	—	—	—	—	—	—	↓	—	—
Striatum 1 day	—	↓	—	↓	↓	↓	—	—	—
Striatum 6 days	—	—	—	—	—	—	—	—	—
Striatum 2 months	—	—	—	—	—	—	—	—	—

The text in the boxes indicates significant differences between control values and results from tissues prepared after chronic ethanol consumption.

↑indicates the value from ethanol-treated animals was significantly higher; ↓ indicates the value from control animals was significantly higher; — indicates no significant change.

DA = dopamine, NA = noradrenaline, 5-HT = 5-hydroxytryptamine, DOPAC = 3,4-dihydroxyphenylacetic acid, HVA = homovanillic acid, 5-HIAA = 5-hydroxyindole acetic acid

drinking in alcoholics. The patterns of results seen in the present study at the 6 day and 2-month intervals suggest that there may be a series of progressive changes in VTA function after withdrawal from ethanol consumption. Progressive alterations have been seen after cessation of chronic psychostimulant treatment (47). We have previously reported that in this strain of mice, following chronic ethanol administration by liquid diet, the brain ethanol concentrations fall to undetectable levels (less than 250 μM) by 2 h into the withdrawal period, while the plasma ethanol was approximately 1 mM at 4 h after the withdrawal so there would have been no residual alcohol in the brain at 24 h after the withdrawal (45).

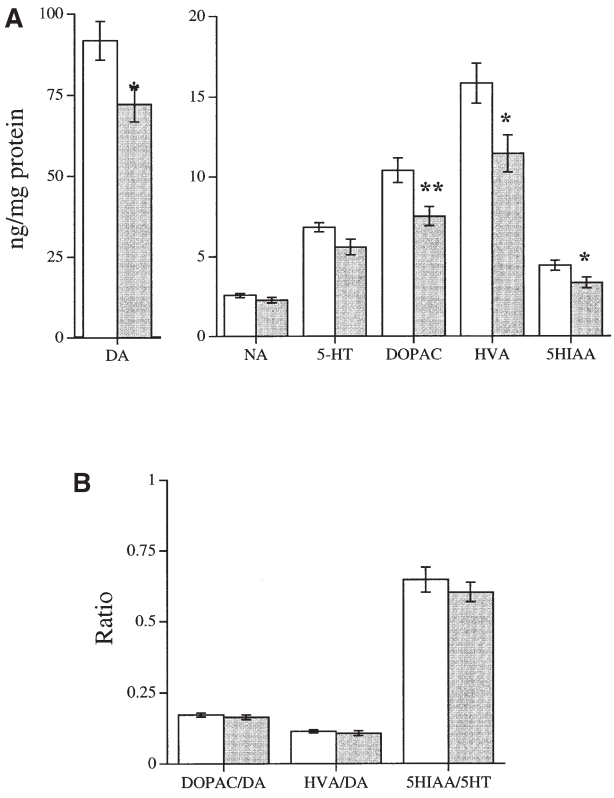

FIG. 4. Concentrations of monamines and metabolites (A) and ratios of monamines to metabolites (B) in striatal tissue at 1 day after cessation of chronic ethanol consumption. Open columns are the control results; shaded columns the results after the chronic ethanol intake. Abbreviations as in Fig. 1.

Although the most important effect of chronic ethanol consumption, as demonstrated by these results, was that changes in dopamine metabolism were evident 2 months after cessation of consumption, they do not explain the increases in sensitization to the locomotor stimulant actions of amphetamine and cocaine seen in our earlier work, as the pattern of the behavioral changes was similar whether the drugs were given for the first time at 24 h, 6 days, or 2 months after ethanol withdrawal (26). It is possible that the decreases in the spontaneous firing of dopamine VTA neurones after ethanol withdrawal were related to the monoamine changes at the 24-h and 6-day intervals, as the decrease in firing rate was more profound at 24 h (2) than at 6 days (1). However, the neuronal changes that decreased the dopamine/metabolite ratio in the VTA did not apparently result in changes in basal firing rates, as there was no difference in spontaneous firing recorded in vitro from VTA neurones at 2 months post-withdrawal (Bailey, unpublished data).

The increases in dopamine and noradrenaline content of the VTA 24 h after ethanol withdrawal might suggest increased catecholamine synthesis. Ortiz et al. (31) showed upregulation of tyrosine hydroxylase, the enzyme involved in the rate-limiting step of catecholamine synthesis, immediately following chronic ethanol treatment, although this study was made earlier in withdrawal when there was still ethanol present in the blood. Increases in brain noradrenaline concentrations in locus coeruleus neurones have also been reported during the acute withdrawal phase (13,15,44), along with elevated basal firing rates of locus coeruleus neurones 24 h after withdrawal (14). By the HPLC method used in the current study, peaks derived from noradrenaline metabolites were masked by the solvent front, precluding measurements of nor-

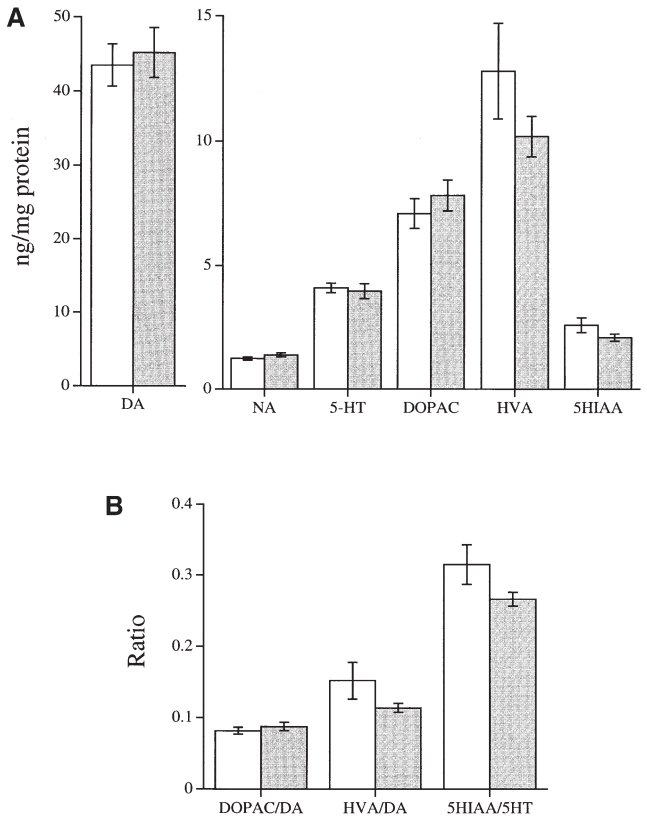

FIG. 5. Concentrations of monamines and metabolites (A) and ratios of monamines to metabolites (B) in striatal tissue at 6 days after cessation of chronic ethanol consumption. Open columns are the control results; shaded columns the results after the chronic ethanol intake. Abbreviations as in Fig. 1.

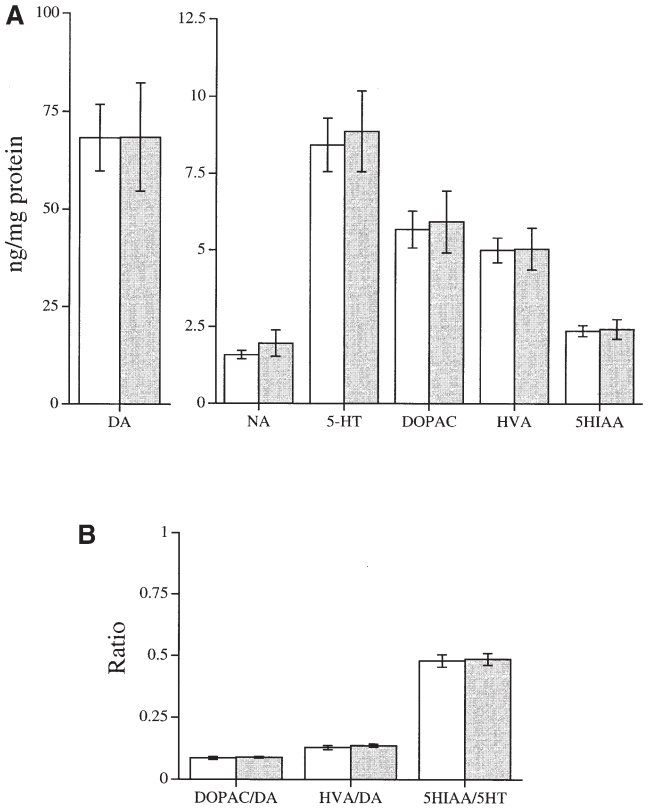

FIG. 6. Concentrations of monamines and metabolites (A) and ratios of monamines to metabolites (B) in striatal tissue 2 months after cessation of chronic ethanol consumption. Open columns are the control results; shaded columns the results after the chronic ethanol intake. Abbreviations as in Fig. 1. $*p < 0.05$ for comparison between controls and ethanol treatment.

adrenaline metabolites; therefore, it was not possible to compare noradrenaline release or turnover in the present study.

The present study also showed significant decreases in dopamine, DOPAC, HVA, and 5-HIAA levels in the striatum, 24 h after cessation of chronic ethanol treatment. As with the VTA, this is the first study to show changes in monoamines and monoamine content in the striatum that outlast the withdrawal syndrome. These decreases in brain content indicate reductions in dopamine turnover and, by implication, release in the nucleus accumbens, as well as a decrease in dopamine synthesis. Previous brain content studies have failed to show consistent changes in dopamine release in the nucleus accumbens or striatum during the acute withdrawal phase, but in vivo microdialysis studies have shown more consistent results in the nucleus accumbens, with Rossetti et al. (37,39) showing decreases in extraneuronal dopamine, DOPAC, and HVA during the acute withdrawal phase in rats. However, these studies showed that the dopamine, DOPAC, and HVA concentrations had returned to control levels when the behavioral signs of withdrawal subsided. This is in contrast with the present study, which showed decrements in dopamine, DOPAC, and HVA brain content in the striatum that outlasted the ethanol withdrawal syndrome. However, the studies by Rossetti et al. used a shorter duration of ethanol treatment, just 6 days, compared with the 3-week treatment in the present study. Many previous studies have shown that longer ethanol treatments result in more profound neurologic changes and Manley and Little (26) found the long-term changes in the effects of amphetamine and cocaine after a 3-week chronic ethanol treatment used in the present study were not seen after only 7 days of ethanol consumption.

The 5-HT neurones terminating in the nucleus accumbens arise in the raphe nucleus and synapse directly onto, and inhibit the firing of, the medium spiny neurones in much the same manner as dopaminergic neurones arising in the VTA (41,42,43). Pistis et al. (34) showed a reduction in the firing rates of dorsal raphe nucleus neurones in the rat during the acute phase of withdrawal from chronic ethanol treatment. A reduction in the firing rates of these neurones would be expected to result in decreased 5-HT release in areas such as the nucleus accumbens, as was suggested by the decreased 5-HIAA concentrations in the striatum 24 h after withdrawal, as seen in the present study.

There were no significant changes in dopamine concentrations or metabolites in the striatum after 6 days or 2 months after cessation of ethanol treatment consumption. This shows that the changes at the 2-month interval were selective for the VTA, but the brain samples were taken from the entire striatum, rather than solely the nucleus accumbens, so it is possible

TABLE 2

THE EFFECTS OF ACUTE INJECTIONS OF ETHANOL, 3 g/kg

	NA	DA	5-HT	DOPAC	HVA	5-HIAA	DOPAC/DA	HVA/DA	5-HIAA/5-HT
Controls									
VTA 45 min	16.79 ± 0.86	14.52 ± 1.69	6.44 ± 0.55	2.82 ± 0.28	2.56 ± 0.23	3.61 ± 0.81	0.211 ± 0.037	0.163 ± 0.032	0.539 ± 0.099
Ethanol									
VTA 45 min	15.44 ± 0.67	7.95** ± 0.93	6.56 ± 0.24	2.98 ± 0.24	2.56 ± 0.23	3.20 ± 0.55	0.384* ± 0.019	0.345** ± 0.050	0.482 ± 0.070
Controls									
VTA 24 h	13.92 ± 0.69	8.48 ± 3.0	7.15 ± 1.03	2.32 ± 0.31	3.09 ± 1.28	2.69 ± 0.32	0.363 ± 0.05	0.355 ± 0.033	0.354 ± 0.037
Ethanol									
VTA 24 h	15.59 ± 0.84	5.45 ± 0.7	5.80 ± 1.29	1.96 ± 0.10	1.53 ± 0.25	2.10 ± 0.53	0.388 ± 0.039	0.294 ± 0.036	0.344 ± 0.028
Controls									
striatum 45 min	2.64 ± 0.54	75.8 ± 7.9	2.55 ± 0.20	6.17 ± 0.55	10.0 ± 0.81	1.89 ± 0.13	0.091 ± 0.007	0.143 ± 0.007	0.746 ± 0.020
Ethanol									
striatum 45 min	2.10 ± 0.27	85.2 ± 9.3	2.73 − 0.18	8.36* ± 0.51	11.06 ± 0.64	2.14 ± 0.14	0.102 ± 0.006	0.135 ± 0.007	0.792 ± 0.042
Controls									
striatum 24 h	2.34 ± 0.15	71.2 ± 5.3	2.10 ± 0.10	8.29 ± 0.80	14.66 ± 1.11	1.82 ± 0.03	0.116 ± 0.003	0.206 ± 0.010	0.879 ± 0.05
Ethanol									
striatum 24 h	2.56 ± 0.13	73.5 ± 3.29	2.53 ± 0.24	8.25 ± 0.44	15.23 ± 0.77	2.22 ± 0.17	0.112 ± 0.003	0.207 ± 0.004	0.894 ± 0.036

Values are mean − SEM, for ng/mg protein, except for the turnover values which are ratios.
*$p < 0.05$, **$p < 0.01$, for comparison between controls and ethanol treatment.

TABLE 3
SUMMARY OF EFFECTS OF ACUTE ETHANOL ADMINISTRATION

	NA	DA	5-HT	DOPAC	HVA	5-HIAA	DOPAC /DA	HVA /DA	5-HIAA /5-HT
VTA 45 min	—	↓	—	—	—	—	↑	↑	—
VTA 24 h	—	—	—	—	—	—	—	—	—
Striatum 45 min	—	—	—	↑	—	—	—	—	—
Striatum 24 h	—	—	—	—	—	—	—	—	—

The text in the boxes indicates significant differences between control values and results from tissues prepared after chronic ethanol consumption.

↑ indicates the value from ethanol-treated animals was significantly higher.

↓ indicates the value from control animals was significantly higher.

— indicates no significant change.

Abbreviations as for Table 1.

that changes in dopamine release in the nucleus accumbens could have been masked by a lack of change in caudate-putamen dopamine release. Nestby et al. (30), in one of the few other studies made at longer times after ethanol withdrawal, found increased dopamine release from accumbal tissue in vitro, prepared 3 weeks after cessation of a chronic ethanol treatment. Alterations in neuronal activity in the VTA, however, as indicated earlier, are likely to have major influences on the reinforcing actions of drugs.

The data from the studies on the acute effects of ethanol showed a profound decrease in dopamine content in the VTA soon after acute ethanol administration, suggesting an increase in dopamine release in the VTA; the majority of dopamine release in the VTA is somatodendritic, rather than axonal. Although these results mirror those seen after the chronic treatment, in that acute alcohol increases the firing rates of dopamine neurones while such firing is decreased after withdrawal, the changes in dopamine levels do not explain the firing rate alterations since somatodendritically released dopamine decreases firing of VTA neurones (5,35).

The present study also showed that after acute administration of ethanol, DOPAC levels were increased in the striatum, suggesting an increase in dopamine release, which is compatible with previous data which showed the same effect (7,28). This finding is also in agreement with the studies showing increased the firing rates of dopamine VTA neurones after acute application of ethanol and in vivo microdialysis studies showing increased levels of dopamine release in the nucleus accumbens by ethanol. The changes in dopamine in the VTA and in DOPAC/dopamine ratio in the striatum are opposite to those after the chronic ethanol administration. this is compatible with theories suggesting neuronal adaptations occur during chronic ethanol intake to counteract the continued acute effects of the drug and persist when ethanol is removed resulting in the opposite changes (24).

There were no effects in monoamine or metabolite content in the VTA or striatum 24 h after administration of a single dose of 3 g/kg ethanol. This time-point was used as an internal control to determine whether or not changes seen at 24 h after cessation of chronic ethanol consumption were a function of the long-term chronic treatment, rather than of the acute effects of ethanol.

In conclusion, the results demonstrate that monoamine or neurotransmitter/metabolite ratios are not only altered for short periods of time after cessation of chronic ethanol consumption, as demonstrated in previous reports, but that changes are apparent as long as 2 months after withdrawal from ethanol consumption. Alterations have therefore been demonstrated in a pathway that is thought to be related to the reinforcing effects of drugs, which continue for a long period into the abstinence phase. This may therefore represent a mechanism for the persistent of altered responses to alcohol during abstinence and may therefore be related to the prolonged nature of alcohol dependence, possibly to relapse drinking.

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