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# Birth insult alters ethanol preference in the adult rat

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**Abstract**

While genetic factors clearly play a role in regulating ethanol intake, the present study considered the possibility that early environmental factors which influence central nervous system development and long-term function might also alter ethanol intake. The specific aim of the study was to test whether alterations in birth condition, namely Caesarean section (C-section) birth and C-section birth with an added period of global anoxia, can affect subsequent ethanol preference in the adult rat. At 5 months of age, groups of experimental and vaginally born control rats were offered free choice between drinking water or various concentrations of ethanol (1–10% v/v) in water across 36 days of testing. Rats that had been born by C-section with 10 or 15 min of added global anoxia showed significant reductions in ethanol preference scores, in comparison to vaginally born controls. For the 10-min anoxia group, ethanol intake was decreased, water intake was increased and total fluid intake remained unchanged relative to values for vaginally born controls, across the entire test period. Although total fluid intake by the 15-min anoxia group also did not differ from that of vaginally born controls, the decreased ethanol preference scores in the 15-min anoxia group were mainly due to increased water intake during some test periods and a combination of reduced ethanol intake and increased water intake during others. Animals born by rapid C-section alone, with no added period of global anoxia, showed reduced ethanol preference only during a few early periods of testing, a much less pronounced effect than that observed for animals with added global anoxia. When animals were given the choice between drinking water vs. solutions of sucrose or NaCl, no group differences due to birth condition were found on measures of sucrose or NaCl preference. Together with reduced ethanol preference, the 10-min anoxia group showed a transient depression of locomotor activity in response to a low dose (0.25 g/kg) of intraperitoneal ethanol, which had no effect on locomotion in vaginally born controls. These results indicate that a relatively subtle alteration in birth condition, compatible with grossly normal development and behavior, is sufficient to alter ethanol preference in the adult rat. 

*Keywords:* Perinatal hypoxia; Ethanol intake; Alcohol drinking; Caesarean section; Development

## 1. Introduction

Alcoholism is a multifactorial disorder, influenced by both genetic and environmental factors. On the basis of twin studies, it has been estimated that genetic factors account for about 1/2 of the variance in liability to alcoholism in males and for about 1/4 of the variance in females (Johnson et al., 1996). Animal modelling, using selectively bred and inbred lines of rodents, has clearly supported the genetic contribution to ethanol sensitivity (reviewed by Crabbe et al., 1994). However, environmental factors must still account for a large proportion of the variance in behavioral responses to ethanol. Rodent models have also been used to provide evidence for influences of certain environmental factors, such as stress (Caplan and Puglisi, 1986; Sprague and Maickel, 1994; Volpicelli et al., 1990) or social isolation (Roske et al., 1994; Wolffgramm, 1990), on propensity to ingest alcohol.

Ethanol interacts with and produces its behavioral effects through actions on a number of neurotransmitter systems including dopamine, serotonin, γ-amino-butyric acid, glutamate and opioids. There is strong evidence that genetic and environmental factors which alter responsivity to ethanol do so, at least in part, through influences on these neurotransmitter systems. In particular, a role for dopamine in influencing ethanol intake through mediation of ethanol's reinforcing properties has received strong experimental support. Pharmacologically, ethanol enhances the firing rate of dopamine neurons and enhances the synthesis, turnover and release of dopamine (Alari et al., 1987; Brodie et al., 1990; Carlsson and Lindqvist, 1973;

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Gessa et al., 1985; Imperato and Di Chiara, 1986). In mice and in an alcohol-preferring (P) line of rats, low doses of ethanol stimulate locomotor activity, an effect blocked by dopamine receptor antagonists (Liljequist et al., 1981; Shen et al., 1995; Waller et al., 1986). Since the rapid time course of this effect follows the time course of ethanol's euphoric effects in man, ethanol-induced locomotor stimulation has been suggested as a predictor of the rewarding effects of ethanol. Another line of evidence implicating dopamine in the control of alcohol-drinking are findings showing alteration in several aspects of dopamine biochemistry in animals bred for enhanced self-administration of ethanol (De Montis et al., 1993; George et al., 1995; McBride et al., 1995; Murphy et al., 1987; Zhou et al., 1995). In other studies, Weiss et al. (1993, 1996) have demonstrated that both alcohol-dependent and naive rats show increased dopamine release in the nucleus accumbens during ethanol self-administration, while Gatto et al. (1994) showed that alcohol-preferring rats will self-administer ethanol directly into the ventral tegmentum, the cell body region from which the meso-accumbal dopamine pathway originates. In addition, either systemic or local central nervous system administration of dopamine receptor agonists, antagonists and partial agonists have been reported to alter voluntary ethanol consumption in rodents (Bono et al., 1996; Hodge et al., 1992, 1993; Panocka et al., 1995; Russell et al., 1996; Samson et al., 1993; Weiss et al., 1990). Studies with humans have also documented an association of alcoholism with alterations in dopamine transporters and D2 receptors and with changes in neuroendocrine measures of dopaminergic function (Dettling et al., 1995; Tihonen et al., 1995; Volkow et al., 1996).

Given the role that neurotransmitters like dopamine appear to play in responses to ethanol, it might be expected that environmental factors influencing development and long-term function of these transmitter systems might also influence alcohol consumption. Perinatal hypoxia, which remains an important occurrence in the human population (Vannucci, 1990; Volpe, 1992), is an environmental factor which can have lasting effects on brain biochemistry. Using a rat model of acute global anoxia during birth, recent studies have documented that animals rendered anoxic during Caesarean section (C-section) birth display long-term alterations in dopaminergic biochemistry and function. In this model, an acute period of global anoxia is produced during birth by isolating the uterus from the pregnant rat dam at term by C-section and submerging it in saline for several minutes. Animals surviving 5–15 min of anoxia thrive and grow up indistinguishable from vaginally born littermates on a gross behavioral level, except for subtle behavioral alterations on specialized testing (Boksa et al., 1995, 1998). Using this model, Bjelke et al. (1991) and Chen et al. (1995) have demonstrated enhanced numbers of tyrosine hydroxylase immunoreactive dopamine cells in the ventral tegmental area and substantia nigra of animals surviving 14–20 min of acute birth anoxia. In in vivo voltammetry studies, adult rats that had been born by C-section, either with or without added acute anoxia, showed enhanced extracellular dopamine responses in the nucleus accumbens following 15 min of tail pinch stress given daily for 3–5 consecutive days, in comparison to vaginally born controls (Brake et al., 1997b). Additionally, adult animals born by C-section, with or without added acute anoxia, show enhanced locomotor responses to low dose amphetamine, in comparison to vaginally born controls (El-Khodor and Boksa, 1997b). We have also demonstrated that following a regimen of chronic mild stress (saline injection once daily for 5 days) animals born by C-section with 15 min of anoxia show a markedly enhanced locomotor response to amphetamine challenge, in comparison to vaginally born control animals (Brake et al., 1997a). Given the postulated role of both dopaminergic neurotransmission and stress responsivity in modulating alcohol consumption, our results suggested that C-section birth and acute global birth hypoxia might also produce long-term effects on ethanol consumption in the rat.

Therefore, the aim of this study was to test the hypothesis that alterations in birth condition, namely C-section birth and C-section birth with an added period of global anoxia, can affect subsequent ethanol preference in the adult rat. For this we have compared free choice intake of ethanol vs. water in adult rats that had been born vaginally, by C-section or by C-section with 10 or 15 min of global anoxia. In addition, since enhanced ethanol consumption has been associated with locomotor stimulant effects of low dose ethanol and attenuated depressant effects of higher dose ethanol in rats (Waller et al., 1986), we also tested the effect of two low doses of ethanol on locomotor activity in rats born vaginally or by C-section with 10 min of global anoxia.

## 2. Materials and methods

### *2.1. C-section and acute global anoxia*

Pregnant Sprague–Dawley rats (Charles River, St. Constant, Quebec) underwent acute global anoxia during a C-section delivery using a modification of the procedure described by Bjelke et al. (1991). Timed pregnant rats at 22 days of gestation (i.e., on the day of birth) were decapitated, an abdominal incision was made and the uterus was quickly isolated from its blood supply and surrounding connective and fatty tissue (10–15 s). (Sacrifice of the dam was by decapitation to avoid the confound of anesthetic use, which may also produce central nervous system depressant effects.) An acute anoxic episode was induced by immersing the intact uterus into a 37°C saline bath for 10 or 15 min (10-min or 15-min anoxia groups). The pups were then delivered and stimulated by gentle tapping until breathing became even (30–40 s). No other means of artificial resuscitation was em-

ployed. The umbilical cord was ligated and the animals placed on a heating pad until given to their surrogate mothers (the rat is able to sustain longer acute periods of hypoxia at birth than is the human neonate (Jilek et al., 1970). Since rats subjected to 10 or 15 min of acute anoxia during C-section began breathing at birth without any form of artificial resuscitation other than palpation, this birth procedure in the rat may produce a 'moderate' hypoxic episode compared to the more severe hypoxic episode compatible with life if vigorous artificial ventilation and resuscitation procedures were employed. Our recently published results quantitating brain lactate, a marker of CNS hypoxia, during the first 24 h of life indicate that this model produces a consistent and reproducible degree of CNS hypoxia in offspring (El-Khodor and Boksa, 1997a)). Survival was 100% following 10 min of birth anoxia and 90–95% following 15-min anoxia. Another group of animals was delivered via C-section with no period of added anoxia (C-section group). Time between sacrifice of the dam and delivery of the last pup in the C-section group was $< 1.5$ min and survival was 100% in the C-section group. Pups born vaginally served as controls. Only male pups were retained for study. Pups from all groups were cross-fostered in mixed litters by the same dam to minimize differential rearing effects; each litter consisted of 12 pups/dam. Animals in the C-section and C-section plus anoxia groups were placed with surrogate dams by 1–2 h after birth. Vaginally born animals were removed from their birth dams and placed with surrogate dams at 2–24 h after birth. Pups were weaned at 21 days of age and grown to adulthood. Animals were group housed up until the time at which ethanol consumption was measured; random combinations of vaginally born, C-sectioned and anoxic animals were housed together in groups of three animals per cage. Animals were maintained on a 12-h light:12-h dark schedule (lights on and off at 0800 h and 2000 h, respectively) with free access to food and water. Free access to food and water continued throughout both experiments 1 and 2; at no time were the animals fluid-deprived. All procedures with animals were performed in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee.

In experiment 1, animals in the vaginally born group were born from 11 different dams, and animals in the C-section, C-section plus 10-min anoxia and C-section plus 15-min anoxia groups were born from 4, 6 and 6 dams, respectively. In experiment 2, vaginally born animals were born from seven different dams and animals in the C-section plus 10-min anoxia group were born from three dams.

### 2.2. Procedure

#### 2.2.1. Experiment 1

At 5 months of age, animals from the four experimental groups (i.e., rats born vaginally, by C-section or by C-section with 10 or 15 min of global anoxia) were allowed a 1-week acclimatisation period to single housing (single housing was required in order to monitor fluid intake from each individual animal. Single housing, in comparison to group housing, has been reported to increase voluntary ethanol intake in rats (Wolffgramm, 1990). Thus, it should be noted that all experimental groups in the current study were tested under conditions of isolation stress, rather than what may be considered 'baseline' group-housing conditions). Following this, each animal was offered free access to two standard water bottles, one of which contained tap water during the entire experiment and the other of which contained 5% ethanol (v/v) for the first 18 days, 2.5% ethanol for the next 6 days, 1% ethanol for the next 6 days and 10% ethanol for the next 6 days. Following this, animals were offered water only for 5 days followed by free choice between water and a solution of sucrose (1.36 g/100 ml) for 6 days. Left–right positioning of the two bottles in each cage was alternated every 3 days to account for possible position preference. Animals were weighed weekly. Intakes of water and of ethanol or sucrose solutions were recorded every 3 days and intakes are expressed as ml/kg body weight per day for each 3-day period. Total fluid intake was calculated as the sum of ethanol and water intake and also expressed in ml/kg body weight per day for each 3-day period. Ethanol preference scores for each 3-day period were calculated as [ethanol intake (in ml/kg body weight per day)/total fluid intake (in ml/kg body weight per day)] $\times$ 100 for each animal.

#### 2.2.2. Experiment 2

A separate cohort of animals born vaginally or by C-section with 10 min of anoxia was generated and tested for locomotor activity in response to two low doses (0.125 and 0.25 g/kg) of ethanol at 5 months of age, in experiment 2. Locomotor activity was tested using lucite activity chambers each equipped with two photoelectric switches and light beam interruptions were recorded via computer. Each rat received a 1-h habituation session in the activity chamber on each of 2 consecutive days before testing began. On the first test-day, each animal received an intraperitoneal injection of saline. Two days later, half of the animals received intraperitoneal injections of 0.125 g/kg ethanol while the other half received injections of 0.25 g/kg ethanol; 3 days later, the animals received a second injection of saline followed 2 days later by an injection of ethanol in the dose they had not yet received. Immediately after each injection, the animal was placed in the activity chamber and locomotor activity was recorded for 1 h. Locomotor activity testing took place between 0900 h and 1400 h and the order of animals tested always alternated a vaginally born animal with an anoxic animal.

Two weeks after locomotor testing, the same groups of animals were singly housed and 1 week later were offered a choice of tap water or 5% ethanol in water to drink for 3 days. This was followed 2 weeks later by a choice of water

or a solution containing sucrose (3.0 g/100 ml) for 3 days. Following 3 further days of water only, animals were given a final 3-day period in which a choice of plain tap water or water containing NaCl (0.6 g/100 ml) was offered. Fluid intakes were recorded every 3 days.

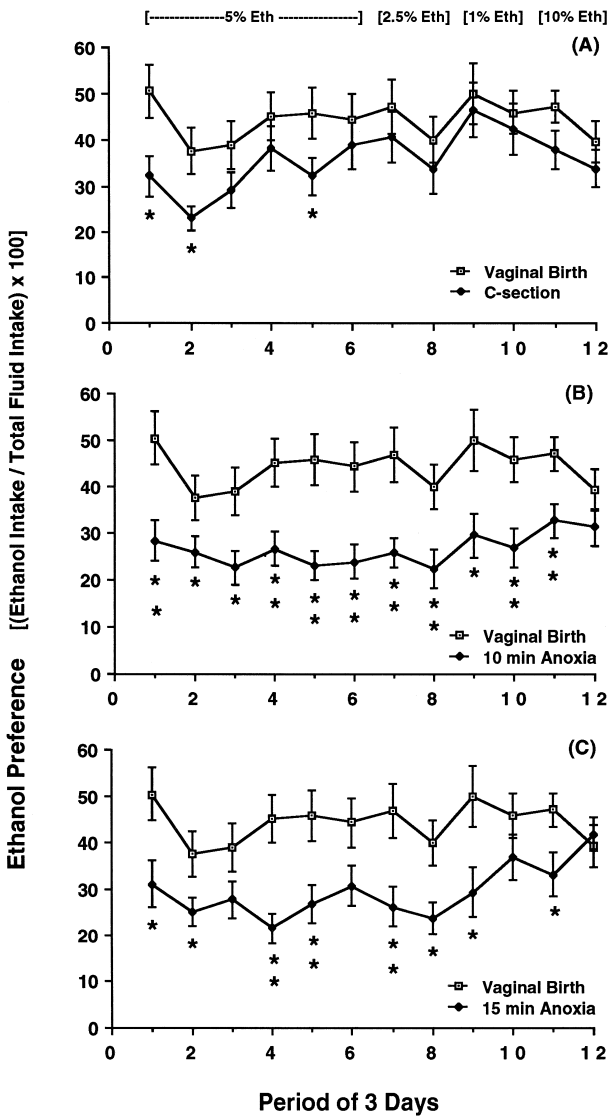


Fig. 1. Ethanol preference under conditions of free choice between ethanol and water, in adult rats born vaginally, by C-section or by C-section with 10 or 15 min of added global anoxia. Rats, that had been born vaginally, by C-section, or by C-section with 10 or 15 min of global anoxia were grown to adulthood and offered a choice of water or the indicated concentration (v/v) of ethanol in water for 12 sequential 3-day periods. Ethanol preference scores were calculated as [ethanol intake/total fluid intake]×100 for each animal, where total fluid intake is the sum of ethanol intake+water intake. Ethanol intake for these calculations was expressed as ml ethanol solution consumed/kg body weight per day, for each period; water intake was expressed as ml water consumed/kg body weight per day. Results are means±S.E.M. from 21, 19, 24, and 20 animals born vaginally, by C-section, by C-section plus 10-min anoxia and by C-section plus 15-min anoxia, respectively. The same values for the vaginally born control animals are shown in (A), (B) and (C) for comparison with experimental groups. Asterisks denote values significantly different from those for vaginally born controls at (**) $P < 0.01$ or (*) $P < 0.05$.

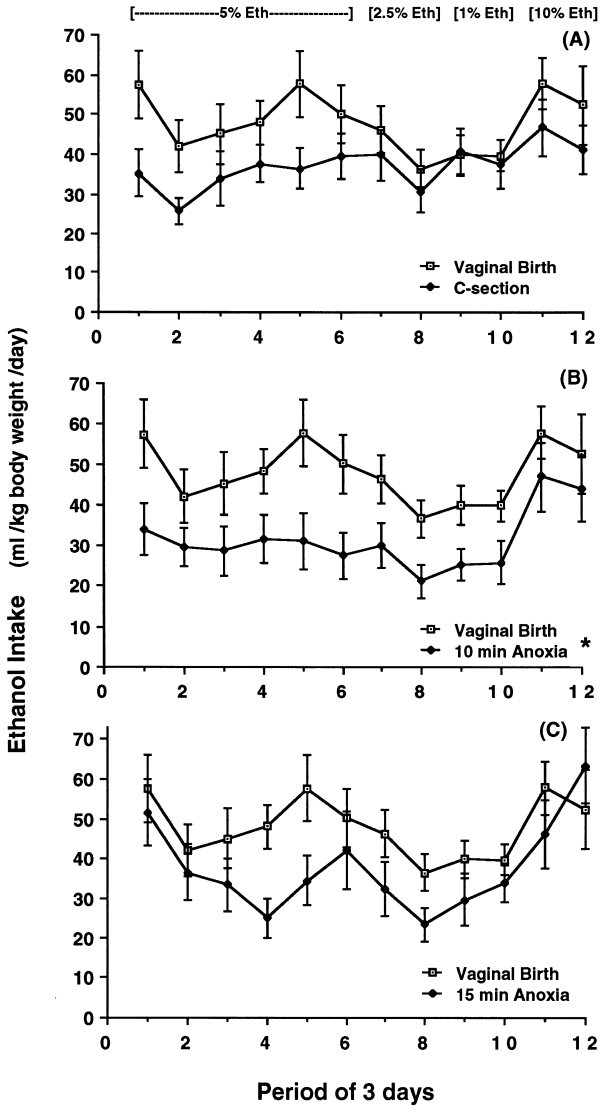


Fig. 2. Ethanol intake under conditions of free choice between ethanol and water, in adult rats born vaginally, by C-section or by C-section with 10 or 15 min of added global anoxia. Rats, that had been born vaginally, by C-section, or by C-section with 10 or 15 min of global anoxia were grown to adulthood and offered a choice of water or the indicated concentration (v/v) of ethanol in water for 12 sequential 3-day periods. Ethanol intake is expressed as ml ethanol solution consumed/kg body weight per day, for each period. Results are means±S.E.M. from the same animals used to generate data in Fig. 1. The same values for the vaginally born control animals are shown in (A), (B) and (C) for comparison with experimental groups. (*) Ethanol intake by the 10-min anoxia group was significantly different ($P < 0.05$) from values for the vaginal birth group, across the 12 periods of testing.

### 2.3. Data analysis

Data obtained from measures taken at sequential time periods were analyzed using two-way repeated measures analysis of variance; differences between birth groups were determined using post-hoc Newman–Keuls tests. Sig-

nificance of differences among birth groups at a single time-point were analyzed by one-way analysis of variance with post-hoc Newman–Keuls tests. Statistical analysis was performed using the pup, rather than either birth litter or surrogate litter, as unit, since experimental manipulations were performed on the pups as individuals (i.e., in the C-section group, individual pups were delivered manually by the experimenter and in the Anoxia groups, pups were placed into a saline bath followed by manual removal of each pup from the isolated uterus).

## 3. Results

### 3.1. Experiment 1

Adult rats, that had been born vaginally, by C-section or by C-section with 10 or 15 min of anoxia, were given a choice of tap water vs. the indicated concentration of ethanol for 12 3-day periods. Ethanol preference scores, expressed as (ethanol intake/total fluid intake) × 100, for each period, are shown in Fig. 1. Two-way analysis of variance of ethanol preference scores throughout the 12 periods indicated a significant effect of birth group ($F(3,80) = 6.439$, $P < 0.0006$) and of period ($F(11,880) = 4.395$, $P < 0.00001$) and a significant group × period interaction ($F(33,880) = 1.498$, $P < 0.036$). Post-hoc Newman–Keuls comparisons based on ethanol preference scores across the 12 periods indicated that both the 10-min anoxia ($P < 0.01$) and 15-min anoxia ($P < 0.01$) groups had decreased ethanol preference scores, in comparison with the vaginally born animals (Fig. 1B,C). When ethanol preference scores for each period were considered separately, the 10-min anoxia group showed significantly decreased ethanol preference scores, in comparison to the vaginally born group, during all periods except the last. The 15-min anoxia group showed significantly decreased ethanol preference scores, in comparison to the vaginally born group, during periods 1, 2, 4, 5, 7, 8, 9 and 11. Ethanol preference scores for the C-section group did not differ significantly from those for the vaginally born group across the 12 periods and separate analysis of each period indicated that scores for the C-sectioned group differed significantly from those for the vaginally born group only

Table 1
Water intake (A) and total fluid (ethanol + water) intake (B) during periods of free choice between ethanol and water, in adult rats born under various conditions

| Period | % Ethanol | Vaginal birth | C-section | 10-min Anoxia[a] | 15-min Anoxia[a] |
|---|---|---|---|---|---|
| | | (A) Water intake (ml/kg body weight per day) | | | |
| 1 | 5% | 52.0 ± 6.1 | 67.3 ± 5.5 | 72.2 ± 4.5 | 80.8 ± 5.4 |
| 2 | 5% | 69.6 ± 6.9 | 79.7 ± 4.6 | 79.6 ± 3.7 | 91.0 ± 7.2 |
| 3 | 5% | 62.7 ± 5.1 | 68.6 ± 3.1 | 76.6 ± 3.0 | 79.9 ± 3.8 |
| 4 | 5% | 60.5 ± 7.1 | 60.8 ± 6.0 | 75.3 ± 3.5 | 78.9 ± 3.8 |
| 5 | 5% | 64.2 ± 7.3 | 71.5 ± 5.1 | 82.1 ± 3.7 | 82.2 ± 5.7 |
| 6 | 5% | 59.9 ± 6.3 | 59.6 ± 5.0 | 77.1 ± 4.7 | 74.5 ± 4.5 |
| 7 | 2.5% | 55.5 ± 7.3 | 58.3 ± 6.5 | 74.4 ± 5.0 | 75.3 ± 5.1 |
| 8 | 2.5% | 54.3 ± 4.9 | 56.8 ± 4.9 | 69.9 ± 5.8 | 68.4 ± 4.3 |
| 9 | 1% | 44.5 ± 7.0 | 46.9 ± 5.4 | 61.2 ± 6.0 | 62.4 ± 4.9 |
| 10 | 1% | 48.4 ± 4.8 | 48.2 ± 4.8 | 64.1 ± 3.9 | 55.9 ± 4.5 |
| 11 | 10% | 62.6 ± 5.4 | 68.5 ± 3.9 | 76.3 ± 4.8 | 75.1 ± 6.9 |
| 12 | 10% | 63.5 ± 3.0 | 69.8 ± 2.7 | 73.8 ± 5.1 | 73.3 ± 3.6 |
| 13 | Water only | 71.7 ± 3.8 | 75.2 ± 2.6 | 78.5 ± 5.6 | 75.7 ± 3.4 |
| | | (B) Total fluid intake (ml/kg body weight per day) | | | |
| 1 | 5% | 109.5 ± 8.2 | 102.7 ± 8.1 | 106.3 ± 7.5 | 132.5 ± 14.2 |
| 2 | 5% | 111.6 ± 7.9 | 105.4 ± 5.5 | 108.0 ± 5.3 | 127.7 ± 12.1 |
| 3 | 5% | 107.9 ± 8.4 | 102.4 ± 6.7 | 105.4 ± 7.5 | 120.1 ± 11.1 |
| 4 | 5% | 108.6 ± 7.0 | 98.7 ± 6.6 | 107.0 ± 7.0 | 104.1 ± 7.1 |
| 5 | 5% | 122.0 ± 11.4 | 108.2 ± 7.5 | 113.1 ± 9.4 | 116.7 ± 9.7 |
| 6 | 5% | 110.1 ± 6.8 | 99.3 ± 5.3 | 104.6 ± 7.8 | 116.6 ± 11.9 |
| 7 | 2.5% | 101.8 ± 8.1 | 98.1 ± 8.1 | 104.6 ± 9.3 | 107.9 ± 9.8 |
| 8 | 2.5% | 90.9 ± 3.4 | 87.7 ± 4.2 | 91.1 ± 7.0 | 91.8 ± 5.7 |
| 9 | 1% | 83.8 ± 5.4 | 87.6 ± 6.2 | 86.4 ± 7.2 | 92.2 ± 8.1 |
| 10 | 1% | 88.2 ± 3.2 | 85.8 ± 3.6 | 90.0 ± 5.7 | 89.8 ± 3.7 |
| 11 | 10% | 120.5 ± 9.3 | 115.4 ± 7.2 | 123.2 ± 11.8 | 121.1 ± 11.7 |
| 12 | 10% | 116.0 ± 10.0 | 111.0 ± 6.2 | 117.8 ± 11.4 | 136.7 ± 11.9 |

Rats, that had been born vaginally, by C-section, or by C-section with 10 or 15 min of added global anoxia, were grown to adulthood and offered a choice of water or the indicated concentration of ethanol (in water) for 12 sequential 3-day periods. Total fluid intake is the sum of water plus ethanol solution consumed. Results are means ± S.E.M. from the same animals for which ethanol preference and intake data are shown in Figs. 1 and 2.

[a]Water intake significantly different ($P < 0.05$) from values for vaginal birth group, across the first 12 periods of testing. There were no significant group differences in total fluid intake.

during periods 1, 2 and 5 (Fig. 1A). Ethanol preference scores for the C-section group also did not differ significantly from those for the 10 or 15-min anoxia groups across the 12 periods.

Ethanol intakes, expressed as ml ethanol solution consumed/kg body weight per day, for the corresponding periods, are shown in Fig. 2. Two-way analysis of variance of ethanol intake throughout the 12 periods indicated a significant effect of birth group ($F(3,80) = 3.202$, $P < 0.028$) and of period ($F(11,880) = 5.358$, $P < 0.00001$) with no significant group × period interaction ($F(33,880) = 0.944$, $P < 0.560$). Post-hoc comparisons indicated that ethanol intake was significantly reduced in the 10-min anoxia group ($P < 0.05$), in comparison with the vaginally born animals (Fig. 2B), across the 12 periods of testing.

Water and total fluid intakes for the corresponding periods, are shown in Table 1. Two way analysis of variance of water intake throughout the 12 periods indicated a significant effect of birth group ($F(3,80) = 3.939$, $P < 0.011$) and of period ($F(11,880) = 21.413$, $P < 0.00001$) with no significant group × period interaction ($F(33,880) = 1.054$, $P < 0.387$). Post-hoc comparisons indicated that water intake was significantly greater in both the 10-min anoxia ($P < 0.05$) and 15-min anoxia ($P < 0.05$) groups, in comparison with the vaginally born animals, across the 12 periods of testing (Table 1A). There were no significant group differences in total fluid intake throughout the 12 periods of free choice between ethanol and water (Table 1B) nor were there any group differences in water intake during a 13th 5-day period in which only water was offered (Table 1A).

Table 3
Body weights of rats born under various conditions

| Birth condition | Weight (g) | |
|---|---|---|
| | 5 Months of age | 7 Months of age |
| Experiment 1 | | |
| Vaginal birth | 636 ± 13 (21) | 708 ± 16 (21) |
| C-section | 648 ± 17 (19) | 721 ± 23 (18) |
| 10-min Anoxia | 602 ± 18 (24) | 675 ± 19 (23) |
| 15-min Anoxia | 593 ± 12 (20) | 664 ± 14 (20) |
| Experiment 2 | | |
| Vaginal birth | 580 ± 18 (12) | 730 ± 22 (12) |
| 10-min Anoxia | 563 ± 13 (12) | 708 ± 15 (12) |

Results are means ± S.E.M. from the number of animals shown in parentheses. There were no significant group differences in body weight at either age.

When the same four experimental groups of animals were given the choice of water vs. a solution of sucrose (1.36 g/100 ml) for 2 further 3-day periods, no group differences were observed in measures of sucrose preference or total fluid intake (Table 2). In addition, although anoxic animals tended to weigh less than did vaginally born or C-sectioned animals, there were no significant group differences in body weights of animals weighed at 5 months or again at 7 months of age (Table 3).

### 3.2. Experiment 2

Results from experiment 1 indicated that the most pronounced differences in ethanol preference were observed

Table 2
Preference for a solution of sucrose vs. water or of NaCl vs. water during periods of free choice, in adult rats born under various conditions

| Period | Birth condition | Sucrose preference [sucrose intake/total fluid intake] × 100 | Total fluid intake (ml/kg body weight per day) |
|---|---|---|---|
| Experiment 1: (Sucrose = 1.36 g/100 ml) | | | |
| 1 | Vaginal birth | 80.1 ± 5.5 (19) | 95.5 ± 9.3 |
| | C-section | 91.1 ± 2.1 (19) | 120.3 ± 6.0 |
| | 10-min Anoxia | 78.9 ± 5.1 (24) | 108.1 ± 5.5 |
| | 15-min anoxia | 76.1 ± 5.5 (20) | 116.5 ± 9.8 |
| 2 | Vaginal birth | 75.5 ± 5.3 (21) | 101.2 ± 7.2 |
| | C-section | 82.2 ± 3.8 (19) | 121.5 ± 9.0 |
| | 10-min Anoxia | 72.0 ± 5.2 (24) | 111.1 ± 6.5 |
| | 15-min anoxia | 75.8 ± 5.6 (20) | 116.4 ± 9.2 |
| Experiment 2: (Sucrose = 3.0 g/100 ml) | | | |
| 1 | Vaginal birth | 93.9 ± 0.9 (12) | 219.7 ± 21.3 |
| | 10-min Anoxia | 94.7 ± 0.4 (12) | 200.0 ± 22.7 |

| Period | Birth condition | NaCl preference [NaCl intake/total fluid intake] × 100 | Total fluid intake (ml/kg body weight per day) |
|---|---|---|---|
| Experiment 2: (NaCl = 0.6 g/100 ml) | | | |
| 1 | Vaginal birth | 88.2 ± 4.1 (12) | 110.3 ± 6.3 |
| | 10-min Anoxia | 85.1 ± 5.3 (12) | 115.0 ± 9.3 |

Rats, that had been born vaginally, by C-section, or by C-section with 10 or 15 min of added global anoxia, were grown to adulthood and offered a choice of water vs. sucrose solution (1.36 g/100 ml or 3.0 g/100 ml, as indicated) or a choice of water vs. NaCl solution (0.6 g/100 ml) for periods of 3 days. Total fluid intake is the sum of water plus sucrose solution or of water plus NaCl solution consumed for that period. Results are means ± S.E.M. from the numbers of animals shown in parentheses. There were no significant group differences in sucrose preference scores, in NaCl preference scores or in total fluid intake during any period.

in animals born by C-section with 10 min of anoxia in comparison to vaginally born controls. Therefore, in experiment 2, a separate cohort of adult rats that had been born vaginally or by C-section with 10 min of anoxia was tested for locomotor responses to two low doses (0.125 and 0.25 g/kg) of ethanol. Fig. 3 shows the locomotor responses of these animals during the first 10 min after ethanol administration. At a dose of 0.125 g/kg, ethanol had no significant effect on locomotor activity, relative to saline, in either the vaginally born (Fig. 3A) or 10-min anoxia groups (Fig. 3B). However, this dose of ethanol showed a non-significant tendency to enhance locomotion in the vaginally born group. At 0.25 g/kg, ethanol had no significant effect on locomotor activity, relative to saline, in the vaginally born group (Fig. 3C), but produced a transient but significant depression of locomotor activity, relative to saline, from 3–7-min post-injection, in the 10-min anoxia group (Fig. 3D). For the 10-min anoxia group, total locomotor activity counts for the period 3–7-min post-injection were $19.8 \pm 3.2$ (mean $\pm$ S.E.M.) following saline and $11.3 \pm 2.3$ following 0.25 g ethanol/kg ($P < 0.039$).

Three weeks following locomotor testing, animals in experiment 2 were given a free choice between drinking water and 5% ethanol in water for 3 days. Similar to

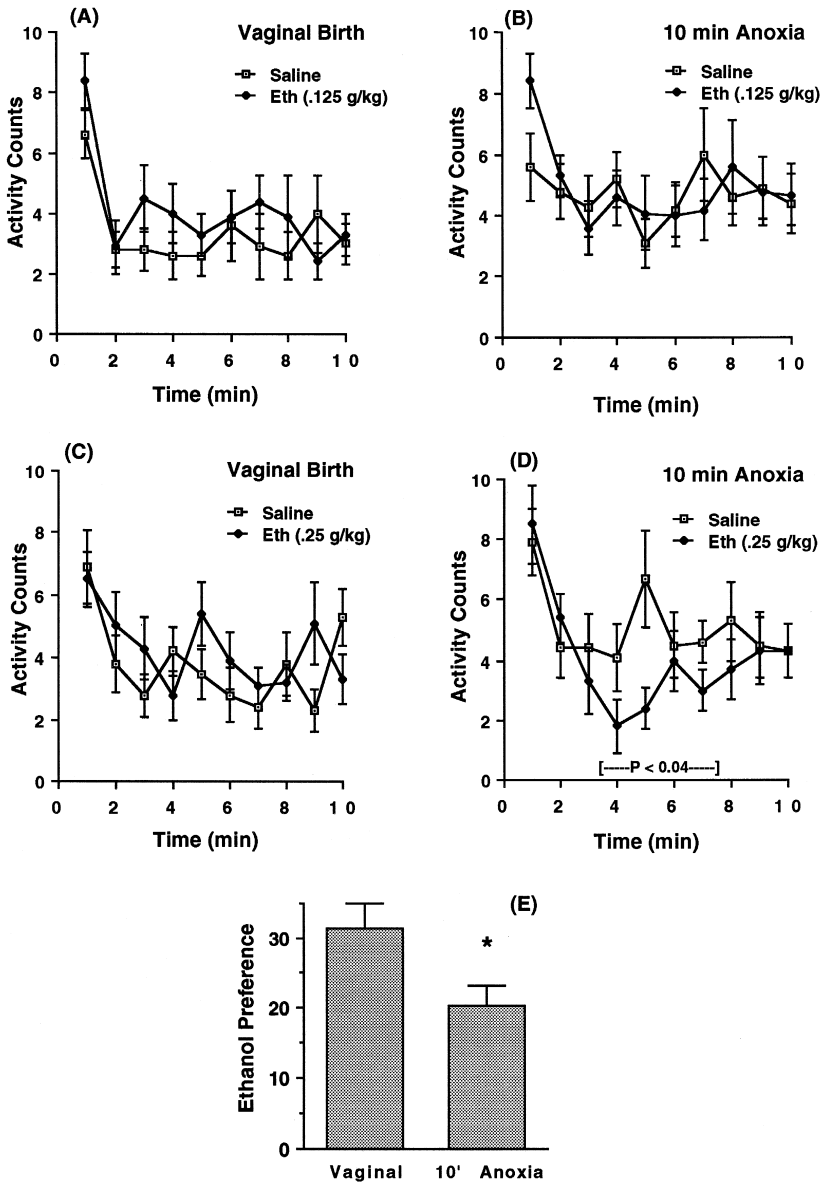


Fig. 3. Locomotor activity in response to low dose ethanol, in adult rats born vaginally or by C-section with 10 min of added global anoxia. Rats were born vaginally (A, C) or by C-section with 10 min of added global anoxia (B, D). At adulthood, rats received intraperitoneal injections of ethanol in the concentrations indicated and locomotor activity was recorded. Three weeks later, rats were offered a choice of water or 5% ethanol in water to drink for 3 days and ethanol preference scores were determined (E). Results are means $\pm$ S.E.M. from 12 animals in each group.

results in experiment 1, the 10-min anoxia group in experiment 2 showed reduced ethanol preference in comparison to vaginally born controls (Fig. 3E). Three weeks later, the same two experimental groups of animals were given the choice of water vs. a solution of sucrose (3.0 g/100 ml) for 3 days, then water vs. NaCl (0.6 g/100 ml) for 3 days. No group differences were observed in measures of sucrose preference, NaCl preference or total fluid intake during these periods (Table 2) (it may be noted that all animals drank an appreciably larger amount of sucrose solution when offered a solution of 3.0 g sucrose/100 ml during experiment 2, in comparison to experiment 1 when 1.6 g sucrose/ml was offered. This is in agreement with previous studies by Stewart et al. (1994) showing a steep increase in sucrose intake over this concentration range in rats offered sucrose solutions vs. water). There were also no significant differences in body weight between vaginally born animals and animals born by C-section with 10 min of anoxia at 5 or 7 months of age, in experiment 2 (Table 3).

## 4. Discussion

The main finding of this study is that rats born by C-section with 10 or 15 min of added global anoxia show reduced ethanol preference as adults, in comparison to vaginally born controls. These findings indicate that variations in birth condition, compatible with grossly normal behavioral development, can permanently alter systems responsible for ethanol preference in comparison to water.

The clearest effects were observed with the 10-min anoxia group. This group showed the most pronounced decrease in ethanol preference scores in comparison to vaginally born animals and showed a significant decrease in absolute amount of ethanol consumed across the entire 36 days of testing in experiment 1. Relative to vaginally born controls, the 10-min anoxia group drank more water to offset the reduced ethanol consumption, such that total fluid intake did not differ from that of controls. The decreases in ethanol preference and ethanol intake in the 10-min anoxia group compared to vaginally born controls are comparable in magnitude to the decreases produced by maximally effective doses of a number of pharmacological agents reported to reduce ethanol intake in rats, for example, bromocriptine (Weiss et al., 1990), {(−)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-*N*-methyl-5-H-benzo[d]naptho[2,1b]azepine} (SCH 39166) (Panocka et al., 1995), *N*-[(8$\alpha$)-2,6-dimethylergoline-8-yl]-2,2-diethylpropanamide (SDZ 208-911) (Bono et al., 1996) and ritanserin (Panocka et al., 1996) (a dopamine receptor agonist, dopamine-$D_1$ receptor antagonist, dopamine receptor partial agonist and serotonin 5-$HT_2$ receptor antagonist, respectively).

Although total fluid intake by the 15-min anoxia group also did not differ from that of the vaginally born controls, the decreased ethanol preference scores in the 15-min anoxia group appeared to be mainly due to increased water intake during some periods and increased water intake combined with a tendency towards decreased ethanol intake during other periods. However, ethanol intake by the 15-min anoxia group was not significantly reduced across the 12 test periods. At first glance, one might predict that the 15-min anoxia group should show changes similar to those observed for the 10-min anoxia group, but greater in magnitude. However, it should be recalled that increasing periods of hypoxia may activate a series of detrimental or compensatory responses, which may alter eventual outcomes. For example, using the same model as in our studies, Dell'Anna et al. (1995) have reported significant increases in Fos-immunoreactive cells in piriform cortex immediately following 2–3 min of birth anoxia, no change with 5–16 min of anoxia and significant decreases with periods of birth anoxia greater than 16 min. In addition, we have demonstrated that global anoxia, as administered in the current model, elicits large increases in circulating norepinephrine and epinephrine (El-Khodor and Boksa, 1997a), which are known to protect against some aspects of hypoxia via a number of systemic mechanisms.

Animals born by rapid C-section alone, with no extra period of global anoxia, showed reduced ethanol preference only during a few of the early periods of testing and no significant overall reduction in ethanol intake, in comparison to vaginally born animals. However, since ethanol preference scores in the C-section group were in between values for vaginally born controls and values for animals born by C-section with 10-min anoxia, this suggests that changes in ethanol preference in the 10-min anoxia group (relative to vaginally born controls) result from the combined effects of both the C-section procedure and the added 10 min of global anoxia. Ideally, one would like to test effects of global birth anoxia in isolation from the C-section procedure by subjecting pups to anoxia during vaginal birth, but this is not technically feasible at present. However, the fact that ethanol preference scores for the C-section group did not differ significantly from those of the 10 or 15-min anoxia groups would suggest that an acute period of global anoxia alone may not be sufficient to reduce ethanol preference.

Previous studies on either unselected rats or rats selectively bred for high oral ethanol preference have shown a positive association between preference for ethanol and for sweet solutions such as saccharin or sucrose and a negative association between preference for ethanol and NaCl solutions (Kampov-Polevoy et al., 1990; Stewart et al., 1994). Our study found no group differences due to birth condition on measures of sucrose preference or NaCl preference. Therefore, in rats born vaginally, by C-section or by C-section with global anoxia, we did not observe an association between ethanol preference and preference for sucrose or saline. Our findings do, however, indicate that the anoxic animals do not have a generalized aversion to

consuming novel tasting fluids since the anoxic (as well as C-section) groups showed the same marked preference for sweet and salty solutions over plain water, that were shown by the vaginally born control animals. Therefore, the reduced ethanol preference on the part of the anoxic animals appears to be relatively specific for ethanol as opposed to solutions of other composition.

Low doses of ethanol are reported to enhance locomotor activity at early times after administration in rodents, although this effect is more pronounced and consistently observed in mice than in rats (Pohorecky, 1977). The locomotor stimulant properties of ethanol have been suggested to model euphoric effects of ethanol in man and have been associated with increased preference for ethanol in animals (Waller et al., 1986). Consistent with this, ethanol, in low doses that have no effect on (0.12 g/kg) or tend to depress (0.25 g/kg) locomotor activity in selectively bred alcohol non-preferring rats, has been shown to stimulate locomotion in alcohol-preferring rats (Waller et al., 1986). These authors further showed that higher doses (0.5–1.5 g/kg) of ethanol significantly depressed locomotor activity in non-preferring rats but tended to stimulate or produce no change in locomotion in the alcohol-preferring line. Consistent with previous literature, effects of low doses of ethanol on locomotor activity were not very pronounced in either vaginally born rats or rats born by C-section with 10 min of global anoxia. However, a dose of 0.25 g ethanol/kg, which had no significant effect on locomotion in vaginally born animals, did transiently but significantly depress locomotion in the 10-min anoxia group. Thus, similar to the findings of Waller et al. (1986), the group of animals showing reduced ethanol preference in our study, the 10-min anoxia group, also showed significantly depressed locomotor activity in response to a low dose of ethanol that had no effect in vaginally born control animals.

One of the original reasons for predicting that birth condition might have effects on ethanol preference was our previous observations that birth condition could produce long-term alterations in dopaminergic transmission. Therefore, it is of interest to compare the current findings on ethanol preference with previous findings on dopaminergic parameters in this model. Significant increases in numbers of tyrosine hydroxylase-immunoreactive neurons have been observed in the substantia nigra and ventral tegmental areas of 4-week-old rats that had been born by C-section with 14–17 min (Bjelke et al., 1991) or 19–20 min (Chen et al., 1995) of global anoxia. The present findings that animals born with 10 or 15 min of anoxia show reduced ethanol preference correlates well with a recent report by Zhou et al. (1995) showing a greater number of tyrosine hydroxylase-immunoreactive neurons (with no change in total neurons) in the ventral tegmental area of alcohol non-preferring rats, in comparison to the alcohol-preferring line. In the same line of alcohol-preferring rats, levels of dopamine have been reported to be decreased in the nucleus accumbens and unchanged in the striatum and frontal cortex (McBride et al., 1995). We, however, have found no significant differences in steady state levels of dopamine (or of serotonin or norepinephrine) in the accumbens, striatum or pre-frontal cortex of animals born by C-section with 15 min of global anoxia in comparison to vaginally born controls (El-Khodor and Boksa, 1997a). Interestingly, we have shown that 2-month-old rats that had been born by rapid C-section alone have significant increases in dopamine levels in the accumbens and striatum and reduced dopamine in the pre-frontal cortex, in comparison to vaginally born controls. However, the C-sectioned group did not show pronounced alterations in ethanol preference in the current study. The probably complex role of dopamine in modulating ethanol consumption is, as yet, incompletely understood. For example, Rassnick et al. (1993) have reported that 6-hydroxydopamine lesions sufficient to produce major depletion of dopamine in the accumbens, frontal cortex, amygdala and olfactory tubercle had no significant overall effect on ethanol self-administration in rats although patterns of responding for ethanol were subtly altered. Nonetheless, reduced ethanol consumption in rats has been reported following administration of a variety of dopaminergic agents, including dopamine receptor agonists, antagonists and partial agonists (Bono et al., 1996; Hodge et al., 1993; Panocka et al., 1995; Russell et al., 1996; Samson et al., 1993; Weiss et al., 1990). As mentioned, we have demonstrated that animals born by C-section with added anoxia show hyperdopaminergic function as adults, particularly under conditions of repeated stress (Brake et al., 1997a,b; El-Khodor and Boksa, 1997b). Thus, it would be of interest to measure ethanol consumption under conditions of chronic stress in these animals. In the current study, starting at 1 week prior to the presentation of ethanol, all animals did undergo the stress of single housing, a condition which has been shown to enhance ethanol consumption in rodents (Roske et al., 1994; Wolffgramm, 1990).

Although much of the discussion in this paper has centered around alterations in dopaminergic mechanisms, it must be emphasized that the birth conditions in this study involved global hypoxia to the entire animal. Clearly, alterations in a large number of mechanisms involved in ethanol consuming behavior or in the metabolism of ethanol remain as candidates that might be responsible for the observed effects on ethanol preference in the anoxic group. In addition, not only the birth insult itself, but the sequelae, either biochemical or behavioral, arising from the initial insult, may contribute to eventual alterations in ethanol preference. Regardless of the mechanism involved, the results show that a brief birth insult, compatible with grossly normal development and behavior, is sufficient to alter ethanol preference in the rat. Animals born with 10 or 15 min of global anoxia did not exhibit generalized malaise or deficits in consummatory behavior as they were of normal body weight and showed no alterations in baseline

water, total fluid, sucrose or NaCl intakes, in comparison to control animals. Previous studies have shown that this model of birth anoxia produces animals that are normal in many aspects of behavior and show alterations only under specific test conditions. For example, we have demonstrated that adult rats born by C-section with 10 or 15 min of anoxia show a subtle deficit in spatial learning under relatively stringent test conditions in the Morris water maze, but no changes in a battery of sensorimotor functions (Boksa et al., 1995). Rats born by C-section with 10 or 15 min of anoxia show no changes in immobility responses during an initial forced swim stress but do show increased immobility, in comparison to vaginally born controls, following repeated exposure to forced swim (Boksa et al., 1998). In addition, no group differences have been observed between animals born vaginally, by C-section or by C-section with anoxia on measures involving novelty or stress including exploratory behavior in an elevated plus maze or approach behavior to food or novel objects in an open field (Boksa et al., 1998).

With regard to comparison of the rat model of birth insult with development of the human CNS, the rat brain is thought to be less mature at birth than is the brain of the human newborn. It has been suggested that, at approximately postnatal day 10, the brain of the rat is at the stage of rapid brain growth, accelerated synaptogenesis, myelinization, and astrocyte proliferation characteristic of the human newborn CNS at term (Romijn et al., 1991). However, direct comparison of developmental stages in humans vs. rats is made difficult, for example, by the recognition that the period of human brain growth and myelinization is quite broad encompassing the last trimester in utero up to the first or second year of life (Dobbing, 1970; Stein and Susser, 1985; Winick, 1970). Nonetheless, inasmuch as parallels can be drawn between humans and rats, findings using models of birth insult in the rat may relate more to premature than to term human brain. In this regard, however, it is of note that prematurely born human infants experience birth anoxia much more frequently than do term infants (Vannucci, 1990; Volpe, 1992). With regard to sensitivity of later developmental periods, it is also noteworthy that environmental manipulation (i.e., brief handling) of early postnatal (day 5–20) rats and early maternal separation in primates have been reported to alter voluntary alcohol consumption in the resulting adult animal (Higley et al., 1987; Hilakivi-Clarke et al., 1991).

In conclusion, our results demonstrate that systems responsible for ethanol preference in the rat are sensitive to and can be permanently altered by a relatively subtle birth insult, compatible with grossly normal development and behavior. It will be of interest to determine if effects of C-section birth with acute global anoxia on ethanol preference extend to other strains and lines (e.g., alcohol-preferring) of rats. If so, this birth insult could serve as an easily producible model to investigate mechanisms providing long-lasting reduction in ethanol preference.

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