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Exposure to ethanol and nicotine during the brain growth spurt Spatial DMP performance in male rats

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Abstract

Male Long–Evans rats were reared artificially and, using a 2 × 2 design, were exposed from postnatal days (PD) 6–9 to ethanol (ET: 6.5 g kg^{-1} day^{-1} "binge" exposure) and/or nicotine bitartrate (NIC: 6 mg kg^{-1} day^{-1} continuous exposure) via gastrostomy tubes. Controls were administered maltose–dextrin in amounts isocaloric to ET and/or sodium bitartrate. A fifth suckled-control group was reared normally. NIC accelerated eye opening on PD 14; whereas ET delayed eye opening and hindlimb support on PD 16. Beginning in postnatal week 7, rats were tested on a spatial delayed matching-to-place (DMP) version of the Morris water maze, which entailed a series of problems, each consisting of search and recall trials, that required the rats to use extra-maze cues to locate a hidden escape platform. In Phase 1 of testing, the ET-exposed groups were impaired in the recall trials, but there was no effect of NIC. A longer encoding time (45 vs. 10 s) improved performance across all groups. In contrast, acute administration of NIC (0.1 mg/kg ip) immediately prior to testing in Phase 2 failed to improve performance in any group. In conclusion, these results confirm previous findings of impaired spatial DMP-task performance in ET-exposed rats and further suggest that these memory deficits are amenable to amelioration.

Keywords: Ethanol; Brain growth spurt; Nicotine; Artificial rearing; Delayed matching-to-place (DMP); Spatial memory; Water maze; Rats

1. Introduction

The use of drugs by the mother during pregnancy can have severe consequences on the development of the offspring, of which effects on the central nervous system are often the most deleterious. Of the licit drugs used during pregnancy, ethanol (ET) and nicotine (NIC) are two of the most common, with the prevalence of smoking being estimated at rates between 20% and 40% of pregnant women (reviewed by Behnke and Eyler, 1993; Cutler et al., 1996) and that of drinking at 49% (Abel, 1998). Furthermore, the use of ET and NIC have been shown to correlate highly, such that abusers of one of these drugs also tend to consume the other heavily (reviewed in Collins, 1996); this association also appears to be true of pregnant women (Cornelius et al., 1994; Fried et al., 1984). Moreover, the increasing incidence of polydrug use (Day and Richardson, 1994) supports the importance of understanding the consequences of combined exposure to ET and NIC on the development of offspring.

Clinical studies report long-term effects of prenatal exposure to ET (Gray and Streissguth, 1990; Mattson and Riley, 1998; Streissguth et al., 1990, 1994; Uecker and Nadel, 1996) and NIC (Fried et al., 1998; reviewed in Lichtensteiger and Schlumpf, 1993; Paulson et al., 1994) on cognition and behavior. Interestingly, a study that showed that ET and NIC acted synergistically to produce learning deficits also showed that infants exposed to NIC alone performed better than controls (Martin et al., 1977). Animal models support the clinical evidence in showing that exposure during development to either ET (Abel, 1996; Goodlett and Peterson, 1995; Greene et al., 1992; Hannigan et al., 1999; Pauli et al., 1995; Tomlinson et al., 1998; West, 1986) or NIC (Slotkin, 1998; Yanai et al., 1992) can have long-term behavioral consequences. With respect to prenatal coexposure, Martin et al. (1982) reported that ET and NIC interacted to affect performance differently on two operant tasks: combined exposure impaired performance

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when shock was the discriminative stimulus, but coexposure to NIC seemed to alleviate ET-induced impairment on an appetitive discrimination task.

An advantage of animal models is that they allow precise control over the timing and degree of exposure. A concern in the literature relating to the effects of fetal alcohol exposure in humans is whether "binge" drinking, leading to intermittent high blood alcohol levels during the third trimester of pregnancy, might lead to cognitive–behavioral deficits in the absence of overt physical abnormalities (e.g., Weinberg and Guerri, 1996). During the third trimester, there is acceleration in brain development known as the "brain growth spurt." In rats, this period of rapid brain growth occurs during the early postnatal period (Dobbing and Sands, 1979). Thus, the artificial-rearing model, in which drugs are administered directly to neonatal rats via gastrostomy tubes, has been used frequently to model binge drinking during the human third trimester (Goodlett and Peterson, 1995; Greene et al., 1992; Tomlinson et al., 1998). An additional advantage of this model is that it affords the opportunity to study the effects of the drug directly on offspring development, independent of maternal physiology. Recently, Chen et al. (1998, 1999) have used artificial rearing to show independent effects of exposure to ET and NIC on brain growth and morphology in rats. Furthermore, Goodlett and colleagues identified postnatal days (PD) 7–9 as the time when the hippocampus in rats is sensitive to binge ET exposure (Goodlett and Johnson, 1997; Johnson et al., in press).

Previous work in our laboratory has shown impairment in performance on the delayed matching-to-place (DMP) version of the Morris water-maze task in artificially reared male rats that were exposed to ET in a binge pattern during the latter part of the brain growth spurt (Girard et al., 2000). The water-maze task requires learning the location of a hidden platform in relation to extra-maze cues. The DMP version of this task assesses memory for recent events, as the platform is moved to a new location for each problem session (see Morris and Frey, 1999). Typically, the first trial of a problem is a search trial that requires finding the hidden platform more or less by chance; escape will be more efficient on the second trial if a place strategy is used in its relocation. We showed that ET-exposed rats were impaired in their ability to relocate the platform on Trial 2. After extensive practice on this task, adult rats that were exposed to ET no longer differed from controls following a short delay (60 s), but the ET-induced deficit remained in a longer-delay condition (2 h). In contrast, Cutler et al. (1996) failed to find an effect of prenatal exposure to NIC on a cued version of the DMP task.

Our first objective in the present study was to replicate our previous findings of ET-induced impairment on the DMP task, and, through the use of a 2×2 experimental design, to extend the question to the combined effects of ET and NIC. The second objective of this study was to ascertain whether the ET-induced, and possibly NIC-induced, impairment on the DMP task would be amenable to amelioration. As clinical research suggests that the encoding of information might be especially vulnerable to prenatal ET exposure (Don et al., 1993; Mattson et al., 1996) and our previous work revealed an effect of extensive practice (Girard et al., 2000), intervention was directed at the encoding of information. In Phase 1, the time allowed to encode the spatial location of the platform was manipulated (10 or 45 s). In Phase 2, we used an acute NIC injection, which has been reported to facilitate attention, as well as the encoding and consolidation of new information (Warburton, 1992). In a review of the animal literature, Levin (1992) reported that, in general, low doses of NIC (0.5 mg/kg or less) tend to facilitate performance on cognitive tasks, especially in animals with memory impairment (Decker et al., 1992; Hodges et al., 1991a,b). Thus, a low dose of NIC (0.1 mg/kg) was used in the present study.

In summary, the main hypotheses addressed by this study were that exposure to ET and to NIC during the brain growth spurt would lead to deficits in male rats on the DMP task that would be ameliorated by (i) increased encoding time and (ii) acute administration of NIC prior to testing. It was further hypothesized that there would be an ET × NIC interaction, such that the combined effect of early exposure to these two drugs would be greater than that predicted by either drug alone.

2. Methods

The present experiment was approved by the Animal Care Committee at the University of Waterloo, in compliance with the Animals for Research Act of Ontario (Revised Statutes of Ontario), and the Guide for the Care and Use of Experimental Animals from the Canadian Council on Animal Care.

2.1. Animals

The rats used in this study were the male offspring of timed-pregnant Long–Evans rats (Harlan Sprague Dawley, Indianapolis, IN). The pregnant dams were obtained at 10–13 days gestation and were housed individually in shoebox cages ($43 \times 21 \times 22$ cm^3) with free access to both tap water and food (#5001, PMI Nutrition International, St. Louis, MO). The rats were maintained at 22 ± 1°C under a reversed 12:12-h light/dark cycle. Litters were culled to 10 pups within 24 h after birth. The males from each litter were assigned randomly to each of five treatment conditions (with no more than 2 pups/litter/group and an initial $n = 14–19$ rats/group).

2.2. Experimental design

The basic experimental design was a 2×2 factorial, with two levels of exposure to ET (0 and 6.5 g kg^{-1} day^{-1}) and (−)-NIC (0 and 6 mg kg^{-1} day^{-1}). As in previous studies

using the artificial-rearing model (Chen et al., 1998, 1999; Girard et al., 2000; Slaweki et al., 2000), these drugs were mixed into a rat-milk substitute (RMS) formula and fed to the rats via gastrostomy tubes (as described below). NIC was administered in the form of a bitartrate salt (Sigma, N5260) in an amount that provided a dose equivalent to 6-mg/kg NIC base. Rats in the no-ET and/or no-NIC control conditions were fed maltose–dextrin substituted isocalorically for ET and sodium ditartrate in the same concentration as the NIC salt. ET was administered using a binge model, i.e., spaced over 4 consecutive feedings out of 12 daily feedings. NIC was provided continuously in each feeding. In addition to the above four groups that were reared artificially, a fifth suckled control group (SC) consisted of pups that were fostered to nursing dams on the day that their littermates were gastrostomized (see below). This group served as a normally reared control group. It should be noted that the SC rats differed from the artificially reared gastrostomy controls (GC, i.e., 0 ET/0 NIC) in terms of both early diet and rearing conditions.

2.3. Artificial-rearing procedure

The artificial-rearing procedure has been described in detail elsewhere (Ward et al., 1998). Briefly, on Day 27 postconception ($\sim$Day 5 after birth), the rat pups were anesthetized with methoxyflurane inhalant (Metofane, Janssen Pharmaceutica, North York, ON) and gastrostomy tubes were inserted. The gastrostomy tube was a 15-cm length of PE-10 intramedic tubing (Clay Adams, Parsippany, NJ) with a small plastic flange at one end. The tube was attached to a short wire contained within Silastic tubing; this tubing was inserted into the mouth, down the esophagus, and out through the stomach wall. The pups were then maintained individually in plastic cups floating in a water bath at $36 \pm 1^{\circ}$C. They were fed RMS formula (see Ward et al., 1998) via PE-50 tubing attached at one end to their gastrostomy tubes and at the other end to an infusion pump (Model #55-4143, Harvard Apparatus, South Natick, MA). The pumps were programmed to deliver the formula for 20 min every 2 h. The first day on the pumps, the pups were fed 29% of the mean body weight and subsequently this percentage increased to 33%. ET and NIC treatments were administered in the RMS from PD 6 to 9 (i.e., 4 days total).

Urine samples were collected for analysis of the NIC metabolite cotinine during the first half of the dark cycle on PD 8. Urine samples of 50 μl were required for radioimmunoassay analysis conducted using a Double Antibody Nicotine Metabolite kit (KCTD1, Diagnostic Products, Los Angeles, CA). Ninety minutes after the start of the last ET feeding on PD 9, 20-μl samples of blood were drawn from the tail of all artificially reared rats. Plasma samples were stored at -80°C for 7 months and analyzed using the method outlined by an enzymatic assay kit (Sigma Diagnostics, St. Louis, MO) to determine blood alcohol concentrations (BAC). Based on our previous study (Girard et al., 2000), BACs were expected to be in the region of 300 mg/dl and thus, as per instructions of the kit, samples were diluted prior to analysis. The pups were maintained on the pumps until PD 12, when they were fostered to nursing dams, with each dam suckling pups from each treatment group.

2.4. Behavioral development

During the fostering period, the effects of early postnatal exposure to ET and/or NIC on physical and behavioral development were assessed. In addition to recording the degree of eye opening on PD 14 and 16, the sensory–motor capacities of the developing pups were assessed on these days. These tasks were adapted from Altman and Sudarshan (1975). The videotaped performance of the animals on these behavioral tasks was scored according to pre-established criteria, as described in detail elsewhere (Wauben et al., 1999). All behavioral testing was conducted with the experimenter blind to the treatment conditions of the rats.

On PD 14, the pups were tested on their capacity to ascend a wire mesh ladder, placed at an angle of 70°, with the base in cold water and bedding from the home cage on the goal platform above. The rats were placed at the base of the ladder and given 2 min within which to reach the platform. Measures included latency (s) to reach the platform, distance climbed (cm), and speed (cm/s).

Two tests were conducted on PD 16: (1) hindlimb support and (2) traversal of a narrow bridge. The first task assessed the degree of support provided by the hindlimbs while suspended from a rope (2 mm diameter, 60 cm length) that was extended horizontally between two poles (30 cm height). The rats were placed with their front paws on the rope and grasping with the hindlimbs usually ensued immediately. The second task measured the ability of the pups to traverse a narrow bridge (60 cm length, 3 cm width; plywood) that connected two elevated platforms (4 cm height). After being placed on one platform (start), the pups were required to cross the bridge in order to reach the goal platform, on which bedding from the home cage was placed. The dependent measures of time (maximum 120 s allowed), distance (cm), and speed (cm/s) were recorded.

2.5. Weaning of the pups

On PD 23–24, all pups were weaned onto solid food (#5001, PMI Nutrition International), which was made freely accessible along with tap water. The rats were housed according to experimental group, with three to five per cage, and maintained at $22 \pm 1^{\circ}$C under a reversed 12:12-h light/dark cycle. The cages were lined with "Beta-Chip" (Northeastern Products, Warrensburg, NY) and black plastic tubing was provided for enrichment. Rats

were weighed weekly until PD 50–52, when behavioral testing in the water maze commenced.

2.6. Behavioral testing

The water-maze apparatus was a circular white plastic tank (152 cm diameter, 50 cm height). The water (23 ± 1°C, 24 cm depth) was made opaque by mixing in soluble, nontoxic, white latex paint to obscure vision of an escape platform (9 × 9 cm) that was positioned 1–2 cm below the surface. The activity [distance swum (cm) and latency (s)] of the rats was recorded by a Videomex-V tracking system (Columbus Instruments, OH).

2.6.1. Phase 1: Manipulation of stay

The first phase of this study was designed to assess the effects of ET and/or NIC on DMP performance and also whether increased encoding time would ameliorate the expected drug-induced deficits. Beginning on PD 51–52, the rats were tested on the DMP version of the Morris water-maze task. Rats received two problems per day, for 12 days. To assess recent memory, the platform location was varied pseudorandomly across problems, such that the same position (out of eight locations) was not repeated within a sequence of three problems. However, within each problem session of four trials, the platform position was held constant and rats were released from each of four start locations (one per trial) in a pseudorandom order. The first trial of each problem was a search trial that required the rats to locate the hidden platform. The subsequent three trials assessed the ability to remember the problem-specific position of, swim to, and mount the escape platform. If a rat failed to locate the platform within 45 s, the rat was directed to it.

In order to manipulate encoding time, the rats were allowed to remain on the platform for either 10 or 45 s at the end of Trial 1. This time provided the rats with the opportunity to learn the location of the platform with respect to extra-maze visual cues. Each rat received one problem per day in each encoding-time condition, with the order of condition counterbalanced across days. Half of the rats received a 10-s stay on Problem 1 and the other half began testing in the 45-s stay condition. The intertrial interval (ITI) between Trials 1 and 2 was 60 s, during which the rats were kept in a warmed holding cage. On subsequent trials (Trials 2–4), the rats were removed from the water maze 10 s after mounting the platform in all problems and the ITI was 30 s.

Previous work in our laboratory suggested that a tendency for control rats to revisit the preceding problem's platform location was reduced in ET-exposed rats (Girard, 1999). Thus, in the current study, the swimming distance in a zone (18 × 18 cm), which included the goal position from the immediately preceding problem, was measured by the Videomex-V system. In addition, the experimenter recorded whether or not each rat entered this zone within the first 10 s on the first trial of each problem.

2.6.2. Phase 2: Pharmacological intervention with NIC

After completion of the first 12 days of testing, the protocol for the next 8 days of DMP testing was modified to address the effects on performance of (−)-NIC. As mentioned previously, low doses of NIC facilitate performance on behavioral tasks (Levin, 1992) and thus, a low dose of 0.1 mg/kg was used in this phase of testing. The rats were tested on one problem per day, with a 10-s stay on the platform for all problems. Fifteen minutes prior to the start of water-maze testing, the rats were injected intraperitoneally with either (−)-NIC bitartrate (equivalent to 0.1 mg/kg NIC) or saline (0.9%). This route of administration (intraperitoneal) has been shown to improve the performance of rats when given 10 min prior to delayed-alternation testing (Nagahara and Handa, 1999). The order of injections was counterbalanced across rats over days, with half of the rats in each group starting with NIC and the other half with saline.

2.6.3. Phase 3: Cued test

As a control measure for sensorimotor and motivational differences among groups, the rats were next tested on a cued version of the Morris water-maze task. On the 21st day of testing, extra-maze cues were eliminated by surrounding the pool with a white curtain. The location of the platform was cued by the attachment of a striped pole (10 cm height, 1.5 cm diameter). This cued test consisted of four trials, with the order of the starting positions assigned pseudorandomly for each rat. The ITI was 30 s between trials and the rats always received a 10-s stay on the platform at the end of each trial.

2.7. Collection of brain tissue

At 3 months of age, the rats received an overdose of anesthesia (0.4 ml × 240 mg/ml ip of Euthanyl) and intracardiac perfusion was performed with 0.9% saline and then with 10% buffered formalin. One week following the perfusion, the brains of the rats were trimmed immediately behind the cerebellum and the flocculi were removed. The whole brains were blotted equally to remove moisture before being weighed.

2.8. Statistical analysis

2.8.1. Water maze performance

The dependent variable of most interest was the swimming distance to find the hidden platform on each of the trials. This measure is preferable to latency, which could potentially be confounded by differences in swimming speed. In this study, Group was a between-subjects factor and Stay, Drug, Trial, and Day were repeated-measures factors. The data were analyzed in accordance with the preplanned questions using SAS to do a series of analyses of variance (ANOVAs). In these analyses, planned contrasts were used to assess the main effects of ET and NIC, their interaction (ET × NIC), and to compare the artificially reared GC group with the normally reared SC group. The

experimental hypotheses predicted that there would be no differences between the groups on Trial 1 of each problem, but that differences would be evident on Trial 2 (in Phase 1), and possibly on Trials 3 and 4. For this reason, analyses were conducted separately on each trial. Furthermore, an effect of Stay was expected on Trial 2 only, as this was the only trial on which stay was manipulated. The alpha level for claiming statistical significance was set at .05.

3. Results

3.1. Survival

Chi-squared analyses comparing the percent survival of the ET (81%), NIC (53%), and ET + NIC (76%) groups to the artificially reared control group (86%) revealed a significantly reduced survival rate in the NIC group only, $\chi^2(1) = 4.42$, $P < .05$. The GC group did not differ significantly from the SC group (100%) in terms of survival, $\chi^2(1) = 2.02$, $P > .05$. In summary, the above mortality left sample sizes of 10–14/group for behavioral testing.

3.2. Body growth and brain weight

A priori contrast analyses of body weights failed to reveal significant main effects of ET, NIC, artificial rearing (GC vs. SC), or an ET × NIC interaction at weaning, and before and after water-maze testing, $P > .05$ (data not shown).

In contrast, analyses of brain weights revealed a significant main effect of ET, such that, on average, the brains of the rats exposed to ET weighed less, $t(55) = -7.843$, $P < .0005$ (see Table 1).

3.3. Biochemical analyses

3.3.1. Urine cotinine concentration

Interestingly, the two NIC-exposed groups differed significantly from each other, with lower levels of cotinine measured in the ET + NIC group (mean ± standard error of the mean (S.E.M.) = 4659 ± 771 ng/ml) than in the NIC group (mean ± S.E.M. = 8651 ± 1605 ng/ml), $t(23) = 2.24$, $P < .05$.

3.3.2. Blood alcohol concentration

Due to lack of normality, these data were analyzed using the Mann–Whitney test. The ET (mean ± S.E.M. = 138 ± 15 mg/dl) and ET + NIC (154 ± 23 mg/dl) did not differ in terms of BAC, $U = 54$, $P = .379$.

3.4. Behavioral development

On PD 14, the eye-opening score, averaged across both eyes, indicated a significant effect of NIC, $F(1,63) = 7.39$, $P < .01$, where the NIC-treated animals were advanced relative to controls (see Table 1). On PD 16, there were significant main effects of ET in delaying the development of eye opening, $F(1,60) = 7.17$, $P < .01$, and in decreasing the overall score on hindlimb support, $F(1,60) = 4.31$, $P < .05$ (see Table 1). None of the other behavioral tests showed statistically significant effects.

3.5. Behavioral testing

Note that in all analyses, results based on latency supported those of swimming distance reported below (data not shown). There were also no significant group differences in terms of swimming speed (data not shown).

3.5.1. Phase 1: Manipulation of stay

An initial mixed-factors ANOVA of Trial 2 data across the 12 days of testing, repeated on Day and Stay, revealed main effects of Group, $F(4,58) = 4.99$, $P = .0016$, Day, $F(11,638) = 42.38$, $P = .0001$, and Stay, $F(1,58) = 3.03$, $P_{\text{one-tailed}} = .0436$, as well as a Group × Day interaction, $F(44,638) = 2.00$, $P = .0002$.

Simple effects analysis at each day indicated differences among groups that were erratic at the outset, but then evolved into consistent main effects of ET, until all groups eventually reached equivalent levels of performance on Day 10 (see below). In contrast to our earlier study (Girard

Table 1
Developmental effects of exposure to ET and NIC during PD 6–9

Group	ET	NIC	ET + NIC	GC	SC
Day 14					
Eye opening	0.14 ± 0.04	0.20 ± 0.06[a]	0.26 ± 0.05[a]	0.08 ± 0.04	0.01 ± 0.01
Day 16					
Eye opening	0.85 ± 0.02*	0.91 ± 0.03	0.82 ± 0.02*	0.91 ± 0.03	0.86 ± 0.02
Hindlimb support[b]	0.78 ± 0.04*	0.82 ± 0.04	0.71 ± 0.04*	0.85 ± 0.04	0.73 ± 0.04
Adult					
Brain weight (g)	1.87 ± 0.04*	2.11 ± 0.02	1.93 ± 0.03*	2.16 ± 0.02	2.14 ± 0.03

Data are expressed as mean ± S.E.M.

[a] Indicates a significant main effect of NIC; scores range from 0 (*closed*) to 1 (*fully open*).

[b] Data represent overall scores, ranging from 0 (*no grasp*) to 1 (*strong grasp by both limbs*).

* Indicates a significant main effect of ET, $P < .0005$.

et al., 2000), in which the adult rats had been tested previously in the water maze as juveniles, the present protocol did not allow the rats the opportunity to habituate to the task procedures prior to testing. Therefore, the performance differences on the first few days of testing in the present study should be considered in the context of an initial training period, which likely involves many factors in addition to mnemonic ability. On this account, the data for the first 3 days were removed from all subsequent analyses, i.e., the following analyses were conducted on the data from the last 9 days only, with data for each subject averaged across days.

These data were first analyzed with a mixed-factors ANOVA on Group, with Trial and Stay as repeated factors. There were significant main effects of Group, $F(4,58)=7.04$, $P=.0001$, and Trial, $F(3,174)=516.78$, $P=.0001$, and a Stay × Trial interaction, $F(3,174)=3.25$, $P=.0232$. These data, collapsed across Stay, are shown in Fig. 1.

Subsequent analyses were performed separately for each trial. As predicted, there were no significant effects of Group on Trial 1, but on Trials 2, 3, and 4, there were significant main effects of ET: $F_{\text{Trial }1}(1,58)=2.90$, $P=.0938$; $F_{\text{Trial }2}(1,58)=19.09$, $P=.0001$; $F_{\text{Trial }3}(1,58)=12.42$, $P=.0008$; $F_{\text{Trial }4}(1,58)=8.55$, $P=.0049$. In all cases, the rats in the two groups that were exposed to ET swam longer distances to reach the platform. In these analyses there was neither a significant effect of NIC nor an ET × NIC interaction.

As predicted, there was also a significant main effect of Stay on Trial 2, $F(1,58)=4.76$, $P=.0333$. As shown in Fig. 2, the 45-s stay on the platform at the end of Trial 1 resulted in shorter swimming distances on Trial 2 in comparison to the 10-s stay condition. The Group × Stay interaction was not significant, $F(4,58)=1.47$, $P=.2223$.

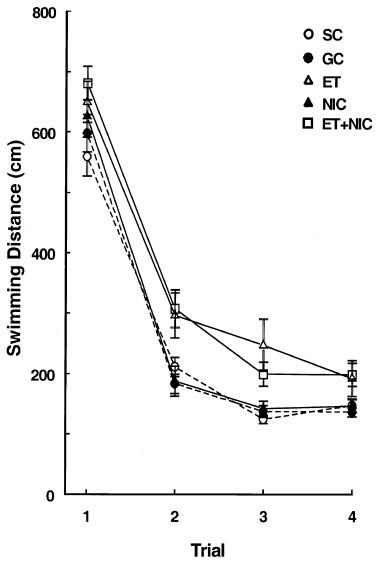

Fig. 1. Effect of postnatal ET and/or NIC exposure on DMP-task performance across trials. Data shown represent group means ± S.E.M.s in terms of swimming distance to reach the hidden platform in Phase 1. All groups performed equally well on Trial 1, $P=.0938$, but the groups exposed to ET (ET, ET + NIC) were impaired at each of the recall trials, $P_{\text{Trial }2}=.0001$, $P_{\text{Trial }3}=.0008$, $P_{\text{Trial }4}=.0049$. Data are collapsed across Stay and Days 4–12.

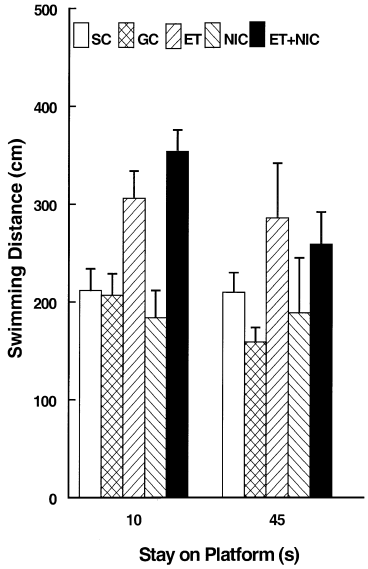

Fig. 2. Effect of the stay manipulation on the distance traveled in Trial 2. Means ± S.E.M.s are plotted for the swimming distance of each group in the 10- and 45-s stay conditions in Phase 1. Longer encoding time improved performance (shorter distance) across all groups, $P=.0333$. Data are collapsed across Days 4–12.

Analysis of the Trial 1 swimming distance in the previous platform zone, expressed as a percent of total distance, revealed a main effect of ET, $F(1,58)=8.09$, $P=.0061$. The swimming path of the ET-exposed groups was relatively less in the vicinity of the goal from the preceding problem. Furthermore, the groups that received ET treatment also entered this zone less often, on average, within the first 10 s of Trial 1, $t(58)=-2.38$, $P=.021$.

As our previous research demonstrated improvement of ET-exposed rats following practice on the DMP task, it was of interest to also examine this issue in the present study. Indeed, there seemed to be equivalent Trial 2 performance among groups by Day 12. Thus, simple-effect analyses were conducted on the Trial 2 swimming distance data, beginning at Day 12 and working backwards until a significant effect was present. These analyses revealed that while the ET-exposed groups were impaired on Day 9, $F(1,58)=5.76$, $P=.0196$, a main effect of ET was absent from Days 10 to 12.

3.5.2. Phase 2: Pharmacological intervention with NIC

In the second phase of testing, there were no significant effects of Group, $F(4,58)=1.89$, $P=.1251$, Drug, $F(1,58)=1.09$, $P=.3015$, or a Group × Drug interaction, $F(4,58)=0.55$, $P=.7029$, in terms of swimming distance on Trial 2 (data not shown).

3.5.3. Phase 3: Cued test

All groups reached the platform on the proximally cued test within comparable distances, $F(4,58)=0.54$, $P=.7066$.

4. Discussion

This study investigated the effects of binge exposure to ET and/or NIC during the brain growth spurt on DMP performance in the Morris water maze. Consistent with predictions, ET-exposed rats were impaired relative to controls in their ability to find the hidden platform on the second trial following the initial search trial. In contrast, early postnatal exposure to NIC failed to affect DMP performance and there were no interactive effects of combined ET + NIC exposure. Increased encoding time improved the ability of adult rats to perform the DMP task, but there was no effect of acute administration of NIC. In summary, the present findings confirm that binge exposure to ET during the brain growth spurt leads to impaired memory for recent events in a spatial-learning context and that this deficit may be amenable to amelioration via behavioral intervention.

The ET-induced impairment on the DMP task replicates our previous findings that demonstrated long-lasting, delay-dependent memory impairment on this task (Girard et al., 2000). Furthermore, the groups did not differ in terms of swimming speed or cued task performance, which control for possible effects on motivation and swimming ability. In our previous study, an ET-exposed group performed as well as controls on the DMP task in a 60-s delay condition following extensive practice (i.e., after they had been tested previously as adolescents and as adults, for a total of 48 problems). In the present study however, the ET-exposed group reached asymptotic performance equivalent to the control groups by Day 10 (Problems 20–21). This discrepancy in acquisition rate may be explained by differences in methodology. Indeed, the rats were allowed a longer time (45 vs. 10 s) to encode the spatial location of the platform during half of the problems in the current study and this behavioral manipulation was successful in improving performance across groups. This study also demonstrated a main effect of early postnatal exposure to ET in delaying the development of eye opening and hindlimb support in juveniles and reducing the brain weight of adult rats.

NIC exposure during the brain growth spurt accelerated the rate of eye opening in rat pups, an effect that is consistent with the literature (Paulson et al., 1994; Seggara and Strand, 1989). The absence of early NIC-induced behavioral effects must be interpreted with caution given the significantly higher rate of mortality in the NIC-exposed group. That is, the pups most sensitive to the early postnatal NIC treatment might have been eliminated from the study, leaving only the "fittest rats" in this group. Nonetheless, the data on the remaining rats did not even show a trend towards an effect.

In addition to a lack of interactive effects on behavior, coexposure to ET + NIC revealed surprising biochemical results. Chen et al. (1997, 1998, 1999) reported that early postnatal exposure to NIC produced lower BACs, and hence reduced effects on brain development, in their coexposed group (as compared to the ET-exposed group). In contrast, the present study revealed no differences in BAC between these two groups. Furthermore, in this study, the combined ET + NIC exposure group had a lower concentration of cotinine than the NIC-only group, whereas Chen et al. reported no significant difference in cotinine levels. However, consistent with our data, acute pretreatment of ET to adult rats has been shown to alter the pharmacokinetics of NIC such that plasma NIC and cotinine levels were lowered (Adir et al., 1980). Factors accounting for the differences between the present results and the findings of Chen et al. might relate to strain (Chen et al. used Sprague–Dawley rats) and/or exposure regimen. Namely, the binge exposure regimen in the Chen et al. (1998, 1999) studies consisted of ET (4 g kg^{-1} day^{-1}) and/or NIC (6 mg kg^{-1} day^{-1}) administered in 2 out of 12 daily feedings from PD 4 to 9. In contrast, in our study a higher total dose of ET (6.5 g kg^{-1} day^{-1}) was administered in 4 daily feedings and, to mimic chain-smoking more accurately, NIC was distributed across all 12 daily feedings from PD 6–9. Another methodological difference between our work and that of Chen et al. is that they administered NIC as the free base, whereas we administered the more water-soluble bitartrate salt. The gastrointestinal absorption of NIC in our work is supported by the urinary cotinine levels, and there is evidence in rats that NIC administered as the bitartrate salt crosses the blood–brain barrier (e.g., Hulihan-Giblin et al., 1990). Further investigation of the effects associated with different exposure regimens might help to elucidate the mechanisms involved in the metabolism of ET and NIC. Moreover, such research will likely aid towards understanding the high association between drinking and smoking, and the potentially different effects on offspring following coexposure to ET and NIC during development.

The mean peak BAC level in the present study (138 mg/dl, ET) was lower than that in our previous study (302 mg/dl in Girard et al., 2000) and the levels found by others (Chen et al., 1998, 1999). This is surprising, because we used the same regimen of ET exposure in the two studies. Another unexpected observation was the lack of effect of acute NIC administration on the performance of the DMP task. Early postnatal exposure to NIC has been reported to alter brain cholinergic systems in adult rats (Miao et al., 1998) and mice (Nordberg et al., 1991). Kelly et al. (1989) demonstrated an increased number and sensitivity of muscarinic receptors in the hippocampus of adult rats that were exposed to ET during the brain growth spurt. Although scopolamine did not differentially impair the place-learning performance of prenatal ET-exposed rats in the Morris water maze (Hannigan et al., 1993), Nagahara and Handa (1999) showed differential effects of scopolamine, pilocarpine, and NIC on the performance of a delayed T-maze alternation task. Of most direct relevance, Nagahara and Handa (1999) reported enhanced memory effects in controls, but not the ET-exposed group, following acute administration of NIC (0.65 mg/kg). Given the above findings, one might expect

differential effects of NIC on DMP-task performance; however, none was found in the present study. Although heterogeneous effects of acute NIC exposure may be explained by differences in genes, doses, and the nature of tasks used, the present finding is best interpreted in terms of level of training (Levin, 1992). As noted above, the ET group was performing as well as controls before the end of the first phase of testing (encoding manipulation) and furthermore, all groups were performing at an asymptotic level by the time the acute NIC intervention was tested (i.e., ceiling effect).

In conclusion, this study replicated our previous finding that repeated binge exposure to ET during the brain growth spurt period impairs the performance of male rats on the DMP version of the Morris water-maze task. In contrast, this study did not support a deleterious effect of early exposure to NIC or an ameliorative effect of acute exposure to NIC at the time of testing. However, increasing the time allowed on the platform improved recall across all groups, suggesting that manipulations aimed at the encoding stage of information processing will likely aid performance on memory tasks in ET-exposed animals.

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