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Neonatal Nicotine Exposure Alters Hippocampal EEG and Event-Related Potentials (ERPs) in Rats

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SLAWECKI, C. J., J. D. THOMAS, E. P. RILEY AND C. L. EHLERS. *Neonatal nicotine exposure alters hippocampal EEG and event-related potentials (ERPs) in rats.* PHARMACOL BIOCHEM BEHAV **65**(4) 711–718, 2000.—A consensus is forming that nicotine can damage the developing rat central nervous system. However, few studies have assessed the electrophysiological effects of neonatal nicotine exposure in rodents in brain regions known to be sensitive to the teratogenic properties of nicotine. In a previous study it was reported that 1.0 and 4.0 mg/kg/day nicotine exposure from postnatal days 4–9, a developmental period corresponding to human third-trimester exposure, significantly altered hippocampal event-related potentials (ERPs) but did not effect cortical ERPs, cortical EEG, or hippocampal EEG. Because alterations in behavior and cortical/hippocampal neurochemistry and morphology have been reported following nicotine exposure, the present study used a higher dose of nicotine during the postnatal period (6.0 mg/kg/day) determine if functional changes in the EEG of these regions might contribute to behavioral changes that have been observed. Male Sprague–Dawley rats were exposed to 6.0 mg/kg/day nicotine via gastric infusion using an artificial rearing, "pup-in-the-cup," technique for 6 consecutive days (postnatal days 4–9). At adulthood, EEG and auditory ERPs were recorded from the cortex and hippocampus. There were no significant differences in EEG or ERPs recorded from the cortex between nicotine-treated and control subjects. Examination of the hippocampal EEG revealed significantly decreased power in the 1–2-Hz frequency band of nicotine-treated rats. In addition, there was a significantly attenuated P300 ERP response to a noise tone in the nicotine-treated rats compared to controls. These data indicate that neonatal nicotine exposure alters functional activity in the hippocampus of adult rats. These effects are likely to be the result of synaptic disorganization in the hippocampus, and indicate that neonatal nicotine exposure exerts teratogenic effects on the developing central nervous system, particularly the hippocampus, which persist into adulthood. © 2000 Elsevier Science Inc.

Neonatal Nicotine EEG Event-related potentials Hippocampus Cortex

PRENATAL or neonatal nicotine exposure has been reported to produce teratogenic effects, resulting in cognitive and behavioral impairments (9,18,21,22,26,31,40,45). Children exposed to nicotine during fetal development are hyperactive (9,40,45), display deficits in attention (31), and exhibit general deficits in cognitive function (18,21,22,26) relative to controls. Analogous effects of early nicotine exposure have been reported using animal models. Rats exposed to nicotine during early development have been shown to be hyperactive (60,66), and are impaired in memory and cognitive function (8,33,34,65). These data suggest that nicotine can disrupt normal central nervous system development in the rat.

Alterations in cortical and hippocampal function may account for some of the behavioral and cognitive deficits observed following exposure to nicotine during early development, because many of the behaviors altered by nicotine exposure are influenced by these brain regions. Behavioral deficits observed in rats following prenatal nicotine exposure, such as impairments in the radial arm maze (33,65), T-maze (33,65), and discrimination learning (36), are also observed

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following lesions/manipulations of the cortex (19,29,41,52) and hippocampus (7,17,49,54,68).The hippocampus and frontal cortex also influence locomotor activity and avoidance learning (5,28,56)— two behaviors that are altered following prenatal nicotine exposure (4,60,66). Neuroanatomical and neurochemical studies have shown that the cortex and hippocampus are altered following prenatal nicotine exposure. In the cortex, there are reports of increased norepinephrine levels and increased norepinephrine binding (43,64), increased choline acetyltransferase activity and increased choline uptake (42), increased nicotinic receptor binding (67), and altered morphology characterized by decreased cortical thickness and decreased dendritic branching (57). In the hippocampus, decreased hemicholinium binding and choline uptake (70), and increased nicotinic receptor binding (67) have been demonstrated following prenatal nicotine exposure.

Few studies have assessed the effects of neonatal nicotine exposure in rodents (15,39,44). However, this time period is of interest because neonatal exposure is analogous to the period of rapid brain growth in the human third trimester commonly referred to as the "brain growth spurt" (10,11). In mice, nicotine exposure during postnatal days (PND) 10–16 does not change baseline locomotor activity at 4 months of age but does attenuate the increased in locomotor activity observed following nicotine challenge (44). Postnatal nicotine treatment in rats from PND8–PND16 has been reported to increase cortical nicotinic binding sites at 115 days of age (39). In the only study to assess the effects of neonatal nicotine exposure on the EEG and event-related potentials (ERPs) recorded from the cortex and hippocampus, neonatal exposure from PND4–PND9 significantly decreased the amplitude of the P3 ERP component in the dorsal hippocampus, but did not affect cortical EEG or ERPs or the hippocampal EEG (15).

A lack of EEG effects following neonatal nicotine exposure, particularly in the hippocampus, was surprising. The hippocampus has been thought to be an area of high sensitivity to teratogens such as alcohol and nicotine (25,35). Further, prenatal and postnatal nicotine exposure have repeatedly been reported to alter hippocampal and cortically mediated behaviors and the morphology and neurochemistry of these regions (42,43,57,64,67,70). If the neurochemical changes that have been reported are responsible for alterations in behavior, functional brain activity as measured by the EEG and ERPs, in these brain regions should be altered following postnatal nicotine exposure. The demonstration of changes in functional brain activity is also important because the neurochemical and neuroanatomical changes reported in these brain regions have not yet been shown to have an effect on electrical activity in the cortex or hippocampus. Therefore, the purpose of this study was to further explore the effects of postnatal nicotine exposure on EEG and ERPs recorded from the cortex and hippocampus using a higher dose of nicotine (6.0 mg/kg/day).

METHODS

Subjects

Sprague–Dawley rats were bred at the San Diego State University Animal Care Facilities. Females were housed overnight with males. Presence for a seminal plug indicated mating. Pregnant dams were then singly housed with ad lib food and water available. The 34 ($n = 34$) male offspring used for this experiment were neonatally exposed to nicotine at the Center for Behavioral Teratology at San Diego State University. Females were not used in this study, because in a previous study differential effects of postnatal nicotine on ERPs or EEG based on gender were not observed (15). Upon arrival at the Scripps Research Institute, rats were housed two/three per cage and maintained under ad lib feeding conditions for the duration of the experiment. Rats were weighed once a week. The light/dark cycle was maintained on a 12 L:12 D cycle (lights on at 0600 h, off at 1800 h). Animal care was in accordance with NIH and institutional guidelines.

Neonatal Nicotine Exposure

Twenty-four hours after birth, litters were weighed and culled to five males, when possible. On postnatal day (PND) 4, individual pups from each litter were assigned to one of the treatment groups: an artificially reared group receiving nicotine in their diet at a dose of 6.0 mg/kg/day (nicotine-treated group, $n = 14$), an artificially reared gastrostomized control group receiving a control diet (GC group, $n = 10$), and sham-surgery animals that remained with the dam as suckle controls (SC group, $n = 9$). Within each litter, no more than one male was assigned to each of the three possible groups.

The gastrostomy and artificial-rearing procedures have been described in detail previously (1,38). Pups designated for the GC and nicotine-treated groups were removed from the home cage on PND4, lightly anesthetized with halothane, and a gastrostomy tube was surgically implanted. Animals assigned to the SC group were removed from the litter on PND4 and exposed to a sham gastrostomy procedure. Following gastrostomy, pups were individually housed in plastic cups (11.0×7.5 cm deep), lined with absorbent wood chips and a piece of artificial fur. The cups floated in a stainless steel tank filled with heated (45°C), aerated water, which kept the inside of the cups at 37°C. Each gastrostomy tube was connected to a 6-ml syringe containing a milk formula (37). Syringes were mounted on infusion pumps (Harvard Apparatus #2265), which delivered the milk formula for 20 min every 2 h, resulting in 12 daily feeding periods. The amount of milk formula (in milliliters) infused each day was equivalent to 33% of the average body weight (in grams) of the artificially reared pups on each given day. Twice a day, the pups were disconnected from the syringes, washed with warm water, weighed, and their anogenital region lightly stroked with a cotton swab to stimulate excretion. The pups' gastrostomy tubes were flushed with distilled water to clear out any remaining stock solution. The pups were then reattached to syringes containing the diets.

Nicotine was added to the stock milk formula of the nicotine-treated group for the first four daily feedings on PND4 through 9, for a total dose of 6.0 mg/kg/day. Milk formula only was delivered to the GC group, and to all groups for the remaining eight daily feedings. On PND10, all groups received milk formula only. On the morning of PND11, the gastrostomy tubes were sealed and trimmed and all pups, including the suckled controls, were bathed in a slurry of water mixed with feces from the foster mother's home cage prior to being placed in the home cage. This procedure virtually eliminates rejection of maternally separated pups from the foster dam (1,15). The pups were weaned at 21 days of age.

Stereotaxic Surgery

After the rats were shipped from San Diego State University, they were maintained under quarantine at the Scripps Research Institute for 6 weeks. Following quarantine, the rats were weighed and handled for 1–2 weeks prior to surgery. All rats were then implanted with screw electrodes in the skull and a bipolor stainless steel depth electrode aimed at the dor-

sal hippocampus (DHPC). Rats were anesthetized with an intraperitoneal injection of 50 mg/kg sodium pentobarbital. A subcutaneous injection of atropine (0.06 mg) was administered to minimize respiratory suppression while anesthetized. Screw electrodes were placed in the skull overlying the frontal cortex (AP 3.0 mm, ML ± 3.0 mm) and the parietal cortex (AP −3.0 mm, ML ± 4.0 mm). A third screw electrode was placed posterior to lambda in the skull overlying and parallel to the cerebellum. Stereotaxic coordinates for the DHPC electrode (AP −3.0, ML ± 3.0, DV −3.0) were determined from the Pellegrino atlas (47). Electrode connections were made to a five-pin Amphenol connector. The entire assembly was anchored to the skull with dental acrylic and anchor screws. Rats were given 2 weeks to recover from surgery before being tested.

Electrophysiological Recording Procedures

Electroencephalograms (EEG) were recorded on a Sensorium Polygraph with a band pass of 0.53–70 Hz. Prior to EEG recording, rats were placed in a BRS/LVE recording chamber and attached to a microdot recording cable. EEG was recorded from a cortical lead (Fctx-Pctx) and a hippocampal lead (DHPC-DHPC). Recording was initiated following a 5-min habituation period. Four hours of EEG were collected and digitized at a rate of 128 Hz. For data analysis, consecutive 4-s epochs of EEG were decomposed with a Fourier transformation over spectra of .25 to 64 Hz. Over a frequency range of 1–20 Hz, 4-s epochs were marked as artifact if their average power was greater than 2000 μvolts squared/octave (cortical lead) and 8000 μvolts squared/octave (hippocampal lead). Artifact epochs were confirmed by visual analysis of the raw EEG. The average percentage of 4-s epochs excluded was less than 10%. The power spectra for each 4-s epoch were then averaged. The averaged spectral data were then compressed into eight frequency bands: 1–2, 2–4, 4–6, 6–8, 8–16, 16–32, 32–50, 1–50 Hz. Mean band power and the predominant frequency for each frequency band from each lead were then calculated as previously described (13).

ERPs were elicited using an auditory "oddball" paradigm similar to those previously used to generate P300s to infrequently presented auditory stimuli in humans and nonhuman primates (12,30,52). In this paradigm, a P300 ERP component is generated in response to infrequently presented auditory stimuli (i.e., "oddball" tones) embedded in a string of frequently present auditory stimuli. In this arrangement, rare tones and white noise bursts generate P300 waves. In addition, a white noise burst serves as a "startle" stimulus, which can be used to assess neurosensory reactivity in the subjects (30). Event-related potentials (ERPs) are recorded immediately following EEG recording. ERP recording sessions lasted approximately 10 min. Auditory stimuli were presented from a speaker attached to the top of the recording chamber (45 cm above the subjects). Three tones were presented during ERP sessions. All tones were presented for 20 msec, with rise and fall times of <1 msec. Standard Tones (1000 Hz square wave, 75 dB) were presented 84% of the time. Rare Tones (2000 Hz square wave, 85 dB) were presented 10% of the time. Noise tones (white noise, 100 dB) were presented 6% of the time. Individual ERP trials were 1000 msec in duration (200 msec prestimulus + 800 msec poststimulus). Each trial was separated by variable time intervals ranging from 500–1000 msec. Standard tone, rare tone, and noise tone presentation was randomized with at least one presentation of a standard tone between each rare tone, no more than six standard tones between each rare tone, and no more than 12 trials between the presentation of noise tones. Each session consisted of 312 individual tone presentations (i.e., 312 trials).

ERP data were digitized at a rate of 256 Hz. Each wave component was quantified on the basis of peak amplitude, latency to peak amplitude from tone presentation, and polarity. Prestimulus baseline activity was determined from average EEG activity 100 msec prior to tone presentation. Each component was identified with an automated peak detection program and confirmed by visual inspection. Movement artifact (i.e., voltages exceeding ±400 μvolts) was identified with an automated computer detection program and eliminated following confirmation by visual analysis. Individual ERP trials for each tone type were then averaged for each subject. ERPs were collected from the cortical and hippocampal leads used for EEG recording. ERP components were identified based on the largest amplitude peak within a specified latency range. Latency windows used to identify ERP components for the cortical lead were: N10 = 5–40 msec, P1 = 30–70 msec, N1 = 60–100 msec, P2 = 150–200 msec, N2 = 225–275 msec, P3A = 375–325 msec, P3B = 350–400 msec. Latency windows used to identify the ERP components for the hippocampal lead were: N10 = 5–30 msec, P1 = 20–60 msec, N1 = 35–65 msec, P2 = 60–90 msec, N2 = 125–200 msec, P3A = 200–275 msec, P3B = 325–400 msec. These data analyses have been previously described (16).

General Procedure

Rats were tested at 4–6 months of age. Prior to recording of EEG and ERPs, rats were exposed to the recording apparatus during two 30-min sessions. During these habituation sessions, rats were placed in the recording apparatus and attached to the Microdot recording cable. Electrophysiological recordings did not take place during these sessions. The 4-h EEG recording sessions were always run from 0600–1000 h. During these sessions, the subjects were placed in the recording chamber while still in their home cage. The recording chamber was equipped with an exhaust fan to mask external noise, and a light that remained on during the recording session. A Plexiglas separator with small holes, which allowed nose pokes, was used to separate the subjects during recording sessions. Immediately following completion of the EEG recording session, ERPs were recorded. The subjects were then removed from the recording chamber and returned to the vivarium. At the end of the experiment, all rats were deeply anesthetized with sodium pentobarbitial (100 mg/kg) and decapitated. The brains were rapidly removed and frozen on dry ice. The brains were then sectioned coronally on a cryostat (60 μm), mounted on a slide, and stained with cresyl violet. A light microscope was used to assess proper depth electrode placement.

Statistical Analysis

Statistical analyses were performed using Systat for the Macintosh and Sigmastat for Windows. Body weight during the neonatal treatment period was analyzed using a two-way repeated measures analysis of variance (ANOVA) with treatment group (nicotine-treated, SC, GC) as a between-subject factor and day as a within-subject repeated measure. Repeated-measures analyses were corrected using the Greenhouse-Geisser correction. When necessary, a Student–Newman–Keuls procedure was employed for post hoc multiple comparisons. All electrophysiological data were analyzed using a one-way analysis of variance (ANOVA) by group. For

all electrophysiological variables assessed, there were no significant differences between the GC and SC groups. As a result, these data were combined and reassessed as a single control group vs. the nicotine-exposed subjects. Statistical significance was set at $p < 0.05$.

RESULTS

During the neonatal treatment period there were significant differences in body weight between the treatment groups, $F(2, 30) = 15.61$, $p < 0.0001$, and a significant interaction of treatment group × day, $F(24, 360) = 3.56$, $p < 0.027$. Body weights of the GC and nicotine-treated subjects were significantly lower than the body weights of the SC group, but there were no significant differences between the GC and nicotine-treated groups. Post hoc comparisons revealed that there were no significant differences in body weight between the groups from postnatal day 4–9. On postnatal day 10, the nicotine-treated group weighed significantly less than the SC group, but the GC group did not significantly differ from either the SC or the nicotine-treated group. From postnatal day 12–30, the body weights of the GC and nicotine-treated rats were significantly lower than the SC group (Fig. 1).

At the start of electrophysiological testing, the rats ranged from 4–6 months old and there were no statistically significant differences in body weight between the nicotine-exposed subjects (561 ± 82 g) and control subjects (587 ± 79 g). One nicotine-treated subject was omitted from the analyses of the hippocampus due to excessive movement artifact. The final number of subjects included in the data analyses of EEG and ERPs from the cortical electrodes were 14 for nicotine-treated and 19 for the control group (SC + GC). For recording from the hippocampal electrodes, there were 13 nicotine-treated animals and 19 controls (SC + GC). Electrode placement in the hippocampus was confirmed histologically for all subjects included in the data analyses.

There were no significant differences in EEG activity between the nicotine-treated and control groups in the cortical lead during the 4-h EEG recording session. In nicotine-treated subjects, power in the hippocampal lead was significantly less than power in the control groups in the 1–2-Hz frequency band, $F(1, 30) = 5.00$, $p = 0.033$. A similar trend towards decreased power in the DHPC was observed across all frequency bands (Fig. 2). There were also statistically significant increases in the predominant frequency of the EEG of the nicotine-treated group compared to the control group (Table 1). The predominant frequencies in the 2–4 Hz band, $F(1, 30) = 6.07$, $p = 0.02$, the 16–32-Hz band, $F(1, 30) = 6.49$, $p = 0.016$, and the 1–50-Hz band, $F(1, 30) = 5.35$, $p = 0.028$, were significantly higher in the nicotine-treated group compared to the controls.

Analyses of ERPs recorded from the Fctx-Pctx lead revealed no significant effects of nicotine treatment on any of the variables assessed. There were significant effects of nicotine treatment on ERPs recorded from the dorsal hippocampus. In the DHPC lead, the amplitude of the P3A wave was significantly, $F(1, 31) = 4.49$, $p < 0.04$, lower in the nicotine-treated group compared to controls (Fig. 3). In addition, there were significant differences between the nicotine and control groups in regards to amplitude differences of the P3A wave in the DHPC in response to the rare tone and noise tone. In the control group, there were significant increases in the amplitude of the P3A wave, $F(1, 31) = 8.18$, $p = 0.008$, when comparing the rare tone and the noise tone (Fig. 3), but a similar response was not found in the nicotine-treated group. The grand averages for the DHPC ERPs presented in Fig. 4 graphically depict the lack of differential P3 ERP response in nicotine treated subjects.

DISCUSSION

The present study is the first to report that early postnatal nicotine exposure significantly alters the spectral power and frequency of the hippocampal EEG of adult rats. Nicotine-treated subjects displayed significant decreases in power in the hippocampus in the low delta band (1–2 Hz) compared to controls, and exhibited a higher frequency in the hippocampal EEG. There was also a significant decrease in P300 amplitude in the nicotine-treated rats, as has been previously observed

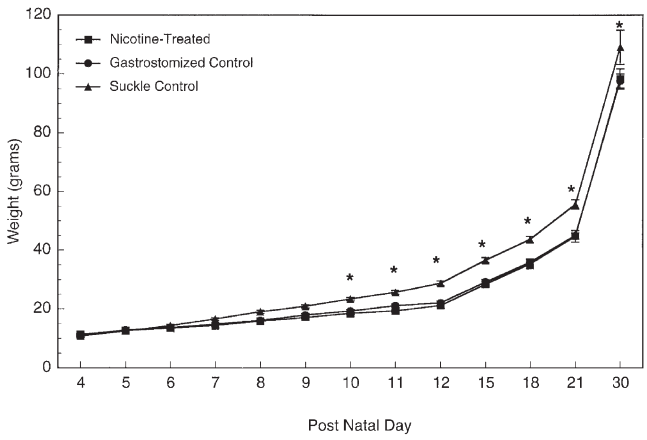

FIG. 1. Perinatal body weight in the nicotine-treated group ($n = 14$), the gastrostomized control group ($n = 10$), suckle control group ($n = 9$). Squares represent the nicotine-treatment group. Circles represent the gastrostomized control group. Triangles represent the suckle control group. Error bars are standard error of the mean. Nicotine-treatment began on postnatal day 4 and continued through postnatal day 9. Asterisks (*) indicate statistically significant difference in the gastrostomized and nicotine-treated groups compared to the suckle control group ($p < 0.05$).

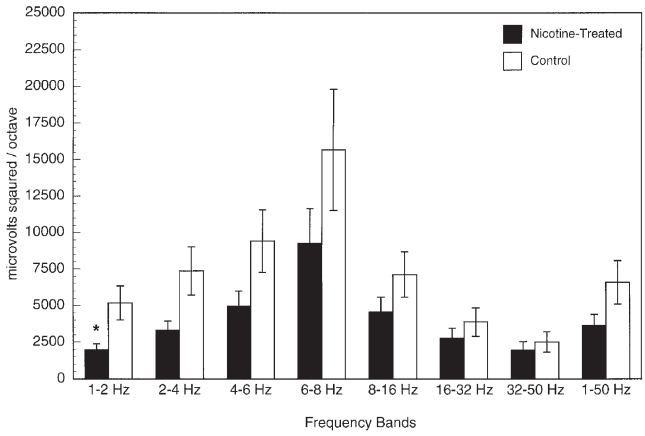

FIG. 2. Spectral power in the dorsal hippocampus in the nicotine-treated ($n = 13$) and control groups ($n = 19$). Solid bars represent the nicotine-treated group and open bars represent the control group. Error bars are standard error of the mean. Control subjects are combined gastrostomized and suckle controls. Asterisks (*) indicate a statistically significant difference from the control group ($p < 0.05$).

TABLE 1

FREQUENCY SHIFTS (MEAN ± SEM) IN THE HIPPOCAMPAL LEAD BETWEEN THE NICOTINE-TREATED ($n = 13$) AND CONTROL SUBJECTS ($n = 19$)

Frequency Band	Nicotine-Treated	Control
2–4 Hz	2.75 ± 0.02*	2.69 ± 0.20
16–32 Hz	19.89 ± 0.17*	19.34 ± 0.14
1–50 Hz	4.38 ± 0.26*	3.67 ± 0.19

Control subjects are combined gastrostomized and suckle controls. Asterisks (*) indicated statistically significant differences from the control group ($p < 0.05$).

(15). The EEG changes represent functional alterations in brain activity of the adult rat that persist long after the cessation of nicotine exposure. These EEG differences could provide the basis for changes in locomotor activity, arousal, or cognitive processing, which have been reported following prenatal and postnatal nicotine exposure. The hippocampal EEG has been shown to be sensitive to changes in locomotor activity, arousal, and classical conditioning (3,6,63). Further, these EEG changes suggest that the morphological and neurochemical changes, which have been observed in the hippocampus following prenatal or postnatal nicotine exposure, may significantly alter functional activity in the hipppocampal EEG.

The hippocampal EEG effects observed in the present study are likely to be attributable to synaptic disorganization in the hippocampus. Roy and Sabherwal (58) have recently reported that at postnatal day 40, after gestational nicotine exposure, pyramidal cells in the CA1 and CA3 regions of the hippocampus have reduced neuronal area and dendritic fields (58). Given that spectral power in the EEG is significantly affected by synaptic organization (23, 46), it is possible that the EEG changes observed in the present study are the result of the synaptic disorganization reported by Roy and Sabherwal. The effects of neonatal nicotine exposure on the hippocampal EEG might also indicate altered arousal. Prenatal nicotine exposure induces hyperactivity in rats (60,66). Delta band power (i.e., 0.5–4 Hz) is typically increased during periods of slow-wave sleep (14,24), and sedatives typically increase power in the delta band (32,55). Therefore, the shift towards less power in the delta band observed in the nicotine-treated rats in the present study could indicate hyperactivity or arousal. However, enhanced arousal would have been expected in the cortical EEG as well, because previous studies have shown stimulants significantly alter the cortical EEG (27,62,69).

The effect of neonatal nicotine on responsivity of the P3 ERP to the noise tone is consistent with our previous investigation (15). Following neonatal exposure to 1.0, 4.0, and 6.0 mg/kg/day, the P3 component response to the noise tone is reduced compared to controls. It is unlikely that this differential response is due to an auditory deficiency, as responses to the rate tone were not different. In controls, P300 amplitude increased 60–100% when comparing the startle to the rare tone. Following treatment with 1.0, 4.0, or 6.0 mg/kg/day, increases in P3 amplitude ranging from 2–10% were observed, but the changes in P3 amplitude across doses is not significantly different. Therefore, the effects of nicotine on P300 amplitude across doses do not appear to be dose dependent within the range tested, suggesting that nicotine's effects on P3 ampli-

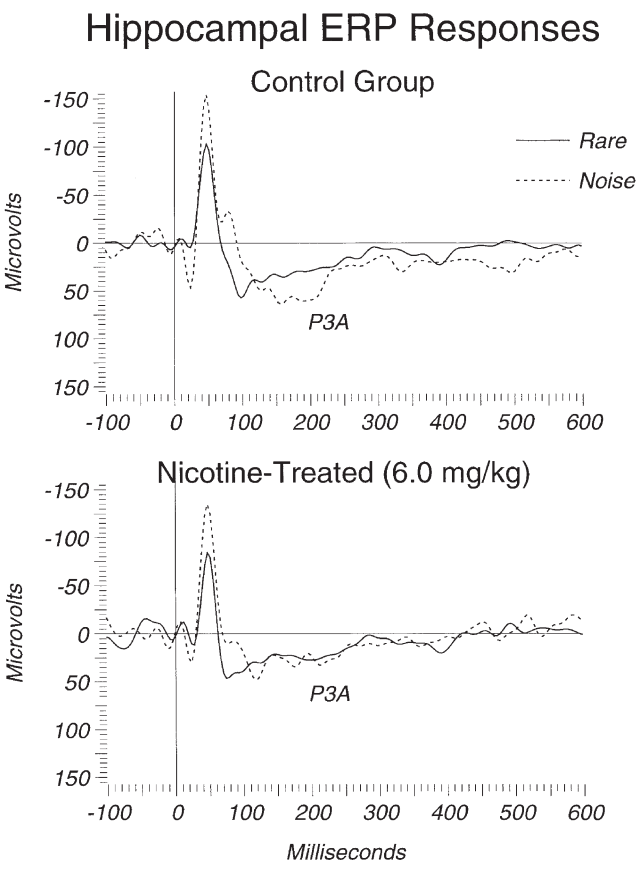

FIG. 4. Grand average comparison of DHPC ERPs in response to rare and noise tones in the nicotine-treated ($n = 13$) and control groups ($n = 19$). Top: control group. Bottom: nicotine-treated group. Control subjects are combined gastrostomized and suckle controls. The solid line in each grand average represents rare tone response. The dashed line in each grand average depicts the noise tone response.

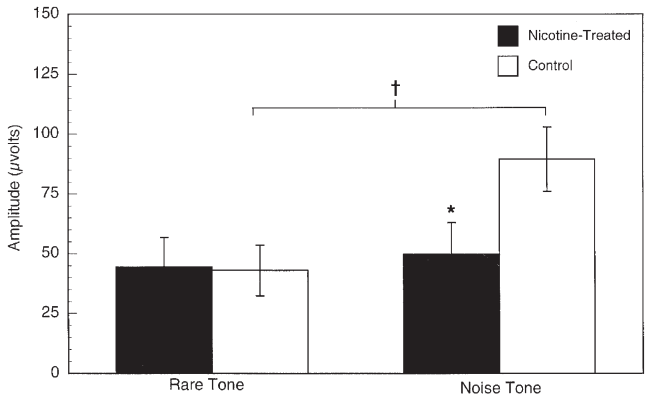

FIG. 3. P3A ERP component amplitudes in the nicotine-treated ($n = 13$) and control groups ($n = 19$) in response to the rare and noise tones. Solid bars represent the nicotine-treated group and open bars represent the control group. Error bars are standard error of the mean. Control subjects are combined gastrostomized and suckle controls. Asterisks (*) indicate statistically significant differences from the control group ($p < 0.05$). Daggers (†) indicate a statistically significant difference between tones.

tude have reached a maximum over this dose range. More importantly, it suggests that the hippocampal P300 ERP component is very sensitive to the effects of nicotine exposure (i.e., altered by 1.0 mg/kg/day), especially when compared to baseline hippocampal EEG, which was not altered until a dose of 6.0 mg/kg/day was administered.

It has previously been reported that rats prenatally exposed to nicotine display decreased prepulse inhibition in response to an acoustic startle stimulus (51). This increased startle response is inconsistent with the decreased ERP response to the noise tone, typically referred to as a "startle ERP," observed in the present study, and with the decreased ability of infants exposed to nicotine during pregnancy to be aroused from sleep by auditory stimuli (20). There are several possible reasons for the discrepancy between the rodent studies, including the age of the subjects at testing (5 weeks vs. 5 months) and the time of nicotine exposure (prenatal vs. neonatal). However, more important is the general effect of early nicotine exposure on "startle" responses and sensory reactivity. The P3 generated has been suggested to be an endogenous neurophysiological response indicative of cognitive processing of salient stimuli (2,50). Taken together, all of these studies indicate that nicotine exposure during early development has a long-lasting effect on neurosensory processing and reactivity.

The lack of effect of neonatal nicotine exposure on EEG or ERPs from the frontal cortex is consistent with our previous study in which lower nicotine doses were employed (15). These data would appear to be inconsistent with the reported neurochemical and neuroanatomical changes reported in cortical regions following prenatal nicotine exposure (42,43,57,64,67); however, many of the neurochemical and/or behavioral effects of prenatal nicotine exposure reported in young rats are not present in the adult rat (61,67,71). Procedural differences could also have contributed to the lack of effects of nicotine on the cortex in the present study. In our study rats were tested at 4–6 months of age and compensatory neural adaptations may have normalized cortical EEG and ERPs. Many studies have also employed prolonged exposure paradigms that lasted for the duration of gestation (42,43,48,57–59,61,64,66), whereas the exposure in the present study was limited to 6 days. In addition, the intragastric route may generate lower brain nicotine levels when compared to intraperitoneal injection or osmotic minipump infusion.

Only three published studies have assessed the effects of postnatal nicotine exposure in rodents (15,39,44). Our own study reported no changes in cortical EEG or ERPs (15). Miao et al. (39) reported increased nicotine binding in the cortex of 115 day old rats. However, it is important to remember that a change in receptor binding need not necessarily lead to altered functional activity or behavior under baseline conditions. During the course of development, neural adaptations may occur, which normalize behavior. Along these lines, Nordberg et al. (44) reported differences in locomotor activity in mice postnatally exposed to nicotine only after acute nicotine challenge. The authors suggested that this difference might be related to decreases in low affinity nicotine bindings sites in the cortex of nicotine exposed mice. Taken together, these data suggest that long-term changes in the neurochemical balance in the cortex could be normalized during development by compensatory changes, but acute nicotine challenge could "unmask" these changes. Therefore, future studies in which adult subjects are challenged with nicotine may reveal altered cortical EEG or ERP responses.

In conclusion, neonatal nicotine exposure results in significant alterations in neurophysiological activity (i.e., basal EEG and ERPs) in the hippocampus. The effects of postnatal nicotine exposure on the hippocampal EEG have not previously been demonstrated, and suggest that some of the behavioral and neurochemical alterations in the hippocampus following nicotine exposure could be related to altered baseline EEG. The hippocampal EEG of nicotine-treated rats displays less power and shifts toward higher frequencies compared to controls. This EEG effect is most likely due to structural alterations within the hippocampus. Decreased neurophysiological P300 responses to a noise tone were also observed, but when compared to our previous study (15), this effect of nicotine exposure on hippocampal P300 amplitude does not appear to be dose dependent. Along with data from other studies, it appears that exposure to nicotine during early development may have a significant effect on neurosensory processing and reactivity. Overall, these data indicate that nicotine exposure in the rat during the equivalent of the third human trimester does have teratogenic effects on the developing central nervous system. Importantly, the effects of nicotine exposure are evident in adult rats suggesting that fetal nicotine exposure can produce lasting changes in neurophysiological function.

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