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The Effects of Perinatal Hypoxia on the Behavioral, Neurochemical, and Neurohistological Toxicity of the Metabolic Inhibitor 3-Nitropropionic Acid

Zbigniew Binienda[1,3], David L. Frederick[1], Sherry A. Ferguson[2], Robert L. Rountree[1], Merle G. Paule[1], Larry Schmued[1], Syed F. Ali[1], William Slikker[1], Jr., and Andrew C. Scallet[1]

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3-nitropropionic acid (3-NPA) neurotoxicity and long-term effects of perinatal hypoxia were evaluated in 18 adult rats. Hypoxia-insulted (I) and noninsulted (NI) rats were delivered by cesarean section. Hypoxic insult was effected by submerging dissected uterine horns in warmed saline for 15 min. NI rats were delivered from the adjacent nonsubmerged horns. At postnatal day 90, I and NI rats were trained to perform tasks thought to measure behaviors dependent upon aspects of time estimation (TE), motivation, and learning. At 12 months of age, rats were injected i.p. with escalating doses of 3-NPA (5 mg/kg/day to a maximum of 30 mg/kg/day) immediately after each test session and sacrificed at the end of treatment. Additional male rats were used as untreated controls. Although 3-NPA produced a dose-dependent impairment of performance in each task, the effects were qualitatively similar for each group. A significant difference between I and NI rats was, however, observed in the TE task where NI rats completed less of the task at high doses of 3-NPA compared to I rats. Compared to untreated controls, dopamine concentrations were decreased in caudate nucleus of both I and NI rats after 3-NPA. Specific areas most frequently damaged included cerebral cortex, hippocampal subfield CA1, thalamus, caudate nucleus, and the cerebellum. Lesions usually were less extensive in the I rather than NI members of a littermate pair, suggesting a possible protective effect of perinatal hypoxia against subsequent 3-NPA neurotoxicity.

Key words: Perinatal hypoxia, 3-nitropropionic acid, neurotoxicity, cerebral energy metabolism, behavior

[1] Division of Neurotoxicology, National Center for Toxicological Research/FIDA, Jefferson, AR

[2] Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research/FDA, Jefferson, AR

3 To whom correspondence should be addressed at Division of Neurotoxicology, NCTR/FDA, HFT-132, Jefferson, AR 72079-9502, USA

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INTRODUCTION

Acute hypoxic conditions associated with brain ischemia-hypoxia lead to the rapid depletion of energy, intracellular calcium accumulation, and disruption of neuronal metabolism (Vannucci, 1990; 1992; Dubinsky, 1993). The accompanying reductions in normal neuronal enzyme activities, including those involved in cellular energy metabolism, may be critical to mechanisms producing neuronal death (Farooqui *et al.*, 1992; Shen *et al.*, 1991). Neurodegeneration of particular brain regions caused by ischemia-hypoxia, i.e., hippocampus and striatum is preceded by massive increases in dopamine concentration followed by an increase in dopamine metabolites upon reoxygenation (Damsma *et al.*, 1990). Behavioral alterations (e.g., in passive avoidance learning behavior observed following cerebral ischemia-hypoxia) have been shown to correlate with histological changes in CA1 subfield of the hippocampus (Yamamoto *et al.*, 1993).

The neurotoxicant 3-nitropropionic acid (3-NPA), found in the food supply as a mold-produced contaminant of sugar-cane (Xingjie *et al.*, 1992), is known to inhibit cellular respiration *in vivo* by irreversible inactivation of succinate dehydrogenase. Succinate dehydrogenase is part of the mitochondrial electron transport chain in the Krebs cycle (complex II; Coles *et al.*, 1979). Therefore, we hypothesized that the effect of 3-NPA would be similar to the effects of ischemic hypoxia since both may act at the cellular level by disrupting the normal production of cellular ATP.

The short-term enzymatic failure of energy production from hypoxia results in neuronal damage in adult rodents (Hornbein, 1991). However, metabolic differences in the uptake of glucose, rate of glycolysis, or production of lactate may result in a greater resistance of the neonate to damage from hypoxia alone (Vannucci, 1992). Neonates can also be protected from hypoxic-ischemic injury by glucose, whereas the effects of hypoxia-ischemia in adults is exacerbated by glucose (Vannucci, 1990; Vannucci and Yager, 1992).

A long-term response (an apparent compensatory increase of cerebral mitochondrial metabolic activity) has been reported following perinatal hypoxia-ischemia (Romijn *et al.*, 1994). Results of recent experimental studies suggest that perinatal hypoxia may paradoxically have some neuroprotective effects on subsequent insults to the CNS when animals reach maturity, perhaps as a result of increased cellular respiration. In support of this hypothesis, exposure to hypoxic conditions was shown to induce tolerance to subsequent episodes of hypoxia-ischemia (Gidday *et al.*, 1994) and resistance to the neurotoxicity of convulsants, e.g., kainate (Pohle and Rauca, 1994). The precise compensatory mechanism by which pre-exposure to hypoxia might protect against subsequent ischemia-hypoxia or neurotoxic episodes is unknown. Elucidation of the nature of this mechanism may have primary importance for neuroprotection and treatment of some neurodegenerative diseases.

The present study was undertaken to assess the neurotoxicity induced by 3-NPA and evaluate the potential modulation of its neurotoxicity by perinatal hypoxia. A better understanding of the toxic effects of 3-NPA could lead to the development of a model for energy-impaired neurodegenerative processes that is more controllable than available hypoxic-ischemic physiological models. The effects of perinatal hypoxia and 3-NPA

treatment were evaluated using neurobehavioral, neurochemical and neurohistological methods.

MATERIALS and METHODS

Animals

Eighteen male rats born to Sprague-Dawley date-mated dams from the NCTR breeding colony were used in the experiment. Rats were individually housed under controlled environmental conditions (temperature 22°C, relative humidity 50%, 12 h light:dark cycle with lights out at 1800 h) in standard plexiglass cages lined with wood chips. Standard rodent chow was available *ad libitum* until PND 70, at which time access to food was gradually restricted so that each rat remained at 80-85% of its PND 70 weight (224.6±7.6 g; mean ± SEM). Water was available at all times except during behavioral test sessions.

Experimental Procedure

Complete details of the procedure are found in Binienda *et al.* (1995). Briefly, sibling rats were delivered by cesarean section as either noninsulted (NI; n=9) or insulted (I; n=9) with hypoxia. Hypoxia was induced at 21 days of gestation by submersion of dissected uterine horns in warmed saline for 15 min (Bjelke *et al.*, 1991). At approximately PND 90, the rats began training to perform one or two of the behavioral tasks. Additional male rats of the same age, remaining under the same environmental conditions and food restriction procedures were used as untreated controls.

Behavioral Assessments

All behavioral test sessions were conducted daily (Monday-Friday) and lasted approximately 50 min, or until the maximum number (120) of reinforcers (45 mg dustless food pellets, Bio-Serve, Frenchtown, NJ) were earned, whichever came first. Ten rats (5-I, 5-NI) were trained to perform both the time estimation (TE) task (40-min) and motivation (MOT) task (10 min), and eight rats (4-I, 4-NI) were trained to perform the learning (LRN) task (50 min).

Time estimation task (TE). Subjects were required to depress a response lever for a minimum of 10 sec, but not longer than 14 sec in order to receive a food reinforcer. Lever holds less than 10 sec or greater than 14 sec were not reinforced. Accurate performance under this schedule is believed to depend on the subject's ability to estimate elapsed time. For a detailed description of this task, often referred to as temporal response differentiation, see Schulze *et al.* (1988).

Motivation task (MOT). Subjects were required to increase the number of lever presses emitted for each subsequent reinforcer (a progressive ratio schedule of food reinforcement). After a reinforcer was earned, the number of lever presses required to obtain the next reinforcer was increased by the number required to obtain the first one. Performance of this task is thought to depend on aspects of the subject's motivation to work for food. See Schulze *et al.* (1988) for a detailed description of this task.

Learning task (LRN). Subjects were required to learn a specific sequence of lever presses (generated randomly each test session), starting with one-lever 'sequences' and incrementing to sequences requiring up to six correct lever presses in order to receive a reinforcer. For a detailed description of this task. often referred to as incremental repeated acquisition (IRA), see Schulze *et al.* (1988).

Behavioral endpoints measured in each task included: percent task completed (total number of reinforcers earned divided by 120 then multiplied by 100), and response rate (lever presses/sec). Accuracy (percent correct responses; TE and LRN tasks only), mean duration of lever hold (TE task only), postreinforcement pause (MOT task only) and breakpoint (the number of lever presses made for the last reinforcer earned in the MOT task) were also monitored.

Drug Administration

At 12 months of age, rats were injected intraperitoneally with escalating doses of 3-NPA (Sigma Chemical Co., St. Louis, MO) immediately following each behavioral test session (Monday-Friday). An initial 3-NPA dose of 5 mg/kg/day was increased by 5 mg/kg/day each week, to a maximum of 30 mg/kg/day during the sixth week.

Neurochemical Assessment

Rats were sacrificed immediately at the end of 3-NPA treatment either by decapitation (for neurochemical analyses) or by perfusion with buffered formaldehyde (for histological assessments). Brains were transferred onto aluminum foil placed in dry ice and dissected into caudate nucleus, frontal cortex, diencephalon, hippocampus, cerebellum, brainstem, and remnant (Glowinski and Iversen, 1966). Whole and regional brain weights were measured. Brain samples were subsequently stored at -70°C. Dopamine (DA), 3,4-dihydroxy-phenylacetic acid (DOPAC), and homovanillic acid (HVA) were assayed by high performance liquid chromatography with electrochemical detection (HPLC/EC) in caudate nucleus, hippocampus, and frontal cortex (Ali *et al.*, 1994).

Neurohistological Assessment

Rats were deeply anesthetized with pentobarbital sodium and perfused through the ascending aorta with about 50 ml of 0.9% saline followed by 500 ml of 4% formaldehyde in phosphate buffer (0.1 M, pH 7.4). Coronal brain sections (50 μm) were stained using a modification of a silver degeneration procedure specific for degenerating axons, terminals, and neurons (Scallet *et al.*, 1988). Cerebellar sections were examined with a novel fluorescent technique for revealing neuronal degeneration (Schmued *et al.*, 1993). Adjacent sections were stained using a gold chloride based technique for the demonstration of myelin in frozen sections (Schmued, 1990).

Statistical Analyses

Univariate and multivariate analyses of variance (MANOVA) with Wilks' Lambda statistics for the MANOVA were applied to the neurochemical and behavioral data. For all statistical analyses, a p value of < 0.05 was considered significant.

RESULTS

Behavioral Results

Effects of hypoxic insult on behavioral task performance prior to and during 3-NPA administration. Both I and NI rats exhibited weight loss during 3-NPA treatment. Ataxic gait was observed but only in NI rats beginning 25 mg/kg/day of 3-NPA treatment. No significant differences between the performance of I and NI rats were detected for any endpoints monitored in the motivation (MOT) or learning (LRN) tasks during baseline (predrug) sessions or at any dose of 3-NPA. A significant difference was noted, however, between the performance of I and NI rats for the time estimation (TE) task during baseline sessions, and this difference was not affected by treatment with 3-NPA (Figure 1). Response rates of I and NI rats performing the TE task did not differ systematically with dose of 3-NPA or during baseline sessions. Similarly, the accuracy and mean duration of lever hold endpoints for the TE task for the I and NI rats did not differ significantly during any phase of the experiment.

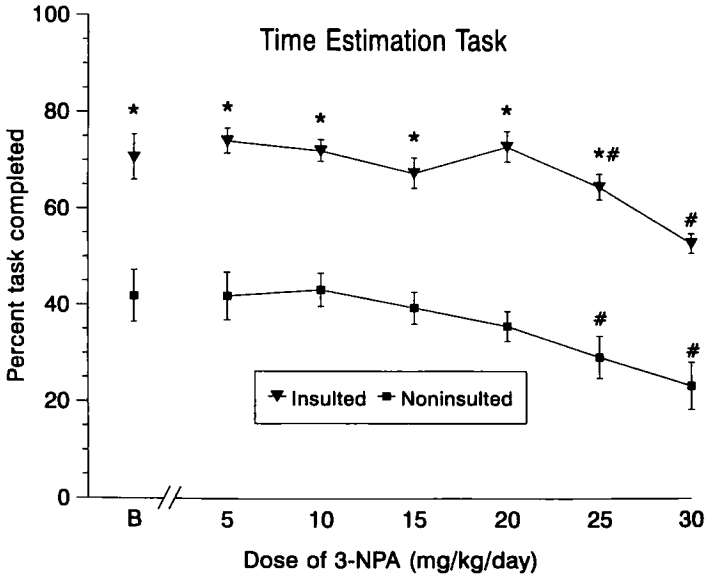

Figure 1. The effects of 3-NPA (multiple and increasing doses) on insulted (I) and noninsulted (NI) rats (n=5 per group) on mean percent task completed during the five preceding baseline (predrug) sessions and at each five day dose exposure for the time estimation task. Data expressed as the mean ± SEM; * denotes significant difference ($p < 0.05$) between I and NI rats; # denotes significant difference ($p < 0.05$) as compared to baseline (B) performance.

Effects of 3-NPA on behavioral task performance of I and NI rats. Results of 3-NPA treatment on behavioral task performance of I and NI rats are summarized in Tables I and II, respectively. As there were no significant differences between I and NI rats on any MOT or LRN task endpoints, data from both groups were combined for the statistical analyses of 3-NPA effects.

Table I. Summary of 3-NPA effects in insulted (I) rats

		Dose of 3-NPA (mg/kg/day)						
Task	Endpoint	B	5	10	15	20	25	30
Time Estimation (TE)	PTC	70.4±6.8#	74.9±5#	71.9±4.2#	67±6.8#	71.8±6.8#	64.6±5.5*#	52±3.7*#
	Resp/sec	.07±.001	.06±.003	.07±.002	.07±.002	.06±.002	.06±.003*#	.06±.007
	ACC	53.8±5	59.6±6	54.8±2.1	54.1±6.1	61.1±5	55.1±3.9	42.7±3.8*
	MDLP	9.5±.41	9.7±.42	9.7±.3	9.2±.41	9.6±.61	9.6±.42	8.7±.73
Motivation (MOT)	PTC	17.8±2.4	17.7±2.1	17.6±2.5	16.9±2.2*	14.9±2.2*	14.2±1.7*	13.3±1.1*
	Resp/sec	.44±.12	.42±.11	.43±.13	.4±.1*	.31±.09*	.28±.07*	.25±.04*
	BP	21.4±2.9	21.2±2.5	21.1±2.9	20.3±2.6*	17.8±2.6*	17±2*	16±1.3*
	PRP	14.3±1.3	12.8±.9	15.3±2.1	15.8±1.5*	20.3±3.7	18.8±1.6*	21.5±2.6*
Learning (LRN)	PTC	62.9±4	69.7±3.3	66.4±3.7	64.7±5.8	60.8±3.4*	58.8±4.8	55.8±3.5*
	Resp/sec	.23±.03	.23±.03	.24±.04	.21±.04	.19±.03*	.18±.02	.14±.01*
	ACC	56.3±4.1	59.3±1.5	57.5±2.5	60.4±5	54.4±2.9	54.5±2.2	66.4±3*

PTC=percent rask completed; ACC=accuray (% correct); MDLP=mean duration of lever press. BP=breakpoint; PRP=post reinforcement pause; * denotes significant difference from baseline (B) ($p<0.05$); # denotes significant difference from noninsulted (NI) rats ($p<0.05$). Data presented as means ±SEM.

Motivation Task. Percent task completed, response rate, and breakpoint were significantly decreased (compared to baseline values) at 3-NPA doses ≥ 15 mg/kg/day, while postreinforcement pause was significantly increased at 15, 25, and 30 mg/kg/day of 3-NPA.

Learning Task. Percent task completed and response rates for both I and NI rats in the LRN task were significantly decreased at 3-NPA doses of 20 and 30 mg/kg/day; accuracy was significantly decreased only at the 30 mg/kg/day dose.

Time Estimation Task. For NI rats the percent task completed endpoint was significantly decreased by 3-NPA at 25 and 30 mg/kg/day. Similar effects were noted in I rats but they were not statistically significant (Figure 1). Response rates of I rats were significantly decreased only at 15 mg/kg/day of 3-NPA. Accuracy (percent of lever presses held for the appropriate duration) was decreased in both I and NI rats at 30 mg/kg/day of 3-NPA, but the mean duration of lever hold was not significantly affected in either group at any 3-NPA dose tested.

Table II. Summary of 3-NPA effects in noninsulted (NI) rats

		Dose of 3-NPA (mg/kg/day)						
Task	Endpoint	B	5	10	15	20	25	30
Time Estimation (TE)	PTC	41.8±10.4	41±11.2	43.1±7.9	39.4±6.2	35.6±4.9	29.2±9.8*	23.3±9*
	Resp/sec	.06±.005	.06±.01	.06±.005	.07±.01	.05±.004	.04±.007*	.04±.009
	ACC	36±10.9	35.5±11.7	35.6±8.5	34.1±9.1	35.6±7.2	32.9±10.4	27.7±11.2*
	MDLP	7.3±1.3	6.7±1.5	7.2±1.1	8.1±2.1	7.6±1	6.8±1.5	5.9±2
Motivation (MOT)	PTC	14.9±1.2	14.8±1.5	15.4±1.4	13.3±1.1*	12.3±1.2*	11.3±1.8*	8.1±2.5*
	Resp/sec	.3±.04	.3±.05	.32±.05	.24±.04*	.22±.04*	.2±.05*	.13±.05*
	BP	17.9±1.4	17.8±1.8	18.5±1.7	16±1.3*	14.7±1.4*	13.6±2.2*	9.7±3*
	PRP	17.1±1.6	17.3±1.4	16.6±1.6	21.8±2.5*	21.1±2.1	30.1±8.1*	23.8±2.5*
Learning (LRN)	PTC	73.2±3.7	63.9±4.6	68.1±6.9	70.5±1.1	56.8±3*	59.3±4.7	40.4±11.6*
	Resp/sec	.24±.02	.24±.01	.25±.02	.23±.02	.23±.02*	.2±.008	.1±.03*
	ACC	61.3±2.8	55.6±6.9	57.8±5.4	60±2.8	48.8±2.1	53.4±5.6	67.7±2.9*

PTC=percent task completed; ACC=accuracy (% correct); MDLP=mean duration of lever press; BP=breakpoint; PRP=post reinforcement pause; * denotes significant difference from baseline (B) ($p<.05$). Data presented as means ± SEM.

Neurochemical Results

Neurochemical results are described in Table III. Compared to untreated controls, DA concentrations decreased significantly in caudate nucleus of I and NI rats (by 19%; $p = 0.03$ and by 25%; $p = 0.006$, respectively). There was a trend toward a DA increase in frontal cortex; however, it did not reach statistical significance. Concentrations of DOPAC and HVA in frontal cortex increased significantly in both I and NI rats compared to the intact controls (DOPAC; controls vs. NI; $p = 0.01$, controls vs. I; $p = 0.0003$, HVA; controls vs. NI; $p = 0.009$, controls vs. I; $p = 0.002$). However DA turnover in the cortex did not change. While most brain region weights in the untreated controls and 3-NPA treated I rats were similar, cerebellar weights in NI rats were reduced by 15% following 3-NPA treatment ($p < 0.001$).

Table III: Dopamine and dopamine metabolites (ng/100mg wet weight of tissue) in different brain regions of adult rats following treatment with multiple and increasing doses of 3-NPA; Mean ± SEM.

Brain region	Dopamine	DOPAC	HVA	HVA + DOPAC / DA
Caudate Nucleus				
Control	1461±31	142±12	71±3	0.145±0.01
Insulted	1185±131*	165±10	86±16	0.226±0.02*
Noninsulted	1100±135*	154±30	94±33	0.220±0.04*
Frontal Cortex				
Control	27.5±7.0	8.4±1.0	5.6±0.5	0.641±0.07
Insulted	62.2±10.5	23.4±4.8*	18.0±2.3*	0.626±0.13
Noninsulted	61.5±29.8	18.0±2.3*	12.0±2.3*	12.0±2.3*
Hippocampus				
Control	8.97±1.2	2.36±2.4	ND	NC
Insulted	9.93±0.6	5.30±1.1	ND	NC
Noninsulted	10.17±0.3	6.74±1.1	ND	NC

* $p < 0.05$ significantly different from control. ND not detected. NC not calculated

Neurohistological Results

Among NI and I rats receiving 3-NPA, there was considerable variability in the severity, extent and even location of the lesions. Degenerating neurons, axons, and terminals were observed throughout several parts of the diencephalon and cerebrum of the 3-NPA treated rats. Prominent among the areas in the cerebrum observed to be moderately damaged were the cingulate cortex and the fibers of the cingulate bundle (Figure 2), the perirhinal cortex (Figure 3), and the caudate nucleus (Figure 4). The hippocampus also was often found to have a scattering of necrotic, argyrophilic pyramidal neurons (Figure 5). In the diencephalon, the thalamus was commonly found to have sustained a very large measure of damage (Figure 6). Examination of the cerebellum revealed extensive lesions within the deep nuclei. A fluorescent stain for neuronal degeneration revealed degenerating neurons (Figure 7 A), while the gold chloride method indicated myelin degeneration as well (Figure 7 B).

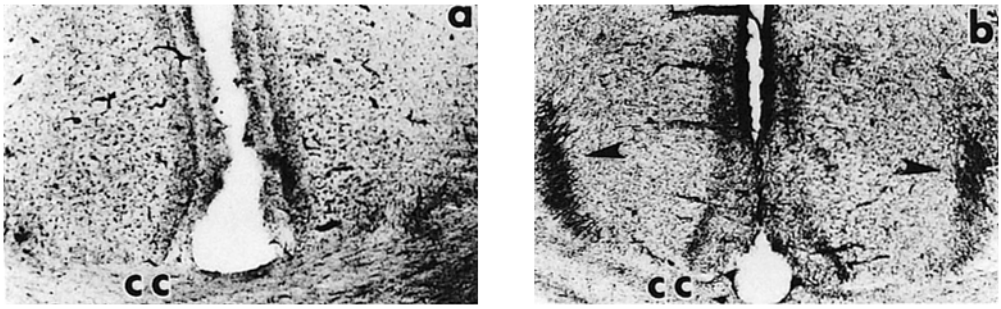

Figure 2. **a)** The photomicrograph illustrates the normal, undamaged appearance of the cingulate cortex and bundle of an I rat despite the 3-NPA dosing regimen (multiple and increasing doses), while **b)** shows the degenerating cingulate bundle (arrows) of the same region of an NI rat following 3-NPA treatment. Nadler-Evenson silver degeneration stain technique. **cc** = corpus callosum Magnification 45X.

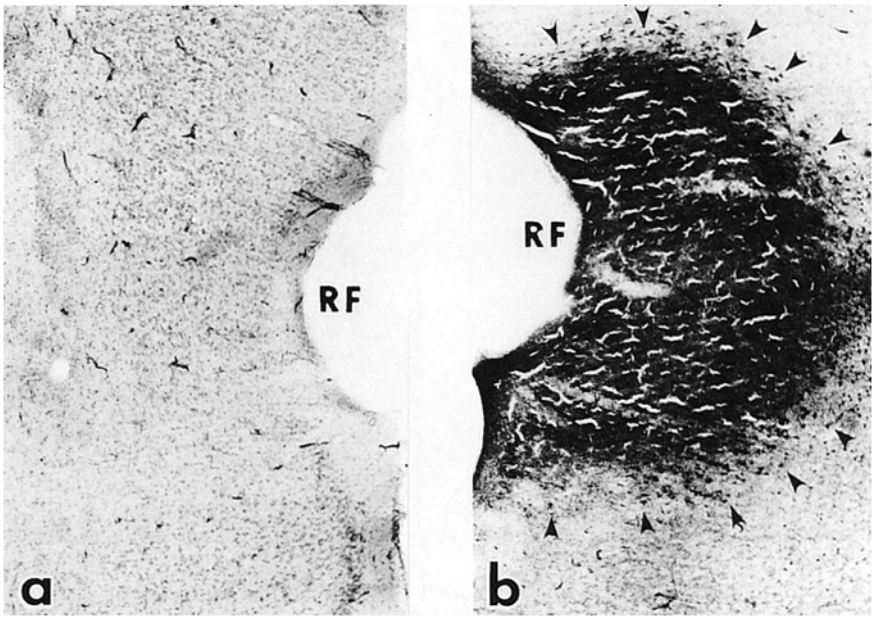

Figure 3. **a)** The perirhinal cortex is distinguished based on the proximity of the rhinal fissure (RF) in an undamaged rat brain after 3-NPA treatment (multiple and increasing doses). **b)** The perirhinal cortex of this NI rat is heavily damaged and necrotic following similar 3-NPA treatment. Individual argyrophilic neurons (arrows) surround a core of completely necrotic tissue. Nadler-Evenson silver degeneration stain technique. Magnification 45X.

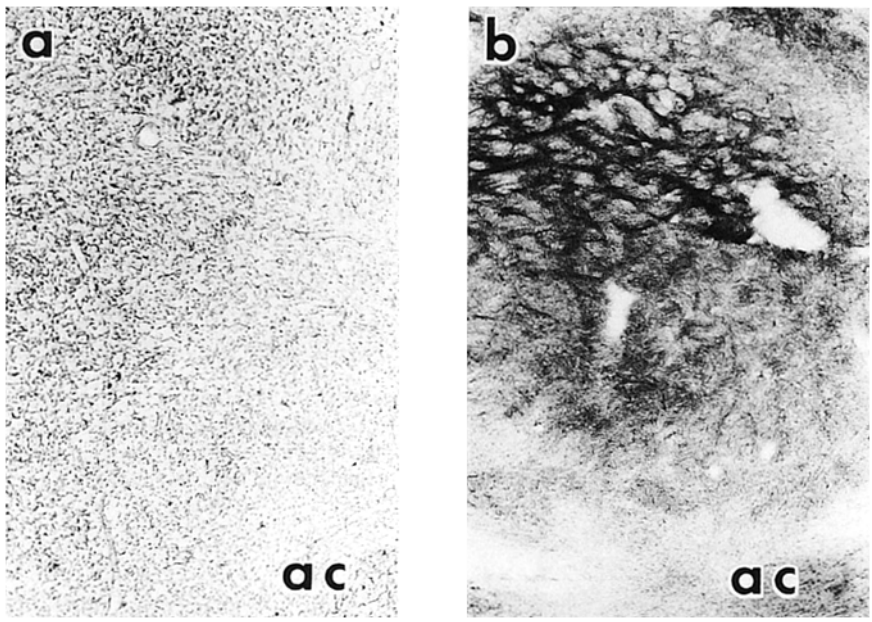

Figure 4. **a)** The caudate nucleus of this I rat after 3-NPA treatment (multiple and increasing doses) remained normal in appearance, whereas in **b)** up to about 30% of the area of the caudate nucleus in the NI rat was necrotic in appearance following 3-NPA treatment. Nadler-Evenson silver degeneration stain technique. ac = anterior commissure. Magnification 34X.

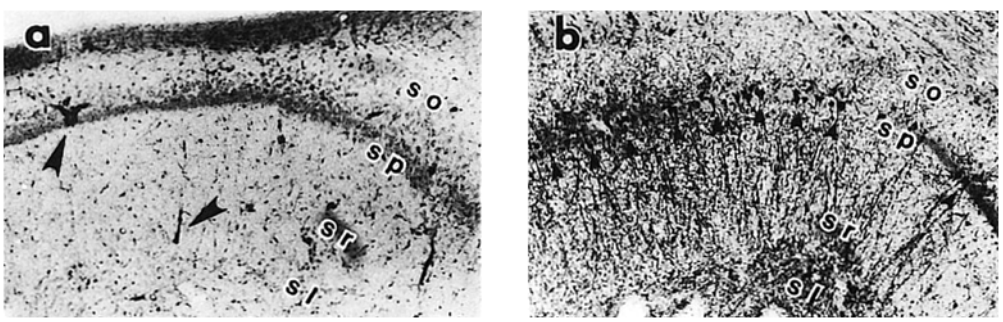

Figure 5. **a)** The hippocampus (region CA1) of the I rat is normal in appearance after 3-NPA treatment (multiple and increasing doses). Only a few blood vessels (normally argyrophilic) are visible (large arrows). **b)** This NI rat has a number of necrotic CA1 pyramidal neurons (small arrows), together with their dendrites showing dense, black silver retention following 3-NPA treatment. Nadler-Evenson silver degeneration stain technique. sl = stratum lacunosum; sr = stratum radiatum; sp = stratum pyramidale; so = stratum oriens. Magnification 68X.

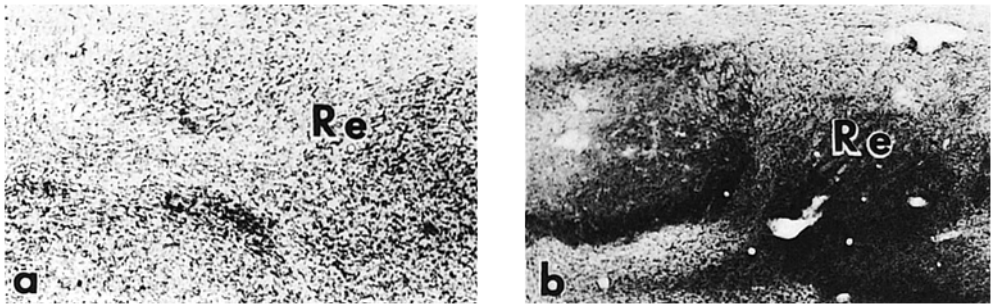

Figure 6. a) The normal appearance of the ventral thalamus is illustrated in this micrograph of an I rat after 3-NPA treatment (multiple and increasing doses). **b)** The NI rat, by contrast, has a large percentage of the thalamus that is completely necrotic and argyrophilic following 3-NPA treatment. Nadler-Evenson silver degeneration stain technique. **Re** = reuniens nucleus thalamus. Magnification 34X.

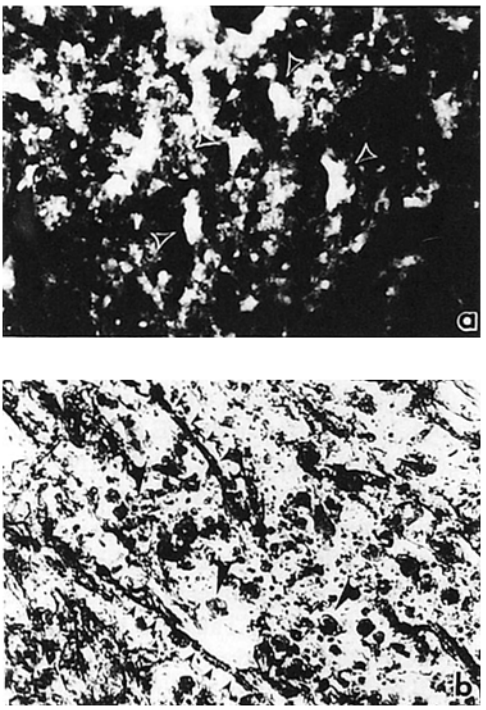

Figure 7. a) Fluoro-Jade, a fluorescent indicator specific for degenerating neurons demonstrates dead neurons (arrows) and cellular debris within the deep nuclei of the cerebellum following 3-NPA treatment (multiple and increasing doses). **b)** Deep nuclei of the cerebellum of 3-NPA treated animals reveals globular degenerating myelin (large arrows) following staining with gold chloride. A few relatively intact linear appearing myelinated fibers (small arrows) can also be detected. Magnification 68X.

Four pairs of I rats and their NI siblings were evaluated by an observer blind to the original treatment groups of the animals using a rating scale for the severity of their lesions. It was determined that in three of the four pairs evaluated, I subjects had less extensive 3-NPA-induced damage than did NI subjects.

DISCUSSION

There is a growing body of evidence suggesting that the effects of perinatal hypoxia-ischemia on subsequent brain function are manifest as adverse behavioral performance (Lun *et al.*, 1986; Shen *et al.*, 1991, Boksa *et al.*, 1995). However, in the present experiment, baseline (pre 3-NPA treatment) performance of I and NI rats significantly differed only in one of the three behavioral tasks. Additionally, neither group appeared to be more sensitive to repeated 3-NPA treatment than the other, with respect to the doses at which significant changes from baseline were noted. This was evidenced by the observation that 3-NPA produced similar dose-dependent impairments in endpoints monitored for each behavioral task in both groups of rats.

No significant differences in MOT or LRN task performance were noted between I and NI rats. There was a trend toward greater decreases in MOT and LRN task endpoints for NI rats compared to I rats at the 30 mg/kg/day dose of 3-NPA, suggesting that such treatment may have afforded some degree of protection from 3-NPA-induced behavioral impairments, but these effects were not significant (possibly due to the relatively small sample size of each group). In general, both I and NI rats were affected in a qualitatively similar manner by 3-NPA treatment, i.e., treatment with 3-NPA disrupted performance of these tasks in both groups in a dose dependent manner. Using the occurrence of a significant disruption in task performance at doses lower than those affecting other tasks as a criteria for determining relative task sensitivity, the MOT task was more sensitive to disruption by 3-NPA than either the TE or LRN tasks.

The only significant differences between I and NI rats, in terms of task performance or differential sensitivity to 3-NPA, occurred in the TE task. Insulted rats completed more of this task during baseline (nondrug) test sessions than did NI rats. However, no significant differences between I and NI rats were observed for the accuracy or mean duration of hold endpoints. That percent task completed and response rate measures were significantly affected at doses lower than those affecting accuracy indicates that disruption of this task by 3-NPA likely reflects gross behavioral impairments rather than a disruption of the cognitive processes associated with time estimation. Thus, the behavioral results indicate that only the percent completion of the TE task sensitively reflected the effects of perinatal hypoxia. While the performance in all of the tasks was impaired by 3-NPA treatment, none reflected any further effects of perinatal hypoxia.

Other research has shown that hypoxia induces protection from the neurotoxicity of kainic acid or subsequent hypoxic insult in neonatal and adult rats (Pohle and Rauca, 1994; Gidday *et al.*, 1994; Glazier *et al.*, 1994) and gerbils (Miyashita *et al.*, 1994). The histological evaluation in the present study similarly indicates a trend towards resistance to

3-NPA treatment in I rats. Too few animals with well-characterized lesions were available to adequately correlate behavioral performance with the brain damage they sustained as a result of the 3-NPA treatment. The description of the 3-NPA dose-response curve for exposure to escalating doses should permit future studies focusing on selected doses.

Neurochemical alterations in dopamine and its metabolites in the caudate nucleus induced by 3-NPA administration correspond well with those areas of greatest histological damage in most of the 3-NPA-treated animals. The alterations likely represent a combination of the effects of losing dopamine terminals and their postsynaptic targets in these areas, as well as some possible compensation by adjacent undamaged striatal tissue. Perhaps the smaller percentage of damaged cortical tissue accounts for the unchanged dopamine turnover in the cortex compared to the decrease in striatum, which was much more extensively damaged. The histological effects of repeated, chronic exposure to 3-NPA reported in the present study are similar to those previously described by Hamilton *et al.* (1987). In addition to the cerebral damage described by Hamilton and associates, bilateral lesions in the insular cortex and the deep nuclei of the cerebellum were also observed.

CONCLUSIONS

Chronic treatment of rats with 3-NPA was associated with neurotoxicity reflected in neurobehavioral, neurochemical, and neurohistological endpoints. The 3-NPA toxicity appears to be diminished in rats exposed at birth to hypoxia-ischemia. Further study is required to define the long-term relationship between the activity of brain mitochondrial enzymes of energy metabolism following perinatal hypoxia-ischemia.

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