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Elicitation and Modification of The Rabbit's Nictitating Membrane Reflex Following Prenatal Exposure to Cocaine

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ROMANO, A. G. AND J. A. HARVEY. *Elicitation and modification of the rabbit's nictitating membrane reflex following prenatal exposure to cocaine.* PHARMACOL BIOCHEM BEHAV 53(4) 857-862, 1996.—The nictitating membrane (NM) reflex was assessed in adult Dutch-belted rabbits exposed to cocaine in utero. The intensity threshold for eliciting the reflex was increased in cocaine progeny and the amplitude of the reflex was decreased at the lower stimulus intensities. However, cocaine and saline progeny showed equivalent rates of habituation of the NM reflex when tested with a suprathreshold eliciting stimulus. Reliable modification of the NM reflex was obtained when the reflex-eliciting stimulus was preceded by an auditory stimulus at intervals of 100-800 ms. Cocaine and saline progeny exhibited an increase in the peak amplitude of the reflex, a shortening of the latency of the reflex, and a shortening of the latency to achieve peak amplitude of the reflex as a function of increases in the interstimulus interval. Furthermore, cocaine progeny showed significantly longer response latencies than saline progeny across all interstimulus intervals, although neither the peak amplitude nor the latency to achieve peak amplitude was affected. Thus, prenatal exposure to cocaine affected elicitation of the defensive NM reflex to an aversive stimulus but did not affect the sensorimotor integration necessary for modification of the reflex by antecedent stimulation.

Blink reflex Defensive reflex Habituation Intrauterine cocaine Nictitating membrane Prenatal cocaine Rabbit Reflex modification Sensorimotor integration

RECENTLY, the rabbit has been employed as a model for examining the neurobehavioral effects of prenatal exposure to cocaine (11). Although intrauterine exposure to cocaine had no effect on the gross physical appearance of offspring in this model, such prenatal cocaine exposure produced effects in the CNS not reported in other animal models. Thus, prenatal exposure to cocaine produced an increase in the number of immunoreactive GABA neurons (23,24) and abnormal dendritic structure of pyramidal cells in the anterior cingulate cortex (8,9) of the rabbit. These anatomic abnormalities occurred in all animals examined, were detectable in early infancy, and persisted into adulthood. Neither of the preceding abnormalities was observed in the neighboring visual cortex, indicating that the effect was not a general phenomenon, but rather, was region specific. Neurochemical and behavioral abnormalities have also been noted in the rabbit model of prenatal cocaine exposure. Potassium-stimulated dopamine release was decreased in the frontal and cingulate cortices but not in the striatum of cocaine progeny ranging in age from 10-120 days (22). Striatum however, was not completely spared from the deleterious effects of prenatal cocaine exposure. Prenatal exposure to cocaine markedly attenuated the ability of cocaine to stimulate the coupling of D_1 receptors to their associated G protein, G_s, in the striata of rabbits ranging in age from 10-100 days (21). One potential behavioral consequence of the disruption in D_1-mediated signal transduction in the striatum was noted in preweanling and adult cocaine progeny treated with the indirect dopamine agonist amphetamine. The offspring of saline-injected rabbit dams exhibited stereotyped head bobbing in response to amphetamine, whereas prewean-

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ling cocaine progeny showed virtually no head bobbing in response to amphetamine, and adult cocaine progeny showed markedly attenuated amphetamine-induced head bobbing (17,18). Control experiments implicated the D_1 receptor in mediating amphetamine-induced head bobbing in normal animals.

Additional behavioral anomalies, possibly mediated by a dysfunctional anterior cingulate cortex and related structures, have been reported in rabbits exposed to cocaine in utero. We previously reported that cocaine progeny undergoing concurrent acquisition to visual and auditory stimuli show a normal rate of learning to a light conditioned stimulus (CS) and an accelerated rate of learning to a tone (15,16). We also reported that cocaine progeny were severely retarded in their ability to form a discrimination between a light and a tone when the light served as the CS+ and the tone was the CS− (13,14). However, cocaine progeny acquired a tone CS+/light CS− discrimination at the same rate as controls. All three of these effects on learning were attributed to an attentional deficit in processing stimuli differing in salience. However, not all of the behavioral anomalies we observed could be attributed to a deficit in attentional processing. In this regard, we noted that cocaine progeny given unpaired presentations of conditioned stimuli and a corneal airpuff unconditioned stimulus (US) showed a consistent but nonsignificant reduction in the amplitude of the nictitating membrane (NM) reflex across 10 days of testing (16). Thus, the present study was designed to follow up on our previous results to determine whether the defensive NM reflex was altered by prenatal exposure to cocaine using more sensitive assessment techniques. In one experiment, a US intensity function was generated and animals were subsequently tested for their ability to habituate to a suprathreshold US. In addition, a second experiment examined modification of the NM reflex by an antecedent stimulus. Rabbits typically show facilitation of the NM reflex when that reflex is preceded by a moderate intensity tone at intervals in the range of ≥200–800 ms (4,5,7,25,27). There have been mixed reports of reflex modification effects in other species following prenatal exposure to cocaine (1,2).

METHODS

Subjects

The Dutch-belted rabbits employed in these studies were obtained from a NIDA-supported, core breeding facility at the Medical College of Pennsylvania. All animals, breeders and offspring, were treated in accordance with approved institutional protocols. The colony rooms were illuminated according to a 12 L : 12 D cycle at 23°C, and all animals had free access to food and water. The rabbits were offspring of dams that had been injected with saline or cocaine during pregnancy as previously described (11). On gestational days 8–29, the dams received twice daily injections of saline or cocaine hydrochloride (4 mg/kg) via the marginal ear vein (in a volume of 2 ml/kg) for a total daily dose of 8 mg/kg. Kits were typically delivered on gestational day 30–31. Exposure to cocaine had no effect on time of delivery, litter size, gender ratios, or body weight, or other gross physical features (11). However, a number of neuranatomic, neurochemical, and behavioral abnormalities have been reported following prenatal exposure to cocaine in this rabbit model (8,9,13–18,21–24). The behavioral studies reported here were initiated in offspring that were 90–144 days old.

Apparatus and General Procedure

The apparatus and data acquisition system are described in detail elsewhere (12). Briefly, each animal was placed in a Plexiglas restrainer and fitted with a head mount that supported a potentiometer which was directly coupled to a suture placed in the right NM. The headmount also supported a tube for delivery of an airpuff US to the right cornea. Movements of the NM were transduced to dc voltages and digitized every 5 ms with a resolution of 0.03 mm of NM movement per analog-to-digital count. A response was defined as a 0.5-mm or greater extension of the NM. The animals were tested in illuminated, sound-attenuated chambers with a stimulus and interconnection panel mounted above and in front of the animal. One day before each of the behavioral procedures described below, animals were given one 60-min adaptation session during which no stimuli were presented.

US Intensity Testing and Habituation of the NM Reflex

Eight saline and seven cocaine progeny were given US-alone trials at five different US intensities. The target pressure of the corneal airpuff US was manipulated by holding the source pressure constant while varying airpuff duration. Airpuff durations of 20, 40, 60, 80, and 100 ms yielded target airpuff pressures of 63, 104, 139, 166, and 200 g/cm^2. The different airpuff intensities were presented in random order within each block of six trials. The intertrial interval averaged 60 s (range: 55–65). Each intensity was presented for a total of 10 times. The percentages of NM responses and their amplitudes were recorded for each airpuff duration.

Habituation of the NM reflex was assessed in the same eight saline and seven cocaine progeny 72 h after US intensity testing. Only the 200 g/cm^2 airpuff was employed. A total of 120 airpuffs were presented at a constant intertrial interval of 30 s. Habituation was assessed as alterations in NM response amplitude as a function of repeated airpuff presentations.

Reflex Modification Testing

Tone-induced reflex modification was assessed in two additional groups of seven saline and nine cocaine progeny. Ten US trials were presented at the beginning of a 60-min testing session. These initial trials were used to ensure that consistent responding would be maintained throughout the testing session, as described elsewhere (25). The remaining 72 trials consisted of 12 US trials and 15 tone–US pairings at each of four different interstimulus intervals (ISI's): 100, 200, 400, and 800 ms. The tone measured 90 dB at 1 kHz and was 50 ms in duration. The US was the 100-ms, 200 g/cm^2 airpuff described earlier. The US and tone–US pairings were presented in a quasirandom order with the restriction that no more than three trials of each type or ISI could occur consecutively in a block of 24 trials. The intertrial interval averaged 45 s (range: 40–50). On each trial, the topography of the NM response was quantified in terms of its peak amplitude, latency to achieve the 0.5 mm NM extension criterion, and latency to achieve peak amplitude. All latency measurements were made within a 400-ms window beginning with US onset.

Data Analysis

The data were analyzed with repeated-measures analyses of variance using the SYSTAT statistical package, version 5.0 (26). The repeated measures consisted of the five US durations or intensities in the first experiment, blocks of 10 US trials in

the habituation experiment, and the US-alone trials and four different ISI's in the reflex modification experiment. The α level for all tests was 0.05.

RESULTS

US Intensity Testing and Habituation of the NM Reflex

As shown in Fig. 1, both the percentages and amplitudes of NM responses increased significantly with increases in US intensity. The percentage of NM responses increased from 43% at the lowest US intensity to 85% at the highest US intensity [$F(4, 52) = 15.28$, $p < 0.001$], whereas the amplitude of the reflex increased from 2.07 to 3.15 mm between the lowest and highest US intensities [$F(4, 52) = 2.82$, $p < 0.05$]. Both measures were significantly affected by intrauterine cocaine exposure. Thus, the mean percentage of NM responses collapsed across US intensities was 80% for saline animals compared to 59% for cocaine animals [$F(1, 13) = 5.17$, $p < 0.05$], and response amplitudes averaged 3.22 mm for saline animals and 1.99 mm for cocaine animals [$F(1, 13) = 5.07$, $p < 0.05$]. There was no significant interaction between prenatal treatment condition and US intensity for either the percentage of NM responses [$F(4, 52) = 2.02$, $p > 0.10$] or for NM response amplitude [$F(4, 52) < 1$].

Habituation of the NM reflex was assessed as changes in response amplitude with repeated US presentations (Fig. 2). Response amplitudes showed a significant decrease over the 12 blocks of trials, from an average of 4.6 mm on block 1 to an average of 2.9 mm on block 12 [$F(11, 143) = 11.33$, $p < 0.001$]. However, neither the prenatal treatment condition nor the interaction with the repeated measure was significant; both F values were less than unity.

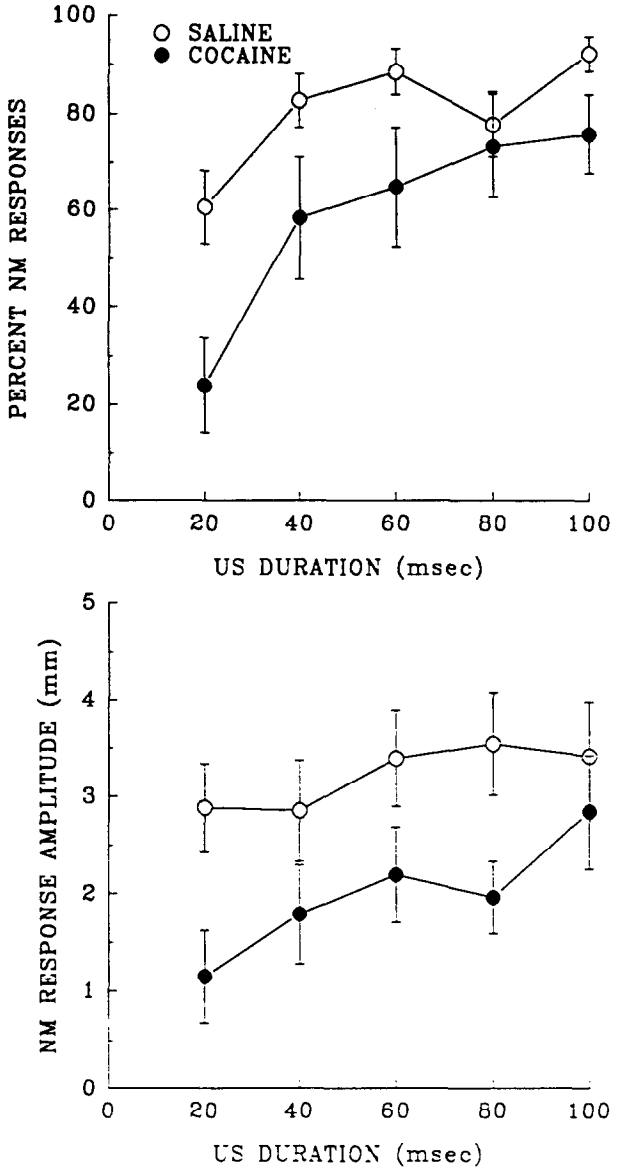

FIG. 1. (Top panel) Mean percentages of NM responses as a function of US duration or intensity for adult rabbits exposed to cocaine in utero and adult, saline progeny. In this and succeeding figures, vertical bars represent ± 1 SEM. (Bottom panel) Mean response amplitudes as a function of US duration or intensity. The 20-, 40-, 60-, 80-, and 100-ms USs corresponded to airpuff pressures of 63, 104, 139, 166, and 200 g/cm^2. Both the percentages of NM responses and their amplitudes were significantly decreased as a consequence of prenatal exposure to cocaine.

Reflex Modification

Figure 3 shows the results of manipulating the ISI on modification of NM response amplitudes. As shown in the top panel of Fig. 3, the amplitude of the NM reflex was facilitated at all ISI's, relative to baseline US-alone trials. Thus, analysis of the raw response amplitudes, shown in the bottom panel of Fig. 3, yielded a significant effect of ISI [$F(4, 56) = 2.75$, $p < 0.05$]. Although the percentage of reflex facilitation appeared to be greater in cocaine progeny (top panel, Fig. 3),

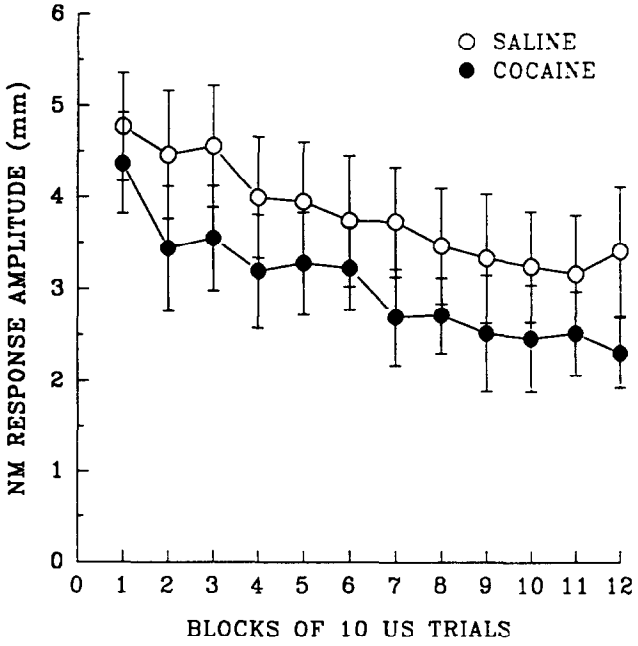

FIG. 2. Mean NM response amplitudes as a function of repeated presentations of a 100-ms, 200 g/cm^2 airpuff US. All animals participated in US intensity testing 72 h previous to the habituation series.

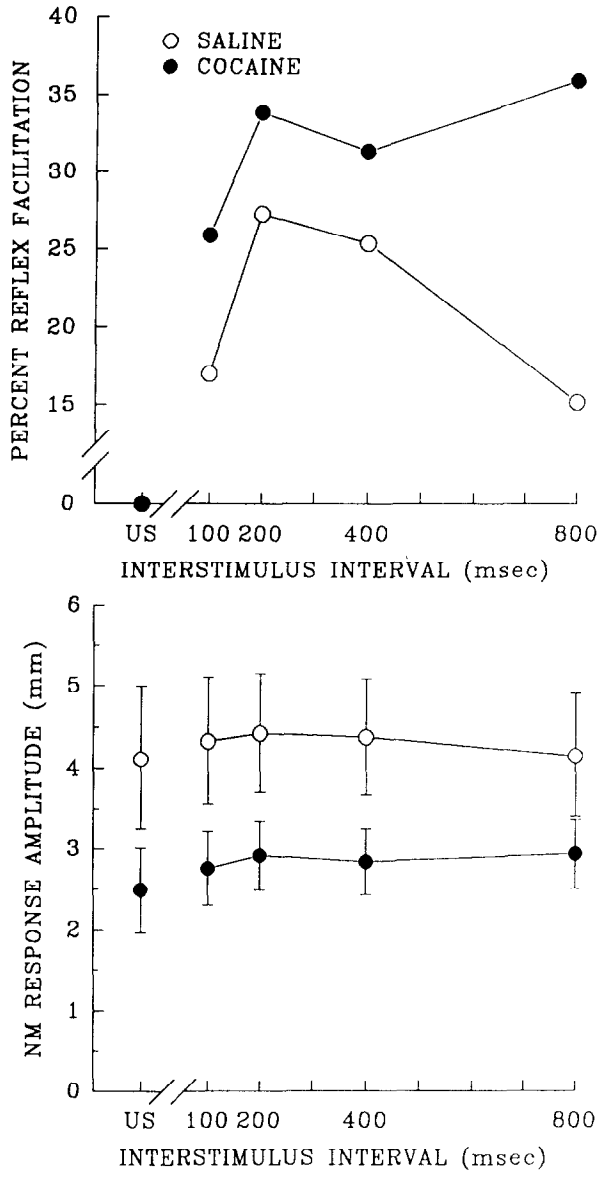

FIG. 3. (Top panel) Reflex facilitation as a function of the tone-airpuff interstimulus interval. Data are expressed as the percentage change in NM response amplitudes obtained on airpuff-alone trials (labelled as US on x axis). SEM's have been eliminated for illustrative purposes. (Bottom panel) Mean response amplitudes (corresponding to top panel) as a function of interstimulus interval. UR amplitudes (mm) were significantly altered as a function of the interstimulus interval.

raw response amplitudes were lower in cocaine progeny compared to saline progeny. However, the difference in response amplitudes did not achieve statistical significance [$F(1, 14) = 3.24$, $p < 0.10$], nor did the interaction between ISI and prenatal treatment condition [$F(4, 56) < 1$].

The effects of prenatal treatment condition and manipulations of the ISI on two temporal measures of NM response topography are shown in Fig. 4. The top panel of Fig. 4 indicates that the latency to achieve 0.5 mm of NM extension was inversely correlated with ISI such that shorter latency responses occurred at the longer ISI's. Furthermore, latencies tended to decrease across ISI's relative to the latencies exhibited on the US-alone trials. Thus, collapsed over groups, the latency to criterion decreased from an average of 104 ms on US-alone trials to an average of 77 ms at the 800-ms ISI. This difference across types and ISI's was statistically significant [$F(4, 56) = 5.83$, $p < 0.001$]. The effect of prenatal treatment condition was also significant [$F(1, 14) = 6.46$, $p < 0.01$], with cocaine progeny achieving the latency criterion an average of 31 ms later than saline progeny (106 vs. 75 ms). However, the interaction between prenatal treatment condition and the repeated measure was not significant [$F(4, 56) = 1.35$, $p > 0.25$].

Latency to achieve peak amplitude was also affected by

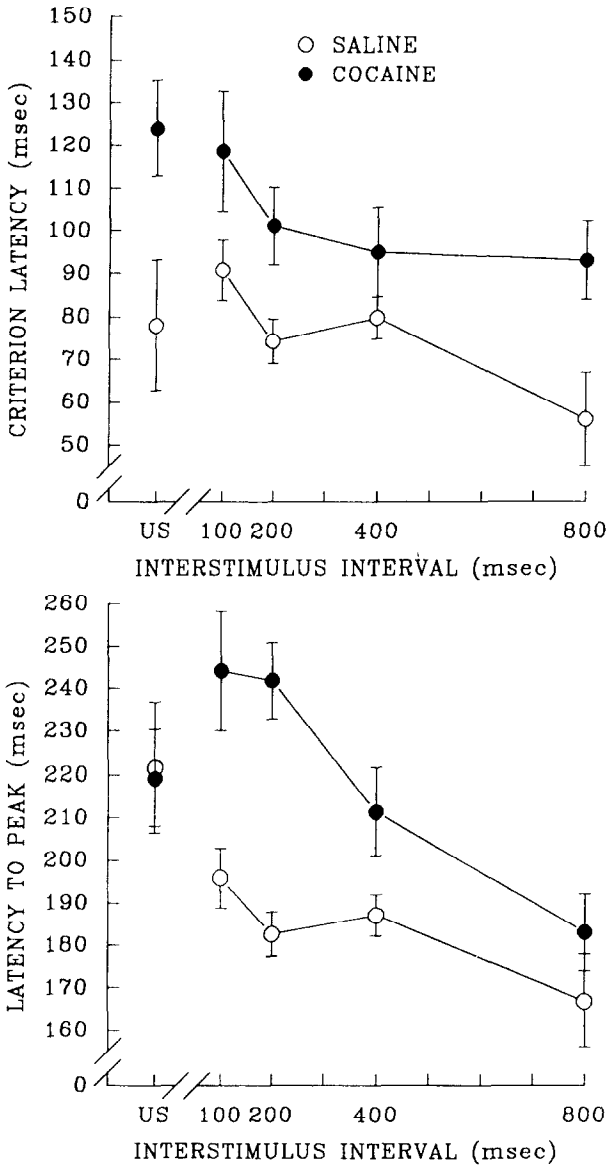

FIG. 4. Mean latency to achieve 0.5 mm of NM extension (top panel) and mean latency to achieve peak amplitude (bottom panel) as a function of interstimulus interval. On the x axis, US refers to the airpuff-alone trials. Prenatal exposure to cocaine significantly increased the latency to criterion but had no effect on the latency to peak amplitude.

manipulations of trial type and ISI, as shown in the bottom panel of Fig. 4. In general, the changes in latency to peak amplitude paralleled those in latency to criterion such that shorter latencies were associated with longer ISI's. This negative relationship was statistically significant [$F(4, 56) = 3.12$, $p < 0.025$]. Although cocaine progeny tended to exhibit longer latencies to peak amplitude than saline animals, (220 vs. 191 ms), this difference in latencies was not statistically significant [$F(1, 14) = 1.39$, $p > 0.25$], nor was the interaction between prenatal treatment condition and the repeated measure [$F(4, 56) = 1.33$, $p > 0.25$].

DISCUSSION

Prenatal exposure to cocaine affected the NM reflex and its modification in a number of ways. First, the intensity threshold for eliciting the reflex was increased as a consequence of prenatal cocaine exposure. Cocaine progeny were less likely to exhibit an NM extension than saline progeny. Second, the amplitude of the elicited response was significantly decreased in cocaine compared with saline progeny. Although both frequency and amplitude differences tended to increase with decreases in US intensity, in neither case did prenatal treatment condition interact significantly with US intensity. Third, reliable habituation to a suprathreshold US was obtained such that response amplitudes decreased significantly over the course of stimulus presentations, but this response decrement did not interact with prenatal treatment condition. Fourth, modification of the NM reflex by an antecedent stimulus yielded mixed results: Cocaine progeny exhibited a nonsignificant reduction in reflex amplitudes but significantly longer latencies than saline progeny across all interstimulus intervals.

The results of US intensity testing and response habituation indicate that the reduced responsiveness to the US demonstrated by cocaine progeny, although a reliable phenomenon over a range of US intensities, is difficult to detect when using suprathreshold stimuli. Nonetheless, the results suggest that prenatal cocaine exposure produced long-term consequences for neurobehavioral function which were detectable as an alteration in an aversive, tactile reflex. Other reflex systems have been examined in other species following prenatal exposure to cocaine, and varying results have been reported. The variability in results is not surprising given that different species, drug dosages, routes of administration, and postnatal ages at testing have been employed. For example, the amplitude of the reflexive eye blink elicited by glabellar tap is increased in human infants exposed to cocaine in utero (1), whereas neither tail-flick latencies of weanling rats nor foot-shock sensitivity of 12-day-old rat pups are affected by prenatal exposure to cocaine (10,20). Mixed results have also been reported for adult animals. Prenatal exposure to cocaine altered tail-flick latencies and foot-shock sensitivity in adult rats (19), but the acoustic startle reflex was either unaffected (3) or yielded inconsistent results (2) following prenatal cocaine exposure. Thus, it appears that human infants and adult rats and rabbits exhibit altered responses to tactile and/or nociceptive stimuli following prenatal exposure to cocaine, but their sensitivity to other, nontactile stimuli may be unaltered.

Modification of the NM reflex by an antecedent stimulus was quantified in terms of its amplitude and latency. Both measures have been shown to be reliably altered by prestimulus conditions in a number of different preparations (6). Similar alterations were seen in the present study. In general, the function relating reflex amplitude to ISI was an inverted U-shape, in agreement with other published reports of modification of the NM reflex in the rabbit (4,5,7,25,27). Moreover, the latency of the reflex decreased with increases in the ISI, in agreement with the only other report of NM reflex modification which used latency as a dependent measure (25). Although reflex amplitude across ISI's was not significantly affected by prenatal exposure to cocaine, the latency of the reflex was significantly increased in cocaine progeny. These results are comparable with those reported for human infants. Cocaine-exposed infants show an increase in the amplitude of the eye blink elicited by glabellar tap, but the effect of antecedent stimulation did not interact with cocaine exposure (1). That is, the amount of change in reflex amplitude as a consequence of antecedent stimulation was the same for normal and cocaine-exposed infants. Similar results were found in the present study: no significant effect on reflex amplitude, a significant increase in reflex latency as a consequence of prenatal exposure to cocaine, but no interaction between prenatal treatment condition and antecedent stimulus conditions. Modification of the acoustic startle reflex of the adult rat is also largely unaffected by prenatal exposure to cocaine (2). Thus, there is no evidence that prenatal exposure to cocaine alters the sensorimotor integration involved in modification of either tactile- or acoustic-elicited reflexes.

In summary, prenatal exposure to cocaine altered the intensity threshold for eliciting the NM reflex in adult rabbits but did not affect the modification of that reflex by an antecedent stimulus. The alteration in the intensity threshold for tactile elicitation of a defensive reflex is consistent with abnormal neurobehavioral functioning following prenatal exposure to cocaine and suggests that additional neuroanatomic and neurochemical abnormalities may be observed in nervous system structures not yet examined.

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