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Prenatal Exposure to Cocaine Disrupts Discrimination Learning in Adult Rabbits

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ROMANO, A. G. AND J. A. HARVEY. *Prenatal exposure to cocaine disrupts discrimination learning in adult rabbits.* PHARMACOL BIOCHEM BEHAV 53(3) 617-621, 1996.—Previous studies had shown that intrauterine exposure to cocaine produces an increase in the number of immunoreactive GABA neurons and abnormal dendritic structure of pyramidal cells in the anterior cingulate cortex, a brain region known to be involved in attentional processes and discrimination learning. Because structural abnormalities might be expected to produce related functional deficits, we examined whether intrauterine exposure to cocaine would affect discrimination learning in adult rabbits. We previously reported that cocaine progeny undergoing concurrent acquisition to visual and auditory CSs show a normal rate of learning to a light CS and an accelerated rate of learning to a tone. Here, we report that adult, Dutch-belted rabbits exposed to cocaine in utero showed impaired discrimination learning when responding to a positive visual cue but not when responding to a positive auditory cue. The nature of the deficit consisted of an impaired ability to acquire learned responses to the visual CS+ rather than in an impaired ability to withhold responses to the auditory CS−. Given that auditory stimuli tend to be more salient than visual stimuli in the normal rabbit, the preceding pattern of results suggests that intrauterine cocaine exposure affected the ability to preferentially attend to less salient but relevant stimuli and to ignore more salient, irrelevant stimuli. More importantly, these results indicate that prenatal exposure to cocaine produces neurobehavioral abnormalities which persist into adult life.

Attention Cingulate cortex Nictitating membrane Pavlovian conditioning Prenatal cocaine Drugs of abuse

RECENTLY, the rabbit has been employed as a model for examining the neurobehavioral effects of prenatal exposure to cocaine (10). 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 central nervous system not reported in other animal models. Thus, prenatal exposure to cocaine produced an increase in the number of immunoreactive GABA neurons (21,22) and abnormal dendritic structure of pyramidal cells (6,7) in the cortical region defined as area 24 in rabbits, anterior cingulate cortex (20). These anatomical abnormalities occurred in all animals examined, were detectable in early infancy and persisted into adulthood. However, neither of the preceding abnormalities had been observed in the neighboring visual cortex, area 17, indicating that the effect was not a general phenomenon, but rather, was region specific.

A number of cognitive and noncognitive functions have been attributed to cingulate cortex (19). Two of the cognitive functions associated with this cortical structure are attention and mnemonic processing (3,18). Positron emission tomography (PET) studies in humans have shown preferential increases in blood flow in anterior cingulate cortex during attention demanding tasks such as the Stroop attentional conflict paradigm (11) and noun-verb word association (13). Electrophysiological and lesion studies in rabbits have suggested that anterior cingulate cortex is involved in attentional and mnemonic processes that are activated during discrimination learning (2-4,14). Multiple units in this brain region show training-induced excitatory discharges in the presence of a CS+ and significantly less activity in the presence of a CS− (3). Moreover, rabbits with lesions confined to anterior cingulate cortex show retarded acquisition of a discriminated avoidance response but are subsequently able to perform at normal criterion levels (3). Given the results of the PET studies in humans and the electrophysiological and lesion studies in rabbits, we hypothesized that the anatomical effects of in utero

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exposure to cocaine should result in abnormal functioning of the anterior cingulate cortex and, therefore in attentional processing, and this dysfunction would be detectable as an impaired ability to discriminate between two stimuli. We previously reported that cocaine-exposed rabbits undergoing concurrent acquisition training with tones and lights show a normal rate of acquisition to the light CS and an accelerated rate of acquisition to the tone (16,17). The latter result suggests that the neurological effects of intrauterine cocaine exposure functionally increased the salience or attentional value of the tone CS. We, therefore, examined how the functional increase in tone salience would affect the rate of discrimination learning when the tone served as the negative cue vs. when the tone served as the positive cue.

METHOD

Subjects

The rabbits were Dutch-belted adult offspring of dams that had been treated with either saline or cocaine (4 mg/kg, IV, twice daily) during gestational days 8-29. Cocaine and saline progeny were 120-144 days old at the start of the experiments. All animals, breeders, and offspring, were treated in accordance with approved institutional protocols. Cocaine-treated dams were not significantly different from saline-treated dams with regard to weight gain, time to delivery, litter size, or litter gender ratios, nor were there any gross physical differences between cocaine and saline progeny (10).

Apparatus and General Procedure

The conditioning apparatus and data acquisition system are described in detail elsewhere (15). Briefly, each animal was placed in a Plexiglas restrainer and fitted with a headmount that supported a potentiometer that was directly coupled to a suture placed in the right nictitating membrane (NM). 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 trained in illuminated, sound-attenuated chambers with a stimulus and interconnection panel mounted above and in front of the animal. Rabbits were trained to discriminate between two conditioned stimuli (CSs): an 800-ms, 75-dB, 1 kHz tone and an 800-ms flashing light produced by interruption of the houselights at a frequency of 10 Hz. The unconditioned stimulus (US) was a 100-ms corneal airpuff exerting a pressure of 200 g/cm^2 measured at the end of the delivery tube. Each training session consisted of 60 trials composed of 30 pairings of the positive cue (CS+) and airpuff US and 30 nonreinforced presentations of the negative cue (CS−). The offset of the CS+ was coincident with the onset of the US. Trials were presented at an average intertrial interval of 60 s (range: 55-65 s) in quasi-random order with the restriction that no more than 3 CS+ or 3 CS− trials could be presented consecutively. A response was scored as a conditioned response (CR) if it occurred within 800 ms of CS+ onset.

In the first experiment, saline (n = 12) and cocaine progeny (n = 9) were trained to discriminate between the flashing light as the CS+ and the tone as the CS−. In the second experiment, two additional groups of saline (n = 7) and cocaine (n = 10) progeny were trained to discriminate between tone as the CS+ and light as the CS−.

Data Analysis

The SYSTAT statistical package (23) was used for all data analyses. The percentages of conditioned responding were analyzed using repeated-measures analyses of variance with prenatal treatment (cocaine vs. saline) as the between-groups variable and Trial-types (CS+ vs. CS−) and sessions as the two repeated measures. Based on a procedure developed by others (4), we also calculated for each animal the number of training sessions required to achieve the first significant discrimination and the number of sessions required to achieve criterion performance. The first significant discrimination was defined as the first session in which the percentage of CRs to the CS+ exceeded the percentage of CRs to the CS− by 25% or more. The criterion session was defined as the second consecutive session when responding to the CS+ exceeded responding to the CS− by 60%.

RESULTS

Light CS+/Tone CS− Training

As shown in Fig. 1, cocaine progeny required seven more sessions than saline progeny to achieve the first significant discrimination. This difference represents a significant retardation in the rate of discrimination learning for the cocaine-exposed animals, $t(19) = 2.83$, $p < 0.025$. A significant difference was also obtained with regard to criterion performance. Cocaine offspring required 19 sessions to achieve criterion performance vs. 14 sessions for saline animals, $t(19) = 2.28$, $p < 0.05$. The source of the retardant effect in cocaine-exposed animals is apparent in the session-by-session acquisition curves, shown in Fig. 2. Cocaine-exposed animals were significantly retarded in their ability to acquire the CR to the light CS+ whereas the ability to withhold responses to the tone CS− was apparently normal. Both the sessions × treatment effect, $F(23, 237) = 1.57$, $p < 0.05$, and the three-way interaction among sessions, treatment, and trial-types, $F(23, 437) = 1.59$, $p < 0.05$, yielded significant effects.

Tone CS+/Light CS− Training

In Experiment 2, saline- and cocaine-exposed animals acquired the tone CS+/light CS− discrimination at approxi-

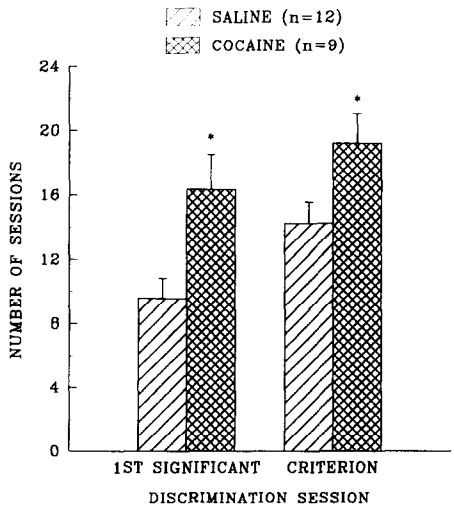

FIG. 1. Number of sessions required to achieve the first significant discrimination and criterion performance using the light CS+/tone CS− discrimination paradigm. The asterisk denotes a significant difference ($p < 0.05$) between cocaine and saline progeny. See the Method section for the definitions of the first significant discrimination and criterion sessions.

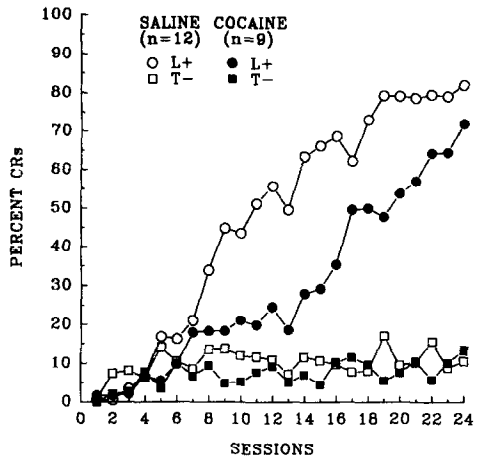

FIG. 2. Percentages of discriminative responses obtained using light as the positive cue (L+) and tone as the negative cue (T−). Adult rabbits exposed to cocaine in utero showed a retarded rate of conditioned response acquisition to the positive cue but were able to withhold responses to the negative cue. Training was terminated after 24 sessions; both groups had achieved criterion performance levels by the 19th session (see Fig. 1).

mately the same rate, as shown in Fig. 3. Analysis of the session-by-session acquisition curves, shown in Fig. 4, indicated that the only significant effects were the main effects of sessions, $F(15, 225) = 31.43, p < 0.001$, trial-types, $F(1, 15) = 98.40, p < 0.001$, and the sessions × trial-types interaction, $F(15, 225) = 23.79, p < 0.001$.

Comparison of Figs. 1 and 3 indicates that the discrimination based on tone as the CS+ was considerably easier to acquire than the discrimination based on light as the CS+, regardless of the prenatal treatment condition. Separate *t*-tests were conducted on the number of training sessions required to achieve the first significant discrimination and criterion performance. Collapsed over treatment condition, significantly fewer sessions, $t(36) = 3.48, p < 0.001$, were needed to achieve the first significant discrimination when tone was the CS+ and light was the CS−. Similarly, fewer sessions, $t(36) = 4.10, p < 0.001$, were needed to achieve criterion performance for animals trained to discriminate between the tone CS+ and light CS−.

DISCUSSION

The impaired discrimination learning observed when the visual cue was the CS+ is consistent with results reported for other discrimination learning paradigms following lesions of area 24, anterior cingulate cortex. Powell and colleagues, using discriminative heart rate and eyeblink conditioning in rabbits, reported that relatively large lesions of cortex, overlapping areas 8, 24, and 32, resulted in smaller amplitude heart rate CRs and impaired discriminative performance in lesioned animals compared to controls (2). However, the lesions had no effect on the acquisition of discriminative eyeblink responding. A subsequent study using more restricted, ibotenic acid lesions of area 24 yielded somewhat different results (14). The magnitude of the decelerative heart rate CR to the CS+ was unaffected by area 24 lesions, but discriminative responding was altered such that decelerative heart rate responding to the CS− was equivalent to responding to the CS+. Although eyeblink responding was not monitored in this last study, other investigators have reported disruptions in learned, discriminative skeletomotor responding following restricted lesions of area 24. Gabriel and colleagues reported that such lesions retarded the development of discriminative avoidance responding as measured by a locomotor response in a running wheel (3,4). Lesions of anterior cingulate cortex disrupted discriminative responding by retarding the acquisition of CRs to the CS+. Lesioned animals were apparently able to withhold responding to the CS− as well as controls, similar to what we observed for cocaine vs. saline offspring. Our results, therefore, suggest that the abnormal dendritic structure and/or the increase in the number of immunoreactive GABA sites in anterior cingulate cortex following prenatal cocaine exposure (6,7,21,22) may be functionally equivalent to a lesion in that cortical structure, at least when learned, skeletomotor behaviors are measured. The latter statement must be further qualified, however, because a deficit in discrimination learning in our paradigm occurred only when the visual cue was the CS+. As discussed below, this modality specificity may be due to differences in salience for auditory vs. visual stimuli and possible interactions among salience differences, attentional processing, and task difficulty.

Most current theoretical treatments of learning acknowledge the influence of stimulus salience on rate of learning (9,12). Stimulus salience is directly related to stimulus intensity when comparing stimuli of the same modality. When comparing stimuli of different modalities, stimulus salience has traditionally been defined in terms of the rate of learning associated with a given CS (8). Thus, given two CSs, the one associated with the faster rate of acquisition is said to be the more salient of the two.

In a previous experiment, using a simpler training paradigm than that employed here, we established that CR acquisition to an auditory CS proceeds faster than acquisition to a visual CS when animals are trained concurrently to both stimuli (16,17). Thus, by definition, the auditory stimulus was the more salient of the two stimuli. The present experiments also suggest that the tone CS is more salient than the light, but the evidence here is not as direct, as it involves comparing acquisition rates across different experiments. Nonetheless, the rate of acquisition of discriminative responding, as measured by the number of sessions required to achieve two different performance criteria, was facilitated in groups trained with the tone as the CS+. Moreover, a comparison of the session-by-session acquisition curves in Figs. 2 and 4 indicates that

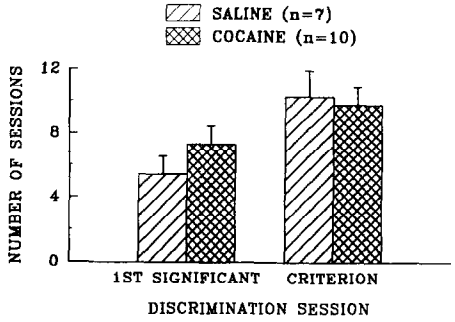

FIG. 3. Number of sessions required to achieve the first significant discrimination and criterion performance using the tone CS+/light CS− discrimination paradigm. The two groups acquired this discrimination at equivalent rates and faster than the light CS+/tone CS− discrimination (compare with Fig. 1).

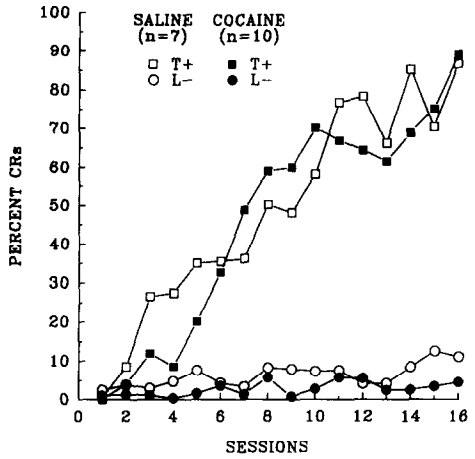

FIG. 4. Percentages of discriminative responses obtained over 16 training sessions using tone as the positive cue (T+) and light as the negative cue (L−). Intrauterine exposure to cocaine had no effect on discriminative responding under these training conditions.

rate of acquisition of CRs to the CS+ was largely responsible for the faster discrimination learning when tone was the positive cue. This last point can best be illustrated by comparing performance in the two tasks on an arbitrarily selected day early in training. For example, on day 10 of L+/T− training (Fig. 2), conditioned responding to the light CS+ averaged 43% for saline animals and 21% for cocaine animals. By contrast, on day 10 of T+/L− training (Fig. 4), conditioned responding in the presence of the auditory CS+ averaged 58% and 70% for saline and cocaine progeny, respectively.

Rate of learning is influenced by other factors related to the CS besides CS salience. The conditioning models of Mackintosh (9) and Pearce and Hall (12) emphasize changes in CS effectiveness as an additional determinant of the rate of associative learning. In the Mackintosh model, CS effectiveness is equated with the attentional value accorded to a CS. In the Pearce-Hall model, CS effectiveness refers only to the ability of the CS to enter into an association. Both models specify that CS effectiveness can change on a trial-by-trial basis and that such changes influence the rate of associative learning on succeeding trials. Because the change in associative strength on Trial n depends upon the change in effectiveness of the CS on Trial $n-1$, both models incorporate, either implicitly (9) or explicitly (12), a storage device for tracking the changes in CS effectiveness. We suggest that such an attentional-mnemonic storage device is deficient in cocaine progeny such that the differential changes in the effectiveness of the CS+ vs. the CS− are not appropriately encoded and/or recalled. In the context of the Mackintosh model, animals learn to attend and respond to the CS+ and to ignore and not respond to the CS−. Apparently, when the CS− is the more salient of the two stimuli, as is the case when the tone is the CS−, cocaine-exposed animals have difficulty in learning and/or remembering to attend to the less salient CS+ such that their rate of CR acquisition to the light CS+ is retarded relative to controls. Stated another way, cocaine progeny have difficulty in learning and/or remembering to preferentially attend to less salient but relevant stimuli (light CS+) when more salient, irrelevant stimuli (tone CS−) occur in the same context. Intuitively, the reverse of this task is far simpler: learning/remembering to attend to more salient stimuli and to ignore less salient stimuli is inherently biased in favor of success due to the differences in salience. Thus, acquisition of a discrimination based on tone as the CS+ and light as the CS− was accomplished faster than the light CS+/tone CS− discrimination for both saline and cocaine progeny. The tone CS+/light CS− discrimination also normalized the performance of cocaine progeny, suggesting that a deficiency in attentional processing can sometimes be masked when the demands of the task are relatively simple. On the other hand, when the demands of the task are made too simple, abnormal attentional processing may again be evident. Thus, cocaine progeny given concurrent acquisition training to tone and light CSs or acquisition only to a tone CS showed more rapid acquisition to the tone than did saline progeny (16,17).

Anterior cingulate cortex was only one of two cortical structures examined that exhibited anatomical abnormalities in our rabbit model of prenatal cocaine exposure (6,7,21,22). However, it is conceivable that a dysfunction in other, related structures may be partially responsible for the pattern of results observed in the present study. Two different laboratories using different behavioral preparations and training procedures have reported that lesions of the mediodorsal nucleus (MD) of the thalamus produce behavioral deficits similar to those produced by area 24 lesions (1,5). Because MD and anterior cingulate cortex are reciprocally interconnected, it is possible that MD activity would also be altered in our rabbit model of prenatal cocaine exposure and may contribute to the behavioral deficit.

Regardless of the mechanism(s) underlying the deficit, the impaired discrimination in adult animals exposed to cocaine in utero is consistent with our hypothesis of a functional deficit in attentional processing. Taken together, the anatomical studies and the present results indicate that prenatal exposure to cocaine produces neurobehavioral deficits that persist into adult life.

ACKNOWLEDGEMENTS

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