Pharmacology Biochemistry & Behavior, Vol. 37, pp. 761–767. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Hyperthermia Sensitizes Rats to Cocaine's Proconvulsive Effects and Unmasks EEG Evidence of Kindling After Chronic Cocaine

G. T. LIVEZEY[1] AND S. B. SPARBER[2]

Department of Pharmacology, University of Minnesota
3-249 Millard Hall, 435 Delaware St. S.E., Minneapolis, MN 55455

Received 1 March 1990

LIVEZEY, G. T. AND S. B. SPARBER. *Hyperthermia sensitizes rats to cocaine's proconvulsive effects and unmasks EEG evidence of kindling after chronic cocaine.* PHARMACOL BIOCHEM BEHAV **37**(4) 761–767, 1990.—In phase I, 64 male and female Sprague-Dawley rat siblings from 8 litters were divided equally among 4 treatment groups; saline plus normothermia (S37), saline plus hyperthermia (S45), cocaine (30 mg/kg) plus normothermia (C37), and cocaine plus hyperthermia (C45) and treated daily from 45–60 days of age. Cocaine plus hyperthermia produced protracted, intense and often fatal convulsions, whereas animals from either treatment alone did not convulse. Subsequently, 12 males, representing all phase I treatment groups equally, were implanted with telemetric transmitters to monitor the EEG and core body temperature in phase II. Survivors of this second phase were exposed to one trial each of saline plus hyperthermia, cocaine plus normothermia, and cocaine plus hyperthermia, in that order. The data obtained suggests that 1) the telemetered EEG and temperature can be used to detect changes reflecting sensitization/kindling in the absence of behavioral expression (convulsions), 2) analysis of EEG power spectral bands and body temperature curves showed that a history of daily cocaine exposure seems to have contributed more than daily hyperthermia to subsequently observed seizure patterns and thermic responses, and, finally, 3) cocaine plus hyperthermia resulted in a shorter latency to convulse and a lower maximal EEG seizure voltage, while increasing the variety, severity and duration of its behavioral expression (convulsion).

Cocaine Hyperthermia Interaction Sensitization Kindling EEG Seizures Power-spectra (FFT) Rat

THE term "kindling" was assigned to the phenomenon of the progressive development (EEG patterns) and expression (convulsions) of seizure activity in response to repetitive, acutely subconvulsive electrical stimulation, particularly in limbic areas (5). A kindling-like mechanism was suggested to describe the progressive sensitization to a variety of behavioral and electrophysiological effects of cocaine following repetitive exposures to the same dose (4). The pattern of the convulsive behavior produced by chronic cocaine administration was highly reminiscent of that produced by electrical kindling or kindling produced by intermittent exposure to heated water-induced hyperthermia (10). As suggested by Post (12), there may be a commonality of mechanism, perhaps characteristic of limbic structures, to explain kindling (sensitization) of behavioral and electrical responses to a wide variety of repetitive stimuli. Hyperthermia has been observed in dogs after chronic daily exposure to cocaine alone (21) and in rats after acute administration of cocaine IV or IP (8). Acute hyperthermia and seizures in response to cocaine have also been reported after human exposure (2). Therefore, it is clinically relevant to consider the concomitant exposure to cocaine and hyperthermia. For the neonate exposed perinatally to cocaine and experiencing serial fevers, or the adult cocaine user who may have a pyrogenic infection, engage in vigorous exercise or experience passive recreational heat exposure (saunas/hot tubs), the risk of a synergistic interaction may far outweigh that of either experience alone.

We wished to determine if evidence of repeated exposure to cocaine and/or hyperthermia can be found in the spontaneous EEG sampled in the absence or presence of drug or overt behavioral expression (convulsions). Further, we examined the body temperature response to hyperthermic water exposure to assess the acute and possible chronic thermoregulatory effects of cocaine. Third, we wished to examine the potential for interactive effects of cocaine and hyperthermia. Finally, we wished to evaluate the telemetry data acquisition system (Data Sciences, Inc., Roseville, MN) in our first application of this technology to a full study.

METHOD

Overview

In phase I, the animals received injections of saline or cocaine

[1]Present address: Department of Obstetrics and Gynecology, University of Nebraska Medical Center, 600 S. 42nd Street, Omaha, NE 68105-1065.
[2]Requests for reprints should be addressed to S. B. Sparber, Department of Pharmacology, University of Minnesota, 3-249 Millard Hall, 435 Delaware St. S.E., Minneapolis, MN 55455.

and exposure to 37°C or 45°C water, once daily for 16 days beginning at the age of 45 days. In phase II, 3 males from each phase I treatment group were implanted with telemetric transmitters for monitoring the EEG and core body temperature and subsequently exposed to saline plus hyperthermia, cocaine plus normothermia, and finally, cocaine plus hyperthermia. Thus, all surviving animals had the same phase II treatment.

Phase I. Eight litters, each consisting of 4 male and 4 female Sprague-Dawley rats (Holtzman, Madison, WI), were divided equally into 4 treatment groups. One male and 1 female of each litter received saline and exposure to 37°C water (S37), or saline and exposure to 45°C water (S45), or cocaine and exposure to 37°C water (C37), or cocaine and exposure to 45°C water (C45). All water exposures were for 4 minutes unless the animal exhibited convulsive behavior. Any animal which began to convulse in the water was immediately removed to prevent drowning. It was anticipated that all animals would receive 16 daily injections and water exposures beginning at the age of 45 days. However, many of the C45 animals convulsed in response to early exposures and several died following subsequent convulsions. As it became evident that the C45 animals would not survive continued exposure if they had convulsed more than once, the remaining rats were removed from this experiment upon the first convulsive episode. Since these litters were born across a span of 7 days, and their first trials were appropriately staggered over a 7-day period, this procedure did not select for animals whose convulsions occurred at later trials. Only one female and four males in the C45 group experienced all 16 trials because they did not convulse.

Apparatus/procedure. In phase I, cocaine and saline controls were exposed to the water bath in pairs. Each animal was first removed from its home cage, weighed, and placed in an individual holding cage for 10 minutes prior to injection. Cocaine/saline pairs received their injections within 30 seconds of each other and were returned to their holding cages for 10 minutes. They were then transferred to a viewing arena (cylinder of clear plastic, height 53 cm, diameter 36 cm, divided vertically into two chambers). At 15 minutes postinjection, the pair was transferred to a similar but smaller divided plastic cylinder (height 53 cm, diameter 18 cm) which rested upon a circular submerged perforated platform that allowed water to flow into and out of each chamber. This cylinder and platform were placed in a glass battery jar (height 30 cm, diameter 39 cm i.d.) and the water maintained at ± 0.1°C of the chosen temperature (37°C or 45°C) by a circulation pump (Model 3052, Lab-Line Instruments Inc., Melrose Park, IL). The water level was adjusted (by transfer to or from the pump vessel) so that the animals could rest their feet on the bottom and keep their head above water without having to swim. After their four-minute water exposure the animals were returned to the dry arena for continued observation. All sessions were videotaped for subsequent behavioral scoring.

Behavior/convulsion scoring. The animals were observed for indications of arousal, stereotypy and/or convulsive behavior in the water and the arena before and after exposure to the water. Behavior/arousal level was scored by a rating scale of psychomotor stimulant effects developed by Ellinwood and Balster (3): 1 = sleep, 2 = inactive/awake, 3 = inplace activity (e.g., grooming), 4 = normal exploration, 5 = fast exploration, 6 = stereotyped locomotion, 7 = fast stereotyped locomotion, 8 = head and/or paw stereotypies, no locomotion, and 9 = dyskinetic/reactive movements (e.g., backing up, seizures, abnormal postures). We have modified the scoring procedure to adjust for the context of our procedure (e.g., certain escape behaviors such as jumping, either while in the bath or immediately following, are context-specific and appropriate, although in an open field or home cage these would not be expected to emerge). Convulsive behavior was scored according to a modification of the seizure classification of Ito et al. (9), described previously (10): 0 = no convulsive behavior; 1 = head or body twitching or jerks; 2 = clonic forelimb convulsion; 3 = rearing (kangaroo posturing); 4 = falling back; 5 = explosive "popcorn-like" running about and/or tonic extensor rigidity. When the animals had recovered (as indicated by their assuming normal posture and beginning to groom and dry themselves) they were returned to their home cages.

Phase II. Twelve males from phase I (four from each of three litters; three from each of the four phase I treatment groups) were implanted with telemetric transmitters to monitor core body temperature and EEG and allowed one week for recovery from surgery. Phase II treatment consisted of one injection of saline and exposure to hyperthermia, three days rest, one injection of cocaine and exposure to normothermia, three days rest, and finally, one injection of cocaine and exposure to hyperthermia.

Surgery. The twelve males were placed on 24-hour food deprivation, pretreated with 2 mg atropine methyl nitrate/kg and anesthetized with 42.5 mg sodium pentobarbital (Nembutal Na, injectable)/kg. The entire surgical procedure was performed according to NIH guidelines as outlined in the *Guide for the Care and Use of Laboratory Animals* (DHEW Publication NIH 85-23, revised 1985). The transmitter was placed within the peritoneum and the bipolar epidural EEG leads were threaded subcutaneously to the top of the skull. Two holes, slightly smaller than the lead wire coil diameter, were drilled in the skull, 2 mm on either side of the midline and 2 mm anterior to lambda. The exposed coiled wire tip was bent back upon iteslf so that the distal end of the coil of wire, with the sharp end buried within the fold, could be compressed and placed into the hole. A small amount of dental acrylic cement was applied to seal the hole and fix the leads to the skull surface. No additional anchor was necessary since there was no significant stress on the lead and the bone grew up to incorporate the wire coil.

Drug/water bath trials. The drug administration and water bath exposure procedures were as previously described for phase I, with two modifications. Since the animals had gained significant weight during phase I and preparation for phase II, the water bath exposure time was increased from 4 minutes to 7 minutes to insure the same body temperature increase in the hyperthermia trials (as determined by preliminary trials with other subjects). Owing to the size of the animals and practical restraints of reception and acquisition of the data (see below), each subject was run individually, instead of paired as in phase I.

Data Acquisition

The behavioral data were recorded by video camera and video cassette recorder (Models PK959, PV8000, Panasonic). All equipment pertinent to acquisition of EEG and temperature data was supplied by Data Sciences, Inc., St. Paul, MN. The implanted telemetric transmitters (Model TA11CTA-F40-L15) sent the digitized data to an individual receiver unit (Model No. CTR86) which was attached to a consolidation matrix (Model No. BCM100), and thus to the data acquisition system (Model DQIII, installed within an American Micro AT computer). During water bath exposure the epidural electroencephalogram (EEG) and core body temperature data were sent by the transmitter to an individual receiver via dual waterproofed wand antennas (Model No. CTR86 with option 4 external antennas). The analog EEG signal from the receiver was viewed with an oscilloscope, with the screen visible to the video camera. The existing prerelease software we used allowed for acquisition, coding and storage of one temperature sample and two seconds of EEG data per ten seconds real time [200 Hz sampling rate, chosen as the minimum or Nyquist fre-

quency to allow spectral analysis from 1–100 Hz (13)].

Drugs

Cocaine HCl, kindly supplied by the National Institute on Drug Abuse, was dissolved in 0.9% NaCl solution and injected IP in a volume of 1 ml/kg of body weight. Control injections were of an equal volume of saline. We have used a dose of 30 mg cocaine/kg, which produces a reliable increase in behavioral arousal, the rapid development of stereotypies with chronic administration, and yet does not produce acute convulsant/toxic effects, as does 60 mg/kg (unpublished observations). We chose a 15-minute period between IP injection and subsequent water bath exposure due to the short ½ life of cocaine and the demonstration of maximal behavioral effects at this time (4).

Data Analyses

The incidence of convulsions and deaths was subjected to the Pearson Chi Square test. The latency to first convulsion, body weights and body temperature were analyzed by ANOVA. Since the rats' temperatures normally fluctuate, a moving average with a period of 6 points (1 minute real time) was used to transform the data for determination of the latency to the peak temperature achieved following hyperthermic bath exposure. Body temperature rise and fall rates were calculated as the slope of the 5 minutes preceding and following the peak temperature, respectively. The peak temperature latency and the slopes from the regression analyses of the rise and fall were subjected to ANOVA. Four 1-minute periods were chosen from the major stages of each phase II trial for analysis of the EEG; preinjection (from 5–6 minutes before injection), postinjection (from 13–14 minutes after injection), the sixth minute in the water bath (from 5–6 minutes after entry into the water), and the first minute out of the bath (beginning after transfer to the dry viewing arena). During each of these 1-minute periods, 6 equispaced 2-second samples of the EEG were stored by the computer. The power spectrum of each EEG sample was computed by performing a Fast Fourier transformation of the linearly tapered autocorrelation function of the EEG data series. The 6 power spectra from each 1-minute period were averaged to give 1 spectrum to represent that time period from 1–100 Hz. Final averaged spectra were divided into 20 Hz bins (1–20, 21–40 . . . 81–100) and 5 Hz bins (1–5, 6–10 . . . 96–100) and the sum of all power within a bin was converted to a percentage of the total power across 1–100 Hz. Total power (summed across 1–100 Hz), and the 20 and 5 Hz bins (both as absolute power levels and as the percent of total power) were analyzed by the Mann-Whitney U-test for chronic effects (group comparisons) and the paired *t*-test for acute effects (time sample comparisons). Values reported in text and tables are the means ± S.D., unless otherwise specified.

RESULTS

Phase I

Body weight gain. Since many cocaine plus hyperthermia (cocaine plus 45°C water: C45) animals were eliminated over the course of the experiment because of convulsions and/or death (see below), we confined our examination of cocaine effects upon weight gain to the normothermia groups (saline plus 37°C water: S37, cocaine plus 37°C water: C37). Comparing the males alone, for trial one the two groups were of approximately equal weights (C37 = 218.4 ± 17.9; S37 = 221.7 ± 26.4 grams). C37 male animals were significantly lighter in weight by trial 11, compared to their saline counterparts (C37 = 266.1 ± 15.9; S37 = 287.6 ± 29.5 grams; $p<0.050$) and remained so through the end of phase I (C37 = 295.6 ± 20.68; S37 = 323.0 ± 33.5 grams; $p<0.035$). No significant differences were noted in the females, suggesting they are less sensitive to cocaine's effect on this parameter, perhaps because of their slower rate of weight gain at these ages.

TABLE 1

COCAINE EFFECT ON PSYCHOMOTOR STIMULANT BEHAVIOR SCORE BEFORE THE FIRST BATH EXPOSURE

Group	Males	Females	Combined
Saline	2.7 ± 0.9	2.8 ± 1.0	2.8 ± 1.1
Cocaine	6.8 ± 0.9	7.9 ± 0.8†	7.4 ± 1.0*

The data are the mean ± S.D. *Cocaine vs. saline, $F(1,30)=234.002$, $p<0.001$. †Sex effect, $F(1,30)=4.479$, $p<0.050$.

Behavior. Behavior just prior to transfer into the water bath showed a rather rapid progression to hyperarousal and stereotypies in all cocaine injected animals. Even in trial 1, most cocaine injected animals had achieved high stimulant scores (see Table 1). With the exception of convulsive behaviors in the C45 group (see below) there were no significant changes with repeated trials.

Convulsion incidence, stage, latency, duration and lethality: Only animals from the cocaine plus hyperthermia (C45) group had convulsions in phase I. Eight out of 9 females and 5 out of 9 males in this group eventually convulsed [13/18 for C45 vs. 0/16 for S37 or 0/16 for C37 or 0/16 for S45 (saline plus 45°C water: S45), Chi square, $p<0.001$]. Furthermore, 5 females and 2 males subsequently died (7/18 for C45 vs. 0/16 for S37 or C37 or S45, Chi square, $p<0.036$). The larger N of 9 in these male and female C45 groups was the result of the substitution by an extra sibling available when their littermate died on the first trial. All first convulsions occurred by the 8th trial. Since the C37 and S45 subjects may have convulsed had they experienced more than 16 trials in phase I, we assigned all animals that had not convulsed by trial 16 a value of 17 for their first convulsive trial. Even with this very conservative estimate, the C45 group convulsed significantly earlier, $F(3,62)=35.160$, $p<0.001$, and, within the C45 group, the females convulsed significantly earlier (mean trial number = 4) than the males (mean trial number = 10), $F(1,16)=4.535$, $p<0.050$. Whether this reflects a true sex difference or a more rapid heat transfer rate due to the smaller mass of the females has yet to be determined. In previous studies, whether kindling was induced by electrical, chemical, or hyperthermic stimuli, kindling was described as a progressive development of convulsive behavior with repeated trials. Yet, what is most striking in our combined cocaine and hyperthermia (C45) animals is that nearly all exhibited advanced stages of convulsive behavior upon their first convulsion, regardless of the number of trials leading up to it. Furthermore, their first convulsion contained a great variety in the type and order of convulsive behavior. Excluding phasic episodes of extensor rigidity, the mean of the convulsion score for their first convulsion was 3.6 ± 1.0 for the males and 4.2 ± 0.9 for the females (out of a maximum of 5). In the C45 group, the mean latency from the beginning of bath exposure to the first convulsive behavior, when it occurred, was 248.8 ± 26.7 seconds for the males and 246.3 ± 20.6 seconds for the females. The mean duration of the first convulsive episode in the C45 group was 155.9 ± 71.9 seconds for the males (N = 5) and 186.8 ± 137.0 seconds for the females (N = 8). Although we had no S45 or C37 animals exhibiting convulsions in phase I of this

TABLE 2

EFFECT OF COCAINE ON THE LATENCY (SECONDS) TO PEAK CORE BODY TEMPERATURE AFTER HYPERTHERMIC BATH EXPOSURE

	Phase II: Acute Treatment	
Phase I: Chronic History	Saline+45°C	Cocaine+45°C
Cocaine (N=4)	625 ± 56.9*	675 ± 56.9*†
Saline (N=5)	556 ± 18.2	608 ± 28.6

The data are the mean ± S.D. *Chronic effect [cocaine vs. saline phase I drug history, $F(1,7)=9.186$, $p=0.019$]. †Acute effect [saline+45°C phase II trial vs. cocaine+45°C phase II trial, $F(1,7)=9.867$, $p=0.016$].

TABLE 3

EFFECT OF COCAINE ON THE RISING AND FALLING SLOPE (°C/MIN) OF CORE BODY TEMPERATURE AFTER HYPERTHERMIC BATH EXPOSURE

	Phase II: Acute Treatment	
Phase I: Chronic History	Saline+45°C	Cocaine+45°C
	Rising Slopes	
Cocaine (N=4)	0.454 ± 0.087	0.393 ± 0.125*
Saline (N=5)	0.543 ± 0.055	0.455 ± 0.064
	Falling Slopes	
Cocaine	−0.305 ± 0.111	−0.205 ± 0.122*
Saline	−0.406 ± 0.080	−0.344 ± 0.090

The data are the mean ± S.D. *Chronic effect [cocaine vs. saline drug history, rising slope $F(1,7)=3.804$, $p=0.092$, falling slope $F(1,7)=9.692$, $p=0.017$]. The apparent acute slowing effect of cocaine did not achieve significance.

experiment for comparison, in previous studies, the duration of the first convulsion following repeated exposure to hyperthermia was 40.0±44.4 seconds in eight mature male rats of the same strain (10).

Phase II. Convulsion incidence, stage, latency, duration and lethality: One C45 animal convulsed spontaneously (i.e., in the absence of handling, drug or hyperthermic stimuli) in its home cage and died prior to the first phase II trial. None of the S37 animals convulsed. One out of 3 S45 animals convulsed. Four out of the 5 remaining animals with chronic cocaine histories (2/3 for C37, 2/2 for C45) convulsed in phase II.

Saline plus hyperthermia (phase II, trial 1) resulted in convulsions for the 2 remaining C45 animals, 1 C37 animal, and 1 S45 animal. One of the 2 C45 animals died subsequent to convulsing (total 9 deaths, all C45; 7 during phase I, 1 spontaneously between phases, 1 during phase II). No convulsions occurred in response to cocaine plus normothermia (phase II, trial 2). Cocaine plus hyperthermia (phase II, trial 3) resulted in convulsions for the same surviving animals as in trial 1, plus an additional C37 animal. The convulsions and deaths in the C45 group suggest that these animals had become sensitized due to their phase I treatment. The convulsions in two C37 and one S45 subjects suggest that phase II treatment unmasked residual effects of phase I (see EEG data below for supporting evidence). The four animals with previous cocaine exposure (C37, C45) exhibited stage 5 convulsive behavior involving extensor rigidity (tonic) with intermittent clonic-tonic involvement of the entire body. The cocaine-experienced animals (C45, C37) began their convulsive behavior while still in the water bath. These observations in the C37 animals upon their first convulsion (in phase II), were consistent with those of the C45 animals first (phase I) and subsequent convulsions. The recovery times (to regain posture and begin grooming behavior) following the phase II saline plus hyperthermia trial, suggested a phase I history effect, with the surviving C45 animal taking longest to recover (S45 = 521 seconds vs. C37 = 581 vs. C45 = 1213 seconds).

Core body temperatures. Maximum core body temperatures were comparable for all animals, reaching an average of 42.2°C, 38.7°C, and 42.5°C, respectively in the phase II saline plus hyperthermia, cocaine plus normothermia, and cocaine plus hyperthermia exposures. Although maximal temperatures were not different, cocaine exposure delayed the rise and fall of temperature, both acutely and chronically. If we consider the latency to peak temperature (see Table 2), we find that 1) the rats with previous cocaine histories in phase I take longer to reach peak temperature, and 2) all the rats took longer to reach peak temperature following acute cocaine challenge in the cocaine plus hyperthermia trial, compared with the saline plus hyperthermia trial. If we consider the rate of temperature change (see Table 3), we find that animals with previous cocaine exposure (C45 and C37 groups) showed a tendency toward slower rise in core body temperature during hyperthermic bath exposure and displayed a significantly slower cooling rate, with or without acute cocaine administration, when compared to their saline experienced counterparts.

EEG. Figure 1 contains both EEG samples and the corresponding power spectra from all phase II trials and time periods in one C37 animal. Examination of the EEG signals from this animal (see Fig. 1, insets) revealed voltage (amplitude) increases of nearly 20-fold when the convulsion was elicited by saline plus hyperthermia. Considerably smaller voltage increases (10-fold) resulted when the convulsion followed cocaine plus hyperthermia. However, the number of discrete convulsive episodes separated by behavioral depression (2 vs. 12) and the total duration of convulsive activity (2 minutes vs. 26 minutes) was much greater with cocaine. It has been suggested that cocaine has both proconvulsant and anticonvulsant properties (16,20). These paradoxical effects may be reflected in the enhanced spread and duration of convulsions and the lowered maximal EEG voltage, respectively (see the Discussion section). The character of individual seizure EEG waveforms covaried with the behavioral expression, and so is beyond a meaningful definitive description here, especially since this report is based upon a limited number of subjects due to phase I toxic interactions between hyperthermia and cocaine.

Power spectra. From visual examination of the serial power spectra (Fig. 1) corresponding to the inset EEG samples from the same C37 animal, we can observe an increase and redistribution of power (voltage density). Note the striking harmonic distribution of power in the cocaine plus hyperthermia trial, as opposed to the saline plus hyperthermia trial.

EEG power spectra quantification. Although most of the spectral power appears to occur in the 1–20 Hz range, animals previously exposed to chronic cocaine (C37,C45 histories) displayed an apparent increase in midrange frequencies (41–60), particularly after cocaine challenge (postinjection period). Therefore, we examined the frequency distribution over the whole 1–100 Hz range.

One of the main goals of the phase II segment of this experiment was to test the utility of the telemetric technology in allowing us to determine if residual (subliminal) effects of chronic cocaine exposure upon EEG activity could be detected and quantified in an objective manner. Since quite varied degrees of convulsive behavior (and deaths) emerged as rats experienced hyperthermia in combination with cocaine administration, we re-

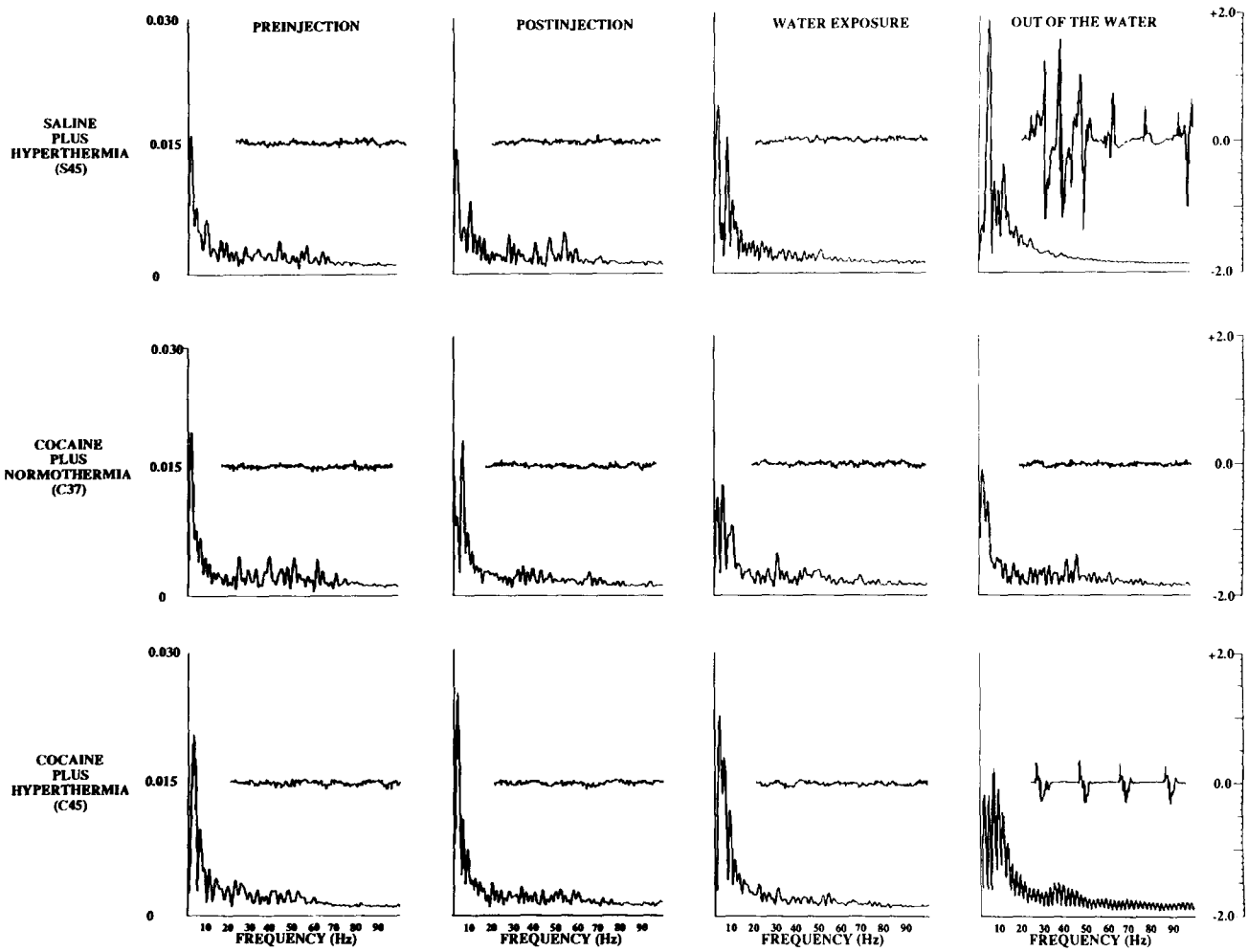

FIG. 1. The serial EEG samples from an animal with a phase I experience of cocaine plus normothermia (C37) are inset upon the corresponding power spectra for the four major time periods within each of the three phase II trials. Each EEG sample represents 2 seconds of real time. The ordinates (on the right) are equal (at ± 2 mV) to demonstrate the voltage change during each trial. Note the complex, yet regular order of the seizure pattern in the last segment (cocaine plus hyperthermia, post bath) which is reflected by the "harmonic" character of the power spectra. Serial power spectra: Each abscissa denotes the frequency range of 1–100 Hz, and all ordinates are absolute power on an equal scale (0–0.030 $mV^2/Hz^{0.5}$). Note both intratrial and intertrial progressions in peak number and complexity, and the added "harmonic" character of predominant frequencies with cocaine. The final time frame, reflecting convulsive activity after cocaine plus hyperthermia exposure, exhibits a striking example of the "combing" morphology which may be specific to epilepsy (see the Discussion section).

stricted our statistical analyses of the effects of cocaine history on the EEG to preinjection baselines before the phase II saline plus hyperthermia trial and three days later, before the cocaine plus normothermia trial (see Table 4). When we grouped the animals according to drug history (saline or cocaine in phase I), we found that total power in the 1–100 Hz range was virtually identical for the two groups for the first baseline sample (phase II, saline plus hypethermia trial, preinjection). Yet, three days after exposure to hyperthermia, those animals with a history of chronic cocaine exposure showed significantly lower total spectral power for the same sampling epoch (cocaine plus normothermia trial, preinjection). So it would seem that the initial hyperthermia challenge was sufficient to unmask a latent effect of chronic cocaine exposure, even under unchallenged (baseline) conditions. Upon closer examination, it was only in the lowest frequencies that a significant decline in absolute power levels was found. In fact, those animals with chronic cocaine histories displayed a significant increase in absolute power levels in the midrange frequencies, possibly reflecting spontaneous spiking activity. To control for the possibility of recording bias in voltage levels, and to confirm the above results, we examined the relative power distribution among frequency bands. Again (Table 5), we found that subjects with a history of chronic cocaine displayed a significantly lower percentage of power in the lowest frequencies, and a significantly higher percentage of power in the midrange. In summary, chronic cocaine exposure produced a decrease in low frequency EEG activity and an increase in intermediate frequency EEG activity, which was detectable in the baseline EEG several days after the first hyperthermia challenge in phase II.

The acute effect of hyperthermia alone was assessed by comparing the first baseline EEG (saline plus hyperthermia, preinjection) with the EEG following removal from the bath (saline plus hyperthermia, out of the water). To separate the acute effect(s) of hyperthermia from possible interactions of chronic history or acute

TABLE 4

THE EFFECT OF CHRONIC COCAINE EXPOSURE ON THE ABSOLUTE EEG SPECTRAL POWER LEVELS

Hz Band	Phase I: Chronic History	Phase II: Sample Time Saline+45°C Preinjection	Cocaine+37°C Preinjection
1–100 Hz	Cocaine (N=4)	0.581	0.573*
	Saline (N=5)	0.599	0.608
1–20 Hz	Cocaine	0.210	0.192*
	Saline	0.227	0.251
1–5 Hz	Cocaine	0.089	0.060*
	Saline	0.117	0.119
41–60 Hz	Cocaine	0.115	0.115*
	Saline	0.098	0.105
46–50 Hz	Cocaine	0.026	0.031*
	Saline	0.023	0.026

All values are the median summed power ($mV^2/Hz^{0.5}$) within the selected frequency band, compared using the Mann-Whitney U-test. *Cocaine vs. saline chronic history, $p<0.050$.

TABLE 5

THE EFFECT OF CHRONIC COCAINE EXPOSURE ON EEG SPECTRAL POWER DISTRIBUTION

Hz Band	Phase I: Chronic History	Phase II: Sample Time Saline+45°C Preinjection	Cocaine+37°C Preinjection
1–20 Hz	Cocaine (N=4)	35.4	33.5*
	Saline (N=5)	39.1	41.3
1–5 Hz	Cocaine	14.5	10.5*
	Saline	18.2	19.6
41–60 Hz	Cocaine	18.7	19.5*
	Saline	16.4	17.3
46–50 Hz	Cocaine	4.5	5.2*
	Saline	3.9	4.3

All values are the median percent of total power within the frequency band, compared using the Mann-Whitney U-test. *Cocaine vs. saline chronic history, $p<0.050$.

convulsive activity, we confined our examination to the S37 group, which had no prior experience with either cocaine or hyperthermia, and which exhibited no convulsions in either phase I or phase II. Consistent with our visual examination of the power spectra, we found a significant enhancement of the 1–20 Hz band both as absolute power levels and relative percent distribution [absolute power, $df=2$, mean X-Y $=-0.100$, paired t value $=-7.796$, p(2-tail) $=0.016$; relative power distribution, $df=2$, mean X-Y $=-0.119$, paired t value $=-6.031$, p(2-tail) $=0.026$].

The acute effect of cocaine alone was assessed by comparing the second trial baseline EEG (cocaine plus normothermia, preinjection) with the EEG following removal from the bath (cocaine plus normothermia, out of the water). The normothermia bath exposure was a control for the exposure to water during the induction of hypethermia. Confining our examination to the S37 group, we found no significant effect in the 1–20 Hz band. However, there was a significant suppression of the relative percent distribution to the 41–60 Hz band [$df=2$, mean X-Y $=0.006$, paired t value $=8.500$, p(2-tail) $=0.014$]. We then examined the 2 C37 animals that did not convulse as a result of the saline plus hyperthermia trial. During the cocaine plus normothermia session, these cocaine-experienced animals showed a significant increase in the relative percent distribution to the 1–20 Hz band ($df=1$, mean X-Y $=-0.058$, paired t value $=-14.500$, $p=0.044$), but no significant effect in the 41–60 Hz band.

DISCUSSION

Cocaine appeared to slow the rise and clearly slowed the fall of body temperature in rats exposed to an exogenous heat source. This may be related to the importance of cutaneous blood flow in the regulation of body heat in the rat and cocaine's vasoconstrictor properties. The rate of temperature change may be more important for a convulsive response than the absolute temperature, below 42–43°C (Klauenberg and Sparber, unpublished observations). Thus, in this sense, cocaine may exert a "protective" effect by slowing the absorption of exogenous heat in our procedure. Yet clinically, most relevant hyperthermic episodes would be "internally" generated, by whatever source, including a convulsion. Therefore, the same cardiovascular (vasoconstriction) effects of cocaine which may slow the body temperature rise in our externally generated model, might hasten temperature rise by reducing the capacity to dissipate heat. The telemetric acquisition of the temperature during the hyperthermic exposure allowed us to observe these dynamic effects.

It would seem from our data that the acute effects of cocaine on EEG activity are in opposition to the chronic effects. Following chronic cocaine administration, the C37 animals demonstrate a reduced activity in the 1–20 Hz band, and an enhanced activity in the 41–60 Hz band (measured in the baseline activity, cocaine plus normothermia, preinjection). Following acute administration, the C37 animals demonstrated an enhancement in the 1–20 Hz band, and the S37 animals (previously cocaine naive) demonstrated a suppression in the 41–60 Hz band. Perhaps this suppression in the 41–60 Hz band in drug naive subjects reflects an acute anticonvulsant effect of cocaine. In the cocaine-experienced animals, the chronic suppression of 1–20 Hz and enhancement of 41–60 Hz may reflect long-lasting effects related to sensitization/kindling. These two chronic effects of cocaine may reflect a decrease in the capacity for synchronization of neuronal activity and an increase in the spontaneous firing of excitatory pathways, since in vitro studies have demonstrated decreased sensitivity of GABA receptors and increased sensitivity of NMDA (glutamate) receptors with kindling (17).

It has been suggested that the paradoxical cocaine effects of reduction of seizure afterdischarge duration and latency to clonus are manifestations of cocaine's monoaminergic and local anesthetic actions, respectively (18,19). Perhaps it is the enhancement of catecholaminergic influences on local inhibitory circuits (GABA) which restricts recruitment of synchronized neuronal firing in the region of seizure origin and thus peak EEG voltage is kept lower after cocaine. The proconvulsant local anesthetic properties, affecting a wide range of systems and brain regions, may allow for many more discrete regions to attain seizure activity with behavioral manifestations and for longer periods of time. This would explain our behavioral and EEG data with respect to the effects of cocaine and hyperthermic stress.

Guegen and Gaches (7) referred to a "combing" morphology in the spectral histograms of epileptic patients, which they suggested was specific for epileptic diseases. Many investigators have turned to newer mathematical approaches in analyzing nonlinear, "dynamic" systems (11), such as the heart (6) and the brain (14,15). These studies refer to the concept of "chaos," which unlike the common definition, actually refers to a complex order.

Using such methods, Babloyantz and Destexhe (1) could show a contrast between epileptic seizures and other synchronous brain states, such as slow wave sleep. By examination of the power spectra of hyperthermia-induced vs. cocaine plus hyperthermia-induced convulsions in our study, it may be suggested that cocaine interferes with the ability to synchronize neuronal activity globally, as reflected in the bifurcated "harmonic" distribution of power peaks. That is to say, the "order" of the seizure activity with cocaine is more complex. As discussed by Rapp (14), it is not suggested that the seizure is the chaotic event, but rather that the "seizure is a corrective response to a previous transition to chaos . . . the neurological analogue to defibrillation . . . the cure rather than the disease." In this context, the increase and prolongation of "harmonic" effects in the EEG spectra following cocaine may explain why these convulsions were so varied and protracted. The bifurcation of power peaks reflects some effect in opposition to the entrainment of brain activity by the seizure, and so, the "cure" was less effective. A more precise mathematical quantification of the effects observed in this study (phase II) will be attempted in future studies.

In summary, the data suggest that: 1) cocaine may affect thermoregulatory mechanisms both acutely and chronically, 2) hyperthermia and cocaine have a synergistic effect on sensitization/kindling, EEG seizure activity, convulsive behavior, and lethality, 3) permanent changes in the power spectra of the EEG, which reflect sensitization/kindling, can be demonstrated in the absence of a convulsion, 4) the quality of the EEG power spectra following cocaine suggests a reduction in the capacity of synchronization, which may explain why the convulsive behavior is highly variable, intense, and prolonged, and, finally, 5) the computer controlled telemetry data acquisition system used provides a reliable and informative approach to research which requires monitoring of freely moving animals.

ACKNOWLEDGEMENTS

Supported in part by USPH grants T32DA07097, DA04979 and NS23289.

REFERENCES

1. Babloyantz, A.; Destexhe, A. Low-dimensional chaos in an instance of epilepsy. Proc. Natl. Acad. Sci. USA 83:3513–3517; 1986.
2. Campbell, B. G. Cocaine abuse with hyperthermia, seizures and fatal complications. Med. J. Aust. 149:387–389; 1988.
3. Ellinwood, E. H.; Balster, R. L. Rating the behavioral effects of amphetamine. Eur. J. Pharmacol. 28:35–41; 1974.
4. Ellinwood, E. H.; Kilby, M. M.; Castellani, S.; Khoury, C. Amygdala hyperspindling and seizures induced by cocaine. In: Ellinwood, E. H., Jr.; Kilbey, M. M., eds. Cocaine and other stimulants. New York: Plenum Press; 1977:303–326.
5. Goddard, G. V.; McIntyre, D. C.; Leech, C. K. A permanent change in brain function resulting from daily electrical stimulation. Exp. Neurol. 25:295–330; 1969.
6. Goldberger, A. L.; West, B. J. Applications of nonlinear dynamics to clinical cardiology. Ann. NY Acad. Sci. 504:195; 1987.
7. Guenguen, B.; Gaches, J. EEG mapping in partial epilepsies. Rev. Electroencephalogr. Neurophysiol. Clin. 16:217–228; 1986.
8. Ishizuka, Y.; Rockhold, R. W.; Hoskins, B.; Ho, I. K. Importance of route of administration in body temperature responses to cocaine in restrained and freely moving SHR and WKY. FASEB J. 3:A419; 1989.
9. Ito, T.; Hori, M.; Yoshida, K.; Shiizu, M. Effects of anticonvulsants on seizures developing in the course of daily administration of pentetrazol to rats. Eur. J. Pharmacol. 45:165–172; 1977.
10. Klauenberg, B. J.; Sparber, S. B. A kindling-like effect induced by repeated exposure to heated water in rats. Epilepsia 25:292–301; 1984.
11. Pool, R. Is it healthy to be chaotic? Science 243:604–607; 1989.
12. Post, R. M. Progressive changes in behavior and seizures following chronic cocaine administration: Relationship to psychosis. In: Ellinwood, E. H., Jr.; Kilbey, M. M., eds. Cocaine and other stimulants. New York: Plenum Press; 1977:353–372.
13. Ramirez, R. W. The FFT: Fundamentals and concepts. Englewood Cliffs, NJ: Prentice-Hall; 1985.
14. Rapp, P. E. Experimental studies of chaotic neural behavior: Cellular activity and electroencephalographic signals. Nonlinear oscillations in biology and chemistry. Berlin: Springer-Verlag; 1985.
15. Rapp, P. E. Oscillations and chaos in cellular metabolism and physiological systems. In: Holden, A. V., ed. Chaos. Princeton: Princeton University Press; 1986:179–208.
16. Russell, R. D. Stripling, J. S. Monoaminergic and local anesthetic components of cocaine's effects on kindled seizure expression. Pharmacol. Biochem. Behav. 22:427–434; 1985.
17. Stelzer, A.; Slater, N. T.; ten Bruggencate, G. Activation of NMDA receptors blocks GABAergic inhibition in an in vitro model of epilepsy. Nature 326:698–701; 1987.
18. Stripling, J. S.; Ellinwood, E. H., Jr. Sensitization to cocaine following chronic administration in the rat. In: Ellinwood, E. H., Jr.; Kilbey, M. M., eds. Cocaine and other stimulants. New York: Plenum Press; 1977:327–351.
19. Stripling, J. S.; Hendricks, C. Effect of cocaine and lidocaine on the expression of kindled seizures in rats. Pharmacol. Biochem. Behav. 14:397–403; 1981.
20. Stripling, J. S.; Gramlich, C. A.; Cunningham, M. G. Effect of cocaine and lidocaine on the development of kindled seizures. Pharmacol. Biochem. Behav. 32:463–468; 1989.
21. Tatum, A. L.; Seevers, M. H. Experimental cocaine addiction. J. Pharmacol. Exp. Ther. 36:401–410; 1929.