

Differential Behavioral Responses to Cocaethylene of Long-Evans and Sprague-Dawley Rats: Role of Serotonin

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KEY WORDS cocaine; dopamine; ethanol; fluoxetine; metabolism; raphe nucleus; rat; strain differences; striatum; ventral tegmental area

ABSTRACT Cocaethylene is a neuroactive metabolite derived from the concurrent consumption of cocaine and ethanol. The effects of cocaethylene on locomotor activity, stereotypy, and rearing in Long-Evans and Sprague-Dawley rats were compared. A single cocaine injection (molar equivalent of 60 $\mu\text{mol/kg}$ cocaethylene, intraperitoneal) elicited a robust series of motor output behaviors, including locomotion, stereotypy, and rearing over a 30-minute testing period in Long-Evans rats. In contrast, cocaethylene administration, under comparable testing conditions, produced no significant changes in locomotor and investigatory behaviors. Because cocaethylene has relatively little impact on serotonin (5-HT) reuptake as opposed to reuptake of dopamine, we pretreated Long-Evans rats with fluoxetine (10 mg/kg; IP), a selective 5-HT reuptake inhibitor. Fluoxetine profoundly augmented cocaethylene-stimulated behaviors in this rat phenotype. To examine whether other rat strains exhibit a similar response to cocaethylene, Sprague-Dawley rats were injected (IP) with cocaethylene and their behavior patterns monitored over a 30-minute testing period. Cocaethylene produced marked locomotor and exploratory behaviors in this strain, suggesting therefore that Long-Evans and Sprague-Dawley rats differ in their response to cocaethylene. To relate these behavioral differences to possible structural differences in the neuronal density of dopaminergic or serotonergic neurons, Long-Evans and Sprague-Dawley brains were evaluated for tyrosine hydroxylase and 5-HT immunocytochemistry. No gross morphological differences in neuronal architecture or density were found in the ventral tegmental area or dorsal raphe nucleus of the two rat phenotypes. These results indicate that two commonly used rat strains show a differential response to cocaethylene and the neurochemical basis for this behavioral difference may be related to synaptic 5-HT bioavailability. **Synapse 26:11–21, 1997.** © 1997 Wiley-Liss, Inc.

INTRODUCTION

The concurrent use of cocaine and ethanol is a common pattern of drug abuse in our society. The pharmacological effects of coingesting powerful psychotropic drugs on the cellular microenvironment are only beginning to be gauged in the clinical setting. For instance, the metabolic interaction of cocaine and ethanol yields the formation of an ethylated derivative of cocaine, cocaethylene, in human liver cells (Boyer and Petersen, 1992; Dean et al., 1991; Hearn et al., 1991; Jatlow et al., 1991). This active metabolite is assumed to enhance cocaine-related morbidity and mortality by inducing necrosis in hepatocytes through oxidative mechanisms (Boelsterli et al., 1993; Dean et al., 1992). In the brain parenchyma, cocaethylene alters the neuro-

transmission of monoaminergic molecules by prolonging the time course of chemical signals across the synapse. For instance, administration of cocaethylene to rats (Bradberry et al., 1993) and nonhuman primates (Iyer et al., 1995) increases extracellular concentrations of dopamine (DA), a molecule that plays an important role in cognition, affect, and movement. Although the predominant neurochemical impact of cocaethylene appears to be on the dopaminergic system, changes in serotonin (5-hydroxytryptamine [5-HT]) reuptake also occur after the administration of the

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metabolite (Bradberry, 1994; Nobiletta et al., 1994). Such synaptic changes in 5-HT, however, are significantly smaller than those observed for DA, especially in brain circuits (e.g., the striatum) implicated in motor activity and arousal (Bradberry et al., 1993). The prevailing view, therefore, is that cocaethylene produces a differential effect on dopaminergic and serotonergic neurons that have a major influence on the regulation of motor behavior. This distinct characteristic in the pharmacology of cocaethylene provides an opportunity to study the relative contribution(s) of DA and 5-HT molecules on cocaethylene-stimulated behaviors, particularly those associated with stereotypy and exploratory behavior. Indeed, interactions between DA and 5-HT pathways are significant because they may be involved in the parallel processing of motor and cognitive arousal in drug-dependent subjects (Chen and Reith, 1993; Iyer and Bradberry, 1996; Yadid et al., 1994).

The role of phenotypic sensitivity to drugs of abuse has received considerable attention as it may help unravel the complex mechanisms that control gene function and therefore drug-induced behavioral responses (Crabbe and Belknap, 1992; Goldman, 1995). In the case of cocaine and ethanol, rodent and human evidence supports the premise that phenotypic sensitivity to these drugs is due, in part, to the genotype of the subject. For example, inbred and selectively bred rat strains (e.g., Fischer and Lewis) have been used to characterize genotype-dependent differences in (i) sensitization (i.e., enhanced-behavioral response); (ii) tolerance (i.e., attenuated-behavioral response); and (iii) differences in DA and 5-HT systems that control the reinforcing effects of cocaine or ethanol (Camp et al., 1994; Kosten et al., 1994; Mocsary and Bradberry, 1996; Tolliver et al., 1994; Wong et al., 1993; Yoshimoto et al., 1992). These data suggest therefore that strain differences in the behavioral responses to drugs of abuse could be related to genotypic differences in brain concentrations of DA, 5-HT or both. It is conceivable then that specific rodent strains may also show differential sensitivity to cocaethylene and may also differ in their neurochemical response to the cocaine metabolite. To test this hypothesis, we examined the behavioral consequences of cocaethylene administration in two common outbred strains of rats: Long-Evans and Sprague-Dawley. It is noteworthy that no attempt has been made to compare these two strains directly, especially in regard to cocaethylene-stimulated behaviors. This is of importance because these two strains are frequently used to assess the behavioral and neurochemical manifestations of drug addiction. In addition, we investigated whether anatomical differences in mesolimbic DA and brainstem 5-HT neurons could be related to strain differences in the behavioral effects of cocaethylene. The dorsal raphe nucleus (DRN) within the brainstem contains most of the perikarya for 5-HT, whose ascend-

ing projections innervate several target structures, including the striatum (caudate putamen and nucleus accumbens).

MATERIALS AND METHODS

Animals

Both female and male Long-Evans and Sprague-Dawley rats were used in all the studies. It should be noted that we have not observed sex differences in the behavioral effects of cocaethylene (see below) and therefore scores from the behavioral rating scale were pooled into a single set of data from both genders. The rats were housed in same-sex groups of two and allowed ad libitum access to food and water in a vivarium with a temperature of 22°C and under a light regimen of 12:12 hours light:dark cycle (lights on at 0600 h). Rat body weights were (means \pm SEM) 263.4 \pm 10.8 g for males ($n = 35$) and 265.7 \pm 5.8 g for females ($n = 40$). Every attempt was made to compensate for extreme gender differences in weight in all behavioral and anatomical studies by matching rats of similar body weight. Behavioral testing procedures were performed between 1100 and 1300 h and were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals, and with approval from the University at Buffalo IACUC.

Drug administration

Cocaethylene and cocaine hydrochloride injections (Research Biochemicals International, Natick, MA) were made on the day of testing by dissolving the drugs in sterile saline. The two drugs were injected intraperitoneally (IP) at a molar concentration of 60 μ mol/kg; a moderate dose that corresponds to 20 mg/kg. Fluoxetine hydrochloride (generously provided by Eli Lilly Laboratories; Indianapolis, IN) was dissolved in distilled water and injected (IP) at a dose of 10 mg/kg. The fluoxetine dose was chosen because it alters the responsiveness of rat forebrain neurons to 5-HT (de Montigny and Aghajanian, 1978) and also because that dose upregulates 5-HT₂ receptor subtypes in limbic structures (Li et al., 1993).

Experimental design

Rats were assigned to different groups and injected with two drugs. The first injection was saline-vehicle (1 ml/kg) or fluoxetine. Five minutes later, a second injection, cocaine or cocaethylene, was administered. Behaviors of the rats were rated blindly 5 minutes before the first injection and every 5 minutes for 30 minutes after the second injection. The behavioral rating scale of Kalivas and colleagues (1988, extended from that of Ellinwood and Balster, 1974) was used because (i) it measures discrete behavioral changes after moderate doses of cocaine, and (ii) it provides an excellent estimate of behavioral activity in rats ranging from explor-

atory behavior to stereotypy. At every observation time, a single score was given on a scale from 1 to 10:

- 1 = asleep or still
- 2 = inactive or in-place activity
- 3 = locomotion (all four feet moving within a 10-second period), rearing or sniffing (>3 seconds duration)
- 4 = any combination of two of locomotion, rearing, or sniffing
- 5 = continuous sniffing for 10 seconds without locomotion or rearing
- 6 = continuous sniffing for 10 seconds with locomotion or rearing
- 7 = patterned sniffing for 5 seconds
- 8 = patterned sniffing for 10 seconds
- 9 = continuous gnawing
- 10 = bizarre disknetic movements or seizures

It should be noted that we never observed scores greater than 6 in the present studies. A subset of the behaviorally studied rats ($n = 4$ per group) were anesthetized and perfused intracardially 30 minutes after the last drug injection.

Immunocytochemistry

The immunocytochemical procedures have been described in detail previously and are only briefly summarized here (Torres and Rivier, 1993). Perfused rat brains were removed from the calvaria and stored in 20% sucrose in 0.01 M sodium phosphate buffer for 3 days. Coronal sections (40–50 μm), cut frozen on a microtome, were collected from both Long-Evans and Sprague-Dawley brains and processed for tyrosine hydroxylase (TH; the rate-limiting enzyme for the biosynthesis of DA) and 5-HT immunocytochemistry. A mouse monoclonal antibody generated against TH in PC12 rat cells recognizing an epitope in the midportion of the TH molecule was obtained from INCStar (Stillwater, MN) and diluted 1:1000 in 4% normal serum, Triton X-100 and bovine serum albumin. The primary antibody has proven to be specific to rodent TH with no cross-reactivity with other catecholaminergic enzymes. Free-floating brain sections were incubated with the antiserum for 72 hours and reacted for the cytoplasmic product with diaminobenzidine tetrahydrochloride (DAB) and 1% H_2O_2 . For the immunocytochemical localization of brain 5-HT, we employed a rabbit polyclonal antibody obtained from Arnel (New York, NY) and diluted 1:500. No cross-reactivity of the primary antibody, as demonstrated by immunoabsorption, has been detected with structurally related indoleamines in brain neurons. Visualization of 5-HT perikarya and neuronal processes was accomplished in a manner similar to that of TH in terms of time of incubation and technical procedures. The brain sections were then washed with KPBS and mounted onto gelatin-chrome

alum-coated slides, dehydrated in graded alcohols, and coverslipped with DPX. Some brain sections were counterstained with neutral red to help identify relevant anatomical structures.

Data analysis

Behavioral activation in response to cocaethylene or cocaine injections was analyzed by a two-way repeated measures ANOVA for drug and strain effects. Differences between specific groups across observation times were subsequently determined by Newman-Keuls post hoc test comparisons with statistically significant differences defined as $P < 0.05$. Analyses were performed on an IBM-compatible computer with statistical analysis software (Jandel Scientific, SigmaStat). Data are presented as the means \pm SEM of behavioral scores. The number of rats ranged from 8 to 22 with approximately equal numbers of female and male rats per experimental condition. Analysis of TH and 5-HT immunoreactivity was performed on coronal brain sections corresponding to plates 37–52 of the rat atlas of Paxinos and Watson (1986). The plates ranged from bregma -4.80 mm to bregma -8.72 mm, depicting the ventral tegmental area (VTA) with landmarks of the medial lemniscus (anterior) and red nucleus parvocellular (posterior). For the DRN, anterior landmarks included the oculomotor nucleus and central gray, and posterior landmarks included the cerebellar lobules, nucleus trapezoid body, and pyramidal tract. Light-microscopy processing of gross TH and 5-HT immunoreactivity was assessed by counting the number of positive perikarya for both cellular phenotypes at a magnification of $\times 20$ through a 10-mm² grid-reticule positioned in the eyepiece of an Olympus microscope. Neuronal profile counts were performed twice by an individual "blind" to each of the experimental conditions. The data are expressed as the means \pm SEM of perikarya bearing the immunoreaction product in three unilateral sections per brain across series of experimental rat groups. No correction factor was employed in this study, and therefore there is the possibility that positive perikarya may have been counted twice (see below).

RESULTS

Effects of cocaine and cocaethylene on behavior: Long-Evans rats

Administration of saline-vehicle followed by a single cocaine injection to Long-Evans rats stimulated activity in all rats tested (Fig. 1). At a dose of 60 $\mu\text{mol/kg}$, cocaine increased motor activity, which was characterized by locomotion, head-bobbing, rearing, and sniffing (i.e., rating scale 4–6); patterns of behavior commonly ascribed to the psychostimulant actions of cocaine (Giros et al., 1996; Kalivas et al., 1993; Kuhar et al., 1991; Torres et al., 1994). The temporal onset of this motor activity was rapid (within 5 minutes of the cocaine injection) and continued past the 30 minutes of

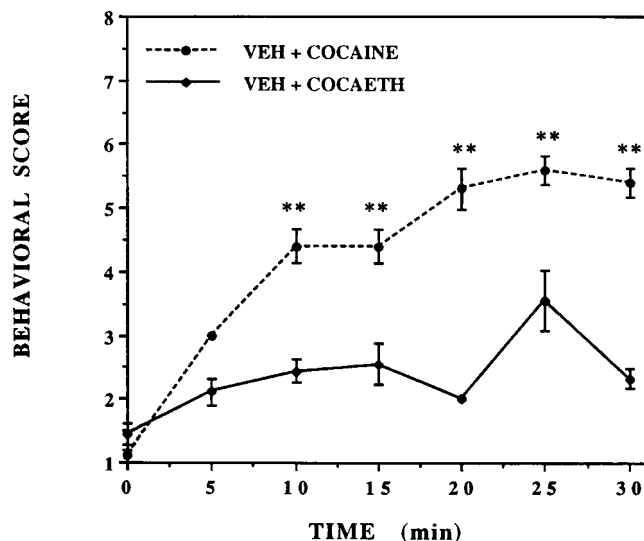


Fig. 1. Behavioral rating after acute coadministration of different drug combinations in Long-Evans rats. Vehicle (veh) injections (1 ml/kg) were followed 5 minutes later by cocaine or cocaethylene (cocaeth) IP injections (60 μ mol/kg). Values represent means \pm SEM. Note that the incidence of cocaethylene-stimulated locomotor activity and rearing behavior in this rat strain is significantly less than that produced by cocaine. ANOVA showed a marked effect of drug ($F_{17, 102} = 37.8$, $P < 0.01$), time ($F_{6, 102} = 37.8$, $P < 0.01$) and drug \times time interaction ($F_{6, 102} = 12.6$, $P < 0.01$). Significantly greater (** $P < 0.01$) than cocaethylene by Newman-Keuls post hoc test comparisons. Group n values: cocaine, $n = 10$; cocaethylene, $n = 9$.

behavioral observations. At this time, locomotion was usually replaced by stereotyped behaviors, including continuous sniffing and rearing in one place (data not shown).

By contrast, when the second injection was cocaethylene, Long-Evans rats exhibited little behavioral activation (Fig. 1). Indeed, 60 μ mol/kg cocaethylene failed to produce a significant effect on locomotor activity or stereotyped behaviors. Furthermore, when some modest locomotion or rearing was finally observed, it was late-developing (about 25 minutes postinjection) and lacked the robustness and intensity of that produced by cocaine (Horowitz et al., 1997).

It should be noted, however, that Long-Evans rats injected with cocaethylene exhibited more behavioral activity ($P < 0.05$) than did rats injected with the saline-vehicle solution (Fig. 3). Therefore, Long-Evans rats showed a differential behavioral response to cocaine and cocaethylene administration, with cocaine producing dramatically greater effects than cocaethylene on specific behavioral profiles typically associated with the function of neurons that regulate motivated behavior and emotion. It is noteworthy that under this behavioral paradigm we failed to detect gender-dependent differences ($P > 0.05$) in behavioral activity among cocaethylene-treated rats. For instance, female Long-Evans rats ($n = 5$) injected with the ethyl homologue of cocaine showed a behavioral rating of 2.6 ± 0.6 (means \pm SEM) at 15 minutes that was similar in

magnitude and intensity to that observed (2.5 ± 0.3) in male rats ($n = 4$).

Effects of fluoxetine pretreatment on cocaethylene-stimulated behaviors: Long-Evans rats

As mentioned above, cocaethylene is less potent than cocaine with respect to blocking 5-HT reuptake in the nucleus accumbens (Bradberry et al., 1993; Bradberry, 1994). Since this structure and its extended circuitries are crucial for the reinforcing and motivational effects of cocaine and ethanol (Koob, 1996), we reasoned that the lack of behavioral activity by cocaethylene in Long-Evans rats may reflect differences in the functional state of the serotonergic system. Therefore, we pretreated Long-Evans rats with fluoxetine, a selective 5-HT reuptake inhibitor. Remarkably, acute pretreatment with fluoxetine augmented cocaethylene-stimulated behaviors in this strain (Fig. 2). It is noteworthy that fluoxetine by itself did not produce spontaneous behavioral activity in this strain (Fig. 3) nor were there gender-dependent differences ($P > 0.05$) when motor behavior was assessed at each 5-minute interval. For instance, at 15 minutes postinjection, female ($n = 12$) and male rats ($n = 10$) showed a behavioral rating of 1.91 ± 0.1 and 1.5 ± 0.2 (means \pm SEM), respectively. That fluoxetine potentiated the effects of 60 μ mol/kg cocaethylene was demonstrated by the rapid development and intense expression of locomotor and stereotyped behaviors (i.e., rating scale 4–6) in this rat phenotype. In fact, a direct comparison of behaviors between fluoxetine/cocaethylene-treated and those of vehicle-saline/cocaine-treated rats (Fig. 2), revealed no marked difference(s) in the relative potency of the behavioral value of these two drug congeners ($P > 0.05$). This is consistent with the contention that cocaethylene is less potent than cocaine in blocking synaptic 5-HT reuptake (Bradberry, 1994), and the hypothesis that 5-HT neurotransmission is crucial for profound locomotor activity (Geyer, 1996).

Effects of cocaethylene on behavior: Comparison between Long-Evans and Sprague-Dawley rats

The results reported in the previous section suggest that cocaethylene has a distinctly different pharmacological profile from that of cocaine in terms of behavioral activation in Long-Evans rats. In order to investigate whether behavioral response differences exist in other rat strains, we administered cocaethylene to Sprague-Dawley rats and rated their behaviors using a 10-point scale as in previous experiments. At a dose of 60 μ mol/kg, cocaethylene produced behavioral activation in Sprague-Dawley rats, which was largely characterized by continuous sniffing with locomotion and rearing (Fig. 4). This motoric output lasted well past the assigned 30 minutes of behavioral observation and

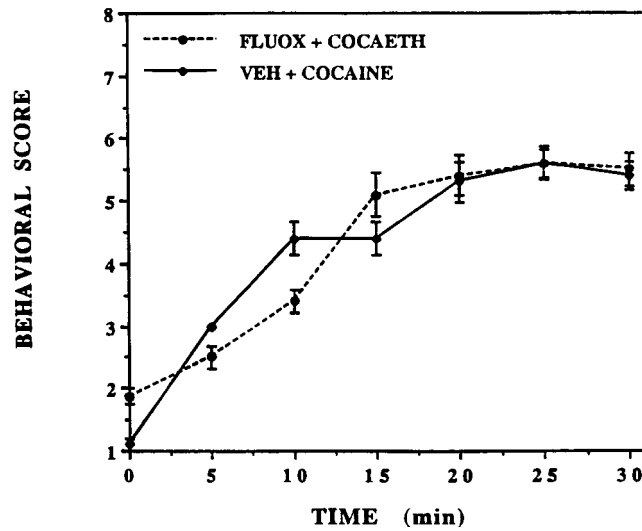
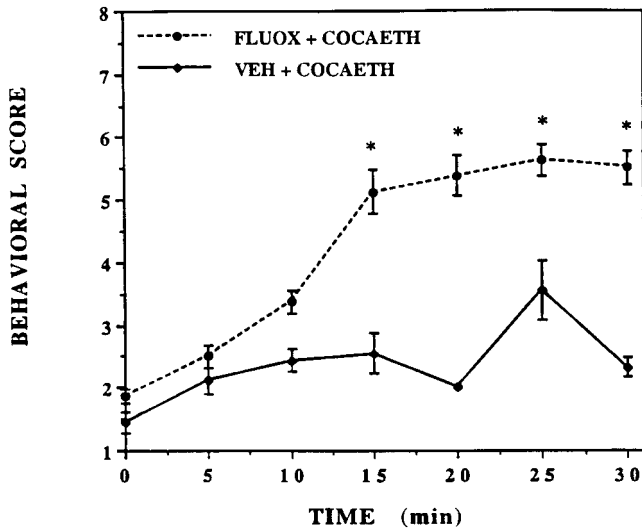


Fig. 2. **Top:** Effects of fluoxetine (fluox; 10 mg/kg) pretreatment on cocaethylene (cocaeth)-stimulated behaviors in Long-Evans rats. All drugs were given IP and the time interval between the two injections was 5 minutes. Note that fluoxetine significantly potentiated locomotor activity and rearing behavior in rats injected with cocaethylene (60 μ mol/kg) as compared to vehicle (veh)-pretreated animals (data obtained from Fig. 1). Values represent means \pm SEM. A two-way repeated measures ANOVA demonstrated a drug ($F_{16, 89} = 82, P < 0.01$), time ($F_{6, 89} = 37.3, P < 0.01$) and drug \times time interaction ($F_{6, 89} = 12.9, P < 0.01$). Significantly greater ($*P < 0.05$) than vehicle pretreatment by Newman-Keuls post hoc test comparisons. Group n values: fluoxetine, $n = 8$; vehicle, $n = 9$. **Bottom:** Effects of fluoxetine pretreatment on cocaethylene-stimulated behaviors as plotted against those produced by vehicle/cocaine treatment. Values represent means \pm SEM. Long-Evans rats exposed to fluoxetine before an acute cocaethylene injection exhibited a behavioral profile almost identical to that observed in rats receiving the parent compound, cocaine. It is noteworthy that the temporal effects of fluoxetine on cocaethylene-stimulated behaviors occurred rapidly (in a matter of minutes) after systemic administration. Overall these results suggest that 5-HT may be a significant factor in priming or facilitating behavioral activation, and provide key information about nondopaminergic mechanisms involved in stereotyped, motoric output behavior(s).

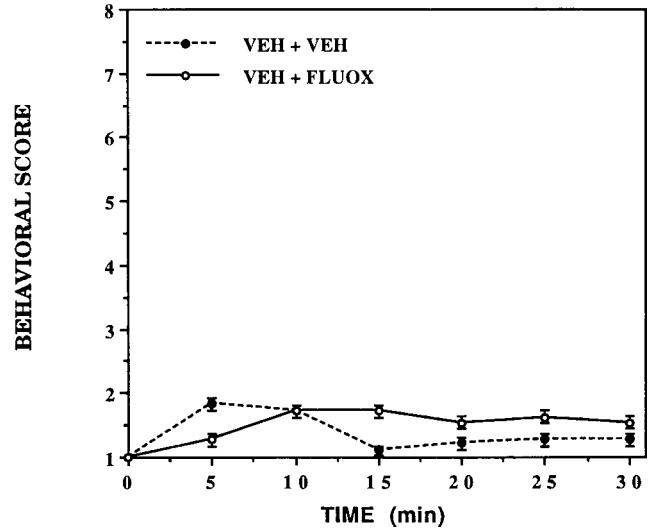


Fig. 3. Lack of behavioral activity in Long-Evans rats exposed to vehicle (veh) (1 ml/kg) or fluoxetine (fluox) (10 mg/kg) treatment over a 30-minute observation period. Values are expressed as means \pm SEM. Although fluoxetine failed to produce any locomotion, rearing or continuous sniffing as observed in the company of cocaethylene, the 5-HT reuptake inhibitor produced its own patterns of behavior as characterized mainly by body flattening. However, fluoxetine-treated rats were alert throughout the test session and could respond to a variety of sensory stimuli. Group n values: vehicle, $n = 18$; fluoxetine, $n = 22$.

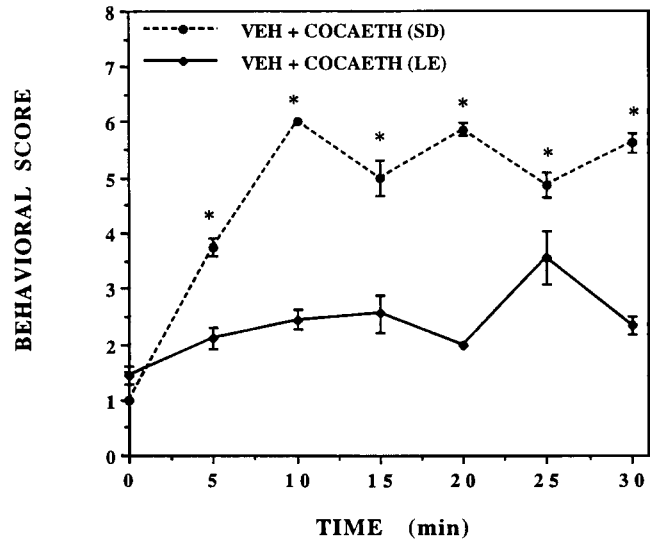


Fig. 4. Behavioral activity of Sprague-Dawley (SD; broken lines) and Long-Evans rats (LE; solid lines) (data obtained from Fig. 1) in response to a single cocaethylene (cocaeth) (60 μ mol/kg) injection. Note the differential sensitivity to the ethanol-dependent, ethylated metabolite of cocaine in the two strains. Values represent means \pm SEM. Profile analysis of motor output was performed between Sprague-Dawley and Long-Evans rats across drug and time variables. A two-way repeated measures ANOVA revealed statistical significance of drug ($F_{2, 84} = 162, P < 0.01$), time ($F_{6, 84} = 44.9, P < 0.01$) and drug \times time interaction ($F_{12, 84} = 11.7, P < 0.01$). Significantly greater ($*P < 0.05$) than Long-Evans rats by Newman-Keuls post hoc test comparisons. Group n values: Sprague-Dawley rats, $n = 8$; Long-Evans rats, $n = 9$.

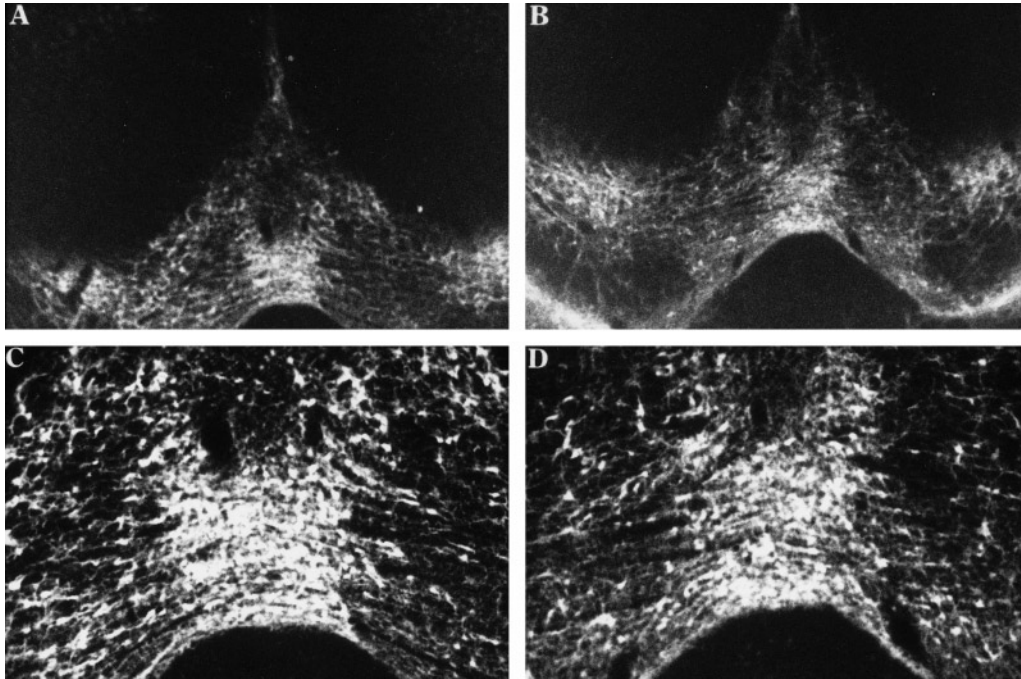


Fig. 5. Reverse-image photomicrographs showing immunoperoxidase labeling for TH-containing neurons in the ventral tegmental area (VTA) and portions of the substantia nigra of (A, C) Long-Evans and (B, D) Sprague-Dawley brains. No significant gross differences in neuronal structure or morphology are evident in the VTA of the two rat strains. The coronal sections are taken from plate 41 (bregma: -5.80 mm) in the rat atlas of Paxinos and Watson (1986). Figures A and B: $\times 4$; C and D: $\times 10$.

gradually phased into stereotyped behaviors (e.g., continuous sniffing and rearing in one place). Indeed, the behavioral profile of Sprague-Dawley rats injected with cocaethylene was similar to that of Long-Evans rats injected with cocaine (see Fig. 1). In a separate, corroborative study, Sprague-Dawley rats ($n = 6$) were injected intraperitoneally with cocaine (molar equivalent of $60 \mu\text{mol/kg}$ cocaethylene) and their behaviors rated as in previous experiments. As expected, cocaine induced a robust sequelae of locomotor activity and rearing behavior that was similar in intensity and magnitude to that exerted by cocaethylene (data not shown). The similarity in behavioral responses to cocaine and cocaethylene in the Sprague-Dawley line and the difference in response to the two drugs shown by Long-Evans rats is of great interest because these results provide relevant data regarding the role of genotype in mediating behavioral responses to an endogenous, ethylated cocaine metabolite, and further buttress the idea that brain 5-HT may be an intrinsic activity-dependent factor that may contribute to strain differences in the behavioral actions of cocaethylene.

Anatomical comparisons between Long-Evans and Sprague-Dawley brains: Mesolimbic dopaminergic and raphe nucleus serotonergic neurons

We have shown that Long-Evans and Sprague-Dawley strains differ in their behavioral sensitivity to

cocaethylene. It is possible that such a difference may be related to quantitative morphological patterns of neuronal density in circuits underlying drug tolerance and dependence. To test this hypothesis, gross morphological comparisons of the VTA, substantia nigra (SN), and DRN of Long-Evans and Sprague-Dawley brains were made by using immunocytochemistry. The general cytoarchitectonic features of the VTA have already been described (Bayer and Pickel, 1990; Hokfelt et al., 1977; Van Bockstaele et al., 1993 and references therein). However, for the sake of clarity, it may be helpful to review some of these characteristics. Light-microscopic observations of brain cross sections revealed numerous TH-labeled perikarya in the VTA (A10 dopaminergic cell group), including those of the parabrachial and paranigral subdivisions (Fig. 5). The majority of these are medium-sized neurons, whose axons bifurcate rostrally to terminate (among other structures) in the nucleus accumbens, caudate putamen, and prefrontal cortex (Hokfelt et al., 1977; Lindvall and Bjorklund, 1974; Zahn and Heimer, 1993). Based upon these features, we found no significant morphological or structural differences between Long-Evans and Sprague-Dawley VTA neurons. In addition, the staining intensity of dopaminergic perikarya and neurite projections (including varicosities) did not differ significantly between the two rat strains. Morphometric profile counts of TH-positive neurons in this midbrain region indeed revealed no strain differences ($P > 0.05$) in the

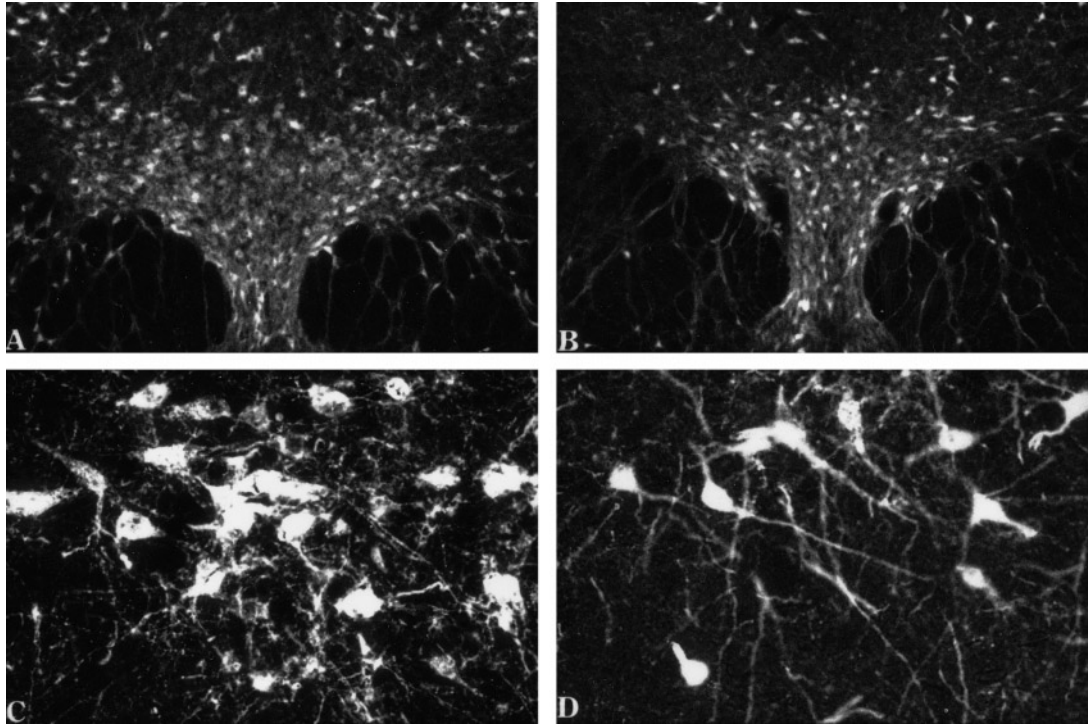


Fig. 6. Reverse-image photomicrographs showing 5-HT-labeled perikarya and processes in the dorsal raphe nucleus of Long-Evans (A, C) and Sprague-Dawley (B, D) brains. The structure and gross morphology of serotonergic neurons appear similar in both rat phenotypes. The aqueduct is at the top of Figures A and B. The coronal sections correspond to plate 48 (bregma: -7.64 mm) in the rat atlas of Paxinos and Watson (1986). Figures A and B: $\times 10$; C and D: $\times 10$.

relative number of these nerve cells (means \pm SEM; $122.0 \pm 20.0/10$ mm² for Long-Evans; $125.2 \pm 29.1/10$ mm² for Sprague-Dawley brains). Dopaminergic axonal distribution to the striatum showed a similar topographical organization in both strains (data not shown).

Peroxidase immunoreactivity for 5-HT was detected in the DRN, particularly along the midline, in both rat strains (Fig. 6). Serotonergic perikarya from this nucleus provide the majority of axonal terminals in the medial prefrontal cortex and nucleus accumbens (Bjorklund et al., 1971; Li et al., 1989; Van der Kooy, 1979). The topographical distribution of 5-HT-positive perikarya was similar to that found in previous studies: a heavy cellular concentration was detected just below the aqueduct and central gray area. No apparent differences in neuronal profile counts, morphology, or structure for this cell type were found between Long-Evans (means \pm SEM; $58.4 \pm 13.2/10$ mm²) and Sprague-Dawley ($51.8 \pm 19.5/10$ mm²) brains. In terms of receptive fields, dense 5-HT varicose (beaded) fibers were seen throughout the core and shell subdivisions of the nucleus accumbens and adjacent septal complex (Fig. 7). The density of these neuronal projections did not appear to differ greatly between the two strains.

DISCUSSION

In the present studies, the effects of cocaethylene on behavioral activity were evaluated in two outbred rat

strains. We found that (i) cocaethylene had a distinctly different pharmacological effect from that of cocaine on motor activity in Long-Evans rats; (ii) cocaethylene-stimulated behaviors in Long-Evans rats were augmented by fluoxetine pretreatment; (iii) cocaethylene treatment in Sprague-Dawley rats resulted in behavioral activation similar to that produced by cocaine in Long-Evans cohorts; and (iv) strain differences in the behavioral responses to cocaethylene did not appear to be related to gross neuronal density in dopaminergic VTA or serotonergic DRN. The results suggest, however, that synaptic 5-HT bioavailability may contribute to strain differences in the behavioral sensitivity to cocaethylene.

Cocaethylene produced expected behavioral activation, including locomotor and investigatory responses, in Sprague-Dawley rats. This is consistent with previous studies showing psychomotor stimulation in rodents after systemic administration of the cocaine analogue (Katz et al., 1992; Prinssen et al., 1996; Schechter, 1994). That cocaethylene has a powerful impact on neural systems associated with sensory arousal is also confirmed in placebo-controlled, double-blind human studies in which administration of the metabolite resulted in episodes of intense euphoria (McCance et al., 1995). These findings agree with the idea that psychostimulants produce a series of behavioral changes that can be readily interpreted in terms of

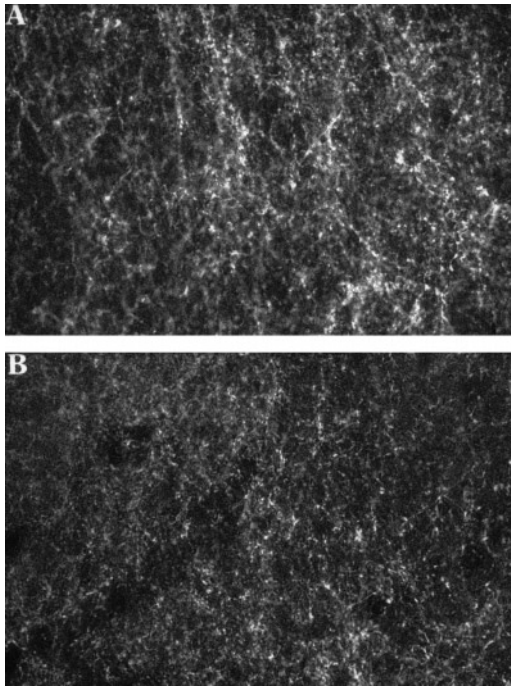


Fig. 7. Reverse-image photomicrographs demonstrating 5-HT immunoperoxidase-labeled fibers in the shell of the nucleus accumbens of (A) Long-Evans and (B) Sprague-Dawley rat brains. Morphologically distinct types of fibers (e.g., thin and thick with greater varicosity) are seen in both coronal sections—plate 15 in Paxinos and Watson (1986)—(bregma: 0.70 mm). The nucleus accumbens is at the right of Figures A and B. Photomicrograph of Figure B includes a greater area of the core subregion of the nucleus accumbens than in Figure A. See Van Bockstaele and Pickel (1993) for the differential density of serotonergic processes in this telencephalic region. $\times 40$.

positive reinforcement (Koob, 1996). Indeed, changes in motor activity and arousal have long been considered the initial phase of the development of substance dependence or addiction. Therefore, it is tempting to speculate that cocaethylene action(s) on sensory and cognitive arousal may be an additional element mediating the maintenance of a polydrug-dependent state. In support of this contention are the facts that cocaethylene (i) is equipotent to cocaine in inhibiting the reuptake of DA (Bradberry et al., 1993; Hearn et al., 1991; Iyer et al., 1995); (ii) stimulates the secretion of stress hormones, such as glucocorticoids, that could potentiate a positive affective state of drug use behavior (Torres et al., 1996); and (iii) could produce adaptations within brain circuits involved in the motivational aspects of drug craving and protracted abstinence (Torres and Horowitz, 1996).

Although we found that cocaethylene produced behavioral activation in rats, the effects of this activation appeared to depend on two factors: rat strain and synaptic 5-HT bioavailability. In our studies, strain-related differences in the effects of cocaethylene were found. A moderate cocaethylene dose produced relatively little behavioral activity in Long-Evans rats, but

an equivalent dose elicited profound and dynamic locomotor activation in Sprague-Dawley rats. These findings suggest that strain differences in sensitivity to the behavioral effects of cocaethylene may be dependent on the ubiquity of genetic influences. Although the precise mechanisms of how genes influence specific responses to cocaine or ethanol are yet to be determined, human and nonhuman studies have made it clear that there are individual, inherent differences in sensitivity to drug abuse and dependence (Crabbe and Belknap, 1992; Elmer et al., 1996; Morse et al., 1995). Indeed, the rapid development of biological and behavioral techniques has greatly facilitated the phenotypic screening of genetically defined populations of rats that are extremely sensitive or insensitive to drugs of abuse. For instance, Lewis (LEW) and Fischer-344 (F344) rats show different behavioral and biochemical responses to cocaine and ethanol. By and large, the LEW rat phenotype exhibits a greater locomotor response and a greater conditioned place preference to systemic cocaine exposure than F344 rats (Camp et al., 1994; Kosten et al., 1994). In addition, LEW and F344 rats differ in their response to hormone secretion (Simar et al., 1996), DA release, TH content and cAMP-dependent protein kinase activity (Beitner-Johnson et al., 1991, 1993). These biochemical characteristics could reflect strain differences in susceptibility to drug abuse and dependence.

Several reasons may account for the behavioral differences of the two rat strains to cocaethylene observed in the present experiments. One may be related to pharmacogenetics. For example, it is conceivable that differences in the availability and distribution of cocaethylene to target brain structures, binding to plasma proteins, metabolic clearance by plasma esterases, or a combination of the above, might account for the discrepant findings. In this context, cocaethylene appears to have a greater metabolic stability than cocaine in baboon brain, thereby contributing to a slower clearance of the metabolite from neurons (Fowler et al., 1992). Perhaps, Long-Evans rats are less sensitive to the behavioral effects of cocaethylene because they possess a metabolic mechanism that diminishes the kinetic activity and distribution of the psychoactive metabolite within neural correlates of motor behavior. Such an explanation could be explored, but would require studies employing an intravenous route of administration to assess whether strain differences to cocaethylene are dependent on metabolic efficiency. A second explanation for the strain differences may be related to synaptic 5-HT actions in the brain parenchyma. Indeed, our results strongly suggest that 5-HT bioavailability is crucial for cocaethylene-stimulated behaviors in Long-Evans rats. Therefore, the functional state of the serotonergic system in brains of Long-Evans rats and the relative lack of impact of cocaethylene on this system may underlie the differences ob-

tained in these studies. For instance, it is conceivable that structural differences in 5-HT release, reuptake transporter domains, membrane receptor density, or signal transduction pathways that regulate 5-HT gene expression could produce an altered behavioral phenotype. Studies addressing these issues are presently in progress.

The fact that fluoxetine intensified the effects of cocaethylene on behavioral activity in Long-Evans rats is worthy of further comment. Fluoxetine is a selective 5-HT reuptake inhibitor that, when administered to mammals, results in the rapid efflux of the monoamine in several subcortical brain structures (Wong et al., 1995). This efflux, which depends on neuronal firing, points to the possibility that cocaethylene (and other psychostimulants) relies on the synaptic presence of both DA and 5-HT to induce locomotor activity and stereotyped behavior. Although most studies have focused on dopaminergic mechanisms underlying the behavioral effects of cocaine and cocaethylene, very little emphasis has been placed on the role of 5-HT. The finding that fluoxetine unmasks the relative contribution of serotonergic systems in promoting and sustaining motoric output after cocaethylene exposure provides a glimpse into some of the neural mechanisms through which drugs of abuse produce locomotor hyperactivity. The importance of 5-HT in mediating the motoric and euphorogenic effects of cocaine and other psychostimulant drugs in rats and humans (Aronson et al., 1995; Broderick, 1993; Carey and Damianopoulos, 1994) indicates that mesolimbic DA function could be directly modulated by the actions of the serotonergic system. Changes in 5-HT bioavailability in drug-sensitive brain structures therefore may define the relative sensitivity of rats or humans to the behavioral effects of a particular drug.

The fact that we have two rat strains exhibiting different behaviors and apparently different neurochemical profiles in response to cocaethylene provided the opportunity to investigate whether behavioral differences in Long-Evans and Sprague-Dawley rats translate into differences in the structural density of dopaminergic or serotonergic neurons. The neuroanatomical results indicate that there was not a significant inter-strain difference in the areal density of these neurons in the VTA or DRN. The possibility exists, however, that the number of neuronal profiles may have been overestimated or underestimated (in either strain) because no correction factor was used. This may not be a serious methodological consideration, as the size of DRN neurons is reported to range from 10 to 30 μm (Van Bockstaele et al., 1993), and our rat brain sections were cut at a thickness of 40–50 μm . This may have diminished the possibility of counting the serotonergic neurons twice in selected tissue samples. Nevertheless, we are currently addressing this issue by using the disector principle, which provides an unbiased numerical

estimate of neuronal particles (West, 1993), and by using an image analysis system calibrated for neuronal size. Taking this potential error and other quantitative limitations of immunocytochemistry into account, the present results suggest that the differences in behavioral response to cocaethylene of Long-Evans and Sprague-Dawley rats may not be based in the absolute number of midbrain dopaminergic or brainstem serotonergic neurons. In addition, we detected no strain differences in TH and 5-HT immunoreactivity in terminal receptive fields of the caudate putamen, nucleus accumbens, or prefrontal cortex. A recent study showed that LEW rat brains exhibited a lower density of TH-positive neurons in the VTA than did F344 brains (Harris and Nestler, 1996), suggesting that the mesolimbic dopaminergic system in F344 rats may contribute, at least in part, to the differences in behavioral response exhibited by these two rat strains. Our studies with Long-Evans (which resemble F344 rats in terms of a hyporesponsive state to psychostimulants) and Sprague-Dawley rats, however, did not yield similar anatomical differences in dopaminergic neurons. The reason(s) for the different results include (i) the rat strains used in the two studies; and (ii) the fact that cocaethylene, rather than cocaine, is the drug that produces the differential behavioral effect in Long-Evans rats. Future studies will have to elucidate these strain differences so that biological variables that promote cocaine and ethanol abuse can be identified.

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