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Isoflurane Anesthesia Interferes with the Expression of Cocaine-Induced Sensitization in Female Rats

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Abstract

Repeated cocaine administration results in a progressive sensitization of behavior which typically occurs more readily in female rats than in males. Our recent studies of rats undergoing surgical procedures revealed that following anesthesia, females sensitized less than males receiving identical repeated cocaine injections. Since isoflurane acts primarily by increasing the effects of the inhibitory neurotransmitter γ -amino butyric acid (GABA) and reducing the effects of the excitatory amino acid glutamate, these amino acids may play more prominent roles in sensitization to cocaine in females than previously understood. In order to examine the effects of isoflurane on cocaine sensitization, we administered cocaine (15mg/kg i.p) or saline to adult male and female Sprague-Dawley rats for 9 days; on day 10, half of the rats were subjected to isoflurane anesthesia and the other half did not receive anesthesia. On day 11, rats were given their last dose of either cocaine or saline. We recorded behaviors for 1 hour on days 1, 9 and 11. Locomotor activity and stereotyped behaviors were quantified using photo beam monitors and the scoring of video tapes respectively. Results indicated that a single exposure to isoflurane significantly dampens the stereotypic behavior associated with repeated cocaine administration in females but not in males. They further suggest that either GABA or glutamate play more prominent roles in cocaine-sensitization behavior in females than in males.

Keywords

Sex differences; isoflurane; cocaine; sensitization

Introduction

The primary molecular targets of isoflurane and other volatile anesthetics are the GABA_A, glycine and kainate receptors with lesser effects (inhibition) on NMDA and nicotinic ACh receptors [26]. Isolated rat cortical neurons exposed to isoflurane increase GABA release and decrease glutamate release, resulting in increased inhibition and decreased excitation overall [24]. Specifically, in rat striatum, GABA has been shown to exert inhibitory control over spontaneous dopamine (DA) release and NMDA-evoked DA release [14]. Using

individual transporter assays in porcine kidney lines, Shahani et al [24] were able to establish differential effects of isoflurane on the DA and norepinephrine (NE) transporter systems; isoflurane inhibited uptake of DA but did not inhibit uptake of NE. Isoflurane also stimulates glycine release, inhibits nicotinic acetylcholine receptors and has a mild stimulatory effect at the 5HT₃ site [26].

It is clear that isoflurane has multiple, significant effects on several neurotransmitter systems. This raises the question of whether isoflurane is the best choice for an anesthetic to be used in experiments focused on the effects of psychostimulants on GABA/glutamate systems. While the majority of general anesthetics (e.g. barbiturates, propofol) have similar effects on GABA and glutamate, some, such as halothane, have more selective effects on specific GABA receptor complexes. It would be interesting to examine the neurotransmitter-specific effects of several volatile anesthetics on cocaine-induced sensitization in male and female rats. Since isoflurane and cocaine exert opposing effects primarily on the GABA_A system, our data would support a prominent role of GABA (and perhaps glutamate) in cocaine-induced sensitization in rats.

In recent experiments in which rats were anesthetized with isoflurane for surgical procedures, we found that females showed diminished sensitization to repeated cocaine compared to males [6]. To verify this effect of isoflurane on cocaine-induced behavioral sensitization, we administered cocaine (15 mg/kg i.p) or saline to adult male and female rats for 9 days; on day 10, half of the rats were subjected to one session of isoflurane anesthesia and the other half served as “sham-anesthesia” controls. On day 11 rats were given their last dose of either cocaine or saline. We recorded the resulting behaviors for 1 hour on days 1, 9, and 11. By assessing the quantity of each behavior we observed, we were able to show the progression of sensitized behaviors with repeated cocaine injections. We were also able to demonstrate that a single dose of isoflurane anesthesia is capable of attenuating the most extreme forms of cocaine-sensitized behavior in females 24 hours later.

Materials and Methods

Adult male and female Sprague-Dawley rats (n=86) (from Charles-River, Wilmington, MA) were housed in a temperature and humidity controlled vivarium with a 12 hour light cycle (lights on at 7AM). Between 11–14 hours, rats were administered cocaine at 15mg/kg/day i.p (weight of the salt) (gift of NIDA through Research Triangle Institute, Research Triangle Park, NC) or saline i.p for 9 days. On day 10, we subjected half of the rats to 45 min isoflurane anesthesia (the time required to insert catheters for a functional imaging (2 deoxyglucose) study). Anesthesia was induced with 5% isoflurane in 100% O₂ and maintained with 2–3% isoflurane in 100% O₂ for the following 45 minutes. The control (sham anesthesia) rats were not given anesthesia but were brought to the laboratory and handled similarly to the anesthetized rats. Cocaine or saline was administered on day 10 four hours following the anesthesia or sham anesthesia. On day 11, rats were given their last injection of either cocaine or saline.

Behaviors were recorded for 1 hour on days 1, 9 and 11. On these days, immediately after injection, rats were placed in a Plexiglas box (42 × 42 × 30 cm with no bedding) equipped

with a Versamax activity monitor (VMRXYZ16; Accuscan Instruments, Columbus, OH) to record locomotor activity. The rats were not given time to habituate to the Plexiglas box; our previous experiments have shown that administration of cocaine at 15 mg/kg (the level used in this study) masks habituation to the environment by increasing activity. In other words, locomotion does not decrease when cocaine-treated animals are placed in a novel environment until the drug wears off. Rats were videotaped using a VHS RCA camcorder (CC4352) for later analysis of 7 different categories of behavior using the computer program Noldus Observer 5.0 (Noldus, Wageningen, The Netherlands). Behavioral categories measured were defined as follows:

1. Quiet: sitting or sleeping without any observable movements
2. Sniffing: movement of the whiskers, but without stereotyped head movements
3. Grooming: repetitive movements of the frontal paws around the head or other parts of the body
4. Rearing: lifting both front paws from the floor for more than 1 second
5. Low intensity stereotypy: repetitive movements of the head side to side usually combined with locomotion
6. Medium intensity stereotypy: faster movements of the head, with or without locomotion
7. High intensity stereotypy: large, rapid circular movements of the head occurring when the animal is not in locomotion

Behaviors were assessed in a time-sampling procedure, with 6-1 min segments throughout the 60 min of observation being scored. The time spent in each behavior during the 6 min of observation (min 9, 19, 29, 39, 49, 59) was totaled for each rat except Rearing which was recorded as number of incidences during the 6 minute intervals. Raters were blind to treatment condition and maintained within and between rater reliability of 95% or better.

All females were subjected to vaginal lavage to determine the phase of the estrous cycle on days 1, 9 and 11 immediately following the behavioral test. All experiments were conducted in accordance with the guidelines established by Institutional Committee for Care and Use of Laboratory Animals and the AAALAS.

A three-way analysis of variance (ANOVA) with factors as sex, anesthesia, and drug (cocaine/saline) with repeated measures for day was conducted for locomotion. Another two-way ANOVA, with sex and anesthesia as factors, was used to assess stereotypy; since stereotyped behaviors are not observed in saline-treated rats, only data from cocaine-treated rats is included. Pre-planned paired T-tests were used to compare time spent in stereotypic behaviors between days 1 and 11. P values at or below .05 were considered significant.

Results

Locomotion

A between-subjects analysis of variance showed a main effect of sex [$F(1, 82) = 6.69, p = .011$] and a main effect of drug (cocaine) [$F(1, 82) = 113.9, p < .001$] (Fig 1), with a sex by drug interaction showing a trend toward significance [$F(1, 82) = 3.55, p = .062$]. Females showed greater locomotion than males on days 1, 9 and 11, thus revealing a more robust behavioral response to cocaine. There were, however, no significant differences in locomotion across days and no interaction of day with sex or isoflurane treatment.

Low Stereotypy

Insufficient stereotypy was observed in saline-treated rat to conduct an analysis. A two-way ANOVA with factors as sex and anesthesia resulted in a significant interaction [$F(1, 31) = 9.03, p = .005$], and a main effect of day [$F(2, 62), G-G = .04$]. In other words, for all groups, low stereotypy decreased across time except in the isoflurane-treated females (fig 2A & B). The time spent in low stereotypy on day 1 vs. day 11 was assessed using T-tests (fig 2). Paired T-tests revealed that non-anesthetized females spent significantly less time in low stereotypy on day 11 than day 1 ($p = .001$) (fig 2A). No significant differences in time spent in low stereotypy between days 1 and 11 were seen for anesthetized males (fig 2B), non-anesthetized males (fig 2A), or for anesthetized females (fig 2B).

Medium Stereotypy

A two-way ANOVA with factors as sex and anesthesia revealed a main effect of sex [$F(1, 33) = 37.98, p < .001$] and a main effect of anesthesia [$F(1, 33) = 9.96, p = .003$]. No significant differences were seen across days. Paired T-tests revealed differences between time spent in medium stereotypy on day 1 vs. day 11; in non-anesthetized males ($p = .034$) and females ($p = .003$) (fig 2C), that is, time spent in medium stereotypy was greater on day 11 than on day 1, thus confirming that repeated cocaine induces a greater expression of higher levels of stereotypy over time in both sexes. Sex differences in the expression of medium stereotypy were seen in the anesthetized groups. Males spent significantly more time in medium stereotypy on day 11 than on day 1 ($p = .027$) (fig 2D), suggesting no effect of isoflurane on behavior. This pattern was not seen in females; medium stereotypy actually decreased between days 9 and 11 and was not significantly different between days 1 and 11.

Body weights and estrous cycles

Analysis of the estrous cycles of all females showed no significant differences between any of the rats (data not shown). There was no significant difference in body weight gain between the saline-treated and cocaine-treated females (data not shown). Males in all treatment groups weighed more than females. There was a significant interaction of day and treatment in the male groups ($p = .02$); males that received cocaine gained less weight over the course of the experiment than saline-treated males (data not shown).

Discussion

While the progression to habitual drug use in male rodents and primates has been studied over the last 20 years, the steps to psychostimulant abuse in females have been less well characterized. Females sensitize more quickly and require lower doses of psychostimulants than males (e.g. [22]). The role of estrogen in cocaine responsiveness was largely established by Hu and Becker [12] who demonstrated that estrogen and not progesterone was effective in restoring sensitized behavior in ovariectomized female rats. Estrogen administration did not have this effect in males. Becker has proposed that estrogens act rapidly on intrinsic striatal GABAergic neurons, decrease the firing rate of collaterals (which inhibit Dopamine (DA) neurons) and thus increase DA release in striatum [1]. Estrogen also enhances DA release by down-regulating pre-synaptic DA D2 receptor function [1]. Estradiol also has robust effects on the glutamate system. Hypothalamic and amygdaloid neurons expressing estrogen receptor α mRNA also express NMDA-2D subunit receptor mRNA, which supports an interaction between glutamate and estrogen in these regions [14]. Estrogen enhances LTP in hippocampal slices and an LTP-like phenomena in VTA has emerged as a sequelae to repeated psychostimulant exposure [8,16]. Therefore, estrogenic enhancement of multiple glutamatergic circuits and of dopaminergic transmission could certainly contribute to the enhanced responses to cocaine in females.

Galanopoulou, Moshe and others have emphasized the sexually dimorphic role of GABA in modulating development of dopaminergic cells within the substantia nigra pars reticulata (SNR) and GABA's role in motor disorders and seizures [9,21]. Females have greater concentrations of GABA and GABA_A receptors in the SNR than males [3]. Estradiol increases ³H muscimol (the prototypical GABA_A receptor agonist) binding in centromedial amygdala, hippocampus and frontal cortex [2,18] suggesting that females may have greater GABA_A receptor densities in these brain areas as well. Estradiol has been reported to have robust facilitory effects on the GABAergic system [11]. Therefore, estrogen effects on GABAergic activity and cocaine responsivity may involve multiple, diverse actions.

In the current experiments, we determined that over the course of the repeated cocaine administration, male and female rats exhibited changes in their behavior marked by progressive stereotypy. In other words, low intensity stereotypy decreased and more severe behaviors such as head shaking and rearing increased (reflected by more time spent in medium intense stereotypy). This intensification of stereotypic cocaine responses, with or without alterations in locomotor activity, is classically defined as sensitization. Behavioral sensitization is associated with a series of alterations in the cortex, striatum (dorsal and ventral) and VTA which result in increased dopaminergic and glutamatergic excitatory amino acid transmission and decreased GABAergic inhibitory transmission in ACC [see [20] for review]. In the VTA and ACC, glutamate transmission is augmented following repeated cocaine administration [13]; consequently, both competitive and non-competitive NMDA (glutamate) receptor antagonists prevent the development of sensitization to cocaine (see Wolf review) [25]. On the other hand, positive allosteric modulators of the GABA_A receptor have been shown to prevent the development and expression of cocaine-induced sensitization [7, 5], inhibit the effects of cocaine on locomotion and on extracellular dopamine in ACC-shell [10], and dampen conditioned place preference for cocaine [17].

Accordingly, blockade of GABA metabolism has been shown to prevent cocaine self-administration [15]. Behavioral sensitization to repeated cocaine administration thus involves reciprocal changes in glutamatergic excitatory activity and GABAergic inhibitory activity overall.

Sensitization is comprised of two independent mechanisms: first, context-dependent sensitization in which environmental stimuli (the environment where the drug is injected) become conditioned stimuli, and secondly, neural adaptations that occur as a direct result of the repeated cocaine injections. Increased glutamate transmission in ACC has been shown to be related to the context-dependent aspects of cocaine-induced sensitization while DA changes in ACC are related to the neurochemical effects of cocaine [19]. Since amino acid transmission is central to context-dependent changes in ACC, it is tempting to speculate that isoflurane's effects are due to dampening of the contextual component of the sensitization response. While the conditions of the current experiment are not optimal for the development of the context specificity of the behavioral response (8 injections occurred in the home cage and only 3 in the test cage), context may nevertheless play a role in our results. It is unlikely that a simple amnesic effect of isoflurane produced a difference in recall of the context in which the drug was administered since manipulations outside of the drug associated context have little effect on retrieval and reconsolidation of conditioned stimuli [23].

This experiment was designed to evaluate the effects of anesthesia on cocaine-induced behavioral sensitization. However, most studies of sensitization utilize daily psychostimulant administration and then challenge the subjects one or more weeks following the termination of administration. While this would be a useful experiment, we would predict that the changes induced by isoflurane would be the same as in the current experiment. While the behavior on the final day of this experiment was assessed within minutes after the last dose of cocaine, there was a 24 hr "withdrawal" period following the exposure to isoflurane. Since isoflurane has been shown to alter GABA receptor mRNA, the withdrawal period may permit changes in multiple GABAergic targets. On the other hand, isoflurane has been found to alter gene expression in the hippocampus and amygdala for at least 2 days in the rat [4]. Since many brain regions may also manifest altered gene expression following isoflurane anesthesia, widespread neurochemical alterations within multiple brain regions, particularly the mesolimbic and nigrostriatal circuits, may contribute to the findings of the present study.

Conclusion

Isoflurane anesthesia dampens cocaine-induced behavioral sensitization in female rats. This study cannot differentiate whether this is due to the sex differences in response to isoflurane or whether the role of amino acid neurotransmitters (especially the GABA_A receptor complex) in cocaine-induced sensitization is more prominent in females. However, our results highlight the role of amino acid neurotransmitters in cocaine-induced sensitization and support an examination of these systems as the basis for the sex differences in sensitization and the neurochemistry of reward.

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ABBREVIATIONS

ACC	nucleus accumbens
ACh	acetylcholine
ANOVA	analysis of variance
DA	dopamine
GABA	Gamma amino butyric acid
LTP	long term potentiation
NMDA	N-Methyl-d-aspartate
SNR	substantia nigra pars reticulata
VTa	ventral tegmental area

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Figure 1.

Locomotion in adult male and female rats administered cocaine or saline for 11 days and undergoing behavioral recording on days 1, 9, and 11. Anesthesia (↑) was administered on day 10 to half of the rats. Females showed greater locomotion than males on days 1, 9 and 11. Males treated with isoflurane showed similar locomotion on days 9 and 11; females treated with isoflurane exhibited reduced locomotion compared to non-anesthetized females, although this trend was not statistically significant.

Stereotypy

Figure 2.

Stereotypy in adult male and female rats administered cocaine or saline for 11 days and undergoing behavioral recording on days 1, 9, and 11. Anesthesia (↑) was administered on day 10 to half of the rats. The time spent in low and medium stereotypy was assessed using paired T-tests. Non-anesthetized females spent significantly less time in low stereotypy on day 11 than day 1, whereas no significant differences between days 1 and 11 were seen for males in either condition or for anesthetized females. In non-anesthetized males and females, there was a significant increase in medium stereotypy from day 1 to day 11, confirming that repeated cocaine induces expression of higher levels of stereotypy over time

in both sexes. Anesthetized males spent significantly more time in medium stereotypy on day 11 than on day 1 showing no effect of isoflurane on behavior. This pattern was not seen anesthetized females, who exhibited less medium intense stereotypy on day 11 than on day 9.