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Molecular mechanisms mediating genetic sensitivity to cocaine-induced convulsions.

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Research report

Molecular serotonergic mechanisms appear to mediate genetic sensitivity to cocaine-induced convulsions

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Abstract

Cocaine-induced convulsions appear to be mediated by serotonin (5-HT) neurotransmission, acting primarily at 5-HT$_2$ receptors. However, this effect of cocaine is attenuated by cocaine binding at sigma and muscarinic M$_1$ and M$_2$ sites. This study examined whether the aforementioned neural sites mediate the nearly two-fold difference in sensitivity to cocaine-induced convulsions across C57BL/6J (6J) and C57BL/6ByJ (6ByJ) mice. Experiment 1 compared 5-HT transporter densities across several brain regions of 6J and 6ByJ mice and cocaine-induced convulsions following pretreatment with the 5-HT reuptake inhibitor fluoxetine. Experiment 2 compared 5-HT$_2$ receptor densities across these mice and cocaine-induced convulsions following pretreatment with the 5-HT$_2$ antagonist cinanserin. There were no differences in 5-HT transporter densities, however, fluoxetine produced a greater facilitation of cocaine-induced convulsions in 6ByJ relative to 6J mice, suggesting that sensitivity to convulsions is mediated postsynaptically. Indeed, 5-HT$_2$ density was higher in 6ByJ relative to 6J mice in the amygdaloid ridge, hypothalamus, and midbrain. In addition, cinanserin attenuated convulsions more potently in 6J relative to 6ByJ mice. There were no differences in the densities or affinities of 5-HT$_1$, muscarinic, or sigma receptors across these strains, suggesting that density of these latter sites does not mediate genetic sensitivity to cocaine-induced convulsions. Since 6ByJ mice are less sensitive to convulsions despite the fact that they have more 5-HT$_2$ receptors, we hypothesized that these mice may exhibit a weaker linkage of 5-HT$_2$ sites to their second-messenger system relative to 6J mice. However, in experiment 3 we demonstrated that 5-HT$_2$-receptor mediated phosphoinositide hydrolysis was higher in 6ByJ relative to 6J mice in the same regions also displaying higher 5-HT$_2$ densities. This study suggests that 5-HT$_2$ receptors mediate genetic sensitivity to cocaine-induced convulsions, further supporting the role of these sites in mediating this toxic effect of cocaine. © 2000 Elsevier Science B.V. All rights reserved.

1. Introduction

Cocaine is an addictive compound that also has toxic consequences, including convulsions and death. We have utilized multiple receptor site analysis to examine which cocaine-binding sites mediate the toxic effects of cocaine. This procedure assumes that a fundamental requirement for the identification of a pharmacologically relevant receptor is a significant correlation between the potency of drugs in producing a response and the affinity of these agents at a particular binding site. Using this procedure, it was determined that serotonin (5-HT) transporters appear to be the primary initial sites of action mediating convulsions produced by cocaine and related compounds [14]. Specifically, the ability of cocaine-like compounds to produce convulsions was highly correlated with the affinity of these drugs for the 5-HT transporter. In fact, drug potency for binding at the 5-HT transporter accounted for 78% of the variance in the potency of cocaine and related compounds for producing convulsions.

Subsequent pharmacological studies also support the

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role of 5-HT neurotransmission in mediating cocaine-induced convulsions. For example, cocaine-induced convulsions are facilitated by pretreatment with the serotonin reuptake inhibitors (SSRI) fluoxetine, paroxetine and citalopram [8,15]. Also, cocaine-induced convulsions are facilitated by coadministration of compounds that increase synaptic levels of 5-HT [18]. Collectively, these binding and pharmacology studies suggest that 5-HT neurotransmission plays an important role in mediating the convulsant effects of cocaine.

Cocaine-induced convulsions appear to be mediated via indirect activation of $5-HT_2$ receptors, since this effect of cocaine is attenuated by pretreatment with the $5-HT_2$ antagonists cinanserin, ketanserin, pirenpirone, mianserin, metergoline, MDL 11939, and methiothepin [10,15,18]. Further, it appears that the $5-HT_{2C}$ receptor subtype may mediate cocaine-induced convulsions, since this effect is facilitated by the preferential $5-HT_{2C}$ agonists 1-(3-triflurormethylphenyl)piperazine (TFMPP), m-chlorophenylpiperazine (mCPP), and 6-chloro-2-(1-piperazinyl)pyrazine (MK212) [9,10].

In an initial pharmacogenetic screen, we found that C57BL/6J (6J) and C57BL/6ByJ (6ByJ) mice differ in their sensitivity to cocaine-induced convulsions, with CD_{50} values being 61 and 100 mg/kg, respectively [9]. This nearly two-fold difference in the CD_{50} values for cocaine-induced convulsions is compelling in light of the fact that 6ByJ mice were derived from a population of previously highly inbred 6J mice that had been isolated on a long-term basis. Based on the close relationship between these strains, the only genetic differences between these mice would be presumed to be due to a rare genetic mutation or fixation of a small number of genes that might not have been fixed at the time of separation of the derivative colony. Therefore, it is likely that sensitivity differences with respect to cocaine-induced convulsions across 6J and 6ByJ mice are mediated by a small number of genes or possibly a single gene locus.

The close genetic relatedness between 6J and 6ByJ mice makes them a good animal model to study the neural mechanisms that mediate genetic sensitivity to cocaine-induced convulsions. Since 5-HT transporters appear to be the primary initial sites of action mediating cocaine-induced convulsions, we first hypothesized that genetic sensitivity to this toxic effect of cocaine may be related to differences between these strains in the density of 5-HT transporters. Therefore, experiment 1 compared 5-HT transporter densities across 6J and 6ByJ mice. The ability of the SSRI fluoxetine to facilitate cocaine-induced convulsions was also compared across these mice.

In addition, because pretreatment with agonist compounds that activate $5-HT_2$ receptors produces a more robust facilitation of cocaine-induced convulsions in 6ByJ relative to 6J mice [9], in experiment 2 we compared $5-HT_2$ receptor densities and affinities across 6J and 6ByJ mice. The ability of cinanserin to attenuate cocaine-induced convulsions was also compared across these mice.

Previous research in our laboratory suggests that cocaine binding to sigma or muscarinic cholinergic receptors plays a protective role in mediating cocaine-induced convulsions. Specifically, the ability of cocaine and related compounds to produce convulsions is inversely related to the affinity of these compounds at sigma or muscarinic receptors [14]. Subsequent pharmacological experiments support these findings, since antagonists at sigma or M1 receptors attenuate this toxic effect of cocaine [15,19]. This inverse relationship between cocaine binding at sigma or M1 receptors and convulsant potencies of cocaine-related drugs suggests that differences in the density and/or affinity of these attenuating sites could explain the differential sensitivity to cocaine-induced convulsions across 6J and 6ByJ mice. Therefore, experiment 2 also compared the density and affinity of sigma and M1 receptors across these mice. The density and affinity of $5-HT_1$ sites was also compared across these mice, in an attempt to rule out the role of the other major family of 5-HT receptor sites in mediating genetic sensitivity to cocaine-induced convulsions.

Because $5-HT_2$ receptors appear to mediate genetic sensitivity to cocaine-induced convulsions, differences in sensitivity to cocaine across these mice could also be related to differences in the functional linkage of $5-HT_2$ receptors to their second-messenger systems. Therefore, experiment 3 compared 5-HT-induced PI hydrolysis across 6J and 6ByJ mice in the same brain regions as used in experiment 2.

2. Materials and methods

2.1. Animals

Experimentally naive male 6J and 6ByJ mice (60–100 days old) were obtained from the Jackson Laboratories (Bar Harbor, Maine). Animals were housed in groups of same-sex littermates with ad libitum access to Purina chow and tap water. All mice were maintained in a temperature-controlled room (26°C) with a 12-h light/dark cycle (07:00–19:00 h lights on). Animals in this study were maintained in facilities monitored by an Institutional Animal Care and Use Committee (IACUC) and the studies were conducted in accordance with the *Guide for Care and Use of Laboratory Animals* provided by the NIH and monitored by the local IACUC.

2.2. Drugs

All tritiated ligands were obtained from New England Nuclear, Inc. (−) Cocaine HCl was obtained from the National Institute on Drug Abuse, cinanserin from Research Biomedicals, Inc., and fluoxetine HCl from Eli Lilly and Co. All compounds were injected intraperitoneally with drug doses expressed as mg/kg base administered in a volume of 10 ml/kg. Cocaine was solubilized in 0.9%

saline and cinanserin and fluoxetine were solubilized in 0.9% saline plus 1% polysorbate-80. The doses of fluoxetine and cinanserin were based on previous research in our laboratory examining the effects of these compounds on cocaine-induced convulsions in 6J mice [15]. In addition, the doses of cocaine were chosen based on previously obtained dose–response curves for cocaine-induced convulsions [9,15].

2.3. Data analyses

For the binding studies, B_{max} and K_d values were analyzed using analysis of variance (ANOVA) with Fisher's PLSD post-hoc tests ($P \leq 0.05$). Behavioral results were first analyzed using one-way ANOVA to examine overall dose-dependent effects. Where significant effects were observed, Dunnett post-hoc tests ($P \leq 0.05$) were used to compare the effects of single doses to control values. CD_{50} (mg/kg) values for convulsions were determined by linear regression analyses of the resultant dose–response curves. The accumulation of PI hydrolysis turnover was analyzed using ANOVA and subsequent Fisher's PLSD post-hoc tests ($P \leq 0.05$) were performed where significant effects were observed.

2.4. Receptor binding

The density and affinity of receptor sites that possibly mediate genetic sensitivity to cocaine-induced convulsions were assessed. Naive 6J or 6ByJ mice were sacrificed by rapid decapitation and their brains were removed and washed in cold saline. The particular brain regions were then dissected and frozen and stored at $-70°C$ until used in the assay procedures. Tissues were homogenized in 20 volumes of the assay buffer, then centrifuged at $30\,000 \times g$ for 10 min. The resulting pellet was washed, recentrifuged, and resuspended in buffer to yield the desired tissue concentration for addition to the assay.

Experiment 1 used eight final concentrations of [3H]paroxetine (3 pM–0.4 nM) to estimate the density and affinity of 5-HT transporters in the brainstem, frontal cortex, hippocampus, midbrain, and striatum of 6J and 6ByJ mice [4]. Nonspecific binding was estimated in the presence of 1 μM citalopram with final assay volumes of 4 ml. Homogenized tissue (1.5 mg original weight per tube) was incubated at room temperature in 4 ml of buffer containing 50 mM Tris, 120 mM NaCl, and 5 mM KCl. The 90-min incubation was conducted at pH 7.8 with a final ligand concentration of 0.2 nM.

Experiment 2 compared the density and affinity of 5-HT$_2$, 5-HT$_1$, sigma, and muscarinic (M$_1$) receptors across 6J and 6ByJ mice. Eight final concentrations of [3H]ketanserin (0.09–12 nM) were used to estimate 5-HT$_2$ receptor density and affinity in the amygdaloid ridge, brainstem, frontal cortex, hippocampus, hypothalamus, midbrain, and striatum of 6J and 6ByJ mice [20]. The density of 5-HT$_2$ sites were also examined in the

amygdaloid ridge and hypothalamus, since preliminary results revealed significant differences in 5-HT$_2$ densities of several other larger brain regions. The amygdaloid ridge was dissected out of the temporal lobe on the ventral surface of the brain. Nonspecific binding was defined by the addition of 0.5 μM cinanserin. Homogenized tissue (4 mg original weight per tube) was incubated at 37°C in buffer containing 50 mM Tris, 120 mM NaCl, 5 mM KCl, 10 mM MgCl$_2$, 2 mM CaCl$_2$, and 0.5 mM EDTA. Drugs were added to assay tubes before incubation at concentrations ranging from 1 nM to 10 mM. The 90-min incubation was conducted at pH 7.8 in a final assay volume of 2.5 ml.

Eight final concentrations [3H]LSD (lysergic acid diethylamide; 0.16–18 nM) were used to estimate 5-HT$_1$ receptor density and affinity in the brainstem, frontal cortex, hippocampus, midbrain, and striatum of 6J and 6ByJ mice. Nonspecific binding was defined by the addition of 1.0 μM serotonin. Homogenized tissue (4 mg original weight per tube) was incubated at 37°C in buffer containing 50 mM Tris, 120 mM NaCl, 5 mM KCl, 10 mM MgCl$_2$, 2 mM CaCl$_2$, and 0.5 mM EDTA. The 30-min incubation was conducted at pH 7.8 in a final assay volume of 2.5 ml.

Eight final concentrations [3H]haloperidol (0.15–20 nM) in the presence of 25 nM spiperone was used to identify sigma receptors in the brainstem, frontal cortex, hippocampus, midbrain, and striatum of 6J and 6ByJ mice. Nonspecific binding was defined by the addition of 100 μM ±SKF-10047 (n-allylnormetazocine). Homogenized tissue (8 mg original weight per tube) was incubated at 25°C in buffer containing 50 mM Tris and 10 mM MgSO$_4$. The 90-min incubation was conducted at pH 7.8 in a final assay volume of 4 ml.

Eight final concentrations of [3H]pirenzepine (0.1–15 nM) were used to identify muscarinic M$_1$ receptors in the brainstem, frontal cortex, hippocampus, midbrain, and striatum of 6J and 6ByJ mice. Nonspecific binding was defined by the addition of 1.0 μM atropine. Homogenized tissue (1 mg original weight per tube) was incubated at 25°C in a 10-mM sodium-phosphate buffer. The 60-min incubation was conducted at pH 7.8 in a final assay volume of 2 ml.

At the end of the incubation period for each experiment, all assay mixtures were filtered through Whatman GF/C filters presoaked with 0.05% polyethylimine, and washed with buffer. Filters were placed into plastic vials and scintillation fluid was added. Vials were shaken for 1 h and radioactivity was measured by liquid scintillation spectrometry. For each experiment, mean B_{max} and K_d values were estimated from at least four separate binding assays and these values were determined using the statistical application PRISM.

2.5. Behavioral observations

Experiment 1 examined the effects of fluoxetine on

convulsions produced by cocaine. Mice received saline or fluoxetine (3, 5.6, 10, 13.3 mg/kg; $n=$at least five per group) and were immediately placed into 30×30 cm^2 cages where they were observed for 15 min prior to cocaine administration. Animals pretreated with saline vehicle served as controls. Changes in the animals' behavior prior to cocaine administration were recorded as observations but were not quantified. Preliminary research in our laboratory indicated that 15 min is sufficient time for maximum or near-maximum drug absorption and activity for all compounds tested [15].

After 15 min, animals received a dose of cocaine previously determined to produce approximately 70% convulsions in control mice [9]. Specifically, 6J mice received a 100-mg/kg dose of cocaine that produced 72% convulsions in control mice, and 6ByJ mice received a 133-mg/kg dose of cocaine that produced 70% convulsions in control mice. Animals were scored for 15 min by a 'blind' observer for the occurrence and latency of any of the following behavioral indices of overt convulsant activity typically produced by cocaine: wild running, clonus, tonus, and clonic-tonic [13]. While behavioral measures such as locomotor activity were not directly measured and quantified for these experiments, observations were made of the animals posture and behavioral patterns during the 15-min period following the initial injection and following cocaine administration. Animals were observed for 15 min, since initial studies in our laboratory indicated that the percentage of convulsions occurring within 15 or 60 min postinjection does not differ, and this effect typically occurs within 2–4 min postinjection.

Experiment 2 examined the role of 5-HT$_2$ receptors in mediating genetic sensitivity to cocaine-induced convulsions. Mice received vehicle or cinanserin (0.3, 1, 3, 10, 30 mg/kg; $n=$at least six per group) and were immediately placed into 30×30 cm^2 cages. Fifteen minutes later, the mice received their respective dose of cocaine and behavioral measures were monitored as described in experiment 1.

2.6. PI hydrolysis

Experiment 3 compared PI hydrolysis turnover in the amygdaloid ridge, frontal cortex, hippocampus, hypothalamus, midbrain, and striatum of 6J and 6ByJ mice. Brain slices were prepared by cross-chopping, using a McIlwain tissue chopper set at 0.35 mm. The slices were preincubated in Krebs–Heinslett Buffer (KHB) containing 118 mM NaCl, 4.8 mM KCl, 1.2 mM KH$_2$PO$_4$, 1.3 mM CaCl$_2$, 1.2 mM MgSO$_4$, 11 mM glucose, and 25 mM NaHCO$_3$. Brain slices were shaken constantly in the presence of KHB bubbled with 95% O$_2$–5% CO$_2$ for 45 min at 37°C with one intermediate change of buffer. The tissue was then incubated in fresh buffer containing 0.3 mM myo-[2-^{3}H]inositol (20–23 Ci/mmol) for 60 min and

then washed three times with fresh KHB. Forty microliters of packed slices were then dispersed into 4-ml plastic tubes containing 225 ml of KHB with 10 mM LiCl for 20 min. Ten μl of 25 mM pargyline was then pipetted into each tube and the slices were incubated for an additional 10 min. The addition of pargyline was based on previous research demonstrating a 16-fold higher induction of 5-HT-induced PI hydrolysis turnover in the presence of this compound [2]. Next, 10 μl of either KHB, 1 mM carbachol, or 10 mM 5-HT was added to the tubes and incubated for an additional 45 min. The agonist doses were chosen based on preliminary dose–response experiments in our laboratory demonstrating that these concentrations produce the maximum reliable induction of PI hydrolysis turnover in mouse brain slices. The agonist stimulation was terminated by the addition of 0.9 ml of chloroform/methanol (1:2, v/v) and the tubes were vortexed well. Further aliquots of 0.3 ml of chloroform and 0.3 ml of water were added and the tubes were revortexed. Following centrifugation at 5000 rpm for 5 min, the supernatants were applied to columns containing 1 ml of Dowex anion-exchange resin (AG, 1×8 formate form, 100–200 mesh, Biorad Laboratories, Richmond, CA). Free inositol was eluted with water, and inositol monophosphate (IP1) was eluted with 0.2 M NH$_4$CO$_2$H/0.1 M HCOOH. Scintillation fleur (Ultima-FloTM AF, Packard Instrument Co.) was then added to the eluted sample and radioactivity was counted using liquid scintillation. To measure the incorporation of [^{3}H]inositol into phospholipids, a 200 μl aliquot of the organic phase was air dried and counted the following day. This procedure was done in order to express the induction of PI hydrolysis as a function of the remaining ligand in the lipid bilayer phase. The induction of PI hydrolysis turnover by agonist stimulation was expressed a function of control values (i.e. vehicle control tubes). Mean levels of PI hydrolysis produced by 5-HT or carbachol were estimated from at least four separate experiments.

3. Results

3.1. Experiment 1: examination of presynaptic 5-HT mechanisms and genetic sensitivity to cocaine-induced convulsions

Fig. 1(top panel) illustrates the density of 5-HT transporter sites across various brain regions in 6J and 6ByJ mice. No differences were found in the densities of 5-HT transporters across any of the regions examined. However, there was a main effect of region ($F_{4,28}=49.7$, $P<0.0001$) that appeared to be due to a higher density of 5-HT transporters in the midbrain section relative to all other regions ($P<0.05$), consistent with an expected high concentration of 5-HT transporters in the cell body region of the raphe nucleus of our midbrain section. Fig. 1(bottom
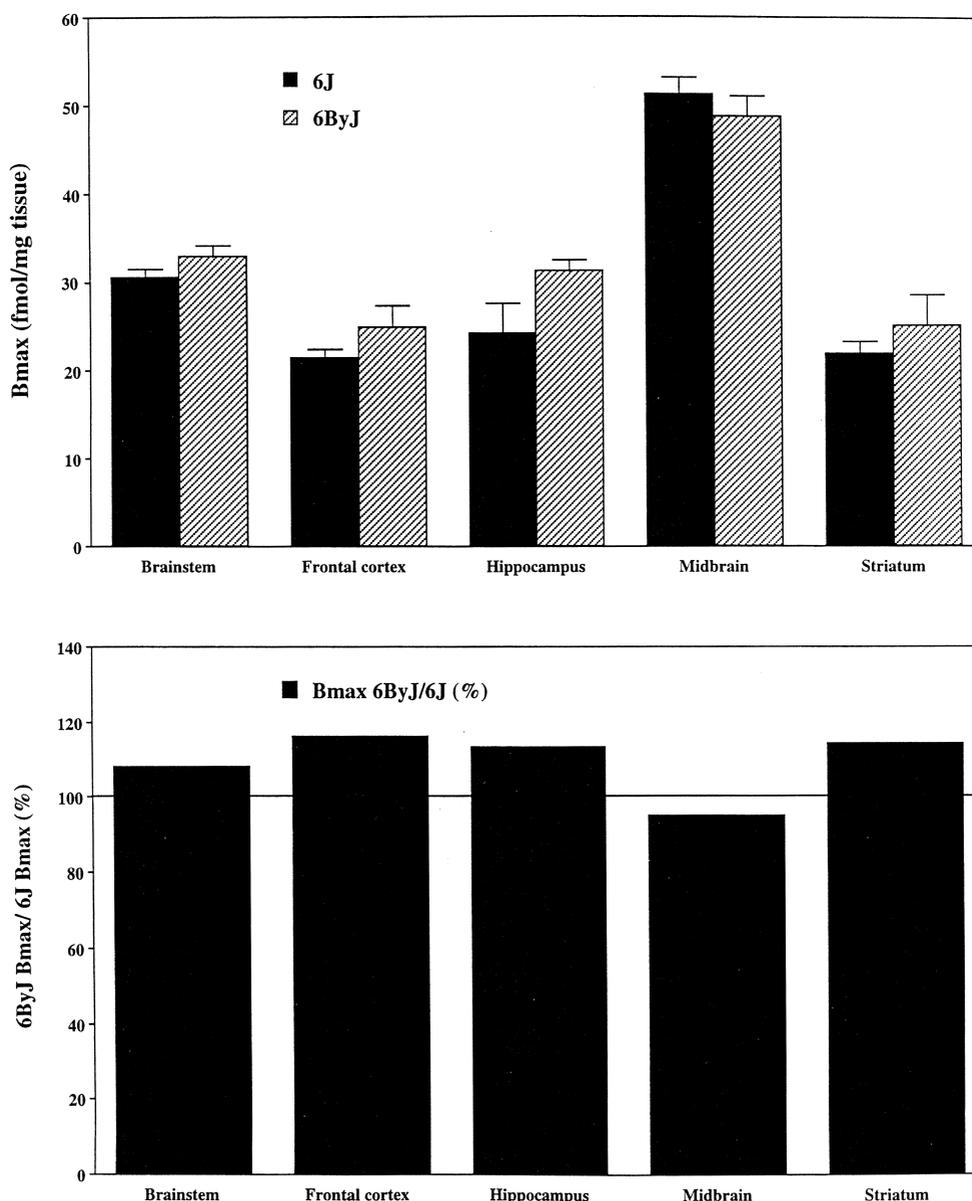
Fig. 1. Estimated density of 5-HT transporters (B_{max} values±S.E.M.) in several brain regions of 6J (solid bars) and 6ByJ (hatched bars) mice. The bottom panel reflects the ratio of B_{max} values in 6ByJ mice expressed as a function of 6J mice, such that 100% reflects the point at which there is no difference in the density of 5-HT transporter sites. Overall, there was no difference in the density of 5-HT transporters across any brain region of 6J and 6ByJ mice.

panel) illustrates the ratio of B_{max} values of 6ByJ mice expressed as a function of 6J mice, such that 100% reflects the point at which there is no difference between the strains, confirming that there was no difference in the density of 5-HT transporters across these strains of mice. Table 1 represents the density and affinity values for 5-HT uptake sites in 6J and 6ByJ mice.

Fig. 2 illustrates the effects of fluoxetine on cocaine-induced convulsions in 6J (circles) and 6ByJ (squares) mice expressed as a percentage of their respective control values. Control mice receiving saline pretreatment exhibited 72% (6J) and 70% (6ByJ) convulsions following

administration of their respective dose of cocaine, consistent with previous findings in our laboratory [9]. Fluoxetine did not significantly facilitate cocaine-induced convulsions in 6J mice although there was a trend for this effect ($F_{2,58}=2.14$, $P<0.12$), and post-hoc analyses revealed a significant facilitation of convulsions at the 13.3 mg/kg dose of fluoxetine ($P<0.05$). In contrast, fluoxetine produced a robust facilitation of cocaine-induced convulsions in 6ByJ mice ($F_{2,32}=3.4$, $P<0.04$). This effect was due primarily to a significant facilitation of convulsions at the 5.6-mg/kg dose of fluoxetine ($P<0.05$). Overall, 6ByJ mice were more sensitive to the effects of fluoxetine on

Table 1
Density (B_{max} values$\pm$S.E.M.) and affinity ($K_d\pm$S.E.M.) of 5-HT uptake sites in several brain regions of 6J and 6ByJ mice[a]

	Paroxetine binding					
	B_{max} values			K_d values		
	6J	6ByJ	6ByJ/6J (%)	6J	6ByJ	6ByJ/6J (%)
Brian stem	30.5±1.0	32.±1.3	108	2.77±0.39	3.38±1.55	122
Frontal cortex	21.4±0.9	24.8±2.5	116	3.57±0.43	3.71±0.38	104
Hippocampus	27.5±1.2	31.2±1.3	113	4.12±1.12	3.12±0.38	104
Midbrain	51.3±2.3	48.7±2.4	95	5.45±1.80	4.51±1.15	82
Striatum	21.8±1.5	25.0±3.6	114	3.79±0.68	3.89±0.50	102

[a] Estimated density (B_{max} values$\pm$S.E.M.) and affinity ($K_d\pm$S.E.M.) of 5-HT uptake sites in several brain regions of 6J and 6ByJ mice. The data is also presented as a ratio of these values in 6ByJ mice expressed as a function of 6J mice, such that 100% reflects the point at which there is no difference in the density of 5-HT transporter sites. Overall, there was no difference in the density of 5-HT transporters across any brain region of these mice.

cocaine-induced convulsions, with a 2.8-fold difference in the ED_{50} values of 6J (4.0 mg/kg) and 6ByJ (11 mg/kg) mice.

Experiment 1 examined whether genetic sensitivity to cocaine-induced convulsions is related to differences in 5-HT transporters. The finding that 5-HT transporter density or affinity does not differ across these mice suggests that genetic sensitivity to convulsions is not related to presynaptic differences in 5-HT transporter densities. However, the finding that fluoxetine produced a more potent facilitation of convulsions in 6ByJ relative to 6J mice, suggests that sensitivity to convulsions across these strains may be mediated via postsynaptic mechanisms.

3.2. Experiment 2: examination of various receptors that may mediate genetic sensitivity to cocaine-induced convulsions

Experiment 2 first examined whether 5-HT$_2$ receptor density or affinity mediates genetic sensitivity to cocaine-induced convulsions. Fig. 3(top panel) illustrates the density of 5-HT$_2$ receptors across various brain regions in 6J and 6ByJ mice. There was a strong trend for a significant main effect of strain, with higher densities of 5-HT$_2$ receptors being observed in 6ByJ relative to 6J mice ($F_{1,88}$=3.4, $P<0.06$). The modest level of this effect appeared to be the result of large variance between regions due to 5–10-fold higher values in the frontal cortex
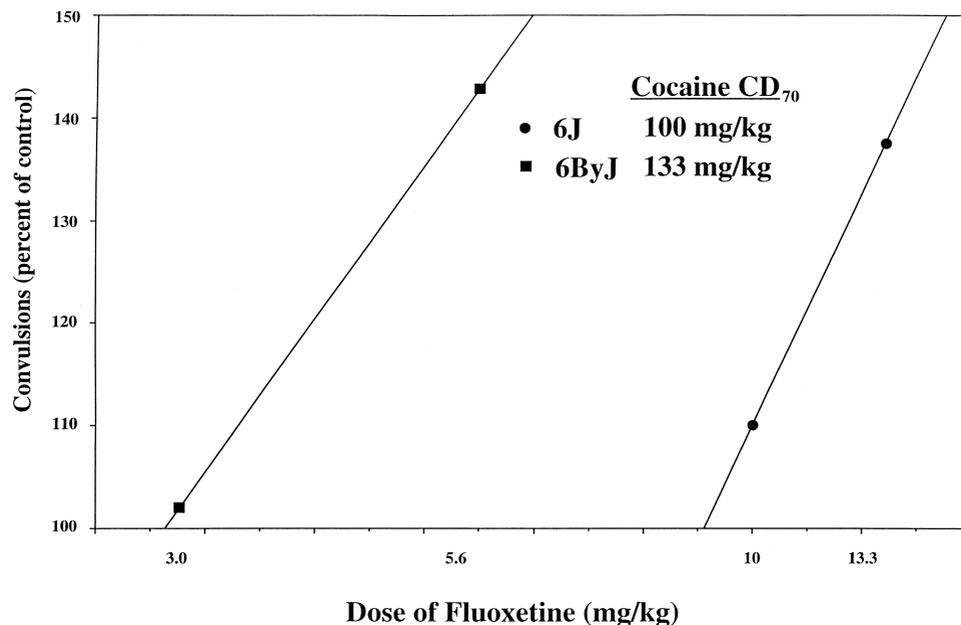

Fig. 2. Dose-dependent effects of fluoxetine pretreatment on convulsions produced by cocaine in 6J (circles) and 6ByJ (squares) mice. Control mice received a dose of cocaine that produced 72% (6J) and 70% (6ByJ) convulsions and the effects of fluoxetine on convulsions are expressed as a percentage of these control values. Fluoxetine appeared to facilitate cocaine-induced convulsions in 6J mice, however, this effect did not reach statistical significance. In contrast, fluoxetine produced a significant and potent facilitation of cocaine-induced convulsions in 6ByJ mice that was significant at the 5.6-mg/kg dose of fluoxetine. It took a 2.4-fold higher concentration of fluoxetine to produce 100% convulsions in 6J (5.6 mg/kg) relative to 6ByJ (13.3 mg/kg) mice.
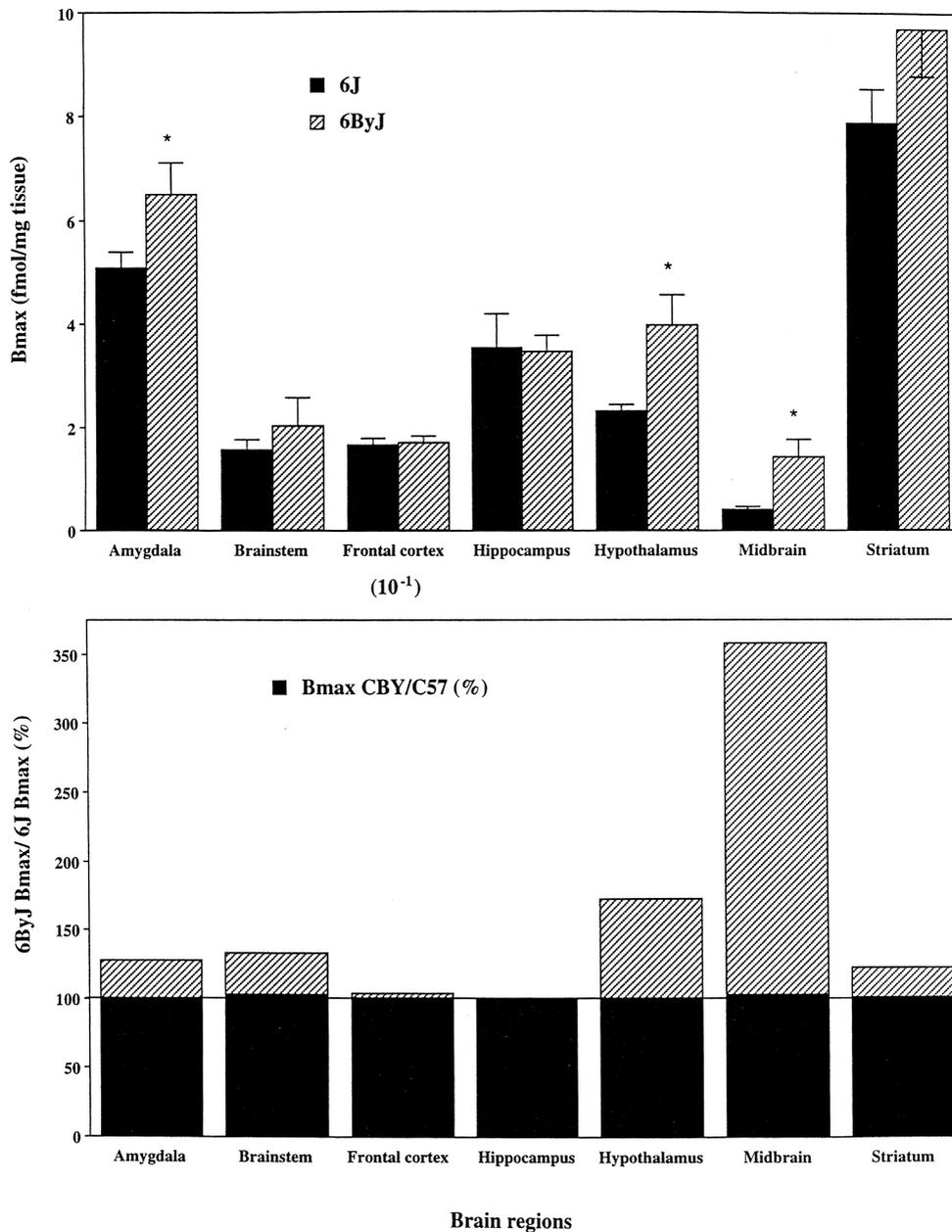
Fig. 3. Estimated density of 5-HT$_2$ receptors (B_{max} values±S.E.M.) in several brain regions of 6J (solid bars) and 6ByJ (hatched bars) mice. The bottom panel reflects the ratio of B_{max} values in 6ByJ expressed as a function of 6J mice, such that values above 100% reflect a higher density of 5-HT$_2$ sites in 6ByJ relative to 6J mice. Overall, there is a higher density of 5-HT$_2$ receptors in 6ByJ relative to 6J mice, and this difference was statistically significant in the amygdaloid ridge, hypothalamus, and midbrain. * Represent a significant difference relative to 6J mice.

relative to other regions. Therefore, the data were subsequently analyzed using a square-root transformation of frontal cortex B_{max} values, which produced a highly significant main effect of strain ($F_{1,88}$=7.0, P<0.01) and region ($F_{6,88}$=75.7, P<0.0001), with higher densities of 5-HT$_2$ receptors being observed in the amygdaloid ridge, striatum, and frontal cortex, relative to the other brain regions (P<0.05). The bottom panel of Fig. 3 illustrates the ratio of B_{max} values of 6ByJ mice expressed as a function of 6J mice, such that values above 100% reflect a

higher density of these sites in 6ByJ relative to 6J mice. This illustrates that in most regions there is a higher density of 5-HT$_2$ receptors in 6ByJ relative to 6J mice. In addition, Table 2 illustrates the density and affinity values of 5-HT$_2$ receptors in 6J and 6ByJ mice, again illustrating a higher density of 5-HT$_2$ sites in 6ByJ relative to 6J mice. The differences in B_{max} values do not appear to be related to the affinity of these sites, since the affinity values for the 5-HT$_2$ receptor do not differ across any brain region of 6ByJ and 6J mice.

Table 2

Density (B_{max} values±S.E.M.) and affinity (K_d±S.E.M.) of 5-HT$_2$ receptors of 6J and 6ByJ mice[a]

| | Ketanserin binding | | | | | |
| | B_{max} values | | | K_d values | | |
	6J	6ByJ	6ByJ/6J (%)	6J	6ByJ	6ByJ/6J (%)
Amygdala	5.07±0.31	6.50±0.61	128*	1.24±0.28	1.37±0.22	110
Brainstem	1.56±0.20	2.01±0.55	129	0.76±0.10	0.81±0.30	106
Frontal cortex	16.4±1.40	16.8±1.40	102	1.48±0.24	1.32±0.15	89
Hippocampus	3.51±0.67	3.46±0.31	100	1.48±0.24	1.32±0.15	108
Hypothalamus	2.30±0.14	3.96±0.60	172*	0.63±0.03	0.70±0.33	111
Midbrain	0.39±0.07	1.40±0.34	358*	1.62±0.65	1.87±0.40	115
Striatum	7.80±0.67	9.67±0.88	124	0.99±0.17	1.19±0.15	120

[a] Estimated density (B_{max} values±S.E.M.) and affinity (K_d±S.E.M.) of 5-HT$_2$ receptors of 6J and 6ByJ mice. The data is also presented as a ratio of these values in 6ByJ mice expressed as a function of 6J mice, such that values above 100% reflects more binding in 6ByJ relative to 6J mice. There is a higher density of 5-HT$_2$ receptors in 6ByJ relative to 6J mice, and this difference was statistically significant in the amygdaloid ridge, hypothalamus, and midbrain. * Represent a significant difference between 6ByJ and 6J mice.

The density and affinity of 5-HT$_1$, muscarinic, and sigma receptors is shown for 6J and 6ByJ mice in Table 3. Although density of these sites differed significantly across several brain regions, there were no strain differences in density or affinity of any of these binding sites, with the exception that there was a significantly higher density of pirenzepine binding sites in midbrain of 6J relative to 6ByJ mice ($P<0.01$).

Overall, the binding results suggest that 5-HT$_2$ sites appear to play a key role in mediating genetic sensitivity to cocaine-induced convulsions. To further examine the role of these receptors in mediating genetic sensitivity to cocaine-induced convulsions, Experiment 2 also compared the ability of the 5-HT$_2$ antagonist cinanserin to attenuate this effect of cocaine across 6J and 6ByJ mice. Fig. 4 illustrates the dose-dependent effects of cinanserin on cocaine-induced convulsions in 6J (circles) and 6ByJ (squares) mice. Control mice exhibited 72% (6J) and 70% (6ByJ) convulsions following administration of their respective dose of cocaine and the data is expressed as a percentage of these control values. The results revealed that cinanserin attenuated cocaine-induced convulsions in 6J and 6ByJ mice ($F_{3,63}=5.7$, $P<0.001$; $F_{3,34}=3.6$, $P<0.02$, respectively). However, it appears that this compound produced a more potent blockade of convulsions in

6J relative to 6ByJ mice. In fact, the attenuating effects of cinanserin were significant at the 10 mg/kg dose in 6J mice, and the 30 mg/kg dose in 6ByJ mice ($P<0.05$). The latter finding is consistent with the three-fold difference in the ED_{50} values obtained for 6J (2.2 mg/kg) and 6ByJ (7.0 mg/kg) mice.

These results demonstrate that 6ByJ mice display a higher density of 5-HT$_2$ receptors and require a higher concentration of cinanserin to attenuate convulsions relative to 6J mice. The finding that 5-HT$_2$ sites mediate genetic sensitivity to cocaine-induced convulsions is consistent with previous research demonstrating agonists that activate 5-HT$_2$ sites produce a more robust facilitation of cocaine-induced convulsions in 6ByJ relative to 6J mice [9], and that 5-HT$_2$ antagonists attenuate this effect of cocaine [10,15,18]. Based on the role of 5-HT$_2$ receptors in mediating cocaine-induced convulsions, it might be expected that mice displaying a higher density of these sites would be more sensitive to cocaine-induced convulsions. However, we found that 6ByJ mice which are less sensitive to this effect of cocaine have more 5-HT$_2$ sites and require a higher dose of cinanserin to attenuate convulsions relative to 6J mice. A weaker coupling of 5-HT$_2$ receptors might explain why 6ByJ mice may be less sensitive to cocaine-induced convulsions despite the higher

Table 3 Estimated density (B_{max} values±S.E.M.) and affinity (K_d±S.E.M.) of Haloperidol, Pirenzepine, and LSD binding sites in 6J and 6ByJ mice. No overall differences were observed in the affinity or density of these sites across 6ByJ and 6J mice.

Density (B_{max} values±S.E.M.) and affinity (K_d±S.E.M.) values of haloperidol, pirenzepine, and LSD binding sites in 6J and 6ByJ mice

| | Haloperidol binding | | | | Pirenzepine binding | | | | LSD binding | | | |
| | B_{max} values | | K_d values | | B_{max} values | | K_d values | | B_{max} values | | K_d values | |
	6J	6ByJ	6J	6ByJ	6J	6ByJ	6J	6ByJ	6J	6ByJ	6J	6ByJ
Brainstem	8.5±0.3	8.9±0.8	4.5±0.7	4.3±0.3	5.80±0.5	4.60±0.5	1.3±0.03	3.1±1.2	2.40±1.1	2.40±1.1	2.3±0.6	1.8±0.7
Frontal cortex	8.6±2.0	8.5±1.2	3.9±0.6	4.5±0.6	62.6±7.9	65.6±02	1.4±0.4	1.8±0.10	4.2±1.0	3.50±0.5	2.2±0.8	1.5±0.4
Hippocampus	5.1±1.1	6.1±1.8	4.5±1.5	4.7±0.5	67.0±2.7	75.2±3.6	1.8±0.2	2.2±0.50	7.3±1.6	10.4±1.5	1.5±0.2	2.2±0.3
Midbrain	8.7±1.2	6.5±0.6	4.0±0.7	4.1±0.9	20.4±4.4	6.6±1.3	2.1±0.5	2.6±0.40	2.8±0.7	4.00±0.6	1.3±0.2	1.6±0.3
Striatum	5.1±0.7	6.0±0.7	4.4±0.2	4.3±0.9	81.0±7.4	94.2±8.3	1.4±0.30	1.4±0.30	3.1±0.6	2.30±0.5	1.5±0.5	0.8±0.2
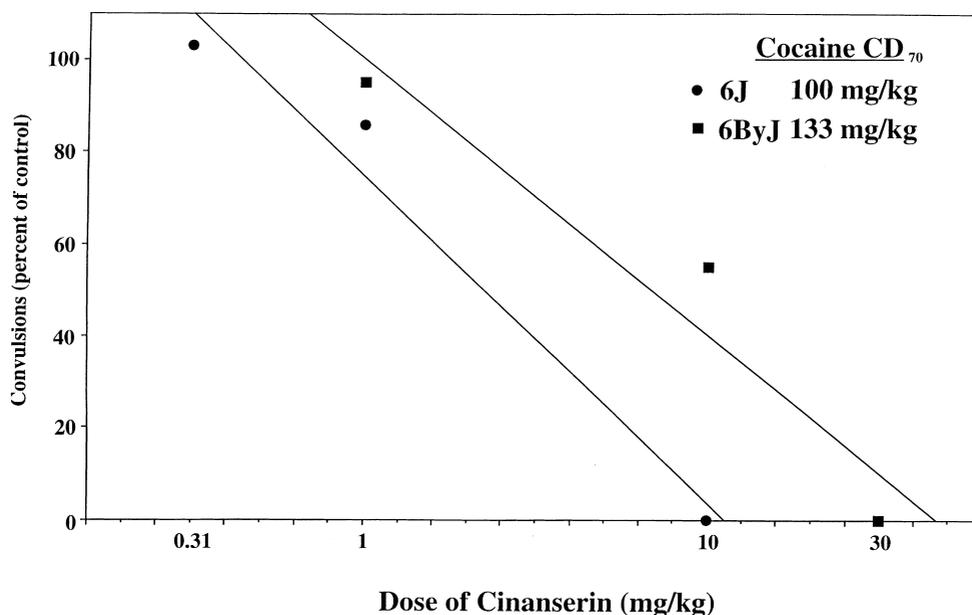
Fig. 4. Dose-dependent effects of cinanserin pretreatment on convulsions produced by cocaine in 6J (circles) and 6ByJ (squares) mice. Control mice received a dose of cocaine that produced 72% (6J) and 70% (6ByJ) convulsions and the effects of cinanserin on convulsions are expressed as a percentage of these control values. Cinanserin attenuated cocaine-induced convulsions in both strains of mice, however, this compound blocked convulsions more potently in 6J (CD_{50}=2.2 mg/kg) relative to 6ByJ (CD_{50}=7.0 mg/kg) mice.

density of these sites. In this way, higher doses of cocaine could be necessary to compensate for a less efficient coupling of 5-HT_2 receptors in 6ByJ relative to 6J mice.

3.3. Experiment 3: examination of 5-HT_2 second-messenger PI hydrolysis and genetic sensitivity to cocaine-induced convulsions

Table 4 illustrates PI hydrolysis values in control conditions and following 5-HT stimulation. IP accumulation (radioactivity measured in the upper phase) is expressed as a percent of the total radioactivity incorporated into lipids (upper+lower phase). Agonist stimulation of PI hydrolysis is expressed as a percentage of control values. Control levels of PI hydrolysis did not differ across any of

the regions in 6J and 6ByJ mice. 5-HT produced an increase in PI hydrolysis turnover in each region relative to control values and the level of this stimulation did not differ across any of the brain regions examined. A main effect of strain was observed, with higher levels of 5-HT-induced PI hydrolysis being observed in 6ByJ relative to 6J mice ($F_{1,66}$=11.1, $P<0.001$). This effect was significantly higher in the amygdaloid ridge, hypothalamus, and midbrain of 6ByJ relative to 6J mice ($P<0.05$), corresponding to regions where higher densities of 5-HT_2 receptors were also observed.

Previous research using 6J frontal cortex slices has demonstrated that 5-HT stimulation produces approximately a 67–80% increase in PI hydrolysis turnover [3], while the present conditions yielded a lower range (30–60%

Table 4
PI turnover produced by 5-HT or carbachol stimulation in various brain regions of 6J and 6ByJ mice[a]

	Control values		5-HT stimulation			Carbachol stimulation		
	6J	6ByJ	6J	6ByJ	6ByJ/6J (%)	6J	6ByJ	6ByJ/6J (%)
Amygdala	80.4±5.1	69.7±5.3	116.7±65.40	169.4±10.4	145*	334.5±26.4	434.8±28.2	130*
Frontal cortex	66.1±6.6	61.5±8.1	123.6±4.40	147.3±11.1	119	257.2±15.5	331.8±19.4	129*
Hippocampus	68.5±7.5	75.0±6.5	158.6±17.0	165.4±18.7	104	330.8±29.7	346.4±45.4	105
Hypothalamus	77.9±3.6	68.4±5.9	128.0±9.50	167.7±12.8	131*	302.1±9.50	370.8±28.1	123*
Midbrain	84.4±11.1	84.5±11.1	140.1±1.20	166.3±9.60	119*	301.9±45.9	259.7±3.8	86
Striatum	78.5±3.9	73.7±5.9	131.7±11.7	129.5±12.1	98	285.1±14.8	319.3±8.5	112

[a] PI hydrolysis turnover (radioactivity measured in the upper phase) is expressed as a percent of the total radioactivity incorporated into lipids (upper+lower phase). Agonist stimulation of PI hydrolysis turnover is expressed as a percentage of control values. Mean levels (±S.E.M.) of agonist stimulation represent at least four experiments in which the obtained ratio for the control values did not exceed 100% and at least a 300% increase from controls was observed following carbachol stimulation. * Represent a significant increase in 6ByJ relative to 6J mice.

increase) of response. Subsequently, carbachol-induced PI hydrolysis turnover was examined (Table 4). Carbachol produced a robust increase in PI hydrolysis in each region and the magnitude of this effect appeared to be related to the region examined ($F_{5,69}=3.9$, $P<0.003$). Regardless of strain, carbachol-induced PI hydrolysis was significantly higher in the amygdaloid ridge (384.6% of control on average) relative to the frontal cortex (294%), striatum (302%), and midbrain (280%; $P<0.05$).

The PI hydrolysis findings are consistent with our binding results, since a higher induction of 5-HT-induced PI hydrolysis was observed in the same regions displaying a higher density of 5-HT$_2$ sites in 6ByJ relative to 6J mice. Additionally, a higher induction of 5-HT-induced PI hydrolysis is consistent with the finding that 6ByJ mice are more sensitive to agonist stimulation of cocaine-induced convulsions relative to 6J mice. Collectively, these findings support the role of 5-HT$_2$ receptors in mediating genetic sensitivity to cocaine-induced convulsions. It was hypothesized that 6ByJ mice might have a weaker functional linkage of 5-HT$_2$ receptors, which could explain why they are less sensitive to the acute convulsant effects of cocaine. However, we found that 6ByJ mice exhibit a higher level of 5-HT-induced PI hydrolysis in regions where they also exhibit a greater density of 5-HT$_2$ receptors relative to 6J mice. Therefore, the PI hydrolysis findings do not provide a clear rationale for why 6ByJ mice are less sensitive to cocaine-induced convulsions despite the fact that these mice have a higher density of 5-HT$_2$ sites relative to 6J mice.

4. Discussion

This study contributes to converging lines of evidence suggesting that 5-HT$_2$ receptors mediate cocaine-induced convulsions. For example, pharmacological studies have demonstrated that cocaine-induced convulsions are attenuated by 5-HT$_2$ antagonists and facilitated by 5-HT$_2$ agonists [9,14,17].

One goal of the present study was to examine whether presynaptic and/or postsynaptic serotonergic mechanisms mediate genetic sensitivity to cocaine-induced convulsions. The finding that there is no difference in 5-HT transporter density or affinity across 6J and 6ByJ mice suggests that the sensitivity to convulsions across these mice is not due to the number and/or affinity of cocaine-binding sites at the 5-HT transporter. It is important to note, however, that the lack of a difference in presynaptic transporter densities does not preclude the role of other presynaptic mechanisms that may influence sensitivity to cocaine-induced convulsions. For example, differences in 5-HT synthesis or rate of 5-HT uptake may influence sensitivity to convulsions across these strains of mice.

This study also examined whether postsynaptic serotonergic mechanisms mediate genetic sensitivity to cocaine-induced convulsions. The role of postsynaptic 5-HT mechanisms was implicated indirectly by the finding that fluoxetine more potently and robustly facilitates cocaine-induced convulsions in 6ByJ relative to 6J mice, despite similar transporter densities and affinities. The hypothesis that postsynaptic 5-HT mechanisms mediate genetic sensitivity to cocaine-induced convulsions was supported by the finding that 6ByJ mice display a higher density of 5-HT$_2$ receptors relative to 6J mice in most brain regions examined. Furthermore, cinanserin more potently blocks cocaine-induced convulsions in 6J relative to 6ByJ mice. The latter finding is consistent with the binding results, since cinanserin produced a more potent attenuation of convulsions in 6J mice, which display fewer 5-HT$_2$ binding sites relative to 6ByJ mice.

The results from our PI hydrolysis turnover experiments further supported the hypothesis that postsynaptic serotonergic mechanisms mediate genetic sensitivity to cocaine-induced convulsions. This is based on the finding that 6ByJ mice have a higher induction of 5-HT-induced PI hydrolysis relative to 6J mice across several regions examined. Importantly, the level of 5-HT induced PI hydrolysis in our study (30–60%), is consistent with behavioral effects related to differences in this second-messenger system. For example, it has been argued that a 30–40% difference in 5-HT$_{2C}$ receptor binding and PI hydrolysis accounts for sensitivity to alcohol preference across genetically distinct strains of rats [11]. In addition, it has been suggested that a 27% increase in 5-HT-stimulated PI hydrolysis following a systemic serotonergic lesion is related to changes in 5-HTP-evoked myoclonus [1]. In the present study, we found a significant difference in 5-HT-induced PI hydrolysis between strains that was observed across three different brain regions. Indeed, this strain difference appeared to be related to the density of 5-HT$_2$ receptors, since a higher 5-HT-induced PI hydrolysis was observed in regions also displaying higher densities of 5-HT$_2$ receptors.

Few studies have attempted to localize the neural regions that mediate the convulsant effects of cocaine. The present finding that there were corresponding differences in 5-HT$_2$ receptor density and PI hydrolysis in the amygdaloid ridge, hypothalamus, and midbrain suggests that these regions may play a role in mediating cocaine-induced convulsions. The role of the amygdala in mediating cocaine-induced convulsions has been implicated in studies demonstrating that cocaine alters sensitization to seizures observed following electrical stimulation of the amygdala, a phenomena referred to as kindling [7,12,17]. To our knowledge, the role of the hypothalamus and midbrain in mediating cocaine-induced convulsions has not been examined. However, the induction of c-fos protein, as a marker of neuronal activity, has been observed in the hypothalamus and midbrain following the expression of electroconvulsive shock-induced seizures [16]. Although

this study suggests that the amygdala, hypothalamus, and possibly the midbrain may mediate cocaine-induced convulsions, further research is needed to localize the neural mechanisms of this toxic effect of cocaine.

The finding that 5-HT$_2$ receptors mediate genetic sensitivity to cocaine-induced convulsions is consistent with previous research in our laboratory. For example, pretreatment with mCPP and MK212 produced a facilitation of cocaine-induced convulsions that was more robust in 6ByJ relative to 6J mice [9]. In fact, the latter study demonstrated that mCPP and MK212 decreased the cocaine CD$_{50}$ by a 2–3-fold greater extent in 6ByJ relative to 6J mice. Based on these findings, it was suggested that genetic sensitivity to cocaine-induced convulsions is mediated by the 5-HT$_{2C}$ receptor subtype, since mCPP is 10-fold and MK212 is 25–50-fold more selective for 5-HT$_{2C}$ receptors relative to 5-HT$_{2A}$ sites [5,6]. The finding that 6ByJ mice are more sensitive to agonist compounds that activate 5-HT$_2$ sites is consistent with the present finding that there is a higher density and second-messenger linkage of 5-HT$_2$ receptors in 6ByJ relative to 6J mice.

Much research suggests that 5-HT$_2$ receptors mediate genetic sensitivity to cocaine-induced convulsions. Based on these findings, it might be expected that mice which have a higher density of 5-HT$_2$ receptors would be more sensitive to the convulsant effects of cocaine. However, we found that 6ByJ mice display a higher density and second-messenger linkage of 5-HT$_2$ sites and are less sensitive to this effect of cocaine relative to 6J mice. Consequently, the relationship between the density and linkage of 5-HT$_2$ sites and relative sensitivity to cocaine-induced convulsions might seem counterintuitive. One possible explanation is that the higher density of 5-HT$_2$ sites in 6ByJ mice may be due to a compensatory upregulation of these receptors in response to lower levels of synthesized 5-HT. If this is the case, it would be expected that 6ByJ mice would require higher doses of cocaine to produce convulsions relative to 6J mice, consistent with previous and present findings. Furthermore, consistent with previous findings, 6ByJ mice should be more sensitive to the effects of 5-HT$_2$ agonists, since these mice have a higher density of these receptors relative to 6J mice. Ongoing research in our laboratory is examining aspects of 5-HT synthesis that may play a role in mediating genetic sensitivity to the toxic effects of cocaine across these mice.

Thus, 5-HT$_2$ sites appear to play an important role in mediating sensitivity to cocaine-induced convulsions across 6ByJ and 6J mice. This is based on the finding that 6ByJ mice have more 5-HT$_2$ sites and require higher doses of cinanserin to block convulsions relative to 6J mice. Furthermore, 6ByJ mice display greater facilitation of cocaine-induced convulsions relative to 6J mice following administration of 5-HT$_2$ agonists [9]. The latter finding is consistent with the higher density and functional linkage of 5-HT$_2$ receptors in 6ByJ relative to 6J mice. These findings may be important for determining the neural

mechanisms that mediate genetic susceptibility to acute cocaine toxicity.

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