Tolerance and cross-tolerance to head twitch behavior elicited by phenethylamine- and tryptamine-derived hallucinogens in mice

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Abstract

The serotonin 5-HT$_{2A}$ receptor is a potential therapeutic target to a host of neuropsychiatric conditions, but agonist actions at this site are linked to abuse-related hallucinogenic effects which may limit therapeutic efficacy of chronic drug administration. Tolerance to some effects of hallucinogens have been observed in humans and laboratory animals, but the understanding of tolerance and cross-tolerance between distinct structural classes of hallucinogens is limited. Here we used the drug-elicited head twitch response (HTR) in mice to assess the development of tolerance and cross-tolerance with two phenethylamine-derived (2,5-dimethoxy-4-iodoamphetamine [DOI] and 2,5-dimethoxy-4-propylthiophenethylamine [2C-T-7]) and two tryptamine-derived (N,N-dipropyltryptamine [DPT] and N,N-diisopropyltryptamine [DIPT]) drugs with agonist affinity for 5-HT$_{2A}$ receptors. Tolerance developed to HTR elicited by daily DOI or 2C-T-7, but not to HTR elicited by DPT or DIPT. DOI-elicited tolerance was not surmountable with dose, and a similar insurmountable cross-tolerance was evident when DOI-tolerant mice were tested with various doses of 2C-T-7 or DPT. These studies suggest that the use of phenethylamine-derived hallucinogens as therapeutic agents may be limited not only by their abuse potential, but also by the rapid development of tolerance which would likely be maintained even if a patient were switched to a different 5-HT$_{2A}$ agonist medication from a distinct structural class. However, these experiments also imply that tryptamine-derived hallucinogens might have a reduced potential for tolerance development, as compared to phenethylamine-derived 5-HT$_{2A}$ agonists, and might therefore be more suitable for chronic administration in a therapeutic context.
Introduction

Hallucinogenic effects exerted by classical ergoline-derived, phenethylamine-derived, or tryptamine-derived drugs are currently understood to be mediated by agonist actions at serotonin (5-hydroxytryptamine, 5-HT) 2A receptors (5-HT_{2A}) (Nichols, 2004; Halberstadt, 2014), a site also implicated in numerous neuropsychiatric conditions including migraine (Berger et al., 2009), eating disorders (Kaye et al., 2005), depression and bipolar disorder (Fountoulakis et al., 2012), anxiety and panic (Graeff and Zangrossi, 2010), psychosis and schizophrenia (Selvaraj et al., 2014), and substance use disorder (Bubar and Cunningham, 2008). As such, medications targeting 5-HT_{2A} could potentially be useful in treating a wide range of conditions, although the hallucinogenic effects of agonist medications would likely limit their clinical utility if chronic administration were required. Nevertheless, interest in using hallucinogenic drugs as therapeutics (e.g., Griffiths and Grob, 2010; Vollenweider and Kometer, 2010) has been growing in recent years, thus, a greater understanding of the pharmacological mechanisms underlying hallucinogenic effects is warranted. In particular, a better understanding of hallucinogen effects following chronic administration may be especially relevant to understanding the long-term efficacy of these drugs as therapeutic agents.

Tolerance to numerous effects of some hallucinogens develops rapidly, evidenced by a progressive decrease in effects over repeated administration of a constant dose. In humans, this phenomenon has mainly been studied using the ergoline hallucinogen lysergic acid diethylamide (LSD) (e.g., Isbell et al., 1959; Isbell et al., 1961), but tolerance to behavioral and physiological effects of phenethylamine-derived hallucinogens can also be readily induced in humans (Angrist et al., 1974; Hollister et al., 1969; Wolbach et al., 1962), presumably mediated by downregulation and desensitization of serotonin 5-HT_{2A} receptors (McKenna et al., 1989; Owens et al., 1991; Smith et al., 1999). Tolerance elicited by tryptamine-derived hallucinogens in man is not
as well characterized, with one early study reporting a lesser degree of tolerance to
psilocybin-elicited effects than to LSD-elicited effects (Isbell et al., 1961), and more
recent experiments failing to demonstrate the development of tolerance to effects of the
structurally-related hallucinogen N,N-dimethyltryptamine (DMT) (Gillin et al., 1976;
Strassman et al., 1996), perhaps due to its apparent inability to desensitize 5-HT$_{2A}$
receptors (Smith et al., 1998).

Because the hallucinogen-induced head twitch response (HTR) in rodents is also
mediated by agonist actions at 5-HT$_{2A}$ receptors (Fantegrossi et al., 2008a; González-
Maeso et al., 2007), it is among the most useful and reliable rodent models for
psychedelic effects. Nevertheless, the role of tolerance in HTR elicited by hallucinogens
as a drug class also remains somewhat unclear since the vast majority of work in this
area has utilized only a single compound, the phenethylamine-derived 2,5-dimethoxy-4-
idoamphetamine (DOI, Figure 1, top left) (reviewed in Canal and Morgan, 2012). In this
regard, Darmani and colleagues (1990; 1992; 1995) have repeatedly demonstrated both
tolerance and supersensitivity to DOI-elicited HTR in male ICR mice, depending on the
specific dose regimen employed. Similarly, male C57BL/6N mice administered DOI
every day for 8 days exhibited a reduced HTR (as compared to the initial effect) on each
day of testing (Dougherty and Aloyo, 2011). Most recently, tolerance to HTR induced by
DOI was observed in DBA/2J mice administered the drug every day, but not every other
day or once per week (Rangel-Barajas et al., 2014). Finally, a remarkable stability in
DOI-elicited HTR following weekly dosing has also been reported in male Sprague-
Dawley rats (Gewirtz and Marek, 2000).

To better understand the ubiquity of tolerance to HTR elicited by distinct
structural classes of hallucinogens, as well as the potential for cross-tolerance among
these chemically diverse 5-HT$_{2A}$ agonists, we studied the effects of acute and chronic
administration of DOI, the structurally-similar phenethylamine 2C-T-7 (2,5-dimethoxy-4-
propylthiophenethylamine, Figure 1, top right), and two tryptamines: \(N,N\)-dipropyltryptamine (DPT, Figure 1, bottom left) and \(N,N\)-diisopropyltryptamine (DIPT, Figure 1, bottom right) on HTR in mice. Acute, single-exposure dose effect curves were determined for all compounds, and maximally effective doses were then chronically administered to study the capacity of each compound to induce tolerance to HTR. Mice made tolerant to HTR elicited by DOI were subsequently tested with various doses of DOI, 2C-T-7 or DPT to examine the surmountability of tolerance or cross-tolerance within and across structural classes. The specific hypotheses tested were that tolerance would develop to chronic administration of all compounds, and that reciprocal cross-tolerance would be observed among the phenethylamines and tryptamines. The major findings of these studies are that phenethylamine-derived 5-HT\(_{2A}\) agonists induce tolerance to HTR while tryptamine-based compounds do not, and that tolerance to behavioral effects induced by DOI is maintained when DPT is substituted, indicating cross-tolerance between these distinct structural classes.

*** INSERT FIGURE 1 APPROXIMATELY HERE ***

**Methods**

**Animals**

Male NIH Swiss mice (Harlan Sprague Dawley, Inc.) weighing 20-25g on delivery were housed 3 animals per cage in temperature-controlled rooms maintained at an ambient temperature of 22±2°C at 45-50% humidity. Lights were set to a 12-h light/dark cycle (experiments were conducted 3-5 hours into the light phase), and animals were fed *ad libitum* with standard rodent chow (Laboratory Rodent Diet #5001) and water for at least 3 days after arrival. Mice were randomly assigned to experimental groups (n=5 or 6 per group), and were free fed for the duration of all experimental manipulations. For all
dose-effect curve determinations, each animal was used in only one experimental observation, but for tolerance studies, the same animals were repeatedly injected, as described below.

Procedure

On experimental days, mice were weighed, marked, and returned to the home cage. Doses were then calculated and prepared for intraperitoneal (ip) injection. For initial dose-effect determinations, individual animals were removed from the home cage, injected with saline (0.01 ml/g), a dose of DOI, 2C-T-7, DPT or DIPT, then placed into an observation cage containing fresh bedding. Ten minutes following this injection, an overhead camera was activated and behavior was recorded for 10 min. For tolerance studies, maximally effective doses of each compound were selected for chronic administration based on the initial dose-effect determinations presented in Figure 2. Each animal was removed from the home cage and injected with DOI (1.0 mg/kg), 2C-T-7 (1.0 mg/kg), DPT (3.0 mg/kg) or DIPT (10.0 mg/kg), then treated as described above. Injections were administered every 24 hours for 5 days (all compounds), every other day for 5 total injections (DOI only), or every seven days for 4 total injections (DOI only). To determine whether or not tolerance to DOI-elicited HTR was surmountable with dose, mice were treated every 24 hours with either saline or 1.0 mg/kg DOI for 3 days, then tested on day 4 with various doses of DOI. For cross-tolerance experiments, mice were treated every 24 hours with either saline or 1.0 mg/kg DOI for 3 days, then tested on day 4 with various doses of 2C-T-7 or DPT. No food or water was available during experimental sessions. In all cases, videotapes were scored for drug-elicited HTR (defined as a rapid rotational jerk of the head which can be distinguished from species-appropriate grooming or scratching behaviors) by at least one observer blind to drug treatment.
Data Analysis

Data are presented as mean ± SEM. In all figures, points without error bars indicate instances where the variance is contained within the data point. For drug-elicited HTR dose-effect determinations (Figure 2), data were not normally distributed and were therefore analyzed by a Kruskal-Wallis one way analysis of variance (ANOVA) on ranks, followed by Dunn’s post-hoc test to compare all drug doses to saline. Tolerance development data were statistically analyzed using a repeated measures one way ANOVA followed by Tukey’s HSD test for all pairwise comparisons (Figures 3 and 4). For tolerance surmountability and cross-tolerance studies, data were not normally distributed, and were thus analyzed by a Kruskal-Wallis one way ANOVA on ranked data, followed by Tukey’s HSD test for all pairwise comparisons (Figures 5 and 6). In all cases, significance was judged at the level of p < 0.05.

Drugs

*R(-)-DOI* was purchased from Sigma Aldrich (St. Louis, MO). 2C-T-7 was obtained from the National Institute on Drug Abuse drug supply program (Research Technology Branch, Research Triangle Park, NC). DPT was synthesized in the Laboratory of Medicinal Chemistry at the NIH/NIDDK and was supplied as a generous gift from Kenner C. Rice, Ph.D. DIPT was synthesized at the Research Technology Branch of the Research Triangle Institute and was supplied as a generous gift by Dr. Bruce Blough. All drugs were dissolved in physiological saline, which, along with all other experimental supplies, was obtained from standard commercial sources. Sonication in warm water was required to dissolve some of the drug concentrations used in these studies, and all injections were administered at a constant volume of 0.01 ml/g.
Results

Dose-effect determinations

Similar to what we have previously observed using this assay, administration of saline elicited very few head twitches (Figure 2, open squares) while DOI elicited a characteristic biphasic dose-effect function (Figure 2, filled circles). DOI-elicited HTR was significantly different from that observed after saline administration at doses of 0.3 (Q=4.033), 1.0 (Q=4.427) and 3.0 mg/kg (Q=3.864, p<0.05 in all cases). A maximum of approximately 18 head twitches was quantified in the 10 minute observation period at a dose of 1.0 mg/kg DOI, which is typically the most effective dose using our procedure (Fantegrossi et al., 2010). 2C-T-7 also elicited a dose-dependent and biphasic HTR (Figure 2, open circles), and the 1.0 mg/kg dose was significantly different (Q=4.230, p<0.05) from saline. A maximum of approximately 16 head twitches was quantified in the 10 minute observation period at a dose of 1.0 mg/kg 2C-T-7, which was also the most effective dose in our previous HTR studies with this drug (Fantegrossi et al., 2005). Similar to the phenethylamine-derived hallucinogens, DPT induced a dose-dependent and biphasic HTR (Figure 2, filled diamonds) with effects significantly different from those elicited by saline at doses of 3.0 (Q=4.244) and 10.0 mg/kg (Q=3.108, p<0.05 for both doses). A maximum of approximately 15 head twitches was quantified in the 10 minute observation period at a dose of 3.0 mg/kg DPT, which was also the most effective dose in our previous HTR studies with this drug (Fantegrossi et al., 2008b). Finally, DIPT administration also produced a biphasic dose effect curve for HTR, but this drug was less effective than all other compounds studied, eliciting significant effects only at the 10.0 mg/kg dose (Q=3.545, p<0.05). A maximum of less than 8 head twitches was quantified in the 10 minute observation period at a dose of 10.0 mg/kg DIPT.

*** INSERT FIGURE 2 APPROXIMATELY HERE ***
**Tolerance development**

Progressive tolerance to DOI-elicited HTR developed with daily administration of 1.0 mg/kg (Figure 3, filled circles), with HTR quantified on days 2-5 significantly different from that observed on day 1 (q=6.786, 11.875, 16.116 and 21.205, respectively, p<0.05 for all comparisons), day 3 significantly different from day 2 (q=5.089, p<0.05), and day 4 significantly different from day 3 (q=4.241, p<0.05). A similar result was obtained with daily administration of 1.0 mg/kg 2C-T-7 (Figure 3, open circles), with HTR quantified on days 2-5 significantly different from that observed on day 1 (q=12.623, 16.831, 21.974 and 22.909, respectively, p<0.05 for all comparisons), day 3 significantly different from day 2 (q=4.308, p<0.05), and day 4 significantly different from day 3 (q=5.143, p<0.05).

In contrast to the phenethylamine-derived hallucinogens, HTR elicited by daily administration of 3.0 mg/kg DPT or 10.0 mg/kg DIPT did not change over the treatment interval (p<0.05). No significant differences from HTR induced on day 1 were achieved on any other day of testing with either tryptamine-derived compound. Importantly, DPT induced a HTR that was approximately equal in magnitude of that of DOI and 2C-T-7 on day 1.

*** INSERT FIGURE 3 APPROXIMATELY HERE ***

Because different regimens of DOI administration have previously been shown to modulate tolerance development (see Introduction), the most effective DOI dose was also administered every other day, or every seven days, in order to further characterize the effects of injection frequency on tolerance to DOI-induced HTR. Interestingly, progressive tolerance to DOI-elicited HTR also developed when 1.0 mg/kg was administered every other day (Figure 3, open circles), with HTR quantified after the 3rd.
4th and 5th injections (i.e., days 5, 7 and 9) significantly different from that observed following the first injection (q=5.787, 9.042, and 13.201, respectively, p<0.05 for all comparisons), but no two consecutive observations were significantly different from one another (p>0.05 for all comparisons). In contrast, no tolerance to DOI-elicited HTR developed when 1.0 mg/kg was administered every seven days (Figure 3, gray circles) (p>0.05 for all comparisons.)

*** INSERT FIGURE 4 APPROXIMATELY HERE ***

**Tolerance to DOI-elicited HTR is insurmountable with DOI test dose**

As demonstrated in Figure 3, daily administration of 1.0 mg/kg DOI for 3 consecutive days produced almost complete tolerance to DOI-elicited HTR on day 4, and this tolerance was maintained on day 5. Therefore, daily administration of 1.0 mg/kg DOI (or saline) for 3 consecutive days was used to induce tolerance to DOI-elicited HTR (or to control for repeated handling and injection), then 0.3, 1.0, 3.0 or 10.0 mg/kg DOI was administered on day 4. A typical biphasic dose-effect function was generated in mice receiving daily saline injections for 3 days, then tested with various doses of DOI on day 4 (Figure 5, filled circles.) In these animals, DOI-elicited HTR was significantly different from that observed after saline administration at doses of 0.3 (q=3.996), 1.0 (q=5.307) and 3.0 mg/kg (q=4.101, p<0.05 in all cases), and the 1.0 mg/kg dose was the most effective dose for eliciting HTR, as usual. In contrast, no HTR significantly different from that observed following saline administration was observed in mice receiving daily 1.0 mg/kg DOI injections for 3 days (Figure 5, open circles) at any tested dose of DOI on day 4 (p>0.05 for all comparisons.) The tolerance to HTR induced by daily 1.0 mg/kg DOI injection for 3 consecutive days was apparent at all active DOI test doses, as HTR observed following administration of 0.3 (q=4.437), 1.0 (q=5.424) and 3.0 mg/kg DOI
(q=4.411) was significantly different between daily saline-treated and daily DOI-treated animals (p<0.05 for all comparisons.)

*** INSERT FIGURE 5 APPROXIMATELY HERE ***

Cross-tolerance to DOI-elicited HTR is insurmountable with dose

Daily administration of 1.0 mg/kg DOI (or saline) for 3 consecutive days was used to induce tolerance to DOI-elicited HTR (or to control for repeated handling and injection), then 0.3, 1.0, 3.0 or 10.0 mg/kg 2C-T-7 was administered on day 4 to assess cross-tolerance within the phenethylamine-derived structural class of hallucinogens. A typical biphasic dose-effect function was generated in mice receiving daily saline injections for 3 days, then tested with various doses of 2C-T-7 on day 4 (Figure 6, left panel, filled circles.) In these animals, 2C-T-7-elicited HTR was statistically different from that observed after saline administration at doses of 1.0 (q=6.561) and 3.0 mg/kg (q=5.059, p<0.05 in both cases), and the 1.0 mg/kg 2C-T-7 dose was again the most effective dose for eliciting HTR. In contrast, no HTR significantly different from that observed following saline administration was observed in mice receiving daily 1.0 mg/kg DOI injections for 3 days (Figure 6, left panel, open circles) at any tested dose of 2C-T-7 on day 4 (p>0.05 for all comparisons.) HTR observed following administration of 1.0 mg/kg 2C-T-7 was significantly different between daily saline-treated and daily DOI-treated animals (q=5.628, p<0.05), but no other between-group comparisons reached statistical significance.

*** INSERT FIGURE 6 APPROXIMATELY HERE ***
To assess cross-tolerance across the phenethylamine-derived and the tryptamine-derived structural classes of hallucinogens, 1.0 mg/kg DOI (or saline) was administered every day for 3 consecutive days to induce tolerance to DOI-elicited HTR (or to control for repeated handling and injection), then 1.0, 3.0, 10.0 or 30.0 mg/kg DPT was administered on day 4. In mice receiving daily saline injections for 3 days, then tested with various doses of DPT on day 4 (Figure 6, right panel, filled circles), a characteristic biphasic dose-effect function was produced, with DPT-elicited HTR significantly different from that observed after saline administration at the 3.0 mg/kg dose (q=5.584, p<0.05). In contrast, no HTR significantly different from that observed following saline administration was observed in mice receiving daily 1.0 mg/kg DOI injections for 3 days (Figure 6, right panel, open circles) at any tested dose of DPT on day 4 (p>0.05 for all comparisons.) HTR observed following administration of 3.0 mg/kg DPT was significantly different between daily saline-treated and daily DOI-treated animals (q=4.491, p<0.05), but no other between-group comparisons reached statistical significance.

Discussion

We initially hypothesized that all four compounds selected for study would produce tolerance to HTR over repeated administrations, and that reciprocal cross-tolerance would be observed between the phenethylamine-derived and tryptamine-derived drugs. Neither of these hypotheses was confirmed by the present studies. We observed dramatic tolerance to HTR elicited by daily administration of DOI or 2C-T-7, but no evidence of tolerance when either DPT or DIPT were administered according to the same schedule. Only a single dose of each compound was studied under conditions of chronic administration, so it is possible that other doses might induce greater or lesser tolerance to HTR than observed here. However, each dose studied under the present
conditions was the maximally-effective dose for each compound, and in the case of DOI, 2C-T-7 and DPT, these doses induced roughly equivalent HTR. Thus, during chronic administration of these three compounds, HTR among all groups were essentially equal on the first day, making it very unlikely that a nonspecific “floor effect” limited tolerance development (although this possibility probably can not be ruled out for DIPT.)

Dose is always an important variable to manipulate in pharmacology, but another important contributor to the different profiles exhibited by the four compounds presented in these studies may be duration of action. Unfortunately, no systematic observations of time course of HTR induced by these substances are available, but effects of phenethylamine-derived hallucinogens are anecdotally longer-lasting than those of tryptamine-derived hallucinogens. For example, Shulgin estimated the duration of action of DOI to be between 16 and 30 hours, and 2C-T-7 effects to last between 8 and 15 hours in humans (Shulgin and Shulgin, 1991). In contrast, using these same methods, the duration of DPT effects were estimated to be between 2-4 hours, and the effects of DIPT were estimated to last between 6 and 8 hours in humans (Shulgin and Shulgin, 1997). It may therefore be the case that the shorter-acting tryptamines could induce tolerance to HTR if administered more frequently than once per day, as drugs with shorter durations of action may require more frequent administration than drugs with longer durations of action to elicit tolerance to their effects. However, it should be noted that Strassman and colleagues (1996) did not observe tolerance to the subjective effects of short-acting DMT in humans even when administered intravenously every 30 minutes for four total injections.

In addition to differences in duration of action, the four drugs used in these studies may also differ in terms of their relative efficacies to activate 5-HT_{2A} receptors. Among other drug classes (i.e., opioids), repeated treatment with equally effective doses of drugs which differ in intrinsic efficacy typically produce different degrees of tolerance
(Duttaroy and Yoburn, 1995; Allen and Dykstra, 2000), with low-efficacy opioids eliciting greater tolerance than high-efficacy opioids (Paronis and Holtzman, 1992;1994). Unfortunately, efficacy estimates for 5-HT$_{2A}$ agonists are rare, and the techniques used to determine relative efficacy at this receptor vary widely. To our knowledge, none of the four drugs utilized in the present studies have ever been directly compared in the same assay. However, a study using a fluorometric imaging plate reader (FLIPR) technique in CHO-K1 cells reported that racemic DOI had 61% the efficacy of 5-HT in this assay (Porter et al., 1999). A more recent study using an assay of non-specific G protein activation in rat brain membranes reported that DPT was 46% as efficacious as 5-HT using these methods, which was identical to the relative efficacy obtained with the DOI analogue 2,5-dimethoxy-4-iodophenethylamine (2C-I) (Nonaka et al., 2007). Thus, based on the available evidence, it is difficult to interpret the present results through the lens of intrinsic efficacy. However, it would appear that DPT is unlikely to exhibit a substantially greater 5-HT$_{2A}$ efficacy than DOI, which would suggest (based on what has been learned through the study of opioid tolerance) that its capacity to elicit tolerance to 5-HT$_{2A}$-mediated effects such as HTR should be greater than or equal to that of DOI, which is the opposite of what we observed in the present studies.

Regarding cross-tolerance, intrinsic efficacy is also a critical determinant among the opioids (Paronis and Holtzman, 1992), but has not been adequately explored with serotonergics. Nevertheless, the finding that a drug which produces no apparent tolerance to a behavioral effect (such as DPT in the present studies) still exhibits a reduced effectiveness in animals made tolerant to DOI-elicited HTR seems to be consistent with mechanistic explanations for tolerance to hallucinogen-induced effects involving downregulation and desensitization of 5-HT$_{2A}$ receptors. A previous study has reported that even a single injection of DOI is sufficient to significantly reduce $B_{max}$ for receptors labeled by 1-(2,5-dimethoxy-4-bromo-phenyl)-2-aminopropane (DOB) or
ketanserin in rat cortex (Pranzatelli, 1991), and chronic DOI administration has repeatedly been demonstrated to downregulate 5-HT$_{2A}$ receptor expression in vivo (McKenna et al., 1989; Owens et al., 1991; Smith et al., 1999). However, in vitro, DOI may produce 5-HT$_{2A}$ upregulation (Akiyoshi et al, 1993), and it appears that the regulatory effects of chronic agonist exposure on 5-HT$_{2A}$ receptors may critically depend on the specific cell line studied (Grotewiel and Sanders-Bush, 1994). Indeed, Roth and colleagues have reported that receptor downregulation and desensitization are dissociable phenomena in vitro (Roth et al., 1995). Despite the obvious complexity of 5-HT$_{2A}$ receptor regulation, it seems likely that the tolerance to HTR induced by DOI (and perhaps by 2C-T-7 as well) is due to progressively reduced receptor expression over the treatment period. In this dampened neuropharmacological context, administration of any 5-HT$_{2A}$ agonist would be expected to produce a reduced behavioral effect, thus, the presently reported cross-tolerance experiments with DOI and DPT would not be inconsistent with what is known about 5-HT$_{2A}$ receptor regulation. Interestingly, the capacity of DPT and DIPT to downregulate or desensitize 5-HT$_{2A}$ receptors has not previously been determined, but would provide a mechanistic explanation for the lack of tolerance observed in the present studies if, like the structurally-related DMT (Smith et al., 1998), these tryptamines also prove incapable of inducing 5-HT$_{2A}$ downregulation.

These studies also provide a systematic characterization of tolerance to DOI-elicited head twitch in another mouse strain, the NIH Swiss mouse. It seems apparent that large strain differences are likely to modulate tolerance development to DOI-elicited HTR, and this may be a fruitful area for further research. As noted in the Introduction, ICR mice exhibit tolerance to DOI-elicited HTR when the drug is administered daily, but supersensitivity when the drug is administered every 48 hours (Darmani et al., 1990; 1992; 1995). In the present studies, we utilized both of these dose regimens, and saw no signs of supersensitivity in the NIH Swiss, similarly to what has been reported in
C57BL/6N mice receiving daily DOI (Dougherty and Aloyo, 2011). In DBA/2J mice, tolerance to HTR induced by DOI was observed when the drug was administered every day, but not every other day or once per week (Rangel-Barajas et al., 2014). This too contrasts with the present profile in NIH Swiss mice, where tolerance was observed with DOI administration occurring every 24 hours or every 48 hours, although the lack of tolerance development with weekly DOI administration is consistent across strains. To our knowledge, there have been no systematic studies of tolerance to hallucinogen-induced HTR across multiple mouse strains, although we have previously reported similar dose effect curves for DOI-elicited HTR between NIH Swiss mice (from two different vendors) and Swiss Webster mice (Fantegrossi et al., 2010). Indeed, the role of strain differences in serotonin pharmacology has not been adequately characterized. In two early studies, BALB/c mice were found to have more 5-HT per gram of dissected brain than C57 bl/10 mice (Maas, 1962), while no differences in brain 5-HT were found when C57B1/6J and DBA/2J mice were compared (Ho et al., 1975). Of particular relevance to the present studies, ddY, ICR, DBA, C3H and C57BL/6 mice were directly compared for HTR induced by tryptamine, and large strain differences were found such that all DBA mice exhibited marked tryptamine-elicited HTR, while only 1 of 20 ddY mice exhibited HTR, and there was no correlation between HTR and tryptamine levels in the brain (Yamada et al., 1987). Most recently, C57BL/6 and DBA/2 mice were both successfully trained to discriminate clozapine from saline, but the non-selective 5-HT₂ receptor antagonist ritanserin elicited clozapine-like stimulus effects only in C57BL/6 mice (Porter et al., 2008), perhaps implying differences in 5-HT₂ receptor expression and function in these two strains. Further research in this area is clearly warranted in order to help synthesize disparate results across laboratories utilizing distinct mouse strains.

In summary, these data suggest that the use of phenethylamine-derived hallucinogens as potential therapeutics may be limited not only by their abuse potential,
but also by the rapid development of tolerance which would likely be maintained even if a patient were switched to a different 5-HT$_{2A}$ agonist medication from a distinct structural class. On the other hand, these experiments imply that tryptamine-derived hallucinogens might have a reduced potential for tolerance development, as compared to phenethylamine-derived 5-HT$_{2A}$ agonists. As research into the therapeutic utility of hallucinogenic drugs proceeds (Nichols, 2014), further characterization of the acute and chronic pharmacological effects of these substances from a basic sciences perspective will likely be required to guide drug development and chronic treatment protocols.

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Authorship Contributions

Participated in research design: Smith and Fantegrossi

Conducted experiments: Smith, Bailey and Williams

Performed data analysis: Smith, Bailey, Williams and Fantegrossi

Wrote or contributed to the writing of the manuscript: Smith, Bailey, Williams and Fantegrossi


Footnotes

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LEGENDS FOR FIGURES

Figure 1. Structures of the phenethylamine-derived (top) and tryptamine-derived (bottom) hallucinogens used in these studies.

Figure 2. Biphasic dose-dependent effects of $R$(-)-DOI (filled circles), 2C-T-7 (open circles), DPT (filled diamonds) and DIPT (open diamonds) on head twitch behavior in NIH Swiss mice. Asterisks indicate significant differences from saline. Abscissae: Dose of drug, expressed as mg/kg on a log scale. Ordinates: Mean head twitches recorded over a 10 minute observation period.

Figure 3. Tolerance development to HTR elicited by daily administration of the most effective doses of DOI (filled circles) and 2C-T-7 (open circles), but not to HTR induced by the most effective doses of DPT (filled diamonds) or DIPT (open diamonds). Asterisks indicate significant differences from HTR observed on day 1, while hash marks indicate significant differences from HTR quantified the previous day. Abscissae: Consecutive day of drug administration. Ordinates: As described in Figure 2.

Figure 4. Tolerance development to HTR elicited by different frequencies of 1.0 mg/kg DOI administration. For ease of comparison, data from daily administration of 1.0 mg/kg DOI have been replotted from Figure 3 (filled circles) without indications of statistical significance, while open circles represent administration of 1.0 mg/kg DOI every other day, and gray circles represent administration of 1.0 mg/kg DOI every seven days. All other graph properties as described in Figure 3.

Figure 5. Tolerance to DOI-elicited head twitch behavior is not surmounted by a higher DOI dose. Filled circles represent mice treated with saline on days 1-3 and tested with various DOI doses on day 4, while open circles represent mice treated with 1.0 mg/kg DOI on days 1-3,
then tested with various DOI doses on day 4. Asterisks indicate significant differences from HTR elicited by saline administration, while hash marks indicate significant differences from saline-treated for each DOI test dose. **Abscissa:** As described in Figure 2. **Ordinate:** As described in Figure 2.

**Figure 6.** Cross-tolerance to head twitch behavior is observed in mice treated with 1.0 mg/kg DOI on days 1-3, then tested with various doses of 2C-T-7 (left panel) or DPT (right panel) on day 4. As with DOI itself (Figure 5), cross-tolerance is insurmountable with increasing 2C-T-7 or DPT dose. All other graph properties as described in Figure 5.
Figure 3

Mean HTR (counts / 10 min) vs. Consecutive days for different treatments:
- 1 mg/kg R(-)-DOI
- 1 mg/kg 2C-T-7
- 3 mg/kg DPT
- 10 mg/kg DIPT

Significance markers:
- *: p < 0.05
- #: p < 0.01
Figure 5

The diagram shows the mean HTR (counts / 10 min) in response to different DOI doses (mg/kg, IP) for two treatment groups: Saline and 1 mg/kg DOI. The x-axis represents the DOI dose, ranging from SAL (control) to 10.0 mg/kg. The y-axis represents the mean HTR counts per 10 minutes.

- **Saline** (●) treatment group:
  - SAL: 0 count
  - 0.3 mg/kg: 0 count
  - 1.0 mg/kg: 12 count
  - 3.0 mg/kg: 10 count
  - 10.0 mg/kg: 8 count

- **1 mg/kg DOI** (○) treatment group:
  - SAL: 0 count
  - 0.3 mg/kg: 4 count
  - 1.0 mg/kg: 8 count
  - 3.0 mg/kg: 2 count
  - 10.0 mg/kg: 0 count

Significance markers:
- * indicates a significant difference compared to the control group (SAL).
- # indicates a significant difference between the two treatment groups.

The bars represent the standard error of the mean (SEM) for each group at each dose level.
Figure 6

Mean HTR (counts / 10 min)

Daily treatment
- Saline
- 1 mg/kg DOI

2C-T-7 dose (mg/kg, IP)

DPT dose (mg/kg, IP)

SAL
0.3 1.0 3.0 10.0
0 2 4 6 8 10 12 14 16

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

*  #