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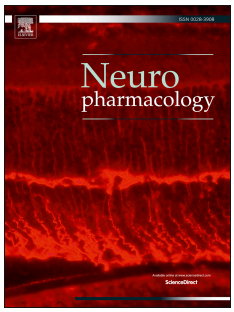

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Correlation between the potency of hallucinogens in the mouse head-twitch response assay and their behavioral and subjective effects in other species

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ABSTRACT

Serotonergic hallucinogens such as lysergic acid diethylamide (LSD) induce head twitches in rodents via 5-HT_{2A} receptor activation. The goal of the present investigation was to determine whether a correlation exists between the potency of hallucinogens in the mouse head-twitch response (HTR) paradigm and their reported potencies in other species, specifically rats and humans. Dose-response experiments were conducted with phenylalkylamine and tryptamine hallucinogens in C57BL/6J mice, enlarging the available pool of HTR potency data to 40 total compounds. For agents where human data are available (n = 36), a strong positive correlation (r = 0.9448) was found between HTR potencies in mice and reported hallucinogenic potencies in humans. HTR potencies were also found to be correlated with published drug discrimination ED_{50} values for substitution in rats trained with either LSD (r = 0.9484, n = 16) or 2,5-dimethoxy-4-methylamphetamine (r = 0.9564, n = 21). All three of these behavioral effects (HTR in mice, hallucinogen discriminative stimulus effects in rats, and psychedelic effects in humans) have been linked to 5-HT_{2A} receptor activation. We present evidence that hallucinogens induce these three effects with remarkably consistent potencies. In addition to having high construct validity, the HTR assay also appears to show significant predictive validity, confirming its translational relevance for predicting subjective potency of hallucinogens in humans. These findings support the use of the HTR paradigm as a preclinical model of hallucinogen psychopharmacology and in structure-activity relationship studies of hallucinogens. Future investigations with a larger number of test agents will evaluate whether the HTR assay can be used to predict the hallucinogenic potency of 5-HT_{2A} agonists in humans.

Keywords: psychedelic, psilocybin, mescaline, *N*,*N*-dimethyltryptamine, DMT, NBOMe, DOM, behavioral model, head shake

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INTRODUCTION

Serotonergic hallucinogens, such as psilocybin, (+)-lysergic acid diethylamide (LSD), and mescaline, remain an enigmatic class of agents. These compounds alter thought, perception, and mood without producing memory impairment, delirium, or addiction (Hollister 1968; Grinspoon and Bakalar 1979). Serotonergic hallucinogens can be divided into three main structural classes: tryptamines, ergolines, and phenylalkylamines. Tryptamine hallucinogens include psilocybin, *N*,*N*-dimethyltryptamine (DMT), *N*,*N*-diethyltryptamine (DET) and *N*,*N*-dipropyltryptamine (DPT). The prototypical hallucinogen LSD is the most important member of the tetracyclic ergoline class. The phenylalkylamine class can be subdivided into phenylethylamines, such as mescaline, 4-bromo-2,5-dimethoxyphenethylamine (2C-B) and 2,5-dimethoxy-4-iodophenethylamine (2C-I), and phenylisopropylamines ("amphetamines"), including 2,5-dimethoxy-4-methylamphetamine (DOM) and 4-bromo-2,5-dimethoxyamphetamine (DOB). In addition, the potency of phenylalkylamine hallucinogens has been found to increase markedly if an *N*-benzyl moiety is added (e.g., 25I-NBOMe) (Braden et al. 2006; Halberstadt and Geyer 2014) or if the ring-substituents are incorporated into rigid furan rings (e.g., bromo-Dragonfly, which is also known as DOB-DFLY) (Parker et al. 1998; Halberstadt et al. 2019b). Phenylalkylamine hallucinogens are relatively selective for the orthosteric binding site of 5-HT_2 subtypes, including 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} sites, whereas tryptamine and ergoline hallucinogens bind non-selectively to most 5-HT receptors (Nichols 2018). In recent years, an increasing number of studies have explored the potential therapeutic effects of serotonergic hallucinogens, with trials focusing on anxiety and depression (Grob et al. 2011; Griffiths et al. 2016; Ross et al. 2016), substance abuse (Johnson et al. 2014; Bogenschutz

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et al. 2015; Johnson et al. 2016), and obsessive-compulsive disorder (Moreno et al. 2006).

Given the likelihood that compounds from the hallucinogen class are therapeutically active, it is becoming more and more important to study the effects and mechanism of action of hallucinogens using preclinical models. Unfortunately, given the complexity, variety, and variability of the effects of hallucinogens in humans, it has been difficult to define animal behavioral tests for hallucinogenic activity (Glennon 1992; Halberstadt and Geyer 2010). Nevertheless, the use of animal models to study hallucinogen effects has enabled the evaluation of hypotheses regarding the neurochemical substrates of their action (reviewed: Fantegrossi et al. 2008a; Halberstadt 2015). The major contribution of 5-HT_{2A} receptor agonism to the effects of hallucinogens was first demonstrated using animal behavioral models (Leysen et al. 1982; Glennon et al. 1984; Wing et al. 1990). Importantly, this mechanism is consistent with evidence from human studies using pharmacological antagonism (Vollenweider et al. 1998; Valle et al. 2016; Kraehenmann et al. 2017; Preller et al. 2017). Animal behavioral models used to study hallucinogens can be divided into two groups: *(a)* models that test for behavior(s) that are analogous to hallucinogen effects in humans (high face validity); and *(b)* models that test for a behavior that does not have a direct human counterpart but appear to involve the same pharmacological mechanism of action as the hallucinogenic effects in humans (high construct validity) (Halberstadt and Geyer 2010). Animal models of hallucinogen effects can be further classified depending on whether they produce *qualitative* or *quantitative* data. All animal hallucinogen models provide a qualitative assessment of activity (i.e., does a test compound mimic the action of serotonergic hallucinogens?). However, certain models can also be used to assess the relative potencies of hallucinogens in a manner that has considerable predictive validity with respect to their potency in humans. The availability of animal models to assess the

in vivo potency of hallucinogens is especially important for studies of their structure-activity relationships (SAR).

Drug discrimination is a cross-species paradigm that is used to classify pharmacological agents based on perceived similarities in interoceptive stimulus effects. In this paradigm, animals are trained to press one of two levers after they receive a training drug, and must press the other lever after they receive saline (or another vehicle control). Once animals are trained to discriminate the training drug from vehicle, challenge experiments can be conducted with other drugs to evaluate whether their interoceptive stimulus effects are perceived as being similar to the cue produced by the training drug. Test agents that induce responding predominantly on the drug lever are said to fully substitute for the training drug. This assay has high pharmacological specificity and can distinguish compounds with different mechanisms of action. Many hallucinogens are capable of serving as discriminative stimuli in rats; their stimulus cues appear to be relatively uniform in nature because members of this drug class produce cross-generalization in drug discrimination studies (Glennon et al. 1983a; Winter et al. 2007; Nichols 2018). In addition to assessing whether test agents produce hallucinogen-like stimulus effects, drug discrimination studies can also be used to compare the relative potencies of hallucinogens. According to Glennon and colleagues, the potency of hallucinogens in the drug discrimination paradigm is significantly correlated with their potency in humans (Glennon et al. 1982a, 1983a, 1983c). Hence, the drug discrimination paradigm has shown considerable utility in studies of hallucinogen SAR (Glennon et al. 1983a; Nichols 2018).

There are some potential drawbacks associated with the use of drug discrimination methodologies to study hallucinogens. Drug discrimination studies require the training drug to be administered repeatedly, which has limited relevance to typical hallucinogen use patterns in

humans and can potentially result in neurochemical and behavioral adaptations (Benneyworth et al. 2008; Marona-Lewicka et al. 2011). Furthermore, drug discrimination studies cannot always reliably distinguish between hallucinogens and non-hallucinogenic 5-HT_{2A} agonists such as lisuride (White and Appel 1982a; Glennon and Hauck 1985; Fiorella et al. 1995a). Some hallucinogens also reportedly have particularly steep dose-response functions in drug discrimination studies, with complete generalization occurring only within a narrow range of doses, making it difficult to detect full-substitution (Glennon et al. 1983a). The latter phenomenon may explain why hallucinogens sometimes fail to elicit full-substitution in rats trained to discriminate hallucinogens (Koerner and Appel 1982; Monte et al. 1997; Helsley et al. 1998; Fantegrossi et al. 2008b; Gatch et al. 2011, 2017). Responses can also be influenced by additional behavioral changes mediated by off-target pharmacological effects of a test compound, complicating analysis.

Another popular hallucinogen behavioral model is known as the head-twitch response (HTR). The HTR is a high-frequency paroxysmal head rotation that occurs in rats and mice after 5-HT_{2A} receptor activation (Halberstadt et al. 2011; Canal and Morgan 2012). The HTR is commonly used as a behavioral proxy in rodents for human hallucinogen effects because it is one of only a few behaviors that can reliably distinguish hallucinogenic and non-hallucinogenic 5-HT_{2A} receptor agonists (Gonzalez-Maeso et al. 2007; Halberstadt and Geyer 2013). For example, LSD induces head twitches in mice and rats, whereas lisuride does not induce the behavior despite acting as a 5-HT_{2A} agonist (Gonzalez-Maeso et al. 2007). Although the HTR is usually assessed by direct observation, we have developed an electronic assessment technique that can detect the behavior with high sensitivity and specificity (Halberstadt and Geyer 2013). These methods have shown great utility for studying novel hallucinogens (Halberstadt and Geyer

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2014; Nichols et al. 2015; Brandt et al. 2016, 2017b, 2019; Halberstadt et al. 2016, 2017a, 2019a, 2019b, 2019c).

Given the potential complexities associated with the drug discrimination paradigm, there is a need for other animal behavioral models that can be used to study the SAR of serotonergic hallucinogens. It may be possible to use the HTR assay for this purpose, but it is not clear whether the paradigm can provide a reliable quantitative assessment of hallucinogen potency. Therefore, studies were conducted to evaluate whether a correlation exists between the potency of hallucinogens in the mouse HTR paradigm and reported potencies (behavioral and subjective) in other species, specifically rats and humans. One set of experiments tested whether a correlation exists between HTR potency in mice and reported hallucinogenic potency in humans. Additional studies were conducted to assess the relationship between ED_{50} values in HTR and drug discrimination studies. The results support the use of the HTR paradigm in studies investigating the SAR of hallucinogens; future work will be undertaken to test whether the generated linear regression models can be used to predict human potency based on HTR data

METHODS

Animals

Male C57BL/6J mice (6–8 weeks old) obtained from Jackson Laboratories (Bar Harbor, ME, USA) were housed in a vivarium at the University of California San Diego, an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)-approved animal facility that meets all federal and state requirements for care and treatment of laboratory animals. Mice were housed up to four per cage in a climate-controlled room on a reverse-light cycle

(lights on at 1900 h, off at 0700 h) and were provided with *ad libitum* access to food and water, except during behavioral testing. Testing was conducted between 1000 and 1800 h. All animal experiments were carried out in accordance with National Institute of Health guidelines and were approved by the University of California San Diego Institutional Animal Care and Use Committee.

Drugs

N,*N*-Diethyltryptamine (DET) fumarate, 2,5-dimethoxy-4-methylamphetamine (DOM) hydrochloride, *N*,*N*-dimethyltryptamine (DMT) fumarate, *N*,*N*-dipropyltryptamine (DPT) hydrochloride, 4-ethyl-2,5-dimethoxyamphetamine (DOET) hydrochloride, *R*-(-)-4-iodo-2,5-dimethoxyamphetamine (*R*-(-)-DOI) hydrochloride, 3,4-methylenedioxyamphetamine (MDA) hydrochloride, *R*-(-)-3,4-methylenedioxyamphetamine (*R*-(-)-MDA) hydrochloride, and 2,5-dimethoxy-4-nitroamphetamine (DON) hydrochloride were obtained from the National Institute on Drug Abuse (Rockville, MD, USA). 2,5-Dimethoxy-α-ethyl-4-methylphenylethylamine (α-Et-2C-D, ariadne) hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO, USA). 5-Methoxy-α-methyltryptamine hydrochloride was obtained from the NIMH Chemical Synthesis and Drug Supply Program (RTI International, Research Triangle Park, NC, USA). 4-Butyl-2,5-dimethoxyamphetamine (DOBU) freebase and *N*-(2-hydroxybenzyl)-4-iodo-2,5-dimethoxyphenethylamine (25I-NBOH) hydrochloride were obtained from Cayman Chemical (Ann Arbor, MI, USA). 4-Chloro-2,5-dimethoxyamphetamine (DOC) hydrochloride, *N*,*N*-diisopropyltyptamine (DIPT) hydrochloride, *N*-(2-methoxybenzyl)-2,5-dimethoxy-4-methylphenethylamine (25D-NBOMe) hydrochloride were available from previous studies

conducted in our laboratories. The identity and analytical purity of the test substances were confirmed by mass spectrometry and nuclear magnetic resonance spectroscopy. Test substances were dissolved in isotonic saline and injected intraperitoneally (IP) at a volume of 5 mL/kg.

Head-twitch response studies

The HTR was assessed using a head-mounted magnet and a magnetometer detection coil (Halberstadt and Geyer 2013, 2014). Briefly, mice were anesthetized, a small incision was made in the scalp, and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a two-week recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between sessions to avoid carryover effects. Test compounds were injected immediately prior to testing and then HTR activity was recorded in a glass cylinder surrounded by a magnetometer coil for 30 min. Coil voltage was low-pass filtered (2–10 kHz cutoff frequency), amplified, digitized (20 kHz sampling rate, 16-bit ADC resolution), and saved to disk using a Powerlab/8SP data acquisition system with LabChart software ver. 7.3.2 (ADInstruments, Colorado Springs, CO, USA), then filtered off-line (40–200 Hz band-pass). Head twitches were identified by trained personnel based on the following criteria: 1) sinusoidal wavelets; 2) evidence of at least three sequential head movements (usually exhibited as bipolar peaks) with frequency ≥ 40 Hz; 3) amplitude exceeding the level of background noise; 4) duration < 0.15 s; and 5) stable coil voltage immediately preceding and following each response.

After magnet implantation, mice were tested in multiple HTR experiments, for up to 4–5 months. Repeated administration of hallucinogens at weekly intervals does not produce

tolerance in the HTR paradigm (Gewirtz and Marek 2000; Rangel-Barajas et al. 2014; Smith et al. 2014). We have confirmed that experimental results obtained using these procedures are stable and replicable over time, both within single cohorts of mice and across multiple independent cohorts (unpublished observations). Experiments were performed between-subjects, with pseudorandomized group assignments, which further reduces the likelihood of carryover effects.

Data analysis

Head-twitch counts were analyzed using one-way analyses of variance (ANOVA). Post hoc pairwise comparisons were performed using Dunnett's test. Significance was demonstrated by surpassing an α-level of 0.05. Half maximal effective doses (ED_{50} values) and 95% confidence intervals (95% CI) for HTR dose-response experiments were calculated by nonlinear regression. Linear regression was used to assess the relationship between ED_{50} values in HTR experiments and potency measures in other species. Statistical and regression analyses were performed using Prism 7.00 (GraphPad Software, San Diego, CA, USA).

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RESULTS

To date, we have tested a variety of hallucinogens in the HTR paradigm (Halberstadt and Geyer 2013, 2014; Brandt et al. 2017b; Klein et al. 2018; Halberstadt et al. 2019a, 2019b). Additional studies were conducted to increase the size of our existing data set. As shown in Table 1, hallucinogens from the phenylalkylamine (DOM, DOET, DOBU, DON, DOC, *R*-(–)-

DOI, MDA, *R*-(–)-MDA, α-Et-2C-D, 25D-NBOMe, and 25I-NBOH) and tryptamine (DMT, DET, DPT, DIPT, and 5-MeO-AMT) structural classes were tested in male C57BL/6J mice and were found to induce head twitches. Similar to other hallucinogens (Fantegrossi et al. 2006, 2008b, 2010; Halberstadt et al. 2019a, 2019b), the responses observed in most of the experiments were non-monotonic with a marked response decrement occurring at high doses (see Figure 1).

A linear regression analysis was performed to determine whether a relationship exists between the potency of hallucinogens in humans and their effects on head-twitch. In our HTR studies with serotonergic hallucinogens, potency is assessed by calculating the dose required to induce a half-maximal response (ED_{50}), which facilitates potency comparisons between compounds. In addition to the HTR data in Table 1, relevant data for other compounds are available from our published and unpublished studies (see Table 2). The human potency data used in the analysis (Table 3) were collected from several sources, including clinical trials, results published by Dr. Alexander Shulgin and Dr. Daniel Trachsel, as well as from websites such as erowid.com and psychonautwiki.org. Human hallucinogenic potency data from those sources are typically reported as a range of effective doses; in those cases, the arithmetic mean of the dose range was calculated and then converted to a total dose in moles. For 36 hallucinogens, a statistically significant positive correlation was found between HTR-derived ED_{50} values and their potency in humans ($r = 0.9448$ [95% CI: 0.8937–0.9717], $p < 0.0001$; Figure 2). Importantly, the hallucinogens included in this analysis cover structurally distinct compounds and include a wide range of human potencies, spanning almost four orders of magnitude.

An additional series of experiments evaluated whether HTR-derived ED_{50} values are correlated with reported ED_{50} values from rat drug discrimination studies. The rat drug discrimination literature contains an extensive dataset of stimulus generalization ED_{50} values for

hallucinogens. One regression analysis was performed using data from rat drug discrimination studies conducted by Nichols and colleagues, who used 0.08 mg/kg LSD as the training drug for the evaluation of the 16 test drugs in Table 4. Although typically a single ED_{50} value was available for each compound, multiple values were occasionally reported in the literature; in those cases, the arithmetic mean was calculated. The linear regression analysis confirmed that ED_{50} values from HTR studies are robustly and significantly correlated (r = 0.9484 [95% CI: 0.8542–0.9823], n = 16, $p < 0.0001$) with drug discrimination ED_{50} values from LSD-trained rats (Figure 3).

Hallucinogens exhibit varying degrees of receptor selectivity and those pharmacological differences can influence their interoceptive stimulus effects, meaning that certain training drugs may produce idiosyncratic results. In contrast to LSD, which produces a compound discriminative stimulus in rats, the stimulus effects of DOM are less complex (Fiorella et al. 1995b). Although strong evidence supports the hypothesis that 5-HT_{2A} activation is an essential component of the stimulus cues evoked by DOM and LSD (Glennon et al. 1983d, 1984), dopamine D_2 receptors may play a secondary role in the response to LSD (Appel et al. 1982a, 1982b; Meert et al. 1989; Marona-Lewicka et al. 2005). Because the HTR induced by hallucinogens appears to be solely mediated by 5-HT_{2A} activation (Schreiber et al. 1995; Halberstadt et al. 2011) — similar to the discriminative stimulus effects of DOM and in contrast to the LSD stimulus cue — we also evaluated the relationship between ED_{50} values in the HTR paradigm and in rats trained to discriminate DOM. This analysis was performed using drug discrimination data published by Glennon and colleagues (Table 5), who trained rats to discriminate 1.0 mg/kg DOM from vehicle (Glennon et al. 1983a, 1988), resulting in relevant data for 21 compounds. Similar to the results from LSD-trained rats, HTR ED_{50} values are

robustly and significantly correlated (r = 0.9564 [95% CI: 0.8937–0.9825], n = 21, $p < 0.0001$) with drug discrimination ED_{50} values from rats trained to discriminate DOM (Figure 3).

In addition to the correlation between HTR- and drug-discrimination-derived ED_{50} values, we noted that some of the potency estimates from head-twitch and drug discrimination studies are nearly identical. For example, LSD induces head twitches in mice with an ED_{50} of 52.9 µg/kg (132.8 nmol/kg) (Halberstadt and Geyer 2013), whereas LSD reportedly substitutes in DOM-trained rats with an ED_{50} of 52 µg/kg (130.5 nmol/kg) (Glennon et al. 1983c). Likewise, the potency of DOM in the HTR experiment from Table 1 (ED_{50} = 1.75 µmol/kg; Table 1) is virtually identical with the potency in the drug discrimination experiment summarized in Table 5 (ED_{50} = 1.79 µmol/kg; Young et al. 1980). To quantify the degree of similarity between drug discrimination and head-twitch data, normalized difference scores were calculated using the formula:

$$\left(\frac{\mathrm{ED}_{50\,HTR} - \mathrm{ED}_{50\,DD}}{\mathrm{ED}_{50\,DD}}\right) \times 100$$

where $ED_{50\,HTR}$ is the median effective molar dose for head-twitch and $ED_{50\,DD}$ is the median effective molar dose for drug discrimination. For the 21 compounds tested in DOM-trained rats, the average normalized difference score was 31.6 ± 5.7% (mean ± SEM; range: 1.8% to 78.6%). For the 16 compounds tested in LSD-trained rats, the average normalized difference score was 330.2 ± 94.4% (range: 22.7% to 1,215.4%). Although the congruence was clearly higher when DOM was used as the training drug compared to LSD, it is difficult to compare the two data sets directly because their composition is heterogeneous. To address this confound, the analyses were repeated using only the subset of compounds that were present in both data sets (2C-B, DET, DMT, DOB, DOET, DOM, LSD, MDA, and mescaline); for those nine compounds, the difference scores were lower in DOM-trained rats (27.3 ± 9.4%) compared to LSD-trained rats

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(99.8 ± 25.7%; t_{16} = 2.608, p = 0.019). We also examined whether the three potency estimates for those nine compounds are correlated using linear regression; the HTR data are correlated with the drug discrimination data obtained using LSD (r = 0.9544 [95% CI: 0.7926–0.9906]) and DOM (r = 0.975 [95% CI: 0.8819–0.9949]) as training drugs. As expected, the ED_{50} values from LSD- and DOM-trained rats are highly correlated (r = 0.9722 [95% CI: 0.8692–0.9943]). Notably, however, the strength of the correlation between the HTR and drug discrimination data is just as strong as the correlation between the two sets of drug discrimination data. Because these analyses included a relatively small number of compounds, the results may not generalize to other members of the hallucinogen drug class. However, many of the compounds are prototypical hallucinogens (i.e., those from which many other hallucinogens are structurally related), increasing the likelihood that the relationship will hold true for other compounds. In summary, the results demonstrate that HTR studies in mice and drug discrimination studies in rats yield remarkably equivalent estimates of hallucinogen potency.

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DISCUSSION

The goal of the present investigation was to assess the reliability and cross-species translatability of hallucinogen potency estimates generated using the HTR paradigm. While head-twitch data have traditionally been used in a *qualitative* manner (i.e., to assess whether compounds produce LSD-like behavioral effects), these studies evaluated whether the HTR assay can also be used to generate *quantitative* measures of hallucinogen activity. Given those goals, we first conducted 16 dose-response experiments to enlarge the available pool of HTR data to 40 total compounds. Compounds were selected based on the availability of relevant data

in rats and humans, as well as by the desire to maximize the range of potencies and structural diversity. These studies confirmed that hallucinogens from multiple structural classes reliably induce head twitches in mice. Next, linear regression was used to evaluate the relationship between median effective doses in the HTR paradigm and potencies in other species including humans. Notably, a strong positive correlation was found between HTR potency in mice and reported hallucinogenic potency in humans. Head-twitch potencies were also found to be correlated with drug discrimination-derived ED_{50} values for substitution in rats trained with either LSD or DOM. All three of the behavioral effects included in the present investigation (HTR in mice, hallucinogen discriminative stimulus effects in rats, and psychedelic effects in humans) have been linked to 5-HT_{2A} activation (Halberstadt 2015; Nichols 2016). Incredibly, hallucinogens induce those three behaviors with remarkably consistent relative potencies. The shared role of 5-HT_{2A} activation in HTR in mice, hallucinogenic drug discrimination in rats and hallucinogenic effects in humans, support strong construct validity of these experimental paradigms. Likewise, the predictive validity of the HTR in mice and drug discrimination are supported by the high correlation between potencies in these paradigms with reported human potencies of hallucinogens. Based on these results, mouse HTR studies with hallucinogens clearly have cross-species translational relevance. Although head twitches are thought to have limited value as a model of hallucinogenesis (i.e., poor face validity) (Canal and Morgan 2012), these findings support the use of the HTR paradigm for predicting potencies of compounds to induce hallucinogenic effects in humans and in SAR studies of hallucinogens. Future investigations will more thoroughly test the utility of HTR studies to understand the pharmacological interactions underlying the effects of hallucinogens in humans.

These studies evaluated whether the HTR assay can provide a reliable assessment of hallucinogen potency in humans. As shown in Figure 2, the positive linear correlation between human hallucinogenic potency and HTR ED_{50} values is extremely robust using linear regression (r^2 = 0.8927). According to a previous study (Glennon et al. 1982a), there is a similar relationship between human potency and drug discrimination-derived ED_{50} values for 14 amphetamine hallucinogens (r^2 = 0.93). Although the coefficient of determination was slightly higher for drug discrimination compared to HTR, it is difficult to compare the results directly because the HTR analysis included compounds from multiple structural classes whereas the study by Glennon et al. (1982a) looked only at amphetamine hallucinogens. To simplify comparison of the relationship between human potency and ED_{50} values derived from head twitch and drug discrimination, we repeated the analyses using the compounds that are shared between Tables 3 and 4 (AL-LAD, DOB-DFLY, 2C-B, 2C-B-FLY, 2C-I, DET, DMT, DOB, DOB-FLY, DOET, DOM, LSD, *SS*-LSZ, MDA, and mescaline). For those 15 compounds, the coefficient of determination was higher for HTR (r^2 = 0.9602) than for drug discrimination (r^2 = 0.9064; Figure S1). In conclusion, the HTR assay provides a quantitative assessment of hallucinogen potency that closely parallels results obtained in humans and in drug discrimination studies. Finding that ED_{50} values for head-twitch are robustly correlated with other measures of activity in humans and rats supports the predictive validity of this paradigm to assess potency relationships between hallucinogens in SAR studies.

The fact that HTR studies can quantify hallucinogen potency with about the same sensitivity as drug discrimination is rather remarkable because it can be challenging to estimate the potency of hallucinogens based on HTR data. The location of the top and bottom of the dose-response curve in HTR studies is not fixed, which can complicate curve fitting using a

regression model. Mice emit head twitches spontaneously (Dursun and Handley 1996) and the baseline rate shows some variability; likewise, the peak HTR rate depends on the particular compound being tested and may be linked to 5-HT_{2A} agonist efficacy (Vickers et al. 2001). In addition, dose-response curves in the HTR assay are non-monotonic, with ascending and descending phases. There is evidence that the two phases of the curve may be mediated by different receptors, with 5-HT_{2A} activation driving the ascending phase and 5-HT_{2C} activation driving the descending phase (Fantegrossi et al. 2010). Nevertheless, despite these complexities, HTR potencies were robustly correlated with activity in rats and humans.

Certain hallucinogens induced head twitches in mice and DOM-like stimulus effects in rats *with potencies that are nearly identical*. Although we expected to find a close relationship between hallucinogen potencies in HTR and drug discrimination, the high degree of similarity between potencies in the two paradigms is remarkable. The potency equivalence, however, may simply reflect the fact that the HTR peaks within the dosage range that is optimal for drug discrimination training. Training doses for drug discrimination must be carefully selected; doses that are too low may not be discriminable, whereas high doses may impair task performance. For LSD, typical training doses range from 80 µg/kg to 120 µg/kg; lower doses are discriminated poorly and higher doses produce considerable behavioral disruption (Cameron and Appel 1973; White and Appel 1982b). Likewise, common doses for DOM training include 1.0 mg/kg (Young et al. 1980) and 1.5 mg/kg (Silverman and Ho 1980). By coincidence, the HTR induced by LSD and DOM peaks at about 100 µg/kg and 1 mg/kg, respectively. Increasing or reducing the training dose in drug discrimination shifts the generalization curve rightward or leftward, respectively (Stolerman et al. 2011), so the degree of potency equivalence observed in our

analyses is probably a consequence of the specific doses used for training in the drug discrimination studies.

Evidence is accumulating that serotonergic hallucinogens from multiple structural classes reliably induce head twitches. Although 2C-I and 2C-B failed to induce the HTR in one study (Moya et al. 2007), those agents were active in follow-up investigations (Halberstadt and Geyer 2014; Elmore et al. 2018; Halberstadt et al. 2019b). Nevertheless, although hundreds of hallucinogens have been discovered to date (Shulgin and Shulgin 1991, 1997; Trachsel et al. 2013), only a small subset have been evaluated in the HTR paradigm. As far as we are aware, these are the first mouse HTR studies conducted with DOET, DOBU, DON, DOC, α-Et-2C-D, MDA, *R*-(-)-MDA, 25D-NBOMe, 25I-NBOH, and DET. Some of the other compounds listed in Table 1 were tested previously by other groups; the present experiments serve to validate the earlier findings. For example, *R*-(-)-DOI (Darmani et al. 1990; Fantegrossi et al. 2010), DOM (Gonzalez-Maeso et al. 2007), DMT (Corne and Pickering 1967; Gonzalez-Maeso et al. 2007; Carbonaro et al. 2015), DPT (Fantegrossi et al. 2008b), DIPT (Smith et al. 2014; Carbonaro et al. 2015), and 5-MeO-AMT (May et al. 2006; Abiero et al. 2019) have been previously shown to induce head twitches in mice. Overall, the results of these studies confirm that 40 hallucinogens from multiple structural classes induce head twitches in mice, demonstrating the high predictive validity of this model.

One complexity associated with the use of the HTR assay to study hallucinogens is that this behavioral paradigm is potentially susceptible to false-positive responses. For example, rolipram, [Met^5]enkephalin, and icilin induce head twitches through non-5-HT_{2A} receptor-dependent mechanisms (Drust et al. 1981; Przegalinski et al. 1981). Indirect 5-HT_{2A} agonists such as fenfluramine, *p*-chloroamphetamine (PCA), and 5-hydroxytryptophan (5-HTP) induce

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head twitches in rodents (Corne et al. 1963; Singleton and Marsden 1981; Darmani 1998) but do not act as hallucinogens in humans (van Praag et al. 1971; Brauer et al. 1996; Turner et al. 2006), However, overdoses of compounds that increase serotonin (5-HT) release can result in 5-HT syndrome, which sometimes includes hallucinations (Birmes et al. 2003; Evans and Sebastian 2007). Nevertheless, it is highly unlikely that any of the compounds tested in the present studies are acting as false-positives in the HTR assay. All of the compounds act as hallucinogens in humans and the vast majority produce DOM- and/or LSD-like stimulus effects in rats. In addition, virtually all of the compounds have moderate to high binding affinity for the 5-HT_{2A} receptor and act as agonists in functional assays; compounds capable of activating the 5-HT_{2A} receptor directly are highly unlikely to produce false-positive responses in the HTR assay.

Some of the compounds in Table 1 deserve comment because they were not included in the human dose correlation due to uncertainties regarding their effective dose range. DIPT is reportedly active at 25–50 mg (Shulgin and Carter 1980) or 25–100 mg (Shulgin and Shulgin 1991). However, at those doses, DIPT distorts auditory perception and does not produce psychedelic effects. Higher doses of DIPT produce more typical LSD-like reactions, but the effective dose range for those effects is not well defined. Likewise, only limited data has been reported regarding the activity of DON in humans. Although DON is reportedly active at oral doses of 4.5 mg (Gomez-Jeria et al. 1986), not much else is known about its effective dose range, so it was not included in the human potency analysis.

Our studies confirm that α-Et-2C-D (also known as BL-3912, 4C-D, or ariadne), the α-ethyl homologue of DOM, induces head twitches in mice. Although α-Et-2C-D does not act as a psychedelic drug at p.o. doses up to 32 mg (Shulgin and Shulgin 1991), subjects administered higher doses (up to 270 mg) reportedly experienced “euphoria and other LSD-like effects”

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(Winter 1980). Consistent with our results, α-Et-2C-D produces full substitution in rats trained to discriminate DOM (Glennon et al. 1983b) and LSD (Winter 1980). However, because the available information on human doses are limited, we chose not to include it in the correlation analyses and linear regression between human hallucinogenic potency and HTR. Although α-Et-2C-D did not induce head or body shaking in cats (Rusterholz et al. 1978), the dose used (1 mg/kg IP) was probably too low to produce a behavioral response. Indeed, we did not observe a significant increase in HTR counts unless mice were treated with 6 mg/kg IP.

Although there was some variability in the data, the strength of the correlation in Figure 2 is notable given how difficult it is to accurately quantify the potency of hallucinogens in humans. As noted above, effective or typical dosage ranges are usually provided, with the lower value representing the minimum dose that produces clearly defined effects and the upper value representing the maximally active level. These dose ranges, however, are really rough approximations. Furthermore, there is frequently considerable variability in human potency data due to the subjective nature of the assessment and the inherent methodological weaknesses associated with non-institutional studies of hallucinogens. Another confounding variable is the route of administration; most of the human potency data used in the present study was based on the oral route of administration, although in several cases p.o. activity is poor and thus alternate routes are used (including sublingual administration and parenteral injections). Despite this limitation, the correlation between HTR and human subjective potency in the present study was high. The strength of the correlation is also notable because the analysis was performed using a mixture of phenylalkylamine, tryptamine, and ergoline hallucinogens. In contrast to phenylalkylamine hallucinogens, which are relatively selective for 5-HT_2 sites, tryptamine and ergoline hallucinogens also produce behavioral effects via 5-HT_{1A} and potentially other receptors

(Halberstadt and Geyer 2011). In one clinical study, pretreatment with the mixed 5-$HT_{1A/1B}$/β-adrenergic antagonist pindolol was reported to markedly potentiate the subjective effects of DMT (Strassman 1996). Since DMT has low affinity for β-adrenergic receptors but nM affinity at 5-HT_{1A} (Deliganis et al. 1991; Ray 2010), these results can be interpreted to suggest that interactions with the 5-HT_{1A} receptor dampen the action of DMT at the 5-HT_{2A} receptor. Indeed, according to our recent studies, the ergoline derivative lysergic acid morpholide (LSM-775) does not induce head twitches unless 5-HT_{1A} receptors are blocked by pretreatment with the antagonist WAY-100,635 (Brandt et al. 2018). We have also shown that 5-HT_{1A} receptor interactions influence the potency of *N*,*N*-diallyltryptamines in the HTR paradigm (Klein et al. 2018). Although the extent to which the 5-HT_{1A} receptor influences the *in vivo* psychopharmacology of hallucinogens is still not entirely clear, these interactions appear to play a similar role in humans and mice given the robust correlation between hallucinogen effects in these species.

There are reportedly significant strain differences in the response of mice to 5-HT_{2A} receptor agonists. The magnitude of the HTR induced by DOI is known to vary across different strains of mice (Weiss et al. 2003; Canal et al. 2010; Rangel-Barajas et al. 2014). For example, the number of head twitches induced by DOI is dramatically larger in DBA/2J mice compared to C57BL/6J mice (Canal et al. 2010). Likewise, C57BL, DBA, ICR, C3H and ddY mice exhibit varying degrees of sensitivity to tryptamine-induced head twitches (Sugimoto et al. 1986; Yamada et al. 1987). Because of these differences, the extent to which our findings with C57BL/6J mice are relevant to other strains is not clear. Future studies will examine whether HTR potencies in other mouse lines are also correlated with activity in humans and rats.

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One unanswered question is whether a linear regression model based on HTR data could be used to estimate the potency of novel 5-HT_{2A} agonists in humans. Given the robust relationship between potency in the HTR assay and activity in humans, follow-up studies are necessary to test the predictive validity of HTR data. Although the present studies were conducted using a structurally diverse series of hallucinogens, it cannot be excluded that the use of a more structurally homogeneous group of hallucinogens would have reduced the variance and increased the coefficient of determination in our regression models. Although interactions with 5-HT_{2A} receptors are known to play an important role in determining the behavioral potency of hallucinogens (Glennon et al. 1984; Luethi and Liechti 2018), factors such as distribution, intrinsic clearance, metabolism, and off-target interactions will also influence their behavioral potency. Although the latter factors are not necessarily uniform within a particular chemical scaffold, they are likely to vary to a much greater extent across different structural classes of hallucinogens. Therefore, although it may be possible to develop a single regression model to predict the potency of hallucinogens in humans based on their activity in mice, it may be more effective to develop discrete models for tryptamines, ergolines, and phenylalkylamines. Although additional HTR data is required to develop suitable regression models, the present studies provide clear evidence supporting the feasibility of this approach.

In summary, these studies confirm that the potency of hallucinogens in the HTR paradigm are highly correlated with their activity in rats and humans. Based on these findings, it may be possible to use HTR assay in mice as a preclinical model of the pharmacological interactions underlying the effects of hallucinogens in humans. It must be emphasized, however, that the HTR assay is not actually modeling the psychedelic or subjective effects of hallucinogens. Despite the robust evidence collected in these experiments, follow-up studies are

necessary to address many unanswered questions regarding the pharmacology of the HTR and its utility as a behavioral model. For example, although 5-HT_{2A} receptor activation appears to mediate the HTR induced by hallucinogens (Schreiber et al. 1995; Gonzalez-Maeso et al. 2007; Halberstadt et al. 2011), there is also evidence that the expression of head-twitch behavior is modulated by activation of 5-HT_{1A} (Darmani et al. 1990; Klein et al. 2018) and 5-HT_{2C} receptors (Vickers et al. 2001; Canal et al. 2010; Fantegrossi et al. 2010; Canal et al. 2013). It is also not clear why certain compounds — most notably lisuride — fail to induce the HTR in mice despite acting as 5-HT_{2A} receptor agonists. Although lisuride appears to be a functionally-selective agonist and may not be capable of activating the specific 5-HT_{2A}-coupled signaling pathways that drive HTR expression (Gonzalez-Maeso et al. 2007), other factors may also be involved (Marona-Lewicka et al. 2002; Cussac et al. 2008). Finally, given the increasing focus on the therapeutic efficacy of hallucinogens in humans, it is intriguing to note that these compounds promote neural plasticity and neurogenesis in rodents (Jones et al. 2009; Lima da Cruz et al. 2018; Ly et al. 2018) and produce positive effects in preclinical models relevant to anxiety, depression, and post-traumatic stress disorder (Catlow et al. 2013; Buchborn et al. 2014; Cameron et al. 2018; Cameron et al. 2019). It would be interesting to determine whether correlations also exist between those effects of hallucinogens and their activity in the HTR paradigm. Although additional studies are clearly warranted, the present findings clearly show that the mouse HTR assay can be used to generate data that are relevant and translatable to other species.

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Table 1. Summary of the head twitch response (HTR) data from dose-response experiments.

Compound	ANOVA	Time (min)	Dose (mg/kg)	*N*	HTR Counts (Mean ± SEM)	ED_{50} mg/kg (95% CI)	ED_{50} µmol/kg (95% CI)
DOM[a]	F(5,23)=17.1, p<0.0001	30	0	5	4.6 ± 0.9	0.43 (0.30–0.62)	1.75 (1.22–2.52)
			0.1	5	17.4 ± 1.7		
			0.3	5	52.6 ± 8.0		
			1	5	118.0 ± 14.5 **		
			3	5	126.8 ± 24.4 **		
			10	4	27.0 ± 8.6		
DOET[a]	F(4,22)=7.68, p=0.0005	30	0	6	7.5 ± 1.7	0.20 (0.11–0.36)	0.77 (0.43–1.37)
			0.1	5	34.8 ± 8.7		
			0.3	5	86.2 ± 9.6 *		
			1	6	122.0 ± 29.3 **		
			3	5	71.0 ± 14.2 *		
DOBU[c]	F(4,26)=36.68, p<0.0001	30	0	7	6.6 ± 1.9	1.17 (0.99–1.38)	4.64 (3.92–5.50)
			0.3	6	13.0 ± 2.5		
			1	6	46.3 ± 6.3 **		
			3	6	111.8 ± 12.4 **		
			10	6	25.5 ± 7.7		
DOC[a]	F(3,18)=23.92, p<0.0001	30	0	6	8.2 ± 1.4	0.32 (0.21–0.47)	1.19 (0.80–1.77)
			0.3	5	64.0 ± 2.0 **		
			1	6	125.3 ± 13.5 **		
			3	5	89.8 ± 16.3 **		
R-(-)-DOI[a]	F(4,23)=25.14, p<0.0001	30	0	7	9.4 ± 1.7	0.24 (0.17–0.33)	0.66 (0.47–0.93)
			0.1	5	27.2 ± 3.1		
			0.3	6	89.2 ± 12.0 **		
			1	5	132.0 ± 17.0 **		
			3	5	97.0 ± 12.8 **		
DON[a]	F(4,20)=7.65, p=0.0007	30	0	5	6.4 ± 2.3	1.31 (0.70–2.43)	4.72 (2.54–8.77)
			0.3	5	17.4 ± 3.4		
			1	5	43.0 ± 1.2		
			3	5	102.8 ± 6.9 **		
			10	5	96.2 ± 34.9 **		
MDA[a]	F(4,22)=3.53, p=0.0227	30	0	6	6.2 ± 1.4	1.46 (0.72–2.98)	6.77 (3.33–13.8)
			0.3	5	6.6 ± 1.2		
			1	5	9.6 ± 1.1		
			3	6	16.7 ± 2.4 *		
			10	5	15.0 ± 5.1		

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R-(-)-MDA[a]	*F*(4,23)=8.11, *p*=0.0003	30	0 0.3 1 3 10	6 5 6 6 5	7.8 ± 1.4 8.6 ± 1.5 15.5 ± 3.2 30.0 ± 6.2 ** 27.6 ± 2.8 **	1.45 (0.82–2.54)	6.71 (3.82–11.8)
α-Et-2C-D[a]	*F*(4,23)=3.95, *p*=0.0139	30	0 3 6 12 24	5 6 5 6 6	9.4 ± 2.6 14.2 ± 4.1 25.4 ± 4.9 * 26.0 ± 2.2 * 14.5 ± 4.0	4.09 (2.70–6.22)	15.8 (10.4–23.9)
25D-NBOMe[a]	*F*(5,28)=10.57, *p*<0.0001	30	0 0.03 0.1 0.3 1 3	6 5 6 6 6 5	6.2 ± 0.8 12.2 ± 2.9 39.2 ± 7.5 45.0 ± 12.5 96.7 ± 17.2 ** 85.6 ± 18.9 **	0.23 (0.12–0.43)	0.64 (0.34–1.22)
25I-NBOH[a]	*F*(5,24)=8.90, *p*<0.0001	30	0 0.03 0.1 0.3 1 3	5 5 5 5 5 5	4.4 ± 1.4 22.8 ± 3.1 48.8 ± 4.7 * 95.6 ± 14.2 ** 64.0 ± 12.6 ** 44.4 ± 17.0	0.085 (0.055–0.132)	0.19 (0.12–0.29)
5-MeO-AMT[a]	F(4,22)=10.65, p<0.0001	30	0 0.1 0.3 1 3	6 5 5 6 5	4.3 ± 0.8 9.4 ± 2.8 11.0 ± 3.0 45.0 ± 11.4 ** 50.4 ± 7.4 **	0.53 (0.25–1.11)	2.21 (1.06–4.60)
DMT[b]	F(4,21)=41.25, p<0.0001	30	0 0.3 1 3 10	6 5 5 5 5	6.5 ± 2.4 11.4 ± 1.8 25.2 ± 3.5 * 65.6 ± 8.6 ** 76.4 ± 6.2 **	1.54 (1.08–2.19)	5.05 (3.55–7.19)
DET[b]	F(5,25)=6.20, p=0.0007	20	0 0.625 1.25 2.5 5 10	6 5 5 5 5 5	3.5 ± 0.5 4.0 ± 1.5 4.6 ± 1.9 13.0 ± 3.6 * 18.8 ± 3.0 ** 13.0 ± 3.7 *	2.28 (1.57–3.30)	6.85 (4.72–9.93)

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DPT[a]	F(4,20)=4.02, p=0.0150	20	0 0.625 1.25 2.5 5	5 5 5 5 5	4.4 ± 0.5 6.4 ± 1.5 7.8 ± 2.7 26.2 ± 7.1 * 24.8 ± 9.0 *	1.90 (1.14–3.19)	6.78 (4.04–11.4)
DIPT[a]	F(4,22)=8.9, p=0.0002	30	0 1.25 2.5 5 10	6 5 5 6 5	6.0 ± 0.4 16.6 ± 4.3 27.6 ± 7.1 53.5 ± 5.3 ** 40.8 ± 12.0 **	2.40 (1.67–3.44)	8.54 (5.96–12.2)

*$p < 0.05$, **$p < 0.01$, significant difference from the vehicle control group (Dunnett's test).
[a]Hydrochloride salt; [b]fumarate salt, [c]freebase.

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Table 2: Summary of the head twitch response (HTR) data that were extracted from our previous publications.

Compound	ED_{50}		Reference
AL-LAD	74.2 µg/kg	174.9 nmol/kg	(Brandt et al. 2017b)
2C-B	0.72 mg/kg	2.43 µmol/kg	(Halberstadt et al. 2019b)
2C-B-FLY	0.57 mg/kg	1.79 µmol/kg	(Halberstadt et al. 2019b)
3C-E	2.36 mg/kg	8.54 µmol/kg	(Halberstadt et al. 2019a)
2C-I	0.83 mg/kg	2.42 µmol/kg	(Halberstadt and Geyer 2014)
3C-P	2.45 mg/kg	8.47 µmol/kg	(Halberstadt et al. 2019a)
DOB	0.23 mg/kg	0.75 µmol/kg	(Halberstadt et al. 2019b)
DOB-DFLY	68 µg/kg	205 nmol/kg	(Halberstadt et al. 2019b)
DOB-FLY	0.22 mg/kg	0.67 µmol/kg	(Halberstadt et al. 2019b)
Escaline	2.94 mg/kg	11.2 µmol/kg	(Halberstadt et al. 2019a)
4-HO-DET	0.44 mg/kg	1.89 µmol/kg	*In preparation*
4-HO-DIPT	1.03 mg/kg	3.46 µmol/kg	*In preparation*
4-HO-MIPT	0.85 mg/kg	2.92 µmol/kg	*In preparation*
4-HO-MPT	0.67 mg/kg	1.92 µmol/kg	*In preparation*
25I-NBOMe	78 µg/kg	0.17 µmol/kg	(Halberstadt and Geyer 2014)
LSD	52.9 µg/kg	132.8 nmol/kg	(Halberstadt and Geyer 2013)
SS-LSZ	52 µg/kg	114.2 nmol/kg	(Brandt et al. 2017b)
MEM	3.75 mg/kg	13.6 µmol/kg	(Halberstadt et al. 2019a)
5-MeO-DALT	2.25 mg/kg	7.3 µmol/kg	(Klein et al. 2018)
Mescaline	6.51 mg/kg	26.3 µmol/kg	(Halberstadt et al. 2019a)
MIPLA	136.4 µg/kg	421.7 nmol/kg	(Halberstadt et al. 2019c)
Proscaline	2.23 mg/kg	8.09 µmol/kg	(Halberstadt et al. 2019a)
Psilocybin	0.38 mg/kg	1.40 µmol/kg	*In preparation*
TMA	3.57 mg/kg	13.6 µmol/kg	(Halberstadt et al. 2019a)
TMA-2	2.79 mg/kg	12.4 µmol/kg	(Halberstadt et al. 2019a)

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Table 3. Human potency data for selected hallucinogens.

#	Compound	Human hallucinogenic potency (dose range in mg)	Arithmetic mean of the dose range		Salt form	Route[a]	Reference
1	LSD	0.06 – 0.2	0.13 mg	326 nmol	Hemitartrate	PO	(Shulgin and Shulgin 1997)
2	AL-LAD	0.08 – 0.16	0.12 mg	343 nmol	Hemitartrate	PO	(Shulgin and Shulgin 1997)
3	*SS*-LSZ	0.1 – 0.3	0.2 mg	487 nmol	Hemitartrate	PO	Erowid.com "common"
4	25I-NBOMe	0.5 – 0.7	0.6 mg	1.29 µmol	Hydrochloride	SL or BUC	Psychonautwiki.org "common"
5	DOB-DFLY	0.2 – 0.8	0.5 mg	1.51 µmol	Hydrochloride	PO	(Trachsel et al. 2013)
6	25I-NBOH	0.5 – 0.9	0.7 mg	1.83 µmol	Hydrochloride	SL or BUC	Psychonautwiki.org "common"
7	25D-NBOMe	0.8 – 1	0.9 mg	2.49 µmol	Hydrochloride	SL or BUC	Psychonautwiki.org "common"
8	DOB-FLY	1		2.99 µmol	Hydrochloride	PO	(Shulgin et al. 2011)
9	*R*-(–)-DOI	1 – 2.3	1.65 mg	5.14 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
10	DOB	1 – 3	2 mg	6.44 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
11	DOC	1.5 – 3	2.25 mg	8.45 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
12	DOET	2 – 6	4 mg	15.4 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
13	5-MeO-AMT	2.5 – 4.5	3.5 mg	17.1 µmol	Freebase	PO	(Shulgin and Shulgin 1997)
14	DOM	3 – 10	6.5 mg	26.5 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
15	DOBU	10		34.7 µmol	Hydrochloride	PO	(Braun et al. 1978)
16	2C-B-FLY	10 – 18	14 mg	43.7 µmol	Hydrochloride	PO	Psychonautwiki.org "common"
17	2C-I	14 – 22	18 mg	52.4 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
18	Psilocybin	10 – 20	15 mg	52.8 µmol	Freebase	PO	(Shulgin and Shulgin 1997)
19	4-HO-DIPT	15 – 20	17.5 mg	59.0 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1997)
20	5-MeO-DALT	12 – 25	18.5 mg	60.3 µmol	Hydrochloride	PO	Erowid.com "common"
21	2C-B	12 – 24	18 mg	60.7 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
22	4-HO-MPT	20 – 30	25 mg	71.8 µmol	Fumarate	PO	Psychonautwiki.org "common"
23	4-HO-DET	10 – 25	17.5 mg	75.3 µmol	Freebase	PO	(Shulgin and Shulgin 1997)
24	4-HO-MIPT	12 – 25	18.5 mg	79.6 µmol	Freebase	PO	(Shulgin and Shulgin 1997)
25	3C-P	20 – 40	30 mg	104 µmol	Hydrochloride	PO	Tripsit.me "common"
26	TMA-2	20 – 40	30 mg	115 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
27	MEM	20 – 50	35 mg	146 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
28	Proscaline	30 – 60	45 mg	163 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
29	3C-E	30 – 60	45 mg	163 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
30	DPT	30 – 70	50 mg	178 µmol	Freebase	IM	(Faillace et al. 1967; Soskin et al. 1973)
31	Escaline	40 – 60	50 mg	191 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
32	DET	50 – 70	60 mg	277 µmol	Freebase	IM	(Boszormenyi et al. 1959; Szara et al. 1966)
33	DMT	50 – 100	75 mg	398 µmol	Freebase	IM	(Szara 1957; Gillin et al. 1976)
34	MDA	80 – 160	120 mg	556 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
35	TMA	100 – 250	175 mg	669 µmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)
36	Mescaline	178 – 356	267 mg	1.08 mmol	Hydrochloride	PO	(Shulgin and Shulgin 1991)

[a]BUC, buccal; IM, intramuscular; PO, orally; SL, sublingually.

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Table 4. Median effective doses (ED_{50}) for hallucinogens in rats trained to discriminate 0.08 mg/kg (+)-lysergic acid diethylamide (LSD) from saline.

Drug	ED_{50} (µmol/kg)	Reference
AL-LAD	0.013	(Hoffman and Nichols 1985)
2C-B	1.13	(Juncosa et al. 2013)
2C-B-FLY	0.31	(Monte et al. 1996)
2C-I	1.04	(Nichols et al. 1994)
DOB	1.06	(Chambers et al. 2003)
	1.12	(Nichols et al. 1994)
	(average = 1.09)	
DOB-DFLY	0.022	(Parker et al. 1998)
DOB-FLY	0.061	(Monte et al. 1996)
DOET	0.69	(Parker 1998)
DET	2.53	(Blair et al. 2000)
DMT	10.2	(Blair et al. 1999)
DOM	0.61	(Oberlender et al. 1995)
	0.89	(Nichols et al. 1991)
	(average = 0.75)	
LSD	0.0275	(Oberlender et al. 1992)
	0.037	(Blair et al. 1999)
	0.040	(Monte et al. 1996)
	0.045	(Nichols et al. 2002)
	0.046	(Hoffman and Nichols 1985)
	0.048	(Monte et al. 1995)
	(average = 0.0406)	
SS-LSZ	0.025	(Nichols et al. 2002)
MDA	4.52	(Parker 1998)
Mescaline	34	(McLean et al. 2006)
MIPLA	0.085	(Huang et al. 1994)

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Table 5: Median effective doses (ED_{50}) for hallucinogens in rats trained to discriminate 1 mg/kg 2,5-dimethoxy-4-methylamphetamine (DOM) from saline.

Drug	ED_{50} (mg/kg)	ED_{50} (µmol/kg)	Reference
2C-B	0.67	2.26	(Glennon et al. 1988)
DET	2.45	9.69	(Glennon et al. 1983c)
DIPT	2.60	9.26	(Glennon et al. 1983c)
DMT	5.80	20.8	(Glennon et al. 1983b)
DPT	2.20	7.83	(Glennon et al. 1983c)
DOB	0.20	0.64	(Glennon et al. 1982a)
DOBU	0.91	3.16	(Glennon et al. 1982b)
DOC		1.2	(Glennon 1989)
DOET	0.23	0.89	(Glennon et al. 1982a)
R-(–)-DOI	0.26	0.73	(Glennon et al. 1982a)
DOM	0.44	1.79	(Young et al. 1980)
DON	0.76	2.75	(Glennon et al. 1982a)
α-Et-2C-D	6.44	24.8	(Glennon et al. 1983b)
LSD	0.052	0.13	(Glennon et al. 1983c)
MDA	1.68	7.79	(Glennon et al. 1982c)
R-(–)-MDA	0.81	3.76	(Glennon et al. 1982c)
MEM	6.33	23.0	(Glennon et al. 1982a)
5-MeO-AMT	0.52	2.16	(Glennon et al. 1983a)
Mescaline	14.64	59.1	(Glennon and Young 1982)
TMA	6.34	24.2	(Glennon and Young 1982)
TMA-2	3.59	13.7	(Glennon et al. 1982a)

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FIGURE CAPTIONS

FIGURE 1. Effect of 2,5-dimethoxy-4-methylamphetamine (DOM), 4-ethyl-2,5-dimethoxyamphetamine (DOET), 4-butyl-2,5-dimethoxyamphetamine (DOBU), and *R*-(-)-4-iodo-2,5-dimethoxyamphetamine (*R*-(-)-DOI) on the head twitch response (HTR). Data are presented as group means ± SEM for the entire 30-min test session. The individual data points for each compound are presented in Table S1. $*p < 0.05$, $**p < 0.01$, significant difference from the vehicle control group (Dunnett's test).

FIGURE 2. Hallucinogen potencies in humans and mice are robustly correlated ($r = 0.9448$). The numbers correspond to the agents in Table 3. Potencies in humans are plotted as log 1/total hallucinogenic dose (in moles); potencies in the mouse head-twitch response assay are plotted as log $1/ED_{50}$ (in moles/kg).

FIGURE 3. Hallucinogen potencies in mice and rats are robustly correlated. *(TOP)* Hallucinogen potencies in the mouse head-twitch response (HTR) assay are correlated ($r = 0.9484$) with published ED_{50} values from rat drug discrimination studies using 0.08 mg/kg LSD as the training drug. *(BOTTOM)* Hallucinogen potencies in the mouse HTR assay are correlated ($r = 0.9564$) with published ED_{50} values from rat drug discrimination studies using 1 mg/kg DOM as the training drug. The drug discrimination data are taken from Tables 4 and 5, respectively. Potencies in mice and rats are plotted as log $1/ED_{50}$ (in moles/kg).

FIGURE S1. Comparison of the relationship between human hallucinogenic potencies and ED_{50} values from rats and mice. (*LEFT PANEL*) Relationship between potencies in humans and mouse head-twitch response (HTR) studies. (*RIGHT PANEL*) Relationship between potencies in humans and published rat drug discrimination studies using 0.08 mg/kg LSD as the training drug (*RIGHT PANEL*). Data for AL-LAD, DOB-DFLY, 2C-B, 2C-B-FLY, 2C-I, DET, DMT, DOB, DOB-FLY, DOET, DOM, LSD, LSZ, MDA, and mescaline are shown. The drug discrimination data are taken from Table 4. Potencies in humans are plotted as log 1/total hallucinogenic dose (in moles); potencies in mice and rats are plotted as log $1/ED_{50}$ (in moles/kg).

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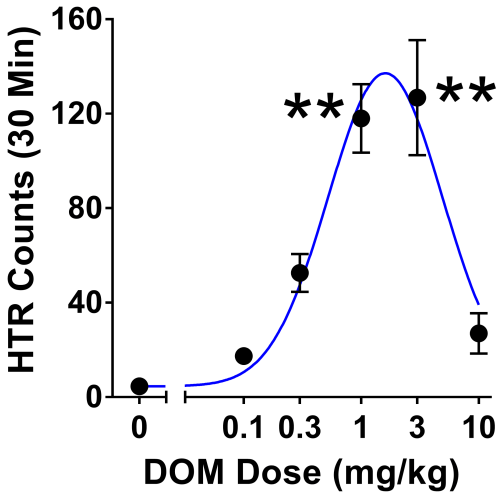
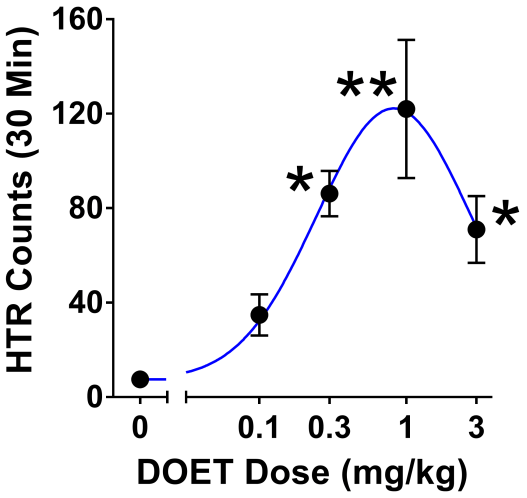
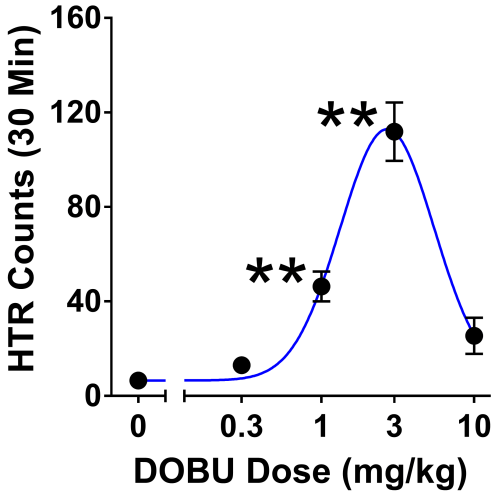
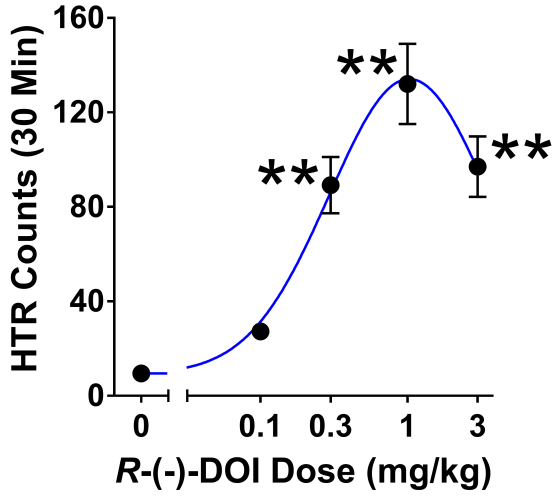
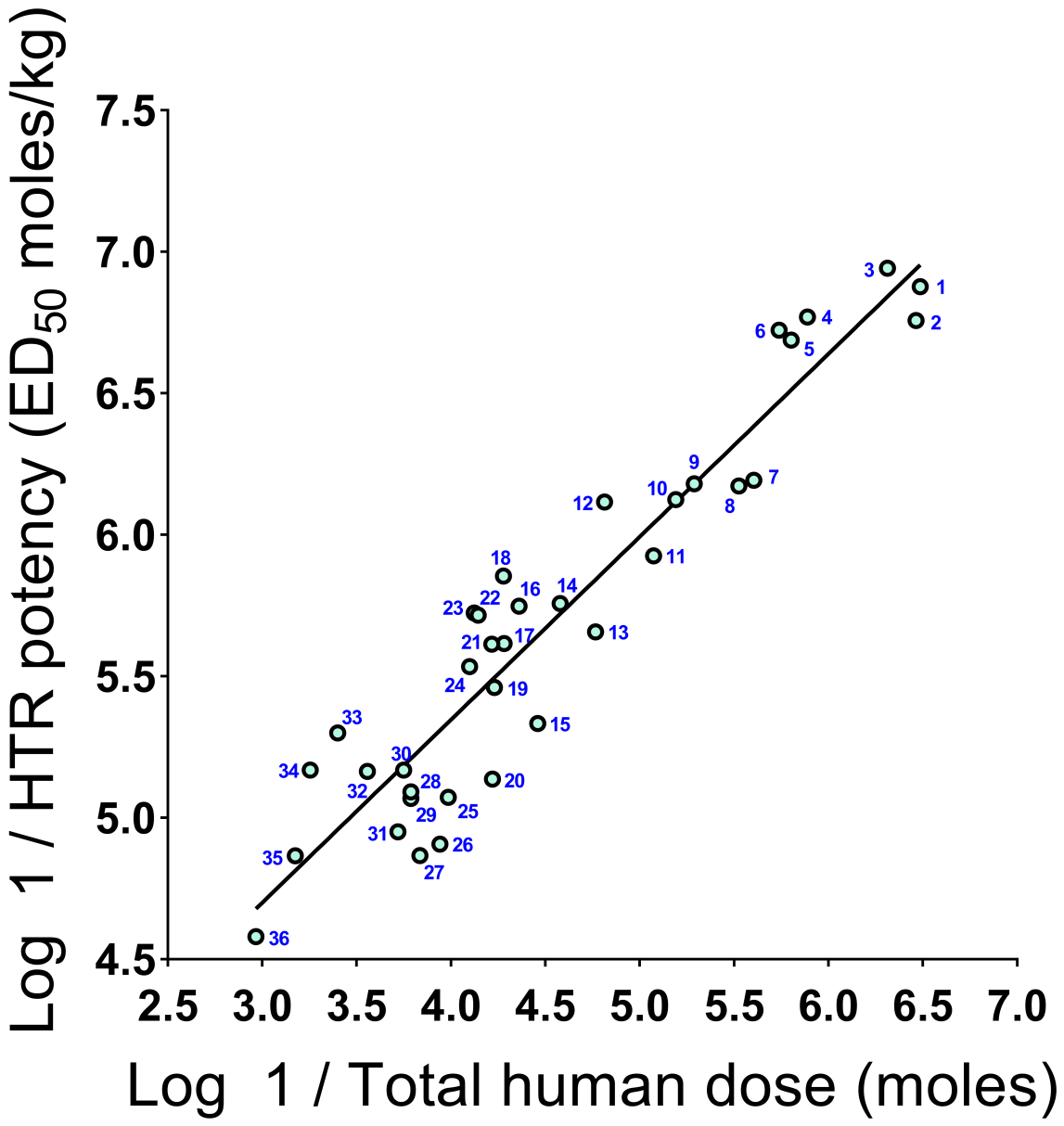

HTR Counts (30 Min)
160
120
80
40
0
**
**
0
0.1
0.3
1
3
10
DOM Dose (mg/kg)


HTR Counts (30 Min)
160
120
80
40
0
**
*
*
0
0.1
0.3
1
3
DOET Dose (mg/kg)


HTR Counts (30 Min)
160
120
80
40
0
**
**
0
0.3
1
3
10
DOBU Dose (mg/kg)


HTR Counts (30 Min)
160
120
80
40
0
**
**
**
0
0.1
0.3
1
3
R-(-)-DOI Dose (mg/kg)


Log 1 / HTR potency (ED50 moles/kg)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
Log 1 / Total human dose (moles)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
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29
30
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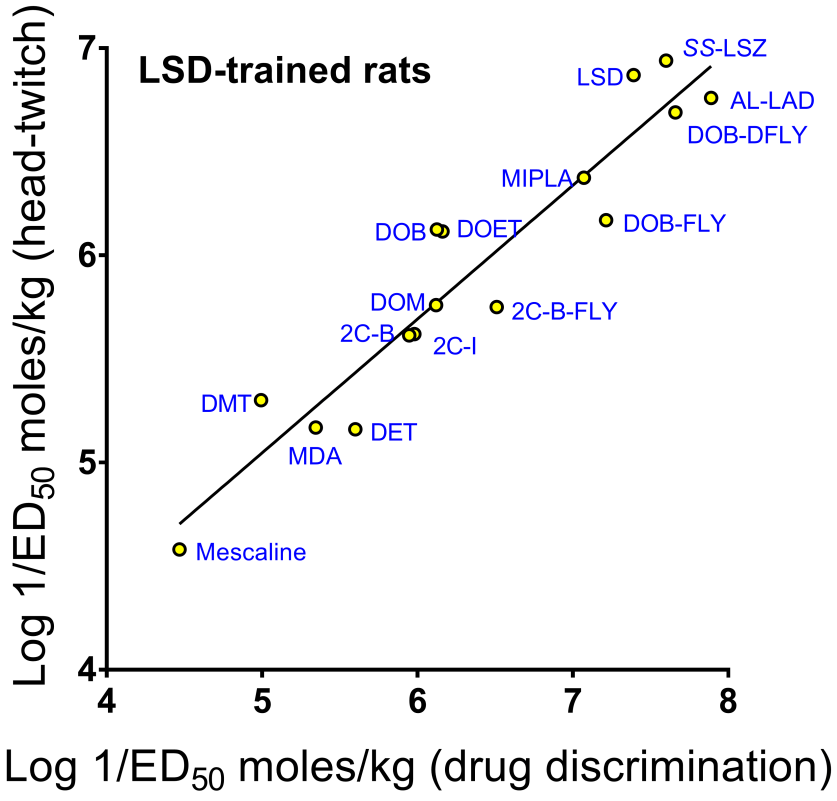
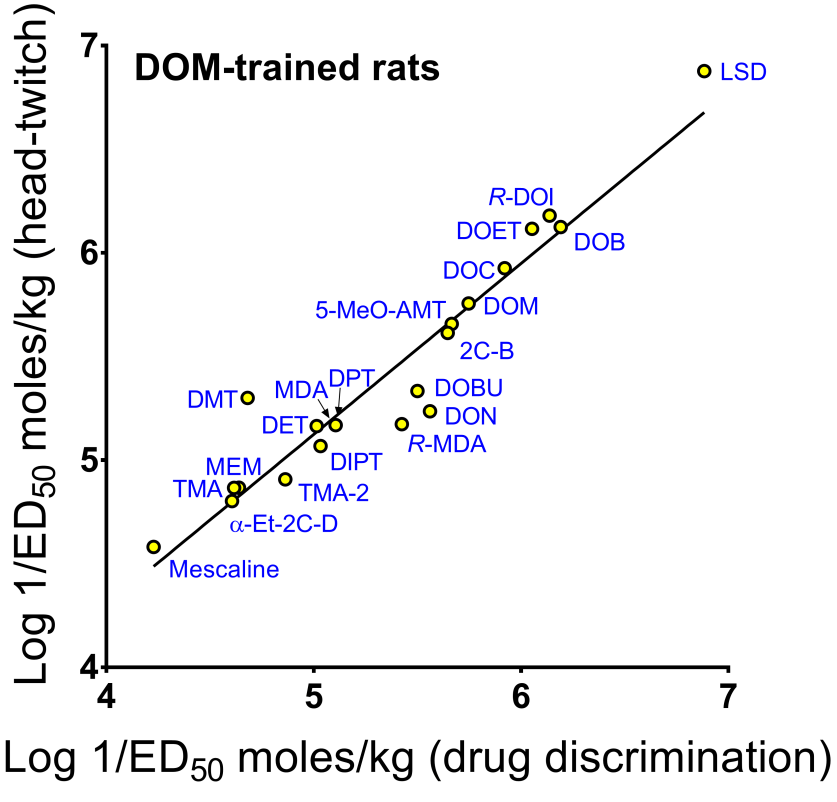
33
34
35
36


LSD-trained rats
SS-LSZ
LSD
AL-LAD
DOB-DFLY
MIPLA
DOB
DOET
DOB-FLY
DOM
2C-B-FLY
2C-B
2C-I
DMT
DET
MDA
Mescaline
Log 1/ED50 moles/kg (head-twitch)
Log 1/ED50 moles/kg (drug discrimination)
4
5
6
7
8


DOM-trained rats
LSD
R-DOI
DOET
DOB
DOC
5-MeO-AMT
DOM
2C-B
DMT
MDA
DPT
DOBU
DET
DON
R-MDA
DIPT
MEM
TMA
TMA-2
α-Et-2C-D
Mescaline
Log 1/ED50 moles/kg (head-twitch)
Log 1/ED50 moles/kg (drug discrimination)
4
5
6
7

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- Serotonergic hallucinogen induce the head-twitch response (HTR) in mice
- HTR potencies in mice are correlated with hallucinogenic potencies in humans
- Hallucinogen potencies in HTR are also correlated with rat drug discrimination data
- Hallucinogen potencies in the HTR assay have significant predictive validity

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CONFLICT OF INTEREST DECLARATION

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

The authors confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

The authors confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

The authors further confirm that any aspect of the work covered in this manuscript that has involved experimental animals has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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Adam L. Halberstadt, Ph.D.

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