HHS Public Access

Author manuscript

J Psychopharmacol. Author manuscript; available in PMC 2020 March 01.

Published in final edited form as:

J Psychopharmacol. 2019 March ; 33(3): 406–414. doi:10.1177/0269881119826610.

Comparison of the behavioral effects of mescaline analogs using the head twitch response in mice

Adam L. Halberstadt[1,2,*], **Muhammad Chatha**[1], **Stephen J. Chapman**[3], **Simon D. Brandt**[4]

[1]Department of Psychiatry, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0804, USA

[2]Research Service, VA San Diego Healthcare System, 3350 La Jolla Village Dr., San Diego, CA 92161, USA

[3]Isomer Design, 4103-210 Victoria Street, Toronto, ON M5B 2R3, Canada

[4]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK

Abstract

Background—In recent years, there has been increasing scientific interest into the effects and pharmacology of serotonergic hallucinogens. While a large amount of experimental work has been conducted to characterize the behavioral response to hallucinogens in rodents, there has been little systematic investigation of mescaline and its analogs. The hallucinogenic potency of mescaline is increased by α-methylation and by homologation of the 4-methoxy group but it not clear whether these structural modifications have similar effects on the activity of mescaline in rodent models.

Methods—In the present study, the head twitch response (HTR), a 5-HT_{2A} receptor-mediated behavior induced by serotonergic hallucinogens, was used to assess the effects of mescaline and several analogs in C57BL/6J mice. HTR experiments were conducted with mescaline, escaline (4-ethoxy-3,5-dimethoxyphenylethylamine) and proscaline (3,5-dimethoxy-4-propoxyphenylethylamine), their α-methyl homologues TMA (3,4,5-trimethoxyamphetamine), 3C-E (4-ethoxy-3,5-dimethoxyamphetamine) and 3C-P (3,5-dimethoxy-4-propoxyamphetamine), and the 2,4,5-substituted regioisomers TMA-2 (2,4,5-trimethoxyamphetamine), MEM (4-ethoxy-2,5-dimethoxyamphetamine) and MPM (2,5-dimethoxy-4-propoxyamphetamine).

Results—TMA induced the HTR and was twice as potent as mescaline. For both mescaline and TMA, replacing the 4-methoxy substituent with an ethoxy or propoxy group increased potency in the HTR assay. By contrast, although TMA-2 also induced the HTR with twice the potency of mescaline, potency was not altered by homologation of the 4-alkoxy group in TMA-2.

Conclusions—The potency relationships for these compounds in mice closely parallel the human hallucinogenic data. These findings are consistent with evidence that 2,4,5- and 3,4,5-substituted phenylalkylamine hallucinogens exhibit distinct structure-activity relationships. These

*Correspondence to: Adam L. Halberstadt, Department of Psychiatry, University of California San Diego, 9500 Gilman Dr., La Jolla, CA 92093-0804 USA. ahalberstadt@ucsd.edu.

Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

results provide additional evidence that the HTR assay can be used to investigate the SAR of serotonergic hallucinogens.

Keywords

psychedelic; head shake; phenylisopropylamine; phenethylamine; animal model

Introduction

Mescaline (3,4,5-trimethoxyphenylethylamine), the active constituent of the peyote cactus (*Lophophora williamsii*), is considered to be a prototypical serotonergic hallucinogen. The first report describing the hallucinogenic effects of mescaline was published by Dr. Arthur Heffter based on self-experiments (Heffter, 1898). Although mescaline has a fairly low potency, with 178–356 mg of the hydrochloride salt being the usual dosage range in humans (Shulgin and Shulgin, 1991), addition of an α-methyl group (Shulgin et al., 1961) or replacement of the 4-methoxy group with an ethoxy or propoxy group (Shulgin, 1978) produces a significant increase in potency. Potency is also increased by rearranging the substituents from a 3,4,5-pattern to a 2,4,5-pattern (Shulgin, 1964). The structures of mescaline analogs and their typical dose ranges in humans are shown in Figure 1.

In recent years, there has been increasing scientific interest into the effects and pharmacology of serotonergic hallucinogens. This interest has been driven, in part, by evidence that hallucinogens may possess therapeutic efficacy in a variety of disorders including anxiety, depression, and substance abuse (Griffiths et al., 2016; Ross et al., 2016; Carhart-Harris et al., 2016; Johnson et al., 2014; Grob et al., 2011). Although human trials with these substances all but ceased after the 1960s, these investigations have cautiously resumed during the last decade.

Another reason for the renewed focus on serotonergic hallucinogens is the large number available as recreational drugs (Gatch et al., 2017; Blough et al., 2014; Brandt et al., 2017b; Klein et al., 2018; Brandt et al., 2016; Halberstadt et al., 2018a). Mescaline and its α-methyl derivative 3,4,5-trimethoxyamphetamine (TMA) are listed in Schedule I of the United Nations 1971 Convention on Psychotropic Substances. A variety of mescaline analogs have been encountered in Europe, including escaline (3,5-dimethoxy-4-ethoxyphenethylamine), proscaline (3,5-dimethoxy-4-propoxyphenethylamine), 3C-E (4-ethoxy-3,5-dimethoxyamphetamine), 3C-P (3,5-dimethoxy-4-propoxyamphetamine), and TMA-2 (2,4,5-trimethoxyamphetamine) (King, 2014; EMCDDA, 2004). TMA-2 was first detected in 1999 (EMCDDA, 2004) and escaline, proscaline, 3C-E and 3C-P were detected in 2013 (EMCDDA, 2014). The identification of escaline has also been reported by researchers in Japan (Kaizaki-Mitsumoto et al., 2016). Another related substance, MEM (4-ethoxy-2,5-dimethoxyamphetamine), appeared as a street drug in Canada in 1986 (Dawson and Awdovich, 1987). Given the potential use of hallucinogens as therapeutic agents and their continuing use, it is necessary to define the pharmacology and structure-activity relationships (SAR) of these substances.

Although rodent behavioral models are routinely used to evaluate the SAR of hallucinogenic drugs (Nichols, 2018; Halberstadt and Geyer, 2018), there has been little systematic

investigation of mescaline analogs in laboratory studies. Mescaline has been tested in a number of drug discrimination (DD) studies (e.g., Glennon and Young, 1982; McLean et al., 2006; Hirschhorn and Winter, 1971; Schechter and Rosecrans, 1972). TMA, TMA-2, and MEM produced full substitution in rats trained to discriminate the hallucinogen 2,5-dimethoxy-4-methylamphetamine (DOM) from saline (Glennon et al., 1982; Glennon and Young, 1982). Conversely, escaline, the 4-ethoxy homolog of mescaline, did not substitute in LSD-trained rats (Monte et al., 1997). The latter finding is surprising because escaline is a hallucinogen in man (Shulgin and Shulgin, 1991).

The goal of the present investigation was to assess the behavioral effects of mescaline and several analogs using the head twitch response (HTR) assay. Serotonergic hallucinogens induce the HTR, a brief paroxysmal head rotation in rats and mice, via activation of the 5-HT_{2A} receptor (Schreiber et al., 1995; Canal and Morgan, 2012; Halberstadt and Geyer, 2014; Halberstadt et al., 2011), which is the site associated with the psychedelic effects of hallucinogens in humans (Valle et al., 2016; Kraehenmann et al., 2017; Preller et al., 2017; Kometer et al., 2013; Vollenweider et al., 1998). The HTR is used as a behavioral proxy in rodents for human hallucinogenic effects because it can reliably distinguish hallucinogenic and non-hallucinogenic 5-HT_{2A} receptor agonists (Gonzalez-Maeso et al., 2007). Although mescaline and TMA induce the HTR in rodents (Corne and Pickering, 1967; Silva and Calil, 1975), other phenylalkylamines with a 3,4,5-substitution pattern have not been evaluated. Given the results of the DD studies, it is important to determine whether escaline can induce the HTR. In addition, it is not clear whether the activity of mescaline analogs in the HTR assay is affected by structural modifications known to alter the potency of 3,4,5-trialkoxyphenethylamines in humans. HTR studies were conducted with mescaline analogs in C57BL/6J mice to examine the effect of the following structural modifications: (a) homologation of the 4-position alkoxy group; (b) addition of an α-methyl group; and (c) relocation of the ring-substituents from a 3,4,5- to a 2,4,5-substitution pattern. The mescaline analogs produced LSD-like behavioral effects *in vivo* and their relative potencies were consistent with human data.

Materials and 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 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 NIH guidelines and were approved by the UCSD Institutional Animal Care and Use Committee.

Drugs

Mescaline hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO, USA). 4-Ethoxy-3,5-dimethoxyphenethylamine (escaline) hydrochloride, 3,4,5-trimethoxyamphetamine (TMA) hydrochloride, and 2,4,5-trimethoxyamphetamine (TMA-2) freebase were obtained from Cayman Chemical (Ann Arbor, MI, USA). 3,5-Dimethoxy-4-propoxyphenethylamine (proscaline) hydrochloride was obtained from Enamine Ltd. (Monmouth Junction, NJ, USA). 4-Ethoxy-3,5-dimethoxyamphetamine (3C-E) hydrochloride, 3,5-dimethoxy-4-propoxyamphetamine (3C-P) hydrochloride, and 4-ethoxy-2,5-dimethoxyamphetamine (MEM) hydrochloride were available from earlier studies. 4-Propoxy-2,5-dimethoxyamphetamine (MPM) trifluoroacetate was synthesized in the laboratory of Jesper L. Kristensen, PhD. 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 head twitch response (HTR) was assessed using a head-mounted magnet and a magnetometer detection coil (Klein et al., 2018; Nichols et al., 2015; Halberstadt and Geyer, 2013; Halberstadt and Geyer, 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. Mice (n = 5–7/group) were injected with drug or vehicle 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 manually 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 (Smith et al., 2014; Rangel-Barajas et al., 2014; Gewirtz and Marek, 2000). 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. Individual experiments were performed between-subjects, with pseudorandomized group assignments, which further reduces the likelihood of carryover effects.

Data Analysis

The entire 30-min recordings were examined for head twitches. Head twitch counts were analyzed using one-way analyses of variance (ANOVA). *Post hoc* pairwise comparisons between selected groups were performed using Tukey's studentized range method. Significance was demonstrated by surpassing an α-level of 0.05.

Median effective doses (ED_{50} values) and 95% confidence intervals (95% CI) for HTR dose-response experiments were calculated by nonlinear regression (Prism 7.00, GraphPad Software, San Diego, CA, USA). A Gaussian distribution (Christopoulos et al., 2001) was used to fit biphasic HTR dose-response data:

$$E = \text{Baseline} + \text{Range} \times e^{-\left[\frac{\log[A] - \text{midA}}{\text{slope}}\right]^2}$$

$$\text{midA} = \log ED_{50} + \text{slope}\sqrt{-\ln(0.5)}$$

In these equations, *E* is the drug effect, *Baseline* is the response in the control group, *Range* is the distance from Baseline to the top of the curve, *[A]* is the dose of the drug, and *midA* is the logarithm of the dose corresponding to the top of the curve.

Results

The first set of experiments examined the response to phenylethylamines with a 3,4,5-substitution pattern. HTR data are shown in Table 1. Mescaline induced the HTR with an ED_{50} of 6.51 mg/kg, which is equivalent to a molar potency of 26.3 μmol/kg. Similar to other hallucinogens (Fantegrossi et al., 2010; Fantegrossi et al., 2005; Fantegrossi et al., 2006; Klein et al., 2018; Halberstadt et al., 2018a), the response to mescaline and most of the other compounds followed an inverted-U-shaped dose-response function. Escaline (the 4-ethoxy homologue of mescaline) and proscaline (the 4-propoxy homologue) also induced the HTR. Escaline (ED_{50} = 11.2 μmol/kg) and proscaline (ED_{50} = 8.09 μmol/kg) were two and three times as potent as mescaline, respectively. Figure 2 shows the time-dependence of the responses induced by mescaline, escaline and proscaline.

The second set of experiments evaluated the effect of α-methylation on the activity of mescaline and its homologs. TMA, the α-methyl derivative of mescaline, induced the HTR with an ED_{50} of 13.6 μmol/kg, making it twice as potent as mescaline. The two higher 4-alkoxy homologues 3C-E (α-methylescaline; ED_{50} = 8.54 μmol/kg) and 3C-P (α-methylproscaline; ED_{50} = 8.47 μmol/kg) were essentially equipotent and both had 50% higher potency than TMA. Figure 3 shows the time-dependence of the responses induced by TMA, 3C-E and 3C-P.

We also examined whether *in vivo* potency is affected by relocating the groups on the aromatic ring from the 3,4,5-substitution pattern to the 2,4,5-orientation. Phenylisopropylamines (amphetamines) were used for these experiments because 2,4,5-

trisubstituted phenylethylamines containing an alkoxy group in the 4-position are only weakly active in man and other species. For example, a 300 mg oral dose of 2,4,5-trimethoxyphenylethylamine reportedly produced a placebo-like response in a self-experiment (Dittrich, 1971). 2,4,5-Trimethoxyphenylethylamine also has limited behavioral activity in rats (Smythies et al., 1967). TMA-2 induced the HTR with an ED_{50} value of 12.4 μmol/kg, making it slightly more potent than TMA. Replacing the 4-methoxy group in TMA-2 with an ethoxy or propoxy group had no effect on potency in the HTR assay: MEM induced the HTR with an ED_{50} = 13.6 μmol/kg, whereas the ED_{50} for MPM was 12.1 μmol/kg. Figure 4 shows the time-dependence of the responses induced by TMA-2, MEM and MPM.

Discussion

The present investigation examined the behavioral effects of phenylalkylamine hallucinogens in mice. While a large amount of experimental work has been conducted over the past few decades to characterize the hallucinogenic activity of mescaline analogs in humans (Shulgin and Shulgin, 1991), most of these substances have not been tested in rodent behavioral assays. Mescaline and the other phenylalkylamine hallucinogens induced head twitches in mice, demonstrating that they produce LSD-like behavioral effects *in vivo*. The potency of mescaline and other 3,4,5-trimethoxy-substituted phenylalkylamines was enhanced by lengthening the 4-methoxy group by one or two methylene units. By contrast, homologation of the 4-position did not increase the potency of 2,4,5-trimethoxy-substituted phenylalkylamines. The potency of mescaline was also increased by the addition of an α-methyl group. Importantly, the potency relationships in mice closely parallel the human hallucinogenic data (Shulgin and Shulgin, 1991). These findings demonstrate that the mouse HTR assay can be used to study the SAR of serotonergic hallucinogens.

Although we did not identify the receptor mechanism responsible for the response to mescaline and other phenylalkylamines in mice, the behavioral effects assessed in this study are likely mediated by 5-HT_{2A} receptor activation. A multitude of studies have demonstrated that the 5-HT_{2A} receptor is responsible for the head twitches induced by hallucinogens, whereas other serotonin receptors can be blocked without eliminating the response (Schreiber et al., 1995; Halberstadt et al., 2011; Halberstadt and Geyer, 2014; Fantegrossi et al., 2010; Vickers et al., 2001; Gonzalez-Maeso et al., 2007). Although compounds such as rolipram, [Met^5]enkephalin, and icilin can induce head twitches through non-5-HT_{2A} receptor-dependent mechanisms (Przegalinski et al., 1981; Drust et al., 1981), almost all of the compounds examined are known to be active at the 5-HT_{2A} receptor (Monte et al., 1997; Glennon et al., 1984; Glennon et al., 1992) and are capable of producing DOM-like stimulus effects in rodents (Glennon et al., 1983a). Hence, there is little reason to believe that the head twitches induced by these compounds are mediated by a mechanism other than 5-HT_{2A} receptor activation.

As noted above, the present results are consistent with the established SAR for phenylalkylamine hallucinogens in humans. The identity of the 4-position substituent is known to play an important role in determining the potency of phenylalkylamines with a 3,4,5-trialkoxy-substitution pattern. A 4-ethoxy or a 4-propoxy group increases human

hallucinogenic potency compared to a 4-methoxy group (Shulgin, 1978; Shulgin and Shulgin, 1991). Based on their typical dose ranges in humans, escaline and proscaline have about five times the potency of mescaline (Figure 1). Likewise, 3C-E has four times the potency of TMA in humans. Homologation of the 4-alkoxy group had a similar effect on potency in the mouse HTR assay. Escaline had twofold higher potency than mescaline in mice, whereas proscaline had threefold higher potency. Although there was less of a potency difference between TMA and its higher homologs, 3C-E and 3C-P clearly had higher potency than TMA in mice.

It is not clear why the presence of an alkoxy group larger than methoxy in the 4-position of 3,4,5-substitued phenylalkylamines increases potency *in vivo*, but there are several potential explanations. First, the presence of an ethoxy or propoxy group in the 4-position of mescaline analogs may have a favorable impact on their pharmacodynamics. If the 4-alkoxy group projects into a complementary hydrophobic region of the binding pocket then its presence may have a favorable effect on 5-HT_{2A} receptor binding. Indeed, escaline (K_i = 2100 nM) reportedly has twofold higher affinity than mescaline (K_i = 5500 nM) for [^{3}H]ketanserin-labeled 5-HT_{2A} receptors in rat frontal cortex homogenates (Monte et al., 1997). A second potential explanation is that ethoxy and propoxy substituents may enhance CNS partitioning. The central activity of hallucinogens is known to be correlated with their lipophilicity (Barfknecht and Nichols, 1975). According to Barfknecht and Nichols (1975), the optimum lipophilicity for a series of hallucinogenic phenylisopropylamines is log*P* = 3.14. Mescaline has a relatively low log*P* value of 0.78, whereas the values for escaline (log*P* = 1.11) and proscaline (log*P* = 1.70) are closer to the optimum level (Nichols and Dyer, 1977; Nichols et al., 1977).

In contrast to the potency increase induced by homologation of the 4-methoxy group in mescaline and TMA, homologation of the 4-methoxy group in TMA-2 has little effect on behavioral potency. MEM and MPM were equipotent with TMA-2 in our HTR studies (see Table 1). Similar potency relationships have been reported for TMA-2, MEM, and MPM in humans (Shulgin, 1978). In the rat DD assay, MEM is actually less potent than TMA-2 in studies using DOM as the training drug (Glennon et al., 1983a). These findings are consistent with previous evidence that the effects of 3,4,5-substituted and 2,4,5-substituted phenylalkylamine hallucinogens are governed by different SAR. These SAR differences are apparent when the methoxy groups of phenylalkylamine hallucinogens are constrained into rigid dihydrofuran rings (Monte et al., 1996; Monte et al., 1997). While activity is maintained by rigidification of the methoxy groups in 2,4,5-substituted phenylethylamines (Monte et al., 1996), constraining the orientation of the methoxy groups in 3,4,5-substituted phenethylamines is detrimental for activity (Monte et al., 1997). Notably, when mescaline is docked into an *in silico*-activated homology model of the 5-HT_{2A} receptor, it adopts a different binding pose than 2,4,5-substituted compounds (McLean et al., 2006). It is therefore possible that homologation of the 4-methoxy group does not uniformly affect the activity of 2,4,5- and 3,4,5-trimethoxy-substituted phenylalkylamines because their alkoxy groups are oriented differently in the binding pocket of the 5-HT_{2A} receptor.

Phenylisopropylamine hallucinogens are typically more potent than their phenylethylamine homologues *in vivo*. For example, 2C-D and 2C-B have approximately 1/10 the potency of

DOM and DOB, respectively, in humans (Shulgin and Shulgin, 1991). In preclinical studies of phenylalkylamine hallucinogens, the presence of an α-methyl group increases *in vivo* behavioral potency (Glennon et al., 1983b; Monte et al., 1996; Halberstadt et al., 2018a) but does not alter 5-HT_{2A} affinity (Johnson et al., 1990; Glennon et al., 1992; Parrish et al., 2005). There are several potential explanations for the potency differences between phenylisopropylamines and phenylethylamines. The α-methyl group likely reduces the metabolic lability of the amine side-chain (Nichols et al., 1991). Phenylisopropylamines are also more lipophilic than phenylethylamines, potentially facilitating their CNS penetration. Indeed, while TMA-2 clearly partitions into the CNS after systemic administration, its α-desmethyl analog 2,4,5-trimethoxyphenylethylamine could not be detected in the brain after IP administration to rats (Cohen et al., 1974).

The effects of phenylalkylamine hallucinogens in the HTR assay are generally consistent with the results of rat DD studies. The following potency relationships were reported in studies using DOM (1.0 mg/kg) as the training drug: TMA-2 (ED_{50} = 3.59 mg/kg) > TMA (ED_{50} = 6.34 mg/kg) > mescaline (ED_{50} = 14.64 mg/kg) (Glennon et al., 1982; Glennon and Young, 1982). Escaline is a notable exception: according to Nichols and colleagues, escaline produced a maximum of 50% substitution when tested in rats trained to discriminate LSD from saline (Monte et al., 1997). It is not clear, however, whether escaline is actually a DD false negative or alternatively whether its inability to produce full-substitution is a consequence of other factors. Some hallucinogens reportedly have particularly steep dose-response functions in DD studies, with complete generalization occurring only within a narrow range of doses, making it difficult to detect full-substitution (Glennon et al., 1983a). Nevertheless, as shown in Table 1, escaline clearly induces head twitches in mice, demonstrating that it can produce an LSD-like behavioral response in a rodent behavioral model.

Similar to other hallucinogens (Fantegrossi et al., 2010; Fantegrossi et al., 2008; Fantegrossi et al., 2005; Fantegrossi et al., 2006; Halberstadt et al., 2018a; Halberstadt et al., 2018b), the HTR induced by mescaline analogues is non-monotonic, with a marked response decrement occurring at high doses. According to one group, the descending arm of the response to the hallucinogen DOI is blocked by the 5-HT_{2C} antagonists RS102221 and SB-242,084 (Fantegrossi et al., 2010), indicating that the response decrement is mediated by 5-HT_{2C} activation. However, other studies with 5-HT_{2C} antagonists failed to corroborate their findings (Canal et al., 2013; Canal et al., 2010). Furthermore, *N*-(2-hydroxybenzyl)-2,5-dimethoxy-4-cyanophenylethylamine (25CN-NBOH), a 5-HT_{2A} agonist with moderate selectivity over 5-HT_{2C} (Jensen et al., 2017; Halberstadt et al., 2016; Hansen et al., 2014), also induces the HTR with a biphasic dose-response (Fantegrossi et al., 2015). The response decrement associated with high doses of 25CN-NBOH is not altered by pretreatment with RS102221 (Fantegrossi et al., 2015), indicating that the inhibition of the HTR at high hallucinogen doses can occur in the absence of activity at 5-HT_{2C}. It is possible that high levels of 5-HT_{2A} activation may provoke countervailing neuronal or behavioral effects that interfere with the expression of the HTR. Further work is required to delineate the mechanisms responsible for the biphasic effects of hallucinogens on the HTR.

Although most of the compounds examined have been evaluated in humans, very little is known about the effects of 3C-P and MPM. MPM reportedly has about the same potency as MEM and TMA-2 (Shulgin, 1978), which is entirely consistent with the potencies of these compounds in the HTR paradigm. Even less is known about the activity of 3C-P in humans. According to websites such as erowid.org and tripsit.me, 3C-P produces hallucinogenic effects, but its active dosage range is uncertain. Nevertheless, in the present studies, 3C-E and 3C-P had virtually identical potencies in the HTR assay; it is reasonable to predict that 3C-P would be active in humans in the same dosage range as 3C-E.

In summary, mescaline and several derivatives induced the HTR in mice. Notably, the potencies of these compounds in mice are consistent with their activity in humans (Shulgin and Shulgin, 1991). Although the HTR assay is widely used to determine whether substances have hallucinogen-like behavioral profiles (Gonzalez-Maeso et al., 2007), few studies have evaluated the relationship between hallucinogen potencies in HTR experiments and their activity in other species including humans. We have now confirmed — at least for one series of phenylalkylamine hallucinogens — that the HTR data are consistent with the established SAR for activity in humans. Hence, although head twitches may have limited value as a model of hallucinogenesis (Canal and Morgan, 2012), the HTR assay may be useful for investigations of the *in vivo* potency relationships of hallucinogens. A preliminary regression analysis, including 7 compounds from the present investigation (mescaline, escaline, proscaline, TMA, 3C-E, TMA-2 and MEM) and 5 agents tested previously (LSD, AL-LAD, DOB, 2C-B and 2C-I; Halberstadt et al., 2018a; Halberstadt and Geyer, 2013; Halberstadt and Geyer, 2014; Brandt et al., 2017a), demonstrated that HTR-derived ED_{50} values are significantly correlated ($r = 0.9761$, $p < 0.0001$) with human hallucinogenic potency data collected by A.T. Shulgin (Shulgin and Shulgin, 1997; Shulgin and Shulgin, 1991). Studies are currently underway in our laboratory to examine the relationship between HTR potency and human hallucinogenic potency for a much larger series of compounds. Importantly, however, the phenylalkylamines examined in these experiments clearly produce behavioral effects similar to those of mescaline and other serotonergic hallucinogens. These findings indicate that the psychopharmacology of these compounds in humans is likely to be similar to mescaline and LSD. Ultimately, clinical trials are necessary to fully characterize the human psychopharmacology of these compounds.

Acknowledgments

The authors are grateful to Mark A. Geyer, Ph.D., for his assistance with these studies. We would also like to thank Jesper L. Kristensen, Ph.D., for synthesizing MPM for use in these experiments.

Funding Acknowledgments

This work was supported by NIDA (R01 DA041336) and the Veteran's Administration VISN 22 Mental Illness Research, Education, and Clinical Center.

REFERENCES

Barfknecht CF and Nichols DE. (1975) Correlation of psychotomimetic activity of phenethylamines and amphetamines with 1-octanol-water partition coefficients. J Med Chem 18: 208–210. [PubMed: 1120990]

Blough BE, Landavazo A, Decker AM, et al. (2014) Interaction of psychoactive tryptamines with biogenic amine transporters and serotonin receptor subtypes. Psychopharmacology (Berl) 231: 4135–4144. [PubMed: 24800892]

Brandt SD, Kavanagh PV, Westphal F, et al. (2017a) Return of the lysergamides. Part II: Analytical and behavioural characterization of N6 -allyl-6-norlysergic acid diethylamide (AL-LAD) and (2'S,4'S)-lysergic acid 2,4-dimethylazetidide (LSZ). Drug Test Anal 9: 38–50. [PubMed: 27265891]

Brandt SD, Kavanagh PV, Westphal F, et al. (2017b) Return of the lysergamides. Part III: Analytical characterization of N(6) -ethyl-6-norlysergic acid diethylamide (ETH-LAD) and 1-propionyl ETH-LAD (1P-ETH-LAD). Drug Test Anal 9: 1641–1649. [PubMed: 28342178]

Brandt SD, Kavanagh PV, Westphal F, et al. (2016) Return of the lysergamides. Part I: Analytical and behavioural characterization of 1-propionyl-d-lysergic acid diethylamide (1P-LSD). Drug Test Anal 8: 891–902. [PubMed: 26456305]

Canal CE, Booth RG and Morgan D. (2013) Support for 5-HT2C receptor functional selectivity in vivo utilizing structurally diverse, selective 5-HT2C receptor ligands and the 2,5-dimethoxy-4-iodoamphetamine elicited head-twitch response model. Neuropharmacology 70: 112–121. [PubMed: 23353901]

Canal CE and Morgan D. (2012) Head-twitch response in rodents induced by the hallucinogen 2,5-dimethoxy-4-iodoamphetamine: a comprehensive history, a re-evaluation of mechanisms, and its utility as a model. Drug Test Anal 4: 556–576. [PubMed: 22517680]

Canal CE, Olaghere da Silva UB, Gresch PJ, et al. (2010) The serotonin 2C receptor potently modulates the head-twitch response in mice induced by a phenethylamine hallucinogen. Psychopharmacology (Berl) 209: 163–174. [PubMed: 20165943]

Carhart-Harris RL, Bolstridge M, Rucker J, et al. (2016) Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. Lancet Psychiatry 3: 619–627. [PubMed: 27210031]

Christopoulos A, Grant MK, Ayoubzadeh N, et al. (2001) Synthesis and pharmacological evaluation of dimeric muscarinic acetylcholine receptor agonists. J Pharmacol Exp Ther 298: 1260–1268. [PubMed: 11504829]

Cohen I, Fischer JF and Vogel WH. (1974) Physiological disposition of beta-phenylethylamine, 2,4,5-trimethoxyphenylethylamine, 2,3,4,5,6-pentamethoxyphenylethylamine and beta-hydroxymescaline in rat brain, liver and plasma. Psychopharmacologia 36: 77–84. [PubMed: 4838506]

Corne SJ and Pickering RW. (1967) A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. Psychopharmacologia 11: 65–78. [PubMed: 5302272]

Dawson BA and Awdovich HW. (1987) Identification of 4-ethoxy-2,5-dimethoxy amphetamine by an NMR shift reagent study. Canadian Soc Forensic Sci J 20: 29–34.

Dittrich A (1971) Alteration of behavioural changes induced by 3,4,5-trimethoxyphenylethylamine (mescaline) by pretreatment with 2,4,5-trimethoxyphenylethylamine. A self-experiment. Psychopharmacologia 21: 229–237. [PubMed: 5095413]

Drust EG, Sloviter RS and Conner JD. (1981) Methionine enkephalin-induced shaking behavior in rats: dissociation from brain serotonin mechanisms. Neuropharmacology 20: 473–475. [PubMed: 7242868]

EMCDDA. (2004) Report on the risk assessment of TMA-2 in the framework of the joint action on new synthetic drugs. Luxembourg: Office for Official Publications of the European Communities.

EMCDDA. (2014) EMCDDA-Europol 2013 Annual Report on the Implementation of Council Decision 2005/387/JHA. Lisbon: Publications Office of the European Union.

Fantegrossi WE, Gray BW, Bailey JM, et al. (2015) Hallucinogen-like effects of 2-([2-(4-cyano-2,5-dimethoxyphenyl) ethylamino]methyl)phenol (25CN-NBOH), a novel N-benzylphenethylamine with 100-fold selectivity for 5-HT(2)A receptors, in mice. Psychopharmacology (Berl) 232: 1039–1047. [PubMed: 25224567]

Fantegrossi WE, Harrington AW, Eckler JR, et al. (2005) Hallucinogen-like actions of 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7) in mice and rats. Psychopharmacology (Berl) 181: 496–503. [PubMed: 15983786]

Fantegrossi WE, Harrington AW, Kiessel CL, et al. (2006) Hallucinogen-like actions of 5-methoxy-N,N-diisopropyltryptamine in mice and rats. Pharmacol Biochem Behav 83: 122–129. [PubMed: 16460788]

Fantegrossi WE, Reissig CJ, Katz EB, et al. (2008) Hallucinogen-like effects of N,N-dipropyltryptamine (DPT): possible mediation by serotonin 5-HT1A and 5-HT2A receptors in rodents. Pharmacol Biochem Behav 88: 358–365. [PubMed: 17905422]

Fantegrossi WE, Simoneau J, Cohen MS, et al. (2010) Interaction of 5-HT2A and 5-HT2C receptors in R(−)-2,5-dimethoxy-4-iodoamphetamine-elicited head twitch behavior in mice. J Pharmacol Exp Ther 335: 728–734. [PubMed: 20858706]

Gatch MB, Dolan SB and Forster MJ. (2017) Locomotor and discriminative stimulus effects of four novel hallucinogens in rodents. Behav Pharmacol 28: 375–385. [PubMed: 28537942]

Gewirtz JC and Marek GJ. (2000) Behavioral evidence for interactions between a hallucinogenic drug and group II metabotropic glutamate receptors. Neuropsychopharmacology 23: 569–576. [PubMed: 11027922]

Glennon RA, Raghupathi R, Bartyzel P, et al. (1992) Binding of phenylalkylamine derivatives at 5-HT1C and 5-HT2 serotonin receptors: evidence for a lack of selectivity. J Med Chem 35: 734–740. [PubMed: 1542100]

Glennon RA, Rosecrans JA and Young R. (1983a) Drug-induced discrimination: a description of the paradigm and a review of its specific application to the study of hallucinogenic agents. Med Res Rev 3: 289–340. [PubMed: 6350763]

Glennon RA, Titeler M and McKenney JD. (1984) Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. Life Sci 35: 2505–2511. [PubMed: 6513725]

Glennon RA and Young R. (1982) Comparison of behavioral properties of di- and tri-methoxyphenylisopropylamines. Pharmacol Biochem Behav 17: 603–607. [PubMed: 6965276]

Glennon RA, Young R, Benington F, et al. (1982) Behavioral and serotonin receptor properties of 4-substituted derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane. J Med Chem 25: 1163–1168. [PubMed: 7143352]

Glennon RA, Young R and Jacyno JM. (1983b) Indolealkylamine and phenalkylamine hallucinogens. Effect of alpha-methyl and N-methyl substituents on behavioral activity. Biochem Pharmacol 32: 1267–1273. [PubMed: 6573879]

Gonzalez-Maeso J, Weisstaub NV, Zhou M, et al. (2007) Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. Neuron 53: 439–452. [PubMed: 17270739]

Griffiths RR, Johnson MW, Carducci MA, et al. (2016) Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. J Psychopharmacol 30: 1181–1197. [PubMed: 27909165]

Grob CS, Danforth AL, Chopra GS, et al. (2011) Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. Arch Gen Psychiatry 68: 71–78. [PubMed: 20819978]

Halberstadt AL, Chatha M, Stratford A, et al. (2018a) Comparison of the behavioral responses induced by phenylalkylamine hallucinogens and their tetrahydrobenzodifuran (“FLY”) and benzodifuran (“DragonFLY”) analogs. Neuropharmacology in press.

Halberstadt AL and Geyer MA. (2013) Characterization of the head-twitch response induced by hallucinogens in mice: detection of the behavior based on the dynamics of head movement. Psychopharmacology (Berl) 227: 727–739. [PubMed: 23407781]

Halberstadt AL and Geyer MA. (2014) Effects of the hallucinogen 2,5-dimethoxy-4-iodophenethylamine (2C-I) and superpotent N-benzyl derivatives on the head twitch response. Neuropharmacology 77: 200–207. [PubMed: 24012658]

Halberstadt AL and Geyer MA. (2018) Effect of Hallucinogens on Unconditioned Behavior. Curr Top Behav Neurosci 36: 159–199. [PubMed: 28224459]

Halberstadt AL, Klein LM, Chatha M, et al. (2018b) Pharmacological characterization of the LSD analog N-ethyl-N-cyclopropyl lysergamide (ECPLA). Psychopharmacology (Berl) in press.

Halberstadt AL, Koedood L, Powell SB, et al. (2011) Differential contributions of serotonin receptors to the behavioral effects of indoleamine hallucinogens in mice. J Psychopharmacol 25: 1548–1561. [PubMed: 21148021]

Halberstadt AL, Sindhunata IS, Scheffers K, et al. (2016) Effect of 5-HT_{2A} and 5-HT_{2C} receptors on temporal discrimination by mice. Neuropharmacology 107: 364–375. [PubMed: 27020041]

Hansen M, Phonekeo K, Paine JS, et al. (2014) Synthesis and structure-activity relationships of N-benzyl phenethylamines as 5-HT2A/2C agonists. ACS Chem Neurosci 5: 243–249. [PubMed: 24397362]

Heffter A (1898) Ueber Pellote. Beitrag zur chemischen und pharmakologischen Kenntnis der Cacteen. Naunyn Schmiedebergs Arch Pharmacol 40: 385–429.

Hirschhorn ID and Winter JC. (1971) Mescaline and lysergic acid diethylamide (LSD) as discriminative stimuli. Psychopharmacologia 22: 64–71. [PubMed: 5119576]

Jensen AA, McCorvy JD, Leth-Petersen S, et al. (2017) Detailed Characterization of the In Vitro Pharmacological and Pharmacokinetic Properties of N-(2-Hydroxybenzyl)-2,5-Dimethoxy-4-Cyanophenylethylamine (25CN-NBOH), a Highly Selective and Brain-Penetrant 5-HT2A Receptor Agonist. J Pharmacol Exp Ther 361: 441–453. [PubMed: 28360333]

Johnson MP, Mathis CA, Shulgin AT, et al. (1990) [125I]-2-(2,5-dimethoxy-4-iodophenyl)aminoethane ([125I]-2C-I) as a label for the 5-HT2 receptor in rat frontal cortex. Pharmacol Biochem Behav 35: 211–217. [PubMed: 2315361]

Johnson MW, Garcia-Romeu A, Cosimano MP, et al. (2014) Pilot study of the 5-HT2AR agonist psilocybin in the treatment of tobacco addiction. J Psychopharmacol 28: 983–992. [PubMed: 25213996]

Kaizaki-Mitsumoto A, Noguchi N, Yamaguchi S, et al. (2016) Three 25-NBOMe-type drugs, three other phenethylamine-type drugs (25I-NBMD, RH34, and escaline), eight cathinone derivatives, and a phencyclidine analog MMXE, newly identified in ingredients of drug products before they were sold on the drug market. Forensic Toxicol 34: 108–114.

King LA. (2014) New phenethylamines in Europe. Drug Test Anal 6: 808–818. [PubMed: 24574327]

Klein LM, Cozzi NV, Daley PF, et al. (2018) Receptor binding profiles and behavioral pharmacology of ring-substituted N,N-diallyltryptamine analogs. Neuropharmacology.

Kometer M, Schmidt A, Jancke L, et al. (2013) Activation of serotonin 2A receptors underlies the psilocybin-induced effects on alpha oscillations, N170 visual-evoked potentials, and visual hallucinations. J Neurosci 33: 10544–10551. [PubMed: 23785166]

Kraehenmann R, Pokorny D, Vollenweider L, et al. (2017) Dreamlike effects of LSD on waking imagery in humans depend on serotonin 2A receptor activation. Psychopharmacology (Berl).

McLean TH, Chambers JJ, Parrish JC, et al. (2006) C-(4,5,6-trimethoxyindan-1-yl)methanamine: a mescaline analogue designed using a homology model of the 5-HT2A receptor. J Med Chem 49: 4269–4274. [PubMed: 16821786]

Monte AP, Marona-Lewicka D, Parker MA, et al. (1996) Dihydrobenzofuran analogues of hallucinogens. 3. Models of 4-substituted (2,5-dimethoxyphenyl)alkylamine derivatives with rigidified methoxy groups. J Med Chem 39: 2953–2961. [PubMed: 8709129]

Monte AP, Waldman SR, Marona-Lewicka D, et al. (1997) Dihydrobenzofuran analogues of hallucinogens. 4. Mescaline derivatives. J Med Chem 40: 2997–3008. [PubMed: 9301661]

Nichols DE. (2018) Chemistry and Structure-Activity Relationships of Psychedelics. Curr Top Behav Neurosci 36: 1–43.

Nichols DE and Dyer DC. (1977) Lipophilicity and serotonin agonist activity in a series of 4-substituted mescaline analogues. J Med Chem 20: 299–301. [PubMed: 836502]

Nichols DE, Oberlender R and McKenna DJ. (1991) Stereochemical aspects of hallucinogenesis In: Watson RR (ed) Biochemistry and Physiology of Substance Abuse, Volume 3 Boca Raton, FL: CRC Press, 1–39.

Nichols DE, Sassano MF, Halberstadt AL, et al. (2015) N-Benzyl-5-methoxytryptamines as Potent Serotonin 5-HT2 Receptor Family Agonists and Comparison with a Series of Phenethylamine Analogues. ACS Chem Neurosci 6: 1165–1175. [PubMed: 25547199]

Nichols DE, Shulgin AT and Dyer DC. (1977) Directional lipophilic character in a series of psychotomimetic phenethylamine derivatives. Life Sci 21: 569–575. [PubMed: 904435]

Parrish JC, Braden MR, Gundy E, et al. (2005) Differential phospholipase C activation by phenylalkylamine serotonin 5-HT 2A receptor agonists. J Neurochem 95: 1575–1584. [PubMed: 16277614]

Preller KH, Herdener M, Pokorny T, et al. (2017) The Fabric of Meaning and Subjective Effects in LSD-Induced States Depend on Serotonin 2A Receptor Activation. Curr Biol 27: 451–457. [PubMed: 28132813]

Przegalinski E, Bigajska K and Lewandowska A. (1981) The influence of rolipram on the central serotoninergic system. Pharmacopsychiatria 14: 162–166. [PubMed: 7291300]

Rangel-Barajas C, Malik M, Vangveravong S, et al. (2014) Pharmacological modulation of abnormal involuntary DOI-induced head twitch response in male DBA/2J mice: I. Effects of D2/D3 and D2 dopamine receptor selective compounds. Neuropharmacology 83: 18–27. [PubMed: 24680675]

Ross S, Bossis A, Guss J, et al. (2016) Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. J Psychopharmacol 30: 1165–1180. [PubMed: 27909164]

Schechter MD and Rosecrans JA. (1972) Lysergic acid diethylamide (LSD) as a discriminative cue: drugs with similar stimulus properties. Psychopharmacologia 26: 313–316. [PubMed: 5073050]

Schreiber R, Brocco M, Audinot V, et al. (1995) (1-(2,5-dimethoxy-4 iodophenyl)-2-aminopropane)-induced head-twitches in the rat are mediated by 5-hydroxytryptamine (5-HT) 2A receptors: modulation by novel 5-HT2A/2C antagonists, D1 antagonists and 5-HT1A agonists. J Pharmacol Exp Ther 273: 101–112. [PubMed: 7714755]

Shulgin AT. (1964) Psychotomimetic amphetamines: methoxy 3,4-dialkoxyamphetamines. Experientia 20: 366–367. [PubMed: 5855670]

Shulgin AT. (1978) Psychotomimetic drugs: structure-activity relationships In: Iversen LL, Iversen SD and Snyder SH (eds) Handbook of Psychopharmacology. New York: Plenum Press, 243–333.

Shulgin AT, Bunnell S and Sargent T. (1961) The psychotomimetic properties of 3,4,5-trimethoxyamphetamine. Nature 189: 1011–1012.

Shulgin AT and Shulgin A. (1991) PiHKAL: A Chemical Love Story, Berkeley, CA: Transform Press.

Shulgin AT and Shulgin A. (1997) TIHKAL: the Continuation, Berkeley: Transform Press.

Silva MT and Calil HM. (1975) Screening hallucinogenic drugs: systematic study of three behavioral tests. Psychopharmacologia 42: 163–171. [PubMed: 1080571]

Smith DA, Bailey JM, Williams D, et al. (2014) Tolerance and cross-tolerance to head twitch behavior elicited by phenethylamine- and tryptamine-derived hallucinogens in mice. J Pharmacol Exp Ther 351: 485–491. [PubMed: 25271256]

Smythies JR, Bradley RJ, Johnston VS, et al. (1967) Structure-activity relationship studies on mescaline. 3. The influence of the methoxy groups. Psychopharmacologia 10: 379–387. [PubMed: 5588333]

Valle M, Maqueda AE, Rabella M, et al. (2016) Inhibition of alpha oscillations through serotonin-2A receptor activation underlies the visual effects of ayahuasca in humans. Eur Neuropsychopharmacol 26: 1161–1175. [PubMed: 27039035]

Vickers SP, Easton N, Malcolm CS, et al. (2001) Modulation of 5-HT(2A) receptor-mediated head-twitch behaviour in the rat by 5-HT(2C) receptor agonists. Pharmacol Biochem Behav 69: 643–652. [PubMed: 11509227]

Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, et al. (1998) Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. Neuroreport 9: 3897–3902. [PubMed: 9875725]

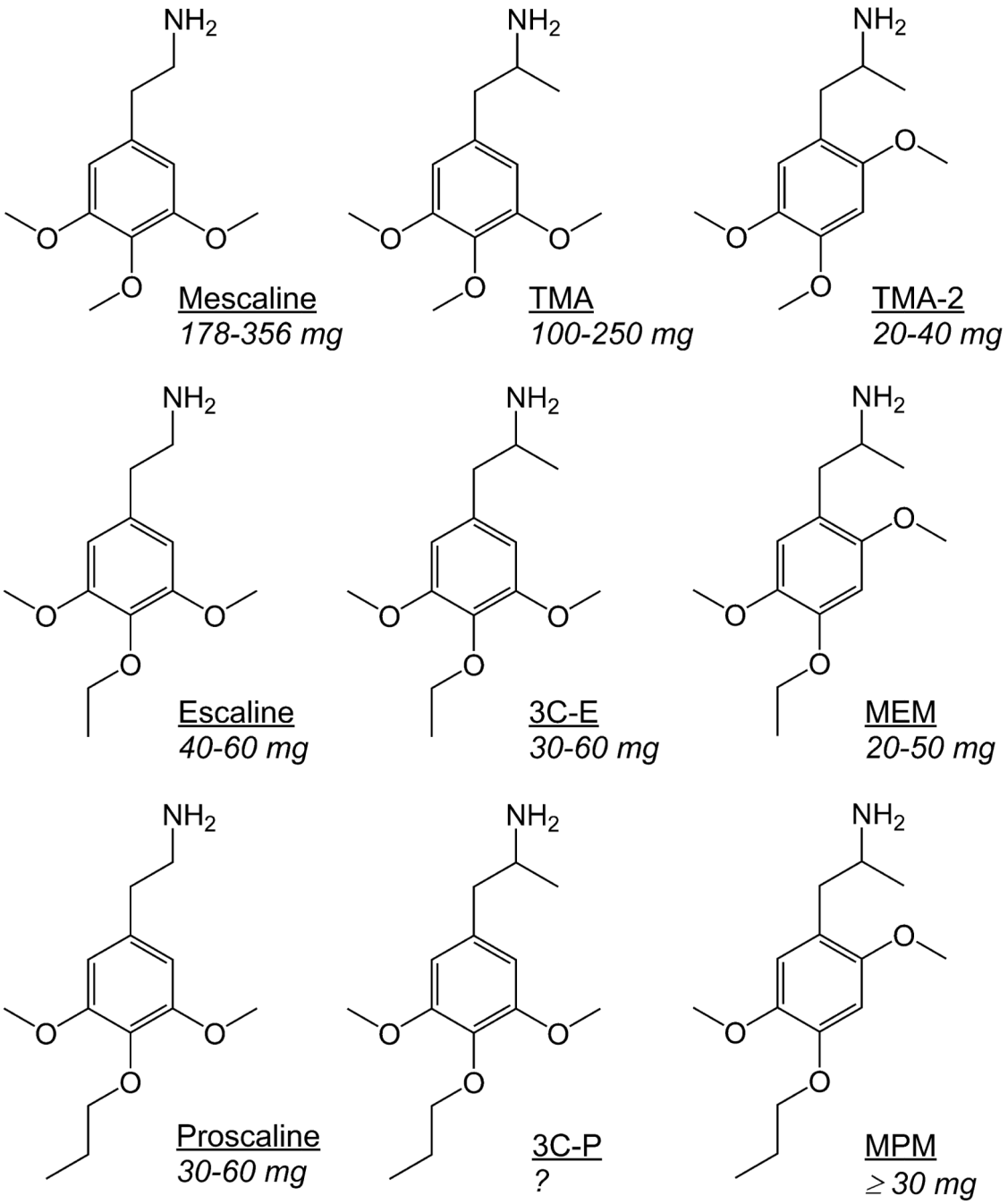

Figure 1.
Chemical structures of the hallucinogens tested in these studies. The typical dose ranges used in humans are indicated (data from: Shulgin and Shulgin, 1991; Shulgin, 1978).

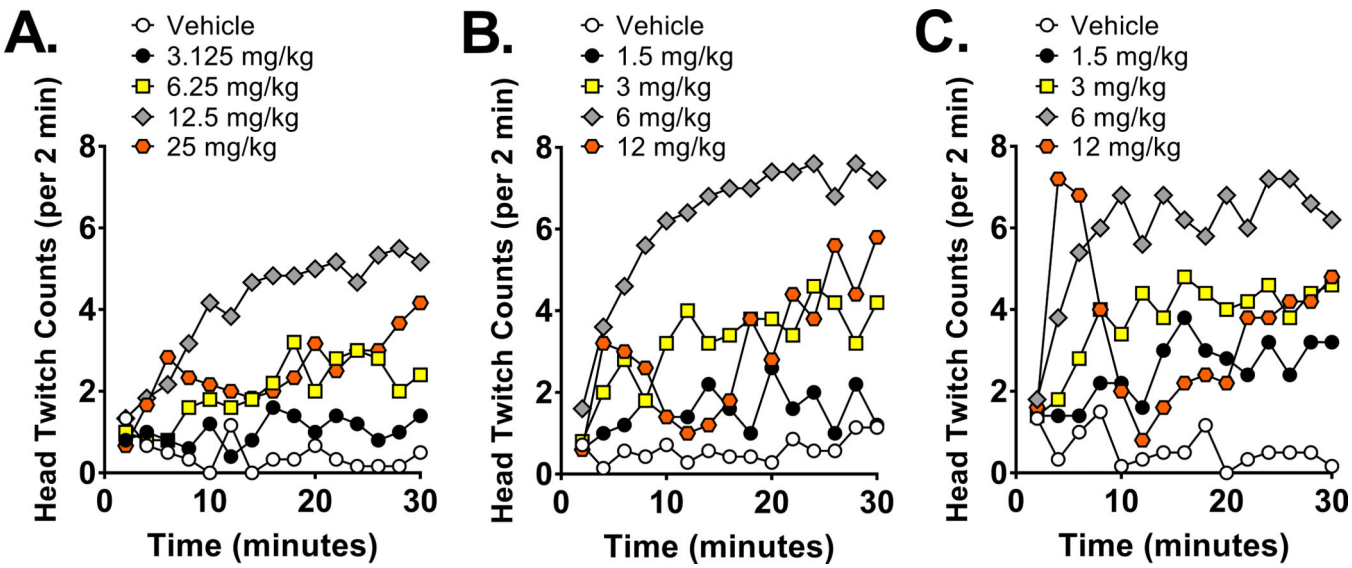

Figure 2.

Time-course of the head twitch response induced by mescaline (*A*) and its 4-position homologs escaline (*B*) and proscaline (*C*). Data are presented as group means in 2-min blocks.

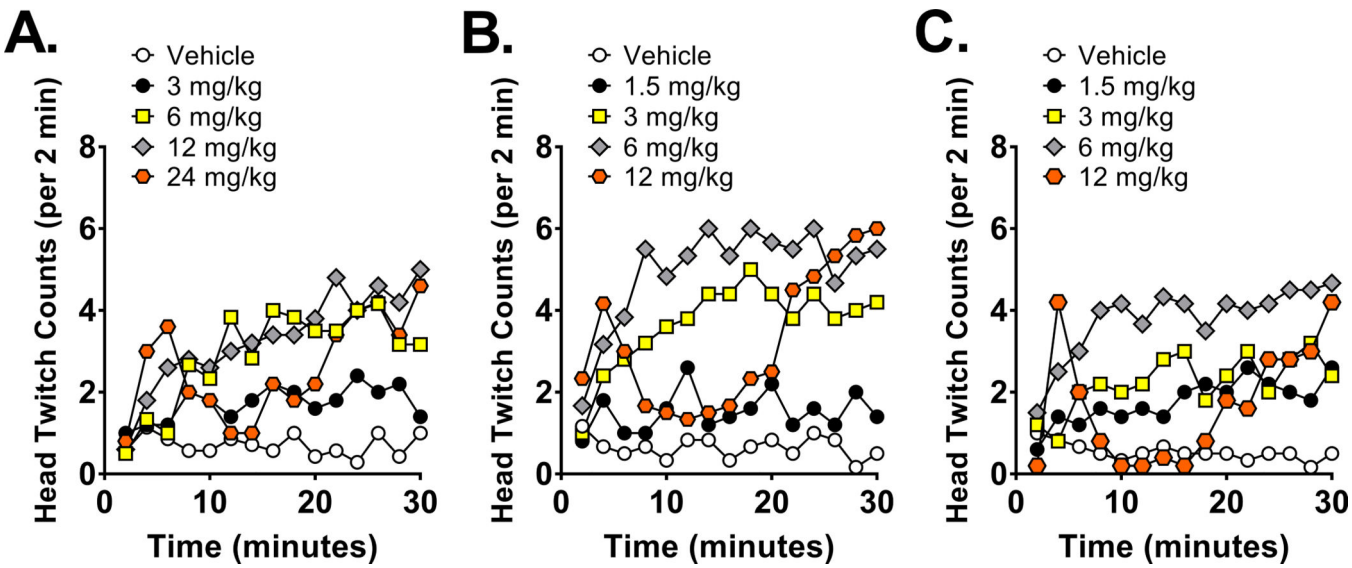

Figure 3.

Time-course of the head twitch response induced by TMA (*A*) and its 4-position homologs 3C-E (*B*) and 3C-P (*C*). Data are presented as group means in 2-min blocks.

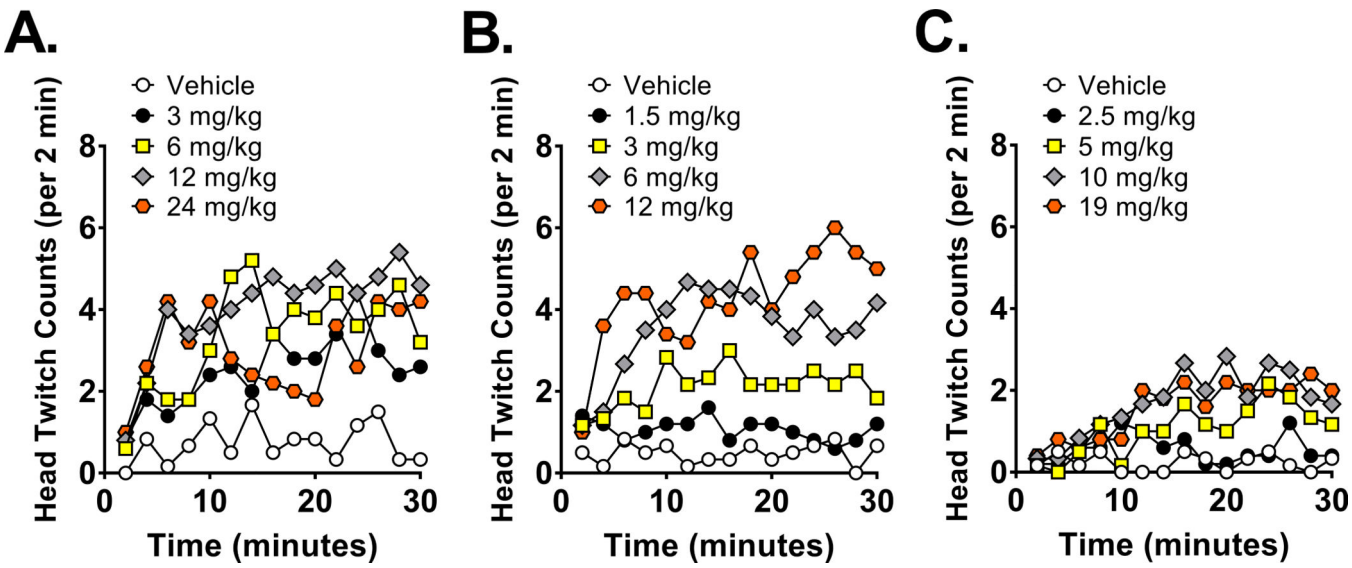

Figure 4.

Time-course of the head twitch response induced by TMA-2 (*A*) and its 4-position homologs MEM (*B*) and MPM (*C*). Data are presented as group means in 2-min blocks.

Table 1.

Summary of the head twitch response (HTR) data.

Compound	One-way ANOVA	Time (min)	Dose (mg/kg)	*N*	HTR Counts (mean ± SEM)	ED_{50} mg/kg (95% CI)	ED_{50} μmol/kg (95% CI)
Mescaline[a]	$F(4,23)=7.54$, $p=0.0005$	30	0	6	6.7 ± 1.0	6.51	26.3
			3.125	5	15.4 ± 3.0	(4.69–9.02)	(18.9–36.4)
			6.25	5	29.6 ± 4.9		
			12.5	6	61.5 ± 8.2 **		
			25	6	37.3 ± 13.4		
Escaline[a]	$F(4,22)=14.95$, $p<0.0001$	30	0	7	8.7 ± 2.8	2.94	11.2
			1.5	5	22.8 ± 4.9	(2.37–3.65)	(9.0–14.0)
			3	5	48.4 ± 6.6 *		
			6	5	92.8 ± 16.4 **		
			12	5	45.4 ± 7.9 *		
Proscaline[a]	$F(4,21)=8.55$, $p=0.0003$	30	0	6	8.8 ± 2.2	2.23	8.09
			1.5	5	37.2 ± 4.4	(1.42–3.10)	(5.13–11.2)
			3	5	56.4 ± 10.6 *		
			6	5	88.2 ± 12.8 **		
			12	5	51.6 ± 16.0 *		
TMA[a]	$F(4,22)=6.32$, $p=0.0015$	30	0	7	10.6 ± 1.4	3.57	13.6
			3	5	26.0 ± 3.6	(2.05–6.19)	(7.84–23.6)
			6	5	44.0 ± 9.8 **		
			12	5	49.8 ± 7.2 **		
			24	5	39.0 ± 9.6 *		
3C-E[a]	$F(4,23)=8.56$, $p=0.0002$	30	0	6	9.8 ± 1.8	2.36	8.54
			1.5	5	22.6 ± 3.9	(1.42–3.10)	(6.02–12.1)
			3	5	55.4 ± 1.7 **		
			6	6	74.3 ± 12.5 **		
			12	6	48.5 ± 13.2 *		
3C-P[a]	$F(4,22)=6.31$, $p=0.0015$	30	0	6	8.0 ± 1.6	2.45	8.47
			1.5	5	26.6 ± 1.9	(1.72–3.51)	(5.92–12.1)
			3	5	33.8 ± 7.0		
			6	6	57.0 ± 13.0 **		
			12	5	25.2 ± 4.3		

Compound	One-way ANOVA	Time (min)	Dose (mg/kg)	*N*	HTR Counts (mean ± SEM)	ED_{50} mg/kg (95% CI)	ED_{50} μmol/kg (95% CI)
TMA-2[b]	$F(4,24)=7.26$, $p=0.0003$	30	0	5	11.6 ± 3.7	2.79	12.4
			1.5	5	23.6 ± 6.2	(1.94–4.01)	(8.61–17.8)
			3	5	38.6 ± 10.1		
			6	5	52.4 ± 6.1 **		
			12	5	60.8 ± 7.1 **		
			24	5	47.4 ± 6.4 *		
MEM[a]	$F(4,23)=12.16$, $p<0.0001$	30	0	6	7.2 ± 1.0	3.75	13.6
			1.5	5	15.2 ± 3.6	(1.88–7.49)	(6.80–27.2)
			3	6	31.5 ± 2.8		
			6	6	52.7 ± 6.1 **		
			12	5	64.8 ± 15.0 **		
MPM[c]	$F(5,27)=5.38$, $p=0.0015$	30	0	6	3.5 ± 0.7	4.44	12.1
			1.25	5	5.0 ± 1.1	(2.74–7.19)	(7.46–19.6)
			2.5	5	8.2 ± 1.7		
			5	6	15.8 ± 1.7		
			10	6	25.3 ± 3.6 **		
			19	5	23.6 ± 9.8 *		

* $p < 0.05$

** $p < 0.01$, significant difference from the vehicle control group (Tukey's test).

[a] Hydrochloride salt

[b] freebase

[c] trifluoroacetate salt.