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Comparison of the behavioral responses induced by phenylalkylamine hallucinogens and their tetrahydrobenzodifuran ("FLY") and benzodifuran ("DragonFLY") analogs

Adam L. Halberstadt[1,2,*], **Muhammad Chatha**[1], **Alexander Stratford**[3], **Matthias Grill**[4], **Simon D. Brandt**[5]

[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]Synex Synthetics BV, Karveelweg 20, 6222 NH Maastricht, The Netherlands [4]Lipomed AG, Fabrikmattenweg 4, CH-4144 Arlesheim, Switzerland [5]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK

Abstract

In recent years, rigid analogues of phenylalkylamine hallucinogens have appeared as recreational drugs. Examples include 2-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine (2C-B-FLY) and 1-(8-bromobenzo[1,2-*b*;4,5-*b*']difuran-4-yl)-2-aminopropane (Bromo-DragonFLY, DOB-DFLY). Although some rigid compounds such as DOB-DFLY reportedly have higher potency than their non-rigid counterparts, it is not clear whether the same is true for 2C-B-FLY and other tetrahydrobenzodifurans. 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 2,5-dimethoxy-4-bromoamphetamine (DOB) and its α-desmethyl homologue 2,5-dimethoxy-4-bromophenethylamine (2C-B), as well as their benzodifuranyl and tetrahydrobenzodifuranyl analogs, in C57BL/6J mice. DOB (ED_{50} = 0.75 μmol/kg) and 2C-B (ED_{50} = 2.43 μmol/kg) induced the HTR. The benzodifurans DOB-DFLY (ED_{50} = 0.20 μmol/kg) and 2C-B-DFLY (ED_{50} = 1.07 μmol/kg) had significantly higher potency than DOB and 2C-B, respectively. The tetrahydrobenzodifurans DOB-FLY (ED_{50} = 0.67 μmol/kg) and 2C-B-FLY (ED_{50} = 1.79 μmol/kg), by contrast, were approximately equipotent with their non-rigid counterparts. Three novel tetrahydrobenzodifurans (2C-I-FLY, 2C-E-FLY and 2C-EF-FLY) were also active in the HTR assay but had relatively low potency. In summary, the *in vivo* potency of 2,5-dimethoxyphenylalkylamines is enhanced when the 2- and 5-methoxy groups are incorporated

*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.

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into aromatic furan rings, whereas potency is not altered if the methoxy groups are incorporated into dihydrofuran rings. The potency relationships for these compounds in mice closely parallel the human hallucinogenic data. The high potency of DOB-DFLY is probably linked to the presence of two structural features (a benzodifuran nucleus and an α-methyl group) known to enhance the potency of phenylalkylamine hallucinogens.

Keywords

5-HT_{2A} receptor; psychedelics; head twitch; new psychoactive substance; NPS; 2C-B-DragonFLY; 3C-B-FLY

1. INTRODUCTION

Serotonergic hallucinogens induce profound alterations of perception, mood, and cognition (Preller and Vollenweider 2018). One group of hallucinogens are based on the phenylalkylamine structural template, including phenylethylamines such as mescaline and 2,5-dimethoxy-4-bromophenylethylamine (2C-B), and amphetamines such as 2,5-dimethoxy-4-bromoamphetamine (DOB) and 2,5-dimethoxy-4-methylamphetamine (DOM). Examples of compounds from these classes are shown in Figure 1. Analogs of phenylalkylamine hallucinogens have also been synthesized where the methoxy groups are incorporated into rigid dihydrofuran or furan rings (Monte et al. 1996; Parker et al. 1998; Chambers et al. 2001). Serotonergic hallucinogens containing 2,3,6,7-tetrahydrobenzo[1,2-*b*;4,5-*b*′]difuran or benzo[1,2-*b*;4,5-*b*′]difuran ring systems are commonly designated by the code-names “FLY” and “DragonFLY”, respectively, because of their structural resemblance to winged insects (Trachsel et al. 2013). These compounds were originally developed to probe the active binding conformation of phenylalkylamine hallucinogens at the 5-HT_{2A} receptor, which is believed to be the primary site of action of (+)-lysergic acid diethylamide (LSD) and other serotonergic hallucinogens (Vollenweider et al. 1998; Kometer et al. 2013; Valle et al. 2016; Kraehenmann et al. 2017; Preller et al. 2017). In recent years, several of these rigid phenylalkylamines have appeared as recreational drugs. According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), the European Union Early-Warning System reported the first detection of Bromo-DragonFLY (DOB-DFLY) and 2C-B-FLY in 2006 and 2007, respectively (EMCDDA 2007,2008; King 2014). DOB-DFLY was also identified in drug exhibits seized in Oregon in 2007 and Australia in 2008 (Anonymous 2007,2008). Recreational use of DOB-DFLY has been linked to multiple fatalities as well as severe adverse reactions including seizures and limb ischemia (Personne and Hultén 2008; Thorlacius et al. 2008; Andreasen et al. 2009; Wood et al. 2009; Nielsen et al. 2010; Corazza et al. 2011; Chavarin et al. 2013; Iwersen-Bergmann et al. 2018).

DOB-DFLY is a highly potent compound. DOB-DFLY has sub-nanomolar affinity for the 5-HT_{2A} receptor, with a K_i value of 0.04 nM for the cloned human receptor labeled with [^{125}I]DOI (Parker et al. 1998). DOB-DFLY is reportedly active orally in humans in the range of 0.2–0.8 mg (Trachsel et al. 2013) and has a slow onset and long duration of action, potentially lasting for up to three days. In comparison, LSD has a typical dosage range of 60–200 μg p.o. (Shulgin and Shulgin 1997). DOB-DFLY also shows rather potent activity in

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rats trained to discriminate LSD from saline (ED_{50} = 22 nmol/kg) (Parker et al. 1998) and is more potent than DOB (ED_{50} = 1.06 μmol/kg) (Chambers et al. 2003). In contrast to DOB-DFLY, relatively little is known about the activity of other hallucinogens containing a benzodifuran ring system.

Tethering the methoxy groups of phenylalkylamine hallucinogens into a tetrahydrobenzodifuran nucleus has little effect on 5-HT_{2A} affinity. For example, DOB (K_i = 22 nM) and DOB-FLY (K_i = 18 nM) have equivalent affinities for 5-HT_{2A} receptors labeled with [^{3}H]ketanserin in rat cortical homogenates (Monte et al. 1996). 2C-B (K_i = 8.6 nM) and 2C-B-FLY (K_i = 11 nM) have similar affinities for cloned human 5-HT_{2A} receptors using [^{3}H]ketanserin as the radioligand (Rickli et al. 2015a; Rickli et al. 2015b). 2C-B and 2C-B-FLY are also essentially equipotent in humans; 2C-B-FLY is active at doses of 10–20 mg orally (Hanna et al. 2008; Green 2013) and 2C-B is active at 12–24 mg (Shulgin and Shulgin 1991). Although little is known about the human psychopharmacology of DOB-FLY, it reportedly produces hallucinogenic effects at 1 mg orally (Shulgin et al. 2011), which overlaps with the 1–3 mg effective dosage range for DOB (Shulgin and Shulgin 1991). Although the presence of a tetrahydrobenzodifuran functionality does not appreciably alter the 5-HT_{2A} receptor affinity or human potency of phenylalkylamine hallucinogens, it does increase their potency in the drug discrimination paradigm. Both DOB and DOB-FLY produce full substitution in rats trained to discriminate 0.08 mg/kg LSD from saline but the latter compound has 18-fold higher potency (Nichols et al. 1994; Monte et al. 1996). Likewise, 2C-B-FLY has almost fourfold higher potency than 2C-B in LSD-trained rats (Monte et al. 1996; Juncosa et al. 2013).

The conflicting data described above led us to compare the potencies of 2C-B and DOB relative to their tetrahydrobenzodifuranyl and benzodifuranyl analogs using the head twitch response (HTR). 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; Halberstadt et al. 2011; Canal and Morgan 2012; Halberstadt and Geyer 2014). The HTR is commonly 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). Similar to the discriminative stimulus effects of hallucinogens, the HTR serves as a behavioral readout of 5-HT_{2A} activation and can be used to compare the *in vivo* potencies of 5-HT_{2A} receptor agonists (Halberstadt and Geyer 2014; Nichols et al. 2015; Brandt et al. 2016; Brandt et al. 2017; Halberstadt et al. 2018; Klein et al. 2018). Although DOB is known to produce head twitches in rodents (Wieland et al. 1990; Gonzalez-Maeso et al. 2007; Moya et al. 2007), it is not yet clear whether 2C-B and other phenylethylamine hallucinogens can reliably induce the behavior (c.f., Moya et al. 2007). In the present investigation, mouse HTR studies were conducted with 2C-B and DOB, their benzodifuranyl and tetrahydrobenzodifuranyl analogs, as well as three novel phenethylamines with rigidified methoxy groups (2C-I-FLY, 2C-E-FLY, and 2C-EF-FLY). The results confirmed that 2C-B and DOB are active in the HTR assay. Furthermore, although the *in vivo* potency of 2C-B and DOB was unaffected by incorporating their 2- and 5-methoxy groups into dihydrofuran rings, incorporating the methoxy groups into fully aromatic furan rings resulted in a significant enhancement of potency.

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2. MATERIALS AND METHODS

2.1. 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 animal care committee.

2.2. Drugs

4-Bromo-2,5-dimethoxyphenethylamine (2C-B) hydrochloride, 4-bromo-2,5-dimethoxyamphetamine (DOB) hydrochloride, 1-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)propan-2-amine (DOB-FLY) hydrochloride, and 1-(8-bromobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)propan-2-amine (DOB-DFLY) hydrochloride were obtained from Cayman Chemical (Ann Arbor, MI, USA). 2-(8-Bromobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine (2C-B-DFLY) hydrochloride was obtained from Lipomed Inc. (Arlesheim, Switzerland). 2-(8-Bromo-2,3,6,7-tetrahydrobenzo[1,2-b:4,5-*b*′]difuran-4-yl)ethan-1-amine (2C-B-FLY) hydrochloride, 2-(8-iodo-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine (2C-I-FLY) hydrochloride, 2-(8-ethyl-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine (2C-E-FLY) hydrochloride, and 2-[8-(2-fluoroethyl)-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl]ethan-1-amine (2C-EF-FLY) hydrochloride were obtained from Synex Synthetics BV (Maastricht, Netherlands). Test substances were dissolved in isotonic saline and injected intraperiotoneally (IP) at a volume of 5 mL/kg.

2.3. Head Twitch Response Studies

The head twitch response (HTR) was assessed using a head-mounted magnet and a magnetometer detection coil (Halberstadt and Geyer 2013,2014; Nichols et al. 2015; Klein et al. 2018). 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. According to experiments performed in our laboratory (data not shown) and literature reports (Nagayama and Lu 1996), the level of illumination does not influence the magnitude of the HTR induced by hallucinogens. 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, and digitized (20 kHz sampling rate) using a Powerlab/8SP with LabChart v 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

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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. Individual experiments were performed between-subjects, with pseudorandomized group assignments, which further reduces the likelihood of carryover effects.

2.4. Data Analysis

The entire 30-min recordings were examined for head twitches, but in some cases a shorter block of time was used for analysis to accommodate compounds with a brief duration-of-action since potency calculations can be confounded by extended periods of inactivity. 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. To determine whether potency differences exist between individual compounds, ED_{50} values were compared using an extra-sum-of-squares *F*-test. Significance was demonstrated by surpassing an α-level of 0.05.

3. RESULTS

The HTR data are presented in Table 1. 2C-B and DOB, as well as their tetrahydrobenzodifuranyl analogs 2C-B-FLY and DOB-FLY, induced the HTR in mice. 2C-B (ED_{50} = 2.43 μmol/kg) and 2C-B-FLY (ED_{50} = 1.79 μmol/kg) were approximately equipotent ($F(1,55)=1.16$, *NS*). The same was observed for DOB (ED_{50} = 0.75 μmol/kg) and DOB-FLY (ED_{50} = 0.67 μmol/kg)($F(1,53)=0.17$, *NS*). Based on these results, tethering the

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methoxy groups of 2,5-dimethoxyphenylalkylamines into a rigid tetrahydrobenzodifuran ring system does not appear to alter their behavioral potency in mice.

In addition to examining the tetrahydrobenzodifuranyl analogs of 2C-B and DOB, the fully aromatic benzodifuranyl analogs were also evaluated. As shown in Table 1, DOB-DFLY (the benzodifuranyl analog of DOB) induced the HTR with an ED_{50} of 204.8 nmol/kg, making it significantly more potent than DOB ($F(1,29)=24.89$, $p<0.0001$). In comparison, LSD had an ED_{50} of 132.8 nmol/kg under equivalent conditions (Halberstadt and Geyer 2013), meaning that DOB-DFLY induces the HTR with 65% of the molar potency of LSD. 2C-B-DragonFLY (2C-B-DFLY), the benzodifuranyl analog of 2C-B, was also active in the HTR assay (ED_{50} = 1.07 μmol/kg) and was more potent than 2C-B ($F(1,59)=5.08$, $p=0.0279$).

HTR counts were also analyzed in 2-min bins to examine the time-course of the responses. There was no interaction between drug and time for 2C-B ($F(70,350)=1.10$, *NS*). Similar to 2C-I (Halberstadt and Geyer 2014), 2C-B induced a relatively constant level of responding during the 30-min test session (Fig. 2A). By contrast, time was a factor in the responses produced by 2C-B-FLY (Drug × Time: $F(70,336)=3.48$, $p<0.0001$), 2C-B-DFLY (Drug × Time: $F(70,392)=2.78$, $p<0.0001$), DOB (Drug × Time: $F(70,378)=5.64$, $p<0.0001$), DOB-FLY (Drug × Time: $F(56,294)=6.12$, $p<0.0001$), and DOB-DFLY (Drug × Time: $F(56,322)=1.45$, $p=0.0266$). For the latter compounds, the responses typically peaked during the first 10 min of the assessment and then plateaued, with the interval between injection and maximal effect being inversely proportional to the dosage (Fig. 2B-F).

Several other phenylethylamines with rigidified methoxy groups were also tested in mice (see Fig. 3). As shown in Table 2, 2C-I-FLY, 2C-E-FLY, and 2C-EF-FLY were active in the HTR paradigm. 2C-E-FLY (ED_{50} = 2.10 μmol/kg) was almost as potent as 2C-B-FLY, whereas 2C-EF-FLY (ED_{50} = 4.37 μmol/kg) and 2C-I-FLY (ED_{50} = 5.12 μmol/kg) induced the HTR with 2- and 3-fold lower potency, respectively.

In order to investigate the influence of α-methyl substitution on the response to phenylalkylamine hallucinogens, we compared the potencies of the amphetamines and their α-desmethyl (i.e., phenylethylamine) homologues. As noted above, DOB has a threefold higher potency than 2C-B, which is a statistically significant difference ($F(1,58)=14.54$, $p=0.0003$). Likewise, DOB-FLY was significantly more potent than 2C-B-FLY ($F(1,50)=12.32$, $p=0.001$) and DOB-DFLY was significantly more potent than 2C-B-DFLY ($F(1,30)=19.00$, $p=0.0001$).

4. DISCUSSION

The present investigation was conducted to assess the behavioral effects of the phenylalkylamines 2C-B and DOB, as well as several analogs incorporating tetrahydrobenzodifuran and benzodifuran ring systems. Consistent with their activity as serotonergic hallucinogens, 2C-B and DOB induced the HTR in mice. DOB was previously shown to produce head twitches (Wieland et al. 1990; Benneyworth et al. 2007; Gonzalez-Maeso et al. 2007; Moya et al. 2007), but as far as we are aware this is the first evidence that 2C-B is active in the HTR assay. The ability of these phenylalkylamines to induce the HTR

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is consistent with their 5-HT_{2A} agonist activity (Chambers et al. 2001; Chambers et al. 2003; Rickli et al. 2015a). Incorporating the 2- and 5-methoxy groups of 2C-B and DOB into aromatic furan rings resulted in a significant increase in potency in the HTR assay. Conversely, incorporating the methoxy groups into dihydrofuran rings did not alter the potency of 2C-B or DOB to an appreciable degree. These potency differences in mice are consistent with the limited human data reported to date. Our findings provide additional evidence that the mouse HTR assay can be used to investigate the SAR of serotonergic hallucinogens.

The absence of a potency difference between 2C-B-FLY and DOB-FLY and their non-rigid counterparts (2C-B and DOB, respectively) is noteworthy. Based on these results, the tetrahydrobenzodifuran nucleus present in the FLY compounds likely matches the active conformation of the methoxy groups in 2,5-dimethoxyphenylalkylamines. As was previously noted by Nichols and colleagues (Monte et al. 1996), when 2,5-dimethoxyphenylalkylamines bind to the 5-HT_{2A} receptor, the oxygen lone pairs of the 2-methoxy group are likely oriented *syn* to the aminoalkyl side-chain, whereas the lone pairs in the 5-methoxy group are likely oriented *anti* to the side-chain. These findings are consistent with the 5-HT_{2A} receptor model proposed by Westkaemper and Glennon (1994) where two serine residues on opposite sides of the binding pocket donate hydrogen-bonds to the methoxy groups seen in 2,5-dimethoxyphenylalkylamines. Subsequent mutagenesis and homology modelling studies identified Ser-159 and Ser-239 as likely candidates to engage the 2- and 5-methoxy groups, respectively (Braden and Nichols 2007; Isberg et al. 2011).

In contrast to the tetrahydrobenzodifurans, the aromatic benzodifurans had significantly higher potency than their non-rigid counterparts. Indeed, DOB-DFLY has about five times the potency of DOB in humans (Trachsel et al. 2013) and also possesses considerably higher affinity for the 5-HT_{2A} receptor than DOB (Parker et al. 1998). With the exception of DOB-DFLY, little is known about the human psychopharmacology of hallucinogens with a benzodifuranyl structure; however, based on the present findings, it is reasonable to anticipate that 2C-B-DFLY would have significantly higher potency than 2C-B and 2C-B-FLY in humans. The relatively high affinity of benzodifuran hallucinogens at 5-HT_{2A} receptors is somewhat unexpected because aromatization of the tetrahydrobenzodifuran ring system should reduce the hydrogen-bonding capacity of its oxygen atoms. There are at least two potential explanations for the affinity increase. First, the orthosteric 5-HT_{2A} binding pocket is a relatively hydrophobic environment, so any detrimental effect of aromatization on hydrogen-bonding may be offset by the increased hydrophobicity of a benzodifuran ring system. Second, the extended tricyclic aromatic ring system in the benzodifurans could increase 5-HT_{2A} affinity by enhancing the Van der Waals interaction known to occur between the aromatic ring of phenylalkylamine hallucinogens and Phe-340 in the binding pocket (Parrish et al. 2005; Braden et al. 2006; Isberg et al. 2011).

In addition to their 5-HT_{2A} affinity, another factor potentially contributing to the relatively high *in vivo* potency of the benzodifurans might be their reported metabolic stability. According to Noble et al. (2018), no metabolism occurred when DOB-DFLY was incubated with pooled human liver microsomes (HLM), pooled human liver cytosol (HLC), monoamine oxidase (MAO), flavin-containing monooxygenase (FMO) or cytochrome P450

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(CYP) isoenzymes. By contrast, DOB, DOI, DOM, and 2C-B-FLY are metabolized by CYP2D6 (Ewald and Maurer 2008; Noble et al. 2018). The benzodifuran nucleus in DOB-DFLY and other DragonFLY compounds may be resistant to hepatic metabolism, potentially resulting in prolonged bioavailability and toxicity.

In contrast to our results, 2C-B failed to provoke the HTR in rats when tested in a previous study (Moya et al. 2007). The failure of 2C-B to induce the HTR is surprising because it acts as a 5-HT_{2A} agonist (Parrish et al. 2005; McLean et al. 2006; Rickli et al. 2015b), fully substitutes in rats trained to discriminate LSD and DOM (Glennon et al. 1988; Juncosa et al. 2013), and produces hallucinogenic effects in humans (Shulgin and Shulgin 1991; Caudevilla-Galligo et al. 2012; Papaseit et al. 2018). Furthermore, several other phenylethylamine hallucinogens, including mescaline (Corne and Pickering 1967; Silva and Calil 1975; Yamamoto et al. 1983), 2C-I (Halberstadt and Geyer 2014; Elmore et al. 2018), 2C-C (Elmore et al. 2018), and 2C-T-7 (Fantegrossi et al. 2005) are known to induce head twitches. Differences in experimental design could have played a role in these discrepant findings. For example, Moya et al. assessed head twitches during the light phase of the 24-h light/dark cycle, whereas our experiments were conducted during the dark phase of the circadian cycle. The magnitude of the HTR is known to vary over the light/dark cycle (Singleton and Marsden 1981; Moser and Redfern 1985; Nagayama and Lu 1996; Darmani 1998). Based on our findings, 2C-B and other phenylethylamine hallucinogens clearly induce head twitches when administered to mice and therefore do not produce false negative results in this behavioral assay.

An additional goal of the present investigation was to compare the *in vivo* potencies of amphetamine hallucinogens and their α-desmethyl homologues. Amphetamine-based hallucinogens are typically more potent than phenylethylamines *in vivo*. For example, DOB is about ten times more potent than 2C-B in humans (Shulgin and Shulgin 1991). Similarly, 3,4,5-trimethoxyamphetamine (TMA) is twice as potent as mescaline (Shulgin et al. 1961). Only a few preclinical studies have investigated the influence of an α-methyl group on the behavioral potency of phenylalkylamine hallucinogens. In drug discrimination studies in rats, TMA is more potent than mescaline (Glennon and Young 1982), DOM has higher potency than its α-desmethyl homologue (Glennon et al. 1983), and DOB exceeds the potency of 2C-B (Glennon et al. 1988). Likewise, DOB-FLY has about four times the potency of 2C-B-FLY in rats trained with LSD (Monte et al. 1996). Our experiments confirmed that DOB, DOB-FLY, and DOB-DFLY have two- to threefold greater potency than their phenylethylamine homologues in mice. The presence of an α-methyl group has little effect on the 5-HT_{2A} affinity of phenylethylamines (Johnson et al. 1990; Glennon et al. 1992; Parrish et al. 2005), so there must be another explanation for these potency differences. The presence of an α-methyl group may increase potency by reducing the metabolic lability of the amine side-chain (Nichols et al. 1991). Deamination by MAO is the primary route of metabolism for phenylethylamines such as 2C-B (Theobald and Maurer 2007; Kanamori et al. 2013) but plays only a minor role in the metabolism of amphetamines (Ho et al. 1971; Ewald et al. 2007; Ewald et al. 2008; Noble et al. 2018). DOB-DFLY was found to be a potent inhibitor of MAO_A (IC_{50} = 0.54 μM) (Noble et al. 2018). 2C-B-FLY, on the other hand, was reported to have a significantly weaker effect on the activity of this enzyme, with IC_{50} values ranging from 19 μM (Wagmann et al., manuscript submitted) to

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27.7 μM (Noble et al. 2018). The enhanced lipophilicity due to the α-methyl group could also potentially enhance CNS penetration. These two factors, either alone or in combination, likely explain why amphetamines have higher potency than phenylethylamines in our HTR studies.

In addition to the potency differences between amphetamines and phenylethylamines in the HTR assay, the amphetamines also induced a greater maximal number of head twitches compared to their phenylethylamine counterparts. For example, as shown in Figure 2, the response to DOB-FLY peaked at 15.6±0.9 (mean±SEM) head twitches/2 min, whereas 2C-B-FLY produced a maximum of 8.8±2.3 head twitches/2 min. In a previous study that examined the relationship between 5-HT_{2A} activation and head twitch for a series of 5-HT_2 ligands, intrinsic efficacy at 5-HT_{2A} was significantly correlated with the maximum number of head twitches but did not influence potency in the HTR assay (Vickers et al. 2001). Importantly, compared to phenylethylamines, amphetamines have higher intrinsic efficacy at cloned 5-HT_{2A} receptors (Nichols et al. 1994; Parrish et al. 2005; Moya et al. 2007). The presence of an α-methyl group reportedly strengthens the interaction of phenylalkylamines with Phe-340 in the 5-HT_{2A} binding pocket via a van der Waals interaction (Parrish et al. 2005), potentially helping to stabilize the receptor in the active conformation. Hence, differences in the magnitude of the HTR produced by amphetamines and phenylethylamines likely reflect differences in their agonist activity at the 5-HT_{2A} receptor, although additional work is necessary to confirm this explanation.

As noted in Section 1, DOB-DFLY is a potent hallucinogen, active in sub-milligram dosages and only marginally less potent than the prototypical agent LSD. The recreational use of DOB-DFLY has resulted in toxicity and fatalities (Personne and Hultén 2008; Thorlacius et al. 2008; Andreasen et al. 2009; Wood et al. 2009; Nielsen et al. 2010; Corazza et al. 2011; Chavarin et al. 2013; Iwersen-Bergmann et al. 2018). As shown by the HTR data, DOB-DFLY is also highly potent in mice, which is not surprising given that it contains two structural features (a benzodifuran ring system and an α-methyl group) capable of enhancing the potency of phenylalkylamine hallucinogens. 2C-B-FLY, by contrast, represents the fully saturated counterpart and does not contain the α-methyl group, and is therefore observed to be considerably less potent than DOB-DFLY. Similar to DOB-DFLY, the recreational use of 2C-B-FLY and other phenethylamine hallucinogens has been linked to adverse reactions. Although some of these cases occurred due to adulteration of samples with a high potency compound such as DOB-DFLY (Chavarin et al. 2013; Iwersen-Bergmann et al. 2018), in other cases the phenylethylamines themselves were the causative agents (Curtis et al. 2003; Drees et al. 2009; Topeff et al. 2011; Sacks et al. 2012; Bosak et al. 2013; Van Vrancken et al. 2013; Stoller et al. 2017). In addition to 2C-B and 2C-I, which are popular phenethylamine hallucinogens (de Boer and Bosman 2004; Caudevilla-Galligo et al. 2012; Burns et al. 2014), 4-ethyl-2,5-dimethoxyphenethylamine (2C-E) is also used recreationally (Topeff et al. 2011; Sacks et al. 2012; Van Vrancken et al. 2013; Woo and Hanley 2013). According to Shulgin and Shulgin (1991), 2C-E is active at doses of 10–25 mg. 4-(2-Fluoroethyl)-2,5-dimethoxyphenethylamine (2C-EF) also reportedly acts as a hallucinogen and is orally active in humans at doses of 6–12 mg with a duration of 12 hours (Shulgin et al. 2011), although information about the extent of recreational use of this substance appears to be lacking. Based on our HTR data, the tetrahydrobenzofurans 2C-I-

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FLY, 2C-E-FLY and 2C-EF-FLY tested in this study for the first time are likely to have LSD-like psychopharmacology. These compounds may appear as new recreational drugs in the future but would likely exhibit relatively low potency compared to DOB-DFLY.

To summarize our findings, 2C-B, DOB, and several structurally rigid analogues induced the HTR in mice. The potency of 2C-B and DOB was increased by incorporating the 2- and 5-methoxy groups into aromatic furan rings, whereas potency was unaffected when the methoxy groups were incorporated into dihydrofuran rings. Indeed, the benzodifuran DOB-DFLY is known to be a highly potent hallucinogen in humans, whereas the tetrahydrobenzodifuran 2C-B-FLY is about as potent as its non-rigid analog 2C-B. In general, the potencies of these compounds in the mouse HTR assay parallel the human hallucinogenic data as well as the established structure-activity relationships for binding to the 5-HT_{2A} receptor. Although head twitches likely 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. Additional studies, however, are necessary to evaluate the predictive and translational value of ED_{50} values generated using this behavioral paradigm.

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- The hallucinogens 2C-B and DOB induced head twitches in mice
- Tethering the methoxy groups into a benzodifuran nucleus increased potency
- Tethering the methoxy groups into a tetrahydrobenzodifuran nucleus did not alter potency
- The benzodifuran hallucinogen Bromo-DragonFLY was highly potent in mice

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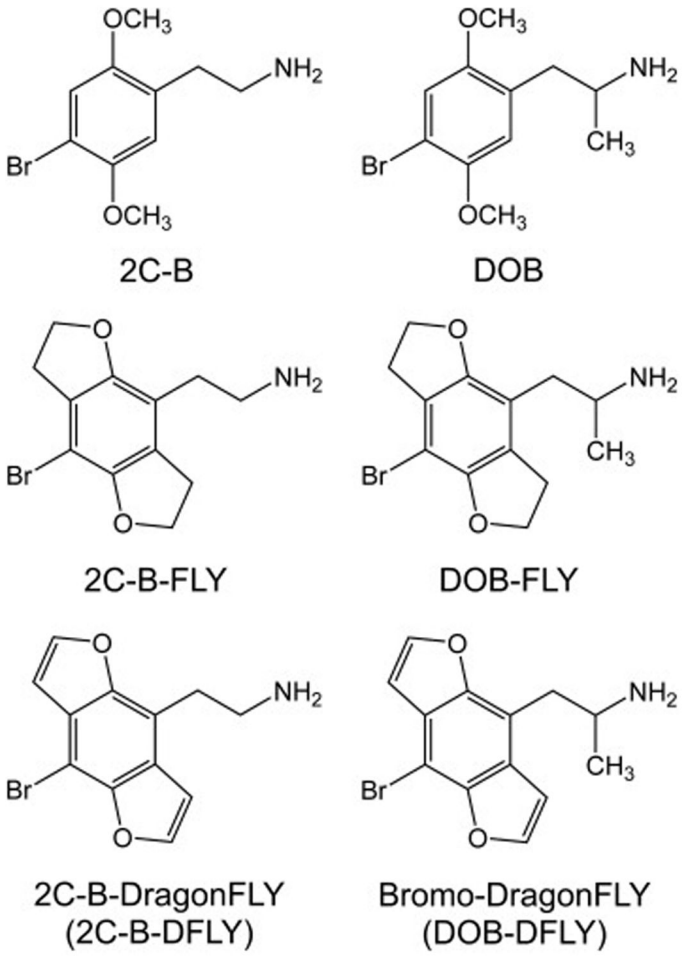


Figure 1.

Chemical structures of the phenylalkylamine hallucinogens 2C-B and DOB, as well as their tetrahydrobenzodifuran and benzodifuran analogs. *Abbreviations:* 2C-B (2,5-dimethoxy-4-bromophenethylamine), DOB (2,5-dimethoxy-4-bromoamphetamine), 2C-B-FLY (2-(8-bromo-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine), DOB-FLY (1-(8-bromo-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)propan-2-amine), 2C-B-DFLY (2-(8-bromobenzo [1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine), DOB-DFLY (1-(8-bromobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)propan-2-amine).

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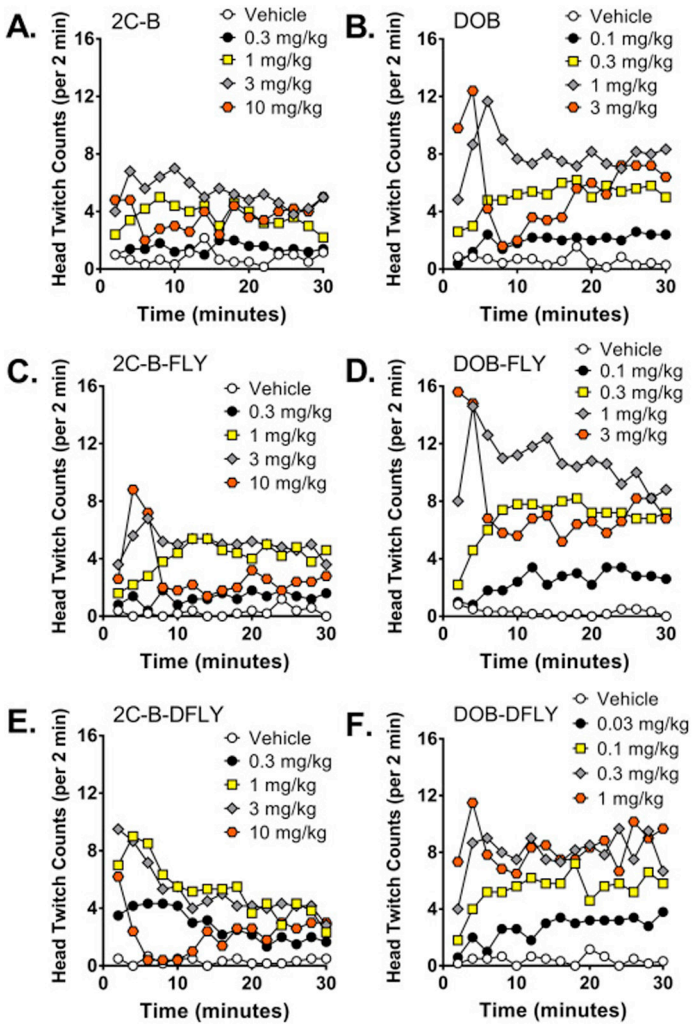


Figure 2.

Time-course of the head twitch response induced by 2C-B (*A*), DOB *(B)*, 2C-B-FLY *(C)*, DOB-FLY *(D)*, 2C-B-DFLY *(E)*, and DOB-DFLY *(F)*. Data are presented as group means in 2-min blocks. Groups receiving low doses were sometimes omitted from the graphs for clarity.

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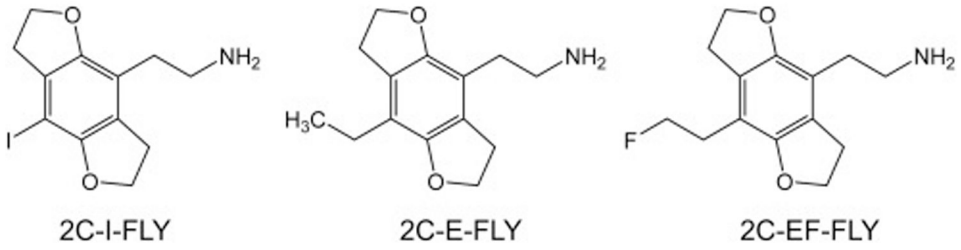


Figure 3.

Chemical structures of novel tetrahydrobenzodifuran hallucinogens. *Abbreviations:* 2C-I-FLY (2-(8-iodo-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine), 2C-E-FLY (2-(8-ethyl-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl)ethan-1-amine), and 2C-EF-FLY (2-[8-(2-fluoroethyl)-2,3,6,7-tetrahydrobenzo[1,2-*b*:4,5-*b*′]difuran-4-yl]ethan-1-amine).

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Table 1.

Summary of the head twitch response (HTR) data for the compounds shown in Figure 1.

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)
2C-B	$F(5,25)=17.60$, $p<0.0001$	30	0	6	11.8 ± 2.8	0.72 (0.48–1.09)	2.43 (1.62–3.66)
			0.1	5	23.8 ± 6.5		
			0.3	5	21.6 ± 4.9		
			1	5	54.6 ± 5.9 *		
			3	5	79.2 ± 9.3 *		
			10	5	55.0 ± 7.2 *		
DOB	$F(5,27)=28.79$, $p<0.0001$	30	0	7	9.1 ± 1.3	0.23 (0.17–0.32)	0.75 (0.55–1.03)
			0.03	5	16.4 ± 4.1		
			0.1	5	29.4 ± 3.5		
			0.3	5	75.8 ± 8.6 *		
			1	6	118.8 ± 15.4 *		
			3	5	85.4 ± 8.2 *		
2C-B-FLY	$F(5,24)=21.22$, $p<0.0001$	30	0	5	4.2 ± 0.6	0.57 (0.40–0.81)	1.79 (1.26–2.54)
			0.1	5	8.8 ± 1.9		
			0.3	5	19.6 ± 4.2		
			1	5	61.0 ± 5.5 *		
			3	5	75.2 ± 9.7 *		
			10	5	45.0 ± 9.8 *		
DOB-FLY	$F(4,21)=15.61$, $p<0.0001$	30	0	6	4.3 ±0.7	0.22 (0.14–0.35)	0.67 (0.43–1.04)
			0.1	5	36.4 ± 3.7		
			0.3	5	101.8 ± 10.3 *		
			1	5	160.2 ± 17.6 *		
			3	5	116.2 ± 31.6 *		
2C-B-DFLY	$F(5,28)=12.88$, $p<0.0001$	30	0	6	5.0 ± 0.3	0.34 (0.22–0.52)	1.07 (0.70–1.66)
			0.1	5	13.0 ± 7.2		
			0.3	6	41.7 ± 6.8		

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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)
DOB-DFLY	$F(4,23)=24.62$, $p<0.0001$	30	0	6	6.2 ± 1.6	0.068 (0.036–0.126)	0.20 (0.11–0.38)
			0.03	5	39.6 ± 1.8		
			0.1	5	80.4 ± 16.6 *		
			0.3	6	118.8 ± 7.6 *		
			1	6	124.5 ± 15.2 *		
			1	6	77.5 ± 11.5 *		
			3	6	77.5 ± 12.9 *		
			10	5	33.6 ± 5.9		

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

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Table 2.

Summary of the head twitch response (HTR) data for the compounds shown in Figure 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)
2C-E-FLY	$F(4,20)=9.29$, $p=0.0002$	30	0	5	5.6 ± 0.9	0.57 (0.32–1.02)	2.10 (1.17–3.77)
			0.3	5	16.2 ± 5.0		
			1	5	90.0 ± 14.2 *		
			3	5	89.4 ± 14.4 *		
			10	5	55.6 ± 20.3		
2C-EF-FLY	$F(5,25)=17.55$, $p<0.0001$	30	0	6	5.7 ± 0.6	1.26 (0.90–1.76)	4.37 (3.13–6.11)
			0.1	5	9.2 ± 2.8		
			0.3	5	12.4 ± 1.8		
			1	5	31.0 ± 6.5		
			3	5	75.8 ± 10.1 *		
			10	5	50.2 ± 11.1 *		
2C-I-FLY	$F(5,26)=26.48$, $p<0.0001$	30	0	6	7.0 ± 1.4	1.88 (1.42–2.50)	5.12 (3.87–6.78)
			0.3	5	10.8 ± 1.9		
			1	5	26.8 ± 3.9		
			3	6	64.3 ± 5.3 *		
			10	5	72.8 ± 8.2 *		
			30	5	14.0 ± 9.3		

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