

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

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Two primary animal models persist for assessing hallucinogenic potential of novel compounds and for examining the pharmacological and neurobiological substrates underlying the actions of classical hallucinogens, the two-lever drug discrimination procedure and the drug-induced head-twitch response (HTR) in rodents. The substituted amphetamine hallucinogen, serotonin 2 (5-HT₂) receptor agonist, 2,5-dimethoxy-4-iodoamphetamine (DOI) has emerged as the most popular pharmacological tool used in HTR studies of hallucinogens. Synthesizing classic, recent, and relatively overlooked findings, addressing ostensibly conflicting observations, and considering contemporary theories in receptor and behavioural pharmacology, this review provides an up-to-date and comprehensive synopsis of DOI and the HTR model, from neural mechanisms to utility for understanding psychiatric diseases. Also presented is support for the argument that, although both the two-lever drug discrimination and the HTR models in rodents are useful for uncovering receptors, interacting proteins, intracellular signalling pathways, and neurochemical processes affected by DOI and related classical hallucinogens, results from both models suggest they are not reporting hallucinogenic experiences in animals. Copyright © 2012 John Wiley & Sons, Ltd.

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A brief chemistry and timeline of DOI

The hallucinogen, 2,5-dimethoxy-4-iodoamphetamine (DOI), is in the broad class of compounds called phenylalkylamines. As the name suggests, phenylalkylamines consist of a phenyl ring attached to an alkyl chain that is attached to an amine. Having a two-carbon chain, the alkyl in DOI is ethyl, rendering phenethylamine. DOI has a methyl group attached to the α carbon of phenethylamine making its core structure, amphetamine (α -methyl-phenethylamine). Attached to the 2 and 5 carbons of the phenyl ring are methoxy groups, and attached to the 4 carbon is iodine. Amphetamine is also named phenylisopropylamine, and thus DOI is interchangeably identified as a substituted amphetamine or phenylisopropylamine. A detailed description of DOI's chemical make-up and its common nomenclature are shown in Figure 1.

DOI possesses one chiral centre and can exist as two enantiomers, (R)-(–)-DOI or (S)-(+)-DOI. Although enantiomers of many compounds exhibit vastly different affinities for their target receptors, this does not appear to be the case for DOI; the (–) enantiomer was reported as having only moderately (~2-fold) higher affinity than the racemate (\pm) or (+) enantiomer for rat cortical serotonin (5-hydroxytryptamine, 5-HT), 5-HT_{2A} receptors^[1,2] and human recombinant 5-HT_{2A} and 5-HT_{2C} receptors,^[3] and had slightly lower (< 2 fold) affinity than the (+) enantiomer for human recombinant 5-HT_{2B} receptors.^[3] There are reports that the behavioural potencies of the enantiomers of DOI, however, are substantially different in mice (induction of an ear-scratch response),^[4] rats (drug discrimination),^[5] and humans (subjective psychedelic effects),^[6,7] with (–)-DOI reported as more potent than (+)-DOI or the racemate. Also, the chemically similar DOM

(2,5-dimethoxy-4-methylamphetamine) was shown to have different potencies to induce hyperthermia in rabbits whether the (–) (more potent) or (+) (less potent) enantiomer was used; the racemate showed an intermediate potency, though somewhat overlapped with the (–) enantiomer.^[8] The greater potency of (–)-DOM was corroborated by behavioural studies in rats.^[9] Regarding the closely related analogue, DOB (2,5-dimethoxy-4-bromoamphetamine), (–)-DOB showed much greater potency and efficacy to activate 5-HT_{2C} receptors in rat choroid plexus compared to (+)-DOB,^[10] an effect recapitulated in rat behavioural studies, wherein (–)-DOB was much more effective than (+)-DOB for substituting for the DOI discriminative cue in rats.^[11]

Of the 2,5-dimethoxy-4 series of substituted amphetamine hallucinogens, synthesis of DOM was reported first in the scientific literature in 1970,^[12] followed later by DOB,^[13] and DOI.^[14] In their recent book, however, Shulgin *et al.* note that Shulgin synthesized DOM in 1963, and its psychedelic effects in humans were discovered in 1964.^[15] The radiolabelled DOI, [¹²⁵I]-DOI, which has high specific activity, was used to identify DOI binding

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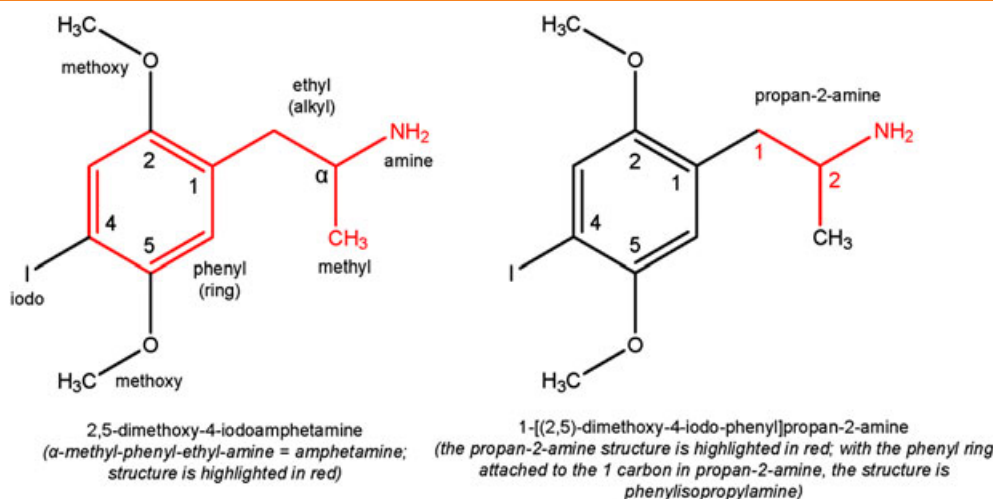


Figure 1. Commercial (a) and IUPAC (b) nomenclature for DOI.

sites in the rat brain in 1987.^[16] Since then, [¹²⁵I]-DOI has become a frequently used tool to label high affinity (or non-hydrolyzable GTP-sensitive) 5-HT₂ (5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C}) receptors for determination of ligand affinities of novel compounds in both recombinant DNA *in vitro* assays and *ex vivo*. Also, competition binding using [¹²⁵I]-DOI to label high affinity receptor binding sites may be part of a first-pass screen to test for 5-HT₂ agonist activity of novel compounds.^[17]

DOI has become one of the most common tools to study mechanisms of classical hallucinogens in animals, with over 100 scientific publications reporting its use since 2000. There are a few reasons for this: (1) of the substituted amphetamine hallucinogens, it is one of the few that is not specifically scheduled in the United States; (2) it is chemically stable even in solution; (3) it is relatively selective for activating 5-HT₂ receptors, activation of which likely underlies the psychoactive effects of classical hallucinogens.^[18]

Pharmacology of DOI

Most discussion regarding the pharmacology of DOI has centered on the 5-HT₂ G protein-coupled receptors (GPCRs) that include 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} subtypes. DOI has high affinity and is a potent agonist for each of the 5-HT₂ receptor subtypes, with respect to the traditional signalling pathways that they share.^[10,19–21] For example, using agonist radiolabelling, the *K_i* values of (±)-DOI for human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors were 0.7, 20, and 2.4 nM, respectively,^[20] and (±)-DOI's agonist potencies for stimulating recombinant human 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor-mediated calcium signalling were 0.9, 1.4, and 7.9 nM, respectively.^[19] DOI has relatively low affinity (*K_i* values greater than 1 μ M) for all other 5-HT receptors investigated.^[22,23] Though DOI's affinity for 5-HT₃ receptors has not been reported to our knowledge, it is likely that DOI also has low affinity for this receptor.^[2] Unique among the 5-HT₂ receptor subtypes, the 5-HT_{2C} receptor can be edited post-transcriptionally to produce more than 20 different isoforms.^[24,25] DOI's potency (both affinity and efficacy) is affected by RNA editing of the 5-HT_{2C} receptor, having lower potency for fully edited compared to non-edited 5-HT_{2C} receptors.^[26,27] RNA editing of 5-HT_{2C} receptors also affects their desensitization and trafficking in the

cell.^[28] Thus, when examining the effects of DOI (and other compounds) at 5-HT_{2C} receptors, the isoform(s) studied should be taken into consideration. This is feasible when assessing recombinant 5-HT_{2C} receptor isoforms *in vitro*, as expression of a specific isoform of the receptor is controlled by the experimenter. *In vivo*, however, it is not possible to determine the specific 5-HT_{2C} receptor protein isoform(s) that are examined in the brain from a wild-type animal using common techniques (e.g. radioligand binding). For example, the relative proportion of different htr2C (genetic nomenclature for 5-HT_{2C}) RNA isoforms that can be assessed by many existing methods does not directly correlate with the relative proportion of different 5-HT_{2C} receptor protein isoforms expressed in the cell membrane.^[29] This challenging issue warrants further attention.

Depending on the signalling pathway examined, DOI has been shown to be, *in vitro*, either a partial or a full agonist for 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors. At recombinant human 5-HT₂ receptors, DOI generally behaves as a potent partial agonist for stimulating 5-HT₂-mediated Ca⁺⁺ currents^[19] (used the 5-HT_{2C}-VSV isoform) and the phospholipase-C pathway, generating inositol phosphates (PLC-IP)^[30–32] (5-HT_{2C} isoform was not identified). DOI is a full agonist for activating 5-HT_{2C} receptors coupled to the phospholipase-A (PLA) signalling pathway,^[31,32] and is a partial agonist for activating 5-HT_{2A} receptors coupled to PLA.^[32] DOI's efficacy for activating 5-HT₂ receptors is also related to receptor expression level, displaying greater efficacy in cells expressing relatively higher receptor densities. For example, DOI behaves as a partial agonist (i.e. 53% efficacy relative to 5-HT) for 5-HT_{2C}-VSV receptor-mediated PLC-IP activity when receptors are expressed at ~300 fmol/mg,^[33] and as a full agonist (i.e. 101% efficacy relative to 5-HT) for 5-HT_{2C}-VSV receptor mediated PLC-IP activity when receptors are expressed at ~20 pmol/mg, suggesting a significant receptor reserve.^[34] *Ex vivo*, DOI exhibits partial agonism at 5-HT₂ receptors, for example at 5-HT_{2A} receptors expressed on GABAergic interneurons in piriform cortex,^[35] and 5-HT_{2C} receptors expressed in choroid plexus.^[10] To our knowledge, DOI's efficacy for activating 5-HT_{2B} receptors in the brain has not been tested, likely owing to their scant expression in the rodent brain.^[36]

DOI's pharmacology at receptors other than 5-HT receptors has received considerably less attention, but warrants discussion. A recent report^[23] presents binding affinity (*K_i*) data for DOI (among many other psychedelic compounds) at more than 40

unique receptors known to be expressed in the brain. DOI exhibited affinities, considered by the author to be noteworthy, for 19 receptors. The highest K_i value presented as noteworthy, however, was 2.72 μM , i.e. DOI's affinity for the M1 muscarinic receptor (shown in the supplementary material), an affinity that is tacitly considered low or arguably not physiologically relevant (but see section on dosing issues). The K_i of DOI was less than 400 nM for only 6 receptors: 5-HT_{2A} (165.4 nM), 5-HT_{2B} (336 nM), 5-HT_{2C} (45.8 nM)^a, α_{2A} -adrenergic (73.7 nM), α_{2B} -adrenergic (340 nM), and β_2 -adrenergic (138.6 nM) receptors.

The reported affinities of DOI for α_2 - and β_2 -adrenergic receptors should be taken into account when considering DOI's behavioural effects. For example, Benloucif *et al.*^[37] showed that DOI (above 12 nmol) increases dopamine release in the anterior striatum of anesthetized rats, an effect that was not blocked by the 5-HT₂ antagonist, ritanserin (4 nmol), suggesting it may be mediated by DOI acting on α_2 -adrenergic receptors, which are known to regulate dopamine release in brain tissue. Also, cortical application of the $\alpha_{1/2}$ adrenergic antagonist, prazosin, blocked the 5-HT releasing effect of DOI in the brain.^[38]

^a Notably, the K_i values of DOI for 5-HT₂ receptors from^[23] are at least 2-fold higher than previous reports^[17,34,39,40] that used [³H]-ketanserin (5-HT_{2A}) or [³H]-mesulergine (5-HT_{2B}, 5-HT_{2C}) as radiolabels, and greater than 10-fold higher than K_i values reported using [¹²⁵I]-DOI.^[20]

The head-twitch response (HTR)

The first drug-elicited HTR

To our knowledge, the first description of a drug-elicited HTR was reported in 1956 by Keller and Umbreit^[41] following intravenous administration of lysergic acid diethylamide (LSD) to mice. They described an easily observed and quantifiable response that could be elicited by a light touch near the back of the head, similar to the reflexive response observed when the ear is touched or pinched (the pinna reflex response), or following LSD administration (which became 'permanent' in a subset of mice following an injection of indole followed by LSD). In their words:

The response consists of a rapid and violent head shaking. . . The head-twitch response does not occur in normal mice, and with a little experience the response is easy to detect. It is only rarely that one is uncertain whether a particular animal possesses the head twitch or not. . . Independent observations by different workers are remarkably consistent, so that this provided a suitable tool for the behavioral studies. (p. 723)

In our experience, the HTR is indeed easily quantifiable, with a high concordance of counts between independent observers, and shows a reliable and remarkably low level of within-subject (when animals are tested repeatedly) and between-subject (across animals within a particular treatment condition) variability. Following this initial observation with LSD, Corne *et al.*^[42] demonstrated in a convincing and comprehensive manner that the serotonin precursor 5-hydroxytryptophan (which is decarboxylated into serotonin) elicits a HTR, and characterized the pharmacology of the response. Several classes of drugs (including serotonin antagonists, antihistamines, and opioids) attenuated the response, MAO inhibitors (e.g. harmine, iproniazid, pheniprazine, and tranylcypromine) potentiated the HTR, whereas other drugs

had no effect (e.g. barbituates). Several years later, Corne and Pickering^[43] demonstrated that a variety of hallucinogens produce the HTR. These drugs included the classical hallucinogens LSD, psilocybin, psilocin, *N,N*-dimethyltryptamine (DMT), and mescaline, as well as drugs such as phencyclidine (PCP), atropine, and yohimbine. Interestingly, there was a correlation between potency of these compounds to elicit the HTR and the dose that was hallucinogenic in humans. Subsequently, a number of serotonergic compounds (predominantly serotonin-releasing agents and direct 5-HT₂ agonists) have been shown to produce the HTR in rodents, including quipazine, 5-MeO-DMT, 5-MeO-DIPT, DPT, 2C-T-7, 1-methylpsilocin, TCB-2, fenfluramine, DOM, MK-212, DOB, ergometrine, and to a lesser extent 2,5-DMA, 2C-D, 2C-B, and 2C-I.^[32,44–51] It should be noted that there are compounds from other pharmacological classes that also produce the HTR (e.g. CB1 cannabinoid antagonists, see the section on Hallucinogenesis).

The first DOI-elicited HTR

To the best of our knowledge, Glennon *et al.*^[5] first studied DOI's behavioural properties in animals (rats), showing that DOI fully substituted for DOM in a drug discrimination task. Two years later, based on a robust correlation between rat 5-HT₂ affinity and behavioural potency, Glennon *et al.* reported that the 5-HT₂ receptor was likely responsible for DOI's discriminative stimulus effects.^[52] With the discovery of 5-HT_{2B} and 5-HT_{2C} receptors, the 5-HT₂ receptor was reclassified as 5-HT_{2A}.^[53,54] Henceforth, when discussing the entire class of serotonin 2 receptors, i.e. 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}, 5-HT₂ will be used. The first study examining the HTR elicited by DOI was reported by Arnt and Hyttel^[55] who used rats, and demonstrated dose-dependent increases in the number of head-twitches; this behavioural effect was blocked by administration of the fairly selective 5-HT_{2A} antagonist ketanserin, as well as the 5-HT_{1A} agonist 8-OH-DPAT. Later studies confirmed 5-HT_{2A} receptors are the primary, direct mediators of the response, while 5-HT_{1A} receptors perform a modulatory function. Many investigators describe the HTR effect in the rat as a 'head shake', however, it appears to be essentially the same response as the HTR in mice, at least in regards to similarity in appearance and 5-HT_{2A} receptor dependence. A report by Darmani *et al.*^[56] was the first to demonstrate a DOI-elicited HTR in the mouse. In this paper, DOI produced dose-dependent increases in HTRs (tested up to 5.0 mg/kg), the (–) isomer was demonstrated to be more effective in eliciting an HTR, and the 5-HT_{2A} antagonists, ketanserin and spiperone, as well as the 5-HT_{1A} agonist, 8-OH-DPAT, attenuated the response. Similar but less commonly studied 5-HT_{2A} receptor-dependent behavioural responses have been elicited in other species including the rabbit (head bobs^[57,58]) and the least shrew.^[59] In contrast, there are several other behavioural responses elicited by serotonergic compounds that are not 5-HT_{2A} receptor dependent. For example, the 'serotonin syndrome' consists of a number of responses (e.g. flat body posture, hind limb abduction, forepaw treading, lower lip retraction, backwards walking) that are primarily a consequence of agonism at 5-HT₁ receptor subtypes.^[60] The 'ear-scratch response' observed in mice (and also elicited by DOI) appears to be primarily mediated by activation of 5-HT_{2C} receptors.^[61]

How to quantify the HTR and dose-related effects

There are several methods that have been used to quantify drug-elicited HTRs. Quantal methods determine the percentage of

animals that show an HTR in a given period of time following particular treatments. This allows a relatively high-throughput method of quantifying the HTR, as large groups of animals can be observed at the same time (e.g. for an extended discussion of these methods see Corne *et al.*^[42]). More commonly, a graded response is obtained simply by counting the number of HTRs observed in a single animal during a testing period; these data are obtained by trained observers either in real time, or video-recorded and later quantified. Depending on the question of interest, either a single animal's behaviour will be observed over time (e.g. to determine the time-course of the drug effect; Figure 2)^[62,63] or more commonly a particular set of pretreatment and observation times are established. As an example, we and other investigators have adopted a protocol in which DOI is administered 10 min before the testing session, and then the mouse is observed for 10 min. These parameters allow for quantification of a robust HTR with consistent frequency throughout the observation period. Numerous studies have demonstrated dose-dependent effects of DOI in rats and mice, such that increasing the doses results in increases in HTRs over a dose range from 0.1 mg/kg (essentially no HTRs) up to 2–10 mg/kg (maximal levels of HTR).^[48,55,56,64–71] Some investigators have reported biphasic dose-effect curves, i.e. increases followed by decreases in HTR frequency with increasing doses. For example, Pranzatelli^[72] describes a 'descending limb' of the dose-effect curve from 12–20 mg/kg in rats, whereas Fantegrossi *et al.*^[73] describe peak HTRs at 1.0 mg/kg, and decreases in HTR frequency at 3.0 mg/kg in Swiss NIH mice. The reasons for the differences in the shape of the dose-effect curve are not known, although species and strain differences are likely to contribute (see extensive discussion of some of these issues below).

Effects of repeated administration

Most studies use a particular experimental subject one time, presumably to eliminate the potential influence of repeated drug administration (i.e. the development of tolerance or sensitization) and repeated exposure to the testing apparatus. However, there

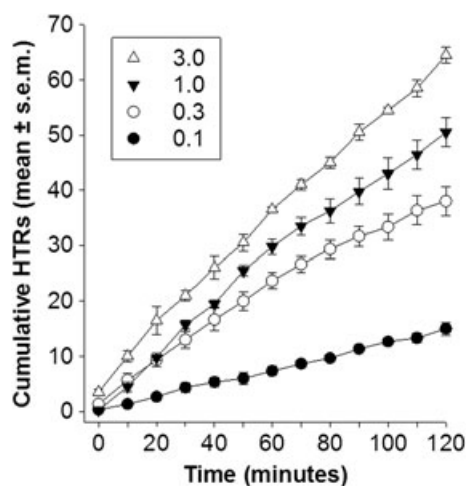


Figure 2. Cumulative number of head-twitch responses (HTRs) following administration of various doses of (—) DOI (mg/kg; IP) to male, C57Bl/6J mice. Each mouse ($n = 4$) was tested with all four doses (in random order) at approximately 1-week intervals. HTRs were counted in 2-min periods, every 10 min, beginning immediately following administration of drug. Note that (—) DOI elicited HTRs began within the first 2-min period (Time 0), the frequency of HTRs were dose-dependent, and HTRs continued throughout the 2-h testing period.

have been a number of studies examining the effects of repeated DOI administration on HTR and subsequent sensitivity to DOI. As observed following the administration of essentially all drugs, the pattern of behavioural effects and change in subsequent sensitivity depend on the dose administered, frequency of administration, and time since the last injection. In early studies, it was demonstrated that a single injection of DOI (2.5 mg/kg) in mice results in tolerance to a second dose administered 24 h later, but 'supersensitivity' (an increase in responsiveness) to a second dose administered 48 h later.^[61,62,70] To further investigate the effects of repeated drug exposure, 2.5 mg/kg DOI was administered daily for 13 days, and separate groups of mice were challenged following discontinuation of the chronic drug treatment. Tolerance immediately developed to the effects of DOI as indicated by ~40–50% decrease in the number of DOI-elicited HTRs following a 24-h period. This tolerance, however, disappeared over a 13-day period such that the number of HTRs increased and returned to control levels by day 6. Following chronic administration, a challenge dose of DOI (2.5 mg/kg), after a 48-h abstinence period, revealed a 'supersensitivity' (i.e. increase in HTR) that lasted for approximately 1 week.^[70] In a more recent paper, Dougherty and Aloyo^[74] administered 1 mg/kg DOI daily for 8 days (and 0.75 mg/kg on the 9th day). Tolerance (responding decreased by ~40–50%) developed following the first injection and was maintained throughout the 9-day testing period. The reasons for the discrepancy between the maintenance of tolerance in this study and the loss of tolerance upon repeated administration in the earlier studies by Darmani *et al.* are unclear; however, the dose used in the earlier studies was significantly higher (2.5 versus 1.0 mg/kg) and different strains of mice (ICR versus C57Bl/6 N) were used (see the section on species and strain differences for further discussion). To our knowledge, no papers have examined the effects of DOI administered repeatedly every 48 h to determine whether the increased sensitivity is maintained over time. However, it has been demonstrated that when DOI is administered at weekly intervals, there appears to be no change in sensitivity over the course of 4 weeks.^[75] This finding is important as it suggests that within-subject experiments could be conducted to allow animals to act as their own controls, potentially increasing statistical power and sensitivity to identifying treatment effects.

Influence of various physiological and environmental factors

Age

Relatively few studies have examined the effects of non-pharmacological factors on the DOI-elicited HTR. With regards to age, the dose-effect curve for DOI was shifted slightly downward (i.e. DOI was less effective) in older (3–3½-month-old) animals relative to younger (5–6-week-old) animals;^[76] however, a relatively limited age range was examined. Darmani *et al.*^[77] examined the ontogeny of various DOI-elicited behaviours and found that the HTR elicited by 1.0 and 2.5 mg/kg became apparent at 18 days of age and remained at high levels through 180 days of age; there were gradual non-significant decreases over time. In a genetic model of aging, the senescence accelerated mouse-prone (SAMP) was significantly more sensitive to DOI (more than twice as many HTRs following 0.3 and 1.0 mg/kg) relative to SAM-resistant mice.^[78] At this point, there are not enough data to draw strong conclusions regarding the role of age in the DOI-elicited HTR, although it has been demonstrated that 5-HT_{2A} receptor levels decrease with age in both humans and rodents.^[79–82]

Sex

Few studies have examined the influence of sex and/or oestrus cycle on the HTR. In the Darmani paper described earlier,^[77] male and female mice were not differentially sensitive to DOI. Using 5-HTP to elicit the HTR, Boulton and Handley^[83] demonstrated that males were more sensitive to the effects of 5-HTP, but that oestrus cycle had no effect on the female mice. There are simply not enough data making direct comparisons to clearly identify the potential influence of sex and hormonal status on the DOI-elicited HTR.

Environmental factors

The effects of housing conditions (e.g. the number of animals per cage) on the DOI-elicited HTR were reported in one study.^[84] Grouped-housed animals (5–6 ddY mice per cage) were significantly less sensitive to (\pm)-DOI (2.5 mg/kg) relative to singly-housed mice (13 vs. 24 HTRs in a 10-min observation period). These data contrast with a study showing that 5-HTP-elicited HTRs are extraordinarily low in singly-housed mice, and the frequency of the 5-HTP-induced HTRs increases along with the number of mice living in a particular group.^[83] However, in the first paper to describe the HTR,^[41] the authors noted that the HTR elicited by LSD was topographically identical to the HTR elicited by 'solitary confinement' (i.e. moving an animal from group-housing to singly-housed conditions). Given that housing conditions and social ranking within a colony can have dramatic effects on the 5-HT_{2A} receptor system,^[85] a systematic study of the effects of these factors on the sensitivity to DOI would be useful.

Yamata *et al.*^[86] examined the effects of a number of 'stressors' on DOI-elicited responses in rats. Tail pinch for 30 min, immobilization for 60 min, and repeated foot shock for 30 min rendered animals less sensitive to DOI. Administration of diazepam (benzodiazepine receptor agonist) 5 min before the immobilization stress prevented this loss of sensitivity to DOI. This effect was blocked by the co-administration of flumazenil (benzodiazepine inverse agonist). Swim stress for 10 min similarly produced a decreased sensitivity to DOI, i.e. 64–73% decrease in HTRs depending on the time of observation.^[87] In apparent contrast to these findings, stress induced by 3 h of restraint enhanced sensitivity to the effects of 1.0 mg/kg (but not 2.0 mg/kg) DOI in rats.^[88] Interestingly, obese Zucker rats that have an overactive hypothalamic-pituitary-adrenal system, resulting in a model of 'hypercorticism' with chronic, high levels of circulating corticosterone (which generally increases in rodents when exposed to stressful conditions) show a dramatically attenuated response to DOI compared to lean littermates.^[89] Finally, a couple of other studies have failed to show any effect of stress on sensitivity to DOI.^[86,90]

Receptor systems involved in the HTR induced by DOI

5-HT_{2A}

Activation of the 5-HT_{2A} receptor is necessary for DOI's (and all other classical hallucinogens) head-twitch-inducing effects in rodents.^[18] Corporates of studies support this claim, which is undisputed, and which has been thoroughly reviewed elsewhere.^[18,91–93] Briefly, the HTR in mice and rats (and the mole-like shrew) induced by DOI (and other serotonin-releasing compounds) is blocked by a plethora of relatively selective 5-HT_{2A} antagonists; mice lacking the 5-HT_{2A} receptor do not show an HTR to DOI; and restoration of 5-HT_{2A}

receptors to cortical (presumably glutamate) neurons in 5-HT_{2A} receptor knockout mice restores the ability of DOI to induce an HTR in mice.^[94] Furthermore, blockade of 5-HT_{2A} receptors by one of the most selective 5-HT_{2A} receptor antagonists, M100907,^[95] occludes the discriminative stimulus effects of psychedelic amphetamines in rodents^[18] and monkeys,^[96] suggesting the 5-HT_{2A} receptor underlies the discriminative stimulus effects of psychedelic amphetamines across species. Finally, modulation of the DOI HTR by any class of compounds, noted in the next several sections, can be blocked by selective 5-HT_{2A} antagonists. Thus, the 5-HT_{2A} receptor is necessary for the DOI HTR, and most of the following receptors, and neurotransmitter systems are best considered modulators of the DOI HTR.

5-HT_{2B}

Owing to its relatively low expression level in the brain,^[36,97,98] the 5-HT_{2B} receptor has not received much of the limelight as a possible regulator of the DOI-induced HTR. We are unaware of papers reporting effects of selective 5-HT_{2B} receptor antagonists on the DOI HTR. The DOI HTR-inducing effects have also not been described in 5-HT_{2B} receptor knockout mice. Interestingly, although the ergoline analogue, lisuride, has 5-HT_{2A} receptor agonist activity, it does not produce the HTR, and some (but not all) studies show that it is a potent antagonist of 5-HT_{2B} receptors.^[99] Other studies suggest it is worth considering the 5-HT_{2B} receptor as a potential modulator of the behavioral effects of DOI. For example, like 5-HT_{2A} and 5-HT_{2C} receptors, 5-HT_{2B} receptor affinity of hallucinogenic phenylisopropalamines is highly correlated with their human hallucinogenic potency.^[20] Also, recent studies with rats show that the 5-HT_{2B} receptor is required for the anorexic effects of the serotonin releaser dexfenfluramine^[100] and for the serotonin-releasing and hyperlocomotion effects of 3,4-methylenedioxymethamphetamine (MDMA),^[101] findings that argue brain 5-HT_{2B} receptors may be involved in cognitive and/or behavioural effects of psychoactive drugs.

5-HT_{2C}

Concerning their putative roles in the behavioural effects of psychedelic phenylisopropalamines, of the three 5-HT₂ receptors, most contention emanates from the 5-HT_{2C} receptor. A number of studies have substantiated a role, albeit a modulatory one, for the 5-HT_{2C} receptor.^[65,102,103] Like the other 5-HT₂ receptors, the potency of phenylisopropalamines is highly correlated with their affinities for 5-HT_{2C} receptors.^[20] Also, the hallucinogenic potency of DOI, DOB, DOM and MDA decreases in parallel with decreasing efficacy to activate 5-HT_{2C} receptors.^[10] The (–)-DOM discriminative stimulus-potentiating effects of citalopram, furthermore, are attenuated by the highly selective 5-HT_{2C} antagonist, SB242084,^[104,105] and mCPP, considered a selective, albeit poorly selective, 5-HT_{2C} agonist (relative to 5-HT_{2A} receptors), also potentiated the discriminative stimulus effects of (–)-DOM.^[106]

Regarding the HTR in rats, direct frontal cortex injections of mCPP induced an HTR, an effect that was attenuated by systemic pretreatment with the selective 5-HT_{2C} receptor antagonist SDZ SER-082.^[107] Interestingly, mCPP was more potent and produced more HTRs than DOI when injected centrally,^[107] though its affinity and efficacy for stimulating 5-HT₂ receptors is substantially lower than DOI's.^[22] Also intriguing, when administered systemically, mCPP actually attenuates HTRs induced by a number of 5-HT_{2A} agonists, including DOI.^[108,109] Nevertheless, both the 5-HT_{2B/2C} selective antagonist/inverse agonist, SB206553,^[110] and

the 5-HT_{2C} antagonist, SB242084, attenuated the HTR elicited by DOI by 50% in C56BL/6J and DBA/2J mice, and the DOI HTR was attenuated by ~50% in 5-HT_{2C} knockout mice, relative to their wild-type littermates.^[65] Moreover, 5-HT_{2C} antagonists also attenuated the discriminative stimulus effects of (+/-)-DOI in C57Bl/6J mice.^[111] Collectively, these data suggest that activation of 5-HT_{2C} receptors regulates the HTR and a portion of the behavioural effects of phenylisopropalamines in mice and rats. Nevertheless, caveats and conflicting data abound.

A role for the 5-HT_{2C} receptor in mediating some of the cognitive and behavioural effects of psychedelic amphetamines is usually downplayed due to observations of opposing actions of 5-HT_{2A} and 5-HT_{2C} receptors.^[73,112] Moreover, SB242084 did not affect a rat's ability to discriminate DOI,^[11] and did not block the HTR in rats elicited by DOI,^[64,113] nor did SDZ SER-082,^[107] or the selective 5-HT_{2B/2C} receptor antagonist SB 200646A.^[114] Furthermore, behavioural tolerance to DOI after chronic administration (i.e. decreased discriminative stimulus effect), paralleled a decrease in 5-HT_{2A} receptor-binding sites in the cortex and claustrum, yet was not associated with a decrease in 5-HT_{2C} receptor-binding sites in the striatum or choroid plexus, determined by autoradiography.^[11] It should be noted, however, that quantification of 5-HT_{2C}-receptor binding sites (which are much fewer in number than 5-HT_{2A} receptor binding sites) in the cortex, which is arguably the site of action for the discriminative stimulus effects of DOI,^[115] could not be ascertained with this method.^[11]

Adding to these findings were reports that the selective 5-HT_{2C} agonists (relative to 5-HT_{2A}), Ro 60-0175 and CP 809 101,^[116,117] did not induce an HTR in rats on their own,^[113,117] and actually dose-dependently decreased the (-)-DOI-elicited HTR in Swiss NIH mice (Ro 60-0175)^[73] and rats (CP 809 101).^[117] When co-administered with SB242084 to putatively unmask its agonist activity for 5-HT_{2A} receptors, Ro 60-0175 produced an HTR, though at a lower frequency than the HTR induced by DOI.^[113] Based on these data, and that SB242084 pretreatment in Swiss NIH mice can potentiate the maximum HTR induced by DOI by ~20%,^[73] it is inferred that 5-HT_{2C} receptor activation suppresses the psychoactive effects of DOI. Further elaborating on this point, counts of HTRs with increasing doses of DOI in Swiss NIH mice reveals an inverted-U shaped dose-response curve, and experiments combining M100907 or SB242084 with (-)-DOI suggest that the ascending part of the curve, at low DOI doses (from 0.3 to 1 mg/kg), is due to activation of 5-HT_{2A} receptors, while the descending part of the curve, at high doses (from 1 mg/kg to 10 mg/kg), is due to increasing activation of 5-HT_{2C} receptors.^[73] How these data are reconciled with the similar agonist potencies and efficacies of DOI for both 5-HT_{2A} and 5-HT_{2C} receptors, and the reports of linear increases in hallucinogenic effects in humans with increasing doses of DOI, was not addressed. These data may suggest, however, that the HTR response observed may not map to hallucinogenic activity in humans. Nevertheless, in C57Bl/6J and DBA/2J mice, which show a more robust HTR to DOI than Swiss NIH mice, the DOI HTR dose-response curve increased linearly then plateaued at doses up to 12.8 mg/kg.^[65] In this set of experiments, mice appeared sick at higher doses of DOI; thus, large doses were administered to only a few mice (unpublished observations). Similar linear DOI HTR dose-response curves up to 5 mg/kg have also been reported in albino ICR mice.^[56] Again, strain and species differences appear to be major factors to consider when interpreting DOI HTR data.

It is also worth noting that 1-methylpsilocin is a 5-HT_{2C} agonist, with more than 50 times greater affinity and potency for activating human 5-HT_{2C-INI} compared to 5-HT_{2A} receptors.^[118] yet causes a dose-dependent increase in the HTR in C57Bl/6J mice,^[47] suggesting that 5-HT_{2C} receptor activation (if the pharmacology of 1-methylpsilocin at human receptors is recapitulated at mice receptors) does not suppress 5-HT_{2A} receptor-mediated HTRs. Finally, the novel indazole 5-HT₂ agonist, AL-38022A, showed several-fold higher potency for human 5-HT_{2C} receptors (isoform unspecified), compared to 5-HT_{2A} and 5-HT_{2B} receptors, and yet fully generalized to the DOM stimulus in both rat and monkey drug discrimination studies.^[96]

5-HT₁

In the first papers describing the DOI-elicited HTR in rats and mice, the 5-HT_{1A} agonist 8-OH-DPAT was shown to attenuate the response.^[55,56] This finding was replicated a number of times,^[64,69,109] including demonstration that direct infusion of 8-OH-DPAT into the medial prefrontal cortex inhibits the effects of systemically administered DOI.^[107] These findings have been extended to a number of relatively selective 5-HT_{1A} agonists, with varying degrees of efficacy, including buspirone, flesinoxan, MKC242, S14671, and S14506.^[64,69,84] The effects of the non-selective 5-HT_{1A/2} agonist 5-MeO-DMT are interesting in that this compound can produce HTRs when administered alone.^[47] When combined with DOI, however, 5-MeO-DMT dose-dependently decreases the number of DOI-elicited HTRs,^[56] an effect attributed to 5-MeO-DMT's agonist activity for 5-HT_{1A} receptors. Antagonism of the 5-HT_{1A} receptor (e.g. with WAY-100635) fails to alter the DOI-elicited HTR.^[69,84,119] Endogenous 5-HT_{1A} receptor activity, thus, may be too low to affect DOI-induced 5-HT₂ receptor activity.

Other 5-HT receptors and the serotonin transporter

In general, it appears that selective serotonin transporter inhibitors (i.e. SSRIs), such as fluoxetine, imipramine, fluvoxamine, and citalopram, fail to alter the DOI-elicited HTR.^[63,120] However, if the target of these compounds (i.e. the serotonin transporter) is genetically deleted, the ability of DOI to elicit HTRs is greatly reduced and/or eliminated.^[119,121] This effect is attributed to adaptations, e.g. desensitization and decreased expression of 5-HT_{2A} receptors, due to life-long elevated levels of serotonin.

Chronic administration of serotonin, norepinephrine transporter inhibitors (i.e. SNRIs) decrease the DOI-elicited HTR.^[122,123] Similarly, repeated exposure to MDMA, which acts partially through interactions with the serotonin, dopamine, and norepinephrine transporters, may alter sensitivity to DOI. Although one early paper suggested no lasting effect of MDMA administration,^[124] a more recent report found that repeated MDMA treatment resulted in an enhanced sensitivity to DOI at a higher (3.0 mg/kg) but not a lower dose (0.5 mg/kg).^[66]

Administration of quipazine, a mixed 5-HT₂/5-HT₃ agonist results in a robust HTR in both mice and rats.^[64,125,126] Co-administration of the 5-HT₃ receptor antagonist, ondansetron, does not substantially alter the DOI-elicited or quipazine-elicited HTR;^[120] we recently replicated this finding in our lab using C57Bl/6J mice (unpublished observations). These data suggest that the 5-HT₃ receptor may not alter 5-HT₂ receptor function. It is worth noting that some early studies suggested that quipazine was hallucinogenic in man,^[127] and a recent case report noted that

quipazine produced a full hallucinogenic response when it was administered with a 5-HT₃ receptor antagonist, presumably to prevent nausea/vomiting associated with 5-HT₃ receptor activation.^[15]

The 5-HT₇ receptor has been generating increasing interest as a potential target for drug development for the treatment of psychiatric disorders, including schizophrenia.^[128,129] To our knowledge, no selective 5-HT₇ compound has been tested in the DOI-induced HTR procedure, although all antipsychotic compounds are active in this model.

Glutamate

There has been considerable interest in the role of glutamatergic systems in the DOI-elicited HTR. Much of this interest stems from the putative involvement of 5-HT₂ receptors and glutamatergic systems in schizophrenia,^[130] and that the DOI-elicited HTR may be relevant as a model of schizophrenia. To this end, numerous studies have examined pharmacological manipulations of both the ionotropic NMDA and AMPA receptors, and the metabotropic mGluR receptor systems. For example, antagonism of the NMDA receptor by MK801 or ketamine,^[131–133] or antagonism of mGluR_{2/3} autoreceptors by LY341495^[75,134] consistently increase prefrontal cortical levels of glutamate and enhance the effects of DOI, indicated by increased HTRs elicited by a particular dose or shifts in the DOI dose–response curve to the left and upwards.

An equally compelling story has emerged regarding glutamatergic interventions that block the effects of DOI. Activation of mGluR_{2/3} autoreceptors with LY379268 and LY354740, which presumably leads to decreased extracellular levels of glutamate, decreased sensitivity to DOI.^[75,134] Similarly, administration of the glutamate release inhibitor riluzole or the glutamate analog theanine (which functions as a competitive antagonist) reduces DOI-induced HTRs.^[135] Recent studies have demonstrated that selective AMPA glutamate antagonists, such as GYKI52466, LY203558, and NBWX, attenuate DOI-induced HTRs.^[131,135] To complicate matters, it was recently demonstrated that the mGluR₂-receptor knockout mice are insensitive to HTR-inducing effects of DOI (and LSD).^[136] Although the neuropharmacological mechanisms underlying all of these interactions are not delineated, these data suggest a prominent role for glutamate receptor systems in the behavioural effects of DOI, and a strong interaction between glutamatergic and 5-HT₂ receptor systems. Notably, it was recently discovered that 5-HT_{2A} and mGluR₂ receptors form heteromeric complexes that have unique signalling properties.^[137]

GABA

Several allosteric modulators of the GABA_A receptor have been examined for their influence on the DOI HTR. Both the high efficacy benzodiazepine agonist diazepam and the inverse agonist flumazenil appear to have no influence on DOI-elicited HTRs,^[67,86,120,138] while the partial inverse agonist Ro154513 attenuates the DOI-induced HTR.^[67,138] Furthermore, the direct GABA_A agonist bicuculline attenuates DOI-induced head twitches.^[138] More comprehensive studies directly comparing a range of compounds with various pharmacological actions at this receptor will help elucidate the potential modulatory role of GABA_A receptors. A single paper has examined the role of metabotropic GABA_B receptors in DOI's behavioural effects.^[139] These authors demonstrated that both an agonist, CGP44532, and a positive allosteric modulator, GS39783, attenuated DOI-induced HTRs, which

was reversed by two antagonists, CGP51176 and CGP36742, that had no effect on their own.

Dopamine

Given the potential relevance of the behavioural effects of DOI as a model of schizophrenia, a number of studies have examined the influence of typical, dopamine D₂ antagonist, antipsychotic compounds on the HTR. For example, haloperidol, chlorpromazine, and pimozide inhibit the HTR elicited by DOI.^[64,68,72,120,140,141] Similarly, chronic administration of haloperidol results in a decreased sensitivity to DOI.^[140] Although they possess excellent affinity for D₂ receptors, these compounds are also 5-HT_{2A} receptor antagonists.^[22,142] The more selective D₂ receptor antagonists, raclopride and sulpiride, show inconsistent effects or do not affect the DOI-elicited HTR.^[64,72,141] The dopamine D₁ receptor antagonists, SCH23390 and SCH39166, which also have affinity for some serotonin receptors, inhibit DOI's behavioural effects;^[64,141] these findings are consistent with observations that the D₁ receptor modulates glutamate and serotonin interactions in the prefrontal cortex.^[143] Cocaine, a drug which blocks the reuptake of dopamine, 5-HT, and norepinephrine, attenuates DOI-elicited HTRs.^[71,144,145] Although many of cocaine's behavioural effects have been attributed to activity at the dopamine transporter, Darmani *et al.* have demonstrated repeatedly that attenuation of the HTR by cocaine can be reversed by administration of the 5-HT_{1A} antagonist, alprenolol, or the non-selective α_2 -adrenergic receptor antagonist, yohimbine, suggesting that cocaine's actions are mediated indirectly by increases in 5-HT_{1A} and α_2 -adrenergic activity.^[71,144,145]

Cannabinoids

The role of the cannabinoid system is interesting as the CB1 receptor antagonist SR141716A (Rimonabant) produces an HTR when administered alone.^[146–148] Although SR141716A has no affinity for 5-HT receptors,^[149] it is of interest that the 5-HT_{2A/2C} antagonist SR46349B potentially reversed the effects of SR141716A, suggesting that activation of 5-HT₂ receptors may be related to the behavioural effects of SR141716A.^[147] Pretreatment with all CB1 agonists tested thus far, i.e. THC ($\Delta 9$ and $\Delta 8$), anandamide, HU210, CP55940, and WIN55212 inhibit DOI's behavioural effects.^[146,150] Similarly, indirect cannabinoid agonists such as the anandamide transporter inhibitor AM404 and the FAAH inhibitor URB597, inhibit the HTR elicited by DOI.^[135] These data suggest close interactions between 5-HT₂ and cannabinoid receptors *in vivo*, which recent studies support.^[151,152]

Acetylcholine

Acute and chronic administration of nicotine (direct agonist) and donepezil (acetylcholinesterase inhibitor; indirect agonist), two common drugs used in the treatment of Tourette's Syndrome, attenuate DOI-elicited HTRs. Based on these findings, it has been suggested that the DOI-elicited HTR may be useful as a model of Tourette's Syndrome.^[140,153,154] The neurobiological mechanisms underlying these interactions are unknown, however, chronic administration of nicotine and donepezil results in decreased cortical 5-HT_{2A} receptor density.^[140]

Norepinephrine

The role of the norepinephrine system in regulating effects of hallucinogens has been studied mostly utilizing hallucinogens

other than DOI, for example, 5-MeO-DMT, quipazine, and 5-HTP.^[126,155–158] Fewer studies have examined the role of norepinephrine in regulating the DOI-induced HTR; however, it was demonstrated that prazosin, an $\alpha_{1/2}$ adrenergic receptor antagonist, completely blocks the DOI-elicited HTR.^[141] In this study, behavioural potency of 9 compounds (including prazosine) was significantly correlated with binding at 5-HT_{2A} and α_1 adrenergic receptors. The finding that DOI itself has appreciable affinity for α_2 receptors^[23] suggests that DOI may directly affect the norepinephrine system, which may contribute to the behavioural effects of DOI. We are currently investigating this possibility.

Adenosine

One comprehensive paper has demonstrated a role for adenosine systems.^[159] Adenosine receptors are expressed in practically all neural systems in the brain, and their activation generally suppresses neural activity. For example, activation of adenosine A1 receptors in the cortex reduces glutamate release.^[160] Thus, it follows that the adenosine A1 agonist, N⁶-cyclohexyladenosine, attenuates the DOI-elicited HTR, an effect that is reversed by the adenosine antagonist 8-cyclopentyl-1,3-dipropylxanthine.^[159] Of interest, the antagonist administered in combination with DOI enhances the HTR suggesting either that there is some basal level of adenosine receptor stimulation that modulates 5-HT_{2A} receptor activity or that the drug is non-selective for adenosine A₁ receptors.

Other systems

The mu opioid agonists, morphine and buprenorphine, and various atypical opiates, for example, tramadol and levorphanol, can attenuate the DOI-induced HTR in a naloxone-reversible manner;^[63,161] for an exception see Wettstein *et al.*^[120] The neuropeptide, galanin, was demonstrated to attenuate DOI-elicited HTRs, and completely blocked HTRs elicited by DOB and mescaline.^[162,163] Lastly, a series of papers examined the effects of the compounds rubidium, caesium, and quinine (all compounds that interact with K⁺ channels and likely alter 5-HT release), and all tended to enhance the number of DOI-elicited HTRs.^[164–166]

Summary

Taken together, the data described above suggest a number of neurotransmitter systems can alter behavioural effects elicited by the relatively selective 5-HT₂ agonist, DOI, and highlight the wide-ranging and complex interactions associated with 5-HT₂ receptors.

Neural and cellular systems underlying the psychoactive effects of DOI

Neural systems

McKenna *et al.*^[16] first showed the distribution of DOI binding sites in rat brain using [¹²⁵I]-(-)-DOI; the highest density of binding sites were observed in the cortex, claustrum, and olfactory bulb. Although the entire spectrum of psychedelic effects are likely the result of emergent outputs from communication between several neural systems,^[167,168] several studies employing pharmacological, physiological, molecular, imaging, and/or genetic tools, have provided converging evidence that the cortex is the most primary site of action of the psychedelic effects of classical hallucinogens

in man (e.g. psilocybin^[167,169]), their drug discrimination stimulus effects in rats (e.g. LSD^[115]), and their head-twitch-inducing effects in rats and mice (e.g. DOI^[94,107]). Regarding DOI specifically, there are no studies (of which we are aware) aimed at deciphering its neuroanatomical site of psychedelic action in man, likely relating to its long duration of action, i.e. 16–30 h,^[6] which would render clinical studies arduous to perform. However, based on results from similar classical hallucinogens, the cortex is probably the primary site mediating its hallucinogenic effects in man.

Whether or not there is a specific cortical region(s) that is necessary for producing the psychedelic effects of classical hallucinogens is not fully elucidated, but data point to the cingulate gyri, a neural system located dorsomedially in the cortex, and implicated in decision making, emotion, and pain processing.^[170] Psilocybin increases cerebral metabolic activity in the cingulate cortex in humans.^[169] Also, direct injections of d-LSD into the cingulate cortex of rats dose-dependently substituted for systemic injections of d-LSD in a drug discrimination task; injections outside this region, including rostrally, into the orbital cortex, did not substitute.^[115] Also, direct injections of DOI into the cingulate cortex dose-dependently induced the HTR in rats.^[107] Moreover, restoration of expression of 5-HT_{2A} receptors solely in the cortex, including the cingulate cortex, of 5-HT_{2A} receptor knockout mice restored the ability of DOI to induce an HTR.^[94]

Cellular systems: neurophysiology and neurochemistry

Many more studies have examined the cellular, both physiological and genetic, effects of DOI. In rodents, primates, and humans, 5-HT_{2A} receptors, primary targets of DOI, are highly expressed in cortical structures where they are mostly (but not solely) postsynaptic; fewer 5-HT_{2A} receptors are found in subcortical structures.^[171–174] 5-HT_{2A} receptors are most abundant on glutamatergic pyramidal neurons and GABAergic interneurons of the prefrontal cortex, where they robustly stud somatodendritic regions of layer V.^[171,172,175–178] Thus, much focus, regarding the cellular effects of DOI, has been on layer V of the cortex.

5-HT₂ receptor activation by DOI significantly alters glutamate release and glutamatergic pyramidal neuron activity in the medial prefrontal cortex (layer V, predominantly), an effect that recent studies show is likely post-synaptically mediated and intrinsic to cortical-cortical circuits.^[179,180] Results from both *in vitro* and *in vivo* studies in rats show that DOI increases spontaneous and evoked excitatory currents in layer V pyramidal cells (EPSCs),^[181,182] increases the firing rate of a majority of neurons, but also decreases the firing rate of a significant number of layer V cells^[183] and also unidentified medial prefrontal cortex cells,^[184] reduces low-frequency oscillations, and desynchronizes pyramidal cell discharge from active phases of slow oscillations of medial prefrontal cortex cells.^[179] The majority of these effects are abolished by M100907 or other 5-HT_{2A} antagonists, suggesting they are 5-HT_{2A} receptor-mediated. However, for full disclosure, it should be noted that Puig *et al.*^[183] reported that the DOI-induced increase in firing rate was not reversed by systemic injections of M100907 (up to 0.5 mg/kg) in 33% (3/9) of the cells examined. Furthermore, the excitatory effects of the 5-HT₂ agonist, α m-5-HT, were mostly, yet not completely absent in cortical pyramidal cells from 5-HT_{2A} receptor knockout mice; indeed 23% (9/39) of cells examined from these mice showed measurable α m-5-HT-induced increases in EPSCs that were subsequently blocked by the selective 5-HT_{2C} antagonist, SB242084.^[180] Also, although the increase in EPSCs by α m-5-HT was completely

abolished by M100907 in pyramidal cells from wild-type mice, the concentration used, 300 nM,^[180] was approximately 6-fold higher than its K_i for 5-HT_{2C} (human, INI isoform) receptors.^[3] Thus, the direct neurophysiological effects of DOI in pyramidal cells of layer V of the cortex likely involve activation of both 5-HT_{2A} and 5-HT_{2C} receptors, and subsequent increases in local glutamate release.

DOI also increases 5-HT, dopamine, and acetylcholine release in the frontal cortex, as well as other neural systems in rodents.^[38,185–189] These effects of DOI, however, are likely secondary to activation of 5-HT₂ receptors on cortical pyramidal neurons, which send efferent projections to regulate neurotransmitter release from subcortical 5-HT, dopamine, and acetylcholine-producing neurons. For example, systemic and direct injections of DOI in the frontal cortex increase 5-HT, dopamine, and acetylcholine release there, and the increases are prevented by direct injections of 5-HT₂ antagonists into the frontal cortex.^[38,187–189] Moreover, the DOI-induced increase in cortical dopamine release is absent in 5-HT_{2A} receptor, but not 5-HT_{2C} receptor knockout mice, suggesting this effect is 5-HT_{2A} mediated.^[190] Notably, 5-HT_{2C} receptor knockout mice show, relative to wild-type mice, increased firing of dopaminergic substantia nigra neurons.^[191] Whether this effect is related to 5-HT_{2C} receptors on cells in the substantia nigra or is an indirect effect is unknown to us. Similarly, the increases in 5-HT in the medial prefrontal cortex in rats produced by direct DOI injections were blocked by co-application of the glutamate AMPA receptor antagonist, NBQX, but not SB242084, suggesting the DOI-induced 5-HT release in the cortex is mediated by 5-HT_{2A} receptors on pyramidal neurons; though local 5-HT_{1A} and alpha-adrenergic receptors were also involved.^[38]

Cellular systems: intracellular proteins

At the molecular level, DOI causes strong expression, in cortical layer V neurons, of the immediate early gene *c-fos*, an effect blocked by pretreatment with M100907, but not by SB206553 (up to 6 mg/kg,^[192]), and not observed in 5-HT_{2A} knockout mice,^[193] suggesting it is specifically 5-HT_{2A} receptor mediated.^[192] The induction of *c-Fos* protein in layer V by DOI likely does not, however, mediate its HTR-inducing effects, as the non-HTR-producing 5-HT_{2A} agonist, lisuride, also induces *c-Fos* expression in the cortex.^[94] DOI, but not lisuride, induced cortical expression of *egr-1* and *egr-2*.^[94] Though these genetic markers, also, do not underlie DOI's HTR-inducing effects, as their maximal induction was not observed until an hour after injection of DOI, whereas the HTR to DOI was observed within minutes of injection; furthermore, mice lacking *egr-1* show a normal response to DOI.^[194]

5-HT₂ receptors share a common signalling pathway involving coupling with Gq/11 proteins that leads to activation of phospholipase C β , production of diacylglycerol, release of Ca⁺⁺ from intracellular stores, and modulation of activity of several other intracellular proteins, including PKC and extracellular signal-regulated kinase (ERK).^[195] Mice lacking the Gq protein show a reduction in number, but not an elimination of HTRs induced by DOI.^[196] Although alterations in density of 5-HT₂ receptors are not associated with alterations in the level of Gq proteins,^[197,198] to our knowledge the converse is not known. Nevertheless, these data suggest that G proteins, other than Gq, that interact with 5-HT₂ receptors, including Gi and G12/13 (5-HT_{2C} receptors,^[199,200]) may also contribute to its HTR-producing effect. Also notable, DOI-induced *c-Fos* expression in the medial prefrontal cortex was completely abolished in Gq knockout mice,^[196] providing further evidence that *c-Fos* is not a critical molecular component of the DOI HTR.

Other intracellular contributors to the HTR induced by DOI include PKC γ . Mice lacking the neuronal specific, PKC γ , show enhanced DOI-induced phospholipase C activation and increased number of DOI-induced HTRs, suggesting a suppressive effect of PKC γ on DOI-mediated intracellular functions.^[201] Importantly PKC γ knockout mice do not show alterations in brain 5-HT_{2A} receptor density.^[201]

Several new studies have begun to elucidate the G-protein independent β -arrestin pathway, which is modulated by 5-HT_{2A} receptors. Recently it was shown that the DOI-induced HTR is unaffected by knockout of β -arrestin2 in mice, yet the HTR from the hallucinogens N-methyl-5-HT and 5-MeO-DMT is significantly enhanced in these mice; on the other hand, the HTR induced by the non-hallucinogenic compound, 5-HTP, is severely diminished, relative to wild-type littermates.^[202,203] Moreover, the HTR induced by all of these compounds is blocked by M100907, suggesting unique 5-HT₂-mediated signalling pathways induced by DOI and other HTR producing compounds^[202,203] that may be activated by stabilization of agonist-specific receptor conformations.

Schmid *et al.*^[202] and Abbas *et al.*^[204] showed that DOI induces phosphorylation of ERK in cortical cells. This event, however, does not seem to be necessary for the discriminative stimulus effects of DOI, as systemic injections of SL327 that attenuated cerebral ERK phosphorylation, did not lessen the ability of mice to discriminate DOI from vehicle (Sanders-Bush *et al.*, unpublished observations). Whether blockade of cortical ERK phosphorylation affects the DOI-induced HTR has not been reported to our knowledge.

Recent studies have described a substantial function of scaffolding and other 5-HT₂ interacting proteins in the cellular and behavioural effects of DOI. For example, mice lacking the receptor-scaffolding protein, postsynaptic density protein of 95 kDa (PSD-95), traditionally considered a glutamatergic scaffolding protein, show a reduction, yet not abolishment of the HTR induced by DOI.^[204] PSD-95 knockout mice showed decreases in brain 5-HT_{2A} and 5-HT_{2C} receptor expression (yet not 5-HT_{1A} receptors), and 5-HT_{2C} receptor function, but did not show reductions in 5-HT_{2A} or 5-HT_{2C} mRNA levels. Furthermore, 5-HT_{2A} receptor trafficking to the cortical dendritic compartment in PSD-95 knockout mice was reduced. These data suggested the effect of PSD-95 elimination on the DOI HTR was mediated by reduced 5-HT₂ receptor expression in the cortex.^[204]

Interestingly, other studies have shown that DOI induces region-specific induction of BDNF mRNA, including induction in layer V of the rat cortex,^[205,206] which can be suppressed by the mGlu2/3 agonist, LY354740, and enhanced by the mGlu2/3 antagonist LY341495.^[206] Also, BDNF induces transport of PSD95 to cortical dendritic spines.^[208] In BDNF knockout mice, 5-HT_{2A} receptor binding sites were significantly decreased in the cortex compared to wild-type mice; the HTR to DOI, nevertheless, was not altered in BDNF knockout mice (though the ear-scratch response to DOI was significantly different^[208]). Finally, there are several other 5-HT_{2A} and 5-HT_{2C}-interacting proteins that could regulate the effects of DOI, including PDZ domain-containing proteins, such as Mupp-1, and non-PDZ-domain-containing proteins, such as Arf-1, caveolin, RSK2, and MAPs.^[209]

Caveats: resolution of conflicting data

Species and strain differences

Behavioural differences

There are many discrepancies in the DOI HTR literature that clearly appear related to the animal subject tested. For example,

as shown in Table 1, mice and rats exhibit widely varying numbers of DOI-elicited HTRs within a defined time period, with mice typically showing a greater number of HTRs than rats. There are also large differences, up to 10-fold, between strains within a given species, and even across sub-strains, i.e. C57Bl/6J mice (Jackson Laboratories)^[65,76] compared to C57Bl/6N mice (Harlan Laboratories),^[74] as shown in Table 1. The time between injection of DOI and the start of HTR counting, however, was notably different, i.e. 10 min^[65,76] compared to 0 min,^[74] we have observed that the frequency of HTRs after peripheral injections of DOI increases within the first 2 min (Figure 2), similar to Darmani *et al.*^[56] Thus, these differences may appear more striking than they are. Experimenter variability does not likely account for most of the variance observed, as reports from several experiments directly comparing mouse strains show significant differences in HTR following DOI injections.

Different receptor densities

A recent report noted that mice and rats have different ratios of 5-HT_{2A/2C} receptor binding site densities in the brain, with the former showing a greater ratio than the latter, i.e. relatively more 5-HT_{2A} receptors than 5-HT_{2C} receptors in the mouse brain.^[74] It could be argued that these observations explain some of the variability between DOI HTRs in rats and mice. Some studies have suggested that activation of 5-HT_{2C} receptors inhibits 5-HT_{2A} receptor function induced by DOI (discussed above in the section on 5-HT_{2C} and below in the section, "Dosing issues: problem of non-selectivity"). Following this logic, because there are fewer 5-HT_{2C} receptors potentially suppressing 5-HT_{2A} receptor activity in mice relative to rats, mice exhibit a greater number of HTRs elicited by DOI. Neither 5-HT_{2A} nor 5-HT_{2C} receptor binding site densities, however, explained the differences in DOI-elicited HTRs between C57Bl/6J and DBA/2J mice. DBA/2J mice exhibited an almost 2-fold greater frequency in DOI-induced HTRs relative to C57Bl/6J mice, yet had approximately equivalent 5-HT_{2A} and 5-HT_{2C} receptor binding site densities in brain areas examined,^[65] though not reaching statistical significance, a closer look at the data, however, suggest that 5-HT_{2A} receptors in the posterior striatum may be more dense in DBA/2J than C57Bl/6J mice. As suggested previously,^[11] autoradiography techniques are relatively poor for deciphering subtle, but potentially important, differences in receptor binding site density.

The fact that there are substantially greater numbers of 5-HT_{2A} receptors, compared to 5-HT_{2B} and 5-HT_{2C} receptors, expressed in the brains of most species, is pertinent when considering the relative contribution of these receptors to the effects of DOI. Namely, 5-HT_{2A} receptors may have a greater, and more easily detectable, role in DOI's effects simply because they exist in greater numbers and therefore have greater control over neurophysiological processes in regions where the three receptors are co-expressed. Thus, the potential 5-HT_{2B} and 5-HT_{2C} receptor-mediated functions in hallucinogen HTR studies and drug discrimination tasks may be masked by 5-HT_{2A} receptors when all three receptors are activated simultaneously, as occurs with DOI. Considering a hypothetical example, if a mouse model were created that over-expressed 5-HT_{2A} receptors, and another 5-HT_{2B} receptors, and another 5-HT_{2C} receptors, and each was trained to discriminate DOI from saline, it seems logical that 5-HT_{2A} selective antagonists would more readily block the discriminative cue in 5-HT_{2A} over-expressing mice (similar to wild-type mice), but would be less effective in blocking the cue in 5-HT_{2B} over-expressing mice, and likewise for all other iterations.

Table 1. Head-twitch response (HTR) induced by DOI (mg/kg, i.p. or s.c.) across rodent species and strains. Results are averages from published reports. This list is not exhaustive, but is for illustrative purposes. Experiments using doses of, or close to 1.0 mg/kg were chosen for consistency. HTR counts began between 0 and 30 min after DOI injection. Results from observation periods greater than 10 min were divided to obtain a 10-min average. NR (not reported)

Species and strains	Dose, enantiomer	HTRs	References
Rat			
Wistar	1.0-2.5, NR	5-12	[55,90,64]
Wistar	0.22-5.0, (±)	5-6	[109,86]
Sprague-Dawley	1.0-3.0, (±)	4-10	[72,159];
Sprague-Dawley	1.25-3.0, NR	5-9	[114,32,131,66]
Listar	1.0, (±)	8	[113]
OFA S-D	1.0-3.0, (±)	5-7	[120]
Mouse			
SAMP6	1.0, NR	83	[78]
DBA-2J	1.0, (±)	70	[65]
C57Bl/6J (background)	1.0,1.5, (±)	48,68	[119,121]
C57Bl/6J	1.0, (+)	46	[76]
C57Bl/6J	1.0, (±)	30,40	[48,65]
CAST/Ei	1.0, (+)	44	[76]
SAMR1	1.0, NR	33	[78]
129S6/SvEv	2.0, NR	32	[94]
Aston MF1	0.5, (±)	32	[153]
ICR	1.0, (±)	28,29	[67,138]
ICR	1.0, NR	25,26	[68,154]
C3HeB/FeJ	1.0, (+)	28	[76]
Aston MF1	1.0, NR	27	[141]
129Sv	2.0, NR	21	[194]
C57Bl/6N	1.0, NR	19	[74]
Balb/cJ	1.0, (+)	18	[76]
NIH Swiss	1.0, (-)	18	[73]
CBA	2.5, NR	16	[87]
Swiss Webster	1.0, (-)	15	[73]
Albino ICR	1.0, (±)	20,25	[61,146]
Albino ICR	1.25-2.5, (±)	8,15	[4,56]
Albino ICR	2.5, (-)	15	[56]
Albino ICR	1.0, NR	8,9	[140,252]
Albino ICR	2.5, (+)	8	[56]
A/J	1.0, (+)	9	[76]
NMRI	1.0, (±)	8	[236]
Albino Swiss	2.5, (±)	5-9	[122,123,134,139]

Finally, although the translated DNA sequences for 5-HT₂ receptors are ~98% identical between rats and mice, and ~90% identical between mice and humans,^[210] it is not currently known whether selective 5-HT₂ ligands have the same affinities and functions at both mouse and rat 5-HT₂ receptors (discussed below). It is well known that changing even a single residue in a critical region of the transmembrane domain (where most ligands bind) of a G protein-coupled receptor can substantially alter ligand affinity and function (e.g. for 5-HT_{2A} and 5-HT_{2C} receptors^[211,212]). Though these regions of the 5-HT₂ receptors tend to be conserved across species, potentially other, unidentified regions of the receptors that differ between species may be important in determining a given ligand's affinity for and function at these receptors. Notably, the differences between rodent and human 5-HT_{2A} receptors result in considerable differences in the pharmacology of N-1 substituted tryptamines and ergolines.^[209,213,214]

Dosing issues: problem of non-selectivity

The issue of drug dose is a critical one in behavioural and neuropharmacological studies, and is regularly overlooked, as it is sometimes presumed that when a compound is described as 'selective', it means 'specific'. 5-HT₂ receptors share approximately 80% sequence homology in their transmembrane domains, which makes development of very specific 5-HT₂ subtype-selective compounds a tedious endeavour, and thus determination of the 5-HT₂ receptor subtypes involved in the behavioural effects of DOI also an ongoing challenge. Although the antagonists/inverse agonists, M100907, SB206553, and SB242084, which for human receptors, show at least 100-fold selectivity for 5-HT_{2A}, 5-HT_{2B/2C}, and 5-HT_{2C} receptors, respectively, were a practical boon for the field, their relative affinities for 5-HT₂ receptors from a range of laboratory species have not yet been reported. In addition, it is important to keep in mind that although M100907, for example, has extremely high affinity (0.1 nM *K_i*) for the human 5-HT_{2A} receptor, its affinity for human 5-HT_{2C} receptors (~50 nM *K_i*) is not dismissible.

Recent studies suggest that the activities of these newer and commonly used compounds may differ at mouse and rat 5-HT₂ receptors. SB206553, for example, dose-dependently, but partially, attenuated the stimulus effects of 2.5 mg/kg (±)-DOI in a drug discrimination paradigm in C57Bl/6J mice,^[111] findings that did not extend to Sprague–Dawley rats, despite using a lower dose of DOI (0.5 mg/kg) and much higher doses of SB206553.^[11] M100907 completely blocked the DOI stimulus effect in both studies, though a 0.25 mg/kg dose was required for maximal effect in mice, whereas a 0.02 mg/kg dose produced maximal blockade in rats.^[11,111]

A recent neuroendocrine study in Sprague–Dawley rats that included a full dose–response curve showed that SB242084 up to 10 mg/kg did not block corticosterone increases caused by 0.3 mg/kg DOI, yet M100907 completely blocked corticosterone increases caused by DOI with an ED₅₀ value of 0.0035 mg/kg,^[215] suggesting this effect is 5-HT_{2A}-mediated. However, these data should not suggest that all of the behavioral effects of M100907 are solely due to antagonism of 5-HT_{2A} receptors. For example, in a drug discrimination study using Wistar rats, although it was reported that neither SB206553, nor SB242084 at doses up to 10 mg/kg significantly generalized to 0.16 mg/kg M100907, there was approximately 40% generalization with 0.16 mg/kg SB206553 and 20% generalization with 2.5 mg/kg SB242084, yet 0% generalization with the 5-HT_{2B} selective antagonist SB204741.^[216] These data illustrate that the stimulus cue elicited by 0.16 mg/kg M100907 may involve some blockade of 5-HT_{2C} receptors, or may involve other unidentified receptors targeted by these compounds.

As another example, in Wistar rats trained to discriminate the selective 5-HT_{2C} receptor agonist, Ro 60–0175 (2.5 mg/kg), from saline, SB 206553 (0.63 mg/kg) was 100%, SB 242084 (0.63 mg/kg) was 80%, and M100907 (0.16 mg/kg) was 40% effective at blocking the discriminative cue.^[217] It must be emphasized that although touted as a highly selective and potent 5-HT_{2C} agonist, Ro 60–0175 also likely causes behavioural effects mediated by receptors other than 5-HT_{2C}, such as 5-HT_{2A} and 5-HT_{2B} receptors. Comparing affinities for each of the human 5-HT₂ receptors, Ro 60–0175 has highest affinity for 5-HT_{2B} receptors (the 5-HT_{2C}-VSV isoform was tested),^[34] and has greatest potency for activating 5-HT_{2B} receptors.^[19] Moreover, the neuroendocrine stimulating effects (including release of corticosterone) of Ro 60–0175 in Sprague–Dawley rats, which occurred above 2.5 mg/kg, were not blocked by pretreatment with SB242084 (at doses up to 5 mg/kg)

or M100907 (0.01 mg/kg).^[218] Taken together, these data illustrate an often ignored point that 'selective' does not translate to 'specific'. Thus, a titration of doses for any given 5-HT_{2A}, 2B, or 2C selective compound is necessary to begin to determine specific 5-HT₂ receptor subtypes that may be involved in a particular behavioural outcome. Also, many compounds, such as SB206553,^[219] have affinity for off-target receptors and other proteins that may play a role in their behavioural effects.

As discussed in a previous section (5-HT_{2C}), similar dosing issues and discrepant findings are apparent using the DOI HTR model. For example, although Ro 60–0175 at 2.5 mg/kg was an effective training dose in drug discrimination studies,^[217] caused significant neuroendocrine activation,^[218] and significant changes in release of cerebral dopamine^[220,221] in rats, 3.0 mg/kg was ineffective at attenuating the HTR induced by 1.0 mg/kg (–)-DOI; 10 mg/kg was required to attenuate the HTR in mice, and even the highest dose tested, 30 mg/kg, was ineffective at abolishing the HTR in these mice.^[73]

SB242084 up to 3 mg/kg does not block the HTR in Listar rats induced by 0.2 mg/kg (±)-DOI,^[113] yet blocks 50% of the HTRs induced by 1 mg/kg (±)-DOI in C57Bl/6J and DBA/2J mice; SB206553 at 0.3 and 3 mg/kg led to the same result,^[65] a finding (with C57Bl/6J mice) we recently confirmed at a different institution (unpublished observations). Nevertheless, this general finding was not observed in Swiss NIH mice.^[73] One possibility for these discrepant findings is that 5-HT_{2C} antagonists, such as the 5-HT_{2C/2B} antagonist SB206553, block 5-HT_{2A} receptors in C57Bl/6J but not Swiss NIH mice. EEDQ irreversible binding of 5-HT_{2A} receptors, however, was not attenuated by pretreating C57Bl/6J mice with 3 mg/kg SB206553, yet was by pretreatment with 0.25 mg/kg M100907,^[65] suggesting this is not the answer.

Finally, in Wistar rats, SB206553 at 1.8 mg/kg inhibited the HTR induced by the 5-HT_{2/3} receptor agonist quipazine.^[222] Proteins other than 5-HT₂ receptors, including 5-HT₂ interacting proteins^[204,209] and perhaps enzymes, such as monoamine oxidase, are likely involved in regulating the HTR and may differ between species (as noted in studies aimed at deciphering the species differences in toxicity caused by MPTP injections^[223]). Obviously, dose is not the only issue here.

Several pharmacological studies have suggested there are different and often opposing neurochemical functions of 5-HT_{2A} and 5-HT_{2C} receptors; the most widely reproduced observation is that 5-HT_{2A} receptor activation increases, whereas 5-HT_{2C} receptor activation decreases dopamine release in cortex, dorsal striatum, and nucleus accumbens,^[190,220,221] which could suggest that DOI has auto-opposing (or auto-regulating) neurochemical effects. Regarding 5-HT_{2C} receptor function, the 5-HT_{2C} agonist, Ro 60–0175 most effectively decreased dopamine release at concentrations at or above 2.5 mg/kg.^[220,221] Although both 5-HT_{2C} receptor antagonists increased dopamine release, the effects of SB206553 (combined 5-HT_{2B/2C} antagonist) were more robust than SB242084, and the increase in dopamine release following SB206553, but not SB242084, injections in Sprague–Dawley rats were dose-dependent up to 10 mg/kg.^[221] This dose is notably very high, considering that 0.63 mg/kg SB206553 (and SB242084) is sufficient to block 100% (and 80%, respectively) of the Ro 60–0175 (2.5 mg/kg) discriminative cue in rats,^[217] and suggests that receptors in addition to 5-HT_{2C} receptors, including nicotinic acetylcholine receptors^[219] and 5-HT_{2B} receptors, may also be regulating the increases in dopamine release by SB206553. Alternatively, the differences between SB206553 and SB242084 may relate to the observations that the former behaves as a 5-HT_{2C} receptor inverse agonist, while the latter behaves as a

partial 5-HT_{2C} receptor agonist, able to attenuate inverse agonist functions of SB206553 (*in vitro*, recombinant receptors, isoform unspecified, in CHO cells, PLC function).^[221] These data suggest that constitutive activity of 5-HT_{2C} receptors may be as important as endogenous 5-HT induced activation of 5-HT_{2C} receptor for regulation of basal dopamine release.

DOI injections (2.5 mg/kg) increased dopamine release in the rat cortex, an effect not observed in 5-HT_{2A} receptor knockout mice, but preserved in 5-HT_{2C} receptor knockout mice.^[190] Curiously, DOI's effects on dopamine release were not potentiated in 5-HT_{2C} receptor knockout mice,^[190] and yet this group reported that increases in the concentration of DOI injected systemically (0, 1.25, 2.5, and 5 mg/kg) produced an inverted U-shaped dose-response curve for dopamine release, and suggested, though did not show, that increased activation of 5-HT_{2C} receptors by DOI mediated the descending part of the curve.^[224]

DOI at 1 mg/kg, as noted previously, reliably induces a HTR that is blocked by 5-HT₂ antagonists, yet this dose was not sufficient to induce increases in acetylcholine in the rat hippocampus; 2 mg/kg was.^[188] Finally, also intriguing are results from neurophysiology studies of DOI. In cortical cells, *ex vivo*, DOI at a concentration of 300 nM, well above its reported *K_i* and EC₅₀ values (*in vitro*) for 5-HT₂ receptors,^[19] had no effect on electrochemical activity; 1 µM was needed to alter activity, and 30 nM M100907 blocked the effects.^[35]

Drug-specific stabilization of receptor conformations

It is becoming increasingly clear that different ligands that bind to the same GPCR induce or stabilize unique receptor conformations, and furthermore, that the conformational changes may be different when receptors are expressed in different cell types.^[225] The stabilization of unique receptor conformations by different ligands positions the receptor to have selective interactions with G-protein subtypes or even with signalling proteins other than G-proteins (e.g. β-arrestin); these unique interactions confer activation (or inactivation) of potentially several specific cellular signalling pathways, a phenomenon known as functional selectivity or biased agonism.^[32,226] Thus, as described by Kenakin,^[227] G-protein coupled receptors are no longer viewed as 'on-off switches', but are more akin to 'microprocessors' regulated by interacting proteins and ligands that bind them.

Therefore, designation of a ligand simply by its efficacy as an agonist (partial or full), antagonist (competitive or non-competitive), or inverse agonist of a particular receptor, is not adequately informative without specifying the relative endpoint that is measured. For example, a ligand at 5-HT_{2A} receptors could be a full agonist for the recombinant rat 5-HT_{2A} receptor-Gq-PLC-IP pathway plus a partial agonist for the 5-HT_{2A}-G12-PLD pathway when the receptor is expressed in Chinese hamster ovary cells. When examined in the rat claustrum *in vivo*, however, the same ligand may show weak partial agonist activity for the 5-HT_{2A} receptor-Gq-PLC pathway, and no activity at the 5-HT_{2A}-G12-PLD pathway; thus the ligand may actually behave as an antagonist of endogenous serotonin. Many of the conflicting data regarding DOI's behavioural pharmacology may be explained by unique functionally selective activity at several receptors, expressed *in vivo*, on different neural cells, on physically distinct parts of the cells, across species and strains.

Most 5-HT₂ receptor agonists that are hallucinogenic are partial agonists, with similar potencies and efficacies (for stimulation of PLC-IP and others) for 5-HT_{2A} and 5-HT_{2C} receptors. So, why are other 5-HT_{2A/2C} agonists, such as the phenylpiperazine, mCPP,

and the benzazapine, lorcaserin^[19,228–230] generally considered non-hallucinogenic? That is, why don't these compounds produce psychedelic effects comparable to LSD? Besides having notably poorer potency for activating either or both 5-HT_{2A} and 5-HT_{2C} receptors, another argument (discussed in detail previously) is that 5-HT_{2C} receptor activation suppresses (or alters) 5-HT_{2A} receptor function, and 5-HT₂ agonists lacking LSD-like effects are generally considered relatively more potent and efficacious 5-HT_{2C} receptor agonists compared to 5-HT_{2A} receptors. This argument seems untenable, though, simply because hallucinogens that produce LSD-like effects are 5-HT_{2C} agonists; if 5-HT_{2C} receptors suppressed hallucinogenic effects, LSD wouldn't be hallucinogenic, a point lost in many discussions. It should be noted, also, that several phenylpiperazines fully substituted for the mCPP stimulus in rats, yet LSD and (–)-DOM only partially substituted. The efficacy of phenylpiperazines for stimulating 5-HT_{2C} receptor-linked PLC signalling, moreover, was not directly related to their stimulus generalization effects.^[231] Thus, these data suggest that the stimulus effects of phenylpiperazines are not mediated by the primary 5-HT_{2C} receptor signalling pathway.

Regardless, a more parsimonious explanation is that certain 5-HT₂ receptor-activating compounds, such as DOI and psilocin, cause specific conformations of 5-HT₂ receptors that lead to LSD-like psychedelic effects.^[94,232] Moreover, although the 5-HT₂ agonists mCPP, lorcaserin, and quipazine, are often considered 'non-hallucinogenic', they actually do produce perceptual, cognitive, and emotional changes that may be considered 'hallucinogenic'.^[15,233,234] These effects, however, are generally not considered LSD-like. Finally, it is noteworthy that novel, partial 5-HT_{2A/D2} receptor agonists also attenuate the DOI-elicited HTR.^[235,236] These observations provide further support for the idea that DOI potentially stabilizes a 5-HT_{2A} receptor conformation unique from other 5-HT_{2A} receptor agonists. It would be interesting to know whether pre-treatment with LSD or DOM attenuates the DOI-induced HTR, as the outcome could potentially strengthen the argument for ligand-specific receptor conformations. Molecular modelling and computational studies of different 5-HT_{2A} agonists docked in the 5-HT_{2A} receptor binding pocket would also help clarify this point.

Animal models

Animal models of human behaviour or psychiatric disorders are typically used to mimic some aspect of the human condition. As is true for all models it is imperative to clearly and explicitly identify the purpose of the model – what are you trying to 'model'? The degree to which any particular animal model is useful can then be assessed through various forms of validity. The purpose of a particular model can vary, and may include:

- mimicking an entire behavioural or psychiatric syndrome;
- modelling a particular behavioural phenomenon or psychiatric symptom, i.e. an endophenotype of a disease; and
- predicting the outcome a particular experimental intervention (e.g. an environmental, behavioural, pharmacological, or neurobiological manipulation).

Model validity

Although a wide variety of validity types have been described, one common categorization of validity for assessing a model of 'normal' or 'pathological' behaviour includes the following:

- **Face validity** refers to the phenomenological similarities between the behaviour of interest in the animal and the behaviour (pathological or not) of the 'real-world' human; that is, do the behaviours resemble each other on the 'face' of it. This is not required for an animal model, and is oftentimes detrimental as it can imply inaccurately similar underlying mechanisms in the model and the human condition.
- **Predictive validity** refers to the ability of the model to predict something about the human condition; that is, does the outcome of testing in the animal model allow you to predict the response in humans? This is the most important and desirable aspect of a model, and is oftentimes assessed by similar outcomes of neurobiological or pharmacological interventions in the animal model and humans.
- **Etiological validity** refers to situations in which the animal and the human condition are "caused" by the same mechanism; that is, the etiology of the behavioural or psychiatric phenomenon is identical. Advances in the study of genetic vulnerability to various psychiatric disorders and our ability to manipulate the genetic makeup of animals are leading to increases in model development along these lines, although this presumes that we know the cause of a particular disorder (which is often not the case).
- **Construct validity** refers to the notion that the animal model is measuring what it was intended to measure; that is, there is close functional homology between the behavioural phenomena in the animal model and the human condition. This requires a high degree of theoretical and/or conceptual understanding of the phenomena, and although much can be learned about the behavioural or pathological condition of interest, construct validity is not necessary for an effective animal model.

What do DOI-elicited HTRs model?

The DOI-elicited HTR has been considered as an animal model of a variety of behavioral and psychiatric conditions including hallucinogenesis, schizophrenia, obsessive-compulsive disorders, Tourette's syndrome, and more generally as a model of 5-HT₂ function/activity. Although the DOI-elicited HTR has some validity as a model for each of these conditions, it is likely most suitable as a model of 5-HT₂ receptor activity, as described below.

Hallucinogenesis

Many hallucinogenic 5-HT₂ receptor agonists produce the HTR in rodents, and early animal studies of hallucinogens suggested that this response may be a model of hallucinating.^[43] The consistently reproduced observations that 5-HT₂ receptor antagonists, such as those that block the hallucinogenic effects of classical hallucinogens, such as psilocybin,^[169] completely block the hallucinogen-induced HTR bolster support for the HTR as a model of hallucinating, and for 5-HT₂ receptor activation as the underlying mediator of hallucinogenesis. Moreover, non-hallucinogenic compounds with 5-HT_{2A} agonist activity, for example, lisuride, fail to produce the HTR.^[94] The HTR model itself (excluding the DOI-elicited HTR model), however, falls short of directly modeling hallucinations in humans, because a much larger list of compounds, including but not limited to the serotonin precursor, 5-HTP, the 5-HT releasing agent, fenfluramine, and the cannabinoid receptor antagonist, SR141716A, that are known to lack hallucinogenic activity in man, nevertheless produce the HTR in rodents.^[43,51,147,237] Also, compounds, including DMT, that are hallucinogenic in man do not reliably produce the HTR.^[46]

However, the HTR is a good model of 5-HT₂ receptor activation in the rodent, as to our knowledge 5-HT₂ receptor antagonists have blocked the HTR induced by every compound tested, and all compounds of which we are aware that have been shown consistently to be potent^b and selective agonists for 5-HT_{2A},^{2B}, and ^{2C} receptors induce the HTR. Nevertheless, the DOI-elicited HTR itself may still be useful as a tool to investigate (as best as the rodent brain will permit) neural mechanisms that may produce or underlie hallucinogenic effects caused by psychedelic drugs.

For reasons similar to the HTR model, the rodent drug-discrimination model also fails to model hallucinogenesis. For example, both LSD and DOM stimuli generalize with the non-hallucinogenic compounds, fenfluramine and lisuride,^[238,239] and much of the stimulus effect of lisuride is likely mediated by 5-HT_{2A} receptor activation.^[239] In other words, 5-HT_{2A} receptor activation in the rodent by lisuride and LSD may produce similar cognitive effects in rodents, i.e. rodents may not always be able to distinguish hallucinogenic (LSD) from non-hallucinogenic (lisuride). Moreover, there is some evidence that activity at 5-HT_{1A} receptors can modulate the discriminative stimulus effects of hallucinogens (e.g. 5-HT_{1A} agonists produce partial substitution and enhance, while antagonists can partially block the effects of LSD),^[240,241] yet results from a plethora of studies suggest 5-HT_{1A} receptor activation does not cause hallucinogenic effects in humans.^[18,92] Indeed, antagonism of 5-HT_{1A} receptors by pindolol potentiates the psychological responses to DMT in humans.^[242] Again a disclaimer arises, as pindolol may have weak partial agonist activity at 5-HT_{1A} receptors *in vivo*. In conclusion, subjective (i.e. drug discrimination) as well as observable (i.e. HTR) behavioural effects observed in rodents after administration of hallucinogens are better conceived as pharmacological outcomes, and not models of hallucinating.

^bnotably DMT has relatively poor affinity for 5-HT_{2A} receptors,^[40] yet we could not find in the literature DMT's affinity for 5-HT_{2C} receptors.

Schizophrenia

Shortly after the discovery of psychedelic hallucinogens, similarities between the subjective effects of these compounds and the perceptual effects associated with schizophrenia and related psychosis-related disorders were noted.^[243,244] Early reports suggested that administration of hallucinogenic drugs to animals could function as a model of schizophrenia.^[245] It may be that drug-elicited head-twitches are a simple model of the 'positive symptoms' (in particular, hallucinations) associated with schizophrenia rather than a model of the entire syndrome of schizophrenia. This would be a classic example of an animal model that initially is proposed as a model of the entire syndrome of interest, which is later tapered down to potentially being a model of an endophenotype of a particular syndrome.^[246]

Current conceptions of the pathophysiology of schizophrenia have been derived largely from pharmacological studies and can be summarized as follows. Dopamine agonists such as amphetamine, 5-HT_{2A} receptor agonists that are psychedelics (e.g. LSD), and glutamate NMDA receptor antagonists including PCP, MK-801, and ketamine can all produce psychosis-like behavioural and subjective effects in humans. Conversely, dopamine D₂ antagonists, 5-HT_{2A} receptor antagonists, and glutamate mGluR_{2/3} receptor agonists are to some extent effective antipsychotic medications.

Data obtained using DOI-elicited HTRs and pharmacological challenges are generally consistent with this notion. In particular,

much data suggests that glutamate NMDA receptor antagonists exacerbate the HTR, mGluR_{2/3} receptor agonists and antagonists attenuate and enhance the HTR respectively, 5-HT_{2A} antagonists block the HTR, and dopamine D₂ receptor and D₁ receptor antagonists block the HTR. Post-mortem study of brains from schizophrenic patients,^[137,247] and PET studies of untreated, first-episode psychotic patients demonstrated altered levels of 5-HT_{2A} receptors which appear to corroborate these findings.

Of particular interest regarding the use of this model as a model of schizophrenia is that essentially every effective antipsychotic medication used in humans is effective in attenuating the HTR elicited by DOI administration. That is, there seem to be relatively few 'false negatives' – drugs that are useful as antipsychotic medications that fail to block the HTR. While this is an interesting set of findings, one issue that has recently become apparent is that blockade of the DOI-elicited HTR in mice does not necessarily translate to antipsychotic activity in humans. This is most clearly demonstrated by clinical studies examining the highly selective 5-HT_{2A} receptor antagonist M100907, which has extraordinary potency at blocking the DOI-elicited HTR. Although originally developed as a novel antipsychotic,^[248] M100907 *apparently* failed in clinical trials for the treatment of schizophrenia.^[249] Although we believe that positive results in DOI-elicited HTR behavioural procedures may be indicative of antipsychotic activity, these results highlight the fact that findings obtained from one animal model are not enough to draw strong conclusions about pharmacotherapeutic implications. Rather, convergent evidence from the use of many animal models is required. So although M100907 was not effective in human trials, the fact that it may show up as a 'false positive' in DOI-elicited HTR studies does not necessarily indicate the model is useless. Rather, this animal model represents one tool at researchers' disposal that may help to identify a desired preclinical profile indicative of antipsychotic activity in humans.

One particularly interesting study exploring the relationship between DOI-elicited HTR and schizophrenia was one in which the effects of various other symptoms commonly associated with the disorder, i.e. poorly-structured sleep and suppressed endogenous opioid function, were assessed in combination with DOI-elicited HTRs.^[250] In this study, the effects of sleep disturbances were modelled by a period of sleep deprivation and suppressed opioid function was induced by administration of the opioid antagonist naloxone. Neither treatment alone had effects on the sensitivity of HTRs to DOI. The combination, however, of sleep deprivation and naloxone treatment significantly enhanced sensitivity to DOI.

Epidemiologically, gestational exposure to infection is associated with high risk of developing schizophrenia in adult offspring.^[251] Another recent and interesting paper using mice demonstrated that maternal influenza viral infection increased sensitivity to DOI (approximately twice as many HTRs) in the adult (but not prepubertal) offspring.^[136] Continued exploration of the model in this matter may provide substantial etiological validity that could contribute to the development of novel treatments for schizophrenia.

^cThis compound is currently being evaluated for the treatment of insomnia and treatment resistant depression: www.clinicaltrials.gov.

Obsessive-compulsive disorder (OCD) and Tourette's syndrome

OCD incorporates a variety of related behavioural pathologies, and Tourette's syndrome is typically considered as part of the OCD spectrum. One symptom associated with these disorders is the presence of a tic, and the DOI-elicited HTR has been described as an analogous behavioural outcome. As an animal model

of tic disorders, the DOI-elicited HTR seems to have some face validity (i.e. the HTR may look like a tic). It is possible, although unknown at this point in time, that there is some etiological validity if tics are indeed produced by an over-stimulation of 5-HT_{2A} receptors. Importantly, the model appears to have some predictive validity in one direction: the only medications that appear to be useful in treating tics, such as haloperidol (primarily a dopamine D₂ antagonist), atypical opioids (levorphanol, tramadol), nicotine (acetylcholine agonist), and donepezil (acetylcholine esterase inhibitor; indirect acetylcholine agonist), are effective in suppressing DOI-elicited head-twitches.^[63,140,153,154,252] Unfortunately the preclinical literature and clinical outcome research exploring the potential relationship between DOI-elicited HTR and OCD/Tourette's syndrome is relatively limited, so firm conclusions cannot be made at this time.

Involvement of 5-HT_{2A} receptor activity and status of the 5-HT_{2A} system

It may be relatively safer to suggest that the HTR elicited by DOI and related compounds can be effectively used as *in vivo* behavioural models to study 5-HT_{2A} receptor pharmacology, however as noted above, drugs from many pharmacological classes can alter DOI-elicited HTRs. Thus, if a novel drug attenuates or enhances the DOI-elicited HTR, one cannot with certainty conclude anything about the pharmacological mechanism of action.

A number of investigators have used sensitivity to DOI (measuring the HTR) as a measure of the functional status of an animal's 5-HT_{2A} receptor system. That is, the animal is exposed to some environmental, pharmacological, or genetic manipulation, and the state of the 5-HT_{2A} system is inferred from the number of HTRs elicited by DOI administration. In many instances, sensitivity to DOI is highly correlated with the number and/or function of 5-HT_{2A} receptors; for example, Rinaldi-Carmona *et al.*^[253] demonstrated that chronic administration of a novel 5-HT_{2A} antagonist upregulated 5-HT_{2A} receptor density and increased sensitivity to DOI (see Dougherty and Aloyo^[74] for a more comprehensive discussion of repeated drug administration, changes in receptor density, and sensitivity to DOI). It should be noted, however, that there are situations (e.g. across mouse strains) where sensitivity to DOI does not correlate with 5-HT_{2A} density.^[65] Thus, interpretations about the 5-HT_{2A} receptor system following a single experiment with DOI should be corroborated using additional models to provide convergent evidence regarding a particular scientific question.

Summary

The DOI-elicited HTR has been used as an animal model of a variety of behavioural and psychiatric conditions. As described above, there is reasonable evidence that these models may have some predictive validity, especially regarding pharmacological interventions in each case. However, simply because a model has some form of validity, one should not infer that the model incorporates all aspects of the behaviour or condition under investigation. That is, DOI-elicited HTRs should not be considered hallucinogenic episodes, or a way to examine the entire syndromes called schizophrenia, Tourette's syndrome, or obsessive-compulsive disorder. Similarly, the neuropharmacology of DOI, along with the pharmacology of essentially every other psychoactive drug ever examined is relatively complex. Furthermore, activity in the head-twitch model itself is not de facto proof of action at the 5-HT_{2A} receptor as described above. Rather these procedures can be useful as one of many tools for examining a

particular aspect of these disorders, and that progress will only be made when there is convergent evidence using a number of models.

Conclusions

The DOI-induced HTR is a relatively straightforward behavioural and pharmacological procedure to set up and quantify, and can be used to ask interesting questions about neuropharmacological mechanisms and complex behavioural and psychiatric conditions. Various unresolved issues remain regarding behavioural neuropharmacological effects of DOI; therefore, there is much reason to continue using this procedure. There is increasing interest in the therapeutic potential of both 5-HT_{2A}, as well as 5-HT_{2C}, receptor agonists and antagonists for a variety of conditions. In addition to various psychiatric disorders mentioned above (e.g. psychosis-related disorders and OCD), the 5-HT₂ receptor system has been identified as a desirable drug target for the treatment of depression, anxiety, post-traumatic stress disorder, obesity, numerous pain conditions (inflammation, neuropathic pain, cluster headaches), cardiovascular disease, and various sleep disorders (recent reviews^[254,259]). Hallucinogenic 5-HT₂ receptor agonists, in addition, are receiving renewed attention as potential psychotherapeutic agents.^[260,261] Others have noted, however, that 5-HT_{2A} receptor activation may potentially limit the therapeutic properties of other neuropsychiatric medications (e.g. SSRIs), suggesting combination therapies that include 5-HT_{2A} antagonists.^[262] Notably, there is not a lot of support to develop selective 5-HT_{2B} agonists, as activation of 5-HT_{2B} receptors in heart tissue is associated with valvular heart disease.^[263,264] Given the great potential for therapeutic agents targeting 5-HT₂ systems, relevant animal models need to be used and improved, in order to advance drug discovery programs. The DOI-induced HTR is one model that can help contribute to this effort.

In conclusion, DOI's hallucinogenic effects may be likened to a symphony orchestra conducted in the brain, with the receptors it activates, the sections or instruments. With this analogy, DOI-induced activation of 5-HT_{2A} receptors sets the melody of the experience, and the other instruments, such as 5-HT_{2B}, 5-HT_{2C}, and potentially adrenergic receptors, play accompanying roles. With each receptor an instrument, DOI has a number of them to play. To argue that performance of only one instrument, i.e. the 5-HT_{2A} receptor, produces the total psychedelic experience while the other instruments are plugged in and turned on but not played, seems indefensible. In other words, as pointed out by other researchers,^[18,93,238] activation of 5-HT_{2A} receptors, may be necessary, but not sufficient to produce the full range of effects of DOI.

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