HHS Public Access

Author manuscript

Neurosci Lett. Author manuscript; available in PMC 2023 April 19.

Published in final edited form as:

Neurosci Lett. 2022 September 25; 788: 136836. doi:10.1016/j.neulet.2022.136836.

Differences across sexes on head-twitch behavior and 5-HT_{2A} receptor signaling in C57BL/6J mice

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Abstract

Psychedelics, also known as classical hallucinogens, affect processes related to perception, cognition and sensory processing mostly via the serotonin 5-HT_{2A} receptor (5-HT_{2A}R). This class of psychoactive substances, which includes lysergic acid diethylamide (LSD), psilocybin, mescaline and the substituted amphetamine 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), is receiving renewed attention for their potential therapeutic properties as it relates to psychiatric conditions such as depression and substance use disorders. Current studies focused on the potentially clinical effects of psychedelics on human subjects tend to exclude sex as a biological variable. Much of the understanding of psychedelic pharmacology is derived from rodent models, but most of this preclinical research has only focused on male mice. Here we tested the effects of DOI on head-twitch behavior (HTR) – a mouse behavioral proxy of human psychedelic potential – in male and female mice. DOI elicited more HTR in female as compared to male C57BL/6J mice, a sex-specific exacerbated behavior that was not observed in 129S6/SvEv animals. Volinanserin (or M100907) – a 5-HT_{2A}R antagonist – fully prevented DOI-induced HTR in male and female C57BL/6J mice. Accumulation of inositol monophosphate (IP_1) in the frontal cortex upon DOI administration showed no sex-related effect in C57BL/6J mice. However, the pharmacokinetic properties of DOI differed among sexes – brain and plasma concentrations of DOI were lower 30 and 60 min after drug administration in female as compared to male C57BL/6J mice. Together, these results suggest strain-dependent and sex-related differences in the behavioral and pharmacokinetic profiles of the 5-HT_{2A}R agonist DOI in C57BL/6J mice, and support the importance of studying sex as a biological variable in preclinical psychedelic research.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Keywords

Psychedelics; Classical hallucinogens; Serotonin 5-HT_{2A} receptor; G protein-coupled receptor (GPCR); Sex differences; Head-twitch behavior

1. Introduction

Classical psychedelics, such as lysergic acid diethylamide (LSD), psilocybin, mescaline and 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane (DOI), have been recognized for their capacity to profoundly dysregulate various mental domains, particularly sensory perception and thought processes [20,44]. Most of the previous studies in rodent models [22,43] and human subjects [9,47] focused their efforts on the effects that occur within minutes to hours after psychedelic administration, with the aim of elucidating the receptor target and signaling pathways involved in their hallucinogenic properties. Importantly, more recent clinical trials suggest that psychedelics may represent a promising novel treatment strategy for patients with depression and other psychiatric conditions. Several studies have reported fast-acting and lasting clinically relevant antidepressant effects upon a single psilocybin administration in combination with psychological support [8,12]. However, the hallucinogenic properties of classical psychedelics preclude their use in daily clinical practice. It is therefore clear that we need a better understanding of the molecular and neural circuit mechanisms underlying the behavioral effects of these drugs with the final goal of developing safer therapeutic alternatives [6,7].

Studies in rodent models and human subjects suggest that most of the acute effects observed directly after psychedelic administration are mediated via activation of the serotonin 5-HT_{2A} receptors (5-HT_{2A}Rs), particularly those expressed in forebrain pyramidal neurons [21,32]. Alternative monoaminergic G protein-coupled receptors (GPCRs), such as serotonin 5-HT_{1A} [4], serotonin 5-HT_{5A} [23] and dopamine D_2 [40], may also play a role in some of the hallucinogenic properties of this family of psychoactive substances. Nevertheless, the implication of 5-HT_{2A}R-dependent signaling mechanisms in the post-acute effects of psychedelics on preclinical phenotypes that model therapeutically relevant outcomes remains a topic of intense debate. Thus, it has been reported that the effects of DOI or dimethyltryptamine (DMT) on frontal cortex dendritic spine density and acceleration of contextual fear extinction were reduced by the 5-HT_{2A}R antagonist ketanserin or absent in 5-HT_{2A}R knockout mice [17,39]. However, the same 5-HT_{2A}R blocker was unable to fully prevent the post-acute effects of psilocybin on stress-induced hedonic behavior [28] and growth of dendritic spines in the frontal cortex [48]. Some of the most recent studies focused on psychedelic action have included both sexes in their samples [5,41,45,48]. Additionally, it has been reported that the psychedelic 5-HT_{2A}R agonist DOI differentially affects prepulse inhibition of the startle – a model of sensorimotor gating – in male and female 129S6/SvEv mice [52], whereas the 5-HT_{2A}R antagonist volinanserin allosterically augmented the antinociceptive effect of the opioid receptor agonist oxycodone in male but not female C57BL/6J mice [50]. Although these findings suggest sex-related differences in 5-HT_{2A}R-dependent behaviors, the majority of the previous studies focused on the psychedelic properties of 5-HT_{2A}R agonists were carried out exclusively in male rodents

[32]. Similarly, while both male and female human participants are generally included in the cohorts to test the effects of psychedelics on behavioral and neuroimaging responses, sex and/or gender as independent experimental variables are usually excluded from the statistical analysis [13,24,53].

This gap in our knowledge related to sex-specific effects of psychedelics is particularly relevant considering that sex and/or gender affects the subjective responses following use of psychoactive substances such as morphine and cocaine [18]. There is also an enhanced vulnerability of women to develop alcohol-related diseases which may be due to sex-related differences in metabolism and gastric tissue activity [2]. Additionally, the subjective peak changes induced by 3,4-methylenedioxy-methamphetamine (MDMA, or ecstasy) were reportedly more intense in women than in men – these included MDMA-induced perceptual changes, thought disturbances, and fear of loss of body control [37].

Head-twitch behavior (HTR) has been demonstrated as a behavioral proxy of human psychedelic potential. This rodent (mouse and rat) behavior – characterized by a side-to-side movement of the head – is exacerbated upon psychedelic administration, and is not induced by other psychoactive drugs such as cocaine, phencyclidine or amphetamine [27,32]. Furthermore, the behavioral potency of different psychedelics on HTR correlates with the psychedelic potency determined in human subjects [25]. Several previous findings based on either pharmacological [26] or gene editing [21,22,33] tools suggest that the 5-HT_{2A}R is the main responsible for the effect of psychedelics on HTR. Consequently, this particular behavior is widely used as a rodent model of classical psychedelic action, yet potential sex-related differences on its manifestation have never been directly evaluated.

Here we characterized the effect of DOI on HTR in two commonly used strains of male and female mice, evaluated *in vivo* 5-HT_{2A}R-dependent signaling, and tested the pharmacokinetic properties of this phenethylamine psychedelic across sexes. Our data report sex-related differences in the manifestation of DOI-induced HTR as well as changes in DOI distribution across sexes.

2. Materials and methods

2.1. Materials and drugs

For HTR and IP_1 assays, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) hydrochloride and (*R*)-(+)-α-(2,3-dimethox-yphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperinemethanol (volinanser in, also known as M100907) were purchased from Sigma-Aldrich. Drugs were dissolved in saline (0.9 % NaCl) to the appropriate volume (5 μl/g body weight), and administered intraperitonally (i.p.). Vehicle-treated condition denotes injection (i.p.) of saline (0.9 %) to the equivalent volume of the drug administered. For pharmacokinetic studies, analytical references DOI and (±)-2,5-dimethoxy-4-bromophenethylamine (2-CB) were purchased from Cerilliant Corporation. Acetonitrile, ammonium acetate, ethyl acetate, formic acid, hexane, methanol, sodium hydroxide and water were purchased from Fisher Scientific. All other chemicals were obtained from standard sources.

2.2. Animals

All assays were performed on adult (8 – 14 week-old) male and female C57BL6/J (Jackson Laboratories) and/or 129S6/SvEv (Taconic Biosciences) mice. For HTR assays, animals were bred in-house following previous purchase of breeding trios (C57BL6/J mice from Jackson Laboratories, and 129S6/SvEv from Taconic Biosciences). All HTR experiments were carried out in adult (8 – 12 week-old) male and female littermates. Pharmacokinetic and IP_1 assays were performed on adult (pharmacokinetics, 8 week-old; IP_1, 10 – 14 week-old) C57BL6/J male and female mice received from Jackson Laboratories and acclimated to the vivarium for two weeks. Sex classification of all mice was based on anogenital distance [30]. Animals were housed (2 – 5 mice per cage) on a 12 h light/dark cycle (lights on 6 a.m. to 6 p.m.) at 23 °C with food and water *ad libitum*, except during behavioral testing. All experiments were conducted in accordance with the National Institutes of Health (NIH) guidelines, and were approved by the Virginia Commonwealth University Animal Care and Use Committee. Behavioral testing took place between 9 a.m. to 6 p.m. All efforts were made to minimize animal suffering and the number of animals used.

2.3. Head-twitch response (HTR)

HTR assays using magnetic ear-tagging were performed as previously reported [15,16]. Our previous findings also indicated a high correlation between the automated detection of HTR and the visually detected videotaped HTRs [15,16]. Testing occurred no more than once per week with at least 7 days between test sessions. On test days, mice were placed individually into the coiled chamber for 30 min to acclimate to the environment and determine baseline HTR. Subsequently, DOI (0.5 or 2 mg/kg) or vehicle was administered immediately prior to HTR recording, whereas volinanserin (0.032 mg/kg) or vehicle was administered 15 min prior to DOI. Doses of DOI and volinanserin were selected based on prior HTR studies [14,33,50,52].

2.4. IP_1 accumulation

IP_1 accumulation assays in mouse frontal cortex samples were performed as previously reported [14]. Briefly, mice were sacrificed by cervical dislocation 60 min after DOI (2 mg/kg) or vehicle administration, and bilateral frontal cortex (bregma 1.90 to 1.40 mm) was dissected and immediately frozen at − 80 °C until analysis.

2.5. DOI distribution

Blood and brain tissue samples were obtained from male and female mice following DOI (2 mg/kg) administration. Mice were decapitated 30 min, 1 h and 4 h after drug administration; controls were mock-injected mice. Ultra-high-performance liquid chromatograph tandem mass spectrometer (UHPLC-MS/MS) assays in blood and frontal cortex samples were performed as previously reported [15,49,50].

2.6. Statistical analysis

Statistical significance of experiments involving two or more groups and two or more treatments was assessed by two-way (or three-way) ANOVA followed by Bonferroni's *post hoc* test. Statistical significance of experiments involving three or more groups was

assessed by one-way ANOVA followed by Bonferroni's *post hoc* test. One-way and two-way ANOVA analysis was performed with GraphPad Prism software version 9. Three-way ANOVA analysis was performed in R v.4.1.0 environment using the aov function. The level of significance was set at P = 0.05. All values in the figure legends represent mean ± S.E.M.

3. Results

3.1. DOI-induced HTR in male and female C57BL/6J mice

We first tested the effect of the psychedelic DOI on HTR in male and female C57BL/6J mice. Low (0.5 mg/kg) and moderate (2 mg/kg) doses of DOI were chosen based on previous findings with this classical psychedelic [33,52]. In time-course assays (Fig. 1A), three-way ANOVA analysis (0–90 min) revealed significant main effects of time, sex, and dose, as well as a significant interaction between time and dose, and sex and dose (Table 1).

This effect of time and treatment with either dose of DOI on HTR was also evident when both sexes were analyzed separately (0–90 min) (Fig. 1A) (two-way ANOVA – 0.5 mg/kg DOI vs vehicle in males: treatment $F[1,175] = 735.3$, $P < 0.001$; time $F[6,175] = 20.25$, $P < 0.001$; interaction $F[6,175] = 32.25$, $P < 0.001$) (two-way ANOVA – 2.0 mg/kg DOI vs vehicle in males: treatment $F[1,175] = 1430$, $P < 0.001$; time $F[6,175] = 56.80$, $P < 0.001$; interaction $F[6,175] = 72.15$, $P < 0.001$) (two-way ANOVA – 0.5 mg/kg DOI vs vehicle in females: treatment $F[1,161] = 1074$, $P < 0.001$; time $F[6,161] = 34.36$, $P < 0.001$; interaction $F[6,161] = 39.20$, $P < 0.001$) (two-way ANOVA – 2.0 mg/kg DOI vs vehicle in females: treatment $F[1,189] = 576.1$, $P < 0.001$; time $F[6,189] = 20.97$, $P < 0.001$; interaction $F[6,189] = 22.15$, $P < 0.001$).

No differences across sexes in response to vehicle administration were observed (0–90 min) (Fig. 1A) (two-way ANOVA – male vs female in vehicle mice: sex $F[1,224] = 0.19$, $P > 0.05$; time $F[7,224] = 13.66$, $P < 0.001$; interaction $F[7,224] = 1.14$, $P > 0.05$).

Additionally, the difference in HTR among sexes was corroborated on the collapsed HTR counts over the peak effect during the first 30 min following DOI (or vehicle) administration (Fig. 1B) (one-way ANOVA – DOI effect in males: $F[2,36] = 237.9$, $P < 0.001$) (one-way ANOVA – DOI effect in females: $F[2,36] = 82.48$, $P < 0.001$) (two-way ANOVA – DOI effect in both sexes: treatment $F[2,72] = 211.9$, $P < 0.001$; sex $F[1,72] = 9.39$, $P < 0.01$; interaction $F[2,72] = 2.92$, $P = 0.06$), as well as during 45–90 min after drug or vehicle administration (Fig. 1C) (one-way ANOVA – DOI effect in males: $F[2,36] = 78.25$, $P < 0.001$) (one-way ANOVA – DOI effect in females: $F[2,36] = 55.42$, $P < 0.001$) (two-way ANOVA – DOI effect in both sexes: treatment $F[2,72] = 116.9$, $P < 0.001$; sex $F[1,72] = 7.29$, $P < 0.01$; interaction $F[2,72] = 3.96$, $P < 0.05$),

To assess the role of the 5-HT_{2A}R in HTR across sexes, a potent and relatively selective 5-HT_{2A}R antagonist – volinanserin (0.032 mg/kg) [10] – was administered 15 min prior to DOI (2 mg/kg). This dose of volinanserin was selected based on previous findings [33]. In time-course assays (Fig. 2A), three-way ANOVA analysis (0–90 min) revealed significant main effects of time, sex, and treatment, as well as a significant interaction between time and treatment, and sex and treatment (Table 2).

This effect of time and DOI/volinanserin treatment on HTR in both sexes was also evident when both sexes were analyzed separately (0–90 min) (Fig. 2A) (two-way ANOVA – DOI and/or volinanserin vs vehicle in males: treatment $F[2,105] = 407.3$, $P < 0.001$; time $F[6,105] = 23.93$, $P < 0.001$; interaction $F[12,105] = 29.26$, $P < 0.001$) (two-way ANOVA – DOI and/or volinanserin vs vehicle in females: treatment $F[2,105] = 195.3$, $P < 0.001$; time $F[6,105] = 8.13$, $P < 0.001$; interaction $F[12,105] = 8.98$, $P < 0.001$). *Post hoc* analysis also showed a difference between DOI and vehicle, but not between volinanserin + DOI and vehicle (males: DOI vs vehicle, $P < 0.001$; volinanserin + DOI vs vehicle, $P > 0.05$) (females: DOI vs vehicle, $P < 0.001$; volinanserin + DOI vs vehicle, $P > 0.05$).

As before (see Fig. 1A, above), no differences across sexes in response to vehicle administration were observed (0–90 min) (Fig. 2A) (two-way ANOVA – male vs female in vehicle mice: sex $F[1,90] = 0.24$, $P > 0.05$; time $F[8,90] = 7.74$, $P < 0.001$; interaction $F[8,90] = 0.22$, $P > 0.05$).

Additionally, as above (see Fig. 1B and Fig. 1C), differences across sexes were observed with the sum of HTR during the first 30 min following administration of DOI and/or volinanserin (or vehicle) (Fig. 2B) (one-way ANOVA – DOI and/or volinanserin effect in males: $F[2,15] = 379.5$, $P < 0.001$) (one-way ANOVA – DOI and/or volinanserin effect in females: $F[2,15] = 52.23$, $P < 0.001$) (two-way ANOVA – DOI and/or volinanserin effect in both sexes: treatment $F[2,30] = 139.7$, $P < 0.001$; sex $F[1,30] = 5.83$, $P < 0.05$; interaction $F[2,30] = 5.45$, $P < 0.01$), as well as during 45–90 min post drug or vehicle administration (Fig. 2C) (one-way ANOVA – DOI and/or volinanserin effect in males: $F[2,15] = 37.43$, $P < 0.001$) (one-way ANOVA – DOI and/or volinanserin effect in females: $F[2,15] = 26.45$, $P < 0.001$) (two-way ANOVA – DOI and/or volinanserin effect in both sexes: treatment $F[2,30] = 50.21$, $P < 0.001$; sex $F[1,30] = 9.78$, $P < 0.01$; interaction $F[2,30] = 5.88$, $P < 0.01$).

3.2. DOI-induced HTR in male and female 129S6/SvEv mice

To evaluate potential strain-dependent variations, we tested the effect of DOI on HTR in male and female 129S6/SvEv mice. Based on our previous HTR findings with this particular strain of mice [52], as well previous publications by other groups showing reduced locomotor activity in 129S6/SvEv mice as compared to other strains, including C57BL/6J animals [38], we assessed time-course HTR assays during the first 30 min after DOI (0.5 or 2.0 mg/kg) or vehicle administration. As above with C57BL/6J mice (see Table 1), three-way ANOVA analysis (0–30 min) showed significant main effects of time and dose, as well as a significant interaction between time and dose (Fig. 3A and Table 3). However, three-way ANOVA analysis (0–30 min) also showed absence of sex-related effects on DOI-induced HTR in 129S6/SvEv mice (Fig. 3A and Table 3).

This effect of time and treatment with either dose of DOI on HTR in both sexes was also evident when both sexes were analyzed separately (0–30 min) (Fig. 3A) (two-way ANOVA – 0.5 mg/kg DOI vs vehicle in males: treatment $F[1,30] = 52.93$, $P < 0.001$; time $F[2,30] = 10.18$, $P < 0.001$; interaction $F[2,30] = 10.28$, $P < 0.001$) (two-way ANOVA – 2.0 mg/kg DOI vs vehicle in males: treatment $F[1,30] = 87.74$, $P < 0.001$; time $F[2,30] = 20.43$, $P < 0.001$; interaction $F[2,30] = 20.95$, $P < 0.001$) (two-way ANOVA – 0.5 mg/kg DOI vs vehicle in females: treatment $F[1,30] = 58.55$, $P < 0.001$; time $F[2,30] = 9.29$, $P < 0.001$;

interaction F[2,30] = 18.51, P < 0.001) (two-way ANOVA – 2.0 mg/kg DOI vs vehicle in females: treatment F[1,27] = 71.50, P < 0.001; time F[2,27] = 12.02, P < 0.001; interaction F[2,27] = 20.04, P < 0.001).

No differences across sexes in response to vehicle administration were observed (Fig. 3A) (two-way ANOVA – male vs female in vehicle mice: sex F[1,40] = 2.24, P > 0.05; time F[3,40] = 5.15, P < 0.01; interaction F[3,40] = 0.83, P > 0.05).

The absence of sex-related differences in 129S6/SvEv mice was corroborated on the collapsed HTR counts over the peak effect during the first 30 min following DOI (or vehicle) administration (Fig. 3C) (one-way ANOVA – DOI effect in males: F[2,15] = 19.67, P < 0.001) (one-way ANOVA – DOI effect in females: F[2,14] = 23.12, P < 0.001) (two-way ANOVA – DOI effect in both sexes: treatment F[2,29] = 42.75, P < 0.001; sex F[1,29] = 0.49, P > 0.05; interaction F[2,29] = 0.14, P > 0.05).

3.3. Spontaneous HTR in both sexes of C57BL/6J and 129S6/SvEv mice

We next explored potential changes in spontaneous HTR (*i.e.*, HTR in the absence of psychedelic administration) in both sexes of C57BL/6J and 129S6/SvEv mice (Fig. 4). There was an absence of sex differences on DOI-induced HTR in either C57BL/6J or 129S6/SvEv mice. However, spontaneous HTR was significantly reduced in 129S6/SvEv as compared to C57BL/6J male and female animals (two-way ANOVA: sex F[1,108] = 0.07, P > 0.05; strain F[1,108] = 4.52, P < 0.05; interaction F[1,108] = 0.01, P > 0.05).

3.4. DOI-induced IP_1 accumulation in the frontal cortex of male and female C57BL/6J mice

We previously reported that the density of the 5-HT_{2A}R was reduced in the frontal cortex of female C57BL/6J mice as compared to male littermates. This was assessed by [^{3}H]ketanserin binding assays in membrane preparations of frontal cortex samples [50]. Here we found a similar trend in a separate cohort of male and female C57BL/6J mice (data not shown). To further evaluate potential sex differences related to 5-HT_{2A}R-dependent signaling, we tested the accumulation of IP_1, a downstream effector of the $G_{q/11}$ signaling pathway *in vivo*, in the frontal cortex of male and female C57BL/6J mice. As expected based on our previous finding using this functional assay with alternative 5-HT_{2A}R agonists [14], DOI-treated animals showed greater accumulation of IP_1 in the frontal cortex. However, no sex-related differences were observed (Fig. 5) (two-way ANOVA: DOI effect F[1,12] = 33.99, P < 0.001; sex F[1,12] = 0.59, P > 0.05; interaction F[1,12] = 0.10, P > 0.05).

3.5. Distribution of DOI in blood and frontal cortex of male and female C57BL/6J mice

We evaluated whether these sex differences on DOI-induced HTR were related to pharmacokinetic variations between male and female mice by testing the distribution of DOI in plasma and the CNS. Mice were administered DOI (2 mg/kg) and blood and frontal cortex samples were collected at different time-points after drug administration. Control group (t = 0) were mock-injected mice. As previously reported in male C57BL/6J mice [15], concentrations of DOI were higher in brain samples as compared to blood (Fig. 6).

Similarly, the absorption of DOI was fast, and reached its maximal concentration during the first ~ 30 min in frontal cortex (Fig. 6A) and blood (Fig. 6B) tissue samples in both male and female mice. Interestingly, concentrations of DOI were higher in brain (Fig. 6A) (two-way ANOVA: sex $F[1,22] = 10.00$, $P < 0.01$; time $F[3,22] = 58.97$, $P < 0.001$; interaction $F[3,22] = 1.73$, $P > 0.05$) and blood (Fig. 6B) two-way ANOVA: sex $F[1,24] = 14.34$, $P < 0.001$; time $F[3,24] = 54.61$, $P < 0.001$; interaction $F[3,24] = 4.41$, $P < 0.05$) samples of male as compared to female C57BL/6J mice.

4. Discussion

Most of the previous studies on the effect of classical psychedelics on mouse HTR did not include sex as an experimental variable. To the best of our knowledge, our findings represent the first evidence of strain-dependent and sex-related differences in the behavioral response to psychedelics in mice.

Using two separate doses of the phenethylamine psychedelic DOI, we show that the number of HTRs was more abundant in female as compared to male C57BL/6J mice. This sex-dependent effect was not observed in male and female 129S6/SvEv mice. Previous findings convincingly demonstrate that the 5-HT_{2A}R antagonist volinanserin eliminates DOI-induced HTR [16,33], but these studies were carried out in male mice only. Here we validate that volinanserin also fully prevents HTR induced at two individual doses of the psychedelic DOI in female C57BL/6J mice. Accumulation of IP_1 in the frontal cortex as a functional readout of 5-HT_{2A}R activation upon DOI administration was comparable among sexes, whereas a sex-specific effect was observed for the pharmacokinetic properties of DOI in blood and brain samples, where concentrations of DOI were lower in female as compared to male C57BL/6J mice.

Although psychedelics pronouncedly exacerbate HTR, this side-to-side movement of the head is also observed as a spontaneous behavior in the absence of psychedelic administration [27]. Here we report that the frequency of spontaneous HTR (tested during the time-course assays before DOI or vehicle administration) was similar between sexes in both C57BL/6J and 129S6/SvEv mice. This suggests that the sex effect on DOI-induced HTR is contingent on strain-specific differences. Similarly, it has been previously suggested [15,16,21,31,33,42,52], but not formally proven, that spontaneous HTR is lower in 129S6/SvEv as compared to strains such as C57BL/6J and CD1. Our findings show a significant sex-independent reduction in the counts of spontaneous HTR in 129S6/SvEv as compared to C57BL/6J mice. We also corroborate that DOI-induced HTR is reduced across sexes in 129S6/SvEv mice versus C57BL/6J animals. Similar findings have been reported related to ambulatory motor activity, with large strain and robust sex effects, as well as higher rearing and locomotor activity in C57BL/6 female mice [35]. Our findings also correlate with previous studies suggesting that 129S6/SvEv mice present lower locomotor, rearing and exploratory activity [36]. It will be interesting explore the effects of sex and strain on the outcomes of DOI and other structural classes of psychedelics with alternative phenotypes that model a potential therapeutic action such as reduction of immobility time in the forced-swim test, acceleration of fear extinction, and lasting alterations in dendritic spine structure and chromatin organization [17]. Furthermore, the extent and magnitude of sex and strain

differences in the response to psychedelics could be assessed in genetically diverse outbred rodents to explore potential genotype-phenotype relationships [19].

As mentioned above, we previously reported that the density of 5-HT_{2A}R is reduced in the frontal cortex of female mice, as compared to male C57BL/6J littermates [50]. Pharmacokinetic sex-related differences may potentially be linked to compensatory mechanisms that underlie at least some of the differences in DOI-induced HTR between male and female C57BL/6J mice. However, DOI-accumulation of IP_1 in the frontal cortex was comparable between both sexes. Although the potential role of this 5-HT_{2A}R-dependent signaling pathway in HTR remains unexplored, a potential explanation may be related to the presence of receptor reserve by which orthosteric agonists need to activate only a small fraction of the existing receptor population to produce the maximal response [34].

Stress and other environmental factors affect DOI-induced HTR in mice [29]. To minimize the influence of environmental stressors on our experimental outcomes, animals tested in the HTR assays were littermates - these included both sexes of C57BL/6J and 129S6/SvEv mice. Further assays are needed to evaluate whether environmental factors such as maternal behavior or shipment differentially affect sex-specific 5-HT_{2A}R-dependent processes such as frontocortical IP_1 accumulation and distribution of DOI. Similarly, additional investigation will test for interactions with estrous cycle status [54], as well as the role of ovarian hormones [3], estradiol [51] and BDNF [11] in regulating 5-HT_{2A}R expression in female rodents.

The phenomenon of female preponderance in depression has been well-reported across different cultures. However, this concept is also challenged by higher rates of suicide and addictive behaviors in males, and a longer life-span in females [1,46]. Considering the therapeutic potential of psychedelics in several psychiatric disorders [8,12,32], our findings highlight the need of more comprehensive preclinical and clinical study designs that evaluate effects of psychedelics across sexes with the goal of developing personalized approaches to help determine the optimum dose and timing of psychedelic therapies.

Acknowledgements

This work was supported in part by the National Institutes of Health (NIH) grants R01MH084894 (J.G.-M.), R01MH111940 (J.G.-M.), P30DA033934 (J.L.P), and T32MH020030 (J.Y. and M.d.l.F.R.).

Data availability

Data will be made available on request.

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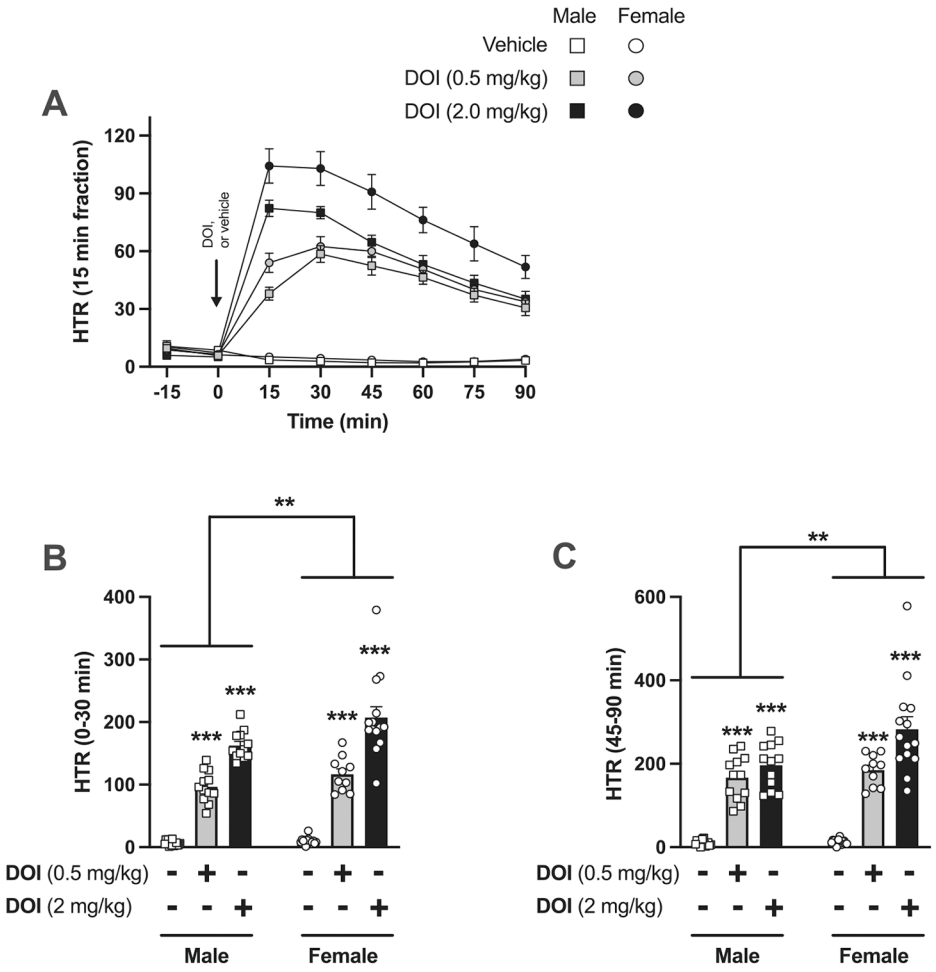

Fig. 1.

Effect of DOI on HTR in male (n = 12–15) and female (n = 10–15) C57BL/6J mice. A) Time-course showing HTR counts in 15 min blocks corresponding to different doses of DOI (0.5 mg/kg and 2 mg/kg) or vehicle. Back arrow shows the administration time-point (t = 0). B) Sum of HTR during the first 30 min after DOI or vehicle administration. C) Sum of HTR during 45–90 min after DOI or vehicle administration. Two-way ANOVA followed by Bonferroni's *post hoc* test (**P < 0.01, ***P < 0.001). For a three-way ANOVA analysis, see Table 1. Data show mean ± S.E.M.

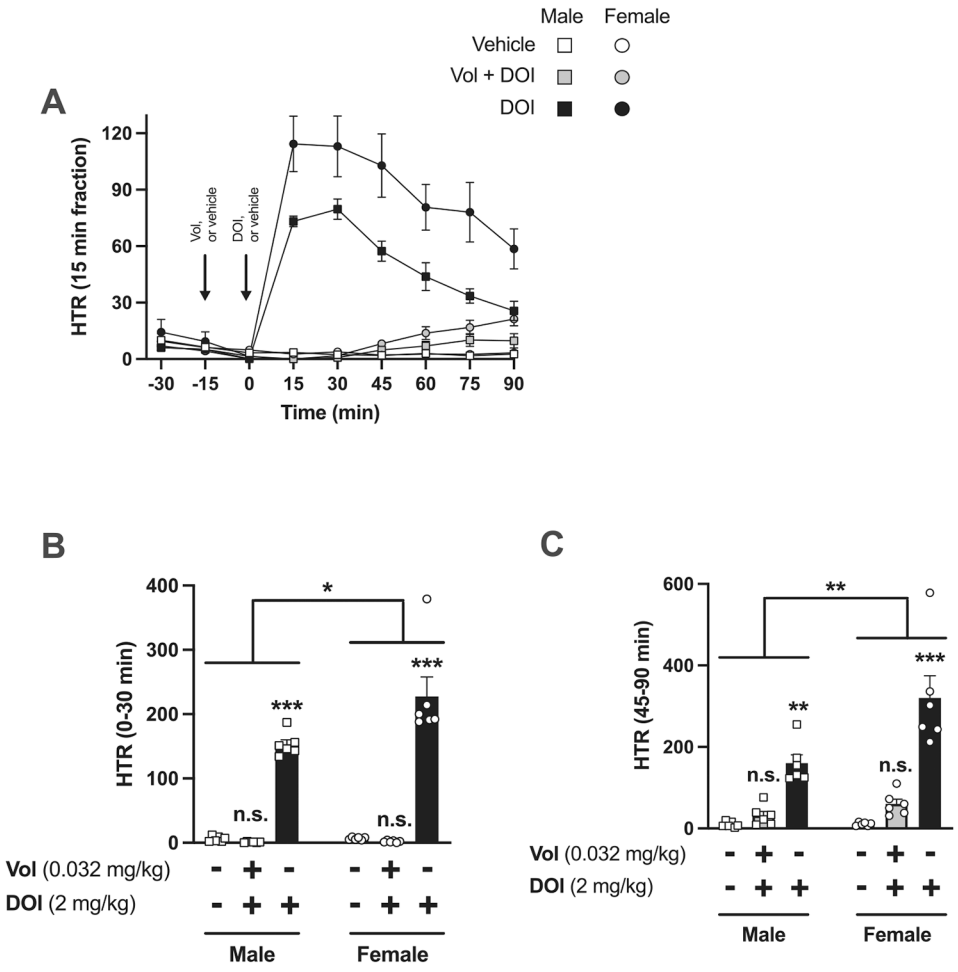

Fig. 2.

Effect of volinanserin (vol) on DOI-induced HTR in male (n = 6) and female (n = 6) C57BL/6J mice. A) Time-course showing HTR counts in 15 min blocks corresponding to mice injected with DOI (2 mg/kg) or vehicle 15 min after being injected with volinanserin (0.032 mg/kg) or vehicle. Back arrows show the administration time-point of volinanserin/vehicle (t = −15) and DOI/vehicle (t = 0). B) Sum of HTR during the first 30 min after DOI or vehicle administration. C) Sum of HTR during 45–90 min after DOI or vehicle administration. Two-way ANOVA followed by Bonferroni's *post hoc* test (*P < 0.05, **P < 0.01, ***P < 0.001, n. s., not significant). For a three-way ANOVA analysis, see Table 2. Data show mean ± S.E.M.

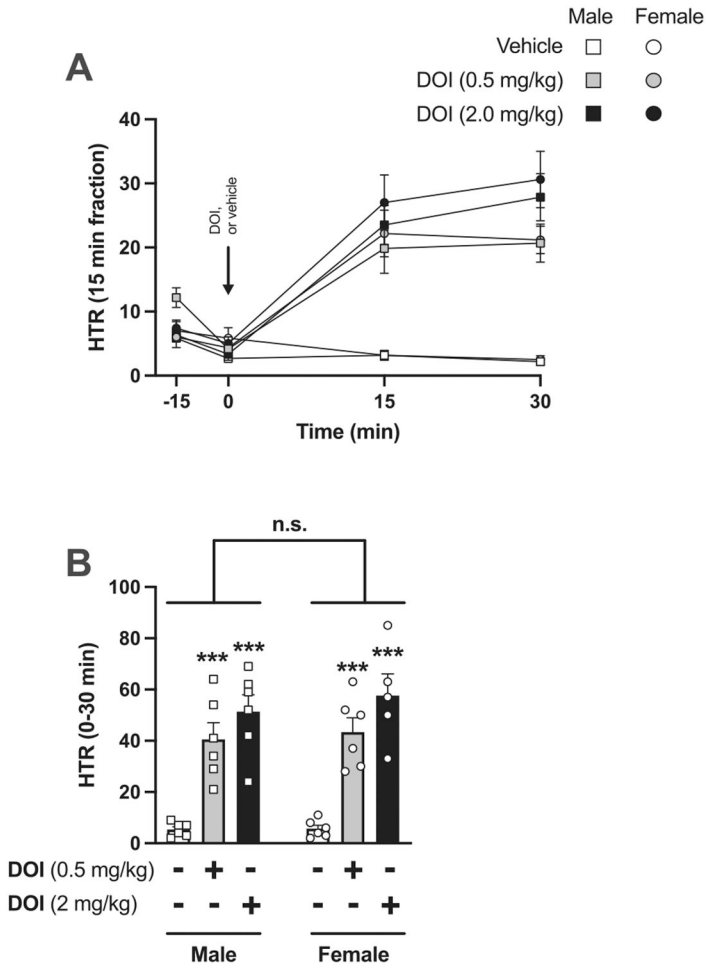

Fig. 3.

Effect of DOI on HTR in male (n = 6) and female (n = 5–6) 129S6/SvEv mice. A) Time-course showing HTR counts in 15 min blocks corresponding to different doses of DOI (0.5 mg/kg and 2 mg/kg) or vehicle. Back arrow shows the administration time-point (t = 0). B) Sum of HTR during the first 30 min after DOI or vehicle administration. Two-way ANOVA followed by Bonferroni's *post hoc* test (***P < 0.001, n.s., not significant). For a three-way ANOVA analysis, see Table 3. Data show mean ± S.E.M.

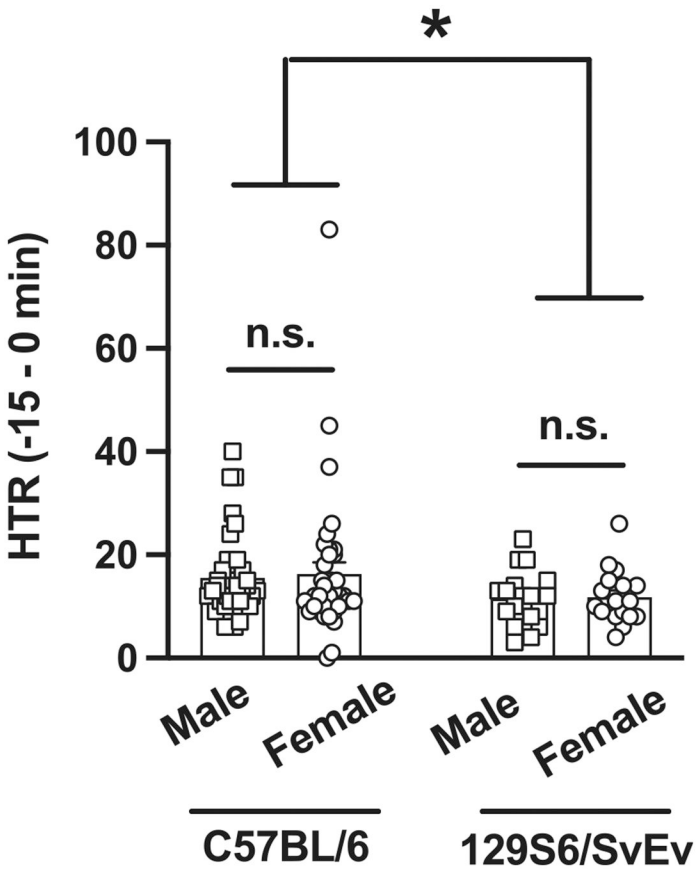

Fig. 4.

Spontaneous HTR in both sexes of C57BL/6J (n = 38–39) and 129S6/SvEv (17–18) mice. A) Total HTR counts during the 15 min before DOI or vehicle administration (see also Fig. 1 and Fig. 3). Two-way ANOVA followed by Bonferroni's *post hoc* test (*P < 0.05, n.s., not significant). Data show mean ± S.E.M.

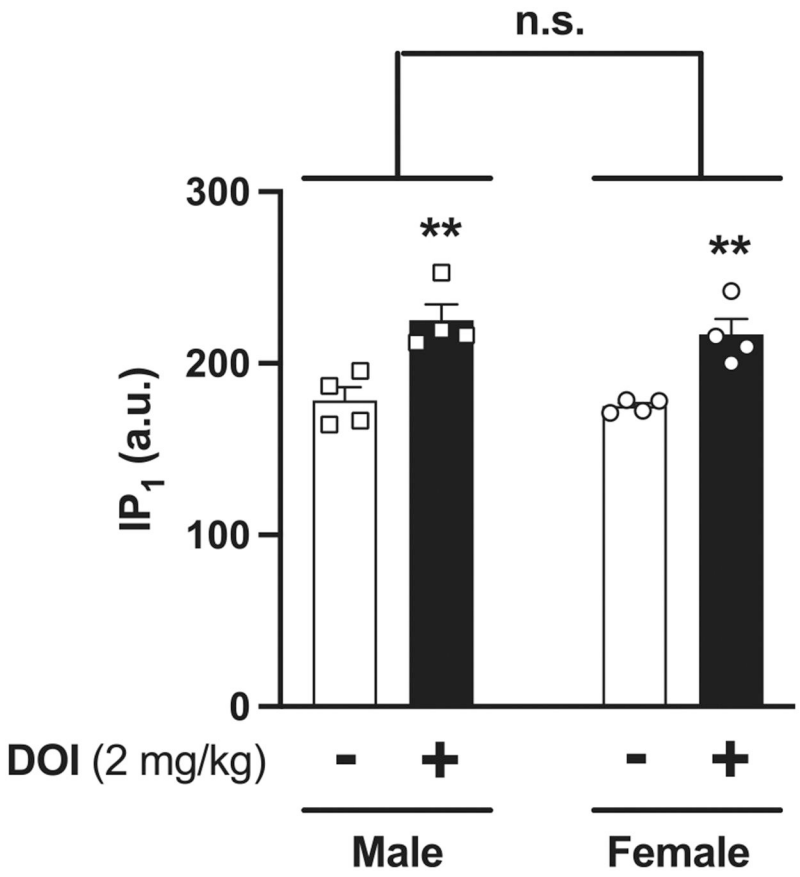

Fig. 5.

Effect of DOI on IP_1 accumulation in the frontal cortex of male (n = 4) and female (n = 4) C57BL/6J mice. A) Samples were collected 60 min after DOI (2 mg/kg) or vehicle administration. Two-way ANOVA followed by Bonferroni's *post hoc* test (**P < 0.01, n.s., not significant). Data show mean ± S.E.M.

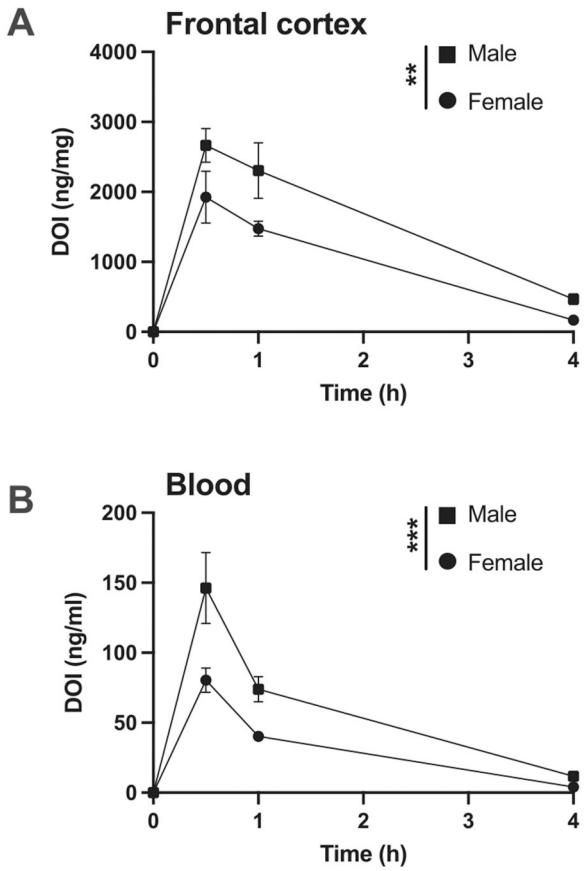

Fig. 6.

Brain and blood concentrations of DOI after a single administration (2 mg/kg) in male (n = 4) and female (n = 3–4) C57BL/6J mice. A) Frontal cortex concentrations of DOI 0.5, 1, and 4 h after a single administration. B) Blood concentrations of DOI 0.5, 1, and 4 h after a single administration. Two-way ANOVA followed by Bonferroni's *post hoc* test (*P < 0.01, ***P < 0.001). Data show mean ± S.E.M.

Table 1

Three-way ANOVA analysis of the effect of DOI on HTR (0–90 min) in male and female C57BL/6J mice (see also Fig. 1A).

Source of variation	ANOVA	p value
Time	F[6,469] = 77.66	p < 0.001
Sex	F[1,469] = 109.66	p < 0.001
Dose	F[2,469] = 631.74	p < 0.001
Time × Sex	F[6,469] = 4.10	n.s.
Time × Dose	F[12,469] = 28.74	p < 0.001
Sex × Dose	F[2,469] = 17.22	p < 0.001
Time × Sex × Dose	F[12,469] = 0.51	n.s.

n.s., not significant.

Table 2

Three-way ANOVA analysis of the effect of volinanserin on DOI-induced HTR (0–90 min) in male and female C57BL/6J mice (see also Fig 2A).

Source of variation	ANOVA	p value
Time	$F[6,210] = 19.13$	$p < 0.001$
Sex	$F[1,210] = 46.77$	$p < 0.001$
Treatment	$F[2,210] = 414.93$	$p < 0.001$
Time × Sex	$F[6,210] = 1.32$	n.s.
Time × Treatment	$F[12,210] = 22.29$	$p < 0.001$
Sex × Treatment	$F[2,210] = 31.99$	$p < 0.001$
Time × Sex × Treatment	$F[12,210] = 1.06$	n.s.

n.s., not significant.

Table 3

Three-way ANOVA analysis of the effect of DOI on HTR (0–30 min) in male and female *129S6/SvEv* mice (see also Fig. 3A).

Source of variation	ANOVA	p value
Time	F[2,87] = 53.84	p < 0.001
Sex	F[1,87] = 1.07	n.s.
Dose	F[2,87] = 74.07	p < 0.001
Time × Sex	F[2,87] = 0.10	n.s.
Time × Dose	F[4,87] = 19.22	p < 0.001
Sex × Dose	F[2,87] = 0.20	n.s.
Time × Sex × Dose	F[4,87] = 0.21	n.s.

n.s., not significant.