Behavioral and genetic analysis of the effects of the psychedelic 2,5-dimethoxy-4-iodoamphetamine (DOI) in *C. elegans*

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ABSTRACT: Psychedelics show promise in treating depression, PTSD, and substance use disorder, prompting research into their mechanisms of action. Most studies use rodent models, but genetic tools can be challenging to apply. Invertebrate models, like *C. elegans*, offer a cost-effective alternative with short generation times and genetic tractability. This study examined the worm's response to the psychedelic 2,5-dimethoxy-4-iodoamphetamine (DOI) by assessing three serotonergic behaviors. DOI exposure did not affect locomotion speed or egg-laying but inhibited feeding. Interestingly, this effect was independent of serotonin receptors, suggesting DOI may act through alternative pathways. These findings indicate *C. elegans* can serve as a useful model for studying psychedelic drug effects, potentially revealing novel mechanisms beyond the serotonergic system. Further research could help clarify these pathways, improving our understanding of the therapeutic potential of psychedelics and refining their efficacy in treating neuropsychiatric disorders.

KEYWORDS: nematode, locomotion, egg-laying, feeding, serotonin

■ INTRODUCTION

Growing preclinical (Catlow *et al.*, 2013; Cameron *et al.*, 2018; de la Fuente Revenga *et al.*, 2021; Hesselgrave *et al.*, 2021) and clinical evidence (Carhart-Harris *et al.*, 2016, 2018; Davis *et al.*, 2021) suggest that psychedelics are effective in treating conditions such as depression, post-traumatic stress disorder, and substance use disorder (Vollenweider and Kometer, 2010; Carhart-Harris and Goodwin, 2017; Kyzar *et al.*, 2017). Nevertheless, the mechanism of action of psychedelic drugs remains unclear.

A vigorous effort is underway to develop alternative psychedelics with enhanced efficacy (Cameron *et al.*, 2021; Lewis *et al.*, 2023; Duan *et al.*, 2024). At present, this effort is proceeding with a limited understanding of the genetic and biochemical signaling pathways activated by psychedelics. Most of this research utilizes *in vivo* and *in vitro* approaches in rodent models (Olson, 2022). Pharmacological probes, which activate or antagonize candidate signaling molecules, play a dominant role. Because of the selectivity concerns regarding pharmacological probes, genetic approaches have also proven to be valuable, especially gene knockouts. However, deploying genetic tools in rodents can be challenging because of high costs, long generation times, and the relative difficulty of epistasis analysis (using double mutants to order genes into pathways). A complementary approach is to use genetically tractable invertebrate models, which are inexpensive, have short generation times, and are amenable to epistasis analysis. Several key genetic and biochemical signaling pathways were first discovered in *Drosophila melanogaster* and *C. elegans* before being recognized as fundamental to human biology including the Notch (Artavanis-Tsakonas, 1988), programmed cell death (Horvitz, Shaham and Hengartner, 1994), RNA interference (Hunter, 1999), and Hedgehog pathways (Parisi and Lin, 1998). A small number of studies have examined the effects of psychedelics on behavior in *Drosophila* and *C. elegans* (Kastritsis and Jacob-Stocker, 1971; Nichols *et al.*, 2002; Hibicke and Nichols, 2022; Ornelas *et al.*, 2024).

C. elegans is an advantageous model for studying the effects of psychedelics on the nervous system. *C. elegans* has a compact nervous system of only 302 neurons, which has been fully mapped. Its neurons utilize essentially all of the signaling molecules forming the neurochemical backbone of the mammalian brain (Hobert, 2013): the neurotransmitters acetylcholine, GABA, glutamate, serotonin (5-HT), dopamine, octopamine (similar to norepinephrine (Mills *et al.*, 2016)), and many neuropeptides (including homologs of oxytocin, vasopressin, and opioids (Garrison *et al.*, 2012; Cheong *et al.*, 2015)). Classic psychedelics are serotonin receptor agonists. *C. elegans* has six 5-HTR genes which encode orthologs of five of the seven serotonin receptor gene families in humans. Four of the six *C. elegans* receptors are GPCRs (*nematode*/HUMAN: *ser-1*/HTR2, *ser-4*/HTR1 & HTR5, *ser-5*/HTR6, *ser-7*/HTR7). The remaining two receptors (*mod-1*, *lgc-50*) are ligand-gated ion channels genetically unrelated to HTR3, the sole ligand-gated serotonin receptors in mammals.

The first step in developing a new model for studying the mode of action of psychedelics is to identify behavioral phenotypes induced by the drug. In this study, we examined the effect of the phenylalkylamine psychedelic 2,5-dimethoxy-4-iodoamphetamine (DOI), a serotonin receptor

agonist, on locomotion, egg-laying, and feeding rate, the three main serotonergic behaviors in *C. elegans*. We tested the hypothesis that DOI acts like serotonin in regulating these behaviors. Toxicity testing showed that DOI was non-toxic up to 3.0 mM. DOI exposure had no effect on locomotion speed or number of eggs laid per worm. In contrast, DOI inhibited feeding in a manner independent of serotonin receptors. We conclude that DOI affects one of three serotonergic behaviors in *C. elegans* and may act on pathways that do not include serotonin receptors.

■ RESULTS AND DISCUSSION

DOI Is Non-Toxic at Low to Moderate Concentrations. We assessed DOI toxicity using a worm survival assay. The worm's body is encased in a thick, collagenous cuticle that acts as a significant diffusion barrier. Accordingly, drugs are often applied at millimolar concentrations. Young adult worms were exposed to DOI in M9 buffer (see Materials and Methods) in the absence of food at various concentrations (in mM: 0.1, 0.3, 1.0, 3.0, 10.0) for 30 minutes; control worms were exposed to vehicle only. After exposure, worms were removed from the solution, placed on a standard culture plate with food, then examined for survival 24 hours later. Spontaneously crawling worms or those that responded to a gentle head touch were considered viable. We found a significant overall effect of DOI concentration on survival (Fig. 1; Table 1, row 1). Post hoc testing showed that survival was indistinguishable from the control group over the range of 0.1 to 3.0 mM; however, at 10.0 mM, survival was significantly reduced (Table 1, row 2). We conclude that DOI is not overtly toxic at concentrations $\leq$ 3.0 mM.

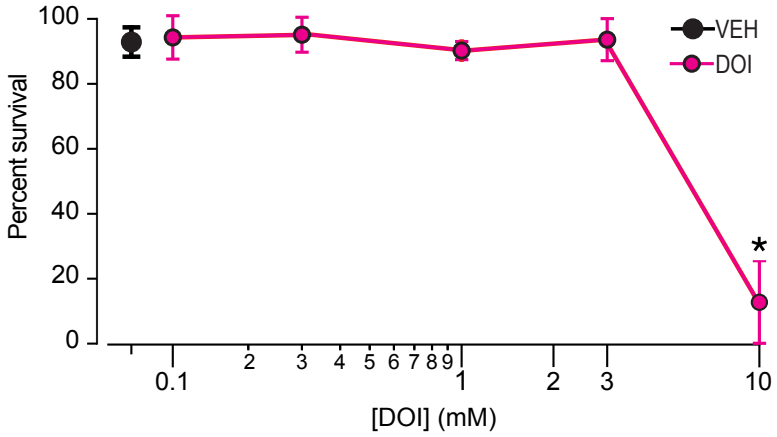


Fig. 1. Worm-survival assay. Mean percent survival 24 hours after exposure to DOI is plotted against DOI concentration. *, significantly different from VEH. Error bars, ±95% CI.

Serotonin suppresses locomotion while stimulating egg-laying and pharyngeal pumping in *C. elegans* (Chase and Koelle, 2007). As DOI is a serotonin receptor agonist, we hypothesized that it would mimic the effects of serotonin on these behaviors.

DOI Does Not Alter Locomotion Speed. Exogenous serotonin (Horvitz et al., 1982; Sawin et al., 2000; Ségalat et al., 1995), or endogenous serotonin released by optogenetic stimulation of

serotonergic NSM neurons (Dag et al., 2023), reduces locomotion speed. To test whether DOI has a similar effect, we exposed young adult worms to M9 solutions containing DOI at various concentrations within the non-toxic range (in mM: 0.1, 0.3, 1.0, 3.0) for 30 minutes; control worms were exposed to vehicle only. After exposure, worms were tracked as they crawled on foodless culture plates. Statistical analysis revealed no effect of DOI dosage on speed (absolute value of instantaneous velocity) (Fig. 2; Table 1, row 3). We conclude that DOI has no effect on locomotion speed. In related work, LSD reduces locomotion speed in worms (Ornelas *et al.*, 2024).

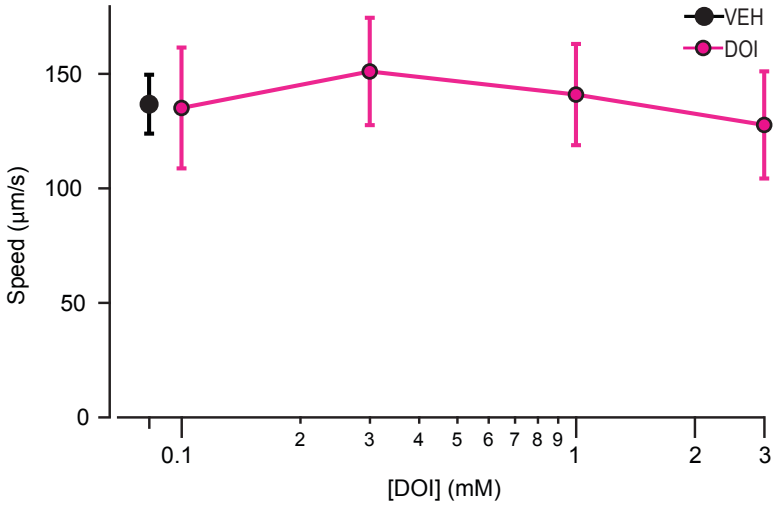


Fig. 2. Effect of DOI on locomotion speed. Mean speed is plotted against DOI concentration. Error bars, ±95% CI.

DOI Does Not Affect Egg-laying. Exogenous serotonin stimulates egg-laying in *C. elegans* (Horvitz et al., 1982; Mendel et al., 1995; Waggoner et al., 1998). To test whether DOI has a

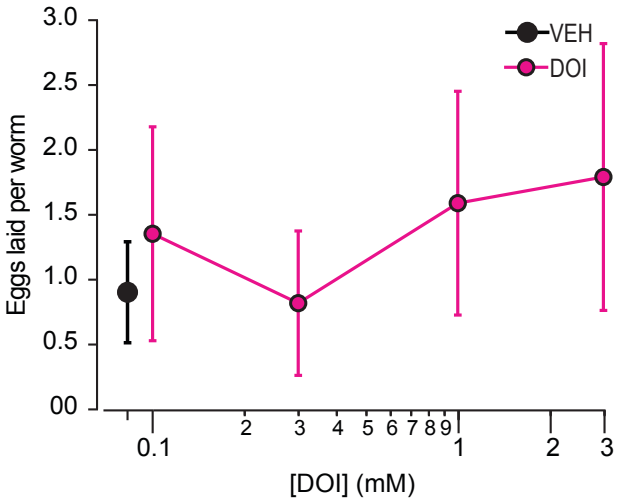


Fig. 3. Effect of DOI on egg laying. Mean number of eggs laid per worm is plotted against DOI concentration. Error bars, ±95% CI.

similar effect, we exposed young adult worms to M9 solutions containing DOI at various concentrations (in mM: 0.1, 0.3, 1.0, 3.0) for 30 minutes; control worms were exposed to vehicle only. We then pipetted the solution onto a foodless culture plate and counted the number of worms as well as the number of eggs laid during exposure. A positive control using serotonin at 1 mM showed a significant increase in egg-laying, indicating that the assay was functional (Supplemental Fig. 1; Table 1, row 4). Statistical analysis revealed no effect of DOI dosage on the number of eggs laid per worm (Fig. 3; Table 1, row 5). We conclude that DOI has no effect on egg-laying. LSD has no effect on egg-laying in *Drosophila* (Kastritsis and Jacob-Stocker, 1971).

DOI Suppresses Feeding. Worms ingest bacterial food through rhythmic contractions of the pharynx, a tube-shaped muscular organ that delivers bacterial particles to the gut. Each pharyngeal contraction is called a pump. Pumping can be activated or accelerated by exogenous serotonin (Horvitz et al., 1982; Niacaris & Avery, 2003; Rogers et al., 2001; Ségalat et al., 1995). Pumping rate is an increasing function of serotonin concentration (Weeks *et al.*, 2018). To test whether DOI has a similar effect on pumping, we exposed young adult worms to M9 solutions containing DOI at various concentrations (in mM: 0.1, 0.3, 1.0, 3.0) for 30 minutes; control worms were exposed to vehicle only. Bacterial food was added to the exposure solution to stimulate pumping. After exposure, worms, along with their exposure solution, were injected into a microfluidic device fitted with electrodes that recorded the electrical signals associated with each pharyngeal contraction (Lockery *et al.*, 2012). Such recordings are called electropharyngeograms (EPGs). At doses exceeding 0.1 mM, DOI suppressed pharyngeal pumping in a dose-dependent manner (Fig. 4A; Table 1, row 6). Thus, the effect of DOI on pharyngeal pumping is opposite to that of serotonin. This result is reminiscent of DOI-induced hypophagia in rodents (Aulakh *et al.*, 1995).

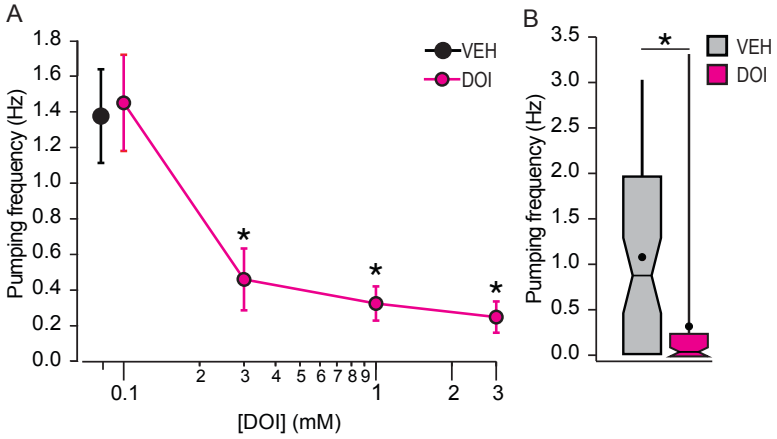


Fig. 4. A. Effect of DOI on feeding rate. Pharyngeal pumping rate is plotted against DOI concentration. *, significantly different from vehicle. Error bars, ±95% CI. B. Effect of 1 mM DOI on feeding rate in a mutant that lacks all chemosensory neuron function.

It is possible that DOI suppresses pumping because it has an aversive taste or smell. For example, the bitter substance quinine suppresses pumping (Li *et al.*, 2012). Accordingly, we

tested the effect of DOI on a mutant strain in which the function of all taste and olfactory neurons was eliminated by a mutation that prevents the formation of chemosensory cilia (Senti *et al.*, 2009). At a DOI concentration of 1 mM, pumping was strongly suppressed in the mutant relative to vehicle-only controls (Fig. 4B; Table 1, row 7). This result indicates that pumping inhibition is not a response to possible aversive chemosensory properties of DOI solutions.

Pumping suppression is part of the normal regulation of pharyngeal activity in which excitatory and inhibitory pathways converge on the pharynx and are likely active simultaneously (Li *et al.*, 2012; Dallière *et al.*, 2016). Excitatory pathways facilitate pumping in response to bacteria or odors that predict them (Li *et al.*, 2012; Liu *et al.*, 2019), whereas inhibitory pathways suppress pumping in response to the absence of bacteria or the presence of aversive compounds such as quinine (Li *et al.*, 2012; Dallière *et al.*, 2016). Components of several inhibitory pathways have been identified. The biogenic amines octopamine and tyramine suppress serotonin-stimulated pumping when applied exogenously (Packham, Walker and Holden-Dye, 2010). These amines are generally considered to be the invertebrate counterparts of the mammalian adrenergic system (Branicky and Schafer, 2009). DOI is polypharmacological, capable of binding to multiple receptor types in addition to serotonin receptors (Ray, 2010). Receptors for octopamine–SER-3, SER-6, and OCTR-1 (Sternberg *et al.*, 2024)–and for tyramine–SER-2 and TYRA-2 (Sternberg *et al.*, 2024)–are thus potential DOI targets. A variety of peptides suppress pumping in dissected pharynges (Rogers *et al.*, 2001; Papaioannou *et al.*, 2005), making their receptors potential targets as well.

Another class of psychoactive drugs, antipsychotics, can suppress pumping in *C. elegans* (Donohoe *et al.*, 2009; Saur *et al.*, 2013). Examples include trifluoperazine, fluphenazine, clozapine, and olanzapine. In the case of clozapine, pumping suppression is reduced in knockouts of the nicotinic acetylcholine receptor ACR-7, which is expressed on pharyngeal muscles. Furthermore, the effects of *acr-7* knockouts can be mimicked by acetylcholine receptor antagonists (Saur *et al.*, 2013). Therefore, clozapine is believed to silence the pharynx through direct activation of ACR-7, causing tetanic contraction of pharyngeal muscles similar to that observed upon bath application of nicotine (Raizen, Lee and Avery, 1995). Thus, an additional mode of action of DOI could be overstimulation of excitatory pathways.

Feeding Suppression Is Intact in Serotonin Receptor Mutants. In mammals, DOI has high affinity for serotonin receptors. To date, DOI is known to bind to two serotonin receptors in *C. elegans*, SER-1 and SER-4, with binding constants similar to that of serotonin (Olde and McCombie, 1997; Hamdan *et al.*, 1999). Although serotonin receptors are associated with the stimulation of pumping, they could, in principle, suppress pumping by excitation block (Saur *et al.*, 2013), like ACR-7. Therefore, we asked whether any of the six *C. elegans* serotonin receptor genes were necessary for DOI-induced suppression of pharyngeal pumping.

Knockout mutants for each serotonin receptor (SER-1, SER-4, SER-5, SER-7, MOD-1, LGC-50) were exposed to DOI and recorded as described above, now at a single DOI concentration (1 mM). There were significant main effects of strain and drug, the latter implying that suppression persisted in the mutants (Fig. 5; Table 1, row 8). Post hoc Brunner-Munzel tests comparing each

mutant to the N2 reference strain revealed main effects of drug but no drug × strain interactions, suggesting that the suppressive effect of DOI is similar in the mutants and reference strain (Table 1, rows 9–14). We conclude that serotonin receptor genes are not individually required for DOI-induced pumping suppression. This result rules out a model in which overstimulation of a single type of serotonin receptor by DOI is necessary and sufficient for pumping suppression. However, it does not exclude the possibility that overstimulation of multiple serotonin receptors is necessary and sufficient to suppress pumping. In related work, the receptors SER-1 and SER-4 have been shown to be required for LSD-induced locomotory slowing (Ornelas *et al.*, 2024).

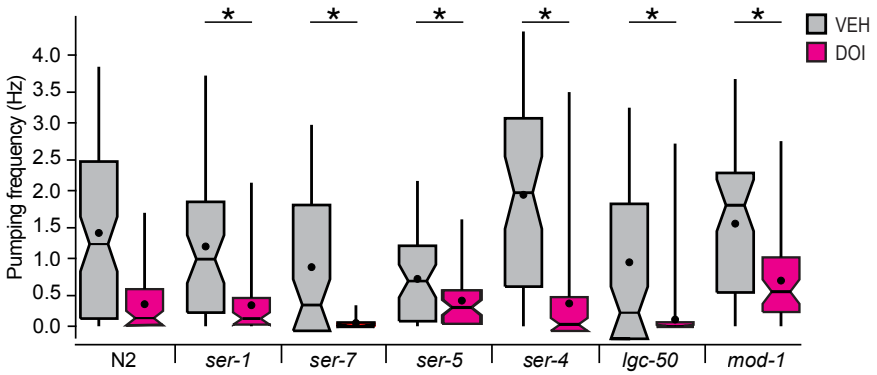


Fig. 5. Effect of DOI on feeding rate in serotonin receptor mutants. *, main effect of DOI in a post hoc two-way ANOVA against N2 with no interaction.

■ CONCLUSION

Our data contradict the hypothesis that DOI acts like serotonin in regulating serotonergic behaviors in *C. elegans.* Serotonin suppresses locomotion while stimulating egg-laying and pharyngeal pumping. In contrast, we detected no effect of DOI on locomotion or egg-laying but observed suppression of pharyngeal pumping. This outcome is surprising because all three behaviors are strongly regulated by serotonin receptors, which are likely targets of DOI.

To explain the effect on pharyngeal pumping, we propose a model in which DOI acts on one or more receptors involved in the worm's native pathways for pumping suppression. These pathways include receptors for octopamine, tyramine, and peptides. Peptide receptors are unlikely candidates because there are no known precedents for DOI binding to such receptors. In contrast, DOI does bind to adrenergic receptors (Ray, 2010), and the worm homologs of these receptors include the octopamine receptors SER-3, SER-6, and OCTR-1. Thus, DOI may activate octopamine receptors to suppress pumping.

However, because octopamine regulates locomotion and egg-laying as well as pharyngeal pumping (Horvitz *et al.*, 1982), the model must account for the fact that, whereas DOI strongly suppressed pumping, we detected no effects on locomotion or egg-laying. Octopamine's effect on locomotion is to activate a "roaming" state, characterized by extended bouts of relatively

fast forward movement (Fujiwara, Sengupta and McIntire, 2002; Ben Arous, Laffont and Chatenay, 2009; Flavell *et al.*, 2013). It is possible that we failed to detect DOI-induced roaming because, on the foodless substrate used in our assay, the worms were already in a roaming state. Octopamine also suppresses egg-laying (Horvitz *et al.*, 1982), which we may not have observed because the baseline egg-laying rate was already low under our assay conditions. Finally, the model requires that if DOI activates serotonin receptors in the worm, these effects are small compared to its activation of octopamine receptors. This model could be tested by examining DOI's effects on serotonergic behaviors in octopamine-receptor mutants.

■ MATERIALS AND METHODS

Cultivation and Strains. Nematodes were grown at 20 °C on nematode growth medium plates (NGM; 51.3 mM NaCl, 1.7% agar, 0.25% peptone, 1 mM $CaCl_2$, 12.9 µM cholesterol, 1 mM $MgSO_4$, 25 mM KPO_4, pH 6) seeded with 200 µL of a saturated *E. coli* (OP50) liquid culture. All experiments were performed on young adult hermaphrodites synchronized by allowing 10-20 gravid hermaphrodites to lay eggs for six to eight hours. Worms were washed and transferred in M9 buffer (3 g KH_2PO_4, 6 g Na_2HPO_4, 5 g NaCl, 1 ml 1 M $MgSO_4$, H_2O to 1 liter). Strains used in this study are given in the table below.

Identifier	Genotype	Source	Figures
Wild type	N2 Bristol	CGC	All
XL344	*daf-19(m86);daf-12(sa204);ntIs[daf-19a::daf-19a;unc-122::dsred]*	This paper	4B
DA1814	*ser-1(ok345)*	CGC	5
AQ866	*ser-4(ok512)*	CGC	5
VV212	*ser-5(vq1)*	CGC	5
DA2100	*ser-7(tm1325)*	Pierce Lab, Univ. Texas, Austin	5
MT9558	*mod-1(ok103)*	CGC	5
AQ4347	*lgc-50(tm3712)*	Schafer Lab, Univ. Cambridge	5

DOI Exposure. 2,5-dimethoxy-4-iodoamphetamine (DOI) hydrochloride (Sigma-Aldrich, D101) solutions were prepared by dissolving DOI in M9. In all assays (toxicity, locomotion, egg-laying, and pharyngeal pumping) 10–15 young adult worms were transferred by picking from a culture plate to a tube containing 50 µL of DOI (0.1, 0.3, 1, 3, or 10 mM) or vehicle (M9 buffer) solution for 30 minutes before behavioral testing.

Toxicity Assay. Following exposure, worms were transferred in liquid to an NGM plate with food. Viability was assessed after 24 hours. Worms were considered to have survived if they

displayed spontaneous locomotion and/or exhibited reversals or head bends in response to gentle nose tap with a platinum wire pick.

Locomotion Assay. Following exposure, approximately 10-15 worms were transferred in liquid to the inside of a 14 mm (I.D.) copper ring placed on an unseeded NGM plate. After 5 minutes of acclimation, worms were imaged at 23.4 µm/pixel using a macro lens (AF Micro-Nikkor 60 mm f/2.8D, Nikon, Japan) and behavior was video recorded for 10 minutes using a microscope camera (HDMI 1080P HD212, AmScope, Irvine, CA) at 30 fps. Movies were down-sampled to 5 fps for analysis. Individual worms were tracked using WormLab (MBF Bioscience, Williston, VT). Tracks were smoothed by Kalman filtering. Average velocity values for each track were computed using custom code written in Igor Pro (Wavemetrics, Lake Oswego, OR).

Egg-Laying Assay. Following exposure, the solution containing worms and eggs was transferred to an unseeded NGM plate. Worms and eggs were counted after the solution was fully absorbed into the medium.

Pharyngeal Pumping Assay. Voltage transients associated with pharyngeal pumping were recorded using a ScreenChip microfluidic system (InVivo Biosystems, Eugene, OR, USA) as described previously (Lockery *et al.*, 2012; Levichev *et al.*, 2023). Worms were incubated for 30 minutes in an M9 suspension of bacterial food (OP50; OD_{600} 2.5) that contained 1 mM DOI or vehicle only. After incubation, worms were loaded into the reservoir of a microfluidic device which was filled with the incubation solution. Individual worms were then sequentially transferred from the reservoir to the recording channel. Worms were acclimated to the channel for one to two minutes before pharyngeal pumping was recorded for five minutes. All recordings were performed within 90 minutes after loading animals. Mean pumping frequency was extracted using custom code written in Igor Pro (Wavemetrics, Lake Oswego, OR, USA).

Statistical Analysis. G*Power Version 3.1.9.6 (Faul *et al.*, 2007) was used to determine the minimum sample size for each experiment, using pilot experiments as a guide. Statistical analyses were performed in R version 4.4.2 (Urbanek *et al.*, 2025). One-way ANOVAs were used to assess the effect of drug concentration on toxicity, locomotion, and egg-laying. As pumping frequencies were not normally distributed, significance in feeding assays was assessed via nonparametric tests. Mann-Whitney tests, corrected for multiple comparisons via the Bonferroni method, were used to assess the effect of drug concentration on pumping frequency. Brunner-Munzel tests (implemented in the R package RankFD) were used to evaluate the role of serotonin receptor mutations in the effect of DOI on pumping frequency. Brunner-Munzel tests (Konietschke *et al.*, 2023), Bonferroni-corrected for multiple comparisons, were also used as post-hoc tests to evaluate the effects of individual mutations. In box-and-whisker plots, the box represents 1st and 3rd quartiles, the notch represents the median, the dot represents the mean, and the whiskers indicate the range of data.

Fig.	Row	Comparison	Test	Number of replicates	Mean ± 95% CI	Statistic	p-value	Signif.	Method	Effect size
1	1	DOI effect on survival in N2: VEH vs [DOI]	ANOVA	VEH, *n* = 15	92.89 ± 4.94					
				DOI 0.1 mM, *n* = 3	94.32 ± 14.64					
				DOI 0.3 mM, *n* = 3	95.16 ± 11.83					
				DOI 1 mM, *n* = 3	90.24 ± 6.14					
				DOI 3 mM, *n* = 3	93.63 ± 14.24					
				DOI 10 mM, *n* = 3	12.74 ± 27.74					
			Main effect of DOI Dose			*F*(5,24) = 53.88	0.000	***		
	Post-hoc tests									
	2	[DOI] 0 vs 0.1 mM	Dunnett's test			*q* = -0.28	0.971			
		[DOI] 0 vs 0.3 mM				*q* = -0.45	0.983			
		[DOI] 0 vs 1 mM				*q* = 0.52	0.768			
		[DOI] 0 vs 3 mM				*q* = -0.15	0.955			
		[DOI] 0 vs 10 mM				*q* = 15.74	0.000	***	Cohen's d	-7.93
2	3	DOI effect on crawling absolute velocity in N2, VEH vs [DOI]	ANOVA	VEH, *n* = 31	136.80 ± 12.85					
				DOI 0.1 mM, *n* = 8	135.12 ± 26.38					
				DOI 0.3 mM, *n* = 8	151.05 ± 23.43					
				DOI 1 mM, *n* = 8	140.99 ± 22.09					
				DOI 3 mM, *n* = 8	127.71 ± 23.40					
			Main effect of DOI Dose			*F*(4,58) = 0.58	0.678			
Supp. Fig. 1	4	Effect of 5-HT on egg-laying: VEH vs 5-HT	t-test	VEH, *n* = 8, 5-HT, *n* = 8	VEH: 0.53 ± 0.70 5-HT: 3.39 ± 1.21	*t*(11.20) =-4.81	0.001	***	Cohen's d	2.4
3	5	DOI effect on egg laying in N2: VEH vs [DOI]	ANOVA	VEH, *n* = 8	0.90 ± 0.40					
				DOI 0.1 mM, *n* = 8	1.35 ± 0.93					
				DOI 0.3 mM, *n* = 8	0.82 ± 0.63					
				DOI 1 mM, *n* = 8	1.59 ± 0.97					
				DOI 3 mM, *n* = 8	1.79 ± 1.16					
			Main effect of DOI Dose			*F*(4,75) = 1.51	0.208			

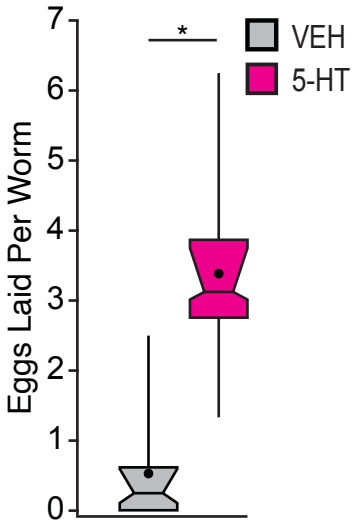
Fig.	Row	Comparison	Test	Number of replicates	Mean ± 95% CI	Statistic	p-value	Signif.	Effect	Effect
4A	6	DOI effect on pumping frequency in N2: VEH vs [DOI]	Wilcoxon rank sum test	VEH, *n* = 84, DOI 0.1 mM, *n* = 65	VEH: 1.38 ± 0.26 DOI: 1.45 ± 0.27	*z* = -0.39	0.653			
				VEH, *n* = 84, DOI 0.3 mM, *n* = 65	VEH: 1.38 ± 0.26 DOI: 0.46 ± 0.17	*z* = 4.99	0.000	***/†††	rank-serial	-0.48
				VEH, *n* = 84, DOI 1 mM, *n* = 65	VEH: 1.38 ± 0.26 DOI: 0.33 ± 0.10	*z* = 4.84	0.000	***/†††	rank-serial	-0.46
				VEH, *n* = 84, DOI 3 mM, *n* = 65	VEH: 1.38 ± 0.26 DOI: 0.25 ± 0.09	*z* = 5.56	0.000	***/†††	rank-serial	-0.53
4B	7	DOI effect on pumping frequency in a chemosensory-blind strain (XL344): VEH vs DOI	Wilcoxon rank sum test	XL344, VEH, *n* = 55	1.08 ± 0.29	*z* = 3.12	0.002	**	rank-serial	-0.35
				XL344, DOI 1 mM, *n* = 55	0.32 ± 0.18					
5	8	DOI effect on pumping frequency in serotonin receptor mutants: N2 vs mutants	Brunner-Munzel Test ANOVA-type statistics (rankFD, Konietschke & Brunner, 2023)	N2, VEH, *n* = 84	1.38 ± 0.26					
				N2, DOI 1 mM, *n* = 65	0.33 ± 0.10					
				ser1(ok345), VEH, *n* = 55	1.18 ± 0.29					
				ser1(ok345), DOI 1 mM, *n* = 55	0.31 ± 0.12					
				ser-7(tm1325), VEH, *n* = 55	0.87 ± 0.25					
				ser-7(tm1325), DOI 1 mM, *n* = 55	0.05 ± 0.02					
				ser-5(vq1), VEH, *n* = 55	1.94 ± 0.38					
				ser-5(vq1), DOI 1 mM, *n* = 55	0.34 ± 0.17					
				ser-4(ok512), VEH, *n* = 55	0.70 ± 0.17					
				ser-4(ok512), DOI 1 mM, *n* = 55	0.38 ± 0.11					
				lgc-50(tm3712), VEH, *n* = 55	0.94 ± 0.29					
				lgc-50(tm3712), DOI 1 mM, *n* = 55	0.10 ± 0.10					
				mod-1(ok103), VEH, *n* = 55	1.51 ± 0.29					
				mod-1(ok103), DOI 1 mM, *n* = 55	0.67 ± 0.16					
			Main effect of Strain			*F*(5.86,665.25) = 15.63	0.000	***		
			Main effect of DOI			*F*(1,665.25) = 181.04	0.000	***		
			Interaction, Strain × DOI			*F*(5.86,665.25) = 3.89	0.000	***		

	Post-hoc tests									
	9	DOI effect on pumping frequency: N2 vs ser1(ok345) ± DOI 1 mM	Brunner-Munzel Test ANOVA-type statistics (rankFD, Konietschke & Brunner, 2023)	N2, VEH, *n* = 84	1.38 ± 0.26					
				N2, DOI 1 mM, *n* = 65	0.33 ± 0.10					
				ser1(ok345), VEH, *n* = 55	1.18 ± 0.29					
				ser1(ok345), DOI 1 mM, *n* = 55	0.31 ± 0.12					
			Main effect of Strain			*F*(1,234.96) = 0.34	0.561			
			Main effect of DOI			*F*(1,234.96) = 57.84	0.000	***/†††	Cliff's Δ	-0.49
			Interaction, Strain × DOI			*F*(1,234.96) = 0.12	0.728			
	10	DOI effect on pumping frequency: N2 vs ser-7(tm1325) ± DOI 1 mM	Brunner-Munzel Test ANOVA-type statistics (rankFD, Konietschke & Brunner, 2023)	N2, VEH, *n* = 84	1.38 ± 0.26					
				N2, DOI 1 mM, *n* = 65	0.33 ± 0.10					
				ser-7(tm1325), VEH, *n* = 55	0.87 ± 0.25					
				ser-7(tm1325), DOI 1 mM, *n* = 55	0.05 ± 0.02					
			Main effect of Strain			*F*(1,200.77) = 26.48	0.000	***/†††	Cliff's Δ	-0.34
			Main effect of DOI			*F*(1,200.77) = 55.24	0.000	***/†††	Cliff's Δ	-0.48
			Interaction, Strain × DOI			*F*(1,200.77) = 1.34	0.249			
	11	DOI effect on pumping frequency: N2 vs ser-5(vq1) ± DOI 1 mM	Brunner-Munzel Test ANOVA-type statistics (rankFD, Konietschke & Brunner, 2023)	N2, VEH, *n* = 84	1.38 ± 0.26					
				N2, DOI 1 mM, *n* = 65	0.33 ± 0.10					
				ser-5(vq1), VEH, *n* = 55	1.94 ± 0.38					
				ser-5(vq1), DOI 1 mM, *n* = 55	0.34 ± 0.17					
			Main effect of Strain			*F*(1,208.87) = 0.15	0.702			
			Main effect of DOI			*F*(1,208.87) = 82.36	0.000	***/†††	Cliff's Δ	-0.55
			Interaction, Strain × DOI			*F*(1,208.87) = 5.98	0.015	**		
	12	DOI effect on pumping frequency: N2 vs ser-4(ok512) ± DOI 1 mM	Brunner-Munzel Test ANOVA-type statistics (rankFD, Konietschke & Brunner, 2023)	N2, VEH, *n* = 84	1.38 ± 0.26					
				N2, DOI 1 mM, *n* = 65	0.33 ± 0.10					
				ser-4(ok512), VEH, *n* = 55	0.70 ± 0.17					
				ser-4(ok512), DOI 1 mM, *n* = 55	0.38 ± 0.11					
			Main effect of Strain			*F*(1,239.45) = 1.35	0.246			
			Main effect of DOI			*F*(1,239.45) = 32.36	0.000	***/†††	Cliff's Δ	-0.4
			Interaction, Strain × DOI			*F*(1,239.45) = 3.64	0.058			
	13	DOI effect on pumping frequency: N2 vs lgc-50(tm3712) ± DOI 1 mM	Brunner-Munzel Test ANOVA-type statistics (rankFD, Konietschke & Brunner, 2023)	N2, VEH, *n* = 84	1.38 ± 0.26					
				N2, DOI 1 mM, *n* = 65	0.33 ± 0.10					
				lgc-50(tm3712), VEH, *n* = 55	0.94 ± 0.29					
				lgc-50(tm3712), DOI 1 mM, *n* =	0.10 ± 0.10					
			Main effect of Strain			*F*(1,194.10) = 24.34	0.000	***/†††	Cliff's Δ	-0.34
			Main effect of DOI			*F*(1,194.10) = 47.62	0.000	***/†††	Cliff's Δ	-0.46
			Interaction, Strain × DOI			*F*(1,194.10) = 0.94	0.334			
	14	DOI effect on pumping frequency: N2 vs mod-1(ok103) ± DOI 1 mM	Brunner-Munzel Test ANOVA-type statistics (rankFD, Konietschke & Brunner, 2023)	N2, VEH, *n* = 84	1.38 ± 0.26					
				N2, DOI 1 mM, *n* = 65	0.33 ± 0.10					
				mod-1(ok103), VEH, *n* = 55	1.51 ± 0.29					
				mod-1(ok103), DOI 1 mM, *n* = 55	0.67 ± 0.16					
			Main effect of Strain			*F*(1,217.06) = 9.11	0.003	**/†	Cliff's Δ	0.16
			Main effect of DOI			*F*(1,217.06) = 47.14	0.000	***/†††	Cliff's Δ	-0.44
			Interaction, Strain × DOI			*F*(1,217.06) = 1.50				

Table 1. Statistical analysis of experimental data. Key: *, p<0.05; **, p<0.01; ***, p<0.001; †, p<0.05 after Bonferroni correction; ††, p<0.01 after Bonferroni correction; †††, p<0.001 after Bonferroni correction.

■ ASSOCIATED CONTENT

Supporting information



Supplemental Fig. 1. Effect of serotonin (5-HT) on egg laying. This experiment serves as a positive control for the experiment of Fig. 3.

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Author Contributions

A.M.W. performed toxicity assays, collected behavioral data, and did statistical analyses. A.D.B performed toxicity assays and collected behavioral data. S.F. performed feeding assays, did the statistical analysis, and generated the figures. S.R.L. directed the research and wrote the paper. A.M.W., A.D.B., and S.F. edited the paper. All authors approved the paper.

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Notes

S.R.L. declares a competing financial interest in InVivo Biosystems, Inc., which manufactures the ScreenChip system. Other authors have no competing interests to declare.

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