Acetylcholine Signaling Genes are Required for Cocaine-Stimulated Egg Laying in *Caenorhabditis elegans*

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Despite the toxicity and addictive liability associated with cocaine abuse, its mode of action is not completely understood, and effective pharmacotherapeutic interventions remain elusive. The cholinergic effects of cocaine on acetylcholine receptors, synthetic enzymes, and degradative enzymes have been the focus of relatively little empirical investigation. Due to its genetic tractability and anatomical simplicity, the egg laying circuit of the hermaphroditic nematode, *Caenorhabditis elegans*, is a powerful model system to precisely examine the genetic and molecular targets of cocaine *in vivo*. Here, we report a novel cocaine-induced phenotype in *Caenorhabditis elegans*, cocaine-stimulated egg laying. In addition, we present the results of an *in vivo* candidate screen of synthetic enzymes, receptors, degradative enzymes, and downstream components of the intracellular signaling cascades of the main neurotransmitter systems that control *Caenorhabditis elegans* egg laying. Our results show that cocaine-stimulated egg laying is dependent on acetylcholine synthesis and synaptic release, functional nicotinic acetylcholine receptors, and the *Caenorhabditis elegans* acetylcholinesterases. Further, we show that cocaine-stimulated egg laying is not dependent on other neurotransmitters besides acetylcholine, including serotonin, dopamine, octopamine, and tyramine. Finally, our data show that cocaine-stimulated egg laying is increased in mutants for the *C. elegans* serotonin reuptake transporter as well as mutants for a 5-HT-gated chloride channel likely expressed in the locomotion circuit. Together, these results highlight serotonergic inhibition of egg laying behavior, functional connectivity between the egg laying and locomotion circuits in *Caenorhabditis elegans*, and possible discrete cholinergic and serotonergic effects of cocaine in the egg laying and locomotion circuits, respectively.

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INTRODUCTION

Cocaine abuse is estimated to account for 900,000 cases of substance use disorder (1) and over 14,000 overdose deaths in the U.S. each year (2). Despite the impact of cocaine abuse on public health, there are no FDA-approved pharmacotherapeutic interventions for either the addictive liability or toxicity associated with cocaine abuse (3). A major impediment to the development of effective pharmacological interventions is the non-specificity of the drug, as pharmacodynamic interventions designed to block the canonical monoamine neurotransmitters implicated in the mode of action of cocaine dopamine (DA), serotonin (5-HT), and norepinephrine (4) have demonstrated limited efficacy in clinical trials (5). Elucidation of the full range of neurotransmitter systems and molecular effectors involved in the mode of action of cocaine could inform the development of novel pharmacotherapies.

A growing body of preclinical research demonstrates an important role for acetylcholine (ACh) in the pathology of cocaine use disorder. Cholinergic signaling influences multiple processes underlying reward and dependence in the mammalian brain, including learning and memory (6), attention (7), and motivation and reward (8). Pharmacological manipulation of cholinergic signaling alters responses in behavioral assays of cocaine reward such as self-administration (9,10), drug reinstatement (11), and conditioned place preference (12). To date, the vast majority of empirical examination of the effects of cocaine on ACh signaling has focused on the changes it induces in dopaminergic efflux (13); however, the direct impact of the drug on the molecular components of cholinergic signaling, including ACh synthetic enzymes, subtypes of nicotinic ACh receptors (nAChRs) and muscarinic ACh receptors

(mAChRs), and ACh degradative enzymes have been the focus of less empirical investigation, necessitating further examination of the direct effects of the drug on the cholinergic signaling.

The *Caenorhabditis elegans* egg laying circuit has served as a tractable genetic and molecular model for the mechanistic underpinnings of various neurotransmitters, including ACh (14) and biogenic amines, 5-HT, DA, octopamine (Oct), and tyramine (Tyr) (15). The circuit consists of two hermaphrodite-specific neurons (HSNs) (16) and six ventral type C neurons (VCs) (17), which innervate the 16 egg laying muscles (18).

ACh plays a crucial and complex role in regulating the egg laying behavior of *C. elegans* (19). The VCs, which are the primary cholinergic neurons in the egg laying circuit, innervate both the vulval muscles and the HSNs (17). Through discrete mechanisms, ACh chronically inhibits and acutely stimulates egg laying behavior. Binding of ACh to nAChRs on the vulval muscles induces their contraction and triggers egg laying (20,21) whereas binding of mAChRs on the HSNs by ACh reduces the release of 5-HT from these neurons, and thereby reduces egg laying (22).

Here we characterize a novel cocaine-induced behavior in *C. elegans*, cocaine-stimulated egg laying, and present pharmacological and genetic evidence for an ACh, nAChR, and (acetylcholinesterase) AChE-dependent mechanism of cocaine-stimulated *C. elegans* egg laying. Crucially, we show that the excitatory effect of cocaine on egg laying in *C. elegans* depends on genes involved in cholinergic neurotransmission, but not on genes involved in the other neurotransmitter systems within the egg laying circuit, including 5-HT, Oct, Tyr, and DA. In addition, we show that cocaine-stimulated egg laying is enhanced in mutants for the *C. elegans* SERT (*mod-5*) and a 5-HT-gated

chloride channel (*mod-1*), supporting a previously published report on the effect cocaine on *C. elegans* locomotion (23) and highlighting the connections between ACh, locomotion, and egg laying in *C. elegans*.

RESULTS

ACh is the only neurotransmitter required for the stimulation of egg laying by cocaine in hypertonic medium

In an effort to add to the current cocaine abuse models available in *C. elegans* (23–25), we set out to observe any strong phenotypes that may result from acute exposure of WT *C. elegans* to cocaine. Our initial observations determined that WT worms cultured on plates containing cocaine overnight showed several behavioral differences compared to untreated controls, including a previously reported modulation of locomotion (23). Specifically, preliminary experiments showed a reduction in the number of eggs in the uterus of WT hermaphrodite animals on plates containing cocaine. These initial observations and the amenability of *C. elegans* egg laying to pharmacological and genetic analysis led us to investigate the effect of cocaine on *C. elegans* egg laying behavior as a model for the genetic and molecular mediators of cocaine action using a quantitative approach.

We next quantified any differences in egg laying in *C. elegans* treated with cocaine by assaying induction of egg laying in liquid hypertonic medium, a well-established method previously used to investigate the stimulation of egg laying by 5-HT and pharmacological agents (16,26,27).

We show that treatment of WT *C. elegans* with cocaine significantly stimulates egg laying in hypertonic medium (6.334±2.193) compared to parallel equimolar sucrose control (0.391±0.600) ($p < 0.0001$, N = 670). These data confirm our initial observation and identify a robust assay for the genetic analysis of cocaine action in worms, cocaine-dependent egg laying.

We next used our newly described behavioral output to identify the main neurotransmitter systems involved in the egg laying response to cocaine. We screened the egg laying response of individual mutants lacking synthetic enzymes for each of the neurotransmitters previously implicated in egg laying (28). The genotypes tested include *tph-1*(*mg280*) (which encodes tryptophan hydroxylase required for 5-HT synthesis) (29), *tdc-1*(*n3419*) (which encodes tyrosine decarboxylase required for synthesis of Tyr) (30), *tbh-1*(*n3247*) (which encodes Tyr beta-hydroxylase required for synthesis of Oct) (15,27,30), *bas-1*(*ad446*) (which encodes an aromatic amino acid decarboxylase required for synthesis of 5-HT and DA) (15,31) and *cha-1*(*pp152*) (which encodes choline acetyltransferase necessary for ACh synthesis) (15,32–34).

We show that the choline acetyltransferase (*cha-1*) is required for cocaine-dependent egg laying (*cha-1*(*pp152*) mean = 0.500±0.332) *vs* (WT mean = 4.82±1.782) ($p = 0.0079$, N = 50) (**Fig. 1A**). Surprisingly, tryptophan hydroxylase (*tph-1*) is not required for cocaine-dependent stimulation of egg laying in hypertonic medium, suggesting that 5-HT, one of the main modulators of egg laying behavior, is not the primary target of cocaine in this model system (*tph-1*(*mg280*) mean = 5.200 ±1.811) *vs* (WT mean = 7.147±2.216) ($p = 0.0952$, N = 50)

Additionally, aromatic amino acid decarboxylase mutants (*bas-1*), tyrosine decarboxylase mutants (*tdc-1*), and Tyr beta-hydroxylase mutants (*tbh-1*) do not exhibit a significant difference in egg laying response to cocaine as compared to WT, suggesting that Tyr, DA, and Oct may be ruled out as main targets of cocaine in this circuit (**Fig. 1C-E**).

Cocaine-stimulated egg laying requires pre-synaptic Ach neurotransmission genes

Suppression of the egg laying response to cocaine in choline acetyltransferase mutants (*cha-1*) suggests that presynaptic cholinergic neurotransmission is necessary for the egg laying response to the drug. To further examine this possibility, we assayed the egg laying of mutants for the transmembrane transporter necessary for packaging of ACh within synaptic vesicles during cholinergic neurotransmission (*unc-17*) (35). We found that the egg laying response of transmembrane transporter mutants to cocaine (*unc-17*(*e245*)) (mean = 0.507±0.109) is suppressed compared to parallel WT control (mean = 5.380±1.293) (p = 0.0079, N = 50) (**Fig. 2A**). The $G\alpha_q$ ortholog *egl-30* is required for multiple neuronal functions, including ACh release from motor neurons (36). We show that the egg laying response to cocaine of $G\alpha_q$ mutants (*egl-30*(*ad806*)) (mean = 2.165±2.014) is suppressed compared to parallel WT control (mean = 5.933±0.778) (p = 0.0159, N = 50) (**Fig. 2B**). Taken together, these results suggest that presynaptic ACh is required for the increased egg laying response to cocaine.

Genes encoding nAChRs, but not mAChRs, are required for the egg laying response to cocaine

To further understand the effect of cocaine on cholinergic signaling in the *C. elegans* egg laying circuit, we tested the egg laying response to cocaine in nAChR and mAChR mutants (*unc-29* and *unc-38,* respectively, encode a beta and an alpha subunit of nAChRs expressed on the vulval muscles) (21,28). Consistent with our finding that cholinergic neurotransmission is required for the egg laying response to cocaine, we show that cocaine-dependent egg laying is significantly reduced in *unc-29* mutants affecting the beta subunit nAChR; (*unc-29 (e1072)*) (mean = 1.460±0.965) *vs* WT control (mean = 5.960±1.744), (p = 0.0079, N = 50) (**Fig. 3A**). Similarly, the egg laying response to cocaine of alpha subunit nAChR mutants (*unc-38*) is suppressed compared to parallel WT control; (*unc-38*(*e264*) (mean = 3.760±1.176) *vs* WT control (mean = 6.460±1.835) (p = 0.0238, N = 50) (**Fig. 3B**). These results suggest that nicotinic receptors expressed on the vulval muscle are required for molecular action of cocaine on egg laying behavior.

To determine if mAChRs are also required for molecular mechanism of cocaine-dependent egg laying, we assessed the response of mutants in the mAchR encoded by *gar-2* (22,28,37). We did not observe any difference in egg laying in *gar-2* mutants when compared to WT controls; (*gar-2*(*ok520*) mean = 3.860 ±0.581) *vs* (WT mean = 5.080±0.988) (N = 50) (**Fig. 3C**).

AChE-encoding genes are required for the egg laying response to cocaine

AChEs catalyze the hydrolysis of ACh after its binding to cholinergic receptors on the postsynaptic membrane (38). Biochemical analysis has revealed multiple genes encoding *C. elegans* AChEs: *ace-1*, *ace-2*, *ace-3*, and *ace-4* (39). The AChEs encoded by *ace-1* and *ace-2* are the major hydrolytic enzymes of ACh whereas the AChE encoded by *ace-3* accounts for a minor proportion of AChE activity and the AChE encoded by *ace-4* is transcribed but does not result in a catalytically active protein (19,39–42). In order to explore all aspects of the relationship between cocaine and cholinergic signaling in the egg laying response, we tested the egg laying response to cocaine in *ace-1;ace-2* and *ace-3;ace-4* double mutants. We found that *ace-1;ace-2* double mutants exhibit a suppressed egg laying response to cocaine (mean = 0.2000±0.187) compared to parallel WT control (mean = 5.2200±2.649) (p = 0.0079, N = 50) (**Fig. 4A**) whereas *ace-3;ace-4* double mutants exhibit an egg laying response to cocaine that does not differ significantly compared to parallel WT control (**Fig. 4B**).

Cocaine induces egg laying independently of 5-HT and of the HSNs

ACh inhibits egg laying through the mAChRs (*gar-2*) expressed on the HSNs by reducing release of 5-HT from these neurons (19,22,28,43). Our results show that cocaine-dependent egg laying is not affected by a mutation in the mAChR encoded by *gar-2* (**Fig. 3C***).* Additionally, HSN neurons are the main serotonergic neurons in the egg laying circuit, and our results suggest that serotonin is not required for the

stimulatory effects of cocaine on *C. elegans* egg laying behavior (**Fig. 1B-C**). Taken together, our data suggest that the mechanism may be independent of the HSN neurons and of the function of serotonin in the egg laying circuit.

To assess the role of HSN in cocaine-dependent egg laying, we quantified the egg laying response to cocaine in animals containing a semi-dominant mutation in the gene *egl-1* shown to trigger HSN cell death (44). We show that mutants lacking the serotonergic HSNs do not exhibit a significant difference in egg laying response to cocaine as compared to WT; (*egl-1*(*n487*) (mean = 7.225±1.394) *vs* WT control (mean = 9.633±4.356) (p = 0.0571, N = 40) (**Fig. 5A**). Our previous results suggest that the serotonergic HSN neurons are not required for cocaine-dependent egg laying but do not eliminate a post-synaptic mechanism requiring 5-HT receptors. We tested the egg laying response to cocaine in mutants with deficits in the four metabotropic 5-HT receptors expressed in the *C.* elegans egg laying circuit. The genotypes tested include *ser-5* (which encodes a $G\alpha_s$-coupled 5-HT receptor) (45), *ser-4* (which encodes an inhibitory $G\alpha_o$-coupled 5-HT receptor)(46), a *ser-1*;*ser-7* double mutant (which encode a $G\alpha_q$-coupled 5-HT receptor and $G\alpha_s$-coupled 5-HT receptor, respectively) (47,48). We show that the *ser-1*;*ser-7* double mutant and *ser-5*, and both exhibit an egg laying response to cocaine that does not differ significantly compared to their respective parallel WT controls, suggesting that the excitatory post-synaptic effects of 5-HT are not required for cocaine-dependent egg laying (**Fig. 5B and D**). Further, our data suggest cocaine does not stimulate egg laying by blocking the inhibitory effects of *ser-4* (**Fig. 5C**).

To investigate all aspects of serotonin neurotransmission, including the previously reported inhibitory effects, we examined whether cocaine-dependent egg laying requires *mod-1,* which encodes a 5-HT-gated chloride channel (49,50). We observed that *mod-1* is required for an inhibitory effect of cocaine, not detected in the WT; (*mod-1*(*ok103*) mean = 18.398±3.153) cocaine-dependent egg laying is increased compared to parallel WT control (mean = 9.760±2.007) (p = 0.0079, N = 50) (**Fig. 5E**). We also screened cocaine-stimulated egg laying in animals carrying a mutation in *mod-5* (which encodes the *C. elegans* SERT) (51). Notably, the egg laying response to cocaine of *mod-5* (mean = 14.760±1.305) is elevated compared to parallel WT control (mean = 9.760±2.007) (p = 0.0079, N = 50) (**Fig. 5F**). These results suggest that cocaine induces 5-HT-dependent inhibitory effects as well as ACh-dependent excitatory effects on egg laying.

Discussion

Despite the severe toxicity and addictive liability associated with cocaine abuse (1,52), its mode of action is not sufficiently understood to produce effective pharmacotherapeutic interventions (5). ACh plays an important role in the mode of action of cocaine (13), but examination of the direct effects of the drug on cholinergic signaling is lacking. Here we present a previously unreported cocaine-induced behavioral phenotype in *C. elegans*, cocaine-stimulated egg laying, and through a candidate screen of individual molecular effectors in the egg laying circuit show that this

phenotype is dependent on ACh signaling genes, namely, *unc-29*, *unc-38*, *unc-17*, *egl-30*, *ace-1* and *ace-2*, and *cha-1*.

Multiple lines of evidence presented in this paper point to the conclusion that cocaine stimulates *C. elegans* egg laying through an ACh, nAChR, and AChE-dependent mechanism. First, animals with deficits in the synthesis of ACh by choline acetyltransferase (*cha-1*) and vesicular packaging of ACh by the transmembrane ACh transporter (*unc-17*) exhibit a suppressed egg laying response to cocaine compared to their respective parallel WT controls whereas animals with deficits in serotonergic (*tph-1*), dopaminergic (*bas-1*), octopaminergic (*tbh-1*), or tyraminergic (*tdc-1*) neurotransmission exhibit an egg laying response to cocaine that is not significantly different compared to their respective parallel WT controls. Second, animals with deficits in nAChRs expressed on the vulval muscles (*unc-29* and *unc-38*) also exhibit a suppressed egg laying response to cocaine compared to their respective parallel WT controls whereas animals with deficits in HSN-expressed mAChR (*gar-2*) exhibit an egg laying response to cocaine that is not significantly different from parallel WT control. Third, the *C. elegans* AChEs encoded by *ace-1* and *ace-2* are required for cocaine-stimulated egg laying.

In addition, our data show that cocaine-stimulated egg laying is increased in mutants for the *C. elegans* 5-HT reuptake transporter (SERT) (*mod-5*), as well as 5-HT-gated chloride channel mutants (*mod-1*), highlighting serotonergic inhibition of egg laying behavior as a consequence of a connection between the *C. elegans* egg laying and locomotion circuits.

Cocaine-induced egg laying in *C. elegans* is dependent on stimulation of nAChRs and *C. elegans* AChEs

The functional role of ACh in *C. elegans* egg laying is complex, as it has both an acute excitatory effect (21) and a chronic inhibitory effect (22). The acute excitatory effect of ACh on egg laying is the result of stimulation of nAChRs expressed on the vulval muscles, as nicotinic agonists stimulate egg laying (53,54), but fail to do so in the absence of vulval muscle expression of genes encoding nAChRs (55). The chronic inhibitory effect of ACh on egg laying is a result of negative feedback from the VCs to the HSNs, via the HSN-expressed mAChR encoded by *gar-2* (22).

Our finding that the egg laying response to cocaine is suppressed in animals with deficits in presynaptic cholinergic neurotransmission (*unc-17* and *cha-1),* as well as postsynaptic deficits in nAChRs (*unc-29* and *unc-38*), but not in animals with functional deficits in other neurotransmitter signaling pathways, suggests that nicotinic cholinergic neurotransmission plays a key role in cocaine-stimulated egg laying.

Interaction between the effect of cocaine on locomotion and egg laying

Our study did not identify any genes involved in serotonergic neurotransmission that are required for the stimulatory effects of cocaine on *C. elegans* egg laying; however, we did observe a statistically significant increase in the egg laying response to cocaine in two serotonergic mutants, including 5-HT-gated chloride channel mutants (*mod-1*) and SERT mutants (*mod-5*).

We propose that the increased egg laying response to cocaine in these mutants may result from interaction between the egg laying and locomotion circuits. Ratiometric calcium imaging of the egg laying circuit shows active and inactive phases that are coordinated with locomotion (20,56,57). In addition to stimulating egg laying through nAChRs expressed on the vulval muscles (19,21,22,28), the VCs slow locomotion preceding egg laying events through cholinergic synaptic contacts with the body wall muscles (20), which is thought hold to the body of the worm in a position that is conducive to egg laying and allow sufficient time for contraction of the vulval muscles. MOD-1 and MOD-5 have been suggested to play an inhibitory role in *C. elegans* egg laying behavior through inhibition of interneurons in the locomotion circuit, which subsequently decrease the activity of the egg laying neurons (45). Notably, cocaine induces a decrease in locomotion speed in *C. elegans* that is dependent on *mod-1* and *mod-5* (23). These results align well with our (**Fig. E-F**) data showing an increased egg laying response *in mod-1(ok103) and mod-5(n3314)* mutants compared to WT and suggest that cocaine may have both serotonergic and cholinergic effects on the locomotion and egg laying circuits, respectively.

Finally, we did not observe a change in egg laying in *tph-1(mg280)* and *bas-1(ad446)* mutant animals which are resistant to the slowing of locomotion speed induced by cocaine (23), potentially suggesting egg laying circuit plasticity in the absence of these enzymes during development.

Cocaine stimulates egg laying independently of HSN and serotonin

Cocaine stimulates egg laying in *C. elegans* independently of serotonergic neurotransmission as neither the HSNs (*egl-1*), 5-HT receptors (*ser-1*;*ser-7*, *ser-4, mod-1*), 5-HT synthetic enzymes (*tph-1* and *bas-1*), nor the *C. elegans* SERT (*mod-5*) are required for stimulation of egg laying by cocaine.

A possible explanation for HSN-independent nAChR-dependent stimulation of egg laying by cocaine is silencing of the VCs and HSNs by high osmotic pressure (20,58). The HSNs and the VCs each play a related, but distinct, role in the stimulation of *C. elegans* egg laying through their neuromuscular innervation of the vulval muscles. 5-HT, released from the HSNs, modulates activity of the vulval muscles by increasing the frequency of spontaneous calcium transits (59). Conversely, ACh, released from the VCs, triggers individual egg laying events by stimulating nAChRs and thereby inducing muscle contraction (14). The activity of both the VCs and the HSNs is depressed under conditions of high osmolarity (20,58); however, the vulval muscles innervated by these motor neurons retain a significant level of activity as measured by calcium transits (58). Therefore, under the high osmolarity conditions in which our egg laying assays were performed, the osmotic silencing of the HSNs and the VCs may be sufficient to negate inhibitory cholinergic feedback while at the same time preserving the potential for nicotinic stimulation of individual egg laying events.

EXPERIMENTAL PROCEDURES

Nematode strains

The WT strain was Bristol N2. The following mutant alleles were used in this study: MT15434: *tph-1(mg280)*; MT13113: *tdc-1(n3419)*; MT9455*: tbh-1(n3247)*; MT7988: *bas-1(ad446)*; PR1152: *cha-1(p1152)*; CB933: *unc-17(e245)*; CB1072: *unc-29(e1072)*; CB904: *unc-38(e264)*; RB756: *gar-2(ok520);* GG201: *ace-1(p1000);ace-2(g72)*; PR1300: *ace-3;ace-4(dc2)*; MT1082: *egl-1(n487)*; DA1084: *egl-30(ad806),* DA2109: *ser-1(ok345);ser-7(tm1325)*; MT9668: *mod-1(ok103)*; MT9772: *mod-5(n3314)*; RB2277: *ser-5(ok3087)*; AQ866: *ser-4(ok512).* Animals were maintained on NGM agar plates with *E. coli* OP50 as a source of food (60). Temperature was controlled at 20 °C.

Egg laying assays and statistical analysis

Egg laying assays were performed according to a protocol by Moresco and Koelle (61). In this study, the egg laying behavior of *C. elegans* hermaphrodites in hypertonic M9 salt solution, which strongly inhibits egg laying in otherwise untreated WT animals (27), was quantified. For each experiment, an aqueous stock solution of cocaine hydrochloride was diluted to the treatment concentration in M9 buffer solution with an equal volume of equimolar aqueous sucrose solution or 5-HT diluted in M9 buffer solution in each of the control conditions. Late stage L4 hermaphrodites of the respective strains were picked ~24 hours before assaying and continued to be cultured on agar plates with an OP50 lawn at 20°C. Day one adult hermaphrodites were isolated into single wells on a 96-well plate containing 35 μl experimental or equimolar control

solutions. After 60 mins, each well was examined, and the number of eggs laid by each animal was recorded. The investigators were blind to both the identity of the treatment solution and the genotype. Each experiment included ten subjects per experimental or control group unless otherwise indicated and each experiment was independently repeated four or five times as indicated. The mean number of eggs laid by each of the replicate treatment groups was treated as a single data point. Statistical analysis was performed using a Mann-Whitney U-test.

Data Availability Statement

The authors affirm that all data necessary for confirming the conclusions of this article are represented fully within the article and its tables and figures.

Chemicals

Aqueous cocaine hydrochloride was supplied by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC, USA). Serotonin hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO).

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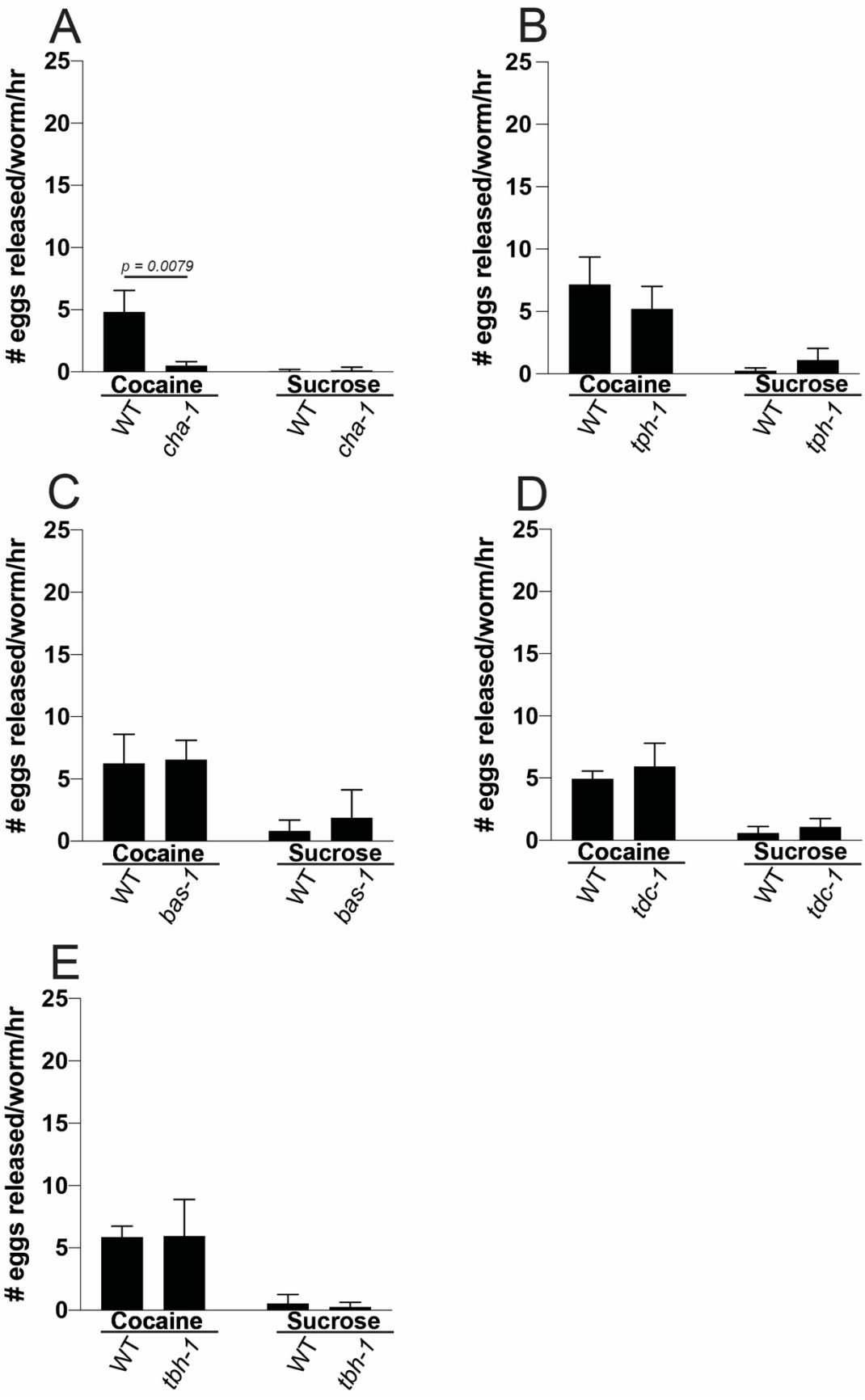

Fig 1: Choline acetyltransferase is required for the egg laying response to cocaine. Egg laying behavior of **A**. *cha-1*, **B**. *tph-1*, **C**. *bas-1*, **D**. *tdc-1*, and **E**. *tbh-1* in 62.5 mM cocaine or a 62.5 mM sucrose control compared to WT. We performed 5 independent experiments with 10 animals in each group. Relevant p values shown for significant differences. Error bars represent SD. Significance as compared to WT control was determined via a Mann-Whitney U-test.

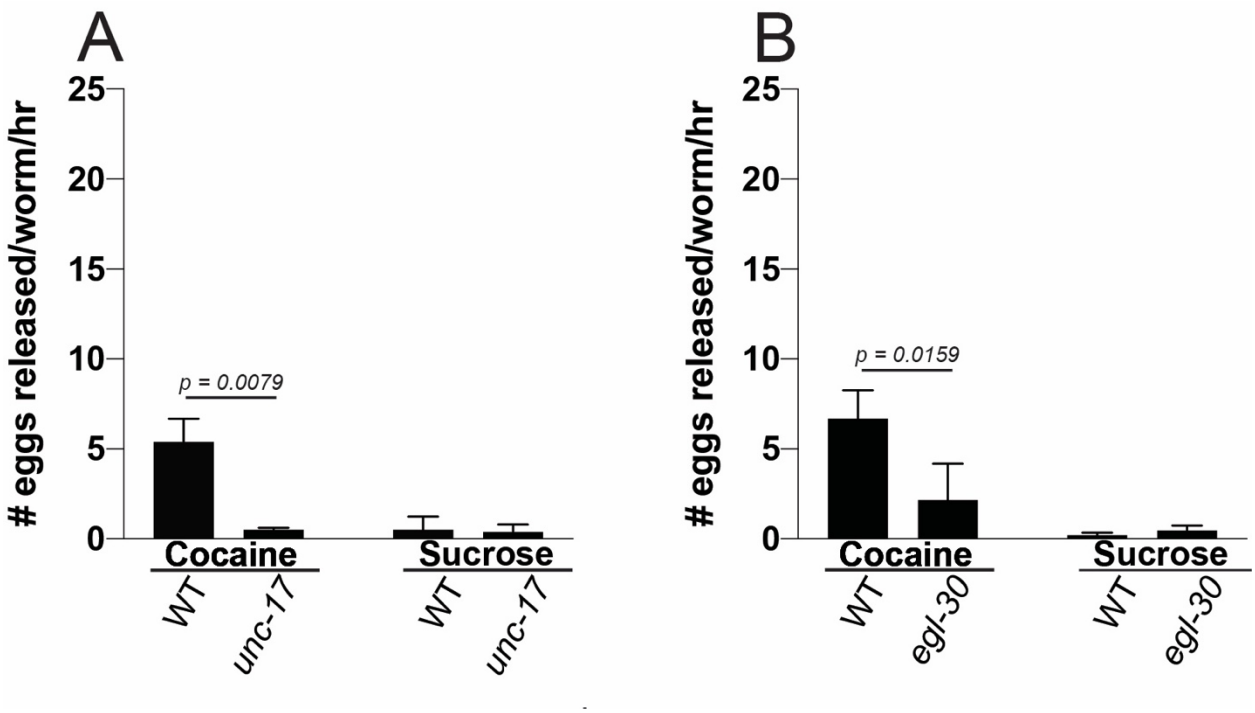

Fig 2: The ACh transmembrane transporter and $G\alpha_q$ are required for the egg laying response to cocaine. A. Egg laying behavior of **A**. *unc-17* and **B**. *egl-30* in 62.5 mM cocaine or a 62.5 mM sucrose control compared to WT. We performed 5 independent experiments with 10 animals in each group. Relevant p values shown for significant differences. Error bars represent SD. Significance as compared to WT control was determined via a Mann-Whitney U-test.

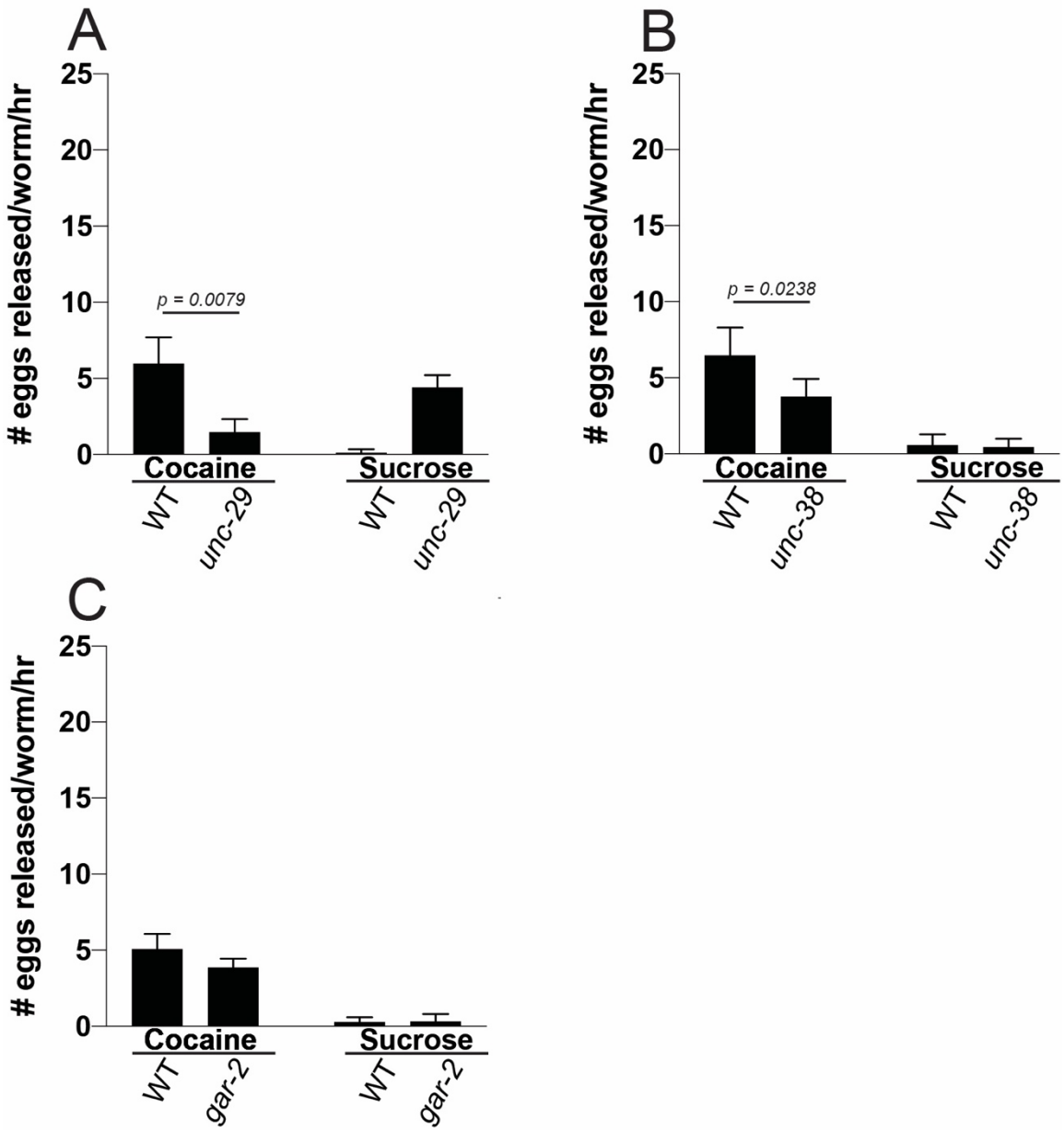

Fig 3: nAChRs are required for the egg laying response to cocaine. Egg laying behavior of **A**. *unc-29*, **B**. *unc*-38, and **C**. *gar-2* in 62.5 mM cocaine or a 62.5 mM sucrose control compared to WT. We performed 5 independent experiments with 10 animals in each group. Relevant p values shown for significant differences. Error bars represent SD. Significance as compared to WT control was determined via a Mann-Whitney U-test.

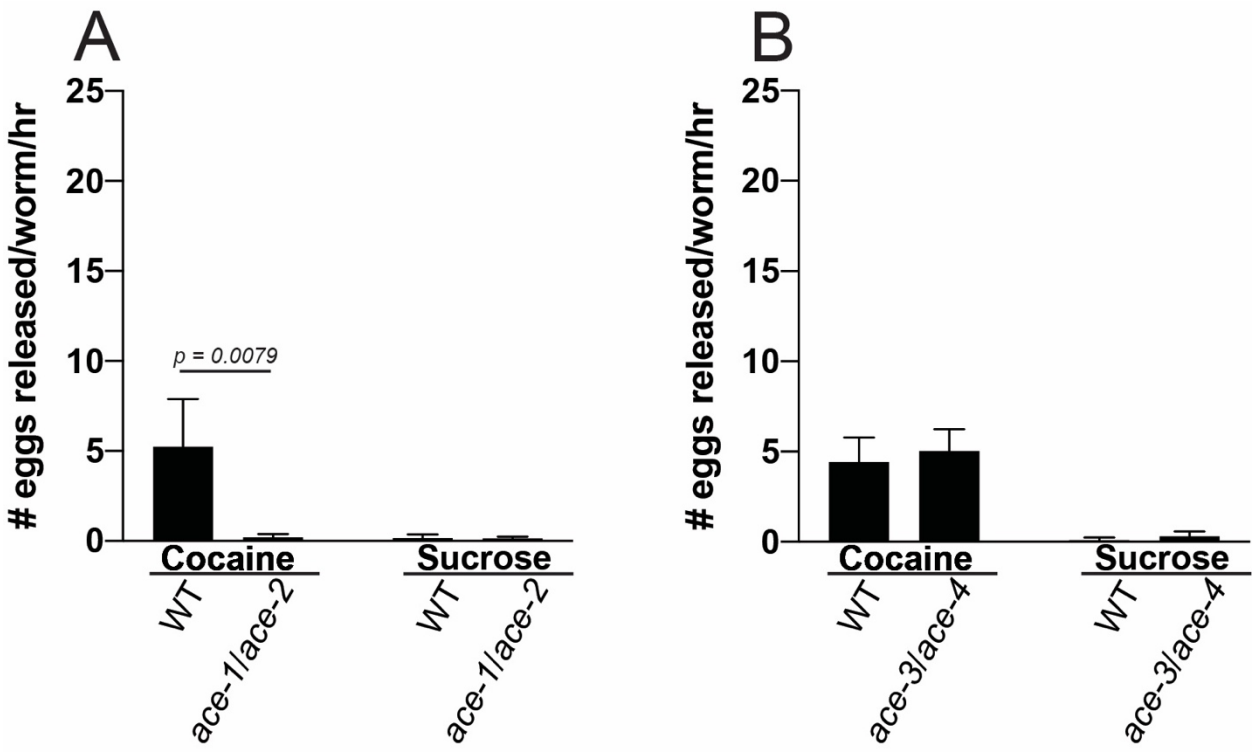

Fig 4: nAChRs are required for the egg laying response to cocaine. Egg laying behavior of **A**. *ace-1;ace-2* and **B**. *ace-3;ace-4* in 62.5 mM cocaine or a 62.5 mM sucrose control compared to WT. We performed 5 independent experiments with 10 animals in each group. Relevant p values shown for significant differences. Error bars represent SD. Significance as compared to WT control was determined via a Mann-Whitney U-test.

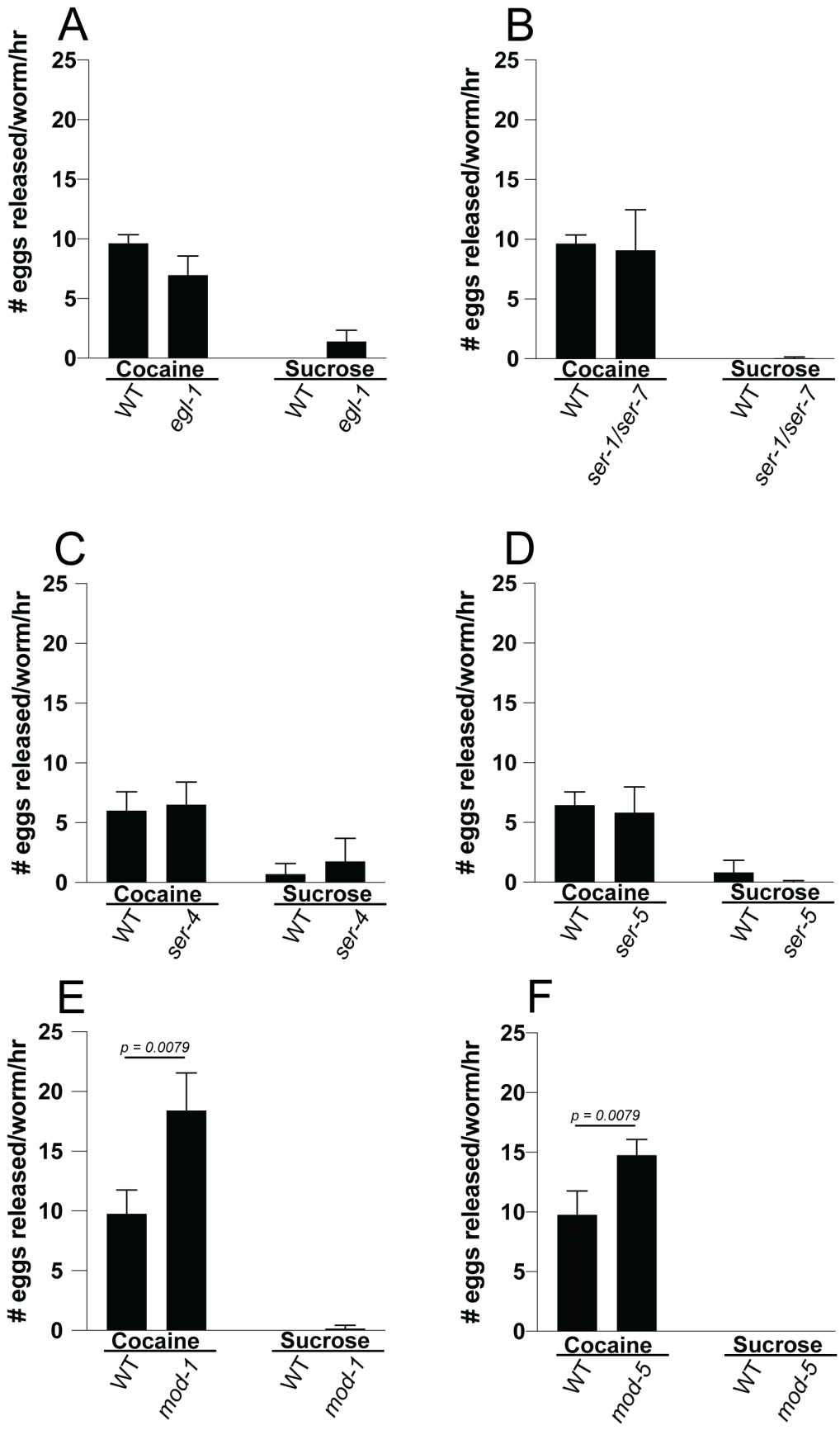

Fig 5: Neither the HSNs nor serotonin receptors are required for the egg laying response to cocaine. Egg laying behavior of **A.** *egl-1*, **B**. *ser-1*;*ser-7*, **C.** *ser-4*, **D**. *ser-5,* **E.** *mod-1,* and **F.** *mod-5* in 62.5 mM cocaine or a 62.5 mM sucrose control compared to WT. We performed 5 independent experiments with 10 animals in each group for assays of *ser-4*, *ser-5*, *mod-1*, and *mod-5* egg laying. We performed 4 independent experiments with 10 animals in each group for assays of *egl-1* and *ser-1*;*ser-7* egg laying. Relevant p values shown for significant differences. Error bars represent SD. Significance as compared to WT control was determined via a Mann-Whitney U-test.