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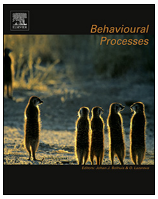

# The effect of sertraline, haloperidol and apomorphine on the behavioural manipulation of slugs (*Deroceras invadens*) by the nematode *Phasmarhabditis hermaphrodita*

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A B S T R A C T

The nematode *Phasmarhabditis hermaphrodita* can infect and kill many species of slugs and has been formulated into a biological control agent for farmers and gardeners. *P. hermaphrodita* can manipulate the behaviour of slugs, making those infected move to areas where the nematode is present. Research suggests *P. hermaphrodita* uses manipulation of biogenic amines to achieve this, however the exact role of serotonin and dopamine needs further elucidation. Here we fed slugs *Deroceras invadens* (uninfected and infected with *P. hermaphrodita*) apomorphine, sertraline and haloperidol and observed their behaviour when given a choice between a *P. hermaphrodita* infested habitat, or a parasite free area of soil. In contrast to their usual *P. hermaphrodita* avoidance behaviour, uninfected *D. invadens* fed sertraline were attracted to the nematodes and conversely those fed haloperidol avoided the nematodes. *D. invadens* fed apomorphine were recorded equally on the control and nematode side. *D. invadens* pre-infected with *P. hermaphrodita* fed sertraline and apomorphine were found significantly more on the side with the nematodes. However, suppressing dopaminergic signalling through feeding with haloperidol abrogated this attraction and slugs were found on both sides. These results demonstrate that serotonin and dopamine are potential regulators of behavioural manipulation by *P. hermaphrodita*.

## 1. Introduction

The ability to manipulate the behaviour of hosts by parasites is common across the tree of life (Hughes et al., 2012; Moore, 2002). How parasites change the behaviour of hosts can be broadly split into 2 main types: those that coerce intermediate hosts to move into areas where they are more likely to come in contact with their definitive host, and those that compel their host to migrate to a habitat which will increase dispersal of offspring (Hughes and Libersat, 2019). The trematode *Leucochloridum paradoxum* is a good example of the former. *L. paradoxum* infects snails (*Succinea* spp.) and makes them less photophobic (as well as producing multi-coloured, pulsating tentacles) and more likely to eaten by their definitive host- birds. The trematode then reproduces in the bird's stomach and eggs are expelled with faeces and eaten by more snails (Wesolowska and Wesolowski, 2013). In contrast, the gypsy moth multicapsid nuclear polyhedrosis virus infects gypsy moths (*Lymantria dispar*) and makes them climb to the top of trees where they will die and the virus replicates and spreads viral particles across the forest floor to infect more caterpillars (Hoover et al., 2011).

Although there are many other examples of viruses, trematodes, protozoa and fungi that influence host behaviour (Hughes et al., 2012; Moore, 2002) there are only a handful of other examples of metazoans such as members of the Nematoda that can alter the behaviour of hosts. We have recently shown the nematode *Phasmarhabditis hermaphrodita* has an unusual ability to change the behaviour of their slug hosts (Morris et al., 2018). *P. hermaphrodita* is a lethal parasite of several commercially important slug species and has been successfully formulated as a biological control agent (Nemaslug®) by BASF-Agricultural Specialities for use by farmers and gardeners (Wilson et al., 1993; Rae et al., 2007). Nematodes are applied to soil where they seek out slugs responding to mucus and faeces (Rae et al., 2006; Nermut' et al., 2012). They then enter through the back of the mantle and kill the slug in 4–21 days and reproduce on the decaying cadaver (Wilson et al., 1993; Tan and Grewal, 2001). *P. hermaphrodita* can provide significant protection against slug damage in horticultural and agricultural crops (Rae et al., 2007).

Many slug species (*Deroceras reticulatum*, *Deroceras invadens* (previously known as *Deroceras panormitanum*), *Arion ater*, *Arion subfuscus* and *Arion hortensis*) avoid areas where *P. hermaphrodita* has been applied (Wilson et al., 1999; Wynne et al., 2016; Morris et al., 2018)

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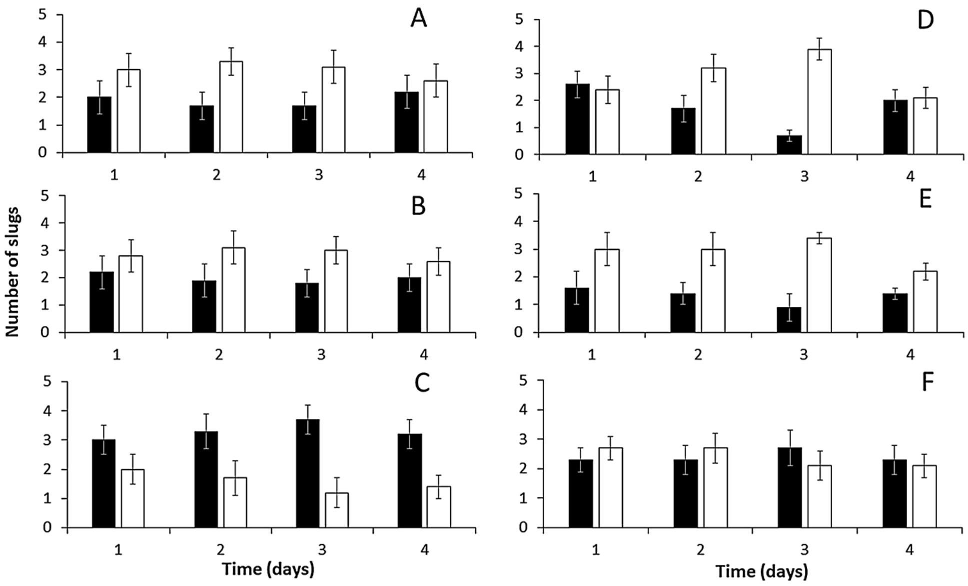

**Fig. 1.** The mean number of uninfected slugs (*D. invadens*) found on the control (water) side (black bars) and the side with *P. hermaphrodita* (white bars) on days 1, 2, 3 and 4. Slugs were fed 10 µM sertraline (A), 10 µM apomorphine (B) or 10 µM haloperidol (C). Slugs pre-infected with *P. hermaphrodita* were fed 10 µM sertraline (D), 10 µM apomorphine (E) or 10 µM haloperidol (F). Bars represent ± one st. error.

however, when infection by the nematode does occur it alters slug behaviour in numerous ways. Infected slugs are slower (Bailey et al., 2003), their feeding is inhibited (Glen et al., 2000), they are unattractive to predatory beetles (Foltan and Puza, 2009) and they move down into soil to die (Pechova and Foltan, 2008). The most striking example of host behavioural manipulation by *P. hermaphrodita*, which cannot be explained simply as a symptom of malaise, is the phenomenon where infected slugs of several species (*D. invadens, A. hortensis* and *A. subfuscus*) are attracted to areas where *P. hermaphrodita* has been applied (Morris et al., 2018). The adaptive reason for this behavioural manipulation is unknown but it is speculated that by moving the slug towards areas with more nematodes this increases the chances of the slug dying and therefore providing food for the nematode to complete its life cycle. This study also showed that uninfected *D. invadens* fed fluoxetine (increasing serotonin levels) were not averted by the nematodes, and were recorded more often on areas with *P. hermaphrodita*. Conversely, slugs infected with *P. hermaphrodita* and fed cyproheptadine (reduced serotonin levels) were no longer attracted to the side with *P. hermaphrodita*. This research showed manipulation of biogenic amines in slugs can produce similar behavioural phenotypes as those caused by *P. hermaphrodita* infection (Morris et al., 2018). We sought to continue this research by examining further the effects of drugs which affect serotonin and dopamine signalling would have on the behavioural manipulation of *D. invadens* when infected with *P. hermaphrodita*. Ultimately, this approach will allow us to gain insight into the molecular mechanism these nematodes have evolved to control gastropod behaviour through pharmacological manipulation.

## 2. Materials and methods

Slugs (*D. invadens*- previously called "*D. panormitanum*" and previously used in Morris et al., 2018 and Wynne et al., 2016) were collected from greenhouses at Liverpool John Moores University. They were placed in non-airtight plastic boxes lined with moist tissue paper, fed lettuce *ad libitum* and stored at 15 °C. Slugs were kept for a minimum of 7 days before use to ensure the slugs were not naturally infected with *P. hermaphrodita*. *P. hermaphrodita* (Nemaslug®) was purchased from BASF-Agricultural Specialties and stored at 15 °C until use. The behaviour of *D. invadens* when exposed to *P. hermaphrodita* was monitored using a standard soil based assay (Wilson et al., 1999; Wynne et al., 2016; Morris et al., 2018). Briefly, 100 g of sterile loam soil (21% water content) was added to separate plastic boxes (24 × 9 x 6 cm). Copper tape was placed around the side of each box to ensure the slugs remained on the soil. To one side (12 x 9 cm) 5 mls of water was even applied over the soil surface and acted as the control. To the other side 120 *P. hermaphrodita* per cm$^2$ were added in 5 mls of water to the other side. *P. hermaphrodita* was applied to each side at 120 nematodes per cm$^2$ as it was the dose that slugs consistently avoided in previous studies (Wilson et al., 1999; Wynne et al., 2016; Morris et al., 2018). Two 4.5 cm diameter pieces of bread were placed on either side of the box. Three millilitres of 10 µM sertraline, 10 µM apomorphine or 10 µM haloperidol (drugs obtained from Sigma-Aldrich, UK, and dissolved in distilled water) were applied to each piece of bread. The concentration of 10 µM for the drugs was used based on studies by Morris et al. (2018). Five *D. invadens* (mean weight = 0.21 ± 0.004 g, n = 270) were added to the middle of the box, sealed and they were stored at $20^0$C. Every 24 h for 4 days the numbers of *D. invadens* were recorded on the nematode and water side and were then placed back in the middle of the box. The experiment consisted of three boxes and the entire experiment was repeated three times (N = 9 replicate boxes; n = 45 slugs). This experiment was also repeated with *D. invadens* previously infected with *P. hermaphrodita*. To infect the slugs 10 *D. invadens* were added to five separate plastic boxes (24 × 9 x 6 cm) filled with 100 g of soil. *P. hermaphrodita* was applied to the entire soil surface at the standard field application rate of 30 nematodes per cm$^2$ (Wilson et al., 1994) and slugs were exposed for 5 days at 15 °C, which has been shown to be a suitable time and number of nematodes to ensure infection (Wilson et al., 1993; Tan and Grewal, 2001; Morris et al., 2018). After 5 days the slugs were used in the same experimental set-up outlined above. A two way repeated measures analysis of variance (ANOVA) was used to compare the numbers of slugs found on the control and nematode side on days 1, 2, 3 and 4.

## 3. Results

When fed sertraline and apomorphine *D. invadens* did not avoid *P. hermaphrodita* and were found significantly more on the nematode side ($P < 0.05$; Fig. 1A, B). In contrast, ingestion of haloperidol made slugs avoid *P. hermaphrodita* with significantly more slugs found on the water side compared to the nematode side ($P < 0.05$; Fig. 1C).

*D. invadens* previously infected with *P. hermaphrodita* fed sertraline and apomorphine were found significantly more on the nematode side ($P < 0.05$; Fig. 1D, E). However, when *D. invadens* infected with *P. hermaphrodita* were fed haloperidol there was no significant difference between the numbers of slugs recorded on the nematode and control sides ($P > 0.05$; Fig. 1F).

## 4. Discussion

Influencing levels of biogenic amines to manipulate the behaviour of hosts is a successful mechanism used by several parasites. For example, the protozoan parasite *Toxoplasma gondii*, which can change the behaviour of rodents making them less photophobic and reducing their fear of cats (their definitive hosts) (Webster, 1994), has the genetic machinery to make dopamine (Gaskell et al., 2009). Inhibition of dopamine through application of haloperidol in infected rats reduces their parasite-induced behaviours (Webster et al., 2006). Similarly, acanthocephalan worms (*Pomphorhynchus* spp.) that infect amphipods makes them congregate at the surface of water to increase the likelihood of being eaten by birds (their definitive host) by altering serotonin levels (Jacquin et al., 2014; Tain et al., 2006). Injection of serotonin in uninfected amphipods makes them graduate to the water surface - similar to their parasite induced behaviour, whereas untreated amphipods avoid the water surface (Tain et al., 2006). Our research suggests that *P. hermaphrodita* can change the behaviour of slugs to cause death so the nematodes can use it as an immediate food source and reproduce on its cadaver (rather than remaining inside the slug and waiting it for it to die – termed ‘necromeny’). We believe that this manipulation behaviour is strongly influenced by levels of serotonin and dopamine. Our reasons are four fold. First, in accordance with Morris et al. (2018) who showed uninfected *D. invadens* fed fluoxetine (which prevents re-uptake of serotonin in cells) were found more on the *P. hermaphrodita* side; we showed that sertraline (another Selective Serotonin Reuptake Inhibitor) produces the same nematode attraction behaviour. Second, uninfected *D. invadens* fed apomorphine (which activates dopamine receptors) no longer were averted from *P. hermaphrodita* and were found in similar numbers on each side - and were even attracted to the nematode side. Third, application of haloperidol to infected slugs (which should graduate to the nematode side) were no longer attracted to the nematodes, presumably as dopamine signalling was antagonised. Fourth, feeding infected slugs (which will move to the side with *P. hermaphrodita*) with cyproheptadine (which antagonises serotonin signalling) makes them no longer attracted to the nematodes. Therefore, we believe the results of this study and that of Morris et al. (2018) strongly implicate the influence of biogenic amines in the behavioural manipulation of *D. invadens* by *P. hermaphrodita*.

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