Title

Multiple tracking and machine learning reveal dopamine modulation for area-restricted foraging behaviors via velocity change in *Caenorhabditis elegans*

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Abstract

Food exploration is an essential survival behavior in organisms. To find food efficiently, many organisms use a foraging strategy called area-restricted search (ARS) wherein individuals first turn more frequently, restricting their search to one area, then turn less frequently, moving along a straight path to widen the search area. Previous research suggests that the nematode *Caenorhabditis elegans* shows ARS behavior by changing turn frequency, and that dopamine is a crucial determinant. However, the effects of dopamine on multiple behavioral parameters have remained unknown. Here, we evaluated turn (pirouette) frequency, moving velocity, and specific area occupancy (cell occupancy) over time by using a multiple-worms tracking system. In the control (mock) experiments, all parameters changed over time, but no changes were observed in experiments with dopamine pre-exposed and dopamine-deficient animals. In inverse reinforcement learning analysis, the value function for specific velocity was found to modulate over time in mock animals only. These results demonstrate that dopamine regulates ARS via changes not only to pirouette frequency change but also to velocity.

Highlights

- *C. elegans* shows ARS behavior, changing its velocity and turn frequency over time.
- Pre-exposure to dopamine inhibits changes in searching behavior.
- A dopamine-synthesis defect inhibits changes in searching behavior.
- Inverse reinforcement learning reveals a change in the value function for velocity.
- A change in value function is absent in dopamine exposed and deficient animals.

Keywords

dopamine; area-restricted search; foraging, behavioral assay; machine learning; inverse reinforcement learning

Abbreviations

Area-restricted search: ARS

Acknowledgements

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Author Contribution

K.A, K.H. and K.O designed the experiments. T.K. performed the experiments. K.A and T.K. performed data analysis; K.A. wrote the original draft of paper and K.A, K.H. and K.O reviewed and edited the paper. K.H. and K.O supervised the work.

Conflict of Interest

No conflict declared.

1. Introduction

Exploring food is an essential survival behavior in organisms. To find food efficiently, many species, from nematode to human, use a strategy called area-restricted search (ARS) [1–6]. In this foraging strategy, animals first search locally, turning more frequently within a restricted area, then search globally, turning less frequently and moving more along straight paths to extend the search area. The nematode *Caenorhabditis elegans* has been shown to use the ARS strategy [1,2,7,8]. While the neuromodulator dopamine has been suggested to play a crucial role in the behavioral shift involved [1], the effect of dopamine on the foraging remains largely unstudied. Previous research indicates that dopamine is important for sharp turns (pirouettes), and that ablation of dopaminergic neurons and treatment with dopamine antagonists inhibit a pirouette-frequency change over time [1]. Although the pirouette frequency is used to evaluate ARS, this parameter does not reliably indicate whether worms are conducting a local or global search, and the effect of dopamine on the searching behavior has therefore not yet been clearly established. Several reports characterize *C. elegans* based on velocity, pirouette frequency, and cell occupancy [7,9,10]. A custom-made tracking system has recently been developed to easily and quickly measure the behavior of multiple worms and to investigate their interaction during chemotaxis [10]. Using this system, we measured several behavioral parameters, including pirouette frequency, velocity, and cell occupancy, with the aim of clearly characterizing the searching behaviors of worms pre-exposed to dopamine, and of mutants with defective dopamine synthesis. We also employed inverse reinforcement learning, a machine learning methods to identify behavioral strategies through value functions that has previously been applied to such data [11–13]. Using these methods, we show that dopamine plays a crucial role in ARS in *C. elegans* not only through its effect on pirouette frequency changes but also on velocity changes.

2. Materials and methods

2. 1. *C. elegans* strains

Worms were cultured at 20°C on nematode growth medium (NGM) agar plates with *Escherichia coli* OP50 bacteria under standard conditions [14]. The N2 strain (wild-type) and *cat-2* (*n4547*) II (MT15620 in in the Caenorhabditis Genetics Center, CGC) were used for the experiments. Hermaphrodites were used for all experiments.

2. 2. Behavioral Assays

Behavioral assays were performed as previously described [10] but without using odor. Assay plates consisted of 8 ml of 1.8% agar, 1 mM $CaCl_2$, 1 mM $MgSO_4$ and 5 mM KH_2PO_4 in 10-cm petri dishes. Worms were washed with S-basal buffer in a microtube with a platinum wire. Next, all worms were transferred to the assay plate with the buffer to enable picking up of each worm. Then, 4 μl of distilled water (rather than S-basal, to avoid the effect of salt taxis) was spotted in the center of another assay plate, and worms were transferred to the spotted water. Water was then removed using Kimwipes until the worms were not swimming. Images were captured with a web camera (HD Pro Webcam C920, Logitech) every second for 31 minutes with a custom-made Matlab program (MATLAB 2016a, MathWorks). Almost all animals were measured for the full period from 0 to 31 min (Fig. 4A).

2. 3. Pre-exposure to dopamine

Worms were cultivated on NGM plates with dopamine and later tracked onto the assay plates (Fig. 1A). Dopamine was dissolved into the NGM solution before solidifying. The NGM plates with dopamine were made and seeded with *E. coli* 24 h before the assay. To prevent the degeneration of dopamine, the plates were covered with aluminum foil sheets. Some young adult worms were transferred to the NGM plates with dopamine 18 h before the assay and were incubated at 20°C covered with aluminum foil sheets. In the mock and mutant experiments, worms were transferred to NGM plates without dopamine.

2. 4. Behavioral analysis

The same analysis methods as in previous research were employed [10] using Matlab programs modified from *parallel worm tracker* [15]. For worms that reached the edge

of the plates, tracking data before arrival at the edge were used for analysis. However, most worms were successfully tracked for over 30 min (Fig. 4A). In the analysis, pirouettes were defined as turns with an absolute turning rate > 90°. In Figures 2, 3 and 5, data were classified into early (0–15 min) and late (16–31 min) stages. Cell occupancy was calculated as the number of unique 1 mm^2 areas (cells) that worms visited per minute [7,16]. This parameter indicates how worms search globally. Tracked data were analyzed in each one-minute bin, and cell occupancy was calculated. For the estimation of velocity and pirouette frequency, total distance and total number of pirouettes in all trails during for one stage were divided by tracking period.

2. 5. Inverse reinforcement learning

The scheme for inverse reinforcement learning was used as previous research [13]. To estimate the value function of velocity, the probability distribution of velocity change under passive dynamics was assumed to be Gaussian ($\sigma = 0.0545$, which corresponds to the standard deviation of acceleration over 1 s for all data). The regularization parameter λ was determined as 40. The value function for each animal was estimated using velocity data for a single track. Velocity was equally divided into 20 segments for a rage of 0–0.25 mm/s (over 95 % of all data were contained in these segments). For maximum likelihood estimation, the Newton-CG method employing the *optimize.minimize* function in SciPy (version 1.2.0) on Python 3.5.2 was used.

2. 6. Statistical tests

Statistical analysis was performed using Dunnett's test (Fig. 4A; R version 3.5.1. with the glht function in the multcomp library), Welch's t-test with holm correction (Fig. 4B–D; Excel 2016 with the TTEST function, and R version 3.5.1. with the p.adjust function) and a paired t-test (Figs. 2, 3, 5; Excel 2016 with the TTEST function). To compare the value function between the early and late stages, only the value at 0.013 mm/s was used to avoid multiple comparisons. The velocity value influences the adjacent velocity value, and *vice versa*, because of the smoothness constraint [13]. This interaction increases the familywise error rate and induces Type I error [17,18], so only the values at 0.013 mm/s were compared. The number of assays (N) and animals (n) for all analyses were: mock: N = 6, n = 27; 40 μM: N = 5, n = 29; 200 μM: N = 6, n = 32;

400 μM: N = 6, n = 40; 4 mM: N = 6, n = 37; *cat-2* (*n4547*): N = 4, n = 18. *cat-2*, 4 mM: N = 6, n = 31.

3. Results

3. 1. Pre-exposure to dopamine suppresses area-restricted search behavior

To investigate the role of dopamine on food-searching behavior, worms with dopamine pre-exposure were tracked for 30 min. In previous research [1], the small assay plate diameter (5 cm) limited tracking and analysis of the trails [7]. Therefore, 10-cm diameter plates were used in this study and enabled the successful tracking of most worms for over 30 min (Fig. 4A). Next, worms were pre-incubated overnight on plates containing dopamine concentration ranging from 40 μM to 4 mM with food. After pre-exposure to dopamine, worms were transferred to assay plates without food, and trails were captured and analyzed (Fig. 1A, see also Materials and Methods). Under mock conditions, worms showed typical ARS behaviors, searching first locally then globally [1,2,7] (Fig. 1B). However, pre-exposure to dopamine suppressed these behaviors. To quantify this effect, pirouette frequency was first evaluated. When comparing the early and late stages (first 15 min and last 15 min), animals decreased pirouette frequency over time under mock conditions (Fig. 2A, mock). This result is consistent with previous research [1]. For further analysis, velocity and cell occupancy were evaluated. Cell occupancy describes how widely worms search [7]. Both velocity and cell occupancy were also found to have increased (Fig. 2B, C, mock). These results clearly indicate that worms changed searching behaviors from local to global. Moreover, a decreasing in pirouette frequency and an increasing in velocity indicate a shift in searching behavior. Conversely, pre-exposure to dopamine inhibited changes in all parameters over time at dopamine concentrations above 400 μM (Fig. 2B, C). These results explicitly demonstrate that an overdose of exogenous dopamine suppresses ARS behavior and that dopamine plays an important role in changing food-searching behavior in *C. elegans*.

3. 2. Dopamine-synthesis deficient mutant suppresses area-restricted search behavior.

The role of dopamine in worms lacking endogenous dopamine production was investigated. The *cat-2* mutant, which cannot synthesize dopamine, was used [19–21]. As expected, the mutant did not display any changes in searching behaviors (Fig. 1C). Further quantification showed that it was unable to modulate pirouette frequency,

velocity and cell occupancy (Fig. 3). These results demonstrate that endogenous dopamine is necessary to change search behaviors.

The effect of dopamine on behavior itself was investigated (Fig. 4). In the assay, behavioral change was not observed with dopamine pre-exposure, but velocity and cell occupancy were decreased in the *cat-2* mutant. Moreover, dopamine exposure for *cat-2* mutant clearly rescued these decreases. These results suggest that dopamine could help maintain high velocity and cell occupancy, and that increasing dopamine levels may change food-searching behavior.

3. 3. Inverse reinforcement learning reveals changed value function in wild type, but not in dopamine pre-exposed and synthesis-deficient animals.

Inverse reinforcement learning was introduced for further analysis. This method enables the evaluation of behavioral strategy based on the value function [11–13]. In the framework of reinforcement learning, the agent (the worm) interacts with the environment to maximize the reward, and changes its behavior (the action) to obtain the reward. Changing behavior incurs a cost; there is a tradeoff between the reward and the cost. The tradeoff, described by the value function, corresponds to the behavioral strategy [11–13]. Inverse reinforcement learning can be estimated by the value function obtained from behavioral data; in this case, the worm changes its velocity to optimize the search. Therefore, the value function of velocity indicates the optimal velocity for a worm's searching behavior. When this method was applied to the velocity data under mock conditions, the value at low velocity (0.013 mm/s in Fig. 5) was found to be high in the early stage, and lower in the late stage (Fig. 5). This result indicates that worms want to maintain a low velocity in the early stage only, which is consistent with the velocity change in the mock animals (Fig. 2B). Moreover, corresponding to the velocity change, both animals pre-exposed to dopamine and dopamine-deficient animals did not show change in the value function at low velocity over time. These results strongly support the notion that changing velocity is an important factor for ARS and that dopamine is necessary for shifting searching behavior.

4. Discussion

Our results demonstrate that dopamine is necessary to switch searching behaviors from local to global in worms pre-exposed to dopamine and in dopamine-deficient mutants. As in previous research [1], pirouette frequency changes were evaluated. The present study further affirmed the hypothesis and also carried out investigations of cell occupancy and velocity. The derived value functions, estimated by inverse reinforcement learning, strongly support a change in behavioral strategy. A velocity change correlated to dopamine has not previously been reported in the context of ARS.

By modulating pirouette frequency, worms can efficiently perform a global search [1,2,7,8]. Our results also reasonably explain searching strategy by showing that velocity is also important in searching behaviors, with faster movements allowing wider exploration. While previous research only considered path structure [1], the present study shows that other parameters also affect searching behaviors.

Previous research shows that dopamine has crucial roles in food related behaviors, through pirouette in foraging and slowing in response to food [1,22–25]. The results of the present study show that dopamine is important in modulating velocity while foraging, and suggest that dopamine may keep both the velocity and cell occupancy high. As dopamine is also known as an essential factor for regulating locomotion rate [21], it may act to shift a search from local to global.

Pre-exposure to dopamine concentrations above 400 μM inhibited changes in almost all parameters over time. A pirouette frequency change was observed with pre-exposure to 400 μM dopamine (Fig. 2A). In previous research [1] , a dopamine concentration above 1 mM was needed to prevent any pirouette frequency change, suggesting that a higher concentration of dopamine is needed to inhibit a pirouette frequency change than to inhibit a velocity change. These results suggest that a separate pathway may modulate pirouette frequency and velocity.

Inverse reinforcement learning was applied to identify behavioral strategy. Machine learning approaches, such as inverse reinforcement learning, have recently been used to understand behavioral data [11–13,26,27]. These methods could be powerful tools in understanding various behavioral strategies from novel points of view.

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335

Figure Legends

Fig 1. Foraging behaviors under dopamine modulation. (A) Experimental scheme. Pre-exposed dopamine concentrations: 0 mM (mock), 40 μM, 200 μM, 400 μM and 4 mM. (B) The representative tracks of animals on one assay with pre-exposure to no dopamine (left, mock), 200 μM dopamine (middle), and 4 mM dopamine (right). Each animal is represented by a different color. (C) The representative tracks on one assay of mutants with a dopamine synthesis defect, *cat-2* (*n4547*). Each animal is represented by a different color.

Fig 2. Evaluation of area-restricted search behaviors with pre-exposure to dopamine. (A) Pirouette frequency during the early (from 0 to 15 min, gray bars) and late (from 16 to 31 min, white bars) stages with pre-exposure to dopamine (from 0 to 4 mM). (B) Velocity during the early and late stages with pre-exposure to dopamine. (C) Cell occupancy during the early and late stages under with-exposure to dopamine. Error bars indicate the standard deviation of the mean (SEM). Paired t-test, $^{**}p<0.01$, $^{*}p<0.05$.

Fig 3. Evaluation of area-restricted search behaviors on dopamine-deficient mutants. (A) Pirouette frequency during the early (from 0 to 15 min, gray bar) and late (from 16 to 31 min, white bar) stages in the *cat-2* mutant (*cat-2* (*n4547*) II). (B) Velocity during the early and late stages in the *cat-2* mutant. (C) Cell occupancy during the early and late stages in the *cat-2* mutant. Error bars indicate the SEM. Paired t-test, $p>0.05$, not significant.

Fig. 4. Effect of dopamine on behaviors. Total time (A), pirouette frequency (B), velocity (C), and cell occupancy (D) for each track of worms in the experiments. The experiments were performed with worms pre-exposed to dopamine from 0 to 4 mM dopamine, dopamine-synthesis defective mutants (*cat-2* (n4547) II) and the mutants exposed to 4 mM dopamine. Error bars indicate the standard error of the mean (SEM). Welch's t-test with holm correction, ***$p<0.001$.

Fig 5. Value function of velocity. The average of value functions of each worm in the mock, pre-exposure to dopamine (from 0 to 4 mM), and the *cat-2* mutant (*cat-2* (*n4547*) II) are shown. Dotted lines are value functions in the early stage, and solid lines are ones in the late stage. Error bars indicate the SEM. Only the values at 0.013 mm/s are compared by a paired t-test. *$p<0.05$.

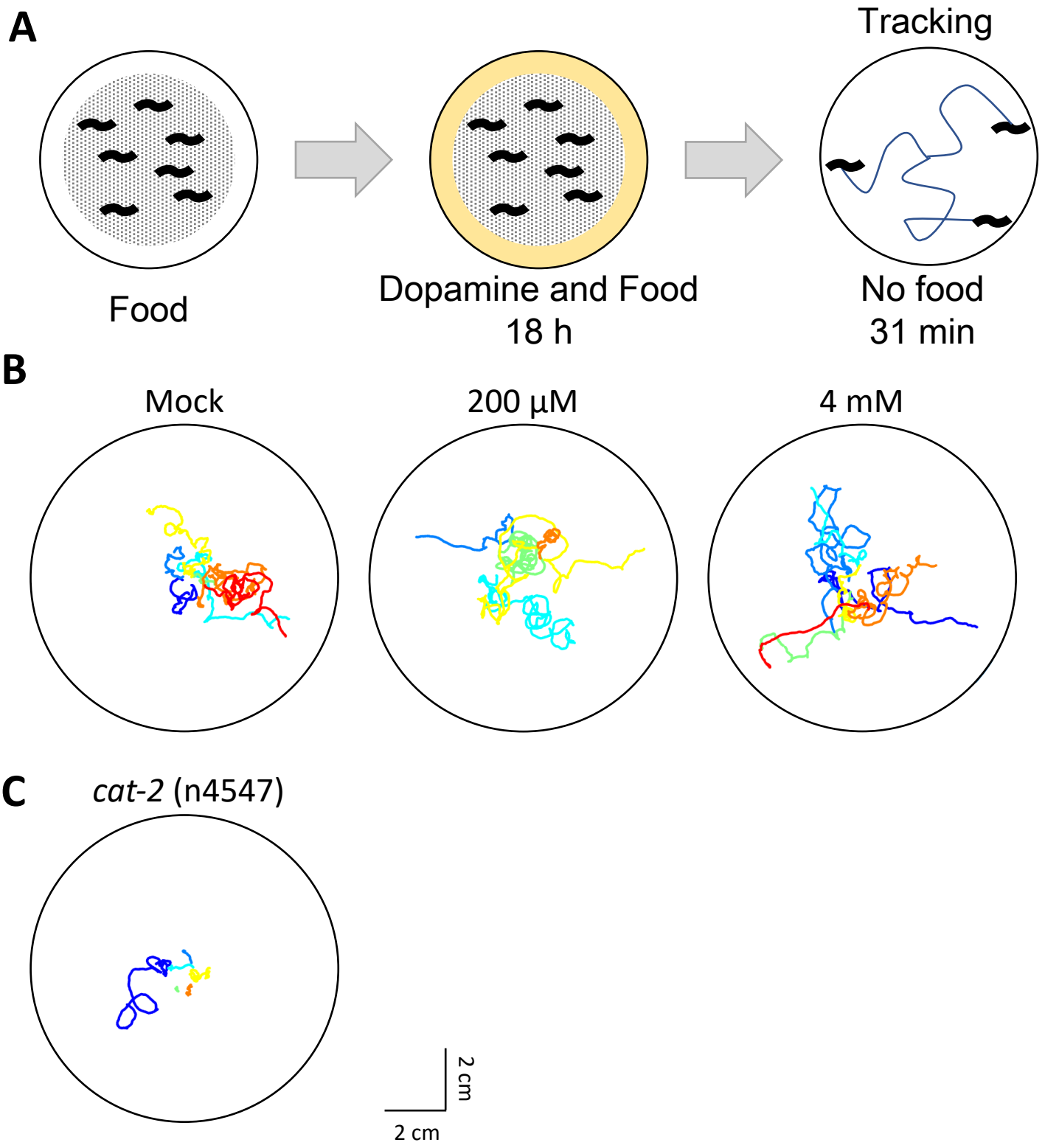
A
Tracking
Food
Dopamine and Food
18 h
No food
31 min
B
Mock
200 µM
4 mM
C
cat-2 (n4547)
2 cm
2 cm

Fig. 1

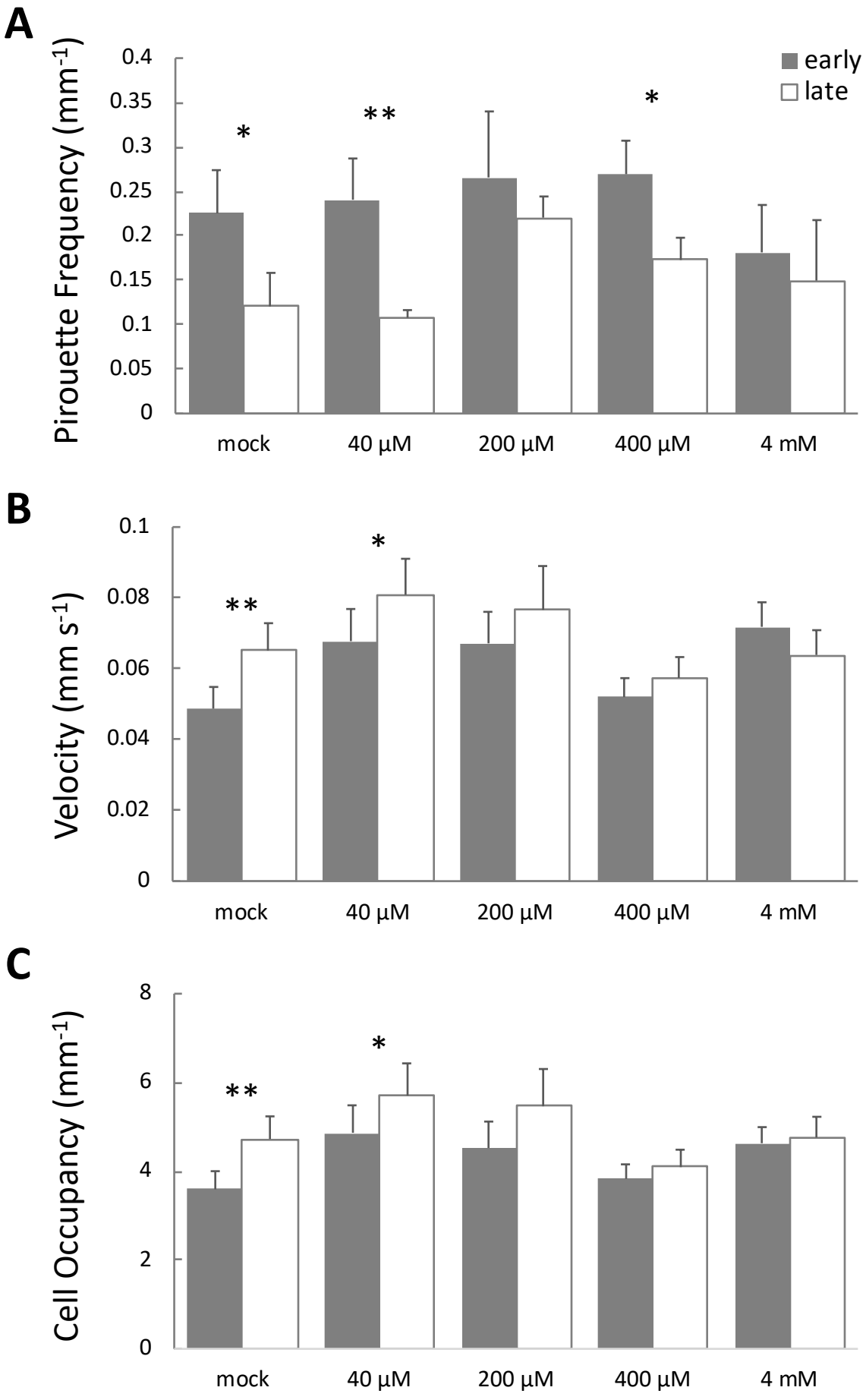
A
Pirouette Frequency (mm-1)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
early
late
*
**
*
mock
40 µM
200 µM
400 µM
4 mM
B
Velocity (mm s-1)
0.1
0.08
0.06
0.04
0.02
0
**
*
mock
40 µM
200 µM
400 µM
4 mM
C
Cell Occupancy (mm-1)
8
6
4
2
0
**
*
mock
40 µM
200 µM
400 µM
4 mM

Fig. 2

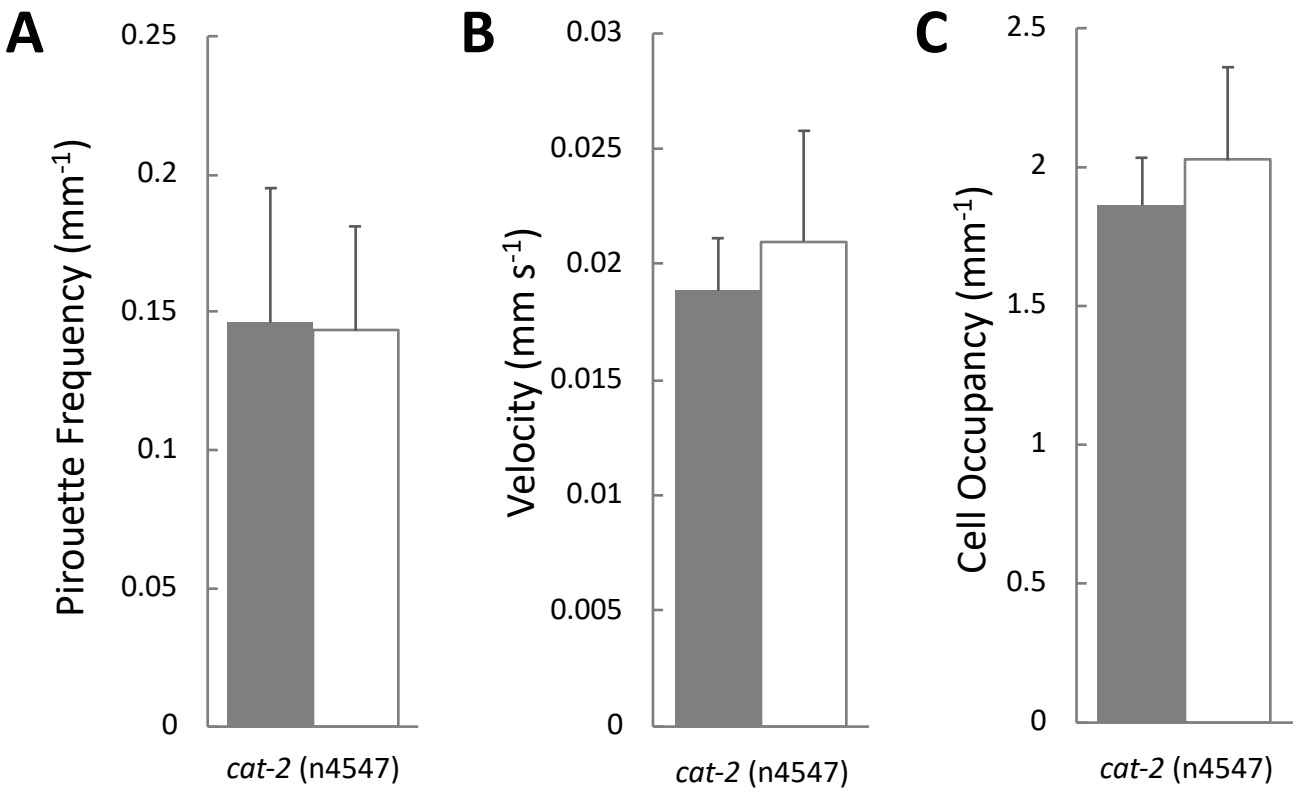
A
Pirouette Frequency (mm-1)
0.25
0.2
0.15
0.1
0.05
0
cat-2 (n4547)
B
Velocity (mm s-1)
0.03
0.025
0.02
0.015
0.01
0.005
0
cat-2 (n4547)
C
Cell Occupancy (mm-1)
2.5
2
1.5
1
0.5
0
cat-2 (n4547)

Fig. 3

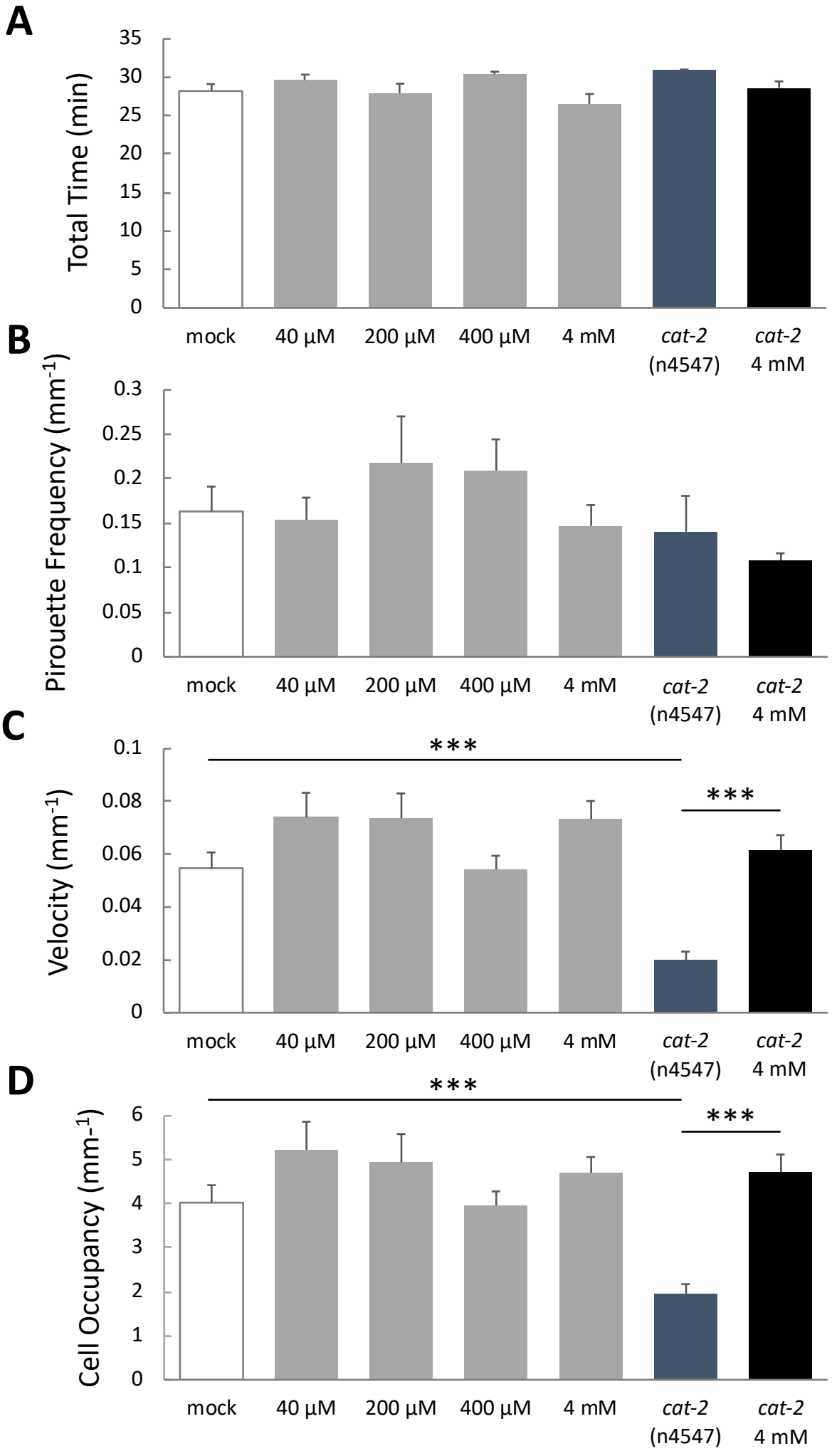
A
Total Time (min)
35
30
25
20
15
10
5
0
mock
40 µM
200 µM
400 µM
4 mM
cat-2 (n4547)
cat-2 4 mM
B
Pirouette Frequency (mm-1)
0.3
0.25
0.2
0.15
0.1
0.05
0
C
Velocity (mm-1)
0.1
0.08
0.06
0.04
0.02
***
***
D
Cell Occupancy (mm-1)
6
5
4
3
2
1
***
***

Fig. 4

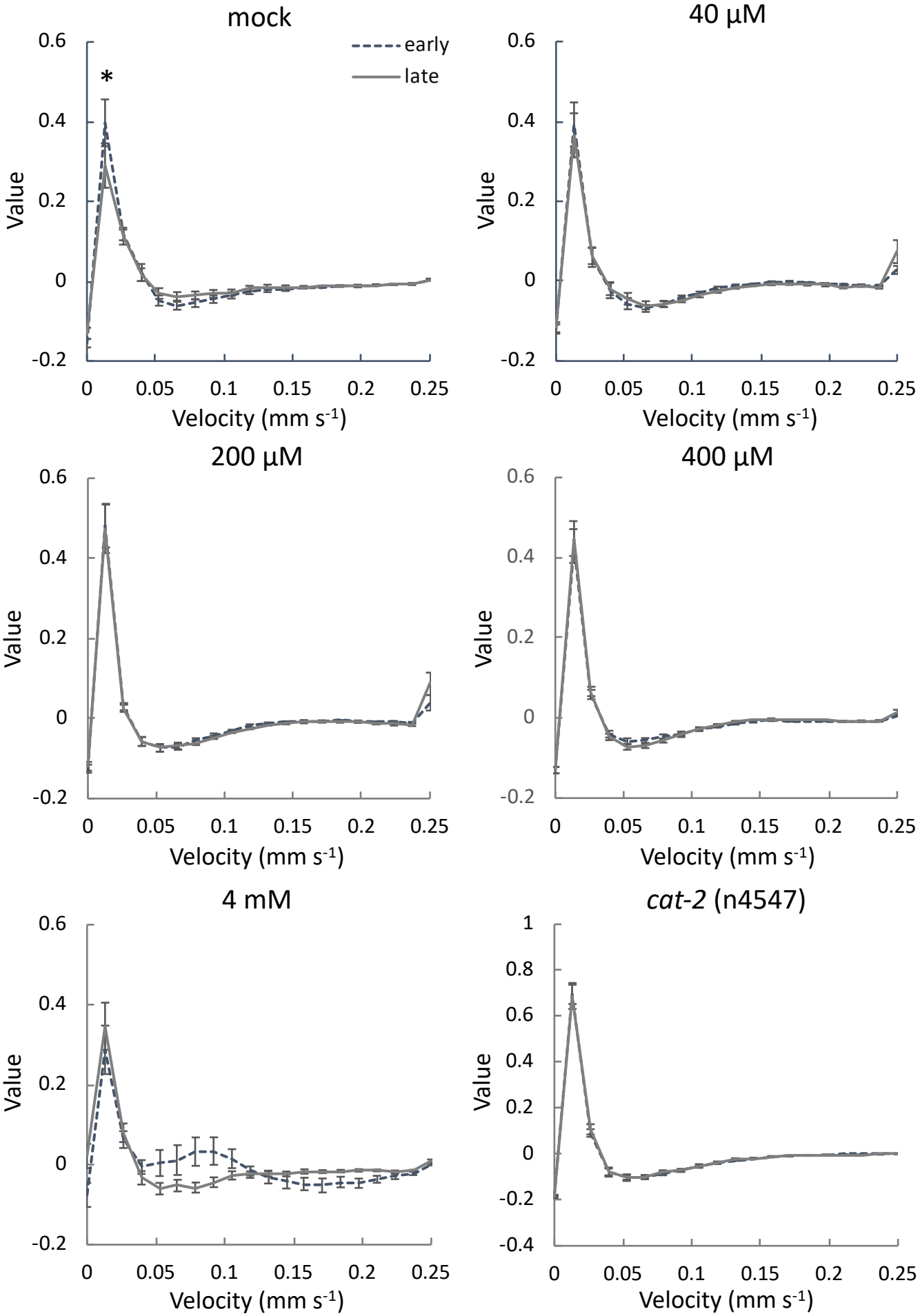
mock
early
late
*
Value
Velocity (mm s-1)
40 µM
200 µM
400 µM
4 mM
cat-2 (n4547)

Fig. 5