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Chemotactic behavior of *Giardia lamblia* and *Trichomonas vaginalis* toward nutrient sources

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Abstract

Chemotaxis is the phenomenon of sensing external concentration gradients by cells and the cellular movement towards or away from the cells. While there have been intensive studies on prokaryotes, little research has been conducted on the chemotaxis in flagellated eukaryotes such as *Giardia lamblia* (*G. lamblia*) and *Trichomonas vaginalis* (*T. vaginalis*). The current study uses a two-chamber assay to discuss the motility of *G. lamblia* and *T. vaginalis* toward simple sugars. The cells were observed moving toward the sugars in a concentration and time-dependent manner. Further, the cell movements were independent of change in osmolarity. Experiments compared the motility of the parasites grown in TYI-S-33 medium and TYI-S-33 medium without glucose (starvation media). It was noted that the starved cells showed a better chemotactic response toward the carbohydrates than the non-starved cells.

Keywords: *Giardia lamblia*; *Trichomonas vaginalis*; chemotaxis; chemoattractant; two-chamber assay; osmolarity

Introduction

Chemotaxis is the directed movement of organisms in a direction towards or away from chemical stimuli according to a concentration gradient. The mechanism of chemotaxis is well characterized in bacteria, where it is known to be mediated by signal transduction via membrane receptors called methyl-accepting chemotaxis proteins and cytoplasmic proteins called Che proteins. A two-component signaling system regulates the movement of the bacterial flagella rotor, leading to the movement toward the chemoattractant (Adler, 1975; Stock, Robinson and Goudreau, 2000; Collins *et al.*, 2018; Korolik, 2019; Hida *et al.*, 2020). The phenomenon of chemotaxis in eukaryotic systems has been thoroughly studied in immune system cells. For example, leukocytes and neutrophils move toward the site of injury because the injured cells/tissue release chemokines (Sullivan and Mandell, 1983; Eddy *et al.*, 2000; Hannigan *et al.*, 2001; Schenkel, Mamdouh and Muller, 2004; Phillipson *et al.*, 2006).

Among the lower eukaryotes, the process of chemotaxis has been described in *Dictyostelium* which is known to be chemotactic towards cyclic adenosine monophosphate (cAMP). The migration in these organisms is via the formation of pseudopodia. Chemotaxis in these cell types occurs via the modulation of actin dynamics to form cellular extensions and cause movement. When it comes to flagellated protozoans, the information regarding chemotaxis is scarce and the mechanism is unknown. Several protozoans have been shown to demonstrate directional motility towards certain stimuli. *Trypanosoma brucei* procyclic forms show positive chemotaxis to actively growing *Escherichia coli* due to an unknown diffusible substance as shown by social motility assays on semi-solid agarose assays (DeMarco *et al.*, 2020). However, the bloodstream forms of the parasite do not show chemotaxis towards host chemokines (Alfituri *et al.*, 2019). The promastigote form of *Leishmania*, the causative agent of leishmaniasis or kalazar, displays positive chemotaxis towards the sugars glucose and fructose as shown by a two-chamber capillary assay (Díaz *et al.*, 2013). Additionally, using

optical tweezers, the strength, and directionality of the force generated to move toward different glucose gradients were analyzed (Pozzo *et al.*, 2009). The human infective metacyclic promastigotes also show directional movement toward a macrophage-derived stimulus (Findlay *et al.*, 2021).

Free-living protozoa such as *Paramecium caudatum* and *Tetrahymena* have also shown chemotaxis to several compounds. *Paramecium* responds to various external stimuli like divalent ions and Guanosine triphosphate (GTP) (Clark, Hennessey and Nelson, 1993; Francis and Hennessey, 1995; Hennessey, 2005). It is also known to show avoidance reactions to odorants and tastants like allyl isothiocyanate, eugenol, piperine, and quinacrine, whereas *Tetrahymena* responded additionally to capsaicin, menthol, chloroquine, and quinine (Rodgers, Markle and Hennessey, 2008). The chemotactic responses are brought about by receptor-mediated membrane depolarization/hyperpolarization with calcium-based action potentials translating to the ciliary movement (Sehring and Plattner, 2004). In *Tetrahymena*, G-protein-coupled receptors (GPCR)-mediated signaling is vital for chemotaxis to proteose peptone and lysophosphatidic acid (Lampert, Coleman and Hennessey, 2011).

In *Giardia lamblia* (*G. lamblia*) and *Trichomonas vaginalis* (*T. vaginalis*), there haven't been many studies on understanding the chemotactic properties of these organisms. A recent study exploring the host-parasite interactions between *T. vaginalis* and vaginal epithelial cells demonstrated that *T. vaginalis* infection can induce the secretion of galectin-3 from host cells which can bind to parasites' cell surface and promote aggregation of cells on the epithelial cell surface (Hsu *et al.*, 2023). They also report increased motility of the parasites upon coculture with vaginal epithelial cells indicating that the parasites may be responding to host factors to promote rapid migration to the epithelial cell surface. These studies indicate the likely crucial involvement of chemotaxis in establishing infection in the host.

The growth media for the parasites is vitamin-rich and contains cysteine, ascorbic acid,

sugars, salts of potassium and iron, etc. *Giardia* specifically requires bile salt as it colonizes the small intestine. When it moves from the stomach to the small intestine to the colon, the perturbation in bile concentration causes the parasite to change its form (cyst to trophozoite to again cyst respectively). Meanwhile, *Trichomonas* requires vitamin-rich media to grow. The media composition of these organisms is complex with specific requirements according to their niche. Additionally, the growth media for the parasites contain glucose, which is the primary source of carbohydrates for ATP generation. Given the complex nature of host-parasite interactions, it is likely that pathogens recognize and respond to multiple signals within the host microenvironment. Out of these, a group of chemicals that the pathogens would likely respond to are carbohydrates since both the parasites utilize glucose as a major source of energy. These essential requirements show us that chemotaxis may help organisms move toward their physiological niche.

Understanding whether these organisms are capable of chemotaxis would immensely aid in gaining insights into their biology and pathogenesis. Here, a simple two-chamber assay was used to identify whether the organisms *G. lamblia* and *T. vaginalis* are capable of chemotaxis towards simple monosaccharides and disaccharides. The assay was previously described in a study to assess chemotaxis to monosaccharides in *Leishmania spp* (López *et al.*, 2021). The two-chamber assay setup (Figure 1) was assembled as follows: the "outer chamber" consisted of the sterile wells of a 96-well plate, while the "inner chamber" was formed by the tips attached to a multichannel pipette. Cells were resuspended in a buffer and added to the outer chamber, while the inner chamber was filled with the experimental substance (buffers or carbohydrate solutions). The 96-well plate containing the cells was positioned on an adjustable clamp stand stage, and the multichannel pipette with the experimental substance was secured to the stand. The stage was then adjusted so that the tips of the pipette dipped approximately 2 mm into the wells of the 96-well plate. This setup was

incubated for varying time periods. After incubation, the solutions from the inner chamber were transferred to clean wells of the 96-well plate. Chemotaxis was assessed by counting the number of cells that migrated into the inner chamber, using a Neubauer hemocytometer. This assay was utilized to examine whether *G. lamblia* and *T. vaginalis* are capable of motility toward various carbohydrate solutions.

Methods

Cell Culture

G. lamblia WB-C6 (assemblage A) cells (obtained from Dr. Sehgal's lab, PGIMER, Chandigarh) were cultured in TYI-S-33 media at 37˚C supplemented with 10% ABS and subcultured every 48 h. The log phase parasites were harvested by chilling on ice for 20 min to dislodge the parasites, followed by centrifugation at 700 x g for 10 min. *T. vaginalis* G3 cells (The strain was kindly provided by Prof. Daman Saluja and Dr. Manisha Yadav, ACBR, New Delhi) were cultured in TYI-S-33 medium supplemented with 10% ABS and 2.5% Diamond Vitamin mix and log phase parasites were subcultured every 24 h as elaborated (Singh *et al.*, 2018).

Two-chamber assay for chemotaxis

The assay used for chemotaxis was adapted from Lopez et al. with minor modifications (López, Díaz et al. 2021). The two-chamber assay was set up as shown in Figure 1. Briefly, a multichannel pipette with the tips containing the desired chemoattractant solution (inner chamber) was attached to a clamp stand. The cells (*G. lamblia / T. vaginalis*) were centrifuged at 700 x g and the pellet was washed with Phosphate-buffered saline (PBS) and resuspended in the following Hepes based buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 mM pH 7.3; NaCl, 132 mM; KCl, 3.5 mM; $CaCl_2$, 1 mM and $MgCl_2$, 0.5 mM) to give a dilution of 3.5 x 10^6 cells/mL. 200 µL of this cell suspension was added to the wells of a 96-well plate (outer chamber). The tips of

multichannel pipette were then dipped into the wells containing the cells up to a distance of ~2 mm. The distance was adjusted using an adjustable stand. The setup was incubated for the desired time periods following which, the solutions in the tips were released into clean wells and the number of cells that travelled into the tips were counted using a Neubauer's haemocytometer. All chemoattractant solutions were made in the Hepes buffer mentioned above at the desired concentrations. The chemoattractants and their respective concentrations are listed in Table 1.

Statistical analysis

Results were reported as Mean ± SD. The biological and technical replicates of the experiments were performed. Grouped data were statistically analyzed using one-way or two-way ANOVA. For one-way ANOVA, Dunnett's multiple comparisons test was used, and for two-way ANOVA, Tukey's multiple comparisons test was applied. All analysis was done using GraphPad Prism 8.0.

Results

G. lamblia *and* T. vaginalis *respond to monosaccharides and disaccharides in a concentration-dependent manner*

The two-chamber assay was performed as depicted in Figure 1a to examine whether the parasites are attracted towards the monosaccharides, glucose, fructose, and galactose. The monosaccharide solutions were made in the Hepes buffer used to resuspend the cells, resulting in 3.5 x 10^6 cells/mL, as described in the methods section. Hepes buffer was used for control. All three monosaccharides were tested using the concentrations of 25 mM, 50 mM and 100 mM. The assay was set up for a time point of 40 min. As shown in Figure 2a and 2b, both the parasites clearly showed a significant attraction towards all the monosaccharides in a concentration-dependent manner with the maximum number of cells moving towards 100 mM of the monosaccharide. The mean number of *G. lamblia* cells that

moved towards glucose was 1.5 x 10^4 , which was higher than those that responded to fructose and galactose (~0.5 x 10^4 cells) whereas, *T. vaginalis* cells showed a higher response to fructose (~1.9 x 10^4 cells) as compared to glucose and galactose (~1.2 x 10^4 cells).

Similarly, the assay was performed to examine the movement of cells towards the disaccharides, maltose, lactose, and sucrose at the concentrations of 25 mM, 50 mM, and 100 mM for 40 min. Both the parasites were observed to respond to all the disaccharides in a concentration-dependent manner as shown in Figure 2c and 2d. Among the disaccharides, maltose and lactose attracted the maximum number of *T. vaginalis* cells (3 x 10^4 cells) compared to sucrose. While *G. lamblia* cells also responded to all the disaccharides, the number of cells moving toward the sugars was less in comparison to *T. vaginalis*.

To understand whether the cells could grow in the presence of these carbohydrate sources, a growth curve experiment was performed with the two parasites with these sugars. The cells grown in media without carbohydrate source were used as a control. Both the parasites were observed to grow in the presence of the monosaccharides and disaccharides provided in the media, as compared to the control cells grown without any sugar as shown in the growth curve represented in supplementary data (Figure S1). This indicates that the parasites may utilize these carbohydrates as nutrient sources which could explain their taxis towards carbohydrates.

G. lamblia *and* T. vaginalis *respond to monosaccharides and disaccharides in a time-dependent manner*

A time-course experiment was performed with the two-chamber assay to assess the time taken by the cells to respond to different monosaccharides and disaccharides. The assay was performed for different time points ranging from 10 min to 60 min using a 100 mM concentration of all the monosaccharides and disaccharides. As observed in Figure 3, a time-dependent increase in the movement towards monosaccharides (Figure 3a and 3b) was

monitored for both parasites. However, the time taken to respond to each sugar was different in both *G. lamblia* and *T. vaginalis*. As observed in Figure 3a, in the assay with 100 mM glucose, with *T. vaginalis*, there was a time lag of 20 min before the number of cells that travelled towards the chemoattractant increased. However, by 30 min, a significantly greater number of cells, i.e. approximately 1.5 x 10^4 cells moved towards the monosaccharides. The maximum number of cells traveling towards the chemoattractant was seen at the 40 min time-point with 100 mM fructose in *T. vaginalis*. The highest number of *G. lamblia* cells, roughly 1.5 x 10^4 cells, migrated towards glucose, followed by galactose in 40 min. However, a comparable number of cells moved towards fructose in 60 min.

Interestingly, in the time-course experiment with disaccharides, both parasites respond significantly earlier to the disaccharides than monosaccharides, as can be seen in Figure 3c and 3d. The number of cells that responded to disaccharides was also markedly higher in comparison to monosaccharides. In the case of sucrose, 2 x 10^4 *T. vaginalis* cells traveled towards it which remained similar throughout the time period, whereas around 3 x 10^4 cells migrated toward both maltose and lactose. In *G. lamblia*, there was an increase in the response to the disaccharides sucrose and lactose with respect to time (~2 x 10^4 cells) whereas the number of cells responding to maltose remained similar (~1.5 x 10^4 cells) throughout the 60 min period.

Cell movement is specific to the carbohydrate and independent of the osmolarity of the solution

These experiments were performed to assess whether the *G. lamblia* and *T. vaginalis* cells responded to the sugars or to change in osmolarity caused by the addition of sugar to the buffer. Towards this, the buffer composition was modified to match the osmolarity produced by the addition of 100 mM of glucose (HEPES, 30 mM pH 7.3; NaCl, 160 mM; KCl, 5 mM; $CaCl_2$, 1 mM and $MgCl_2$, 0.75 mM) and the two-chamber assay was performed to assess the

movement of parasites towards the modified buffer. It was observed that the cells did not respond to the buffer with higher osmolarity (395 mOsm/L) (Figure 4a). Additionally, the cells also did not respond to metronidazole (100 µg/mL), the drug used to cure the infections caused by these organisms.

Additional experiments were performed to confirm our observations regarding the movement of cells towards carbohydrates. First, the assay was also performed when the cells were resuspended in 100 mM glucose solution, and the tips (inner chamber) contained 100 mM glucose solution. In this case, the cells did not move towards the glucose solution in the tips (Figure 4a). Further experiments would shed light on understanding the other aspects of chemotaxis like the preference of cells for one carbohydrate over another.

As shown in Figure 4b, *T. vaginalis* cells, when resuspended in Hepes buffer, showed a high chemotactic response to its growth media. In contrast, when the cells are resuspended in growth media, they did not show a chemotactic response to any of the monosaccharides and disaccharides, implying the cells could sense the most favorable environment of growth media consisting of all the nutrients as opposed to just carbohydrate sources and chose to remain in the favourable condition. Upon resuspending the cells in chemoattractants, their movement towards the growth media was observed. The cells exhibited the highest migration towards the media when resuspended in the buffer, followed by those present in monosaccharides. On the other hand, the *T. vaginalis* and *G. lamblia* cells resuspended in the disaccharides showed the least migration toward the growth media as depicted in Figure 4c and 4d respectively.

Starvation induces the cells to respond faster to glucose

In this assay, *T. vaginalis* cells were cultured in glucose-free TYI-S-33 media for 12 hours. The two-chamber assay was performed with these cells for different periods to examine their chemotaxis towards 100 mM glucose. As shown in Figure 5, the starved cells responded

significantly faster to glucose in comparison to the non-starved cells. The starved cells begin responding to glucose as soon as 10 min, with the maximum number of cells at 30 min. The non-starved cells on the other hand, responded slowly to glucose with the maximum number of cells moved at 50 min. Interestingly, the number of cells migrated drops after reaching the peak. This could be attributed to the diffusion of the chemoattractant from the tips into the wells containing the cells after a particular time point.

Discussion

Chemotaxis in eukaryotes has been described in a multitude of cells ranging from crawling of the amoebae Dictyostelium towards cAMP gradients to migration of fibroblasts towards the site of injury guided by gradients of platelet-derived growth factors. It is a complex process driven by spatial gradients to drive temporal movements. The process can be divided into 3 temporal stages (Ranamukhaarachchi *et al.*, 2024) which involve directional sensing of the chemical gradient, polarization of the cell, and finally motility which results in actual movement of the cell. These movements often involve a dynamic remodeling of the cytoskeleton, either microtubule-based, microfilament-based, or both, which facilitates cell migration towards or away from the substance. Several signaling pathways have been implicated in eukaryotic chemotaxis including those mediated by G protein-coupled receptors and receptor tyrosine kinases. However, the molecular machineries facilitating the coupling of the three modules finally resulting in directional movement is unknown. (Di Cioccio *et al.*, 2004; Jin, 2011; Xu, 2020). In the context of pathogens, research on chemotaxis in flagellated organisms becomes even more pertinent since it would give novel insights into their pathogenesis. As far as protozoan pathogens are concerned, chemotaxis has been demonstrated in Trypanosomes and Leishmania (DeMarco *et al.*, 2020; Shaw *et al.*, 2022). *G. lamblia* and *T. vaginalis* are pear-shaped parasitic protozoans with the former containing four pairs of flagella and the latter containing five flagella. *G. lamblia* is known to show complex

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movement with each pair of flagella moving in a different pattern. In contrast, *T. vaginalis* shows a non-linear jerky movement causing the cell to rotate. Both pathogens establish infection in the host via cytoadherence on intestinal and vaginal epithelial cells. Adhesion to host cells requires the movement of cells across the mucus layers to the epithelial cell surface. This could be achieved by the pathogens' response to concentration gradients of nutrients, hormones, and secreted host proteins within the host microenvironment. A study by B. Sugarman and N. Mummaw, 1988 (Sugarman and Mummaw, 1988) demonstrated the chemorepulsion of *T. vaginalis* from the antimicrobial drug metronidazole. A simple two-chamber assay was used to understand whether *G. lamblia* and *T. vaginalis* are capable of motility towards monosaccharides and disaccharides, which may be used as nutrient sources. The cells were observed to move toward both monosaccharides and disaccharides in a concentration and time-dependent manner. Since *G. lamblia* thrives in the intestine which is a nutrient-rich environment, it provides a possible explanation for the chemoattraction towards various carbohydrates. *T. vaginalis* on the other hand, is a urogenital pathogen and was also observed to move towards all the sugars. The growth curve experiment showed that *T. vaginalis* showed increased cell densities in the presence of both monosaccharides and disaccharides as compared to media without carbohydrates. The parasite is also known to secrete alpha-glucosidase enzymes which can break down disaccharides like maltose (ter Kuile and Müller, 1995). Interestingly, the protozoan also showed increased movement to fructose in comparison to the other monosaccharides glucose and galactose. Fructose is known to be found in the cervical mucus of women and in the semen of human males. (van der Linden *et al.*, 1992). *T. vaginalis* is also known to be present in the semen of infected men. Similarly, other disaccharides like maltose and sucrose are also present in vaginal secretions. The vaginal mucosal environment is highly complex and *T. vaginalis* needs to adapt to the changes in the physicochemical environment throughout the menstrual cycle.

The time-dependent experiments depicted that although *T. vaginalis* cells moved faster toward galactose, the maximum number of cells moved toward fructose. In the case of *G. lamblia*, the number of cells moving toward glucose and galactose was similar whereas the cell response was more or less the same towards fructose in the 60 min time period. The *T. vaginalis* cells were observed to respond to disaccharides within 10 min of the setup with a higher number of cells moving towards maltose. In comparison, *G. lamblia* cells showed comparable movement toward the three disaccharides. Overall, when disaccharides were used as chemoattractants, a larger number of *G. lamblia* and *T. vaginalis* cells traveled toward the tips in a shorter time as compared to when monosaccharides were used. This may be due to the presence of two molecules of monosaccharide in a disaccharide.

To ensure that the movement of the parasites towards the carbohydrates was a specific response, various controls were tested. First, the dependence of cell movement on osmolarity was checked. Cells were resuspended in the buffer and their movement towards the buffer having an osmolarity akin to 100 mM glucose and towards the metronidazole drug was analysed. Negligible chemotaxis was observed. This behavior was anticipated given that metronidazole is used to treat infections caused by these parasites. Another assay was set up with cells resuspended in 100 mM glucose and moving towards 100 mM glucose but no cell movement was observed.

The favorable environment of the cells was checked firstly by resuspending the cells in the buffer and checking their motility towards the growth media. A considerable number of cells showed movement. Secondly, the cells were resuspended in the growth media and the cell movement towards different carbohydrates was noted. No chemotaxis was observed in this case. Based on these observations, it can be inferred that the cells have more affinity to the growth medium. Conversely, when the cells were resuspended in chemoattractants, the movement towards the media was observed. The highest number of cells traveled to the

media when resuspended in the buffer, followed by the cells in monosaccharides. The number of cells traveling towards the growth media was the least when the cells were resuspended in disaccharides. As seen in Figure 2, the movement of cells towards disaccharides was more.

Additionally, a study was done to compare the response of starved and non-starved *T. vaginalis* cells. As compared to non-starved cells, a greater number of starved cells showed movement towards 100 mM glucose in less time. This indicated that the parasites have certain mechanisms to sense the availability of nutrients hence more starved cells moved towards glucose.

Conclusion

A quantitative method to assay chemotaxis in protozoans was demonstrated. The response of *G. lamblia* and *T. vaginalis* towards carbohydrates which are nutrient sources for the organisms was observed. This assay could also be used to examine chemotaxis towards other molecules like hormones and second messengers like cAMP and Ca^{2+}. The underlying mechanism of chemotaxis can be studied. This would be useful in analyzing the behavior of the organisms in their host environment. An insight is given into a basic, yet previously unknown phenomenon in these parasites and holds the potential to spur further investigation into molecular mechanisms involved.

Supporting information. The supplementary material for this article can be found at [DOI].

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Author's contribution. AS conceived and designed the study, conducted experimental work, analyzed the data, interpreted the results, and drafted the manuscript. AK assisted with the conceptualization, data analysis, data curation, interpretation, and validation. SR assisted

with data analysis, interpretation, validation, and manuscript editing. PJ provided cell culture and assisted with data validation and manuscript editing. UT assisted with conceptualization, funding acquisition, supervision, validation, and manuscript review and editing.

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Table 1. Chemoattractants and their respective concentrations used

Chemoattractant		Concentration
Monosaccharides	Glucose	25 mM, 50 mM, 100 mM
	Fructose	25 mM, 50 mM, 100 mM
	Galactose	25 mM, 50 mM, 100 mM
Disaccharides	Lactose	25 mM, 50 mM, 100 mM
	Sucrose	25 mM, 50 mM, 100 mM
	Maltose	25 mM, 50 mM, 100 mM
Drug	Metronidazole	100 µg/mL

Figure 1. Experimental setup. a. Schematic representation of two-chamber assay set-up for chemotaxis. b. Workflow of the two-chamber assay.

Figure 2. Chemotactic response of *G. lamblia* and *T. vaginalis* to monosaccharides and disaccharides. The assay was setup for 40 min and the graphs depict the number of a. *T. vaginalis* and b. *G. lamblia* cells that have traveled to the tips containing 25 mM, 50 mM, and 100 mM glucose, fructose, and galactose. The graphs show the number of c. *T. vaginalis* and d. *G. lamblia* cells that have traveled to the tips containing 25 mM, 50 mM, and 100 mM lactose, sucrose, and maltose. (N=3 independent experiments). Two-way ANOVA was performed and Tukey's test was done for comparison. Here, ns depicts $p > 0.05$, ** depicts $p \leq 0.005$, *** depicts $p \leq 0.0005$ and **** depicts $p \leq 0.0001$.

Figure 3. Time-dependent dynamics of the chemotactic response of *T. vaginalis* and *G. lamblia* to monosaccharides and disaccharides. a. Graph depicting the number of *T. vaginalis* cells moved towards monosaccharides viz glucose, fructose, galactose, and no carbohydrate (Hepes buffer as control) at different time points. A time-dependent increase in response to all carbohydrates is observed. b. Similarly for *G. lamblia cells*, a time-dependent movement toward monosaccharides- glucose, fructose, galactose and Hepes buffer is shown. A significant no. of cells moved toward all the sugars as compared to Hepes buffer (control). c. Graph depicting the number of *T. vaginalis* cells moved to tips containing disaccharides lactose, sucrose, maltose, and no carbohydrate (Hepes buffer) at different times of incubation. A time-dependent increase in response to the all the disaccharidesobserved. d. Correspondingly, the no. of *G. lamblia* cells migrated toward disaccharide at diffrent time point is shown. A time-dependent increase in response to sucrose and lactose is observed, whereas maltose shows no significant change across different incubation times. (N=3 independent experiments). ns $p > 0.05$, **$p \leq 0.005$, ***$p \leq 0.0005$, ****$p \leq 0.0001$.

Figure 4. Chemotactic response of *T. vaginalis* and *G. lamblia* to osmolarity changes and

growth media. a. The graph depicts the number of *T. vaginalis* and *G. lamblia* cells moving to Hepes buffer (295 mOsm/L and 395 mOsm/L), metronidazole drug, and 100 mM glucose when the cells are resuspended in Hepes buffer (295 mOsm/L). There is no chemotactic response in either of the Hepes buffers and metronidazole. Similarly, the cells resuspended in gluocse and moving toward glucose did not show chemotactic response. However, the movement towards 100 mM glucose when cells are resuspended in the Hepes buffer is depicted for comparison (in grey bar). b. Graph depicting the chemotactic response of *T. vaginalis* cells to monosaccharides and disaccharides when the cells are resuspended in growth media. No response is seen in these conditions. In the case of control (shown in pink bar), a movement towards growth media is observed when the cells are resuspended in the Hepes buffer. Graph depicting the chemotactic response of c. *T. vaginalis* and d. *G. lamblia* cells to growth media when the cells are resuspended in various monosaccharides and disaccharides. When resuspended in chemoattractants, fewer cells show movement toward growth media as compared to Hepes buffer. (N=3 independent experiments). ns $p > 0.05$, **$p \leq 0.005$, ***$p \leq 0.0005$, ****$p \leq 0.0001$.

Figure 5. Time-dependent dynamics of chemotactic response of *T. vaginalis* cells to glucose after growth in glucose-free media. When grown in glucose-free media for 12 h, *T. vaginalis* cells respond faster to glucose, within 10 min, whereas, non-starved cells grown in media supplemented with glucose demonstrate a slower response. (N=3 independent experiments). ns $p > 0.05$, **$p \leq 0.005$, ***$p \leq 0.0005$, ****$p \leq 0.0001$.

Figure 1

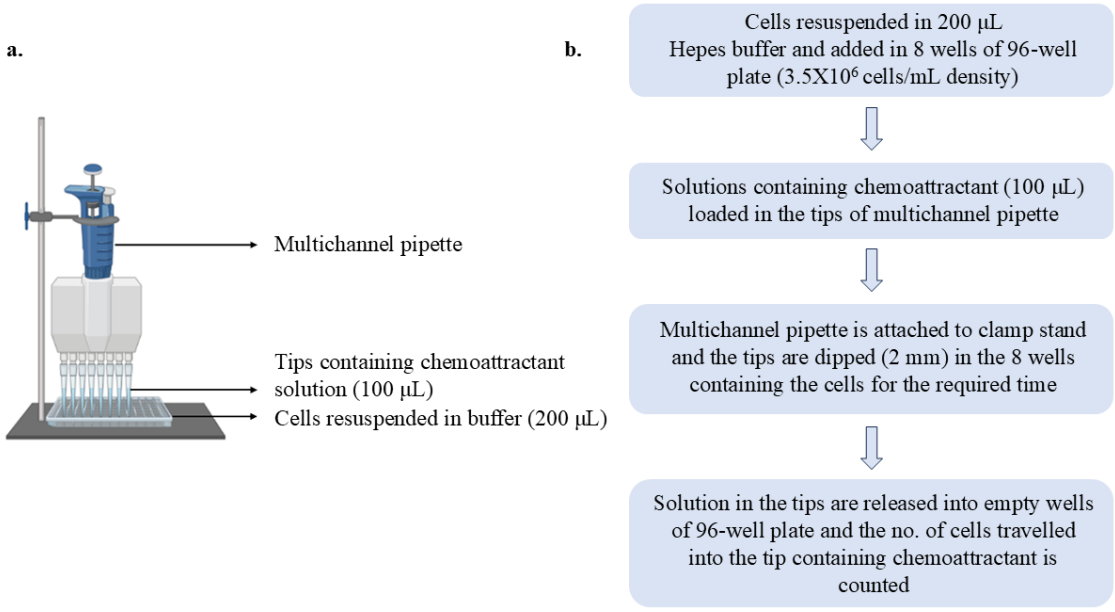


Figure 2

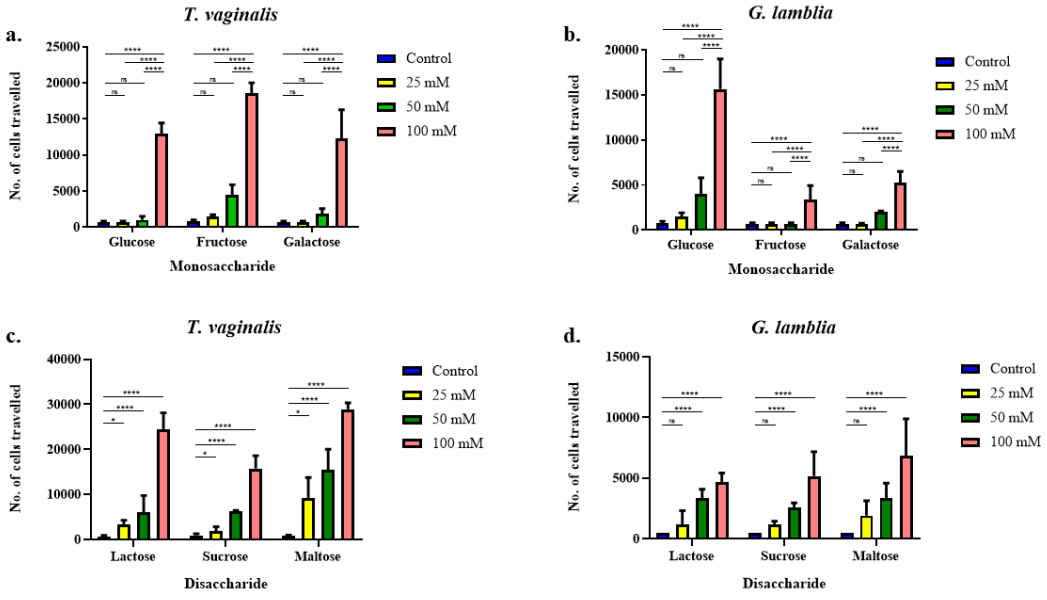


Figure 3

https://doi.org/10.1017/S0031182025000484

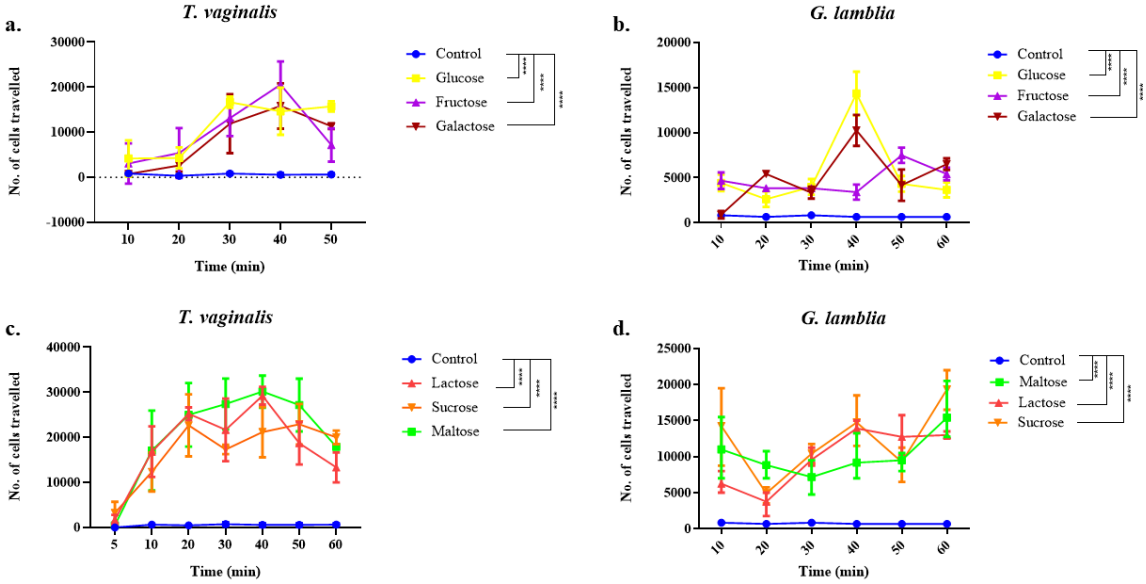


Figure 4

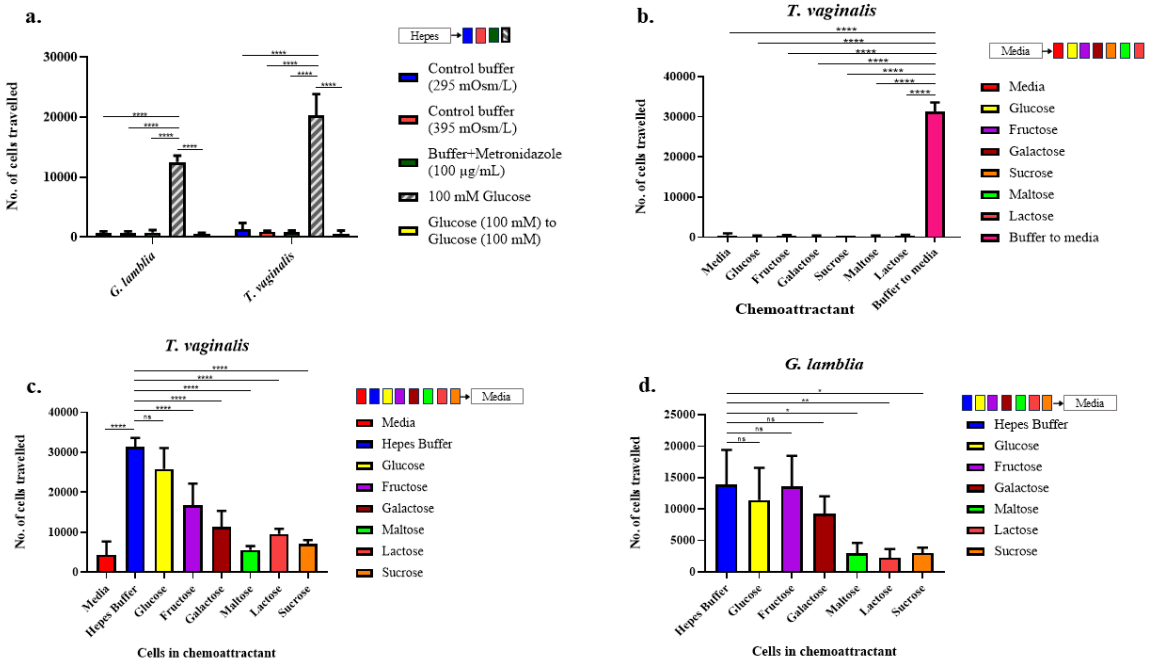
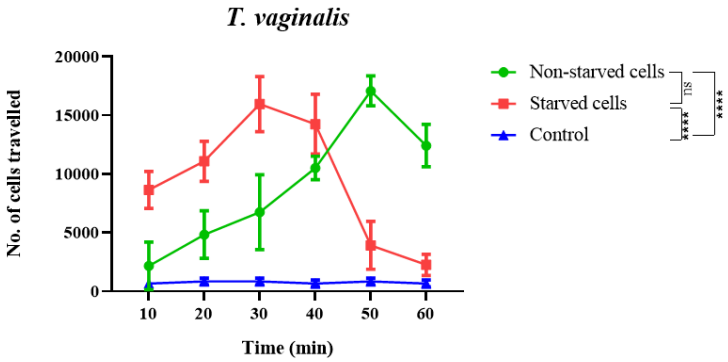


Figure 5

https://doi.org/10.1017/S0031182025000484



Graphical_abstract