

An interdisciplinary approach to investigate collective cell migration in neural crest

Rasa Giniūnaitė¹, Rebecca McLennan², Mary C McKinney², Ruth E Baker¹, Paul M Kulesa^{2,3} and Philip K Maini¹

¹Wolfson Centre for Mathematical Biology, Mathematical Institute, University of Oxford, Woodstock Road, Oxford, OX2 6GG, United Kingdom.

²Stowers Institute for Medical Research, 1000 E 50th Street, Kansas City, MO 64110, USA.

³Department of Anatomy and Cell Biology, University of Kansas School of Medicine, Kansas City, KS, 66160, USA.

Corresponding author: Rasa Giniūnaitė, *e-mail address:* rasa.giniunaite@maths.ox.ac.uk.

Keywords: collective cell movement, neural crest, interdisciplinary studies, mathematical modelling

Abstract

The neural crest serves as a powerful and tractable model paradigm for understanding collective cell migration. The neural crest cell populations are well-known for their long-distance collective migration and contribution to diverse cell lineages during vertebrate development. If neural crest cells fail to reach a target or populate an incorrect location, then improper cell differentiation or uncontrolled cell proliferation can result. A wide range of interdisciplinary studies has been carried out to understand the response of neural crest cells to different stimuli and their ability to migrate to distant targets. In this critical commentary, we illustrate how an interdisciplinary collaboration involving experimental and mathematical modelling has led to a deeper understanding of cranial neural crest cell migration. We identify open questions and propose possible ways to start answering some of the challenges arising.

1 Introduction

Collective migration is a common phenomenon that is observed to arise at many different length scales ranging from the micron level to kilometers. For example, in embryonic development many processes rely on cells moving collectively either in sheets or as individuals (Freshney and Freshney, 2004; Kedrin et al., 2008); in humans, a major area of research is the understanding of crowd dynamics (Marsan et al., 2008; Bellomo and Gibelli, 2015; Bellomo and Bellouquid, 2015); in insects, the phenomenon of swarm dynamics is well-studied (Fetecau and Guo, 2012; Parisot and Lachowicz, 2016; Bernardi et al., 2018a). These are only a small sample of the vast number of examples of this phenomenon. In this Critical Commentary, we narrow our focus to one example, namely, cranial neural crest (NC) cell migration in chick and illustrate how an interdisciplinary approach combining experiments and computational modelling has enabled us to investigate a number of key biological questions concerning their collective migration.

NC cells make a vital contribution to structures that include the head, heart, and peripheral nervous system. NC cells delaminate from the dorsal neural tube and traverse along well-defined migratory routes to precise targets. NC cells exhibit a wide range of collective behaviours depending on the organism and axial level of origin (Figure 1). This great variety of emerging behaviours makes the NC system an important model paradigm for collective cell migration. Therefore, the insights gathered from studying NC cell behaviour are not only crucial to understanding embryogenesis and the reasons for certain developmental defects, but could potentially provide important insights for other collective cell migration systems, including regulation of wound healing, and metastatic cancer progression (Haeger et al., 2015; Hendrix et al., 2007; Kasemeier-Kulesa et al., 2008).

The wide range of potential signalling mechanisms involved in the collective migration of NC cells makes it challenging to identify the key factors that ensure successful invasion (defined here as collective occupation of the whole domain). In addition, there are many different types of cell-cell and cell-tissue interactions, such as cell-adhesion and cell-induced tissue alterations, possibly involved and these have to be carefully investigated. Crucially, the NC cell system is experimentally tractable, and so it is possible to generate the appropriate data to investigate certain signalling mechanisms. However, the consistency of experimental hypotheses in the light of observations can sometimes be challenging to assess due to the sheer number and complexity of the signalling pathways involved. Mathematical

models can incorporate multiple simultaneous and interacting inputs and compute outputs which are beyond our intuitive thinking. Therefore, models can be used to validate experimental hypotheses and help unravel the mechanisms that drive collective cell migration.

Various experimental and computational studies have been carried out to understand the behaviour of distinct NC cell populations in different species (see, for example, the reviews by Theveneau and Mayor, 2010; Kulesa and McLennan, 2015; Schumacher et al., 2016; Szabó and Mayor, 2016; Szabó and Mayor, 2018). These distinct populations of NC cells emerge at different axial locations and, after migration and differentiation, contribute to diverse cell lineages, including cartilage, connective tissue, melanocytes and glia (Le Douarin, 2004; Rogers et al., 2012). Most of the different populations of NC cells are guided by different signalling mechanisms. This is understandable as characteristics such as NC cell-cell adhesion, cell-microenvironment interactions, distances traversed and time to reach targets, all differ depending on the organism and axial level.

Interdisciplinary studies have demonstrated that the combination of contact inhibition of locomotion (CiL, which is a process during which migratory cells momentarily stop upon physical contact and subsequently repolarise to move in the opposite direction) and co-attraction (CoA, a mutual cell-cell attraction at distances larger than a cell diameter mediated by contacts of thin filopodia) mechanisms are sufficient to explain collective migration of cranial NC cells using *Xenopus* and zebrafish research organisms (Carmona-Fontaine et al., 2008, 2011; Woods et al., 2014; Szabó et al., 2016a). *Xenopus* NC cells initially migrate as a cohesive tissue (Alfandari et al., 2010). More recent studies suggest that cell polarisation is also crucial for successful cell invasion but it is naturally induced by CiL and CoA, which are modulated by dynamical interactions of Rac1 and RhoA (Merchant et al., 2018).

Studies of chick NC cell migration reveal that the main migratory driving forces for these cells vary across different axial levels and involve chemotaxis. Simpson et al. (2007) investigated the main mechanisms regulating successful invasion and colonisation of the gut by vagal NC cells in chick embryos. Their mathematical model suggested that cell invasion is driven by a combination of motility and proliferation of the NC cells at the front of the stream, whereas the cells behind the front do not contribute to invasion. These results were verified experimentally and used to provide insights to understand Hirschsprung’s disease, which is characterised by a failure of NC cells to fully migrate throughout the developing gut (Landman et al., 2007). In contrast to Simpson et al.’s (2007) model for chick

vagal NC cells, McLennan et al. (2010) demonstrated that chemotaxis induced by vascular endothelial growth factor (VEGF) plays a fundamental role in chick cranial NC cell invasion. Stromal cell-derived factor (Kasemeier-Kulesa et al., 2010; Theveneau et al., 2010), glial cell-derived neurotrophic factor (Lake and Heuckeroth, 2013), brain-derived neurotrophic factor (Kasemeier-Kulesa et al., 2015), platelet-derived growth factor (Eberhart et al., 2008; He and Soriano, 2013) and fibroblast growth factors (Sato et al., 2011) are other important chemotactic factors in NC cell migration.

The remainder of this paper is organised as follows. In Section 2, we briefly introduce some basic biological knowledge of chick cranial NC cell invasion. In Section 3, we discuss a study that suggests that different cell phenotypes are necessary for successful cell invasion – these have been termed “leaders” and “followers”. In Section 4, we ask the question: how many leader cells are required to ensure robust invasion? The findings that demonstrate these phenotypes to be plastic are presented in Section 5. In Section 6, we present results that suggest cell speed is regulated by an extracellular protein signal (DAN). We conclude, in Section 7, with a short discussion and list some open questions that still need to be addressed.

2 What is known about chick cranial neural crest cells?

Chick cranial NC cells delaminate from the dorsal neural tube, at the midbrain and hindbrain levels, including rhombomere (r) segments of the hindbrain. We focus on the cranial NC cell migratory stream that emerges adjacent to r4 and migrates through the growing embryonic tissue into branchial arch 2 (ba2) (Figure 1). Cranial NC cells differentiate and contribute to cartilage, bone, teeth, connective tissue and cranial neurons (Rogers et al., 2012). Until recently, it has been mostly unclear what the main molecular signals that drive the collective migration of chick cranial NC cells are. An experimental study on the expression of neuropilin genes in these cells (Kulesa and McLennan, 2006) helped to generate a hypothesis that VEGF, to which neuropilins bind, plays an important role in migration. McLennan et al. (2010) investigated this hypothesis and discovered that indeed, VEGF acts as a chemoattractant for NC cells. The absence of VEGF at the target site, or the inability of NC cells to read out the VEGF signal by knockdown of the neuropilin-1 receptor, led to a loss of directed migration and failure of NC cells to invade ba2. These data suggested that local known inhibitory signals that typically

maintain the segregation of cell migratory streams prevent the cells from further dispersion since NC cells collapsed protrusions and remained at the entrance to ba2. McLennan et al. (2010) also demonstrated that NC cells are attracted by VEGF *in vitro* and that migrating cranial NC cells changed direction and moved towards ectopic *in vivo* sources of VEGF placed either adjacent to a cranial NC cell migratory stream or within a stream (McLennan et al., 2015b). They also observed that before the NC cells enter the migratory domain, VEGF protein is expressed throughout the surface ectoderm directly overlying the NC cell migratory pathway and becomes expressed in the ba2 target tissue (McLennan et al., 2010). These findings suggested that a possible explanation for collective NC cell migration is chemotaxis up to gradients in VEGF. To test this hypothesis for collective migration, a combined experimental and mathematical modelling study was carried out in McLennan et al. (2012) and we summarise their results below.

3 Is a cell-induced VEGF gradient sufficient for cranial neural crest cell migration?

The NC cells migrate through a three-dimensional curved domain. It is known that the depth of the domain is about three to five cell diameters. Therefore, to investigate computationally whether a cell-induced VEGF gradient can account for collective chick cranial NC cell invasion, McLennan et al. (2012) used a two-dimensional flat domain as an approximation of the full geometry. In their model, cells were represented as discrete individuals (individual-based model (IBM)), while the VEGF concentration was modelled as a continuum. The cells were modelled as non-overlapping circular discs that move by randomly sampling the VEGF concentration at a certain number of points a fixed distance away from their centre in randomly chosen directions (Figure 2) and then moving towards higher levels of VEGF. This is a simple way to model cells sending out filopodia to sense the VEGF gradient. The elongation of the domain was prescribed, based on empirical measurements (McLennan et al., 2012). McLennan et al. (2012) assumed that VEGF is consumed and/or degraded by the cells, setting up a gradient to which cells respond by becoming polarised and moving up the gradient. In their model for VEGF dynamics, they included VEGF diffusion, production, uptake by cells and dilution due to domain growth. This led to a reaction-diffusion equation on a growing domain. One of the key issues was to choose appropriate boundary conditions. The initial studies assumed that the VEGF

concentration was fixed at zero on the lateral boundaries creating an inward pointing VEGF gradient to ensure that the cells do not leave the domain (McLennan et al., 2012). Later studies used zero flux boundary conditions for VEGF, and the key results of the model were independent of the choice of these two different sets of boundary conditions (McLennan et al., 2015b,a, 2017).

Initially, McLennan et al. (2012) assumed that all the cells are homogeneous in their behaviour. Their computational results revealed that, in this case, the cell stream breaks, meaning that there are large gaps in the stream. This behaviour is due to the fact that, for biologically realistic parameter values, the VEGF gradient is highly localised, moving with the leading cells and away from those cells newly entering the domain (Figure 3, left). Although the model predicted that some cells reach ba2, the experimentally observed intact streams could not be generated to allow a sufficient number of cells to reach their target. Hence, the hypothesis that effective collective cell invasion can result from a cell-induced gradient is not consistent with experimental observations. However, on implementation of a new hypothesis, namely that there are two NC cell phenotypes, “leaders” that generate and move up the gradient of chemoattractant and “followers”, that follow (unspecified) signals from the leaders, the model simulations suggested that successful streaming migration is possible (Figure 3, right).

These model driven hypotheses were verified experimentally using qPCR gene profiling and multiplexed imaging of mRNA expression using a fluorescent hybridisation chain reaction method (McLennan et al., 2012). They observed that the gene expression profiles of cells at the front of the stream (leaders) differed significantly from the profiles of the cells at the back of the stream (followers). The characteristics of followers were verified by showing that *in vivo* knock-down of VEGF did not affect their migration. These findings were further validated by subsequent investigations: for example, the model was used to make predictions on the effects of (i) removing followers, (ii) transplanting leaders from a donor to the back of the stream in a host. In parallel with the simulations, biological experiments were carried out and their results validated the model predictions. However, model predictions for a third experiment – implanting followers from a donor ahead of the host leaders and profiling the cells at a later stage – did not agree with experimental results. The conclusion of this experiment was that cell phenotype is not fixed and that microenvironmental signals, including VEGF, regulate leader/follower cell behaviours and gene expression cell phenotype (see Section 5).

To summarise, this interdisciplinary study showed that the hypothesis of a cell-induced gradient, while verbally elegant and persuasive, was not wholly correct. Instead, the computational model predicted that, in addition, cell phenotype heterogeneity is required, and this prediction was validated experimentally. This new hypothesis leads to several further questions, some of which we now discuss.

4 How many leaders are necessary for successful invasion?

Having established that there appears to be specialised cells (leaders) at the front of the invading stream, a natural question to ask is how many leaders are necessary for successful invasion. However, McLennan et al.'s (2012) model is not sufficiently detailed to answer such a question, as experimental data are not yet at the level of detail required to allow very accurate identification of parameter values. Further, experimental photoablation methods to delete leader cells are difficult to interpret due to collateral damage of surrounding tissue within the cell migratory pathway (Stark and Kulesa, 2007). Therefore, the model is more appropriate for making qualitative predictions, and McLennan et al. (2015a) used their model to investigate how invasion depended on the proportion of cells that are leaders. The model predicted that it is possible for cells to invade *ba2* for a wide range of leader cell numbers, but that beyond a certain (small) number of leaders, the total number of cells in a stream will decrease (Figure 4).

To test these predictions, McLennan et al. (2015a) performed single-cell gene expression profiling using real-time qPCR. They discovered regional differences in gene expression and identified a unique stable molecular signature of the cells at the front of the migrating NC stream. *HAND2* and *BAMBI*, both known for their importance in development (Holler et al., 2010; Onichtchouk et al., 1999), were the two genes highly expressed by the cells within the most invasive front of the stream, and their overexpression resulted in alterations of migratory patterns, consistent with the predictions of the model.

The question of the optimal number of leaders in a group of collective individuals is one of general interest and has been addressed in a number of biological systems, for example, lateral line in zebrafish embryos (Colak-Champollion et al., 2019), angiogenesis (tip and stalk cells as leader and followers, respectively) (Palm et al., 2016), border cells (Cai et al., 2016), and bees (Bernardi et al., 2018b), to name only a few.

5 Is cell phenotype predetermined?

As mentioned in Section 3, the experiments of McLennan et al. (2012) suggested that cells may switch their phenotype in response to VEGF. This finding was further investigated in McLennan et al. (2015b).

To investigate the importance of plasticity in cell phenotype, McLennan et al. (2015b) updated their computational model to include phenotype switching. They assumed that cell phenotype is determined by the gradient of VEGF, with cells that are exposed to gradients above a threshold value for a certain time becoming leaders while those in low gradients become followers. A natural question to ask when implementing such a mechanism is whether the timescale of cell phenotype switching has a significant effect on migration efficiency. Computational modelling predicted that switching times from leader to follower and vice versa should be similar to each other to ensure optimal migration efficiency.

To test the hypothesis of the plasticity of gene expression, McLennan et al. (2015b) performed experiments in culture to test the effect of the addition and removal of VEGF. They observed rapid (of the order of a few minutes) changes in relevant gene expression. This result validated the modelling assumption that cell phenotype is determined by VEGF concentration. Further, previous studies have shown the plasticity of lead NC cells to reroute to a head target in response to either physical barriers placed in front of a migratory stream (Kulesa et al., 2005) or laser ablation of leader cells (Richardson et al., 2016).

The model was then used to predict the outcome of an *in vivo* experiment in which cells behind the front were exposed to VEGF. The prediction was that these cells would become leaders, break away from the initial stream, and move towards the new source of chemoattractant. This prediction was validated experimentally (McLennan et al., 2015b). Note that these observations differ from the CiL and CoA model that proposes no role for chemoattractant in the collective migration of NC cells (Carmona-Fontaine et al., 2008, 2011), but require a constrained 2D boundary in order for cells to propagate in a forward directed manner (Szabó et al., 2016b).

6 What influences neural crest cell speed?

The studies described above help us to understand how a number of different factors conspire to play key roles in NC cell migration. One issue with the model is that it is possible for some leader cells to invade too far ahead of the stream and therefore not be able to signal to followers, causing stream break up (leader cells moving too fast might eventually leave followers behind, Figure 3, right). This observation suggests that cell speed needs to be tightly controlled. McLennan et al. (2017) observed that, in the early stages of invasion, DAN, a BMP inhibitor, is expressed within the paraxial mesoderm adjacent to the dorsal neural tube and extending in the antero-posterior direction along the hindbrain. As the NC cells moved through the migratory domain, DAN expression decreases where NC cells have migrated through. *In vitro* experiments showed that cells tended to be inhibited from moving on stripes of a matrix on which DAN protein was present. A detailed study showed that knockdown of DAN led to enhanced cell speed and directionality, while overexpression of DAN significantly reduced the number of invading cells. From these observations, McLennan et al. (2017) concluded that DAN may act to reduce cell speed close to the neural tube, in order to maintain better stream cohesion.

To test the validity of this hypothesis using the model, McLennan et al. (2017) simulated scenarios with changes in DAN expression incorporated as changes in cell speed in the region adjacent to the neural tube. The results were consistent with experimental observations and, indeed, reduction in cell speed corresponding to overexpression of DAN led to inhibition of cell invasion, whereas downregulation led to increased distances travelled by a few leader cells, but the stream broke more frequently (Figure 5).

As mentioned in the Introduction, NC cells serve as an important paradigm for cell invasion. In particular, NC cells are the precursors of many of the most invasive cancers, such as melanoma. McLennan et al. (2017) confronted melanoma cells with DAN *in vitro*, and observed that they avoided the DAN-rich regions. From this observation, they concluded that DAN could be a candidate inhibitor of human metastatic melanoma. This study provides an example of where a deeper understanding of what controls and regulates cell behaviour in developmental biology could have a significant impact on our understanding of certain diseases.

Maiuri et al. (2015) have demonstrated using an interdisciplinary approach that cell persistence is robustly coupled to cell migration speed via actin flows in many different

cell types, such as pigment epithelial or dendritic cells, and propose this as a universal mechanism. Our recent studies have revealed that a water channel protein AQP-1 also regulates NC cell speed and affects invasive abilities (McLennan et al., 2019).

7 Discussion

In Alan Turing’s seminal 1952 paper (Turing, 1952) he states of his model, “This model will be a simplification and an idealization, and consequently a falsification. It is to be hoped that the features retained for discussion are those of greatest importance in the present state of knowledge.” A corollary of this statement is that if a model is successful, it will change the present state of knowledge, thus requiring us to re-evaluate and modify the model with this new knowledge – a process now commonly known as “predict-test-refine-predict”. In this Commentary, we have showcased this process in the context of cranial NC cell migration. We have shown how the integration of experiment with theory can lead to new insights into fundamental processes in biology, with mathematical modelling used to test experimental hypotheses, generate new hypotheses and make predictions that can then be tested experimentally. Crucial to this collaboration (see Figure 8 for more information about our collaborative group) is the identification of well-defined problems that can be investigated by both modelling approaches, namely experimental models (in this case, chick) and theoretical models (in this case, a hybrid computational model). Having defined such a problem, the computational model simulation study and the biological experimental study are carried out in parallel, but with no communication during this process to ensure complete independence between each study. Only when the studies have been completed are they compared with each other. Using this approach, we showed how a mathematical model was able to refute the experimental hypothesis that successful cranial NC cell invasion is the result of a single cell type responding to a cell-induced chemotactic gradient but, instead, that heterogeneity in cell response (phenotype) is required. This new mathematical model led to predictions that were invalidated experimentally, suggesting that cell phenotype was not fixed. The resultant modified model gave rise to predictions that were then consistent with experimental results that manipulated the chemoattractant gradient. Furthermore, the model was able to make correct predictions on the results of manipulating cell phenotype, and suggested a role for the BMP inhibitor DAN that is expressed within the initial subregion of the cranial NC cell migratory pathway.

Current computational models, including the one we discussed in this review, are mainly used to produce qualitative predictions. Faster and more powerful quantitative techniques in both biological data acquisition and data storage, manipulation, and presentation are likely to lead to more refined models that could be used to make more accurate quantitative predictions. To illustrate this paradigm, we use the example of the insight that distinct cell phenotypes are required for collective cell migration, as discussed in Section 3. The necessity of distinct leader and follower phenotypes is a qualitative prediction, and detailed gene expression analysis has to be performed to investigate phenotypic differences across cells. Recent measurements of the whole transcriptome suggest a continuum of cell states, from the most leader-like cells to the most follower-like cells. Schumacher’s (2019) computational model supported the continuum of cell states hypothesis. This study is a promising example of how refined biological data can be used to clarify modelling assumptions to ensure more quantitative predictions.

7.1 Future directions

There are many questions that still remain to be answered and we discuss some of these here.

7.1.1 Results of stream manipulations

With the present model described in this Commentary, there are still a number of tests that can be performed. For example, our mathematical model, developed based on McLennan et al.’s (2015a,b, 2017) model, can make predictions on what would happen if other manipulations were carried out on the streaming cells. We illustrate this with two cases. Firstly, we use the model to predict what would happen if, after 15 hours, cells were restricted from entering the domain. Figure 6 shows the model prediction, that the restriction of cell influx does not impede the cells at the front, which continue to invade the domain as in the control case. This observation suggests that crowding at the back of the stream is not significant for a successful invasion by the cells at the front of the stream.

Let us now consider what would happen were we to ablate cells from the middle of the stream. Figure 7 shows the model prediction after removal of the cells in the middle of the stream (at a distance more than $150\mu\text{m}$ but less than $200\mu\text{m}$ after 15 hours). In this case, we observe that the proximal segment of followers do not manage to catch up with the

followers ahead of them, and remain moving randomly close to the neural tube. The follower cells in the most distal subregion of the abandoned segment cannot switch phenotype to leaders because of the lack of a VEGF signal. The stream breaks and collective invasion is entirely disturbed.

The above examples are purely qualitative predictions of the outcomes of manipulations made within the stream. In order to reach rigorous conclusions on the effects of these alterations, more detailed computational studies have to be performed; for example, the restriction of cell influx at different times or the removal of a different number of cells. However, we present them to highlight computationally tractable alterations of the system that can potentially shed light upon the underlying mechanisms crucial for successful invasion.

7.1.2 Within stream signalling

In our computational model, we assume that follower cells are guided by the leader cells forming chains with leaders at the front. The cells in a chain take on the direction of a leader at the front of that chain. However, we do not know the type of signal to which these follower cells respond. For example, are leader cells producing a chemical signal to which follower cells respond? If so, what is the signal and how it is relayed down the stream? Alternatively, leader cells could be boring a tunnel through or remodelling the extracellular matrix making a path for the followers. There is experimental evidence for this (McLennan et al., 2019) but there is the question of how polarity is determined along this tunnel. Leaders could also be aligning the extracellular matrix or even producing components of it. Alternatively, the leaders may set up mechanical guidance cues to which the followers respond, a mechanism which is present in other collective cell migration phenomena, such as angiogenesis (Sottile, 2004; McCoy et al., 2018). It has been shown that enteric NC mesoderm stiffens during the period of colonisation (Chevalier et al., 2016) and that in *Xenopus*, tissue stiffening triggers NC cell migration (Barriga et al., 2018). The present model cannot distinguish between different signalling possibilities, and so further model refinement which accounts explicitly for other chemical signals or extracellular matrix mechanical cues is required to investigate these questions. This should be accompanied by a parallel experimental study to investigate whether, for example, followers express receptors for possible signals from the leaders.

7.1.3 How does cell response to guidance cues change along the stream?

McLennan et al. (2015a,b) assumed that cells behave differently in their response to signalling cues, depending on VEGF concentration, and hypothesised two cell phenotypes. They imposed a generic within stream signalling mechanism which could originate from chemical or mechanical gradients. However, their model is too coarse-grained to be able to distinguish between these possibilities and it is most likely that cells have the ability to respond to multiple signalling cues. Moreover, as mentioned above, Schumacher (2019) has shown that a model comprising a continuum of phenotypic states is consistent with the experimental results described in the Morrison et al. (2017) paper. Further biomarkers for different cell phenotypes along the stream and their behaviour are required to understand the importance of heterogeneity in successful invasion.

7.1.4 What constricts cells to the stream?

It is observed that NC cells primarily move within a “corridor” (see, for example, the review by Kulesa et al. (2010)). It has been shown that in *Xenopus*, where cranial NC cell migratory streams are adjacent to one another, the cells are confined in streams by cell-cell interactions through Eph/ephrin signalling (Smith et al., 1997) and cell-tissue interactions through Versican (Szabó et al., 2016a). Recently, we have discovered several membrane-bound and secreted factors expressed distinctly within the chick cranial paraxial mesoderm in NC cell exclusion zones that, in preliminary results, inhibit NC cell movements, suggesting redundant mechanisms to prevent stream mixing. Computational modelling could be used to demonstrate whether stream confinement is regulated by external factors, or by the ability of the NC cells to remain within a confined group.

7.1.5 The role of domain growth?

In the model we presented, domain growth was assumed to be spatially uniform but changing in time (growing exponentially initially before tapering off). This was motivated by measurements of the total length of the domain (McLennan et al., 2012). However, our recent experimental findings suggest that domain growth is not spatially uniform but has a spatial pattern (McKinney et al., 2019). Naturally, we question whether this makes migration more robust. Could there be a feedback loop between the migrating NC cells and

the microenvironment, in which the former controls domain growth? Alternatively, is spatially non-uniform domain growth necessary for other processes occurring in development, and so must NC cell migration be robust to such non-uniformities? Due to the challenges in manipulating domain growth experimentally, computational modelling provides a natural mean to directly investigate the effect of changes in domain growth profiles on the migration of cells.

7.1.6 The role of geometry

The above model has considered a flat two-dimensional domain, but in reality, cells are moving through a three-dimensional curved domain. A natural question to ask is whether the above model is a good approximation to that. This is a question that is amenable to computational analysis but has not yet been investigated.

7.1.7 How do we integrate bioinformatics with mechanism?

Typically, in mathematical biology, gene expression data are derived and theoreticians are asked to then determine meaning and mechanism. In the study of McLennan et al. (2012), this process was reversed – mechanistic modelling predicted gene expression variation, and this was verified experimentally. A key challenge in all areas of biology is how to integrate data from across spatial and temporal scales to inform models, to generate predictions and drive new experiments to allow us to gain further mechanistic insights.

7.1.8 Is there a unifying theory for collective cell migration?

As stated in the Introduction, collective cell migration is ubiquitous in biology. In the NC system, there is a wide variety of stream behaviours depending on the axial level of origin (e.g. cranial, vagal, or trunk), and on the model organism. A natural question to ask is: are there certain universal principles that must be obeyed for successful collective cell invasion, and do these variations simply correspond to different biological ways to satisfy these principles? There is the need for directed cell motion to invade distal targets collectively – this can arise from some polarising agent, be it chemical or mechanical. Then, there has to be signalling between cells to ensure collective movement. Cells may also need some external signals to confine them to a certain region. In the case of the NC, where there

is also domain growth, does the growth have to be regulated by the migrating cells in some way? Are there other hallmarks of collective cell migration?

It should be possible to understand the different ways in which biology performs these tasks (see, for example, the reviews by Theveneau and Mayor, 2010; Schumacher et al., 2016; Szabó and Mayor, 2016; Szabó and Mayor, 2018). From a mathematical point of view, the challenge is to develop a generalised model that encompasses all these features. This would then allow us to see how the different modes of migration we observe are related to each other, and may also predict other modes of motion yet to be seen.

The challenge of modelling collective behaviour in general (including ecology and human interactions) has also led to significant mathematical advances in multiscale modelling and the analyses of these models, ranging from cellular automaton to partial differential equation models (see, for example, the review by Giniūnaitė et al. (2019), and collected articles in the special issues Kanso et al. (2015), Bellomo and Brezzi (2015)).

In summary, we have shown how mathematical modelling, combined with custom-designed experiments, can lead to new biological insights. The recent advances in imaging techniques offer the real possibility of further tight integration of modelling with experiment, with the exciting potential of allowing us to fully exploit these data and accelerate biological discovery.

Acknowledgements

RG is funded by the Engineering and Physical Sciences Research Council (EP/G03706X/1). PMK would like to thank the Stowers Institute for Medical Research for their generosity. REB is a Royal Society Wolfson Research Merit Award holder, would like to thank the Leverhulme Trust for a Research Fellowship.

References

- Alfandari, D., Cousin, H., and Marsden, M. 2010. Mechanism of xenopus cranial neural crest cell migration. *Cell Adhesion & Migration*, 4(4):553–560.
- Barriga, E. H., Franze, K., Charras, G., and Mayor, R. 2018. Tissue stiffening coordinates morphogenesis by triggering collective cell migration in vivo. *Nature*, 554(7693):523.

- Bellomo, N. and Bellouquid, A. 2015. On multiscale models of pedestrian crowds from mesoscopic to macroscopic. *Communications in Mathematical Sciences*, 13(7):1649–1664.
- Bellomo, N. and Brezzi, F. 2015. *Traffic, crowds, and dynamics of self-organized particles: New trends and challenges*. World Scientific.
- Bellomo, N. and Gibelli, L. 2015. Toward a mathematical theory of behavioral-social dynamics for pedestrian crowds. *Mathematical Models and Methods in Applied Sciences*, 25(13):2417–2437.
- Bernardi, S., Colombi, A., and Scianna, M. 2018a. A discrete particle model reproducing collective dynamics of a bee swarm. *Computers in Biology and Medicine*, 93:158–174.
- Bernardi, S., Colombi, A., and Scianna, M. 2018b. A particle model analysing the behavioural rules underlying the collective flight of a bee swarm towards the new nest. *Journal of Biological Dynamics*, 12(1):632–662.
- Cai, D., Dai, W., Prasad, M., Luo, J., Gov, N. S., and Montell, D. J. 2016. Modeling and analysis of collective cell migration in an in vivo three-dimensional environment. *Proceedings of the National Academy of Sciences*, 113(15):E2134–E2141.
- Carmona-Fontaine, C., Matthews, H. K., Kuriyama, S., Moreno, M., Dunn, G. A., Parsons, M., Stern, C. D., and Mayor, R. 2008. Contact inhibition of locomotion in vivo controls neural crest directional migration. *Nature*, 456(7224):957–961.
- Carmona-Fontaine, C., Theveneau, E., Tzekou, A., Tada, M., Woods, M., Page, K., Parsons, M., Lambris, J., and Mayor, R. 2011. Complement fragment C3a controls mutual cell attraction during collective cell migration. *Developmental Cell*, 21(6):1026–1037.
- Chevalier, N., Gazquez, E., Bidault, L., Guilbert, T., Vias, C., Vian, E., Watanabe, Y., Muller, L., Germain, S., Bondurand, N., et al. 2016. How tissue mechanical properties affect enteric neural crest cell migration. *Scientific Reports*, 6:20927.
- Colak-Champollion, T., Lan, L., Jadhav, A. R., Yamaguchi, N., Venkiteswaran, G., Patel, H., Cammer, M., Meier-Schellersheim, M., and Knaut, H. 2019. Cadherin-mediated cell coupling coordinates chemokine sensing across collectively migrating cells. *Current Biology*, 29(15):2570–3579.
- Eberhart, J. K., He, X., Swartz, M. E., Yan, Y.-L., Song, H., Boling, T. C., Kuerth, A. K., Walker, M. B., Kimmel, C. B., and Postlethwait, J. H. 2008. MicroRNA Mirn140 modulates Pdgf signaling during palatogenesis. *Nature Genetics*, 40(3):290.

- Fetecau, R. and Guo, A. 2012. A mathematical model for flight guidance in honeybee swarms. *Bulletin of Mathematical Biology*, 74(11):2600–2621.
- Freshney, R. I. and Freshney, M. G. 2004. *Culture of Epithelial Cells*, volume 10. John Wiley & Sons.
- Giniūnaitė, R., Baker, R., Kulesa, P., and Maini, P. 2019. Modelling collective cell migration: neural crest as a model paradigm. *Journal of Mathematical Biology*.
- Haeger, A., Wolf, K., Zegers, M. M., and Friedl, P. 2015. Collective cell migration: guidance principles and hierarchies. *Trends in Cell Biology*, 25(9):556–566.
- He, F. and Soriano, P. 2013. A critical role for PDGFR α signaling in medial nasal process development. *PLoS Genetics*, 9(9):e1003851.
- Hendrix, M. J., Seftor, E. A., Seftor, R. E., Kasemeier-Kulesa, J., Kulesa, P. M., and Postovit, L.-M. 2007. Reprogramming metastatic tumour cells with embryonic microenvironments. *Nature Reviews Cancer*, 7(4):246–255.
- Holler, K. L., Hendershot, T. J., Troy, S. E., Vincentz, J. W., Firulli, A. B., and Howard, M. J. 2010. Targeted deletion of Hand2 in cardiac neural crest-derived cells influences cardiac gene expression and outflow tract development. *Developmental Biology*, 341(1):291–304.
- Kanso, E. and Saintillan, D. 2015. Special issue editorial: Emergent collective behavior: From fish schools to bacterial colonies. *Journal of Nonlinear Science*, 25(5):1051–1052.
- Kasemeier-Kulesa, J., M Kulesa, P., McLennan, R., H Romine, M., and Lefcort, F. 2010. CXCR4 controls ventral migration of sympathetic precursor cells. *Developmental Biology*, 344:473.
- Kasemeier-Kulesa, J. C., Morrison, J. A., Lefcort, F., and Kulesa, P. M. 2015. TrkB/BDNF signalling patterns the sympathetic nervous system. *Nature Communications*, 6:8281.
- Kasemeier-Kulesa, J. C., Teddy, J. M., Postovit, L.-M., Seftor, E. A., Seftor, R. E., Hendrix, M. J., and Kulesa, P. M. 2008. Reprogramming multipotent tumor cells with the embryonic neural crest microenvironment. *Developmental Dynamics*, 237(10):2657–2666.
- Kedrin, D., Gligorijevic, B., Wyckoff, J., Verkhusha, V. V., Condeelis, J., Segall, J. E., and Van Rheenen, J. 2008. Intravital imaging of metastatic behavior through a mammary imaging window. *Nature Methods*, 5(12):1019.

- Kulesa, P. and McLennan, R. 2006. In vivo analysis reveals a critical role for neuropilin-1 in chick cranial neural crest cell migration. *Developmental Biology*, 1(300):227–239.
- Kulesa, P. M., Bailey, C. M., Kasemeier-Kulesa, J. C., and McLennan, R. 2010. Cranial neural crest migration: new rules for an old road. *Developmental Biology*, 344(2):543–554.
- Kulesa, P. M., Lu, C. C., and Fraser, S. E. 2005. Time-lapse analysis reveals a series of events by which cranial neural crest cells reroute around physical barriers. *Brain, Behavior and Evolution*, 66(4):255–265.
- Kulesa, P. M. and McLennan, R. 2015. Neural crest migration: trailblazing ahead. *F1000 Prime Reports*, 7.
- Lake, J. I. and Heuckeroth, R. O. 2013. Enteric nervous system development: migration, differentiation, and disease. *American Journal of Physiology - Gastrointestinal and Liver Physiology*, 305(1):G1–G24.
- Landman, K. A., Simpson, M. J., and Newgreen, D. F. 2007. Mathematical and experimental insights into the development of the enteric nervous system and Hirschsprung’s disease. *Development, Growth & Differentiation*, 49(4):277–286.
- Le Douarin, N. M. 2004. The avian embryo as a model to study the development of the neural crest: a long and still ongoing story. *Mechanisms of Development*, 121(9):1089–1102.
- Maiuri, P., Rupprecht, J.-F., Wieser, S., Rupprecht, V., Bénichou, O., Carpi, N., Coppey, M., De Beco, S., Gov, N., Heisenberg, C.-P., et al. 2015. Actin flows mediate a universal coupling between cell speed and cell persistence. *Cell*, 161(2):374–386.
- Marsan, G. A., Bellomo, N., and Egidi, M. 2008. Towards a mathematical theory of complex socio-economical systems by functional subsystems representation. *Kinetic and Related Models*, 1(2):249–278.
- Mayor, R. and Etienne-Manneville, S. 2016. The front and rear of collective cell migration. *Nature Reviews Molecular Cell Biology*, 17(2):97–109.
- McCoy, M. G., Wei, J. M., Choi, S., Goerger, J. P., Zipfel, W., and Fischbach, C. 2018. Collagen fiber orientation regulates 3d vascular network formation and alignment. *ACS Biomaterials Science & Engineering*, 4(8):2967–2976.

- McKinney, M. C., McLennan, R., Giniūnaitė, R., Baker, R. E. B., Maini, P. K., Othmer, H. G., and Kulesa, P. M. 2019. Neural crest cells bulldoze through the microenvironment using Aquaporin-1 to stabilize filopodia. *bioRxiv*: <https://www.biorxiv.org/content/10.1101/738542v1>.
- McLennan, R., Bailey, C. M., Schumacher, L. J., Teddy, J. M., Morrison, J. A., Kasemeier-Kulesa, J. C., Wolfe, L. A., Gogol, M. M., Baker, R. E., Maini, P. K., et al. 2017. DAN (NBL1) promotes collective neural crest migration by restraining uncontrolled invasion. *Journal of Cell Biology*, 216(10):3339–3354.
- McLennan, R., Dyson, L., Prather, K. W., Morrison, J. A., Baker, R. E., Maini, P. K., and Kulesa, P. M. 2012. Multiscale mechanisms of cell migration during development: theory and experiment. *Development*, 139(16):2935–2944.
- McLennan, R., McKinney, M. C., Teddy, Jessica M, M. J. A., Kasemeier-Kulesa, J. C., Ridenour, D. A., Manthe, C. A., Giniūnaitė, R., Robinson, M., Baker, R. E., Maini, P. K., and Kulesa, P. M. 2019. Neural crest cells bulldoze through the microenvironment using Aquaporin-1 to stabilize filopodia. *bioRxiv*: <https://www.biorxiv.org/content/10.1101/719666v1>.
- McLennan, R., Schumacher, L. J., Morrison, J. A., Teddy, J. M., Ridenour, D. A., Box, A. C., Semerad, C. L., Li, H., McDowell, W., Kay, D., et al. 2015a. Neural crest migration is driven by a few trailblazer cells with a unique molecular signature narrowly confined to the invasive front. *Development*, 142 11:2014–25.
- McLennan, R., Schumacher, L. J., Morrison, J. A., Teddy, J. M., Ridenour, D. A., Box, A. C., Semerad, C. L., Li, H., McDowell, W., Kay, D., Maini, P. K., Baker, R. E., and Kulesa, P. M. 2015b. VEGF signals induce trailblazer cell identity that drives neural crest migration. *Developmental Biology*, 407(1):12–25.
- McLennan, R., Teddy, J. M., Kasemeier-Kulesa, J. C., Romine, M. H., and Kulesa, P. M. 2010. Vascular endothelial growth factor (VEGF) regulates cranial neural crest migration in vivo. *Developmental Biology*, 339(1):114–125.
- Merchant, B., Edelstein-Keshet, L., and Feng, J. J. 2018. A Rho-GTPase based model explains spontaneous collective migration of neural crest cell clusters. *Developmental Biology*, 444:S262–S273.
- Morrison, J. A., McLennan, R., Wolfe, L. A., Gogol, M. M., Meier, S., McKinney, M. C., Teddy, J. M., Holmes, L., Semerad, C. L., Box, A. C., et al. 2017. Single-cell transcrip-

- tome analysis of avian neural crest migration reveals signatures of invasion and molecular transitions. *Elife*, 6:1–27.
- Onichtchouk, D., Chen, Y.-G., Dosch, R., Gawantka, V., Delius, H., Massague, J., and Niehrs, C. 1999. Silencing of TGF- β signalling by the pseudoreceptor BAMBI. *Nature*, 401(6752):480–485.
- Palm, M. M., Dallinga, M. G., van Dijk, E., Klaassen, I., Schlingemann, R. O., and Merks, R. M. H. 2016. Computational screening of tip and stalk cell behavior proposes a role for apelin signaling in sprout progression. *PLOS One*, 11(11):1–31.
- Parisot, M. and Lachowicz, M. 2016. A Kinetic Model for the formation of Swarms with nonlinear interactions. *Kinetic and Related Models*, 9(1):33.
- Richardson, J., Gauert, A., Montecinos, L. B., Fanlo, L., Alhashem, Z. M., Assar, R., Marti, E., Kabla, A., Härtel, S., and Linker, C. 2016. Leader cells define directionality of trunk, but not cranial, neural crest cell migration. *Cell Reports*, 15(9):2076–2088.
- Rogers, C., Jayasena, C., Nie, S., and Bronner, M. E. 2012. Neural crest specification: tissues, signals, and transcription factors. *Wiley Interdisciplinary Reviews: Developmental Biology*, 1(1):52–68.
- Sato, A., Scholl, A. M., Kuhn, E., Stadt, H. A., Decker, J. R., Pegram, K., Hutson, M. R., and Kirby, M. L. 2011. FGF8 signaling is chemotactic for cardiac neural crest cells. *Developmental Biology*, 354(1):18–30.
- Schumacher, L. J. 2019. Neural crest migration with continuous cell states. *Journal of Theoretical Biology*, pages 1–7.
- Schumacher, L. J., Kulesa, P. M., McLennan, R., Baker, R. E., and Maini, P. K. 2016. Multidisciplinary approaches to understanding collective cell migration in developmental biology. *Open biology*, 6(6):160056–66.
- Simpson, M. J., Zhang, D. C., Mariani, M., Landman, K. A., and Newgreen, D. F. 2007. Cell proliferation drives neural crest cell invasion of the intestine. *Developmental Biology*, 302(2):553–568.
- Smith, A., Robinson, V., Patel, K., and Wilkinson, D. G. 1997. The EphA4 and EphB1 receptor tyrosine kinases and ephrin-B2 ligand regulate targeted migration of branchial neural crest cells. *Current Biology*, 7(8):561–570.

- Sottile, J. 2004. Regulation of angiogenesis by extracellular matrix. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1654(1):13–22.
- Stark, D. A. and Kulesa, P. M. 2007. An in vivo comparison of photoactivatable fluorescent proteins in an avian embryo model. *Developmental Dynamics: an official publication of the American Association of Anatomists*, 236(6):1583–1594.
- Szabó, A. and Mayor, R. 2016. Modelling collective cell migration of neural crest. *Current Opinion in Cell Biology*, 42:22–28.
- Szabó, A. and Mayor, R. 2018. Mechanisms of neural crest migration. *Annual Review of Genetics*, 52:43–63.
- Szabó, A., Melchionda, M., Nastasi, G., Woods, M. L., Campo, S., Perris, R., and Mayor, R. 2016a. In vivo confinement promotes collective migration of neural crest cells. *Journal of Cell Biology*, 213(5):543–555.
- Szabó, A., Melchionda, M., Nastasi, G., Woods, M. L., Campo, S., Perris, R., and Mayor, R. 2016b. In vivo confinement promotes collective migration of neural crest cells. *Journal of Cell Biolopgy*, 213(5):543–555.
- Theveneau, E., Marchant, L., Kuriyama, S., Gull, M., Moepps, B., Parsons, M., and Mayor, R. 2010. Collective chemotaxis requires contact-dependent cell polarity. *Developmental Cell*, 19(1):39–53.
- Turing, A. 1952. The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London*, 237:37–72.
- Woods, M. L., Carmona-Fontaine, C., Barnes, C. P., Couzin, I. D., Mayor, R., and Page, K. M. 2014. Directional collective cell migration emerges as a property of cell interactions. *PLOS One*, 9(9):1–10.

8 Figure captions

Figure 1: Migration of NC cells in the chick embryo, ba1-3 – branchial arch 1-3, respectively, ov – otic vesicle. The image is taken after NC cells reach ba2. The scalebar is 50 microns (white).

Figure 2: McLennan et al. (2012) model schematic. The yellow circle corresponds to a leader cell, the white circle to a follower. The white and yellow short cell protrusions represent filopodia and c is the VEGF concentration. The elongation of the rectangle corresponds to domain growth. The leader cell senses the gradient in c and moves forward, while the follower cell senses the leader and adopts its direction of motion.

Figure 3: McLennan et al.’s (2012) model simulations: left computational model with one cell phenotype; right computational model with two cell phenotypes. Yellow – leader cells, white – follower cells, chemoattractant concentration represented by colour bar. For each case, the left boundary of the domain is the neural tube, and the right-hand side boundary is the end of the domain which corresponds to $ba2$. It can be seen that in the left panel (in which there is only one cell phenotype) cells are stranded at the neural tube, while in the right panel (with two cell phenotypes) they invade. The length units are μm . Figure reproduced with permission from McLennan et al. (2012).

Figure 4: Cell counts versus distance migrated for five different leader fractions (f_L). It can be seen that in all cases cells invade, but that a small number of leaders ensures higher cell density all along the stream. Figure reproduced with permission from McLennan et al. (2015a).

Figure 5: McLennan et al.’s (2017) model simulations. Collective NC cell migration can be facilitated by DAN expression through regulation of cell speed within range of the dorsal neural tube. (a) No reduction in speed: the model simulated is as in McLennan et al. (2015a,b) on a widened domain with cell speed $40 \mu\text{m}/\text{h}$. Leader NC cells are in yellow, follower cells are in green, and the background blue colour shows the VEGF chemoattractant concentration. (b) Moderate reduction in speed: inside the area representing DAN expression (red dashed rectangle), cell speed is reduced in proportion to DAN concentration. The minimum cell speed is $30 \mu\text{m}/\text{hrs}$. (c) Modelling increased DAN: the simulation is set up as in (b), but the cell speed is now reduced down to $10 \mu\text{m}/\text{hrs}$ (at peak concentration of DAN). Figure reproduced with permission from McLennan et al. (2017).

Figure 6: Snapshot of the simulations at $t = 24\text{hrs}$ of the model developed based on McLennan et al.’s (2015a,b, 2017) model and described in the first part of Section 3. (a) Control case. (b) Limited cell influx. Yellow circles correspond to leader cells, green circles correspond to follower cells, and c is the concentration of chemoattractant. We restrict the influx of cells, so that no cells enter the domain after 15hrs.

Figure 7: Snapshots of simulations of the model developed based on McLennan et al.’s (2015a,b, 2017) model and described in the first part of Section 3. Yellow circles correspond to leader cells, green circles correspond to follower cells, and c is the concentration of chemoattractant. The cells in the middle of the stream (at distance more than $150\mu\text{m}$ but less than $200\mu\text{m}$) are removed after 15 hrs. Follower cells do not manage to catch up with the leaders and the stream breaks.

Figure 8: This decade-long interdisciplinary collaboration began through discussions, lasting several months, between the experimental group (Rebecca McLennan and Paul Kulesa, main picture, left) and the theoreticians Philip Maini and Ruth Baker (main picture, right). The mathematical modelling has been carried out by three students who were/are part of a special interdisciplinary doctoral training program that exposes students from a variety of scientific backgrounds to a wide range of areas in the biological and mathematical sciences before they chose the appropriate department in which to do their doctorate. Louise Dyson (top right) and Rasa Giniūnaitė (bottom right) were trained as mathematicians, while Linus Schumacher is a physicist. All three students spent time in the laboratory at the Stowers. Louise’s thesis developed the prototype agent-based model and also carried out detailed mathematical analysis of the system. She is now a permanent faculty member with a joint appointment at the Mathematical Institute, and the School of Life Sciences, at the University of Warwick. Linus extended the model and also carried out gene expression analysis and more detailed studies of collective cell behavior. He is now a Chancellor’s Fellow at the MRC Centre for Regenerative Medicine, University of Edinburgh. Rasa is presently doing her doctorate, focusing on extending the study on domain growth. The group photograph was taken during the annual visit that the experimental group makes to Oxford and it is at these meetings that we compare experimental results with model predictions and discuss/design new experiments that can be done with the theoretical model and also in the lab – the results of these are then compared at the next meeting with no discussion in between in case it compromises the comparison (of course we continue to discuss other parts of the collaboration).

9 Figures

Figure 1

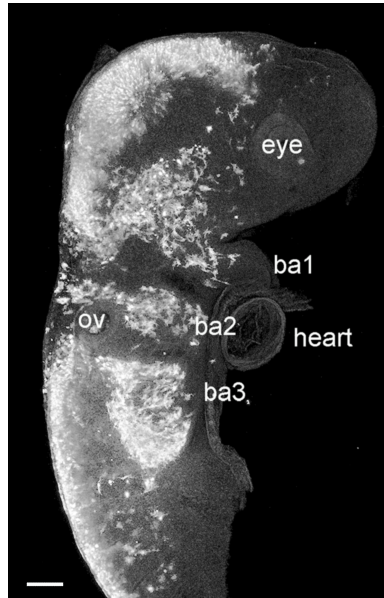


Figure 2

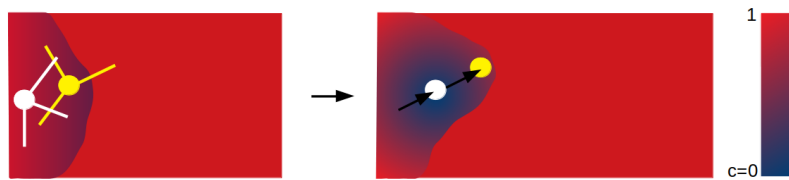


Figure 3

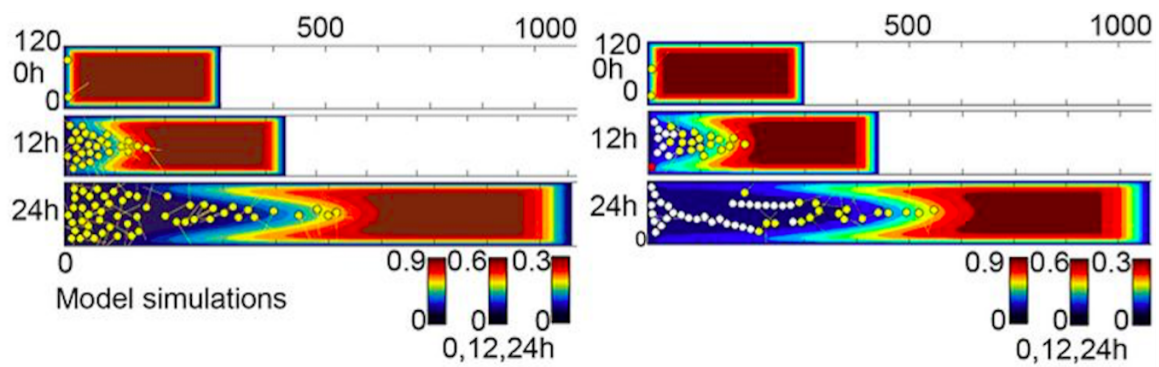


Figure 4

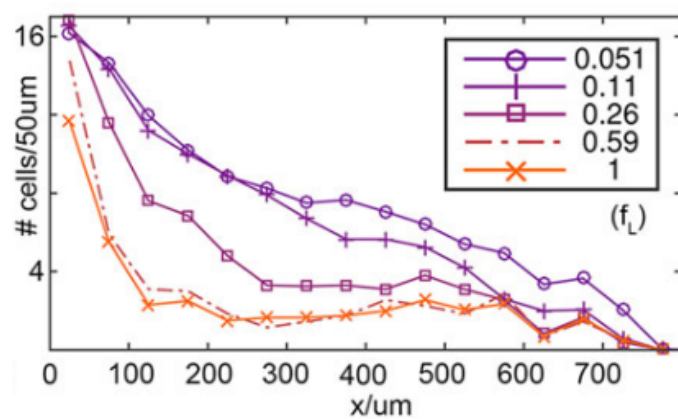


Figure 5

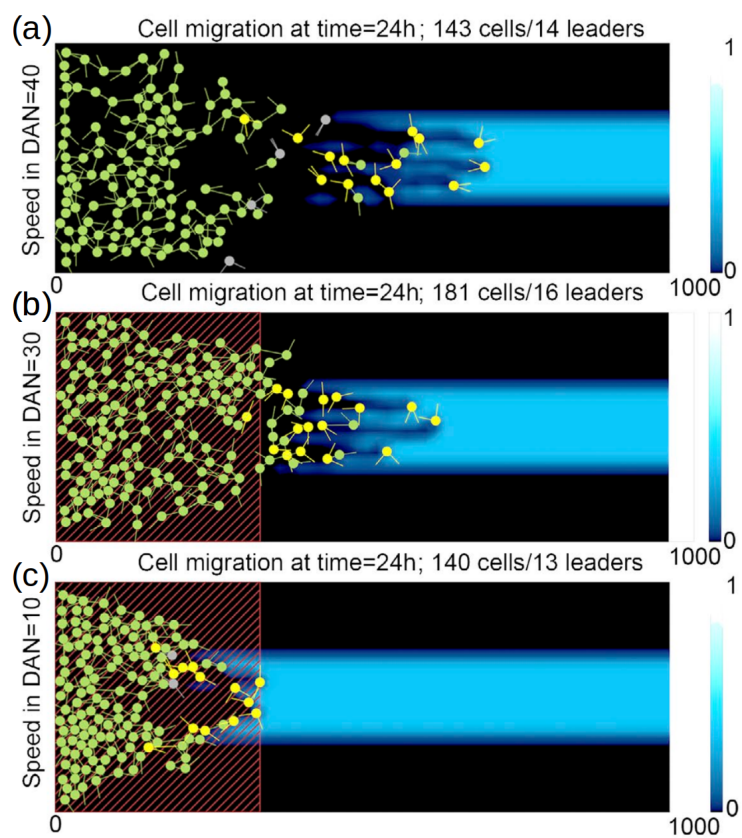


Figure 6

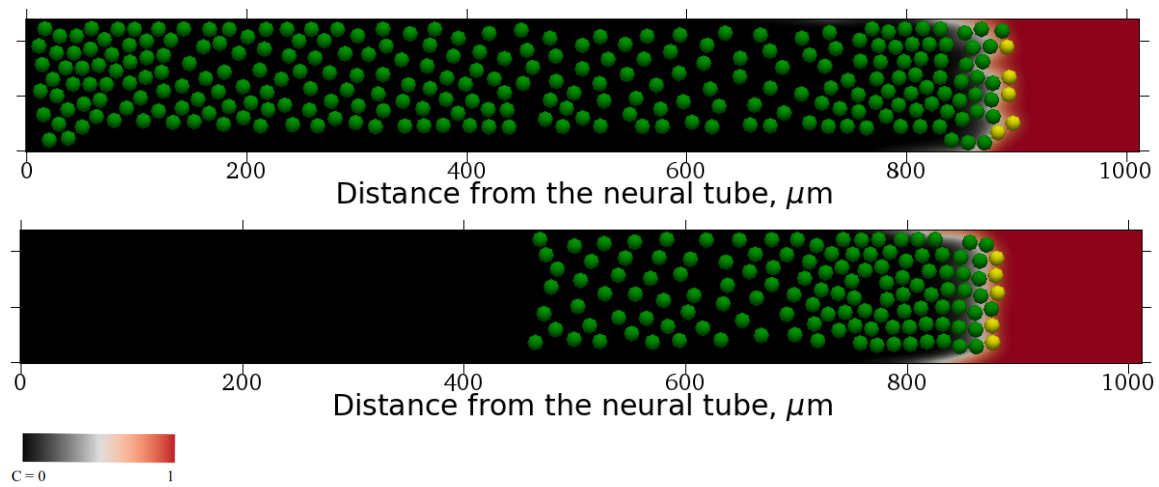


Figure 7

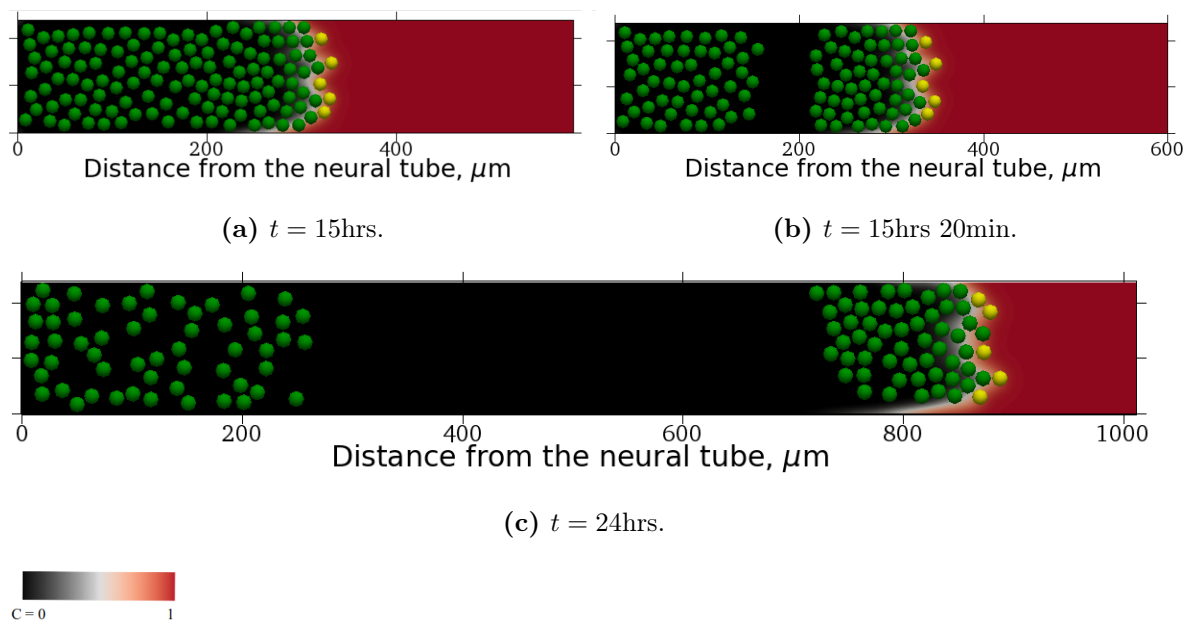




Figure 8