



**HAL**  
open science

# In vivo Neural Crest Cell Migration Is Controlled by “Mixotaxis”

Elias H Barriga, Eric Théveneau

► **To cite this version:**

Elias H Barriga, Eric Théveneau. In vivo Neural Crest Cell Migration Is Controlled by “Mixotaxis”.  
Frontiers in Physiology, 2020, 11, 10.3389/fphys.2020.586432 . hal-03031064v2

**HAL Id: hal-03031064**

**<https://hal.science/hal-03031064v2>**

Submitted on 17 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 ***In vivo* Neural Crest cell migration is controlled by “mixotaxis”**

2 Running title: neural crest mixotaxis

3

4 Elias H Barriga<sup>2\*</sup> and Eric Theveneau<sup>1\*</sup>

5 <sup>1</sup> Centre de Biologie du Développement (CBD), Centre de Biologie Intégrative (CBI), Université de

6 Toulouse, CNRS, UPS, Toulouse, France.

7 <sup>2</sup> Instituto Gulbenkian de Ciência, Mechanisms of Morphogenesis Lab, Oeiras, Portugal.

8

9 \* Corresponding authors: eric.theveneau@univ-tlse3.fr, ebarriga@igc.gulbenkian.pt

10

11

12 Keywords: directed cell migration, neural crest, morphogenesis, durotaxis, chemotaxis, haptotaxis,  
13 galvanotaxis, electrotaxis, mixotaxis

14

15 **Abstract**

16 Directed cell migration is essential all along an individual's life, from embryogenesis to tissue  
17 repair and cancer metastasis. Thus, due to its biomedical relevance, directed cell migration is currently  
18 under intense research. Directed cell migration has been shown to be driven by an assortment of  
19 external biasing cues, ranging from gradients of soluble (chemotaxis) to bound (haptotaxis) molecules.  
20 In addition to molecular gradients, gradients of mechanical properties (duro/mechanotaxis), electric  
21 fields (electro/galvanotaxis) as well as iterative biases in the environment topology (ratchetaxis) have  
22 been shown to be able to direct cell migration. Since cells migrating *in vivo* are exposed to a challenging  
23 environment composed of a convolution of biochemical, biophysical and topological cues, it is highly  
24 unlikely that cell migration would be guided by an individual type of 'taxis'. This is especially true since  
25 numerous molecular players involved in the cellular response to these biasing cues are often recycled,  
26 serving as sensor or transducer of both biochemical and biophysical signals. In this review, we confront  
27 literature on *Xenopus* cephalic neural crest cells with that of other cell types to discuss the relevance  
28 of the current categorization of cell guidance strategies. Furthermore, we emphasize that while  
29 studying individual biasing signals is informative, the hard truth is that cells migrate by performing a  
30 sort of "mixotaxis", where they integrate and coordinate multiple inputs through shared molecular  
31 effectors to ensure robustness of directed cell motion.

32

### 33 **Introduction**

34 Finding a solution to trigger directed cell migration is simple. An external signal that cells can  
35 interpret needs to be spatially organized. Then, cells can use that signal to generate a front-rear  
36 polarity allowing directional movement along that cue. Very much like drivers following road signs.  
37 Many inputs (e.g. chemical, mechanical, electrical, topological) can be shown to fulfil this function in  
38 controlled and simplified experiments [1; 2; 3]. However, living systems were not engineered by a  
39 designer to strictly follow a set of specifications in a logical manner that is then validated by external  
40 quality controls. Instead, *in vivo* migrating cells are often exposed to an overwhelming range of inputs  
41 which may at best appear to have no obvious hierarchy and at worst to be contradictory; yet, the  
42 migratory response of cells to such convoluted environments still logical. In addition, each polarity cue  
43 may not be as neatly organized as it would in an *in vitro* assay. Further, some cells may display a given  
44 migratory behavior while their neighboring tissues do not. Hence, there may be cooperation,  
45 coordination and/or competition between directionally migrating cells and the activities of their  
46 neighbors. Furthermore, a given input may lead to different responses in different cell populations  
47 within the same time window indicating that the directional information is not carried by the signal  
48 itself but generated as a result of the interplay between cells and a given signal or set of signals (we  
49 discuss examples hereafter). This can be equated to how geneticists view the phenotype as a result of  
50 the interaction between a genotype and the local environment of an organism. Yet, for cells willing to  
51 undertake directed migration, it all comes down to two simple facts: i) cells need to propel themselves  
52 and ii) establish and sustain a front-rear polarity. This means that all inputs have to be somewhat  
53 integrated by a cell for a directional behavior to emerge. In groups of cells, intercellular communication  
54 may in addition lead to emerging properties such that what a cell collective does may differ from what  
55 a single cell would do in a similar context [4]. Hence, unveiling the mechanisms that control directed  
56 cell migration in its full complexity could have countless impacts in our understanding of intricate  
57 morphogenetic events. In addition, a more integrative approach to directed cell migration would help  
58 designing effective ways to hinder cancer metastasis, improve wound healing or contribute to new

59 methods for *ex vivo* organ patterning in the context of regenerative medicine. In this review, we used  
60 the *Xenopus* cephalic neural crest (NC) cells, an embryonic stem cell population that collectively and  
61 directionally migrates [5], as an example to discuss the complexity of the control of directed cell  
62 migration. We address first how motility is initiated in NC cells before discussing the strategies  
63 displayed by cells in order to bias their motion and perform directed cell migration. Drawing parallels  
64 between NC results and findings about directed cell migration in other cell types, we propose some  
65 working hypotheses for signal integration and the emergence of directional motion.

66

### 67 **The neural crest, EMT and the onset of cell motion**

68 NC are induced during mid to late gastrulation stages at the interface between the neural and  
69 non-neural ectoderm and between the epidermis and mesoderm (FIGURE 1). They later leave the  
70 dorsal neuroepithelium to collectively migrate throughout the developing embryo. Anterior NC cells  
71 make an outstanding contribution to the head morphology and sensory structures by providing  
72 cartilage and bones, meninges that surround the brain, smooth and striated muscle cells and tendons  
73 as well as pigments cells among other structures [6]. In addition, NC cells cooperate with placodal cells  
74 to form the cephalic peripheral nervous system [4]. Cranial placodes are discrete thickenings of the  
75 ectoderm that produce some of the neurons that in turn form the cranial ganglia [7]. The rest of the  
76 neurons and the glial cells are provided by the cephalic NC cells [8]. NC cells are an extremely powerful  
77 model to investigate cell migration. Their timing and pattern of migration has been documented in  
78 multiple species allowing comparative studies [9]. In chicken, mice and *Xenopus* embryos, NC cells can  
79 be manipulated *in vivo* and *ex vivo*, thanks to well-defined culture conditions. This has allowed  
80 researchers to perform in-depth cell and molecular biology studies. Whereas in genetically tractable  
81 species (e.g. zebrafish and mouse), transgenic lines have been generated for long-term observation  
82 and targeted molecular manipulation of these cells. In addition, the first part of NC cell migration

83 occurs superficially, especially in cephalic regions, permitting direct observation of cell behavior by  
84 time-lapse cinematography in fish, chick or amphibians.

85         The first step toward directed motion is for cells to acquire motile capabilities. NC cells initiate  
86 migration by undergoing epithelial-mesenchymal transition (EMT). EMT leads to a qualitative and  
87 quantitative remodeling of adhesive properties, cytoskeleton dynamics and cell polarity such that cells  
88 have transient adhesions to one another, display faster membrane dynamics and go from apicobasal  
89 polarity associated with epithelial state to a front-rear polarity associated with motility. EMT is  
90 performed by a series of non-obligatory steps such that cells that initiate EMT do not systematically  
91 complete it [10] and is reversible [11]. EMT in *Xenopus* NC cells is better described as a partial EMT  
92 with cells migrating at high cell density with frequent transient physical contacts via functional  
93 adherens junctions, as recently discussed [5].

94         Canonical EMT is controlled by an array of transcription factors whose expression is detected  
95 many hours before NC migration is initiated. In *Xenopus*, cephalic NC migration starts around stage 19-  
96 20 when the neural folds closure nears completion to form the neural tube. Nonetheless, the  
97 expression of key EMT transcriptional regulators such as Snail2 or Twist1 starts in NC cells at stages 12  
98 and 14, respectively. One of the main targets of these factors is the cell-cell adhesion receptor E-  
99 cadherin (CDH-1) whose expression only starts to decrease in the NC at around stage 17.5 [12],  
100 suggesting that Snail2 and Twist1 may not be recruited to the E-cadherin promoter or that they may  
101 not even be active until stage 17.5. One way to control transcription factors' activity is to regulate their  
102 entry into the nucleus. Intriguingly, in mammalian cell lines, Twist has been shown to be imported to  
103 the nucleus when cells are exposed to stiff substrates [13; 14]. In this situation, EphA2 is activated in a  
104 ligand-independent manner and leads to the phosphorylation of Twist via LYN kinase. This frees Twist  
105 from its cytoplasmic anchor G3BP2 and allows it to enter the nucleus. This is particularly interesting in  
106 the context of NC development because the onset of NC migration in *Xenopus* has been linked to the  
107 local increase of stiffness underneath the cephalic crest generated by the convergent extension

108 movement of the mesoderm towards the midline of the embryo [15]. In addition, Twist expression is  
109 under the control of the Hif signaling pathway which also controls the expression of CXCR4, the  
110 receptor for the chemokine CXCL12/Stromal cell-derived factor 1 (Sdf1) [16]. Interestingly, in renal  
111 carcinoma cells, Hif1 $\alpha$  and CXCR4 have been shown to take part in a feed forward loop for nuclear  
112 translocation such that, via a direct physical interaction between the two proteins, nuclear  
113 accumulation of CXCR4 favors entry of HIF-1 $\alpha$  and HIF-1 $\alpha$  then further promotes CXCR4 expression  
114 [17]. Thus, one can propose a model in which Hif-1 $\alpha$  primes NC cells for EMT and directional migration  
115 by regulating Twist and CXCR4 expressions until mesoderm stiffness reaches a threshold suitable for  
116 migration. Twist1 is not the main and certainly neither the only EMT-associated NC transcription factor,  
117 however, to date it is the most likely candidate to mediate a 'rapid' response to environmental cues.  
118 Another example is that of Sox10, this transcription factor constantly shuttles between the nucleus  
119 and the cytoplasm and docks at the surface of mitochondria [18; 19]. However, experimental  
120 assessment of Sox10's function ties it to lineage decisions rather than NC migration in *Xenopus* [20;  
121 21]. Whether Sox10's nuclear localization is also mechanically controlled remains to be explored. In  
122 any case, controlling the emergence of cell motility does not explain directionality *per se*. Such cell  
123 intrinsic motility needs to be iteratively biased to sustain directed motion. The rest of this review is  
124 dedicated to the various cues that might bias NC directed motion.

125

## 126 **Chemotaxis**

127 The directional migration of NC cells could be explained by chemotaxis, the ability of cells to  
128 follow gradients of soluble guidance cues (FIGURE 2A). As mentioned, NC and placodes cooperate to  
129 form the cephalic peripheral nervous system. Interestingly, NC and placodes interact early on during  
130 head morphogenesis and this interaction is crucial for directional migration of NC cells [22; 23]. Prior  
131 to the onset of NC migration, NC and placodes are located in adjacent domains of the lateral ectoderm.  
132 NC are on either side of the neural plate and the placodes are surrounding the NC domains and the

133 anterior neural plate, forming a horseshoe-shaped zone (FIGURE 1). Placodes secrete CXCL12 that  
134 promotes cell-matrix adhesion and motility via activation of Rac1 in NC cells [4; 22; 24]. The presence  
135 of this chemokine stimulates migration such that NC move towards the CXCL12-producing placodes.  
136 When NC cells and placodes make a physical contact they exhibit contact-inhibition of locomotion  
137 (CIL), an active repolarization process upon cell-cell contact that leads to cells moving away from each  
138 other [25]. However, placodes and NC cells do not have the same migratory capabilities. Placodes at  
139 this stage are epithelial, located in the deep layer of the ectoderm and are barely motile. NC cells being  
140 more active, they are systematically the ones filling local gaps between cells generated by the CIL  
141 response. This creates a bias that favors lateral migration of the crest cells towards the placode  
142 domain. Thus, once NC migration is initiated there is a progressive shift of the placodal cells  
143 laterally/ventrally that displaces the source of CXCL12. This has been proposed to generate a feed  
144 forward loop driving directed movement of both cell populations from medial to lateral [22] (FIGURE  
145 1). At first glance, this mechanism explains the directional movement of the NC cells and the  
146 progressive redistribution of placodes during head morphogenesis via a combination of CXCL12-  
147 dependent chemotaxis and heterotypic contact-inhibition between NC and placodes. So, what is  
148 missing?

149         There are several caveats. First, we infer lots of *in vivo* directional migratory behaviors and  
150 mechanisms (chemotaxis, haptotaxis, ratchetaxis, durotaxis) from *in vitro* data which in general show  
151 that cells have the ability to interpret and follow such signals. Nonetheless, clear demonstration of  
152 their actual implication in directed cell migration *in vivo* is tough, owing to the complex nature of native  
153 environments. Some of these directional cues are also not easy to distinguish from one another. In  
154 particular, it is difficult to assess whether cells undergo chemotaxis (soluble signal) vs haptotaxis  
155 (bound signal) *in vivo*. For instance, CXCL12 and VEGFA, common examples of putative NC chemotactic  
156 cues [4; 26], are capable of binding to the extracellular matrix and we still do not understand whether  
157 their physiological relevance is linked to a soluble or a bound state. Also, graded distribution of a signal  
158 is not a proof that cells are detecting it or reading it. In the case of CXCL12 and VEGFA such unequivocal



159 proof of graded distribution of the protein along migratory paths has not been obtained. Moreover,  
160 while CXCL12 is a powerful chemotactic factor for NC *in vitro* [4], its spatial distribution is dispensable  
161 *in vivo* as it primarily acts by promoting adhesion to the extracellular matrix rather than giving clear  
162 direction to the cells [24]. This has been demonstrated by showing that *in vivo* directed NC migration  
163 can occur in the absence of CXCL12/CXCR4 signaling if Rac1 is homogenously and iteratively activated  
164 in NC cells to allow for cell-matrix adhesions to form [24]. This suggests that CXCR4-CXCL12 may work  
165 as a chemokinetic factor (promoting motility via cell-matrix adhesion) rather than a chemotactic one  
166 (biasing directionality). If CXCR4-CXCL12 signaling does not provide a directional bias what are the  
167 mechanisms ensuring sustained directed motion and how does CXCR4-CXCL12 integrate with them?

168

#### 169 **Durotaxis**

170 Durotaxis is the directed motion of cells according to local gradients of rigidity (stiffness) with  
171 cells moving from compliant to rather stiff regions of a given substrate [27]. For example, in *Xenopus*,  
172 cell proliferation drives local changes in brain tissue stiffness, creating local gradient that are followed  
173 by axons of developing neurons [28]. Given that NC cells are able to sense differences in rigidity and  
174 that stiffness of the underlying mesoderm is a key factor for the initiation of NC migration [15], one  
175 could also propose that there might be a gradient from dorsal to ventral promoting stiffness-  
176 dependent directional migration. The main driver of this observed increase of stiffness is the local  
177 accumulation of mesodermal cells underneath the NC domain [15]. In the trunk, the medio-dorsal  
178 mesoderm aggregates as somites and thus is denser than the ventro-lateral mesoderm (FIGURE 2).  
179 Therefore, if there is a cell density associated gradient of mesoderm stiffness it would be oriented  
180 ventro-dorsally which is opposite to the direction of trunk NC migration. In the head, where mesoderm  
181 does not form somites, such spatial distribution of cell density and stiffness has not been assessed so  
182 far. Though, published data suggest that the emergence of such a gradient is unlikely owing to the high  
183 degree of mechanical heterogeneities observed in that region [15]. Yet, even if true, such gradient of

184 stiffness leading to durotaxis could not be seen as an absolute signal that would restrict any kind of  
185 cell movement in a dorsoventral manner. While cephalic NC cells are migrating ventralward, the  
186 surface ectoderm is moving dorsalward to accompany dorsal neural tube closure. In addition, myeloid  
187 cells (macrophages) are undergoing random migration from the cardiac region to survey the entire  
188 developing embryo [29]. Myeloid cells migrate as single cells, display extensive dispersion and cross  
189 areas that NC cells are completely unable to use. Thus, during head morphogenesis, there are  
190 concomitant migration events (e.g. epidermis, neural crest, myeloid cells) that follow different  
191 directions despite sharing a common environment. This highlights the importance of considering the  
192 interaction between cells and the environment as the main driver of cell behavior rather than intrinsic  
193 cell motility.

194           Mechanosensing of the substrate requires functional cell-matrix adhesions. Thus, in NC cells,  
195 the putative distinction between durotaxis and chemotaxis/chemokinesis downstream of CXCR4 is  
196 further blurred by the fact that CXCL12 regulates cell-matrix adhesion [24]. This does not mean that  
197 CXCR4 is involved in mechanosensing in NC cells. Instead, we could see CXCR4 signaling as priming cells  
198 to undergo mechanosensing by allowing them to functionally interact with the matrix. Interestingly,  
199 cell-matrix adhesion in cephalic NC cells also involves cadherins [30; 31]. There is an indirect role such  
200 that contact-dependent cell polarity primes NC cells to respond to CXCR4 signaling [4]. But there is also  
201 a direct role of cadherins. During migration, inhibiting E-cadherin affects adhesion to fibronectin rather  
202 than cell–cell adhesion [30] and cadherin-11 actively contributes to the formation of focal adhesion  
203 [31]. This means that we should regard EMT as a way to coordinate the quantitative and qualitative  
204 changes in cell-cell and cell-matrix adhesions rather than as a mechanism for cell dispersion in which  
205 loss of cell-cell adhesion and motility would be regulated in parallel and as purely cell autonomous  
206 properties.

207           Cadherins take part in regulating cell-matrix adhesions (directly and indirectly) and cell-matrix  
208 adhesions are needed to sense substrate stiffness. In turns, when substrate stiffness reaches a

209 threshold it promotes Twist nuclear entry which favors cadherin repression. One wonders about the  
210 molecular control of such intricate feedback loops. It could also mean that what has been labelled as  
211 CXCR4-dependent chemotaxis might be part of a global change of adhesive property taking place  
212 during EMT that prepares cells for efficient stiffness sensing. The existence of stiffness gradients  
213 around the cephalic NC cells is still highly speculative. But do cells need such spatially organized  
214 mechanical cue to promote directed movement? If so, how could we distinguish durotaxis from the  
215 so-called chemotaxis?

216

### 217 **Confinement, topological biases and ratchetaxis**

218 During EMT, cells pass from stable to transient cell-cell adhesions and this favors cell dispersion  
219 *in vitro*. This is further accentuated by CIL that biases cell's front-rear polarity such that cells move  
220 away from cell-cell contacts. However, *in vivo*, cephalic NC cells migrate at high cell density and do not  
221 undertake widespread dispersion despite EMT and CIL. The reason for this is that NC cells actively  
222 sense and follow each other via complement factor C3a signaling [32] and are constrained by their  
223 surrounding tissues physically and chemically [33]. When NC cells initiate migration, there are several  
224 epithelial structures around them such as the neural plate/tube, the eye, the epidermis and the cranial  
225 placodes. Placodes, as discussed above, are slowly displaced by NC cells such that they organize as  
226 discrete structures forming dorsoventral corridors restricting NC migration (FIGURE 1). This is  
227 reinforced by the fact that placodes are also the source of negative regulators of NC motility such as  
228 semaphorins rendering their vicinity non-permissive for migration [24; 34]. Interestingly, physical and  
229 chemical confinement together with intrinsic motility, CIL and mutual attraction are sufficient to drive  
230 directed NC migration even in absence of a stiffness gradient or a chemotactic cue [33; 35].

231 Another putative level of signaling interplay in this context is related to the fact that CXCR4 can  
232 physically interact with C3aR, the receptor of C3a, the chemokine mediating NC cell gregarious  
233 behavior [32]. C3 signaling can enhance CXCR4 signaling and both receptors colocalize in lipid rafts [36;

234 37; 38]. Interestingly, lipid rafts are mechanosensitive [39]. Thus, stiffness of the mesoderm underlying  
235 cephalic NC cells may also modulate a putative C3aR/CXCR4 cooperative signaling by promoting lipid  
236 raft remodeling. C3a-dependent mutual attraction increases the likelihood of transient cell-cell  
237 contacts. These contacts are known to block Rac1 activity at the site of transient junctions but also to  
238 promote an overall increase of Rac1 level in the cells [32]. In addition, CXCR4 also promotes Rac1  
239 activity and Rac1 is a key factor in protrusion and focal adhesion formation in cephalic NC cells [4].  
240 Thus, a lack of mutual attraction might also reduce the ability of NC cells to sense substrate stiffness  
241 (by lowering the ability to polarize and form cell-matrix adhesions) and might act a selection  
242 mechanism to prevent extensive migration of cells that are unable to properly interact with one  
243 another. A similar hypothesis could be drawn from the fact that N-cadherin-deficient cephalic NC cells  
244 disperse better *in vitro* but fail to polarize efficiently, do not migrate extensively *in vivo* and show signs  
245 of weaker cell-matrix adhesion [40]. This is even more relevant knowing that, in other cells, N-cadherin  
246 junctions can be regulated by the association of N-cadherin with lipid rafts and F-actin [41]. Thus, cross-  
247 regulating cell-cell interaction (N-cadherin and C3) and cell-matrix adhesion (Rac1, CXCR4) in a  
248 stiffness-dependent manner during collective cell migration may be a robust way to ensure that only  
249 functional cells can efficiently travel together to their final location.

250 Another level of integration could be mediated by proteases. *Xenopus* NC cells express Matrix  
251 Metalloproteinase MMP14 (a.k.a. MT1-MMP) [42; 43]. Interestingly, MMP14 can cleave Fibronectin  
252 [44] the main substrate of cephalic NC migration but also inactivates CXCL12 by removing a few of its  
253 N-terminal aminoacid [45]. This is even more interesting knowing that CXCL12 exhibit a high binding  
254 affinity for Fibronectin [46]. Therefore, *Xenopus* cephalic NC cells could use MMP14 to remodel  
255 Fibronectin (e.g. organization, density), release CXCL12 from the matrix (haptotaxis vs  
256 chemotaxis/chemokinesis) and inactivate CXCL12. This would further crosslink CXCR4-dependent cell-  
257 matrix adhesion with mechanosensing and blurs the lines between chemo and haptotaxis.

258           Xenopus cephalic NC cells are clearly exposed to a topologically biased environment at the  
259 onset of migration favoring ventralward migration. The medial part of the embryo with the neural  
260 plate/tube acting as an epithelial obstacle which releases several inhibitors of migration and a lower  
261 content in fibronectin than the lateral regions [24] is definitively an unfavorable territory for migration.  
262 However, it is unclear if *in vivo* cells experience repeated geometrical or mechanical anisotropy in  
263 environment organization known to generate ratchetaxis [47]. A more relaxed view of this concept  
264 relies on repeated topological anomalies (e.g. repetition of narrow and large spaces) that cells have to  
265 cross [48]. An important difference between topological bias and confinement as discussed above and  
266 ratchetaxis or its declinations is the scale at which these mechanisms act. The aforementioned  
267 chemical/physical topological bias acts at tissue scale, defining broad domains that are unsuitable for  
268 migration, whereas ratchetaxis occurs at the single cell level or subcellular level biasing individual cell  
269 polarity and cytoskeleton dynamics. We currently do not have tools to investigate whether ratchetaxis  
270 and the likes are indeed physiologically relevant for Xenopus NC cell migration. A detailed analysis of  
271 extracellular matrix composition and organization over time as well as a clear quantification of the  
272 roughness index of the NC migratory environment would need to be performed with modern tools.  
273 Even if repeated topological biases at microscopic scale would be observed it is unclear how such  
274 biases would be implemented and maintained in 4D throughout head morphogenesis to sustain  
275 directed NC migration over time. In addition to MMP14 discussed above, MMP2, 3, 7, 9, 11, 13, 15, 16,  
276 18, 20, 24 and 28, as well as multiple ADAMs, are expressed by cephalic NC cells or produced by the  
277 environment they cross during migration (see [49], Table 1 in [50] and references therein). Thus, in this  
278 context, the likelihood of relatively stable and iteratively distributed topological or mechanical cue (a  
279 requirement for ratchetaxis) along the dorsoventral path of cephalic NC migration appears quite low.

280

281 **Galvano/Electrotaxis**

282 Another mechanism that can generate directed cell motion is the detection of electric fields,  
283 known as galvanotaxis (or electrotaxis). Interestingly, in mammalian cell lines, lipid rafts were shown  
284 to take part in galvanotaxis [51] and electric fields also affect the GSK3 $\beta$ -dependent polarization of the  
285 Golgi apparatus [52] which helps organizing the non-centrosomal microtubule network, a key player  
286 in front-rear cell polarity [53]. GSK3 $\beta$  is required for cephalic NC migration in *Xenopus* [54] and is a  
287 known regulator of Snail cytoplasmic-nuclear shuttle [55]. Thus, by regulating C3aR/CXCR4 carrying  
288 lipid rafts and GSK3 $\beta$ , electric fields might be acting on multiple levels during *Xenopus* NC cell  
289 migration: EMT, front-rear polarity, cell-cell and cell-matrix adhesions. The ability of trunk NC cells to  
290 undergo galvanotaxis was shown using quail, *Xenopus* and axolotl embryos trunk neural tube explants  
291 *in vitro*, which were sometimes cultured for days before fields were applied [56; 57; 58; 59; 60].  
292 However, to our knowledge, electrotaxis has not been assessed in primary cephalic *Xenopus* NC cell  
293 culture. Some of the behaviors described in the literature appear to be somewhat artefactual with cells  
294 permanently elongated perpendicularly to the applied field. One of the reason may be the strengths  
295 of the applied electric fields used ranging from 100 to 600 mV/mm [60; 61] which are 4 to 22 times  
296 higher than what has been measured *in vivo* in *Xenopus* [62]. Indeed, from early in development, the  
297 *Xenopus* embryo has a transepithelial potential and electrical currents [62]. An anteroposterior  
298 gradient is detected from the blastopore and applying electric fields to nullify it led to developmental  
299 defects such as failure of anterior neural tube closure and reduced head development. Noticeably, it  
300 led to expulsion of cells from the blastopore which might indicate that the anteriorward displacement  
301 of mesoderm is partially affected. Given that this movement is crucial to generate a stiff environment  
302 for cephalic NC cells to migrate [15], one could propose that the observed head defects in embryos  
303 with nullified electric fields might be due to a partial failure of cephalic NC migration linked to improper  
304 mesoderm development. As for the other putative guiding mechanisms discussed, electric fields will  
305 not be a one-size-fit-all cue. While most cell types exposed to electric fields seem to migrate towards  
306 the cathode, some, such as macrophages, seem to prefer the anode [63]. Also, as discussed for the

307 other taxis, some of the cellular structures required for sensing and implementation of a polarity bias  
308 at the single cell level are not specific to electric fields as an input (e.g. lipid rafts, cell surface receptors).

309

## 310 **Conclusion**

311 All these interplays are mind blowing and place us, as experimentalists, in a chicken and egg  
312 situation. Hierarchy between signals and pathways is difficult to dissect because of the numerous  
313 cross-regulations taking place during migration itself. Exposure to chemokines is needed for cell-matrix  
314 adhesion. Cell-matrix adhesions are needed for motility and mechanosensing. Mechanosensing  
315 controls nuclear shuttling of transcription factors. These factors control expression of adhesion  
316 molecules and cytoskeleton components which in turn feedback into cell polarity, etc. Therefore,  
317 rather than being driven by competing guidance strategies, cephalic NC cells seem to iteratively use  
318 the molecular machinery of cell motility and adhesion to read the various signals at their disposal. This  
319 blurs the lines between the different kinds of taxis even if for most of them the initial cue is clearly  
320 identifiable (e.g. chemokine, rigidity, electric field). This may mean that an understanding of the  
321 complexity of an *in vivo* morphogenetic process such as NC cell migration requires a systems biology  
322 approach with contribution from multiple disciplines to integrate studies in which cues, genes or  
323 pathways are handled one at a time. We can think of it as studying the role that each individual LEGO  
324 piece plays in forming a bigger structure. Taking a single piece out is extremely powerful to gather  
325 information about it. However, at some point, one needs to try to fit all pieces together. The added  
326 difficulty is that in the regulation of *in vivo* cell migration each LEGO piece has melted and started to  
327 blend with several of its direct neighbors.

328 Our aim with this review is to raise awareness about artificial distinctions between supposedly  
329 different modes of cell guidance. In that context, we (as a community of NC researchers) should always  
330 keep in mind that the signal we are looking at in a given project may actually influence other inputs.  
331 The reason for that is that NC cells are exposed to multiple signals and may have evolved to use them

332 all at once, not one by one. That is already a fact based on published data but we probably  
333 underestimate it. Thus, we might need to systematically assess what knocking down one input does  
334 “outside” of its expected canonical function and with that in mind, design appropriate controls for our  
335 experimental approaches. We believe that the point we are making here invites the field to leave the  
336 current comfort zone and to address directed cell migration both in the context where it takes place  
337 and with the complexity it deserves.

338

339

#### 340 **Acknowledgements**

341 Work in ET lab is supported by Fondation pour la Recherche Medicale (FRMAJE201224), the Midi-  
342 Pyrenees regional council (grant 13053025), the CNRS and Universite Paul Sabatier. EHB is supported  
343 by a La Caixa Junior Leader grant (94978) and by Calouste Gulbenkian Foundation I-411133.01.



344 **References**

- 345 [1] L. Capuana, A. Bostrom, and S. Etienne-Manneville, Multicellular scale front-to-rear polarity in  
346 collective migration. *Current opinion in cell biology* 62 (2020) 114-122.
- 347 [2] M. Zhao, B. Song, J. Pu, T. Wada, B. Reid, G. Tai, F. Wang, A. Guo, P. Walczysko, Y. Gu, T. Sasaki, A.  
348 Suzuki, J.V. Forrester, H.R. Bourne, P.N. Devreotes, C.D. McCaig, and J.M. Penninger,  
349 Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma  
350 and PTEN. *Nature* 442 (2006) 457-60.
- 351 [3] K. Zhu, N.R. Hum, B. Reid, Q. Sun, G.G. Loots, and M. Zhao, Electric Fields at Breast Cancer and  
352 Cancer Cell Collective Galvanotaxis. *Scientific reports* 10 (2020) 8712.
- 353 [4] E. Theveneau, L. Marchant, S. Kuriyama, M. Gull, B. Moepps, M. Parsons, and R. Mayor, Collective  
354 chemotaxis requires contact-dependent cell polarity. *Developmental cell* 19 (2010) 39-53.
- 355 [5] N. Gougnard, C. Andrieu, and E. Theveneau, Neural crest delamination and migration: Looking  
356 forward to the next 150 years. *Genesis* (2018) e23107.
- 357 [6] E. Dupin, S. Creuzet, and N.M. Le Douarin, The contribution of the neural crest to the vertebrate  
358 body. *Advances in experimental medicine and biology* 589 (2006) 96-119.
- 359 [7] G. Schlosser, Development and evolution of vertebrate cranial placodes. *Developmental biology*  
360 389 (2014) 1.
- 361 [8] E. Theveneau, and R. Mayor, Collective cell migration of the cephalic neural crest: the art of  
362 integrating information. *Genesis* 49 (2011) 164-76.
- 363 [9] E. Theveneau, and R. Mayor, Neural crest delamination and migration: from epithelium-to-  
364 mesenchyme transition to collective cell migration. *Developmental biology* 366 (2012) 34-54.
- 365 [10] J. Yang, P. Antin, G. Berx, C. Blanpain, T. Brabletz, M. Bronner, K. Campbell, A. Cano, J. Casanova,  
366 G. Christofori, S. Dedhar, R. Derynck, H.L. Ford, J. Fuxe, A. Garcia de Herreros, G.J. Goodall,  
367 A.K. Hadjantonakis, R.J.Y. Huang, C. Kalchauer, R. Kalluri, Y. Kang, Y. Khew-Goodall, H. Levine,  
368 J. Liu, G.D. Longmore, S.A. Mani, J. Massague, R. Mayor, D. McClay, K.E. Mostov, D.F.  
369 Newgreen, M.A. Nieto, A. Puisieux, R. Runyan, P. Savagner, B. Stanger, M.P. Stemmler, Y.  
370 Takahashi, M. Takeichi, E. Theveneau, J.P. Thiery, E.W. Thompson, R.A. Weinberg, E.D.  
371 Williams, J. Xing, B.P. Zhou, G. Sheng, and E.M.T.I. Association, Guidelines and definitions for  
372 research on epithelial-mesenchymal transition. *Nature reviews. Molecular cell biology* 21  
373 (2020) 341-352.
- 374 [11] D. Pei, X. Shu, A. Gassama-Diagne, and J.P. Thiery, Mesenchymal-epithelial transition in  
375 development and reprogramming. *Nature cell biology* 21 (2019) 44-53.
- 376 [12] E. Scarpa, A. Szabo, A. Bibonne, E. Theveneau, M. Parsons, and R. Mayor, Cadherin Switch during  
377 EMT in Neural Crest Cells Leads to Contact Inhibition of Locomotion via Repolarization of  
378 Forces. *Developmental cell* 34 (2015) 421-34.
- 379 [13] S.C. Wei, L. Fattet, J.H. Tsai, Y. Guo, V.H. Pai, H.E. Majeski, A.C. Chen, R.L. Sah, S.S. Taylor, A.J.  
380 Engler, and J. Yang, Matrix stiffness drives epithelial-mesenchymal transition and tumour  
381 metastasis through a TWIST1-G3BP2 mechanotransduction pathway. *Nature cell biology* 17  
382 (2015) 678-88.
- 383 [14] L. Fattet, H.Y. Jung, M.W. Matsumoto, B.E. Aubol, A. Kumar, J.A. Adams, A.C. Chen, R.L. Sah, A.J.  
384 Engler, E.B. Pasquale, and J. Yang, Matrix Rigidity Controls Epithelial-Mesenchymal Plasticity  
385 and Tumor Metastasis via a Mechanoresponsive EPHA2/LYN Complex. *Developmental cell*  
386 (2020).
- 387 [15] E.H. Barriga, K. Franze, G. Charras, and R. Mayor, Tissue stiffening coordinates morphogenesis by  
388 triggering collective cell migration in vivo. *Nature* 554 (2018) 523-527.
- 389 [16] E.H. Barriga, P.H. Maxwell, A.E. Reyes, and R. Mayor, The hypoxia factor Hif-1alpha controls  
390 neural crest chemotaxis and epithelial to mesenchymal transition. *The Journal of cell biology*  
391 201 (2013) 759-76.

- 392 [17] Y. Bao, Z. Wang, B. Liu, X. Lu, Y. Xiong, J. Shi, P. Li, J. Chen, Z. Zhang, M. Chen, L. Wang, and Z.  
393 Wu, A feed-forward loop between nuclear translocation of CXCR4 and HIF-1alpha promotes  
394 renal cell carcinoma metastasis. *Oncogene* 38 (2019) 881-895.
- 395 [18] S. Rehberg, P. Lischka, G. Glaser, T. Stamminger, M. Wegner, and O. Rosorius, Sox10 is an active  
396 nucleocytoplasmic shuttle protein, and shuttling is crucial for Sox10-mediated  
397 transactivation. *Molecular and cellular biology* 22 (2002) 5826-34.
- 398 [19] Z. Mou, A.R. Tapper, and P.D. Gardner, The armadillo repeat-containing protein, ARMCX3,  
399 physically and functionally interacts with the developmental regulatory factor Sox10. *The*  
400 *Journal of biological chemistry* 284 (2009) 13629-40.
- 401 [20] Y. Aoki, N. Saint-Germain, M. Gyda, E. Magner-Fink, Y.H. Lee, C. Credidio, and J.P. Saint-Jeannet,  
402 Sox10 regulates the development of neural crest-derived melanocytes in *Xenopus*.  
403 *Developmental biology* 259 (2003) 19-33.
- 404 [21] S.M. Honore, M.J. Aybar, and R. Mayor, Sox10 is required for the early development of the  
405 prospective neural crest in *Xenopus* embryos. *Developmental biology* 260 (2003) 79-96.
- 406 [22] E. Theveneau, B. Steventon, E. Scarpa, S. Garcia, X. Trepast, A. Streit, and R. Mayor, Chase-and-  
407 run between adjacent cell populations promotes directional collective migration. *Nature cell*  
408 *biology* 15 (2013) 763-72.
- 409 [23] M.D. Culbertson, Z.R. Lewis, and A.V. Nechiporuk, Chondrogenic and gliogenic subpopulations of  
410 neural crest play distinct roles during the assembly of epibranchial ganglia. *PloS one* 6 (2011)  
411 e24443.
- 412 [24] F. Bajanca, N. Gougnard, C. Colle, M. Parsons, R. Mayor, and E. Theveneau, In vivo topology  
413 converts competition for cell-matrix adhesion into directional migration. *Nature*  
414 *communications* 10 (2019) 1518.
- 415 [25] B. Stramer, and R. Mayor, Mechanisms and in vivo functions of contact inhibition of locomotion.  
416 *Nature reviews. Molecular cell biology* 18 (2017) 43-55.
- 417 [26] R. McLennan, J.M. Teddy, J.C. Kasemeier-Kulesa, M.H. Romine, and P.M. Kulesa, Vascular  
418 endothelial growth factor (VEGF) regulates cranial neural crest migration in vivo.  
419 *Developmental biology* 339 (2010) 114-25.
- 420 [27] C.M. Lo, H.B. Wang, M. Dembo, and Y.L. Wang, Cell movement is guided by the rigidity of the  
421 substrate. *Biophysical journal* 79 (2000) 144-52.
- 422 [28] A.J. Thompson, E.K. Pillai, I.B. Dimov, S.K. Foster, C.E. Holt, and K. Franze, Rapid changes in tissue  
423 mechanics regulate cell behaviour in the developing embryonic brain. *eLife* 8 (2019).
- 424 [29] Z.N. Agricola, A.K. Jagpal, A.W. Allbee, A.R. Prewitt, E.T. Shifley, S.A. Rankin, A.M. Zorn, and A.P.  
425 Kenny, Identification of genes expressed in the migrating primitive myeloid lineage of  
426 *Xenopus laevis*. *Developmental dynamics : an official publication of the American Association*  
427 *of Anatomists* 245 (2016) 47-55.
- 428 [30] C. Huang, M.C. Kratzer, D. Wedlich, and J. Kashef, E-cadherin is required for cranial neural crest  
429 migration in *Xenopus laevis*. *Developmental biology* 411 (2016) 159-71.
- 430 [31] R.P. Langhe, T. Gudzenko, M. Bachmann, S.F. Becker, C. Gonnermann, C. Winter, G. Abbruzzese,  
431 D. Alfandari, M.C. Kratzer, C.M. Franz, and J. Kashef, Cadherin-11 localizes to focal adhesions  
432 and promotes cell-substrate adhesion. *Nature communications* 7 (2016) 10909.
- 433 [32] C. Carmona-Fontaine, E. Theveneau, A. Tzekou, M. Tada, M. Woods, K.M. Page, M. Parsons, J.D.  
434 Lambris, and R. Mayor, Complement fragment C3a controls mutual cell attraction during  
435 collective cell migration. *Developmental cell* 21 (2011) 1026-37.
- 436 [33] A. Szabo, M. Melchionda, G. Nastasi, M.L. Woods, S. Campo, R. Perris, and R. Mayor, In vivo  
437 confinement promotes collective migration of neural crest cells. *The Journal of cell biology*  
438 213 (2016) 543-55.
- 439 [34] H.H. Yu, and C.B. Moens, Semaphorin signaling guides cranial neural crest cell migration in  
440 zebrafish. *Developmental biology* 280 (2005) 373-85.
- 441 [35] A. Szabó, E. Theveneau, M. Turan, and R. Mayor, Neural crest streaming as an emergent  
442 property of tissue interactions during morphogenesis. *PLoS computational biology* 15 (2019)  
443 e1007002.

- 444 [36] M. Honczarenko, M.Z. Ratajczak, A. Nicholson-Weller, and L.E. Silberstein, Complement C3a  
445 enhances CXCL12 (SDF-1)-mediated chemotaxis of bone marrow hematopoietic cells  
446 independently of C3a receptor. *Journal of immunology* 175 (2005) 3698-706.
- 447 [37] M.Z. Ratajczak, R. Reza, M. Wysoczynski, J. Yan, and J. Ratajczak, Modulation of the SDF-1-CXCR4  
448 axis by the third complement component (C3)--implications for trafficking of CXCR4+ stem  
449 cells. *Experimental hematology* 34 (2006) 986-95.
- 450 [38] M. Wysoczynski, M. Kucia, J. Ratajczak, and M.Z. Ratajczak, Cleavage fragments of the third  
451 complement component (C3) enhance stromal derived factor-1 (SDF-1)-mediated platelet  
452 production during reactive postbleeding thrombocytosis. *Leukemia* 21 (2007) 973-82.
- 453 [39] D.E. Fuentes, and P.J. Butler, Coordinated Mechanosensitivity of Membrane Rafts and Focal  
454 Adhesions. *Cellular and molecular bioengineering* 5 (2012) 143-154.
- 455 [40] S. Kuriyama, E. Theveneau, A. Benedetto, M. Parsons, M. Tanaka, G. Charras, A. Kabla, and R.  
456 Mayor, In vivo collective cell migration requires an LPAR2-dependent increase in tissue  
457 fluidity. *The Journal of cell biology* 206 (2014) 113-27.
- 458 [41] M. Causeret, N. Taulet, F. Comunale, C. Favard, and C. Gauthier-Rouviere, N-cadherin association  
459 with lipid rafts regulates its dynamic assembly at cell-cell junctions in C2C12 myoblasts.  
460 *Molecular biology of the cell* 16 (2005) 2168-80.
- 461 [42] T. Garmon, M. Wittling, and S. Nie, MMP14 regulates cranial neural crest epithelial-to-  
462 mesenchymal transition and migration. *Developmental dynamics : an official publication of*  
463 *the American Association of Anatomists* (2018).
- 464 [43] M.L. Tomlinson, P. Guan, R.J. Morris, M.D. Fidock, M. Rejzek, C. Garcia-Morales, R.A. Field, and  
465 G.N. Wheeler, A chemical genomic approach identifies matrix metalloproteinases as playing  
466 an essential and specific role in *Xenopus melanophore* migration. *Chemistry & biology* 16  
467 (2009) 93-104.
- 468 [44] F. Shi, and J. Sottile, MT1-MMP regulates the turnover and endocytosis of extracellular matrix  
469 fibronectin. *Journal of cell science* 124 (2011) 4039-50.
- 470 [45] G.A. McQuibban, G.S. Butler, J.H. Gong, L. Bendall, C. Power, I. Clark-Lewis, and C.M. Overall,  
471 Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-  
472 1. *The Journal of biological chemistry* 276 (2001) 43503-8.
- 473 [46] A.J. Pelletier, L.J. van der Laan, P. Hildbrand, M.A. Siani, D.A. Thompson, P.E. Dawson, B.E.  
474 Torbett, and D.R. Salomon, Presentation of chemokine SDF-1 alpha by fibronectin mediates  
475 directed migration of T cells. *Blood* 96 (2000) 2682-90.
- 476 [47] D. Caballero, J. Comelles, M. Piel, R. Voituriez, and D. Riveline, Ratchetaxis: Long-Range Directed  
477 Cell Migration by Local Cues. *Trends in cell biology* 25 (2015) 815-827.
- 478 [48] A. Reversat, F. Gaertner, J. Merrin, J. Stopp, S. Tasciyan, J. Aguilera, I. de Vries, R. Hauschild, M.  
479 Hons, M. Piel, A. Callan-Jones, R. Voituriez, and M. Sixt, Cellular locomotion using  
480 environmental topography. *Nature* 582 (2020) 582-585.
- 481 [49] L. Christian, H. Bahudhanapati, and S. Wei, Extracellular metalloproteinases in neural crest  
482 development and craniofacial morphogenesis. *Critical reviews in biochemistry and molecular*  
483 *biology* 48 (2013) 544-60.
- 484 [50] N. Gougnard, E. Theveneau, and J.P. Saint-Jeannet, Dynamic expression of MMP28 during  
485 cranial morphogenesis. *Phil Trans R Soc Lond B Biol Sci.* (IN PRESS) IN PRESS (2020).
- 486 [51] B.J. Lin, S.H. Tsao, A. Chen, S.K. Hu, L. Chao, and P.G. Chao, Lipid rafts sense and direct electric  
487 field-induced migration. *Proceedings of the National Academy of Sciences of the United*  
488 *States of America* 114 (2017) 8568-8573.
- 489 [52] L. Cao, J. Pu, and M. Zhao, GSK-3beta is essential for physiological electric field-directed Golgi  
490 polarization and optimal electrotaxis. *Cellular and molecular life sciences : CMLS* 68 (2011)  
491 3081-93.
- 492 [53] J.C.M. Meiring, B.I. Shneyer, and A. Akhmanova, Generation and regulation of microtubule  
493 network asymmetry to drive cell polarity. *Current opinion in cell biology* 62 (2020) 86-95.
- 494 [54] S.G. Gonzalez Malagon, A.M. Lopez Munoz, D. Doro, T.G. Bolger, E. Poon, E.R. Tucker, H. Adel Al-  
495 Lami, M. Krause, C.J. Phiel, L. Chesler, and K.J. Liu, Glycogen synthase kinase 3 controls

496 migration of the neural crest lineage in mouse and *Xenopus*. *Nature communications* 9  
497 (2018) 1126.

498 [55] I. Muqbil, J. Wu, A. Aboukameel, R.M. Mohammad, and A.S. Azmi, Snail nuclear transport: the  
499 gateways regulating epithelial-to-mesenchymal transition? *Seminars in cancer biology* 27  
500 (2014) 39-45.

501 [56] R. Nuccitelli, T. Smart, and J. Ferguson, Protein kinases are required for embryonic neural crest  
502 cell galvanotaxis. *Cell motility and the cytoskeleton* 24 (1993) 54-66.

503 [57] H. Gruler, and R. Nuccitelli, Neural crest cell galvanotaxis: new data and a novel approach to the  
504 analysis of both galvanotaxis and chemotaxis. *Cell motility and the cytoskeleton* 19 (1991)  
505 121-33.

506 [58] R. Nuccitelli, and T. Smart, Extracellular Calcium Levels Strongly Influence Neural Crest Cell  
507 Galvanotaxis. *The Biological bulletin* 176 (1989) 130-135.

508 [59] R.F. Stump, and K.R. Robinson, *Xenopus* neural crest cell migration in an applied electrical field.  
509 *The Journal of cell biology* 97 (1983) 1226-33.

510 [60] M.S. Cooper, and R.E. Keller, Perpendicular orientation and directional migration of amphibian  
511 neural crest cells in dc electrical fields. *Proceedings of the National Academy of Sciences of*  
512 *the United States of America* 81 (1984) 160-4.

513 [61] R. Nuccitelli, and C.A. Erickson, Embryonic cell motility can be guided by physiological electric  
514 fields. *Experimental cell research* 147 (1983) 195-201.

515 [62] K.B. Hotary, and K.R. Robinson, Endogenous electrical currents and voltage gradients in *Xenopus*  
516 embryos and the consequences of their disruption. *Developmental biology* 166 (1994) 789-  
517 800.

518 [63] Y. Sun, B. Reid, F. Ferreira, G. Luxardi, L. Ma, K.L. Lokken, K. Zhu, G. Xu, Y. Sun, V. Ryzhuk, B.P.  
519 Guo, C.B. Lebrilla, E. Maverakis, A. Mogilner, and M. Zhao, Infection-generated electric field  
520 in gut epithelium drives bidirectional migration of macrophages. *PLoS biology* 17 (2019)  
521 e3000044.

522

523

524 **Figure legends**

525 Figure 1. Overview of Neural Crest migration

526 **a-c.** Diagrams depicting the position of NC cells (shades of brown to magenta) with respect to the  
527 placodal region (light blue) at pre-migration (stage 18), early migration (stage 21) and late migration  
528 (stage 25). EMT is progressively implemented as NC migration proceeds. Brown NC cells are more  
529 epithelial while magenta star-shaped NC cells are more mesenchymal. Top and bottom rows shows  
530 lateral views and dorsal views, respectively. Orientations and structures are indicated on the figure.  
531 Ot. ves., otic vesicle.

532

533 Figure 2. Neural Crest “mixotaxis”

534 **a,** The classical view of cephalic NC cell directed cell migration in *Xenopus laevis*. NC cells become  
535 motile via EMT and exhibit a collective behavior (collective cell migration (CCM)) due to a balance  
536 between dispersion (contact-inhibition of locomotion (CIL)) and mutual attraction (or co-attraction,  
537 CoA). Placodes, located in the lateral ectoderm, produce CXCL12, a well-known chemoattractant. NC  
538 cells express the main CXCL12 receptor, Cxcr4. NC are migrating towards latero-ventral territories due  
539 to CXCL12-dependent chemotaxis. **b,** The current view of cephalic NC cell directed cell migration in  
540 *Xenopus laevis* in which CXCL12, by promoting cell-matrix adhesion, contributes to defining permissive  
541 areas for cell migration in the context of a biased distribution of topological features. These include  
542 chemical and physical cues and requires a minimal stiffness of the surrounding tissue for cell migration  
543 to proceed. The main difference with the classical view is that precise and biased spatial distribution  
544 of secreted molecules is dispensable. **c,** A speculative view of what the actual control of cephalic NC  
545 cell directed cell migration in *Xenopus laevis* might look like with the inclusion of additional features  
546 such as a hypothetical graded distribution of stiffnesses (Durotaxis) and electric fields (Galvanotaxis)  
547 at tissue scale as well as iterative biases in topography at cellular and subcellular scales (Ratchetaxis).  
548 While most of these features can be experimentally disentangled under controlled *ex vivo*

549 experiments, none of these cues relies on a specific set of molecular sensors and effectors but rather  
550 share downstream signal transduction machineries leading to cell adhesion and polarity. Therefore, *in*  
551 *vivo*, each input (e.g. chemical, mechanical, electrical) is likely to extensively feed into the others  
552 leading to the exciting idea that, in their native environment, NC cells may achieve directed migration  
553 by performing a sort of “mixotaxis”. See main text for details.