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A stochastic model of adult neurogenesis coupling cell cycle progression and differentiation

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7 Abstract

8 Long-term tissue homeostasis requires a precise balance between stem cell self-renewal and the
9 generation of differentiated progeny. Recently, it has been shown that in the adult murine brain,
10 neural stem cells (NSCs) divide mostly symmetrically. This finding suggests that the required
11 balance for tissue homeostasis is accomplished at the population level. However, it remains un-
12 clear how this balance is enabled. Furthermore, there is experimental evidence that proneural
13 differentiation factors not only promote differentiation, but also cell cycle progression, suggesting
14 a link between the two processes in NSCs. To study the effect of such a link on NSC dynamics, we
15 developed a stochastic model in which stem cells have an intrinsic probability to progress through
16 cell cycle and to differentiate. Our results show that increasing heterogeneity in differentiation
17 probabilities leads to a decreased probability of long-term tissue homeostasis, and that this effect
18 can be compensated when cell cycle progression and differentiation are positively coupled. Using
19 single-cell RNA-Seq profiling of adult NSCs, we found a positive correlation in the expression levels
20 of cell cycle and differentiation markers. Our findings suggest that a coupling between cell cycle
21 progression and differentiation on the cellular level is part of the process that maintains tissue
22 homeostasis in the adult brain.

23 1 Introduction

24 It is now widely accepted that a precise balance of stem cell self-renewal and differentiation is
25 required to maintain long term tissue homeostasis. There are two strategies to achieve this task:
26 asymmetry on the cellular or on the population level [1, 2, 3]. In mammals, the applied home-
27 ostatic strategies are different for every regenerating tissue, among which are the skin, intestine,
28 lung, blood, bone marrow, heart, testis, uterus and mammary gland [4, 5, 6]. For instance, ap-
29 proximately 85% of the stem cells in the skin divide asymmetrically [7], whereas stem cells in the
30 intestine divide mostly symmetrically [8]. The mammalian brain also regenerates [9], whereas it
31 remains unclear which homeostatic strategy is applied. For a long time, it has been thought that
32 the strategy to maintain tissue homeostasis in the adult brain would rely on asymmetry on the
33 cellular level [10]. This thinking is likely to have originated from the fact that during embryo neu-
34 rogenesis, primary neural progenitors in the ventricular zone (VZ) divide mostly asymmetrically
35 [11]. However, Obernier et al. have recently shown that stem cells in the adult brain divide mostly
36 symmetrically [12]. This implies that for adult neurogenesis the strategy to maintain homeostasis
37 is population asymmetry rather than asymmetry on the cellular level. Although the sustainment
38 of adult organs via population asymmetry is a well-established concept and is found in other tis-
39 sues, there are still many open questions. For example, how can a stable stem cell population be
40 achieved when stem cells divide exclusively symmetrically?

41 Homeostasis via population asymmetry could, for instance, be achieved by limited access to a
42 stem cell niche in combination with a short-range cell fate determining signal [2, 13]. Conversely,
43 if the stem cell population asymmetry is based on an internal regulation that is either intrinsic
44 or coupled to an extrinsic signaling, it is unknown what mechanisms could govern the process. Is
45 a molecular regulator enabling a balanced stochastic cell fate decision for a stem cell population?
46 Interestingly, an experimental study revealed a molecular link between cell cycle progression of neu-
47 ral progenitors and neuronal differentiation through the proneural gene *Ascl1* [14]. Furthermore,
48 recent results have shown that the transcription factor *Ato* in *Drosophila*, regulating the expression
49 of *Ase*, the non-mammalian homolog of *Ascl1*, has a dual role in proliferation and differentiation
50 of neural progenitors [15]. These findings suggest that the cell cycle progression and differentiation
51 of embryonic NSCs are regulated in a coupled manner through the expression of proneural genes.
52 At present it remains unclear, whether cell cycle progression and differentiation of adult NSCs is
53 also regulated in a coupled manner, and, in addition to that, whether such a coupled regulation
54 would have an effect on tissue homeostasis.

55 Over the years, a number of insightful studies on renewal dynamics of stem cells have been
56 conducted, ranging from models on stem cell dynamics in general [16, 17, 18, 19, 20, 21, 22, 23]
57 to brain specific models [24, 25, 26, 27, 28, 29, 30, 31, 32]. The latter were used to explore

58 specific characteristics of neurogenesis, such as the role of fate determining signaling factors during
59 development [28], or the migration of neuroblast cells to the olfactory bulb in adults [26]. Other
60 models explored the conditions for homeostasis without further specifying the studied regenerating
61 tissue [17, 19, 20, 21, 22, 23]. For instance, the dynamics of mutant accumulation in homeostatic
62 tissues over time has been explored, showing that type and number of mutants depend on the
63 division-tree length [23]. Especially the role of feedback signals controlling the balance between
64 stem cell self-renewal and differentiation was investigated. It was shown that homeostasis based on
65 stochastic processes needs to be regulated by negative regulatory feedback loops [20]. Furthermore,
66 it was found that, depending on the applied feedback network, symmetric stem cell divisions can
67 both stabilize or destabilize the homeostatic state of a population [21].

68 In order to study how a coupling between cell cycle progression and differentiation on the single
69 cell level affects homeostasis, we developed a model in which stem cells have an intrinsic probability
70 to progress through cell cycle and to differentiate. In our model, stem cells divide symmetrically,
71 representing for instance adult NSCs [12]. We chose a stochastic approach as it allows to study
72 how fluctuations that emerge at the cellular level affect population dynamics. With our model, we
73 investigated the dynamics of stem cell populations with uncoupled and coupled cell cycle activity
74 and differentiation. We evaluated how robust populations are maintained and how many pre-
75 differentiated cells are produced. Furthermore, we analyzed single-cell RNA-Seq data of adult
76 NSCs for a qualitative comparison to our simulation results.

77 This paper is organized as follows. In Section 2 we define our model and discuss the biological
78 background underlying our assumptions. In Section 3 we present the results of our simulations
79 (Section 3.1 and 3.2) and the RNA-Seq profiling analysis (Section 3.3), offering biological interpre-
80 tation of the results. In Section 4 we discuss our findings.

2 Methods

2.1 Modeling a stem cell

We developed a stochastic model of stem cell dynamics in which the fate of a stem cell is determined by its probability to progress through cell cycle within a time frame Δt and its probability to differentiate.

In our model, we assign each stem cell a factor ϵ , where ϵ corresponds to:

$$\begin{cases} \epsilon : & \text{probability to progress through cell cycle within } \Delta t, \\ (1 - \epsilon) : & \text{probability to not progress through cell cycle within } \Delta t, \end{cases} \quad (1)$$

with $\epsilon \in [0, 1]$. The cell cycle activity ϵ is inverse proportional to the cell cycle time τ_{cc} and given by $\epsilon = \frac{\Delta t}{\tau_{cc}}$. Further, each stem cell has a

$$\begin{cases} \alpha : & \text{probability to differentiate, and a} \\ (1 - \alpha) : & \text{probability to maintain stemness,} \end{cases} \quad (2)$$

with $\alpha \in [0, 1]$.

Recent findings revealed that the divisions of NSCs in adult mice are mostly symmetrical [12]. Our model mimics the dynamics of adult NSCs by allowing stem cells to divide only symmetrically, either into two stem cells (symmetric proliferative division) or into two pre-differentiated cells (symmetric differentiative division). If a stem cell does not progress through cell cycle, it remains unchanged while keeping its typical stem cell properties. The dynamics of a stem cell are described by a discrete-time Markov chain, where each iteration has a set of 3 states: symmetric proliferative division, symmetric differentiative division and no division. A graphical representation of one iteration is shown in Figure 1(a). The probability of differentiation and self-renewal are given by $\epsilon\alpha$ and $\epsilon(1 - \alpha)$, respectively.

2.2 Modeling a stem cell population

Several factors can lead to inhomogeneous populations, e.g. the spatial distribution of stem cells or biomolecular signaling. Our model allows for extrinsic and intrinsic sources of heterogeneity by assigning an individual value pair of cell cycle activity and differentiation probability (ϵ_k, α_k) to every stem cell. In case of a symmetric proliferative division, the daughter cell does not inherit the properties of its mother cell, being in line with an experimental study that revealed independent cell cycle times between mother and daughter cells in the rat retina [33].

We investigated stem cell populations with uncoupled and coupled cell cycle and differentiation probabilities. The probabilities (ϵ_k, α_k) for every stem cell of a population were generated according to a multivariate normal distribution $\mathbf{X} \sim \mathcal{N}_2(\mathbf{k}, \mathbf{\Sigma})$ with vector $\mathbf{k} = (\mu_\epsilon \mu_\alpha)^\top$ and covariance matrix

$$\mathbf{\Sigma} = \begin{pmatrix} \sigma_\epsilon^2 & c\sigma_\epsilon\sigma_\alpha \\ c\sigma_\epsilon\sigma_\alpha & \sigma_\alpha^2 \end{pmatrix}. \quad (3)$$

The mean values μ_ϵ, μ_α and standard deviations $\sigma_\epsilon, \sigma_\alpha$ determine the normal distribution of cell cycle activities and differentiation probabilities, respectively. The correlation coefficient $c \in [-1, 1]$ defines the type of coupling between cell cycle activity and differentiation.

For our simulations, we assumed average cell cycle times of $\tau_{cc} \simeq 20h$, which is close to measured cell cycle times for stem cells in the ventricular-subventricular zone (V-SVZ) of the adult mammalian brain [34]. We chose a time step of $\Delta t = 1h$. Figure 1(b) shows an exemplary histogram of cell cycle activities $\{\epsilon\}$ and the corresponding histogram of cell cycle times $\{\tau_{cc}\}$. We chose σ_ϵ such that the resulting distribution of the cell cycle times $\{\tau_{cc}\}$ resembles experimental measurements [33, 35]. Consequently, about 90% of all stem cells have a cell cycle time of $(20 \pm 10)h$, while the remaining ones are slow cycling stem cells that accumulate over time.

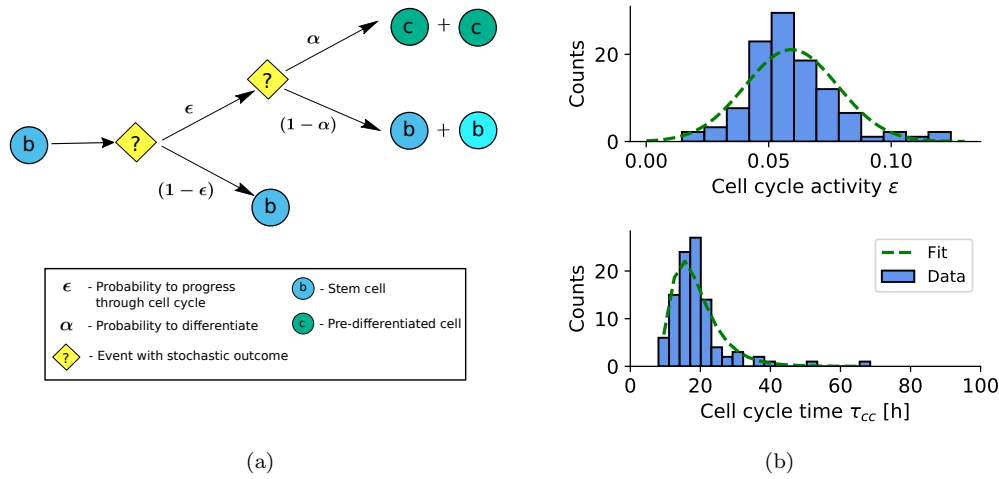


Figure 1: (a) *Schematic representation of one iteration step.* The probability of differentiation and self-renewal are given by $\epsilon\alpha$ and $\epsilon(1 - \alpha)$, respectively. (b) *Exemplary histogram of cell cycle activities $\{\epsilon\}$ and corresponding histogram of cell cycle times $\{\tau_{cc}\}$, with normal and log-normal fit, respectively.*

120 To study the dynamics of a stem cell population in a tissue, we summed over all stem cells at
 121 each iteration step to track the stem cell population size. The expected net change of the stem cell
 122 population B after one time step is given by

$$\Delta B = B_{t+1} - B_t = \sum_{k=1}^{B_t} \epsilon_k \cdot (1 - 2\alpha_k), \quad (4)$$

123 where B_t is the number of stem cells before the iteration step. For homeostasis, we assumed the
 124 stem cell population to be constant in size:

$$\langle \epsilon_k (1 - 2\alpha_k) \rangle_{k,t} = 0. \quad (5)$$

125 For all relevant parameter areas in our study, the differences in the solution for $\langle \alpha_k \rangle_k = \mu_\alpha$ between
 126 the statistically dependent and independent case, are negligible. Thus, we solve $\langle \epsilon_k \rangle_k \cdot \langle 1 - 2\alpha_k \rangle_k = 0$
 127 to determine μ_α for the homeostatic case. The non-trivial solution is $\mu_\alpha = 0.5$, which implies that
 128 the stem cells are on average as likely to maintain stemness as to differentiate. We analyzed the
 129 average stem cell population dynamics for the non-homeostatic cases and found that a deviation
 130 from the homeostatic conditions ($\mu_\alpha \neq 0.5$ and $\mu_\epsilon > 0$) leads to a measurable change in the stem
 131 cell population size within approximately two days (Section A.1).

132 To evaluate and compare our simulations, we analyzed the number of differentiated daughter
 133 cells and the probability to maintain a stem cell population (robustness). We defined the differen-
 134 tiation rate D as the number of pre-differentiated cells C that were generated on average per time
 135 step, normalized by the initial stem cell population size B_0 :

$$D = \frac{C_{t_{end}}}{t_{end} \cdot B_0}, \quad (6)$$

136 with t_{end} being the simulation time. We simulated the stem cell population dynamics for different
 137 t_{end} and we set $B_0 = 50$. For every t_{end} , we simulated $n = 300$ populations and evaluated how
 138 many stem cell populations \tilde{n} could maintain their size, i.e. were neither lost ($B_{t_{end}} = 0$) nor did
 139 overgrow ($B_{t_{end}} \geq 2B_0$) within the given time period t_{end} . We calculated the robustness R as
 140 follows:

$$R = \frac{\tilde{n}}{n}. \quad (7)$$

141 2.3 Analysis of single-cell RNA-Seq data

142 We analyzed public single-cell RNA-Seq data of adult NSCs [36] for a qualitative comparison to
 143 our simulation results. From the RNA-Seq data, we extracted cell cycle and differentiation marker
 144 expression levels, which we related to the cell cycle activity ϵ and differentiation probability α in
 145 our model. The goal of this analysis was, to extract information on the coupling between cell cycle
 146 activity and differentiation of adult NSCs.

147 For our analysis, we plotted the expression levels of cell cycle markers (*Mki67*, *Mcm2*, *Ccnd1*
 148 [36, 37, 38]) and differentiation markers (*Ascl1*, *Neurog2*, *Neurod2* [34, 39, 40]) on a \log_2 scale
 149 against each other, excluding data points with zero expression for both marker types. We then
 150 computed the Pearson correlation coefficient (PCC) and did a bootstrapping analysis to evaluate
 151 the accuracy of the determined PCC.

152 3 Results

153 3.1 Uncoupled cell cycle progression and differentiation

154 First, we studied the dynamics of stem cell populations with uncoupled cell cycle activity and
 155 differentiation. To do so, we generated probabilities as described in Section 2.2 with various
 156 σ_α and $c = 0$. Two exemplary distributions are shown in Figure 2(a) and further exemplary
 157 distributions are shown in Figure A.2. We simulated the dynamics of two stem cell population types
 158 with distinct differentiation heterogeneity levels ($\sigma_\alpha = 0.02, \sigma_\alpha = 0.08$) for different simulation
 159 times t_{end} . We found that for both population types the average differentiation rates D are not
 160 significantly changing for different simulation times, while a population with higher differentiation
 161 heterogeneity tends to generate more differentiated progeny (Figure 2(b), top). Evaluating the
 162 robustness R , we observed that for both differentiation heterogeneity levels, the probability to
 163 maintain a population decreases for increasing simulation times (Figure 2(b), bottom). Although
 164 the robustness of populations with lower differentiation heterogeneity decreases significantly slower,
 165 there is no life-long homeostasis for a population with uncoupled cell cycle and differentiation
 166 activities.

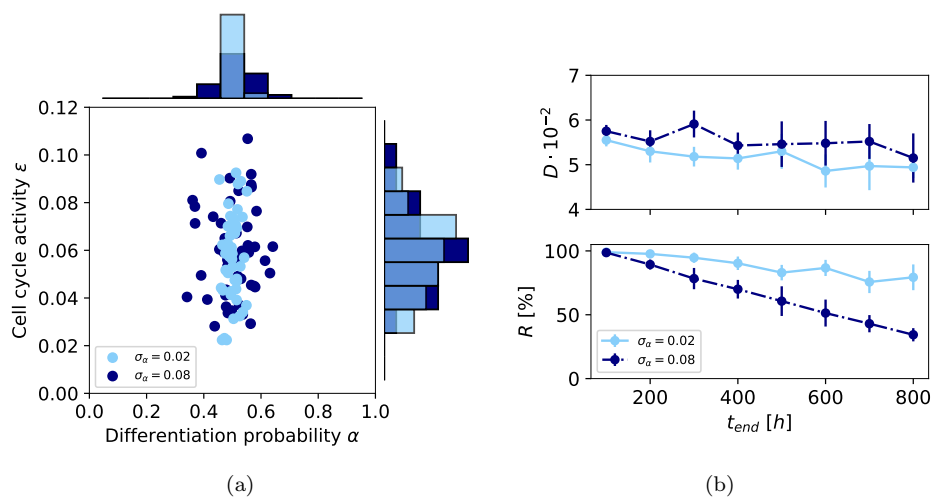


Figure 2: (a) Two exemplary stem cell populations with uncoupled cell cycle and differentiation and distinct differentiation heterogeneity levels. The histograms show an overlay of cell cycle activity and differentiation probability distributions, respectively, where the bars for $\sigma_\alpha = 0.02$ are transparent. (b) Average differentiation rates D (top) and average robustness R (bottom) of two stem cell population types with uncoupled cell cycle and differentiation for different simulation times t_{end} . The level of heterogeneity affects the probability for maintaining homeostasis significantly, while the production of differentiated progeny is not affected.

167 Focusing on long-term simulations ($t_{end} = 800h \simeq$ one month), we observed that the level of
 168 heterogeneity in the differentiation probabilities has a strong impact on homeostasis: the higher
 169 the differentiation heterogeneity level, the lower the probability to maintain a stem cell population
 170 (Figure 3(a)). Furthermore, the probability for overgrowth among the unstable cases increases
 171 from around 50% for $\sigma_\alpha = 0$ to 100% for $\sigma_\alpha = 0.12$. Both results are likely to be caused by
 172 the dynamics of stem cells that have a high cell cycle activity and are more likely to maintain
 173 stemness: these cells cause a stem cell population to (over-)grow and their number increases
 174 with increasing differentiation heterogeneity. Moreover, the long-term simulations revealed that
 175 the differentiation rates D of populations with a robustness $\geq 10\%$ do not significantly change
 176 for various differentiation heterogeneity levels (Figure 3(b)). However, stem cell populations with
 177 heterogeneous differentiation probabilities tend to be larger and thus have on average slightly higher
 178 differentiation rates than stem cell populations with homogeneous differentiation probabilities.

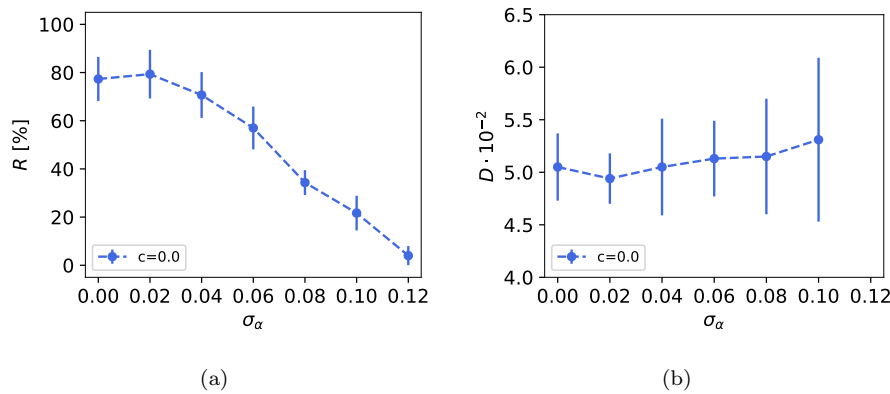


Figure 3: Long-term ($t_{end} = 800h$) robustness and differentiation rates of stem cell populations with uncoupled cell cycle and differentiation for various σ_α . (a) The higher σ_α , the lower the probability for a population to be maintained. (b) The level of heterogeneity in the differentiation probabilities does not affect the differentiation rates significantly. More heterogeneous differentiation probabilities induce the generation of more differentiated progeny.

179 3.2 Coupled cell cycle progression and differentiation

180 Having analyzed uncoupled stem cell populations, we investigated stem cell populations with cou-
 181 pled cell cycle and differentiation activities. Again, we generated probabilities as described in
 182 Section 2.2 with various σ_α and $c \neq 0$. Two exemplary distributions are shown in Figure 4(a) and
 183 further exemplary distributions are shown in Figure A.2. We studied the dynamics of stem cell
 184 populations with coupled cell cycle and differentiation activities and $\sigma_\alpha = 0.08$. We found that
 185 for all simulation times, a negative coupling results in significantly higher average differentiation
 186 rates D than a positive coupling (Figure 4(b), top). Evaluating the robustness R , we observed
 187 that for both, populations with positive and negative coupling, the probability to be maintained in
 188 the long term is lower than in the short term (Figure 4(b), bottom). The probability to maintain
 189 homeostasis decreases significantly slower for populations with a positive coupling than for popu-
 190 lations with a negative coupling. However, there is no life-long homeostasis for a population with
 191 coupled cell cycle and differentiation activities.

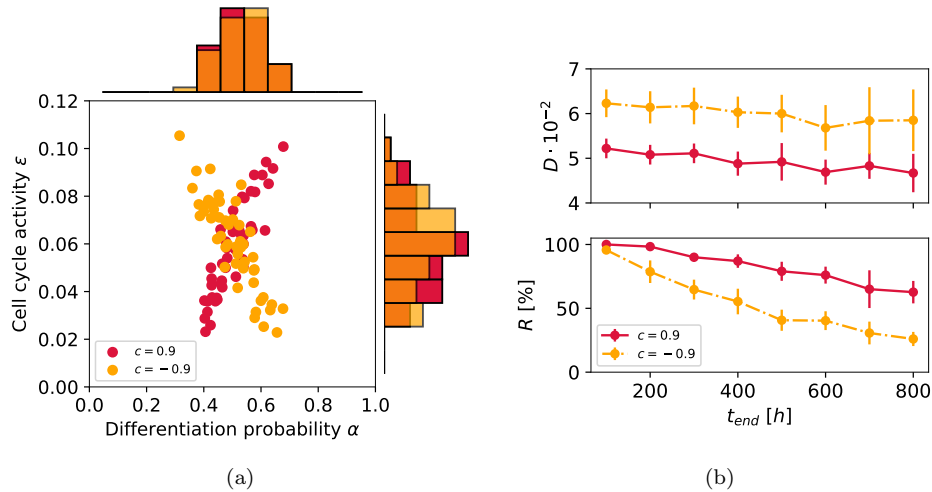


Figure 4: (a) *Two exemplary coupled stem cell populations with positive and negative correlation. The histograms show an overlay of cell cycle activity and differentiation probability distributions, respectively, where the bars for $c = -0.9$ are transparent.* (b) *Average differentiation rates D (top) and average robustness R (bottom) of two coupled stem cell population types for different simulation times t_{end} . A negative coupling increases differentiation rates, while a positive coupling increases the probability for maintaining homeostasis.*

192 Focusing on long-term simulations ($t_{end} = 800h \simeq$ one month), we observed that the type of
 193 coupling between cell cycle activity and differentiation has a strong impact on homeostasis. A
 194 positive correlation increases the probability for a tissue to be maintained in the long term (Figure
 195 5(a)) and decreases its average differentiation rates D (Figure 5(b)). In contrast, a negative
 196 correlation decreases the probability for a tissue to be maintained in the long term. At the same
 197 time, it results in the highest average differentiation rates D for stem cell populations with a
 198 robustness $\geq 10\%$. Furthermore, we found that the probability for overgrowth among the unstable
 199 cases merely depends on the differentiation heterogeneity level and is independent of the coupling
 200 type. Stem cell populations with a coupling between cell cycle activity and differentiation mainly
 201 consist of two subpopulations: slow dividing stem cells and fast dividing stem cells, which are
 202 either more likely to maintain stemness or more likely to differentiate. For populations with a
 203 negative coupling, the slow dividing stem cells are more likely to differentiate and the fast dividing
 204 stem cells are more likely to maintain stemness, which causes populations to (over-)grow. At the
 205 same time, because these stem cell populations are larger, they enable the generation of more
 206 pre-differentiated progeny compared to populations with a positive coupling. For populations with
 207 a positive coupling, the fast dividing stem cells are more likely to differentiate, while the cells that
 208 are more likely to maintain stemness divide slowly, which enables robustness of a population in
 209 the long term.

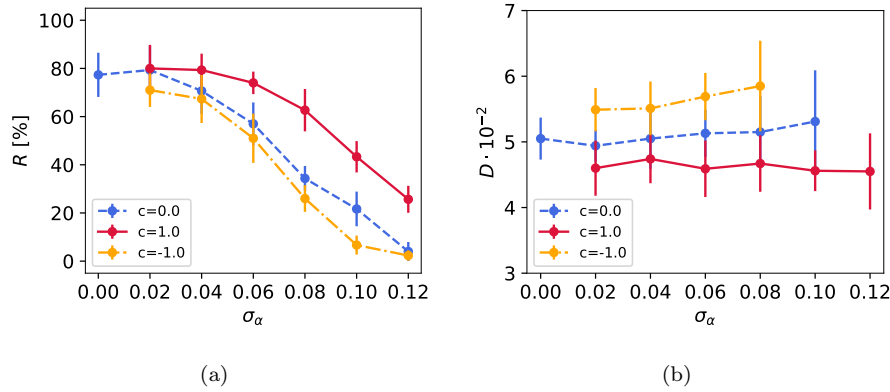


Figure 5: Long-term ($t_{end} = 800h$) robustness and differentiation rates of stem cell populations with coupled cell cycle and differentiation for various σ_α . (a) A positive coupling increases the probability for maintaining homeostasis. (b) A negative coupling increases differentiation rates, while a positive coupling lowers them.

210 3.3 Single-cell RNA-Seq data of adult murine NSCs

211 In our simulations, we observed the most robust stem cell populations for a positive correlation
 212 between cell cycle activity and differentiation (Figure 5(a)). We also found a positive correlation
 213 between cell cycle and differentiation marker expression levels in the adult murine SVZ (Figure
 214 6(a)). This finding indicates that an adult NSC population is likely to prioritize its robust main-
 215 tenance rather than a high amount of differentiated progeny. We saw this positive correlation
 216 confirmed when we analyzed the subpopulation of stem cells which have non-zero expression levels
 217 in both, cell cycle and differentiation markers (A.3).

218 An underlying mechanism for a coupling of cell cycle and differentiation on the molecular level
 219 could be driven by proneural genes regulating both processes in a coupled manner (Figure 6(b)).
 220 Determining which molecular players might be involved in such a regulation will require further
 221 experimental investigation.

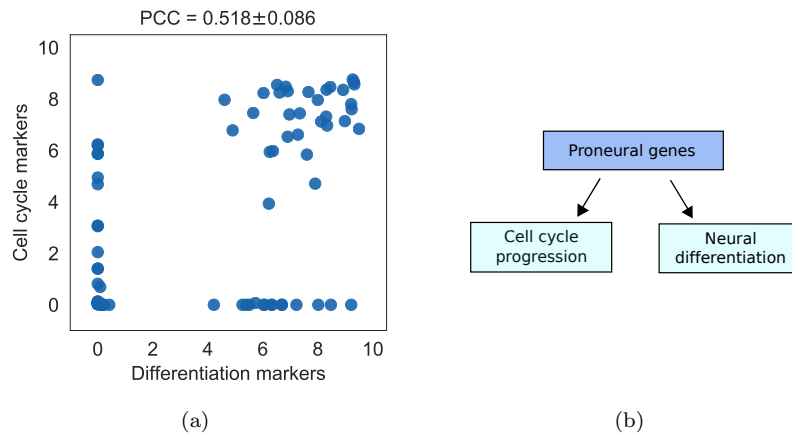


Figure 6: (a) Analysis of single-cell RNA-Seq data of adult mouse NSCs [36] reveals a positive correlation between cell cycle and differentiation marker expression levels. (b) Schematic representation of a possible underlying molecular mechanism of a coupled regulation of cell cycle progression and differentiation.

222 4 Discussion

223 Homeostasis is an important mechanism for the sustainment of adult tissues during life. Various
224 organs regenerate via tissue homeostasis, and there is evidence that also complex organs like the
225 brain make use of it. Here, we presented a stochastic model to study stem cell dynamics in the
226 adult brain. We focused on the interplay between cell cycle activity and differentiation on the
227 cellular level and its impact on stem cell population dynamics. We also analyzed single-cell RNA-
228 Seq data of adult NSCs for a qualitative comparison to our simulation results. In the following,
229 we present and discuss the main findings of this study.

230 Our simulations showed that the probability to maintain homeostasis decreases with increasing
231 differentiation heterogeneity. Furthermore, we found that a coupling of cell cycle and differentiation
232 has an effect on stem cell population dynamics, and, depending on whether the coupling is positive
233 or negative, increases or decreases the robustness of homeostasis in an adult tissue, respectively. A
234 coupling between cell cycle activity and differentiation leads to the generation of two subpopulations
235 within a stem cell population. There are slow dividing and fast dividing stem cells, which are either
236 more likely to maintain stemness or more likely to differentiate. In a population with a positive
237 coupling, the fast dividing stem cells are more likely to differentiate and the slow dividing stem
238 cells are more likely to maintain stemness. This combination leads to an increase in the typical
239 lifetime of a stem cell pool. How subpopulations within a stem cell population could be generated
240 remains unclear. For instance, limited access to a stem cell niche in combination with a short range
241 signal can possibly generate two or more subpopulations of stem cells.

242 Although a positive coupling between cell cycle and differentiation leads to a significantly higher
243 long-term robustness for heterogeneous differentiation probabilities, it is also advantageous for a
244 stem cell population to keep the heterogeneity level of differentiation low. Based on our theoretical
245 analysis, we cannot conclude which of these scenarios is probably the case in the adult brain. Other
246 studies report heterogeneous gene expression amongst stem cells within a population, suggesting the
247 existence of differentiation heterogeneity within a NSC population. For instance, there is evidence
248 for heterogeneity in gene expression among hematopoietic stem cells [41, 42, 43]. Furthermore,
249 heterogeneity among the NSCs in the mammalian brain prevails in terms of proliferation dynamics
250 and regional identity [44]. A central question is, whether the heterogeneity is a reflection of intrinsic
251 differences of NSCs or is caused by an external signal [10].

252 Single-cell RNA-Seq is a powerful method to compare gene expression levels of cells within a
253 population. Our qualitative analysis of single-cell RNA-Seq data of adult NSCs showed a positive
254 correlation between the expression of cell cycle markers and differentiation markers, indicating that
255 cell cycle activity and differentiation are indeed coupled. As RNA-Seq data has several limitations,
256 such as providing a snapshot of gene expression in time and allowing for a relative quantification
257 of gene expression levels only, we can neither conclude that the differentiation probabilities are
258 heterogeneous and positively coupled nor that they are rather homogeneous. Nonetheless, the
259 idea that both, cell cycle progression and differentiation, are controlled by the same molecular
260 players could explain a positive correlation between these two key components of homeostasis.
261 The proneural gene *Ascl1*, associated with differentiation of neuronal progenitor cells [14], could
262 be part of a molecular network that regulates cell cycle progression and differentiation of NSCs in
263 a coupled manner. The existence and the details of such a regulating network will require further
264 experimental investigation, as well as the identification of additional molecular players.

265 In this study, our focus was on exploring the effect of a coupled internal regulation of cell cycle
266 activity and differentiation on stem cell population dynamics. Explicit regulating factors are not
267 part of our model, as they were intensively explored in former studies [17, 21]. As there is no
268 long-term homeostasis without external control, we propose that feedback signaling among the
269 stem cells or signaling from the stem cell niche are the building blocks to maintain a stem cell
270 population. In addition, a coupling of cell cycle activity and differentiation of stem cells gives a
271 stem cell population an internal robustness and is thus likely to be part of a homeostatic strategy.
272 Furthermore, a certain, if only small, amount of adult NSCs dividing asymmetrically can further
273 increase the stability of homeostasis, as well as stem cell quiescence.

274 If the balance between stem cell self-renewal and differentiation gets out of equilibrium, the

275 stem cell population suffers overgrowth or depletion of the stem cell population, resulting in fatal
276 consequences for the organ such as tumorigenesis [3, 45, 46]. Our simulation results have shown
277 that a stem cell population whose cell cycle activity and differentiation are negatively correlated is
278 rather unstable: it tends to overgrow, while producing many differentiated cells at the same time.
279 This results in the question whether in case of disease, a regulating molecular mechanism might
280 possibly be altered and adopt a state that is similar to the negatively coupled one. In summary, in
281 terms of neurogenesis and neural diseases related to tissue overgrowth, the molecular link between
282 cell cycle progression and differentiation in NSCs is worthwhile to study further.

283 **Materials**

284 **Code Availability**

285 For simulations and data analysis we used Python programming language (Python Software Foun-
286 dation, <https://www.python.org/>).
287 Our code is available on <https://github.com/astopka/StochasticModelNSC.git>.

288 **Data Availability**

289 For the analysis of single-cell RNA-Seq data we used the public dataset from [36] and the processed
290 data was downloaded from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67833>.

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416 A Appendix

417 A.1 Average stem cell population dynamics

418 We studied the average stem cell population dynamics, assuming constant average stem cell prop-
 419 erties $\epsilon = \langle \epsilon_k \rangle_k$, $\alpha = \langle \alpha_k \rangle_k$ for any time. Equation (4) then becomes

$$\frac{dB}{dt} = [\epsilon \cdot (1 - 2\alpha)]B(t) \quad (8)$$

420 with the solution

$$B(t) = B_0 \cdot e^{[\epsilon \cdot (1 - 2\alpha)]t}. \quad (9)$$

421 We characterized the evolution of a stem cell population by bringing the initial population size
 422 B_0 in relation to the final population size $B_{t_{end}}$:

$$\rho_B = \frac{B_{t_{end}}}{B_0}. \quad (10)$$

423 There are three possible scenarios for the evolution of a stem cell population over time: it can be
 424 constant in size ($\rho_B = 1$), grow ($\rho_B > 1$) or shrink ($\rho_B < 1$), where the two latter cases occur under
 425 non-homeostatic conditions (Figure A.1). We analyzed the non-homeostatic cases and found that
 426 even a minimal deviation from the homeostatic cell cycle and differentiation activity leads to a
 427 vast change in the stem cell population size within the time of two average cell cycles ($t_{end} = 40h$).
 428 Both, overgrowth and depletion, become stronger with an increasing average cell cycle activity
 429 $\langle \epsilon_k \rangle_k$.

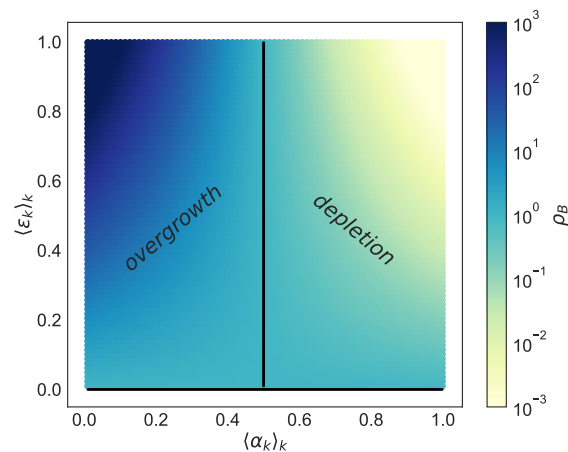


Figure A.1: Average stem cell population dynamics for the homeostatic cases (black lines) and the non-homeostatic cases for $t_{end} = 40h$.

430

A.2 Exemplary distributions of stem cell probabilities

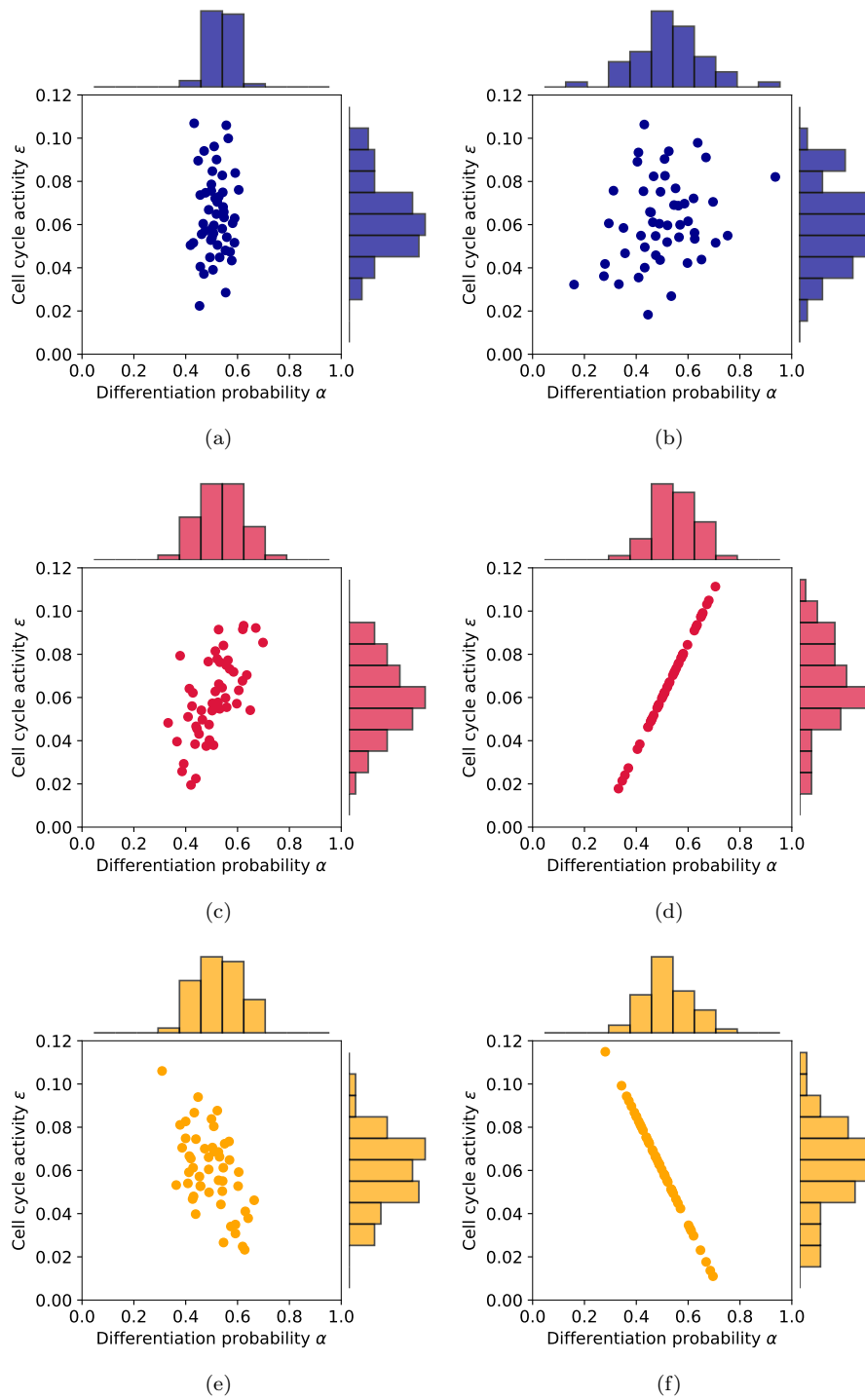


Figure A.2: (a) *Uncoupled probabilities with $\sigma_\alpha = 0.04, c = 0$.* (b) *Uncoupled probabilities with $\sigma_\alpha = 0.12, c = 0$.* (c) *Coupled probabilities with $\sigma_\alpha = 0.08, c = 0.5$.* (d) *Coupled probabilities with $\sigma_\alpha = 0.08, c = 1.0$.* (e) *Coupled probabilities with $\sigma_\alpha = 0.08, c = -0.5$.* (f) *Coupled probabilities with $\sigma_\alpha = 0.08, c = -1.0$.*

431 A.3 Subpopulation in single-cell RNA-Seq data of adult murine NSCs

432 Here, we analyzed the subpopulation of cells that do have non-zero expression levels in both gene
433 marker sets. For this analysis, we excluded cycling cells that do not express differentiation markers
434 and cells that do express differentiation markers without cell cycle activity. The results show, that
435 also for this subpopulation there is a positive correlation between cell cycle and differentiation in
436 the adult (Figure A.3).

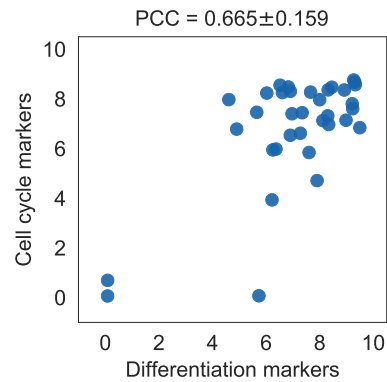


Figure A.3: *Cell cycle marker expression levels against cell differentiation marker expression levels of those adult mouse NSCs [36], that express both, cell cycle and differentiation markers.*