A Physical perspective to understand the mechanism of myelin development

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Abstract
In this study, a hypothesis, which conjectures that an E-field modulates the myelin development, is proposed to establish a new theoretical framework for the study of myelin. This E-field consists of components from three different origins: the resting potential, the action potential, and the dipole potential. Each of them has its changing trend with the axonal calib and the number of myelin lamellae. With detailed analyses of each component, a series of observed unique phenomena of myelin development, such as radial sorting and g-ratio, can be explained. Furthermore, this theory reveals the physical factors modulating the myelination process and builds connections between neural activities and neural development. Finally, a possible experiment is proposed to validate the role of E-field in the myelination process.

Introduction
Myelin is an insulating sheath forming around axons. Its biological function in neural systems and the growing mechanism are the research focus of neuroscience [1-6]. In previous studies, a series of non-trivial experimental observations about the geometry structures of myelin were recorded, including but not limited to the following:

1. The spiraling directions of neighboring myelin sheaths are non-random. The neighboring myelin sheaths on the same axon have the same spiraling direction [7], while the neighboring myelin sheaths on the adjacent axons have the opposite spiraling directions [8-10].
2. For oligodendrocytes (OLs), the inner and outer tongues tend to be located within the same radial quadrant [11-15].
3. The axons of varying calibers tend to have myelin sheaths of the same thickness, resulting in the g-ratio phenomenon [16-18].
4. Only the axon whose caliber is higher than a threshold can be myelinated, resulting in the radial sorting phenomenon [19-21].
5. For Schwann cells (SCs), one SC can only myelinate one axon. If the SC forms the remak bundle, it can never form the myelination even if a large axon is ensheathed [21].

Most of the previous studies about myelin development focused on the function of all kinds of molecules and proteins [2,19,21-26]. However, up to now, non of these studies can provide reasonable
explanations or clues for the phenomena mentioned above. It indicates a blind spot in the mainstream research direction and the current theoretical framework, so the true answer lays out of the current scope.

If we briefly think about these phenomena, it is easy to find the problem of the current mainstream research direction.

1. The non-random spiraling phenomenon. It indicates some form of communication between the spatially closed myelin sheaths, even when they are not physically in contact with each other. This communication can affect myelin growth.

2. The same quadrant phenomenon. It indicates some form of relationship between the inner and outer tongues, which also affect myelin growth.

3. The g-ratio phenomenon. It indicates a correlation between the factor promoting the inner tongue growth and the number of myelin lamellae.

4. The radial sorting phenomenon. It indicates that the myelin can experience the curvature of the axon. Thus, some factor, which can promote or inhibit myelin growth, is determined by the curvature of axons.

5. The different behaviors of SC in myelination and the remak bundle. It indicates that the factor promoting the growth of the inner tongue is affected by the surrounding environment.

The simple reasonings above indicate that the growth of myelin can be modulated by some factors, which can take effect without physical contact. So these factors are not likely chemical or biological, though chemical and biological processes shall be involved during the formation process of these phenomena. Therefore, this study proposes a new theoretical framework that reveals the physical factors of the phenomena mentioned above. This new theoretical framework starts from a straightforward hypothesis.

**Hypothesis-E**

In our previous theoretical model \[1\] for explaining the Peters quadrant mystery \[11-15\], which means the inner and outer tongues of the oligodendrocytes in central nervous systems will be located within the same radial quadrant with an abnormally high probability, it is proposed that the growth of myelin is guided by the electric field (E-field) with a very simple hypothesis (named as Hypothesis-E, "E" refers to "electric"): an external negative E-field promotes the growth, while an external positive E-field inhibits the growth (Figure 1(a)).

Based on this Hypothesis-E, three further hypotheses are proposed to systematically explain the physical origins of a series of morphological characteristics (Figure 1(b-d)) of myelin from a mathematical and physical perspective.
1. Hypothesis-E to explain g-ratio

1.1 g-ratio

The myelin g-ratio is defined as the ratio between the inner and the outer diameter of the myelin sheath. This precise relation between axon diameter and myelin sheath thickness is one of the most enigmatic questions: how is the myelinating glial cell instructed to make precisely the correct number of wraps? Transplantation of oligodendrocytes into nerve tracts containing axons of different sizes demonstrates that the number of wraps is determined by the axon and not by the glial cell because the transplanted glial cells elaborate myelin sheaths appropriate for their new location. A key axonal signal for regulating myelin sheath thickness, the growth factor neuregulin (Ngr1), is now identified by Michailov et al. However, the complete process of controlling the myelin wrapping by the axonal signal remains unclear.
Figure 2 The model to explain $g$-ratio. (a) The cross-section of a myelinated axon in the static condition, the resting potential is equivalent to a voltage source; (b) A section of myelin cross-section with a radial angle of $\theta$; (c) The equivalent circuit modeling the myelin cross-section.

1.2 Hypothesis-$E_N$

The cross-section of a myelinated axon in the static condition, which means no action potential is activating, is shown in Figure 2(a). Since in the resting state, the intracellular terminal is more negative than the extracellular terminal of the axon, the part of the inner tongue facing the axon experiences a negative $E$-field from the axon, as shown in Figure 2(a). This $E$-field from the axon is the driven force making the inner tongue grow and wrap around the axon to form myelination.

So the description of Hypothesis-$E_N$ ("N" refers to "negative") is as follow:

*The inner tongue of myelin is driven by a negative $E$-field from the axon in the resting state. The strength of the $E$-field on the inner tongue is proportional to its growth rate. When the $E$-field is lower than a threshold, the growth of the inner tongue terminates.*

1.3 Modeling the relation between $g$-ratio and the threshold $E$-field

Consider a mature myelinated axon with the number of myelin lamellae as $N$, as shown in Figure 2(b). The axonal radius is $a$, and the thickness of a single myelin lamella is $b$. So the total myelin thickness, $D$, is $b \times N$. Since the whole cross-section is central symmetric, we only consider a sector with a radial angle as $\theta$, as shown in Figure 2(b).

The capacitance, $C$, of each layer is proportional to its area. Since the longitudinal length of each layer is the same, the capacitance, $C$, of each layer is proportional to the arc length $l$:

$$C \propto Area \propto l$$

Then for the $n^{th}$ layer, the capacitance, $C_n$, is proportional to its arc length $l_n$:

$$C_n \propto l_n = \theta \times (a + (n - 1)b)$$

The voltage, $V_n$, on the $n^{th}$ layer is:

$$V_n = \frac{Q_n}{C_n}$$

Here $Q_n$ is the charge on the capacitor. So the voltage, $V_1$, on the first layer is:
\[ V_1 = \frac{Q_1}{C_1} \]

Since all capacitors are connected in series, as shown in Figure 2(c), the two boundary conditions are:

1. The charge on each capacitor is the same, assigned with the value of \( Q \):
   \[ Q = Q_1 = Q_2 = Q_3 = \ldots = Q_N \]

2. The resting potential, \( V_R \), is equivalent to a voltage source connected with these series-connected capacitors, as shown in Figure 2(c), so \( V_R \) is the sum of the voltage on all capacitors:
   \[ V_R = \sum_{n=1}^{N} V_n = \sum_{n=1}^{N} \frac{Q_n}{C_n} = Q \times \sum_{n=1}^{N} \frac{1}{C_n} \]

The charge, \( Q \), on each capacitor is:
   \[ Q = \frac{V_R}{\sum_{n=1}^{N} \frac{1}{C_n}} \]

The voltage, \( V_1 \), on the first layer, which is the inner tongue, is as shown below:
   \[ V_1 = \frac{Q}{C_1} = \frac{V_R}{C_1 \times \sum_{n=1}^{N} \frac{1}{C_n}} = \frac{V_R}{a \times \sum_{n=1}^{N} \frac{1}{(a + (n - 1)b)}} \]  

(1)

As seen, when the voltage potential, \( V_R \), and the thickness of a single myelin lamella, \( b \), are constants, the voltage on the inner tongue, \( V_1 \), is only a function of the number of layers \( N \), axonal radius \( a \), and monotonically decreases with the number of layers, \( N \). Here the threshold E-field proposed in Hypothesis-E_N is defined as \( V_{N-T} \) ("N" refers to "negative" and "T" refers to "threshold"). And the ratio between \( V_{N-T} \) and \( V_R \) is defined as \( \eta_{N-T} \):
   \[ \eta_{N-T} = \frac{V_{N-T}}{V_R} \]  

(2)

Then the criteria for the max number of myelin lamellae \( N_{max} \) is:
   \[ \begin{cases} 
   V_1 \geq V_{N-T} \text{ when } N = N_{max} \\
   V_1 \leq V_{N-T} \text{ when } N = N_{max} + 1 
\end{cases} \]  

(3)

Substitute (1) and (2) into (3) and get:
   \[ \begin{align*}
   &\frac{1}{a \times \sum_{n=1}^{N_{max}} \frac{1}{(a + (n - 1)b)}} \geq \eta_{N-T} \\
   &\frac{1}{a \times \sum_{n=1}^{N_{max}+1} \frac{1}{(a + (n - 1)b)}} \leq \eta_{N-T}
   \end{align*} \]  

(4)

(4) can be further simplified as follow:
   \[ \frac{1}{a \times \sum_{n=1}^{N_{max}} \frac{1}{(a + (n - 1)b)}} \approx \eta_{N-T} \]  

(5)

As seen, the value \( N_{max} \) is a function of \( a \) and \( \eta_{N-T} \), while \( b \) is constant:
   \[ N_{max} = f_1(a, \eta_{N-T}) \]

Then g-ratio is also a function of \( a \) and \( \eta_{N-T} \).
\[ g_{\text{ratio}} = \frac{a}{a + D} = \frac{a}{a + b \times N_{\text{max}}} = \frac{a}{a + b \times f_1(a, \eta N - T)} = f_2(a, \eta N - T) \]

To enable calculating these two functions, we need to obtain the constant of \( b \). Based on previous studies, we set \( b = 17 \text{ nm} \) as a typical value \([29]\). The calculation results of \( g\)-ratio and \( N_{\text{max}} \) is shown in Figure 3(a) \\
& (b).

![Figure 3](image)

Figure 3. (a),(b) \( g\)-ratio, the maximum number of myelin lamellae, \( N \) calculated by model with different \( \eta N - T \). (c) Illustration of the claim: the measured statistical data of \( g\)-ratio shall locate above the \( g\)-ratio curve; (d) The measured statistical data of \( g\)-ratio in publications \([30-33, 18, 34-41]\).

1.4 Results

The calculated \( g\)-ratio, the maximum number of myelin lamellae, \( N_{\text{max}} \), is shown in Figure 3(a) \\
& (b). As seen, the \( g\)-ratio monotonically increases with the axonal radius, \( a \), and saturates at the value of 1.

The general trend agrees with experimental observations \([16-18]\). The curve of \( N_{\text{max}} \) has a decreasing slope with \( a \), approaching a constant value, which is determined by \( \eta N - T \). This explains why the axons with different diameters always tend to have the same myelin thickness. Based on the comparison with the
modeling results, the statistical data, and typical values in previous observations [17,29,42-43], it can be concluded that the $\eta_{N,T}$ is within the range of 1/13~1/19. For $\eta_{N,T}$ within this range, the calculated $N_{\text{max}}$ for typical myelinated axons (radius is 0.7 $\mu$m~10 $\mu$m) is within the range of 15~37, which also quite agrees with actual observations. Noticeably, $N_{\text{max}}$ goes infinite when $a$ approaches zero, which means the axon with a very small diameter shall have infinitely thick myelin, which never happens in the observation. On the contrary, the axons with a radius within the divergence region in Figure 3(b) actually are unmyelinated, which means $N_{\text{max}}$ is zero! We will make a more detailed discussion in the next section.

Figure 3(b) shows the maximum number of myelin lamellae, $N_{\text{max}}$, of an axon, meaning that this number is just an upper limit. Not every axon can have maximum myelination. So the actual measured number of myelin lamellae $N$ should be no more than $N_{\text{max}}$ calculated in Figure 3(b), $N \leq N_{\text{max}}$. Then the g-ratio showed in Figure 3(a) is also the curve of minimum value. All measured data points of the g-ratio shall be higher than the g-ratio curve, as illustrated in Figure 3(c). The measured data points of g-ratio shall be located within a region whose lower edge will form a g-ratio curve shown in Figure 3(a). By fitting the curve of this lower edge, the actual $\eta_{N,T}$ can be obtained. We collected lots of published papers with measured g-ratio data to validate this claim, as shown in Figure 3(d) [30-33,18,34-43]. When the g-ratio measurement contains sufficient data points, a clear edge can be formed (the red fitting curves are plotted by ourselves for a more unambiguous indication).

If our claim is correct, then both averaging and fitting the g-ratio points are not proper methods to process the statistical data. The lower edge, which represents the $\eta_{N,T}$, a critical characteristic of target neuron fibers, shall be the major focus of the data analysis.

1.5 Discussion

1.5.1 Why does the divergence happen?

The condition to achieve $N_{\text{max}}$ is to meet the condition of equation (5), as written again here:

$$\frac{1}{a \times \sum_{n=1}^{N_{\text{max}}} \frac{1}{(a + (n - 1)b)}} \approx \eta_{N,T} \quad (5)$$

However, the limit of $\eta_{N,T}$ when $N_{\text{max}}$ approaches infinite is as follow:

$$\lim_{N_{\text{max}} \to \infty} \eta_{N,T} = \lim_{N_{\text{max}} \to \infty} \frac{1}{a \times \sum_{n=1}^{N_{\text{max}}} \frac{1}{(a + (n - 1)b)}} = \frac{b}{a}$$

As seen, $b/a$ is the lower limit of $\eta_{N,T}$. If the actual $\eta_{N,T}$ is above this lower limit, $V_I$ is lower than $V_{N,T}$ when $N_{\text{max}}$ is a finite number; then the myelin growth stops (eq (6)). However, if the actual is $\eta_{N,T}$ lower than this lower limit, $V_I$ can never be reduced to $V_{N,T}$, whatever $N_{\text{max}}$ is; the myelin growth never stop (eq(7)).

$$\text{when } \frac{b}{a} < \eta_{N,T} ; N = \text{finite number } \quad (6)$$

$$\text{when } \frac{b}{a} \geq \eta_{N,T} ; N \to \infty \quad (7)$$

As seen, the occurrence of divergence is determined by the ratio between the thickness of single-layer myelin, $b$, and the axonal radius, $a$. When $a$ is large enough to meet eq (6), the calculation of $N_{\text{max}}$ is convergent. Otherwise, when $a$ is a small number, which is the case of unmyelinated axons, the divergence happens. A more intuitive modeling result is shown in Figure 4. Since $V_I$ decreases with the growth of myelin lamellae, the ratio of $V_I/V_R$ will decrease with $N$. Then this ratio reaches the value of $\eta_{N,T}$, the curve stops at the value of $N_{\text{max}}$. As seen, the curve of axonal diameter of 0.8 $\mu$m, 1.4 $\mu$m, 2.6 $\mu$m and 6.2 $\mu$m can have finite value of $N_{\text{max}}$. However, when axon diameter is 0.2 $\mu$m, the curve of $V_I/V_R$ approaches $\frac{b}{a} = 0.17$ ($b=17$nm and axonal radius $a=100$nm), which is higher than $\eta_{N,T} = 1/18 \approx 0.056$, the growth cannot be stopped.
Figure 4. The relationship between $N_{\text{max}}$ and $V_1/V_R$. When $b/a<\eta_{N,T}$, $N_{\text{max}}$ is a finite number, otherwise $N_{\text{max}}$ is infinite.

1.5.2 The relation between the divergence region and unmyelinated axons

Since the modeling result can well duplicate the observation results of the g-ratio and myelin thickness at different axonal diameters, we tend to think about the biological meaning hidden behind the divergence region rather than doubting the correctness of the model.

We can firstly think about it from the perspective of experimental observation. It is observed that the myelin thickness is almost the same by varying axonal diameters. However, The number of myelin lamellae suddenly decreases to zero when the axonal diameter is lower than a threshold, breaking the continuous trend. Apparently, some unknown factor, which does not play a role, or at least does not play a major role at the large axonal diameter range, begins to dominant the growth of myelin and forbids the process of myelination. Currently, this unknown factor is not considered in this model.

Then we can also think about it from the perspective of pure reasoning. In our modeling, if the hypothesis-E_N is the exclusive principle to modulate the myelin growth, the axon of a very small diameter shall have infinitely thick myelin, which definitely will not happen in the real world. Evolution also can never allow this to happen in any successful biological system. So a reasonable conjecture is that, during the evolution, some unknown factor, which inhibits the myelin growth, is introduced and only exerts its function when the axonal diameter is lower than a threshold.

Therefore, the reasoning from two perspectives draws the same conclusion: an unknown factor can inhibit the myelin growth and only exerts its function when the axonal diameter is lower than a threshold. We will make a detailed analysis of this unknown factor in Hypothesis-E_D in the next chapter.

1.5.3 An introspection of this model

The most fundamental reason for the g-ratio is that myelin's growth rate decreases with its layers. It means that the promoting factor of myelin growth shall decay with its layers. Meanwhile, it is well-known that the inner tongue is the growing terminal. So this promoting factor shall take effect upon the inner tongue. Moreover, in the g-ratio phenomenon, the myelin thickness is not strongly correlated with the axon caliber. Thus, this promoting factor shall also be weakly affected by the axon caliber. Then we get three boundary conditions to every possibly successful theory/model explaining g-ratio, as below.

1. The factor that induces myelin growth decays with myelin lamellae.
2. This factor must take effect upon the inner tongue.
3. This factor is not, or weakly, affected by the axon caliber.

In our model, the voltage, $V_I$, on the inner tongue meets these boundary conditions. Any alternative theories shall also meet the above-mentioned boundary conditions. Since this $V_I$ is obtained from Hypothesis-E_N, so it is renamed as $V_{EN}$ in the following chapter to avoid confusion with other variables.
2. Hypothesis-ED to explain radial sorting

2.1 Radial sorting

Radial sorting is the process by which Schwann cells choose larger axons to myelinate during development. During this process, SCs proliferate and expand cellular extensions into bundles of unsorted axons to detach individual axons and establish the one-to-one relationship required for myelination [44]. Axons with a diameter of <1 µm remain in bundles, and SCs in contact with these axons differentiate into unmyelinated SCs, called remak bundles [45]. It is widely believed that this radial sorting process is tightly regulated and depends on signals from axons as well as the extracellular matrix [46].

2.2 Hypothesis-ED

The most mysterious feature of radial sorting is that SCs can recognize biologically identical axons just by their calibers. It seems that axons of large caliber possess a promoting factor while the axons of small caliber possess an inhibiting factor to the myelin growth. Based on Hypothesis-E, we can predict that larger axons shall possess a voltage which is more negative, or less positive, than that of smaller axons, which is Hypothesis-ED ("D" refers to "dipole"), described as follow:

When SCs get close to the surfaces of axons, axons of larger caliber will exert a special "E-field," which is more negative than that of the axons of smaller caliber, to the cell membrane of SCs. Thus, SCs tend to grow and wrap on larger axons. When the caliber of axons is lower than a threshold, the amplitude of the negative E-filed is too low to enable the growth of SCs on their surfaces.

2.3 Modeling the relation between the radial sorting and the dipole potential

Now we need to find a candidate for this special negative "E-field," whose amplitude is positively correlated with axonal calibers. One of the candidates, which perfectly fits the characteristics of this particular "E-field" is the dipole potential generated by the lipid bilayer structure of the cell membrane, as shown in Figure 5(a). The lipid bilayer consists of two layers of amphiphilic molecules. The hydrophobic tails of these lipids, which are positively charged, are directed toward the membrane center, while the hydrophilic tails, which are negatively charged, are directed toward the extra- and intracellular fluid. Each amphiphilic molecule is an electric dipole, a group of separated charges with opposite polarities. The potential, also called the dipole potential, generated by this lipid bilayer is shown in Figure 5(a), which has two negative peaks at the extra- and intracellular surface and one positive peak at the membrane center [47-50]. The bending of the cell membrane will break the centrosymmetry of the bilayer structure and change the amplitude of those two negative peaks, which is called the flexoelectric effect [51]. In particular, the amplitude of the left negative peak located at the extracellular surface, named $V_{D,N1}$ ("D" refers to "dipole" and "N" refers to "negative"), will decrease with bending, while the amplitude of the right negative peak located at the intracellular surface, named $V_{D,N2}$, will increase with bending. When the membrane of the SC contacts with the axon surface, a portion of $V_{D,N1}$, labeled as $AV_{D,N1}$ in Figure 5(b), was applied across SC's membrane. As seen, this $AV_{D,N1}$ meets the criteria of growth promotion, which is an external negative E-field. Meanwhile, the amplitude of this $AV_{D,N1}$ increases with the axon caliber and saturates at a certain value, as shown in Figure 5(c). The detailed modeling and calculation process of the dipole potential can be found in Supplementary. As seen, $AV_{D,N1}$ has a sudden decline from the position of about 400 nm, which is roughly the threshold of radial sorting of SCs.

It is known that the surface potential of the cell membrane can influence the binding affinity of the peptide to lipid bilayers [52]. So it is conjectured that the binding affinity between the polarized protein molecules on the membranes of SCs and axons, which are responsible for the interface adhesion, is positively correlated with the surface dipole potential of the axon $AV_{D,N1}$. When the axon caliber is large, $AV_{D,N1}$ is strong enough for the molecules to form the bound; thus, SCs can grow and wrap on these axons to form myelin. But when the axon caliber is lower than a threshold, e.g., 400 nm in Figure 5(c), $AV_{D,N1}$ is insufficient to provide the binding affinity, and the SCs fail to adhere with the axon. Thus, these small axons are unmyelinated. Therefore, the dipole potential from the axon surface is the unknown factor mentioned in the previous chapter.
2.4 An introspection of this model

The most intriguing part of radial sorting is that SCs can identify the biologically identical axons just by their calibers. It indicates that this identification process, which is executed biologically, is initiated by physical signals rather than biological signals. Meanwhile, during the contact with the axon of difference calibers, the only difference can be experienced by SCs is the curvature. So it can be inferred that this physical identification signal is related to the surface curvature. To the best of our knowledge, the dipole potential is the sole physical factor determined by the axon caliber and whose changing trend is consistent with Hypothesis-E. Since this $\Delta V_{D,N1}$ is obtained from Hypothesis-E, it is renamed as $V_{ED}$ to avoid confusion with other variables.

3. Hypothesis-EP to explain behaviors of SCs

3.1 Different behaviors of SCs in myelination and remak bundle

The SCs behave differently in myelination and remak bundles [19]. In the scenario of myelination, an SC will wrap around a large axon with a 1:1 relationship. In the scenario of a remak bundle, an SC can never form myelination, even if a large axon is ensheathed.

3.2 Hypothesis-EP and modeling

In this chapter, another further hypothesis, named Hypothesis-EP (P refers to ”Positive”), is proposed to reveal the criteria of myelination and explain the mechanism of the behavior of SCs. The detailed description is:

*The growth of the inner tongue of myelin is inhibited by a positive E-field during the action potential. The strength of the E-field on the inner tongue is proportional to the growth-inhibiting strength. When the E-field is lower than a threshold, it does not exert its inhibition function.*

So a new perspective combining Hypothesis-EN and EP about how the myelin growth is modulated by E-field is shown in Figure 6(a). $V_P$ and $V_N$ refer to the amplitude of resting potential and the positive peak voltage of the action potential, respectively. The threshold voltage $V_{P,T}$ is the threshold voltage to inhibit myelin growth, while $V_{N,T}$ is the threshold voltage to promote myelin growth. Then the ratio between $V_{P,T}$ and $V_P$ is $\eta_{P,T}$, while the ratio between $V_{N,T}$ and $V_N$ is $\eta_{N,T}$. The area higher than $V_{P,T}$ is the inhibition phase (red area in Figure 6(a)), while the area lower than $V_{P,T}$ is the promotion phase of myelin growth (blue area in Figure 6(a)).

We have made a detailed discussion about $\eta_{N,T}$ and its effect on g-ratio in the previous chapter. In this chapter, we will make a detailed investigation about $\eta_{P,T}$ and its effect on inhibiting myelin growth.
For the condition of case A of one layer of myelin, shown in Figure 6(b-i, ii), the total voltage \( V \) (this voltage can be either the resting potential \( V_R \) or the action potential \( V_A \), "A" refers to "action") is applied on \( C_{1-A} \) and \( C_{2-A} \):

\[
\begin{align*}
C_{1-A} &\propto a; \\
C_{2-A} &\propto 2 \times (a + b); \\
Q_{1-A} &= C_{1-A} \times V_{1-A} = Q_{2-A} = C_{2-A} \times V_{2-A} = Q; \\
V_{1-A} + V_{2-A} &= V;
\end{align*}
\]

Since \( C_{2-A} \) only has a single layer of the cell membrane, the equivalent capacitance shall be doubled compared with the one with double layers of the cell membrane.

Then the ratio between the voltage on \( C_{1-A} \) and \( V \) is:

\[
\eta_A = \frac{V_{1-A}}{V} = \frac{1}{\frac{1}{1 + \frac{a}{2a+2b}} + \frac{1}{1 + \frac{b}{2+2b'}}};
\]

For the condition of case B, the total voltage \( V \) is applied on \( C_{1-B}, C_{2-B}, \) and \( C_{3-B} \):

\[
\begin{align*}
C_{1-B} &\propto a; \\
C_{2-B} &\propto a + b; \\
C_{3-B} &\propto 2 \times (a + b + b'); \\
Q_{1-B} &= C_{1-B} \times V_{1-B} = Q_{2-B} = C_{2-B} \times V_{2-B} = Q_{3-B} = C_{3-B} \times V_{3-B} = Q; \\
V_1 + V_2 + V_3 &= V;
\end{align*}
\]

Here we set the thickness of the second layer is \( b' \), which is different from that of the first layer \( b \).

Since \( C_{3-B} \) only has a single layer of the cell membrane, its equivalent capacitance shall be doubled.

Then the ratio between the voltage on \( C_{1-B} \) and \( V \) is:
\[ \eta_B = \frac{V_{1-B}}{V} = \frac{1}{1 + \frac{a}{a+b'}} = \frac{1}{1 + \frac{a}{2(a+b+b')}}; \]

Since the myelin lamellae are not compact yet at the initial myelination process, \( b \) is a value comparable with \( a \). So here we set the ratio of \( b/a \) is 0.1, which is a typical value and a reasonable approximation, to further simplify the equation of \( \eta_A \) and \( \eta_B \) as below:

\[ \eta_A = \frac{1}{1 + \frac{a}{2+2b'}} \approx 0.88; \]
\[ \eta_B = \frac{1}{1 + \frac{a}{2(a+b+b')}} = \frac{1}{1 + \frac{1.9094}{2(1.1+\frac{b'}{a})}}; \]

As seen, \( \eta_A \) is a constant, meaning that about 88% of the transmembrane voltage, which can be either \( V_R \) or \( V_A \), will be applied onto the adaxonal layer of the myelin. Meanwhile, \( \eta_B \) is a function of \( \frac{b'}{a} \), which is calculated as shown in Figure 7. As seen, \( \eta_B \) increases with \( \frac{b'}{a} \).

**Myelination Region**  **Non-myelination Region**

![Myelination Region Diagram](image)

Figure 7. Calculate result of \( \eta_B \) curve changes with \( \frac{b'}{a} \).

Then let’s consider the situations of the wrapping of the second myelin lamella on a large axon by both a normal SC and a remak SC, as shown in Figure 6(b) and Figure 8(a), respectively. For an SC forming myelination, the condition is similar to Figure 6(b) when \( a \gg b' \). So its \( \eta_B \) is located within the blue region in Figure 7, labeled with myelination region. For a remak SC, the condition is similar to Figure 8(a). Then a large axon is ensheathed by a remak bundle, initially the axon is wrapped by a SC as shown in Figure 8(a-i&ii). When one of the SC terminal tends to further grow and wrap the large axon to form myelin, it will inevitably face the situation shown in Figure 8(a-iii&iv) when \( b' \) is comparative or even larger than \( a \). Thus its \( \eta_B \) is located within a pink region in Figure 7, labeled with non-myelination region.
Figure 8. (a) The scenario when an SC of remak bundle ensheathes a large axon: (i-ii) only one layer of SC is wrapped; (iii-iv) when the SC tries to wrap the second layer; (b) A more decayed action potential induces a shorter inhibitory phase (red region).

Since in Hypothesis-E_P, we have assumed that the positive voltage, \( V_P \), in the action potential can inhibit the myelin growth. Therefore, for a normal SC myelinating a large axon, the inhibiting voltage exerted upon the inner tongue is lower; the promoting factor induced by the negative voltage, which mainly comes from the resting potential, dominates the myelin growth (illustrated in Figure 8(b)). However, for a remak SC, the inhibiting voltage upon the inner tongue is higher. Thus the inhibiting factor dominates the myelin growth, stopping the wrapping of the second layer. Since this inhibitory voltage on the inner tongue, \( V_{P-T} \), is obtained from Hypothesis-E_P, it is renamed as \( V_{E_P} \) to avoid confusion with other variables.

Therefore, based on this model, two phenomena are inevitable to be observed:

1. An SC can only myelinate one axon.
2. Remak SC cannot form myelin, even if a large axon is ensheathed.

Moreover, we can also make a rough estimation of \( \eta_{P-T} \). It is a value located close to the myelination and non-myelination region interface, 0.43–0.46, shown in Figure 7.

3.3 Discussion

This model also indicates potential explanations for other experimental observations, as discussed below.

Firstly, it is contradictory to the conventional understanding of the correlation between neural activities and myelin development. It was widely believed that the action potential is a positive factor in the myelination process, while in our model, it is a negative factor. If our model is correct, it can be predicted that by eliminating the action potential during myelin development, the myelin can grow thicker. Chan et al. have confirmed this hypermyelination phenomenon of oligodendrocytes by muting the action potential \([53]\), which is supporting evidence of our model. It can be foreseen that the same phenomenon can be observed in the experiment of SCs.

Secondly, the frequency of the action potential is also a factor affecting the fate of myelination. When the action potential is activated more frequently, which is the case of sensory fibers, the inhibiting factor tends to dominate, and the axons tend to be unmyelinated. Conversely, when the action potential is activated more rarely, which is the case of motor fibers, the promoting factor tends to dominate, and the axons tend to be myelinated. This may partially explain that the majority of the sensory fibers are unmyelinated while the counterparts of the motor fibers are myelinated \([54]\).

This model also indicates a positive correlation between neural hyperactivity and the degeneration of myelin. It may provide a clue for the neurodegenerative disorders such as Parkinson’s disease, whose
early-stage symptoms, such as hand tremor and muscle stiffness, are the results of uncontrollable hyper-activation of some neurons, while the accompanying symptoms include the demyelination of neurons. At least, these phenomena are not contradictory to our model.

3.4 An introspection of this model

It is seemingly incredible that SCs behave differently to axons of large calibers in myelinated axons and the remak structure scenarios. But, after all, an SC is not a highly intelligent and programmable machine. Therefore, the principle hidden behind the complex phenomena should be simple. First, this principle is related to the inner tongue since this is still a phenomenon about myelin growth. Secondly, this principle is associated with the remak structure. In other words, it is affected by the different conditions of the inner tongue during the formation of the second myelin lamellae. With such a consideration, it is easy to notice that the thickness of the outer layer is quite different during the second layer wrapping, though this is not the sole difference. By leveraging the circuit model of Hypothesis-EN, we can easily acquire the explanatory model shown in Figure 8. Although we cannot claim this is the exclusively correct model, it is highly consistent with the whole theory.

4. A rethinking of the complete model

At the beginning of this study, we have proposed a general hypothesis, called hypothesis-E, which conjectures that the development of myelin is guided by an E-field applied upon the inner tongue. By explaining different phenomena of myelin development, it is concluded that this E-field consists of three components, as summarized below.

1. The component, $V_{EN}$, from $V_R$. Although $V_R$ is almost an identical value for axons of different calibers, its component, $V_{EN}$, applied to the inner tongue changes with both the axon calibre and number of myelin lamellae, explained in Figure 2. Therefore, $V_{EN}$ is a function of both the axon caliber, $a$, and the number of myelin lamellae, $N$:

   $$V_{EN} = f_{EN}(a, N);$$

2. The component, $V_{EP}$ from $V_A$. This $V_{EP}$ functions the same as $V_{EN}$ in the circuit, just with a different waveform. So it is also a function of $a$ and $N$ and changes with the same trend as $V_{EN}$:

   $$V_{EP} = f_{EP}(a, N);$$

3. The component, $V_{ED}$, from the dipole potential of the cell membrane. This component does not change with the number of myelin lamellae, $N$. So it is just a function of $a$:

   $$V_{ED} = f_{ED}(a);$$

The voltage upon the inner tongue, $V_I$, is the sum of these three components:

$$V_I = f_{EN}(a, N) + f_{EP}(a, N) + f_{ED}(a); \ (8)$$

The detailed waveform is shown in Figure 9.

Figure 9. A complete perspective of Hypothesis-E: the total voltage consists of three major components: $V_{ED}, V_{EN}$ and $V_{EP}$.
Since the major target of this study is to establish a new theoretical framework for the mechanism of myelin development, we do not intend to involve an accurately quantitative comparison of the importance of each component. However, a very rough and qualitative analysis can still help us have a better understanding. The amplitude of the dipole potential of the lipid membrane, whose measurement is not an easy task, is estimated within the range of 200–1000 mV \(^{55-57}\). It means \(V_{ED}\), which is just a small portion of the dipole potential as shown in Figure 5, may possess an amplitude of tens of mV, which is a comparative value to \(V_K\) and \(V_A\). Meanwhile, \(V_{EN}\) and \(V_{EF}\) take a small ratio of \(V_K\) and \(V_A\), respectively. Thus, \(V_{ED}\) may take the major portion of \(V_I\). In this scenario, \(V_I\) has no substantial positive part. So a complete Hypothesis-E, which is a corrected version of Hypothesis-E\(_P\) in Figure 6(a), is illustrated in Figure 9 and described below:

**The growth of the myelin is promoted by the negative E-field when it exceeds a threshold, represented by the potential \(V_{NLT}\), and inhibited by the negative E-field when it is lowered than another threshold, represented by the potential \(V_{NLT}\), respectively.**

Meanwhile, the conclusion about the g-ratio explanation in Figure 2 should also be corrected from two perspectives.

The first correction comes from \(V_{ED}\). Previously, only \(V_{EN}\) was considered as the sole origin of the voltage source in Figure 2. Since \(V_{EN}\) slightly decreases with the axonal diameter, the myelin thickness will also slightly decrease with axonal diameter. It means that the axon with a larger diameter shall have a slightly thinner myelin sheath. However, in equation (3), the myelin growth is actually modulated by \(V_I\), which is the sum of three components. Among them, \(V_{ED}\) slightly increases with the axonal diameter (Figure 3(a)), which is opposite to \(V_{EN}\). Since the actual quantitative value of \(V_{ED}\) is unknown, it is unclear how \(V_I\) changes with axonal diameter. The only thing for sure is that the slope of the curve of \(V_I\) versus \(a\) should be very low when \(a\) is large. It means that the myelin thickness shall be weakly correlated with axonal diameters.

The second correction comes from further thinking of the possible influence of \(V_{ED}\). Some studies reported that the axon caliber is weakly correlated with the myelin thickness \(^{16,17,18,36}\). Based on our theory, this scenario means the myelin growth is mainly modulated by \(V_{EN}\), as explained in Figure 2. The number of myelin lamellae is normally lower than 50. However, we also notice that in some studies, it is reported that the myelin can have a perpetual growth, which makes the number of myelin lamellae more than 100 \(^{58,59}\). Meanwhile, in this scenario, a larger axon tends to have thicker myelin. The model in Figure 2 cannot explain this phenomenon. One possible explanation is that \(V_{ED}\) is much higher than \(V_{EN}\); thus, \(V_{ED}\) dominates the modulation of myelin growth. Since \(V_{ED}\) does not decay with the increasing number of myelin lamellae, once \(V_{ED}\) can solely provide the voltage to promote the myelin growth, the myelin can grow perpetually with a constant growth rate, which agrees with the description in a previous study \(^{58}\), quotes here:

*It is, moreover, concluded that myelin production on the average seems to be a perpetual process which, in the fully mature cat, operates at the same rate regardless of axon size.*

### 5. A possible experiment for validation of the theory

It has been validated that axonal cues are not necessary for the myelin wrapping of oligodendrocytes, though they are still necessary for myelin compaction \(^{60}\). It is highly possible that SCs follow the same principle. So we can design an experiment shown in Figure 10 to validate the role of the E-field in myelin development. A mesh of silver micro/nano-wires, 0.2–10 μm in diameter, coated with 1 μm thick parylene as an insulating layer is used as a substitute for the axons with varying calibers. When it is partially immersed in the culture medium, the surface potential can be controlled by the applied voltage, as shown. The oligodendrocytes or SCs can both be cultured with nano-wire in the medium, and the myelination process can be observed by varying the applied voltage. Based on our theory, several phenomena can be predicted as follow:

1. The minimum diameter of the myelinated wire decreases with the increasing amplitude of the negative voltage.
2. When the positive voltage is applied, the myelination process will be inhibited for all wires.
3. If a negative voltage is applied to induce the myelination first, the post-applied positive voltage can induce demyelination (Figure 10(b)).

4. There will be a threshold voltage, \( V_{N1-T} \), to initiate the myelination process.

5. There will be another threshold voltage, \( V_{N2-T} \), to initiate the demyelination.

![Figure 10](image_url)

**Figure 10.** A designed experiment for validation of Hypothesis-E: (a) the experimental setup; (b) Modulate the myelination process by controlling the E-field of the nano-wire.

**Conclusion**

In this study, a hypothesis, which conjectures that an E-field modulates the myelin development, is proposed to establish a new theoretical framework for the study of myelin. This E-field consists of components from three different origins: the resting potential, the action potential, and the dipole potential. Each of them has its changing trend with the axonal caliber and the number of myelin lamellae. With detailed analyses of each component, a series of observed unique phenomena of myelin development, such as radial sorting and g-ratio, can be explained. Furthermore, this theory reveals the physical factors modulating the myelination process and builds connections between neural activities and neural development. Finally, a possible experiment is proposed to validate the role of E-field in the myelination process.

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