"Do Ion Channels Spin?" Update

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The idea that ion channel proteins physically rotate to enhance ion flow through the membrane is reviewed, in light of recent experimental results. Although there is still no definite answer to the question presented in the title, some recent work can be interpreted as supporting this notion. As a bonus, we present a general theory of anesthesia. Anesthetics, and alcohol, are dissolved in the membrane lipids, and (perhaps) do not directly bind to ion channels or pores. Instead, they change the properties of the surrounding lipids, thus compromising the rotations of the pores, and producing the anesthetic effects.

Introduction

About five years ago, this writer put forward the notion that some ion channels, as part of their function, rotate rapidly [1]. On the face of it, the idea may seem unlikely. But if it is correct, the consequences are significant enough to motivate a re-evalution in light of new research conducted in the intervening time. To summarize, there has been a lot of suggestive work, but nothing to either prove the idea true, or definitively rule it out.

In 1952, Hodgkin and Huxley [2] introduced phenomenological equations governing ion flows and pulse transmission down an axon. In their model they use the idea of currents of sodium and potassium being separately controlled, but do not specify an exact mechanism. It might seem that the simplest way of accomplishing this is to have ion-selective holes through the membrane, with gates to control the flows. Nevertheless there was resistance to the notion of an actual pore through the membrane as late as the 1970's, see Bertil Hille's comments in [3]. But by 1977 Hille could draw a cartoon [4] representing the standard picture of a voltage controlled ion channel, which has persisted to the present time. Changes in potential across the membrane act on a voltage sensor, which is mechanically connected to a little door, which it pulls open (or closed).

In the intervening years, there have been enormous advances in determining the actual atomic structure of ion channel proteins. Nevertheless the picture of Fig. 1 has remained largely unchanged. A more modern instantiation of this image [6] appeared in 2003, interpreting the functional structure of a newly resolved potassium channel. A cartoon drawn by Sigworth, discussing this work [5], shows, again, the voltage sensor moves, and mechanically pulls open a gate, a situation conceptually identical to the Hille picture.

"Not so fast!" said other workers in the field, supporting other models of the shape and movement of the voltage sensor. Amongst the candidates, a "sliding helix" model, presaged early on by Armstrong [8, 9] has received broad support, for reviews see for example [10–12]. A recent paper authored by a number of ion channel luminaries even declares a "consensus" on the architecture of the voltage sensor [13]. Still, there remains a mechanical connection between the voltage sensor and a pore gate, not that different from the models of Fig. 1 [14].

The rotating pore picture suggests an entirely different architecture, there is no permanent connection between the voltage sensor, and a gate. Movement of the voltage sensor *removes a constraint*, which allows dynamical motion of

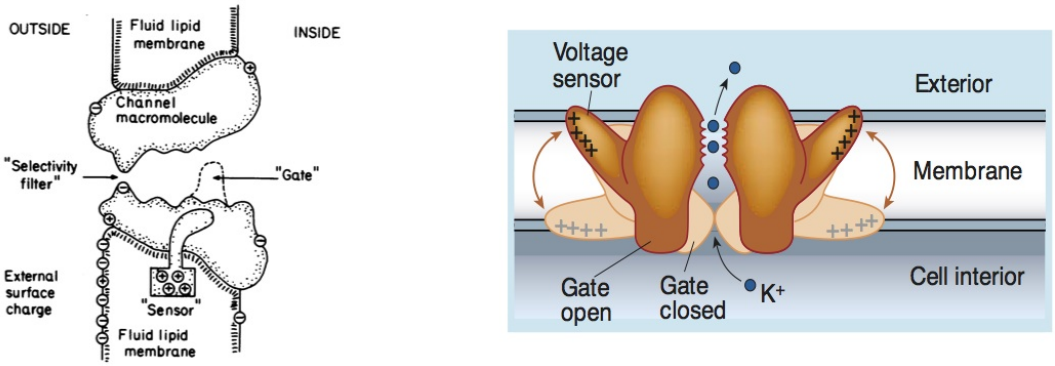

FIG. 1: Left, a 1977 Hille cartoon [4]. Right, a 2003 Sigworth cartoon [5], representing work from the MacKinnon lab [6]. There is a direct mechanical connection between the voltage sensor and the pore gate.

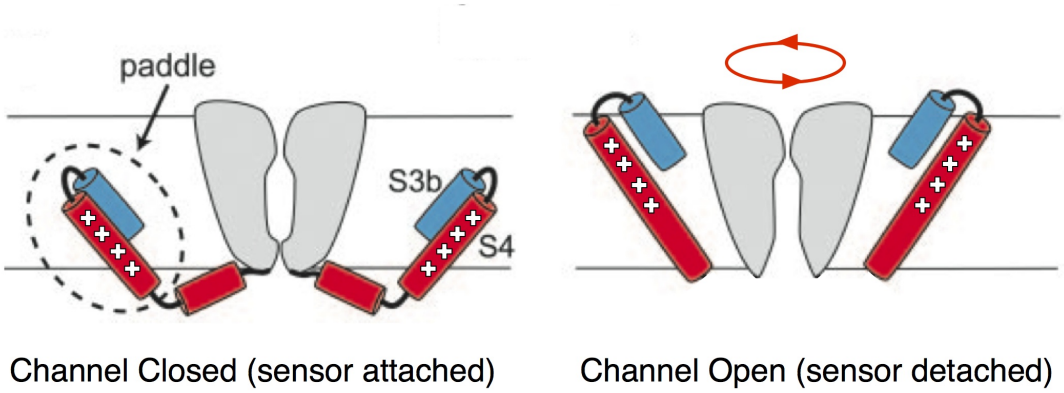

FIG. 2: An alternative ion channel architecture. The sensor moves upward with changes in the electric field, and disconnects from the pore structure. The pore rotor is then free to rotate, and conduction occurs. Figure adapted from Horn [7].

the channel, even a rotation, and ion flow follows. If this is true, the assumption of a solid connection between sensor and gate must be false. So let us try and examine the literature on the connection of the voltage sensor to the rest of the channel protein. It is impossible for an outsider to review a huge and fast-moving field, here is only presented an impression. For something more authoritative, a starting point is Hille's invaluable book [15].

The S4-S5 "Linker"

A common feature of models of voltage controlled ion channels is the "S4-S5 linker" which supposedly mechanically connects the voltage sensors to the pore gate. The use of the word "linker" certainly prejudices the mind towards a picture of a solid connection, and if the rotating pore picture is to be true, this connection must be dissolved. This motivates a careful look at the assumptions underlying the "linker".

When gene sequencing became possible in the 1970's, one could attempt to infer the secondary structure of ion channel proteins by identifying parts of the protein chain that were hydrophilic, and other parts that were hydrophobic. The chain is presumably folded and cleaved during assembly into subunits which form the full channel protein, and the hydrophobic segments will pass through the lipid membrane. Noda et al. accomplished this analysis in 1984 for a sodium channel isolated from the electric eel [16]. They identified six hydrophobic subsequences, which they labeled S1 - S6. They noticed that one of the subsequences, segment S4, contained regularly spaced positive charges. There are four copies of the S1 - S6 which form the full protein, and in their words, "It is attractive to hypothesize that each of the four positively charged segments, possibly in conjunction with each of the negatively charged segments, acts as voltage sensor, thus being involved in an activation gate." They went on to hypothesize that the four copies of the uncharged S5 and S6 forms a pore, though which the ions pass.

This general architecture, four sub-units each composed of six segments, has proven to be quite general, describing voltage-controlled channels for the sodium, potassium and calcium ions. There are variations, sometimes the four sub-units are identical ("homotetramers"), and sometimes varied ("heterotetramers"), for example.

But how does the S4 "sensor" gate the S5-S6 "pore"? The assumption has been that the gating takes place via a

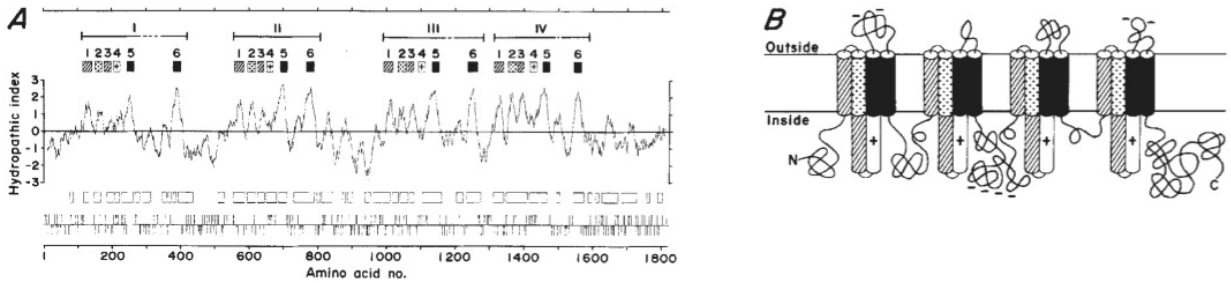

FIG. 3: A 1984 figure from Noda et al. [16]. On the left is the "hydropathy profile" of the genetic sequence, and on the right their proposed transmembrane topology. This architecture has proven to be quite general for voltage-controlled ion channels.

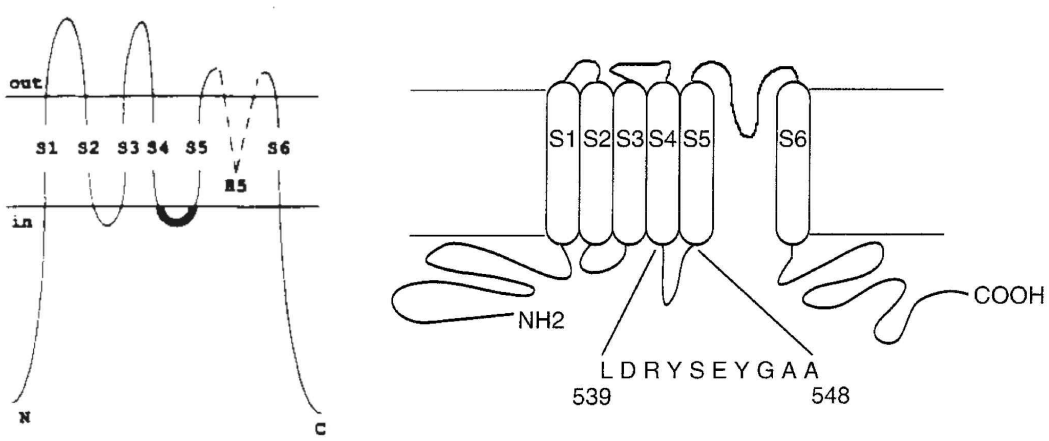

FIG. 4: On the left, a 1991 figure of the "S4-S5 loop" from Isacoff et al [17]. On the right, a 1999 figure of the "S4-S5 linker", from Sanguinetti and Xu [19]. Mutations in the S4-S5 region greatly affect the behavior of potassium and other ion channels.

mechanical connection, the S4-S5 "linker". This was given experimental support by mutation studies, which varied the genetic sequences of the S4-S5 region, and studied the properties of the resulting channels. It was found that, indeed, changes in the S4-S5 sequence had major effects on ion channel function, which one would expect if this was the connection between the sensor and the pore [17, 18]. Fig. 4 reproduces diagrams from two early studies which demonstrated the functional importance of the S4-S5 area. In 1991 this area was referred to as the "S4-S5 loop" [17], by 1999 it had become the "S4-S5 linker" [19].

Now the question becomes, how are strings such as those in Fig. 4 assembled into a three-dimensional structure? There were early hypothetical diagrams [20], see for example Fig. 5, but a more definitive picture had to await atomic resolution imaging. This was forthcoming, notably in a paper by Doyle et al. [21]. (6275 citations and counting.) This imaged the potassium channel from a bacterium, which is not voltage sensitive. However, it was expected that this channel would have a lot in common with the S5-S6 pore region of higher organisms. This was soon shown to be the case, again in the MacKinnon laboratory [6]. These images indicated that the voltage sensors were *not* integrated into the full protein, but rather "...the voltage sensors are self-contained domains, quite independent of the pore except for their specific localized attachments (through the S4-S5 linker) that enable them to perform mechanical work on the pore." [22]. Figure 6, adapted from Lee et al [23], shows the situation, the voltage sensors are separate from the central pore, and in their words, only "loosely adherent".

It should be emphasized that these are not images of living proteins. It is difficult to force a large protein to form a crystal, so that x-ray diffraction imaging can be performed. Forming a crystal from a large protein is somewhat of a black art, requiring detergents, monoclonal antibodies, and other additions to stabilize the crystal. This crystal is then blasted with intense synchrotron radiation from a repurposed particle accelerator.

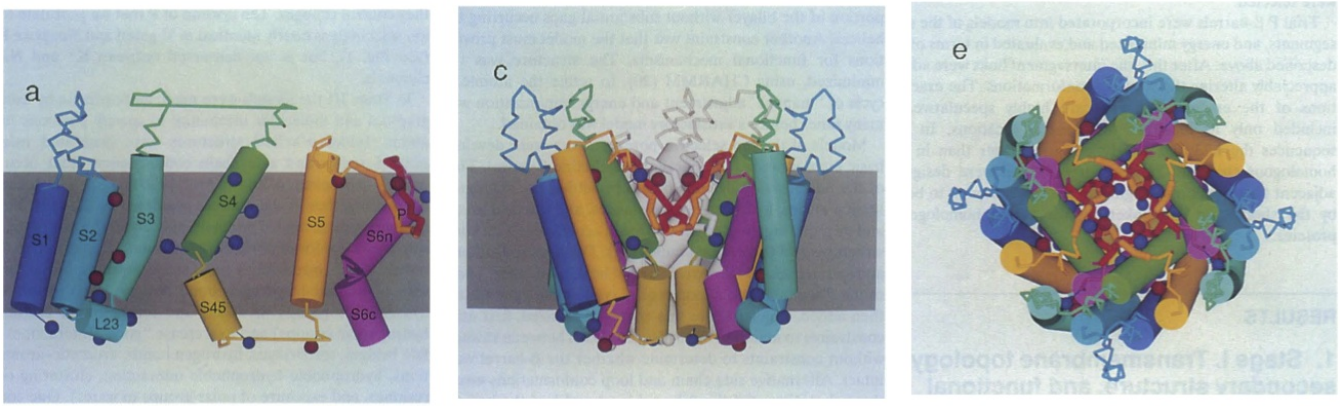

FIG. 5: A hypothetical diagram of a voltage-controlled potassium channel drawn by Durell and Guy in 1992 [20]. To the left is one of the four S1-S6 subsequences, the center is a side view, and the right is a top view of the complete protein.

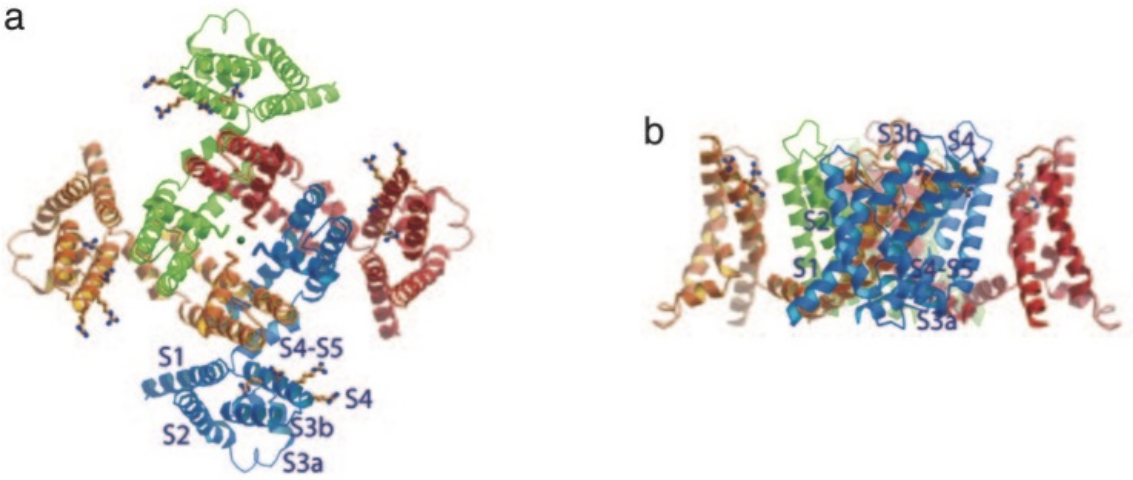

FIG. 6: Top view and side view of a voltage-controlled potassium channel from Lee et al [23] (2005). The voltage sensors are now quite separate from the pore and only "loosely adherent".

It is arguable whether the images so obtained have much resemblance to a "native" conformation, whether they represent an "open" or "closed" state, and so on, the literature is full of such discussions. The same is true for other methods of atomic-resolution protein imaging, such as electron microscopy, or cryo-electron microscopy. However, the technologies and resolution are improving at a spectacular rate.

In an attempt to produce images more likely to be in a "native" conformation, Tsai et al. formed two-dimensional crystals of a sodium channel, and examined them via cryo-electron microscopy [24]. The hope is that the proteins would be more likely to assume positions that they would have in the two-dimensional lipid membrane bilayer. They found that the crystal formed two different types of unit cell, which they suggested corresponded to the open and closed conformations of the channel. The interesting result, from the point of view of the rotating pore hypothesis, is that the S4-S5 region in the "open" conformation was "highly disordered". In other words, in the open conformation, there may be no connection at all between the voltage sensor and the pore.

At a minimum, it has become clear that the picture of the S4-S5 region as a simple mechanical connection may need revision. Without attempting to go through all the rapidly accumulating literature, here are a few suggestive references. (In other words, the writer has cherry-picked the literature for papers supporting his point of view.)

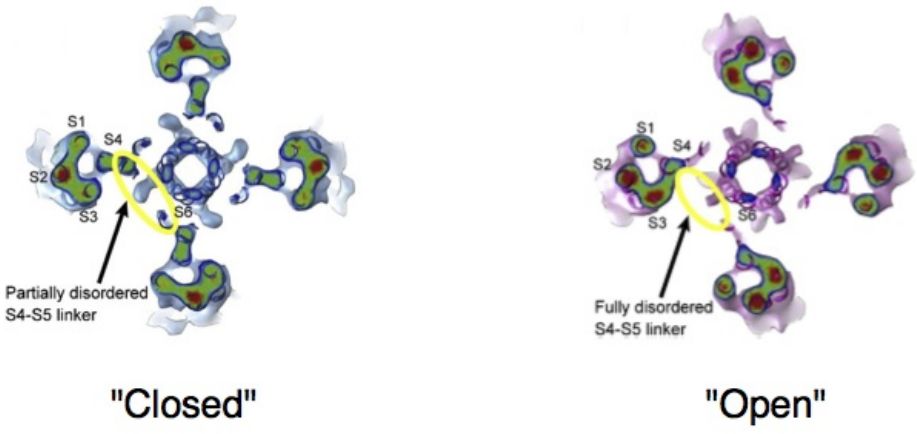

FIG. 7: Two conformations found in a two-dimensional crystal of a sodium channel by Tsai et al [24] (2013). They ascribe one conformation to an open state, and the other to a closed state. The open state has a larger pore opening, and a "fully disordered" S4-S5 "linker". In other words, no connection at all!

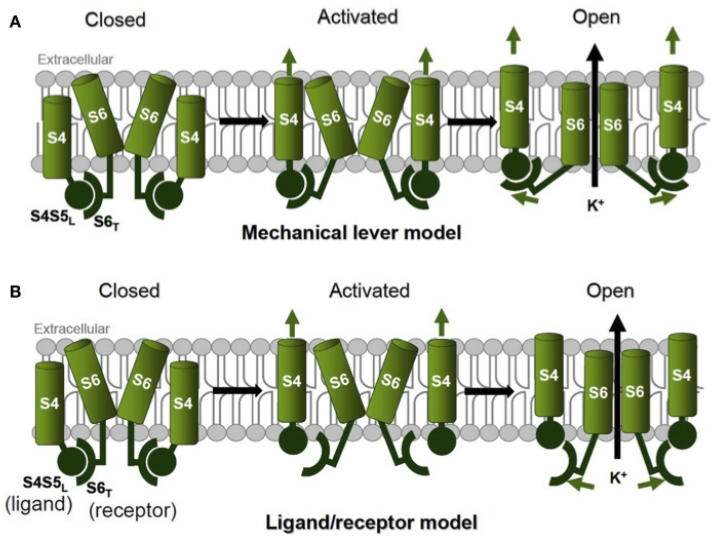

FIG. 8: Work of Choveau et al [25], who contrast a model with a mechanical linkage between the voltage sensors and the pore, with a model where the sensors can actually detach. The S4-S5 "linker" is broken!

In a 2011 study of a potassium channel, Choveau et al. [26] suggested that when the voltage sensor moves to open the pore, it does not do so through a mechanical linkage, but rather "pulls away" from the S5-S6 pore. When the S4-S5 region is connected to the pore, it "stabilizes the closed state", see also [27]. Figure 8 reproduces a diagram from a 2012 review by the same group [25], contrasting a "mechanical lever model" with a "ligand/receptor model". In a recent experiment on a potassium channel, the S4-S5 region was mutated in a way that produced a strong covalent bond between the sensor and the pore, and the channel was completely locked closed [28]. From the rotating pore viewpoint, this is to be expected, if the pore cannot turn, it cannot effectively conduct.

In a remarkable experiment, Lörinczi et al. [29] took the genetic sequence of a potassium channel, and split it into two pieces, around the S4-S5 area. They introduced "stop" and "start" codons at the end of the first and the beginning of the second piece, to insure that two separate proteins were "expressed". In the membrane, the two pieces happily self-assembled into a functional voltage-controlled ion channel, despite there being *no covalent link* between the voltage sensor and the pore, by construction, From the abstract, "These observations indicate that voltage sensing by the S4 segment is transduced to the channel gate in the absence of physical continuity between the modules." They could demonstrate that "the two halves are not covalently bound..." yet the protein could turn on and off as a function of voltage.

In short, these works have all the features of the rotating pore model, except (of course) the rotation...

Lipids as Rotor "Lubricants"

The cell membrane bilayer is composed of a varied population of lipids, which act as a two-dimensional liquid. Functional proteins such as ion channels float in this sea [30], although the actual distribution of various lipids and proteins can be highly organized, into localized sub-populations. In recent years it has become clear that membrane lipids are more than a passive sea, rather they are essential to ion channel function [31]. A particular lipid composition seems to be optimal, for example, cholesterol will quite generally inhibit channel function [32], while some small amount of PIP_2 is required to be present, the latter we now briefly examine.

Among the minor phospholipid constituents of the membrane is PIP_2 (shorthand for phosphatidylinositol 4,5-bisphosphate). Despite its being a small proportion of the membrane lipids, it seems to be essential for the operation of many ion channels, for a review see Suh and Hille [33]. They describe PIP_2 as "a necessary cofactor for ion channel function", mention evidence that "PIP_2 stabilizes the open state" of channels, and speculate that it could act as a "signaling" lipid, ion channels could be conveniently turned on and off by providing or depleting PIP_2.

Still PIP_2 remains somewhat of a mystery, in the words of Hille et al [34], "How does the low-abundance plasma membrane lipid PI(4,5)P2 regulate the activity of so many ion channels?" The literature centers on looking for "binding pockets" where PIP_2 can somehow enable pore opening. However, the "binding" can't be all that strong,

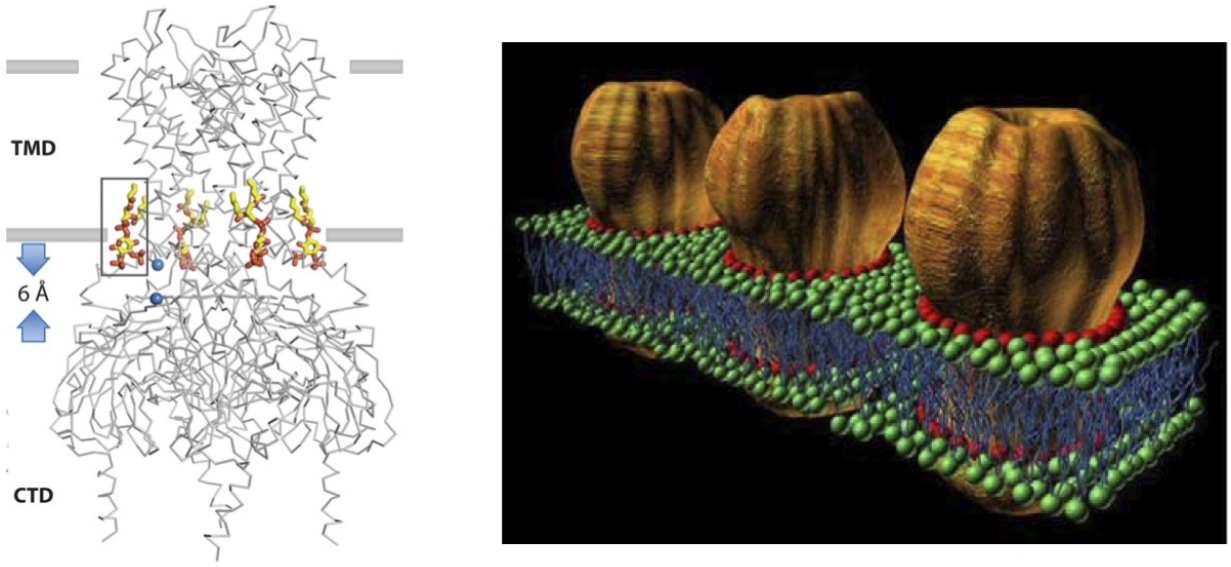

FIG. 9: (left) Figure from Hansen et al. [36]. The potassium channel will not function without some PIP_2 molecules present (one molecule outlined in box). (right) Schematic figure from Barrantes [37]. The nicotinic acetylcholine receptor will not function without one complete layer of lipid around the molecule (single layer indicated in red).

as channels can be reversibly opened and closed by adding and washing out PIP_2, see for example [35, 36].

Within the rotating pore picture, lipids assume a more dynamical function. Perhaps some lipids are "slipperier", more amenable to lowering the friction between a moving protein and static protein and/or the rest of the lipid bilayer. Perhaps PIP_2 acts as a necessary "lubricant", enabling movement of the inner rotor.

Support for this possibility is provided by recent work of Hanson et al. [36]. They performed x-ray diffraction studies on a potassium channel (not voltage-controlled), and found PIP_2 molecules fixed at particular locations between the transmembrane domain (TMD on the left side of Fig. 9) and the cytoplasmic domain (CTD). Their interpretation is in terms of "binding" between the two domains, and a linker "opening" the pore. An alternative interpretation might be that the PIP_2 frees the connection between the putative "rotor" (the TMD) and the "stator" (the CTD), enabling rotation and ion flow.

The right side of Fig. 9 displays another possible "lubrication" scenario, in the nicotinic acetylcholine receptor, a completely different ion channel. These channels open in the presence of a neurotransmitter, and operate muscles, for example. Such channels can occur in dense arrays, and Barrantes [37] has shown that in order for them to function, there must be one complete layer of lipid (indicated in red in the schematic figure) around each channel. Why must this be? The answer, in the rotating pore picture, is clear, the channels rotate, and a lubricating lipid layer is required for smooth rotation.

Why the Rotation?

Now comes the central question, how does a rotating pore enhance the transport of ions from one side of the membrane to the other? First we will point out some problems with the standard view, then suggest how the rotation picture might solve them. However, as the writer still has no convincing simulations, the rotation picture must remain in the realm of speculation, barring experimental support (see below).

Ion channels can conduct at an impressive rate, upwards of 10^8 ions per second, while maintaining ion selectivity. There are two forces available to move ions through the channel, the force due to the electric field across the membrane, and that due to the chemical potential, or difference in concentration of an ion species across the membrane. The electric field has the advantage that in a single-file situation, it can operate globally, on the whole file at once. But the channel often must operate when the membrane is depolarized, or even with an electric field opposing the flow. In that case, it might seem that the channel has to rely on one-dimensional diffusion to maintain the flow.

In Hille's standard reference [15] he estimates the maximum possible ionic flow through a pore by asking how many ions could arrive at a hemisphere covering the pore entrance, by bulk diffusion (page 354). He writes "If we assume that the ion is as mobile in the pore as in free solution" then we can perhaps obtain the observed large ion channel

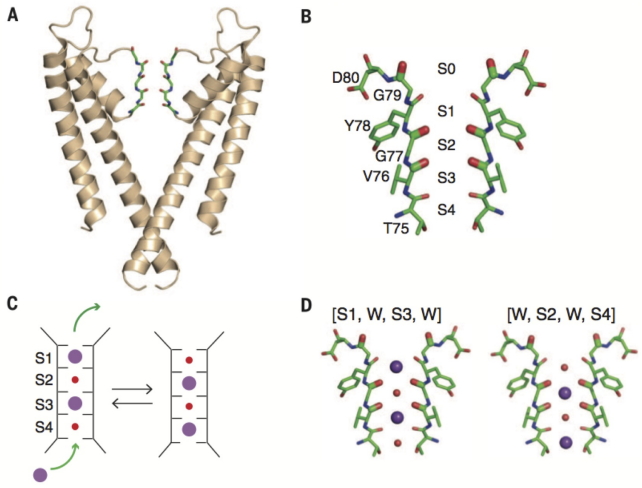

FIG. 10: The KcsA channel, figure from Kratochvil et al. [41]. The panels A and B show the channel, and a closeup of the "selectivity filter"; panels C and D diagram the hypothesized "knock-on mechanism" for transport through the filter. The purple dots represent K^+ ions, the red dots, water molecules. The atomic-level precise single-file flow probably cannot be maintained in the larger lower chamber.

conduction rates.

This assumption is suspect. There is a substantial literature on particles moving single-file through narrow channels [38], and the usual finding is that such motion is subdiffusive. The mean displacement of a diffusing particle in the bulk goes as the square root of time, for a single-file particle in a one-dimensional channel, it goes as time to the 1/4 power [39]. (haven't been able to actually get a copy of the Harris reference).

A classical way around this problem is to invoke a "knock-on" effect [40], ions in a channel are aligned, so that introducing an ion at one end of a chain will quickly pop out the ion at the far end. This is akin to the "Newton's cradle" toy, constructed of a line of swinging spheres. However it would seem that efficient transmission of ions down the channel would require a rather precise alignment of the atoms of the wall, and of the moving ions. If one examines the structure of, for example, the potassium channel [21], these conditions might be met in the selectivity filter, but probably not elsewhere in the channel, see Fig. 10.

Thus we might look elsewhere for a dynamical explanation of the high flux rate through the channel, including a rotation of the channel itself. If this is the case, rotation must somehow enhance flow through the membrane. In Ref. 1, this writer presented a hand-waving argument that the enhancement is a bulk effect, which upon reflection, is pretty bogus. The new and improved theory holds that the rotor provides a mechanical pathway for the chemical potential to propel ions across the membrane, separate from the mechanism of single-file diffusion. At an atomic scale the channel walls are irregular, and in thermal motion. This has the consequence that ion momentum down the channel tends to be rapidly dissipated into the walls, generating friction. A solution to this difficulty would be to have some momentum transferred via a rotor, thus lowering the friction.

We will now construct a somewhat artificial quasi-Hamiltonian model in two dimensions, which nevertheless might illustrate the point. We imagine a reservoir of hard disks, interacting via elastic collisions. There are periodic boundary conditions in the vertical direction. In the horizontal direction, there is a back wall to the left, and a channel extending to the right, see Fig. 11. Any disks that successfully pass through the channel to the right are returned to the chamber at a random position. The magnitude of the velocity is retained, but the direction is randomized. In this way, we construct a system which nominally preserves energy, but features an open flow. We now fix the density of disks in the chamber to an area density of about 1/2, and observe the flux down the channel as a function of channel geometry.

We begin with a smooth channel, of width 3/2 times the diameter of the disks. A histogram of the disk density in the horizontal direction appears above, note that, as expected with smooth walls, the density is nearly uniform along the channel. The flux in this situation is set to a normalized value of 1.

Now the upper wall is retracted, exposing a set of fixed disks, protruding halfway, as shown. This has a number of effects. First, the flux is greatly reduced, to a value less than 1/5 of the smooth wall case, even though the average width of the pore has increased. Also, the density profile declines along the length of the pore, in a roughly diffusive Fickian manner. This is because the motion of the disks in the pore is now chaotic. A new length scale has been introduced, after a certain distance down the pore, a disk has some probability of bouncing backwards, it has "forgotten" that it entered the pore with a momentum in the rightward direction. Now disks must be propelled

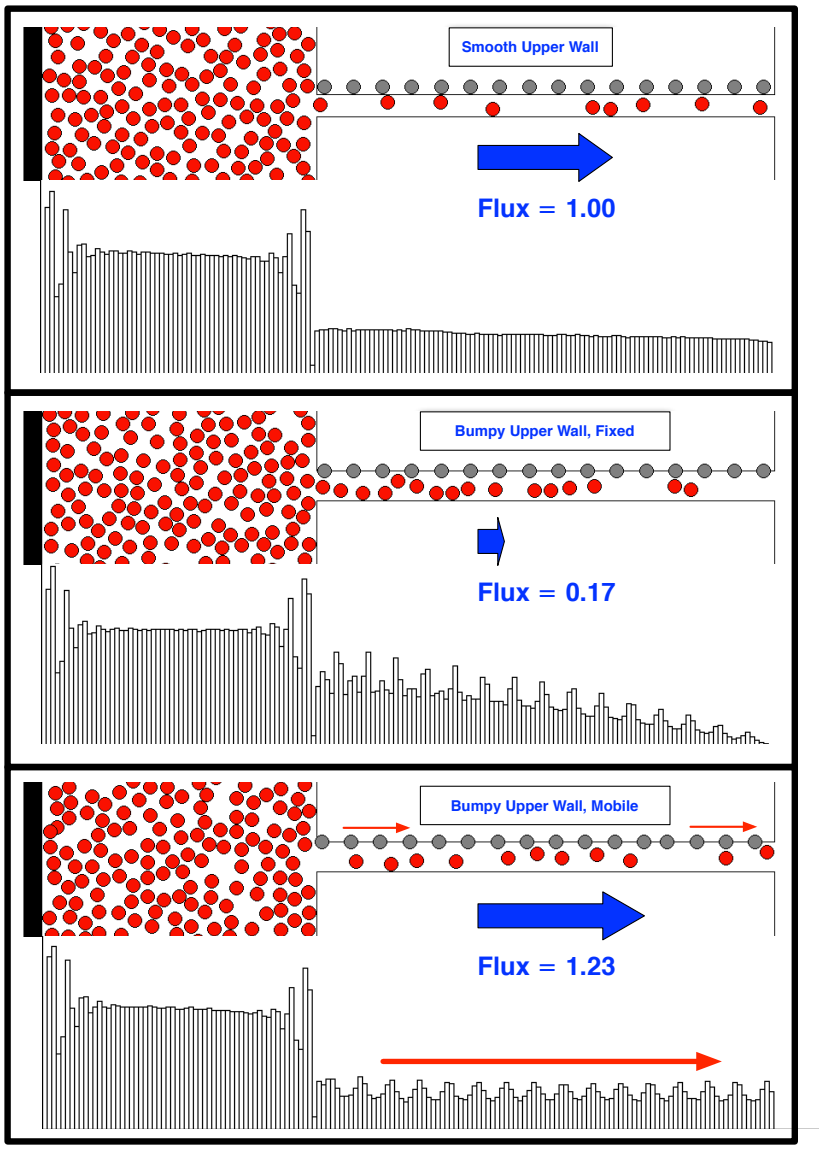

FIG. 11: Hard disk simulation, disks flow from left to right, and are returned to the left-hand chamber when they exit the pore on the right. Top panel - Smooth walls, a typical disk configuration, and a histogram of the average disk density along the horizontal direction. Middle panel - The upper wall is retracted to expose fixed disks. Disk motions in the pore are now chaotic and diffusive. The flux is greatly reduced, and the probability distribution falls off towards the exit. Lower panel - The pattern of disks on the upper wall can now absorb energy from the flow, and move as a "corrugated belt". The flux is restored to a large value, and the probability distribution is more uniform along the channel. The histogram shown is an instantaneous snapshot, the probability distribution within the pore moves as a whole along with the disk pattern on the upper wall.

forward diffusively, by a gradient in the mean density of disks along the pore.

In the third panel, we allow the disks decorating the upper wall of the pore to move in response to collisions. We take the total mass of the upper disks to be 100 times a single free disk, and view them as moving in concert, one can imagine a corrugated belt moving along. When one upper disk reaches the right end of the pore, it is returned to the left end. Now the flux returns to a high value, in fact greater than the first case, even though some energy has been transferred into the motion of the belt. Also, the histogram of disk probability density along the channel has returned to close to uniform.

Why is the flow so greatly enhanced? The moving belt provides an additional pathway for momentum to pass from left to right, one free of the problem of momentum being dumped into the pore walls by collisions.

This is a highly contrived example, but perhaps does show how a dynamic accommodation of the boundaries to the flow can lower dissipation. In the rotor picture, the mechanical boundary fixture moves transversely to the flow.

FIG. 12: Stereo views of the KcsA channel, taken from Doyle et al. [21], for viewing with eyes crossed. The left pair is a side view, the right pair a top view. Note that the helical interior is along the path of an ion (green sphere) as it travels through the pore.

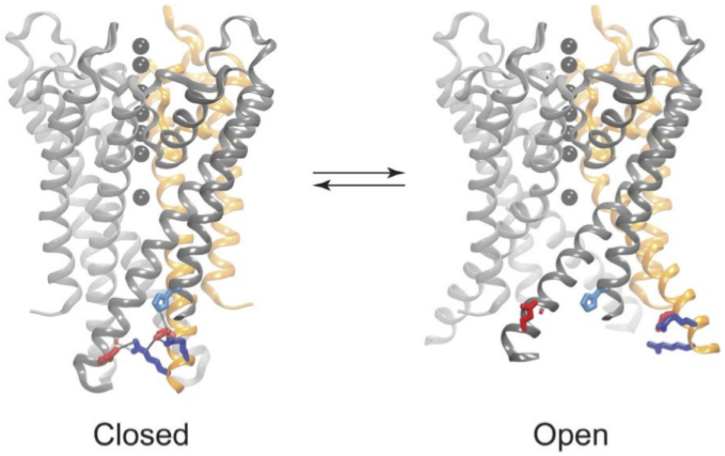

FIG. 13: Hypothetical changes in KscA geometry, as the channel opens, figure from Thompson et al. [42]. The open channel is, roughly, a hollow cylinder with helical ridges along the interior.

Also the rotor moves at a small fraction of the average speed of the flow particles, whereas here the belt moves at a significant fraction of the average disk speed. Nevertheless, the *bulk movement of the average probability distribution driven by the moving boundary* may be a common feature.

With this two-dimensional result in mind, let us examine the structure of the bacterial KcsA channel. This potassium channel was the first to be crystallized, and thus imaged at atomic resolution, by Doyle et al [21]. KcsA is considered the simplest, and a prototype model for all potassium channels. Fig. 12, taken from Doyle et al, shows, in stereo, two views of the KcsA protein crystal. Notice the strong helical geometry of the interior walls. KcsA is gated by changes in pH, and though the mechanism is still an open research question, it is thought that, when the channel opens, the lower portion of the protein opens, giving the channel the overall form of a hollow cylinder, see Fig. 13.

Thus, roughly, we have a hollow cylinder, decorated on the inner wall by four helical ridges. If this structure were free to turn, ions colliding with the walls on their way through the channel would rotate the structure. Note that ions are traveling at thermal speeds, there will be some thousands of collisions with the inner walls during a passage. To repeat the central argument, the rotor might provide an alternative pathway for the chemical potential gradient to propel ions through the channel. Stress can pass through the rotor, rather than being limited to a concentration gradient of ions along the membrane. Ions at the far end of the channel are accelerated by the rotor, as well as by the local concentration gradient.

Clearly a full three-dimensional simulation of flow through such a structure is very much in order, to see if transport is in fact enhanced. To be fair, there do exist large-scale atomic-level simulations of the potassium channel which claim to reproduce the high transmission rate as well as the high selectivity for the potassium ion [43, 44]. These simulations were performed on special-purpose hardware, and are difficult to critique. We can only point out that

1) the possibility of large-scale motions such as a rotation are not built into the simulations, and 2) their proposed mechanisms depend on the existence of an S4-S5 linker, which as argued above, is questionable.

The weakest point of the existing arguments for the rotating pore picture is the lack of a detailed kinetic argument of how rotation increases transport. The chief difficulty stems from the difference in time scales between molecular motions and any "gating", this is a problem for all molecular dynamics simulations of ion pore activity. The strongest argument in favor is perhaps a simple consequence of conservation of angular momentum. As argued in Ref. 1, the torque exerted by a rapid stream of heavy ions on a helical geometry is large, the pore will turn, if it's not nailed down.

How to Detect the Rotation?

The arguments above are suggestive, but hardly definitive, they still could be interpreted within the picture of a static channel pulled open by a linker. Laboratory experiments are required, especially as the rotating pore picture is a radical departure from previous thought.

One might think that the rotation of a large molecule in a biological membrane would be hard to miss. However, even the rotation of, say, a cylindrical protein 20 Å in radius, at fully one megahertz produces molecular motions which are small compared to thermal noise. The velocities of water and ions at body temperature are on the order of hundreds of meters per second, while the periphery of our rapidly spinning molecule moves four orders of magnitude more slowly, around a centimeter a second. Thus the rotation is quite leisurely compared to thermal motions, and, at an atomic level, can only be seen as a small bias on random fluctuations.

The prime example of a rotating molecule in a biological membrane is ATP synthase, the molecular machine which takes the potential difference across a membrane to produce ATP, or, run in reverse, degrades ATP to add to the membrane potential difference. This was conjectured, on theoretical grounds, to include a rotor that mechanically turns, by Paul Boyer [45] around 1980. The first physical demonstration of protein rotation was perhaps that of Sabbert et al [46] in 1996, via a photobleaching experiment. This was followed the next year by the work of Noji et al [47], who attached a fluorescent filament to the rotor, and directly observed rotations through the microscope.

It may not be feasible to directly attach large markers to a putative ion channel rotor. Ion channel proteins are much smaller, and, if they rotate, do so much faster. Further, the ATP synthase motor is designed to operate under load, to perform chemical work, whereas ion channels function to simply get ions across the membrane as quickly as possible. However recently it has become possible to attach a smaller "fluorophore" to a single residue on an ion channel protein, and observe photo-emission from a single molecule, see for example [48, 49]. If such a fluorophore were attached to a rotor, and the photo-emission were polarized, one might be able to observe rotation via changes in the plane of polarization. "Fluorescence polarization microscopy" may be a candidate technology [50]. Yet another might be "2D infrared spectroscopy" [51].

A more indirect observation might be obtained by attaching *two* probes to a single molecule, and measuring the distance changes between them as a function of molecule dynamics. There are at least two technologies capable of doing this. FRET (Förster resonance energy transfer) uses non-radiative coupling between two fluorophores to determine distance, and DEER (double electron-electron resonance) uses microwave pulses to find the coupling, and thus the distance, between two electron spin labels. Both techniques have begun to be used in studying ion channels, see for example [52] (FRET) and [53] (DEER).

An experimental strategy would then be to attach one label to one of the subunits on the "rotor", and another near the (presumably static) latch, near the "linker", as schematically illustrated in Fig. 14. If the channel is in fact spinning while conducting, it would come to rest in one of four positions when the channel is closed. If the channel is a symmetric "homotetramer" (and the labels do not interfere), there will be a 1/4 probability that the two labels will be close when the rotor is static. Thus we would look for "multiple conformations" in the closed state.

There are to the writer's knowledge so far no experiments which watch FRET or DEER signals from an ion channel while the channel is actually conducting ions across a membrane. But the two references mentioned above come close, changing a bacterial potassium channel between putative "open" and "closed" conformations, by changing the chemistry of the environment. The last sentence of [53] reads "Finally, the broadening of the distance distribution when KcsA opens has revealed the dynamic nature of the lower gate suggesting that upon activation, the lower gate populates multiple conformationally open states." A spinning rotor would certainly generate multiple states!

The rotation, if it exists, is fairly certainly experimentally detectable, but one would have to look for it. The writer would very much enjoy participating in such an experiment.

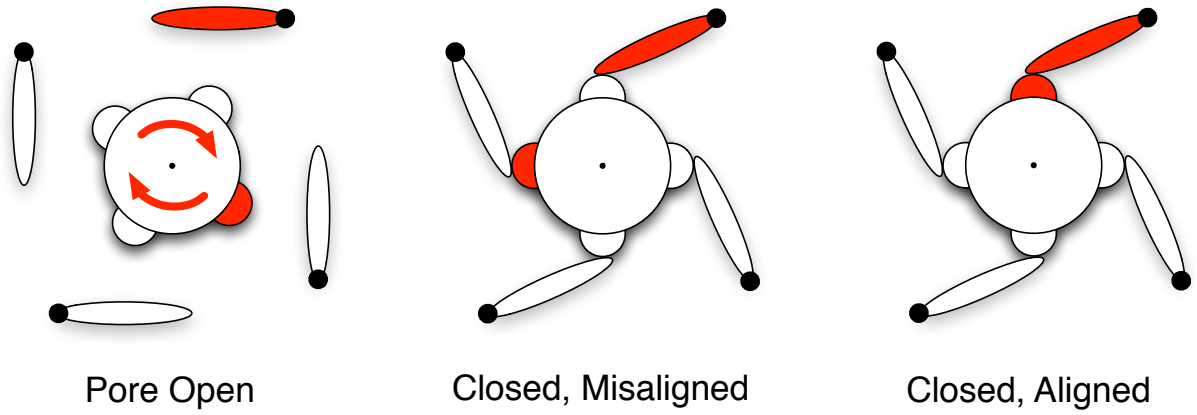

FIG. 14: Schematic of an experiment which would support pore rotation. A marker is placed on one of the voltage sensors, and another on one of the pore domains. When the two markers are close, a signal can be detected, perhaps via EPR, or FRET. When the pore is open, and rotating, the signal strength has a broad distribution. When the pore is closed, the rotor is fixed, and there is a 1/4 probability that the marked sensor and marked pore domain are adjacent, leading to a strong signal.

Evolutionary Considerations

In searching for an evolutionary path from an inanimate world to the present one, populated by giraffes and jellyfish, a path which a card-carrying materialist assumes must exist, there are problems. One is that all evidence of the evolutionary history of, for example, ion channels, has been gobbled up by more efficient new life forms. Another is the sheer intimidating complexity of living systems, matter organized right down to the atomic level (at least). Most attempts to reconstruct the Last Universal Common Ancestor (LUCA) arrive at an entity which already has in place sophisticated cellular machinery, including DNA and RNA, and significantly, the rotary molecule ATP synthase [54, 55]. It seems even possible that this rotary motif arose earlier in prebiotic or "mesobiotic" forms than the genetic machinery.

If one performs a web search for, for example, "ATP synthase", one often finds a Creationist website, often remarkably good. This writer found several useful, as an ignorant physicist trying to learn some biology, for example [56]. The message of such websites is direct. They will describe in some detail a system, like ATP synthase, which is present in every known living system, and essential, yet is so intricate that it seems impossible for it to spontaneously spring into existence. The message effectively is, "Evolve this, suckers!" (this is viXra, we can speak freely).

The Creationists have a point that is not easily dismissed. The problem is first causes, and finding a path to the present. For a Creationist, that's not a problem. One might admit that the mainstream science answer to that question is, "A long time ago, there was a big explosion. Boom!". And that the giraffes and jellyfish followed, through unspecified paths. This is intellectually not very satisfying, and no better than a directly theological explanation. But seemingly all that is left for us to do is to find the various paths.

One such path might be from rotating ion channels, to rotating ATP synthase. If it in fact is the case that ion channels spin, and they do so because of a simple dynamical advantage, then the leap to a rotating ATP machine becomes much more feasible. One can imagine first a simple tube conducting ions, and then the tube acquiring a twist, leading to better conduction. The survival advantage of any increase in transport rate is extreme, affecting speed of perception, and speed of muscle movement.

Of course one still has to explain the Krebs cycle, and a hundred other impossibly complicated yet necessary features of current living systems. There remains as well a central mystery, which may be beyond explanation, the drive to organize down to the lowest length scale possible, to branch out again and again, to fill all possible space.

Life is a state of matter.

A General Theory of Anesthesia, How Alcohol and Anesthetics Work

The effects of alcohol on human consciousness are well-known, and in many cases, welcome. The deliberate consumption of ethanol for its psychological effects goes back to prehistory, and is not limited to the human species. Despite the long history and familiarity, the mechanism of action of a common glass of wine remains unclear.

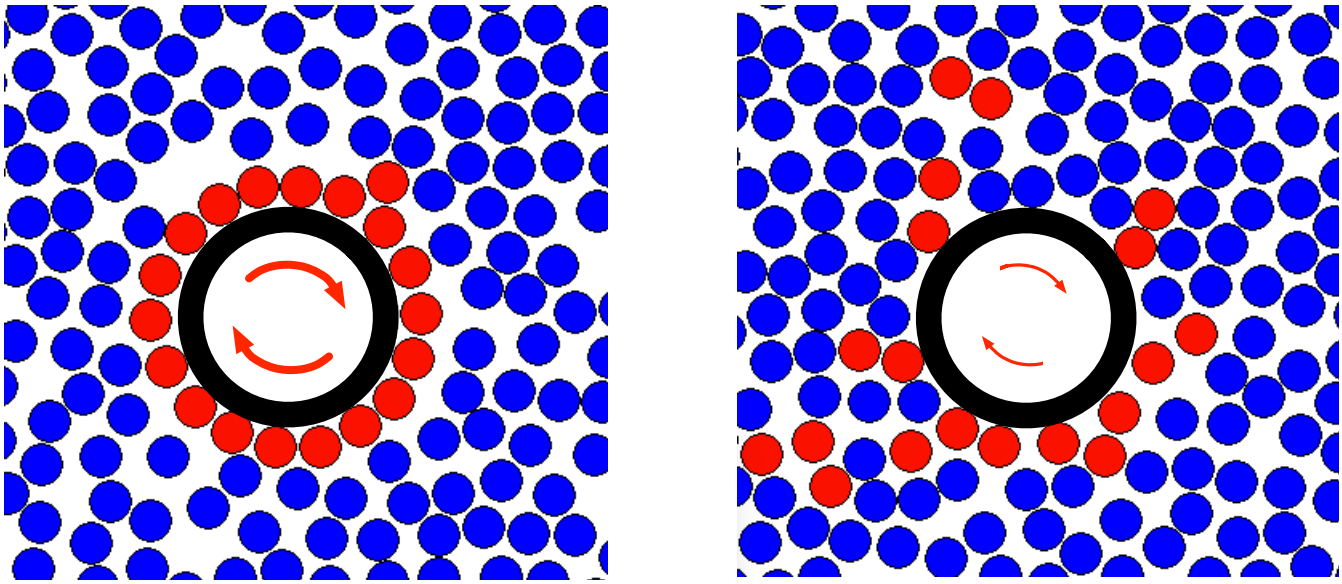

FIG. 15: Anesthetics and alcohol may compromise the phase transition that keeps bulk lipids and "lubricant" lipids compartimentalized. Without the optimized lubricating lipids in place, rotation, and ionic flow, is slowed.

Equally unclear is the operation of general anesthetics, which have a more profound effect than alcohol. A variety of substances, from ether to xenon, can render a human completely insensible, and, importantly, in a reversible fashion. There is apparently no particular "ether receptor", or "ethanol receptor", in the sense that there is a morphine receptor [57].

A strong hint as to what is going on was provided independently by Meyer and Overton, around the year 1900. They noted that there is often a direct correlation between the concentration of a chemical required to produce anesthesia, and the solubility of that chemical in olive oil. This has prompted a number of theories suggesting that anesthetics somehow change the material or mechanical properties of the lipid cell membrane. Although such theories are apparently somewhat out of favor, they have the advantage that they "explain" why a variety of seemingly unrelated chemical agents can produce anesthesia.

We suggest here a modern update of that view. Recent research shows that cell membranes are not composed of a single lipid species, but rather an entire population. These can segregate into "rafts", and in particular can be localized around large proteins floating in the membrane, for example, ion channels [58]. It seems increasingly clear that ion conduction, and other conduction occurring across the membrane, is a highly dynamic process, involving both proteins and the membrane lipids [59].

To put forward a specific model, this writer proposed that conducting ion channels are in a state of rotation. This perhaps outlandish notion is supported by some indirect evidence, see [1] and above. If this is the case, we might expect that in the ecology of membrane lipids there are some that are specialized to lubricate protein rotation, or other dynamical motions. These lubricants would be of course localized near the moving proteins.

The simple argument is then, that anesthetics generally act to disrupt the local populations of lipids in the membrane, moving lubricating liquids away from their sites of action next to proteins, see Fig. 15. Thus conduction will broadly and generally be affected, usually reduced. When the anesthetic agent is removed, the lipid population can return to its original distribution.

The more general hypothesis is that anesthetics and alcohol disrupt the spatial distribution of specialized lipids, and thus standard membrane function. This would affect any number of models of protein-lipid dynamics, not just the spinning pore picture. One interesting line of thought holds that phase transitions in lipids, from a raft or gel phase to a two-dimensional liquid, are affected by anesthetics [60]. This 2007 work of Heimburg and Jackson notes the experimental fact that high pressure inhibits anesthetic action, and explains it as "a consequence of the pressure-induced elevation of the melting point in lipid membranes". More anecdotally, many air travelers have noted that the effect of a cocktail is enhanced in the low-pressure environment of a commercial airliner.

Particularly relevant to the image presented in Fig. 15 is the "lipid domain theory of general anesthesia" [61]. In a 2003 paper, Veatch and Keller showed that there commonly is a mixing-demixing transition between different species of lipids in a membrane as a function of temperature, and presumably other thermodynamic variables. This transition occurs in physiologically relevant mixtures of lipids, at near-physiological temperatures. Later work [62] developed the

idea that anesthetics act by affecting the mixing-demixing phase transition point. Specifically, they act by lowering the critical temperature for the transition, and thus to "reduce lipid-mediated heterogeneity", a notion consistent with the picture of Fig. 15.

The Meyer-Overton view, that anesthesia is largely mediated by the lipid membrane environment, remains controversial. For example, a recent paper studied the effects of anesthetics on single-species lipid bilayers and concluded that anesthetics "function through direct interactions with the channel protein with little, if any, contribution from changes in bulk lipid bilayer properties. Our findings further suggest that changes in lipid bilayer properties are not involved in clinical anesthesia." [63]. However such criticisms perhaps miss the point, the work cited above focusses on *mixtures* of lipids, which then can undergo a *phase transition* between mixed and separated states.

Summary

A thorough discussion of even a single type of ion channel is out of reach here, these notes are just limited to the suggestion that rotation may be part of the story. But at a minimum, there exists a strong argument that, in the case of voltage-controlled channels, the voltage sensors *detach* from the central part of the protein, thus *removing a constraint* which enables some dynamical motion which then enhances ion flow. It is argued, on the basis of geometrical simplicity, and the apparent helical form of the interior ionic pathways, that this dynamical motion is a rotation. However it is possible, and consistent with some of the above arguments, that the motion is some type of fluttering, not involving rotation.

Let's watch the literature as it unfolds, it should be interesting. Finally, regarding the rotating pore notion, true or false, the writer quotes the immortal words of Miss Anne Elk [64], "Ahem. This is my theory. My theory which is mine."

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