Is Consciousness Dissectible? Acute Slice Electrophysiology and a Bayesian Interpretation of Neural Correlates of Consciousness

Richard König[1,2,3,*], Alexander Mirnig[3,4], Ludwig Aigner[1,2], and Thomas M. Weiger[5]

[1]*Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg Austria*

[2]*Spinal Cord Injury and Tissue Regeneration Center Salzburg, Paracelsus Medical University Salzburg, Austria*

[3]*Department of Ecology and Evolution, Neurosignaling Unit, University of Salzburg, Austria*

[4]*Center for Human-Computer Interaction and Department of Computer Sciences, University of Salzburg, Austria*

[5]*Division of Cellular and Molecular Neurobiology, Department of Cell Biology, University of Salzburg, Austria*

Abstract: The acute brain slicing method has become one of the foundations of modern neuroscience research. It is a laboratory technique in electrophysiology, which allows the study of electrical properties directly on a freshly prepared slice of animal brain tissue. During recording and/or stimulation, the acutely isolated brain slice is artificially kept "alive" up to many hours after the animals' death. During an acute brain slice preparation, cortical and subcortical areas, which are suggested to correlate with conscious experience in humans, such as the claustrum and the thalamus, are dissected. In this paper, we investigate whether scientific statements can be made regarding the likelihood that some neural activities on the brain slice still support consciousness or degrees thereof.

We exemplarily demonstrate how acute slices are produced and provide own electrophysiological data combined with a short literature review. Subsequently, we introduce the concept of Neural Correlates of Consciousness (NCC) and apply conditional probabilities inferred from Bayes´ theorem, in order to draw from it an informed hypothesis on the likelihood that specific neural activities that sustain on the slice still correlate with some form of conscious experience. We propose that the probability that there is something that is it like to be, even on the acutely isolated brain slice, is similar to the likelihood that certain mental states correlate with certain brain activities in a healthy human subject, depending on the robustness of the underlying NCC.

Keywords: Neural correlate of consciousness (NCC), Acute slice electrophysiology, Philosophy, Empirical bayes methods, Conditional probability.

1. INTRODUCTION

What happens to your inner subjective experience, your conscious state that you are in at that very moment, when suddenly an ice-cold vibrating razor blade dissects exactly those brain regions suggested to be the neural correlate of your specific conscious state? Does the razor blade only cut your (mostly still) living brain tissue apart or is it also cutting away content of your conscious experience slice-by-slice? What if this dissection were so gentle that 300 µm thick brain sections could be produced, where many cells inside this acutely isolated brain slice still show intact membrane properties and neuronal network participation? What would happen to your conscious experience in such a situation? Would your consciousness fade away gradually, slice after slice? Or would this preparation directly and immediately lead to an irreversible loss of all your conscious states at once, after the first cut? In other words: Is consciousness dissectible?

At first glance, this question seems to be rather vague and based on too speculative premises in order to be answered directly, but it bears a degree of intuitive plausibility that renders it worth investigating. Hundreds of well-trained electrophysiologists use the acute slicing method to deepen our understanding of brain physiology on a daily basis [1-5] whilst the underlying basic question of what happens to an individuals' conscious states and contents is still unanswered. Recently, a method was capable to extend the lifespan of an acutely produced murine brain slice to more than 36 hours following preparation [3], which lends further relevance to the issue of whether any scientific statements can be made regarding the likelihood that some form of conscious experience might preserve on so produced brain slices.

In this paper, we discuss, based on empirical findings, how much cellular activity is expected to

*Address correspondence to this author at the Institute for Molecular Regenerative Medicine, Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCITReCS), Paracelsus Medical University, Strubergasse 22, 5020 Salzburg, Austria; Tel: +43 662 2420-80811; Fax: +43 662 242 080 009;
E-mail: richard.koenig@pmu.ac.at

E-ISSN: 2409-3564/17

persist on the acutely isolated brain slice and, as more speculative but empirically informed extrapolations, what the likelihood may be that these cellular activities still correlate with some form of conscious experience. We address the first issue of this work - how much activity persists on the isolated brain – in section 2. Here we exemplarily show how acute slices are produced and provide own electrophysiological data combined with a short literature review for these illustrations. In section 3, we introduce the concept of neural correlates of consciousness (NCC) from Koch and Crick [6] and survey whether any NCC-candidate might preserve their biological function after the acute brain slice preparation. To speculate on the likelihood that specific neural activities that sustain on the slice still correlate with some form of conscious experience, we apply conditional probabilities inferred from Bayes´ theorem; based on the work of Bernroider [7], Bialek [8] and Choe [9]. We close with a critical valuation of the NCC-approach in general and state that given our formal representation the likelihood that there is something that is it like to be, even on the acutely isolated brain slice, is similar to the likelihood that certain mental states correlate with certain brain activities in a healthy human subject – depending on the robustness of the underlying NCC.[*]

2. ACUTE SLICE ELECTROPHYSIOLOGY

Based on the pioneering work of Henry McIlwain [10-12], the acute brain slice preparation has become one of the most commonly used methods in neurophysiology [13, 14]. During an acute rat or mouse brain slice preparation (see Figure **1**), following anesthesia, (A) the brain (white arrow) is removed from the skull, (B) sliced on a vibratome, (C) and finally transferred to a recording device. There are various protocols available to produce viable acute brain slices [4, 15] for several cell types and brain regions (D) predominantly for mice and rats, like the cerebellum [16], the hippocampus [17], the piriform cortex [18], the barrel cortex[19], the ventromedial prefrontal cortex [20], the thalamus [21], the hypothalamus [22], the corpus callosum and the motor cortex [23], the olfactory bulb [24], the brainstem [25], different dopaminergic neurons of the midbrain [26-28], the amygdala [29], and parts of the basal ganglia [30], such as the substantia nigra [31]. Notably, a new recovery incubation system was capable to extend the lifespan of an acutely produced brain slice to more than 36 hours following preparation [3]. This leads to the question of how much cellular activity is reported to persist on so produced acutely isolated brain slice.

As exemplarily demonstrated in Figure **2**, many different types of brain cells are still intact after the acute slice preparation. For example, our whole-cell recordings visualized in Figure **2** on the acute mouse brain slice of principal neurons in the cerebral cortex and the hippocampus are confirmed by single cell measurements carried out in anesthetized (alive) mice *in-vivo* (*e.g*. [32]). Moreover, our recordings of immature neurons parallel *in-vitro* recordings of cultured immature neurons (*e.g*. [33]). Even oligodendroglial progenitor cells, studied on the brain slice, mirror the functional properties of oligodendroglial progenitor cells recorded *in-vivo* (*e.g*. [23, 34]). Taken together, cell-specific properties and activities are sustained on the slice, at least to the time-point of measurement.

A substantial body of electrophysiological research affirms the viability of the acute slice method to study not only single cells, but also physiological characteristics down to the level of synapses (*e.g*. [35-38]), or up to the level of neural networks. One of the most prominent network properties are long-term potentiation (LTP) and long-term depression (LTD). LTP and LTD are suggested to build the cellular basis of learning [39-42]. Both have been described and studied on acute brain slices of the hippocampus (eg. [41]), the neocortex [43, 44], the amygdale [45], different mid-brain areas [46], the cerebellum [47, 48], and the striatum [49]. The ability of a cellular microcircuit to learn and memorize specific stimulations (*e.g*., LTP or LTD) determines that a sufficient amount of synapses and cells within this cellular circuit must be functioning properly and keep specific spike trains structured to still encode for specific information, following the acute slice preparation.

But is the remaining array of brain activity on the slice sufficient to correlate with conscious experience? Is there a *something* that is it like to be inside of a fresh slice of brain tissue? In every slice? What is already known about the function of the brain regions that the

[*] Note: No animal experiments were carried out for the specific purpose of this paper. All electrophysiological data shown in this paper were obtained for a different research project (König *et al*., in preparation; Title: *Membrane physiology of immature neurons in the piriform cortex: Evidence for non-proliferative functional neurogenesis?*), where all guidelines for animal handling and care were followed and nationally approved (66.019/003-W).

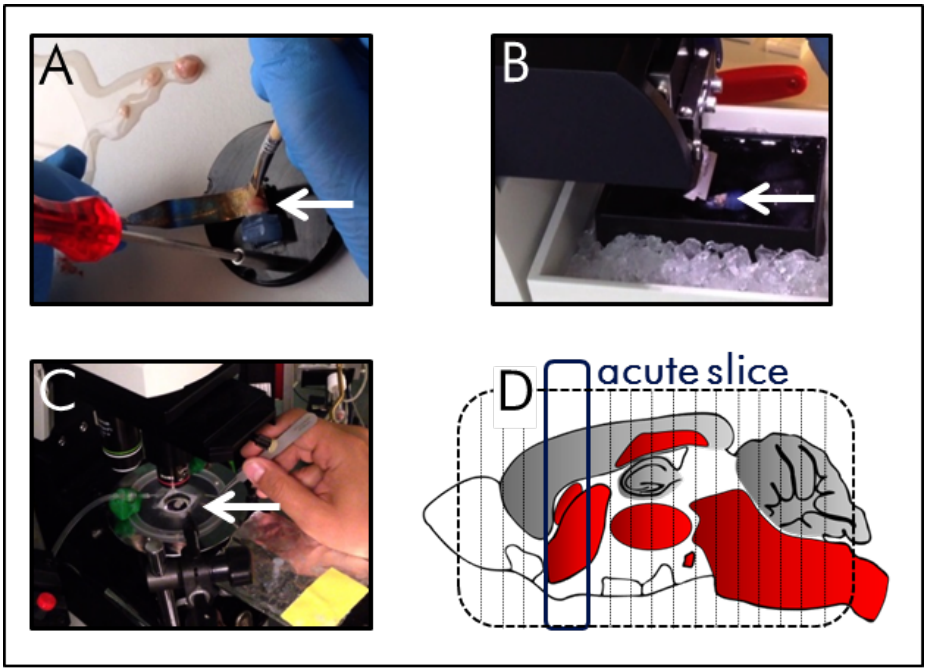

Figure 1: Acute slice preparation visualization. (**A**) The rodent brain (white arrow) is removed from the scull and (**B**) sliced by the use of a vibratom. (**C**) So produced brain slices are transferred to a measurement device (*e.g.* an upright microscope for single-cell patch clamp experiments). (**D**) Vital brain slices of rodents can be produced of many brain region of interest, like the claustrum or other brain regions suggested to correlate with conscious experience in humans (red areas).

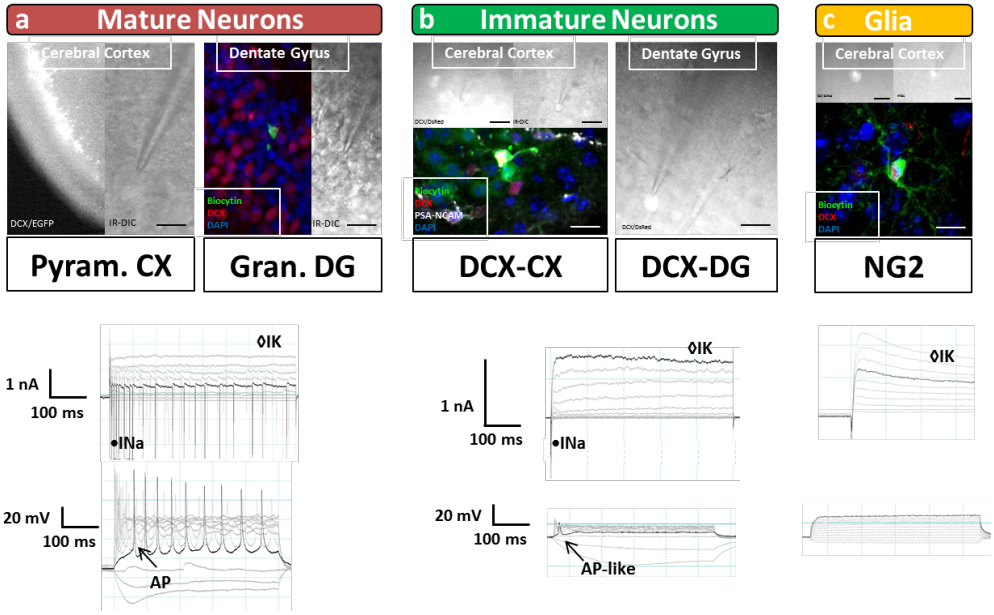

Figure 2: Single cell whole cell patch-clamp recordings performed on acutely isolated brain tissue as a method to characterize various cell types or to identify specific maturation stages. (**a**) On the slice, mature neurons, like the pyramidal cells of the cerebral cortex or dentate gyrus granular cells of the hippocampus, elicit strong sodium (I_{Na}) driven inward currents (upper recording) and action potential firing rates (lower recording) can be increased by the induction of current steps (*e.g.* nine 40 pA current steps, 50ms). (**b**) Doublecortin (DCX) expressing immature neurons as well as their maturation stages on their way to functional integration can be traced (I_K = potassium current). (**c**) Glial cells, like NG2-expressing oligodendroglial progenitor cells or polydendrocytes, can be functionally identified by the absence of action potential firing, their low membrane resistance, and potassium driven outward currents (I_K) with a strong inactivation over time. Scale bars: 50 µm.

acute slice preparation is dissecting? In the following section, we will approach this question with the help of the so-called *Neural Correlates of Consciousness*.

3. NEURAL CORRELATES OF CONSCIOUSNESS

A neural correlate of consciousness (NCC) is defined as the "minimum neural mechanism sufficient for any one specific conscious percept" [6]. This definition presumes that *there is something that it is like to be* and this *something* is then termed ´consciousness´, without further details or sub-definitions. While this "what is it like-ness" seems not be reducible to physical processes, the inner subjective states of an organism might directly correlate with the activity of specific brain regions or functions [6, 50-52]. Thus, NCC are an elegant way to enable investigations of various brain regions and functions – the neural correlates (NC) – directly, while the last 'C', the conscious experience, is studied only indirectly as a mere correlation. While this simple working definition is not without some conceptual problems [53, 54] the use of the NCC approach has led to noteworthy advantages in the neuroscience of consciousness in the last number of decades.

The neurobiological quest for consciousness has led to some promising NCC candidates. Nowadays, the neuroscientific community differentiates between the general/full NCC, a content-specific NCC and the background conditions that must be fulfilled for being conscious at all. A general NCC is defined as the full NCC including all neural substrates enabling conscious experiences in their entirety, regardless their specific contents. A content-specific NCC is the brain regions or functions, which determines a particular conscious experience. These differentiations led to a variety of promising candidate brain regions to correlate with conscious experiences in humans, as outlined in Figure **3**. It must be noted, however, that no single area or activity has yet been conclusively identified to correlate with conscious experience, neither in humans nor in non-human animals (reviewed in [51]).

4. NCC-CANDIDATES

Lesions of the cerebellum were shown to have no effect on conscious experiences in human patients [56, 57], which excludes the cerebellum as a NCC in general, a content-specific NCC as well as a background condition of a NCC. More relevant for the search of NCC, lesions in the brainstem were reported to cause immediate coma [58] by damaging the reticular activating system [59] in humans. However, brainstem activity alone is insufficient to sustain consciousness in a clinical sense, since human patients with substantial damages in their frontal cortices but intact brainstem function were often reported to be in a vegetative state [58]. Thus, it was suggested that the brainstem might be a necessary background condition for conscious experience [51], but neither a content specific NCC, nor a full NCC.

Some basal ganglia nuclei are likely NCC-candidates. These nuclei are strongly interconnected with the cerebral cortex, thalamus, and brainstem. Interestingly, the basal ganglia are commonly reported to play a central role in cognition [51] and lesions in the basal ganglia were documented to lead to an emotionless state [60, 61] in humans. This suggests the basal ganglia to play an important role for conscious experience. However, one report from a human being with bilateral basal ganglia hemorrhagic lesions found no significant alternation in the level of consciousness [62]. Thus, it remains unclear whether the basal ganglia contribute to consciousness in

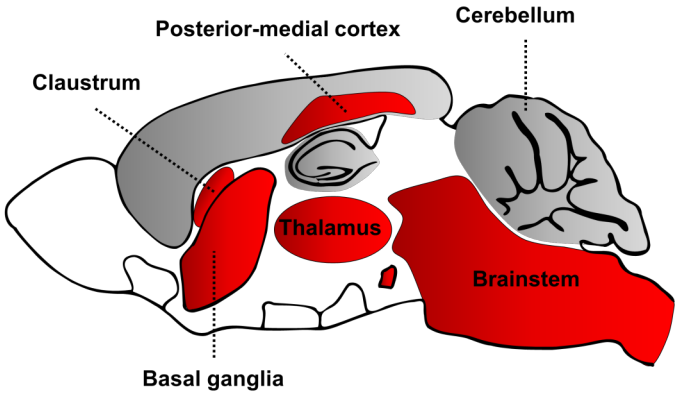

Figure 3: Plausible anatomical structures previously suggested to correlate with conscious experience [55], approximated on a schematic mouse brain (sagittal). It should be noted that the various NNC candidates are primarily deduced from human studies. Different brain regions are only shown schematically in this illustration; their location is approximated and many cannot be found on the same sagittal plane, neither in humans, nor in mice.

general or if the basal ganglia functions are necessary for specific conscious contents.

Another prominent NCC candidate is the claustrum, which is a thin, irregular sheet of neurons closely attached to the neocortex, considered by some researchers to be part of the basal ganglia [63]. The claustrum is bidirectionally connected with most cortical regions [64]. Thus, it was hypothesized by many that the claustrum plays a crucial role for information integration [65-67] and is, therefore, necessary for conscious experience in general [50]. Further highlighting the role of the claustrum to support consciousness in general, the direct electrical stimulation of the claustrum in one human study disrupted consciousness reversibly [68]. Accordingly, the function of the claustrum is often believed to be the NCC in general.

Beside the claustrum, one of the most prominent and controversial structures supposed to correlate with conscious experience are the various thalamic regions. Many experts propose that at least some thalamic cell clusters represent critical enabling factors for consciousness in general [69-72]. Nevertheless, other studies were unable to confirm a central role of the thalamus for conscious experience, in non-human animal lesion experiments [73-75], in brain injured human patients [76], as well as in human patients in a vegetative state [77, 78]. Accordingly, the role of the thalamus for conscious experience remains elusive.

Based on the still existing uncertainties of other NCC postulates, a further NCC "hot zone" was proposed recently: the posterior-medial cortex [51]. Compared to other NCC candidates, this highly interconnected cortical region showed the strongest correlation between decreases in brain activity and loss of consciousness in recent human brain imaging studies [77, 79]. Further studies are necessary to illuminate the possible correlation between the posterior-medial cortex activity and conscious experience in humans and non-human animals.

Taken together, there are some likely candidates of brain structures which activities are expected to correlate, in one way or another, with consciousness in humans. On some of these brain structures electrophysiological measurements on acutely isolated brain slices were already carried out in non-human animals [20, 26-28, 30, 49]. By a combination of both, the NCC-approach and current knowledge regarding the biology of acute sliced brain tissue, we further discuss in the following section what can be expected to happen to consciousness and its (presumptive) neural correlate by the use of Bayes´ probability theorem.

5. BAYES´ THEOREMS AND THE LIKELIHOOD OF NCCS ON SLICES

The usual NCC-research approach begins with a subject of any kind, as simplified in Figure **4a**. Let us assume this subject to be an awake human adult without any pathology or other peculiarities. There is good reason to assume that this subject bears some conscious states of any form, denoted as A. Most such awake "standard subjects" are able to communicate their conscious states, which is strengthening the assumption that some form of A is given in the subject under investigation as well. The task, then, is to identify and characterize a measurable physical signal B given A. Mathematically spoken, the aim of scientific consciousness research might be described by a function f, such as

$$f : A \rightarrow B.$$

While f is assumed to be a highly complex function, an NCC researcher simply postulates that there is a correlation between A and B, without further stating what the correlation actually is; and whenever A, B is present and B can be characterized scientifically. With this simple but effective approach, neuroscientists were able to come up with the remarkable list of NCC-candidates reviewed in the previous section.

For an acute brain slice, there are not as many reasons to assume that any forms of conscious experience or degrees thereof, as was the case for the awake human adult. Moreover, we cannot expect any form of verbal or non-verbal report about an inner mental state from a brain slice. To define the likelihood that conscious experience is present on the slice (Figure **4b**) we must rely on the measurable physical signals only and compare them to the physical signals obtained using the standard NCC-mode described in Figure **4a**. This indicates that the reliability of predicting A given just B for non-responding entities depends exclusively on the robustness of the prior defined NCC B given A). To avoid a circular argument, such as $(A \rightarrow B) \rightarrow (B \rightarrow A)$, we here suggest the use of probability statements.

More precisely, we want to estimate the probability of an event A occurring, when it is known that an event

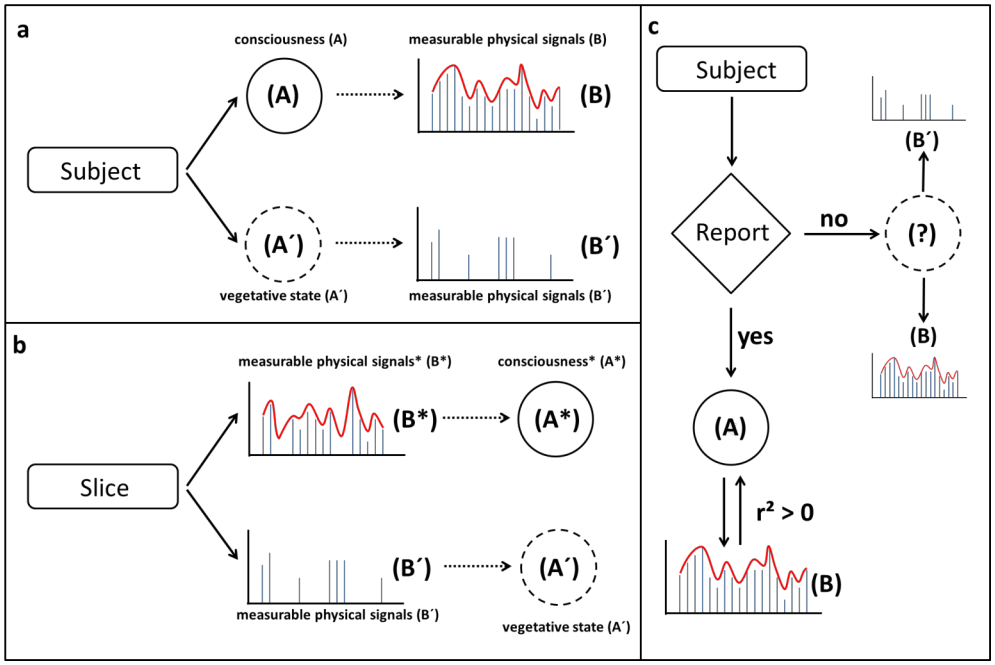

Figure 4: Simplified flowcharts of the basic principles of the NCC-approach. (**a**) Imaging a subject (*e.g.* an awake human being) is asked about its mental state. If there is good reason to assume the presence of some certain forms of consciousness (A), then certain physical signals (B) might be identified and attributed to correlate with A. This defines B as a possible NCC. (**b**) If the entity of investigation is unable to report about its inner mental states (*e.g.* an acute brain slice), the source for scientific statements about possible conscious states (A*) have to be grounded on certain physical signals (B*). (**c**) The slice-problem highlights the problem of reportability in NCC-research. While a system that can report about its inner mental state helps to identify any correlations (r^2) between the physical signals and the conscious states, systems that are unable to report about conscious sates (*e.g.* brain slices, humans in vegetative states, non-human beings of certain physical complexities etc.) are conceptually problematic.

B has occurred. This probability is called a *conditional probability* [7, 80, 81] and is denoted by

$$P(A/B).$$

In our case, this term can be read as "the probability P that a specific conscious state A is occurring, given that a particular physical signal B has already occurred". This conditional probability can, in principle, be calculated using Bayes´ theorem, which can be expressed by the following equation [8, 9]:

$$P(A/B)=\frac{P(B/A)P(A)}{P(B)}.$$

Now imagine a hypothetical experiment on one human subject. Let there be 100 trials so that $N=100.$ Say that in half of these trials, the imaginary subject is conscious, while in the other 50 trials, the tested person is unconscious. Let us also assume that a particular physical signal B has already occurred in 40 of the 50 trials, in which the imaginary subject was conscious. Let us further assume that B also occurred in 10 trials, in which the subject was unconscious, since there still is – as reviewed in the previous section - no neural correlate perfectly correlating with consciousness. These hypothetical assumptions might be denoted and estimated by

$$P(A/B)=\frac{P(B/A)P(A)}{P(B)}=\frac{P(A\cap B)}{P(B)}=\frac{\frac{40}{50}*0.5}{0.5}=\frac{4}{5}$$

so that if B is a predefined NCC-candidate and A a certain conscious state: $P(A/B)>P(A)$.

With this, we can conclude, based upon nothing but the intuitively plausible assumptions made above, that the estimated conditional probability that conscious states occur on the slice, given that specific physical signals occur, is not zero. As a consequence, if an (almost) ideal NCC could be identified, there is a formally valid motivation and justification to use "just" the presence of a neural correlate (NC) to predict the occurrence of a conscious state (C), without the need of actual reportability. Thus, it might be that for every entity an (almost) ideal NCC might bear some predictive power to predict the presence of certain mental states, given certain physical signals; even for entities like humans in unclassified comas or non-

human animals. While this intuition is shared by many neuroscientists, it is now more than a mere intuition. It is a plausible and formally sound prediction, based on elementary probability theory.

6. DISCUSSION

We applied conditional probabilities inferred from Bayes´ theorem to speculate on the likelihood that specific neural activities sustained on an acutely isolated brain slice correlate with some form of conscious experience. Our formal analysis shows that there is a convincing way to predict conscious states from predefined NCC, even for beings unable to report about their inner mental states. Thus, our application can be seen as a potential extension of the NCC-approach. The formal analysis can further be used to highlight and treat certain conceptual obstacles for the practicability of NCC in general.

Reportability is a crucial necessity to identify any potential neural correlate. If a report about an inner mental state can be correlated with certain physical activity, repetition and an increase of the sample size (*e.g.*, include more human participants) may help to correlate, indirectly, this physical activity with the (reported) mental state, as schematically illustrated in Figure **4c**. One minor but noteworthy problem of the NCC approach is that the study design must ensure that neural correlate reports are not mistaken for NCC. A prominent suggested solution to this problem is to determine the neural correlates of stimulus reportability without any prior assumptions about its relationship to consciousness [82] first, before proceeding with the actual NCC studies proper. Nevertheless, subjects who are unable to communicate about their mental states constitute a serious conceptual problem (Figure **4c**).

As stated above, the claustrum is believed to be one of the most likely brain regions to support conscious experience in humans. Can we state whether an acutely isolated brain slice of a mouse containing claustral tissue bears some form of consciousness? Based upon its connectome across various mammals and its activity, the claustrum has been proposed as one of the cornerstones of sensory integration [65, 83, 84]. Accordingly, the claustrum entails extensive large-scale neuronal projections to cortical and subcortical regions [67, 84-87]. Roughly 85% of all claustral neurons are long-range projection neurons in humans with their pyramidal somata evenly distributed throughout the body of the claustrum [88, 89]. These excitatory neurons have spiny dendrites with axons projecting out of the claustrum and have cell body diameters of 15–29 µm. Depending on the angle and plane of the produced brain slice, most of their long-range axons are destroyed during acute brain slicing. It is unknown whether the relatively small somata of these pyramidal cells in a 300 µm thick brain slice die instantly or stay functional – and if they do stay functional, for how long (several seconds, several tens of seconds, or several minutes). However, about 15% of claustral cells have cell body diameters of 10–15 µm, are aspiny, and have axons that do not project outside the body of the claustrum, as evidenced by human and cat studies [88, 90]. Many of these aspiny neurons most likely stay functional following the preparation. Still, the remaining functionality of the claustrum might not fulfill the assumption that a physical signal correlating with conscious states in an alive organism B is sufficiently similar to the measurable physical signals B^* on the acute slice. To answer the question whether consciousness is, at least to some degree present on such a claustral slice, it must be empirically shown that at least some $B \sim B^*$. As soon as there is sufficient empirical data fulfilling this criterion, a conditional probability can be calculated in principle.

One general objection to our considerations might be that consciousness, with all its richness, is a purely human phenomenon. While our present work is mainly focused on the formal structure of a possible statement regarding consciousness in non-responsive beings, rather than giving a concrete answer regarding consciousness on (*e.g.*,) a murine brain slice, we consider it an objection that is to be taken seriously. If we were postulate the above claim as true, then a rodent might not, or not to a sufficient extent, share the quality of human conscious experience. Therefore, a projection of a human consciousness into an acutely sliced rodent brain would be misleading. In answer to this objection, we emphasize that the neural substrates of *e.g.*, emotions do not appear to be confined to cortical structures and that, as the Cambridge Declaration on Consciousness postulates it, “humans are not unique in possessing the neurological substrates that generate consciousness” [91]. In addition, as visualized in Figure **3** most prominent NCC candidates are subcortical brain regions present in both, humans and rodents. Accordingly, in our opinion, there is “compelling evidence for evolutionarily shared primal affective qualia” between mice and man. While the richness and resolution of a visual percept of humans might outperform the spectrum of a mouse’s visual experience, the primal “what it is like-ness” might be

similar. Moreover, the richness and resolution of an odor percept of a mouse might outshine the human capacity to discriminate different aromas. Nevertheless, the "what is it like to smell something" is presumably comparatively similar. Therefore, we assume that while the richness of percepts might differ between mice and man, the inner subjective experience for "what is it like to have the brain sliced acutely" is a matter of degree and not of category. Moreover, the concept of degrees of consciousness accounts, in our opinion, also to humans with reduced, or not yet developed, cognitive capacities, like humans suffering on neurodegenerative diseases, split-brain patients, or even infants.

7. OUTLOOK

While NCC is a potent tool of today's neuroscience of consciousness we highly encourage to further investigate the causal relation between the mind and its body. We highly encourage philosophy and neuroscience to merge together even more tightly in the near future, so that one day we may use neural causation of consciousness instead of neural correlates of consciousness.

ACKNOWLEDGMENTS

We would like to acknowledge Anne and Daniel Mazuga for their intellectual and literary input on this paper, as well as Bruno Benedetti and Christina Kreutzer for their technical support regarding electrophysiology and histology, respectively. Moreover, we would like to thank Sébastien Couillard-Després and Manfred Tscheligi to provide the authors with sufficient intellectual freedom to make this publication possible and Gustav Bernroider for perfect editorial support and his outstanding intellectual encouragement.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

HUMAN AND ANIMAL RIGHTS AND INFORMED CONSENT

No animals were tested or sacrificed for the present study.

ABBREVIATIONS

Neural Correlate of Consciousness (NCC), pyramidal cortical neuron (Pyram. CX), granular cell of the dentate gyrus (Gran. DG), cortical doublecortin reporter positive immature neuron (DCX-CX), doublecortin reporter positive immature granular cell of the dentate gyrus (DCX-DG), NG2 expressing oligodendroglial progenitor cell (NG2), enhanced green fluorescence protein expressing cells, under the doublecortin (DCX) promotor (DCX/EGFP), infrared-dichromatic interference contrast (IR-DIC), membrane resistance (Rmem), resting membrane potential (Em), sodium current (35), potassium current (IK), action potential (AP).

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Received on 20-12-2016 Accepted on 29-12-2016 Published on 12-05-2017

http://dx.doi.org/10.15379/2409-3564.2017.05