Hypothesis: Initiating new paths to study mechanisms of anxiety disorders and chronic pain for effective treatment development.

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Abstract

Prominent scientists classified mental health drugs and its research as a disappointment or even a failure, which lead to the decision of some drug companies to draw away their money from psychiatric drug treatment research, while mental health disorders now claim more lives than automobile accidents each year. Furthermore, because of the expeditious rise in mental health disorders worldwide, the economic health of developing and developed nations will depend on controlling the growth and the costs of mental illnesses. There have been many attempts to explain how we ended up in this situation. Many of these theories boil down to the hypothesis that the lack of the concept of a "self" in research animals means that they can't consciously experience fear, and are therefore not an adequate model to investigate mental health drugs. We hypothesize that the lack of consciousness in animals is not a hurdle to study anxiety and chronic pain mechanisms for effective treatment development, because it is not exclusive to humans. Based on evolutionary data we theorize, that the first step to generate a multicellular organism was introspection and self-awareness. The first multicellular organisms were the biota of the Ediacaran period. They lived in a time when no competition or predators existed, and based on research they show no evidence of interactions with each other, or engagement with their environment. Their challenge was the organization and compartmentalization of different cell functions in order to become a functioning whole organism, which requires inner awareness to perform intentional self-monitoring. Excluding consciousness as an obstacle for effective treatments, we propose that two main problems are not addressed correctly in current research: 1) accurate dissection of acute vs chronic states of disorders, 2) specific to anxiety we need to examine pain-free fear memory in more detail. We propose experiments and mechanisms that should be examined in order to develop novel effective treatments. Addressing the first point we suggest alternative fear-inducing stimuli to examine anxiety disorders mechanisms in rats with and without pain receptor

knockdown (Nav1.7 knockout) after the chronic state of anxiety is established. To address chronic pain under exclusion of acute pain, we propose to first induce chronic pain and after its establishment, one needs to knock down pain receptors such as Nav1.7 to examine mechanisms that became independent of an acute painful input. Additionally, one should examine if pain-free knock out mice have different or no fear memory induced by other anxiety generating stimuli than pain. We propose that one should examine epimutations and identify epigenetic biomarkers for these chronic disorders in which biotechnologies analyzing genome-wide detection of disorder related patterns of DNA methylation and histone modifications should be used. Novel research using epigenetic drugs for mental health disorder treatments are showing promising results. Therefore, designing accurate strategies with novel approaches will enable us to develop more chronic specific, and effective treatments, that will hopefully regain hope, trust and funding for our important undertaking.

Introduction

The World Health Organization's report estimates the global cost of mental health disorders in 2010 was at nearly \$2.5T, which includes the loss of income due to unemployment, expenses for social supports, and indirect costs due to chronic disability that often begins early in life. An increase to over \$6T by 2030 is projected. The economic health of developing and developed nations will depend on controlling the growth in costs from mental illnesses. Even worse is, that mental health disorders now claim more lives than automobile accidents each year and leaders in the mental health field believe that our mental health drugs and its research turned out to be a disappointment or even a failure. Adding to this predicament, some drug companies already started to draw away their money from psychiatric drug treatment research, because they see no future in novel profitable drug treatments.

There is a trend to explain the lack of effectiveness of current mental health drugs such as anxiolytics, with the construct of consciousness, which hypothesizes being exclusive to humans. Consciousness is discussed by numerous philosophers and people throughout human history. One of the most well-known expressions on this topic is "*Cogito, ergo sum*" by René Descartes, which is translated into English as "*I think, therefore I am*". Descartes wrote this phrase in his work "*Discourse on the Method*" and

in his later work "Principles of Philosophy". Descartes himself explained, "*we cannot doubt of our existence while we doubt....*"¹.

There are many more definitions of consciousness, but we will use this term as an organism being in an awake state experiencing an inner awareness, meaning that one is aware of its own representation and performs intentional self-monitoring and evaluation, which is defined as introspection². The definition of "self" is to be consciously aware of one's self. Many claim that the only organisms that are able to be conscious are humans. To learn about ourselves we would like to go back in time and look into how we became multicellular organisms.

The first multicellular organisms were the biota of the Ediacaran period. Back then no competition existed. This way of life of abundance and lack of predators lasted for about 300 million years till the Cambrian time. Mark McMenamin named it "The Garden of Ediacara"³. Researchers couldn't find fossils with sensory input systems such as light receptors. As far as we know, they didn't interact with each other and they show no evidence of engagement with their environment, which means that the first information transmission ("nervous") system was for controlling internal coordination, and not sensory-motor control⁴. Therefore, the sensory-motor system came after the system of controlling internal coordination. The nervous system was originally built for self-coordination, and not to interact with the environment. The main challenge was the organism's inner awareness in order to perform intentional self-monitoring and evaluation to organize and define compartmentalization of different cell functions to become a functioning whole organism. Only the rise of predators introduced the necessity of automatized introspection integration to create a fast and efficient response mechanism for survival, that lead to reinforcement of increasingly complex and distinct strategies, which evolved to the diversity and even to the evolution of culture we live in today. To summarize, our conclusion is that conscious self-awareness was the first multicellular organism's feature and should therefore not represent an obstacle for studying anxiety in other organisms than humans.

Another way to describe consciousness is the theory of hysteresis, which was first described in the visual system but named differently "perceptual moment" in 1860⁵ where a flaming torch was swung rapidly showing, that there must be a duration or persistence of visual images that leads to a continuing overlapping of successive perceptual moments

to give a sense of continuity. This is translated into consciousness in general. Various disorders are a too high or too low excitability which leads to abnormal levels of hysteresis⁶. This could be due to malfunction of the inhibitory system and/or intrinsic neuronal modulations of excitability which leads to too low or too high/long upstates introducing aberrant synaptic plasticity modulating and/or influenced by epigenetic mechanisms. This mechanism is also not exclusive to humans.

Adding to our current view of consciousness, Jackson wrote in 1879 about his theory of hierarchical levels in the brain through his observation of aphasia and Freud expanded it to speech⁷. Sometimes patients show regressions of emerging primitive emotional speech. Freud's view of systems rather than isolated centers was very progressive. It was important for the notion of functional systems developed by Lucia 50 years later⁷. The more complex the organism and its culture is, (which enforces each other into developing more complexity) the more hierarchical levels are needed and adding layers just means increasing integration complexity, however it does not mean that an organism with less layers is less or not conscious.

Now let's focus on conscious anxiety, which is claimed to be a human only feature, and mostly defined by the prediction of a threat that is not currently present, and acting as if it would. Many claim that this is not possible to study in animals, because they don't show any sign of having conscious anxiety. We claim, that there is vast evidence that animals make predictions of a future possible threat and behave upon that prediction, and that without this feature it would be almost impossible to survive in an environment full of predators and other threats. There are numerous examples of birds and other animals learning to escape from predators throughout life⁸⁻¹¹, and one example even includes possibly social learning of emotions. When rats eat something that makes them feel noxious or even poisonous they will warn each other with cues (marking the source with a threat related fragrance) for others to not eat the threatening food¹²⁻¹⁴. This requires analysis of a passed or current threat and its integration in order to make future predictions to perform a behavior for future threat prevention not just for itself, but also for others. This behavior doesn't just show a conscious anxiety induced behavior, but it also shows that a rat knows and distinguished its own self from others and knows that it plays its own individual role in a form of culture. There is no sign that this behavior is inherited and preprogrammed, furthermore, the fast learning of threats and its behavioral

adaptation is the reason why we have such difficulties to keep rats at bay as pests around the world. If this behavior was an inherited program it would be inflexible and rats wouldn't be able to strive in such a diverse way around the globe.

Throughout history, especially in medicine, progress only happened by demystification and rationalization, which has to also be applied to our own mind works, since we are part of the natural system only in a more elaborate way. This is true for every organ in our body, which includes the brain.

In order to develop treatments that actually help people affected by mental health disorders, we need to not just examine underlying mechanisms, but also define what we want to achieve. In the case of anxiety disorders, we shouldn't erase trauma or a fearful memory as performed in animals ¹⁵. Who are we to know if this specific life experience is something one should extinguish? Also modifying a traumatic memory into something rewarding and changing a person's value system is highly unethical ¹⁶. The only solution is to help a person to cope and enforce resilience. Already Dr. Oliver Sacks stated that in response to stressors, people go through a phase of adaptation and reorganization, often mobilizing inner resources that have previously lain dormant, and that it is the physician's task to help patients to achieve a new order by being sensitive to these altered orientations.

Additionally, we need to design research that examines accurately the chronic characteristics of anxiety disorders and chronic pain. The transition from acute to chronic disorders appear to occur in discrete pathophysiological and histopathological steps. In a chronic disorder the stimuli that first initiated a response vary, which at some point leads to a defense mechanism that stays active and inflexible regardless of the insult's presence or not.

We first address the research of underlying anxiety mechanisms conundrum. From our standpoint one of the biggest problems is, that the prevalent used anxiety eliciting paradigm for its research is pain based (Pavlovian fear conditioning) ¹⁷. However, a vast number of anxiety disorders are not pain related at all (social anxiety, all kinds of phobia, etc) ¹⁸. There is abundant evidence that one can successfully combine Pavlovian fear conditioning with other stimuli than pain, for example a noxious stimulus ¹⁹. Therefore, we are suggesting to consider alternative fear inducing stimuli to examine anxiety disorders mechanisms to develop effective treatments. Additionally, we propose

experiments in rats with and without pain receptor knockdown (Nav1.7 knockout ²⁰). Knocking down pain receptors is another way to make sure one studies pain unrelated fear mechanisms and its circuitry. Additionally, one should examine if pain free knock out mice have different or no fear memory induced by other anxiety generating stimuli than pain:

1. Response to predator through smell or virtual reality
2. CO2 induced asphyxiation
3. Social stress through dominant aggressive rat with no escape for weaker rat.

In order to develop a treatment that addresses chronic pain, and not just acute pain one needs to exclude acute pain transmission. Therefore, we propose to initially induce pain and after the establishment of its chronic state, one should knock down pain receptors such as Nav1.7 in order to narrow down and study mechanisms involved in chronic pain that became independent of an acute or initially induced painful input.

Suggestions of underlying mechanisms

In general, it is of existential importance to establish the specific roles of distinct brain regions involved in anxiety and chronic pain, but for treatments it is currently not feasible to modulate those brain region's activity specifically in every patient with anxiety disorders or chronic pain for many reasons (examples: deep brain stimulation involves surgery, or the use of other inpatient devices such as transcranial magnetic stimulation ²¹). As a result, we focus in this paper on mechanisms that could be targeted specifically, however, ameliorated with a systemic applied treatment.

One theory regarding anxiety disorders and chronic pain is, that a memory takes over the system and overpowers other memories chronically. A recent study showed that Arc is involved in the inhibition of competing memories ²². The authors established that the Hebbian strengthening of activated synapses and hetero-synaptic weakening of adjacent synapses was performed by AMPA receptor redistribution via targeted expression of Arc. Therefore, we suggest that over activity of Arc would be a possible mechanism in anxiety disorders, in which fear related memories would overwhelm the circuitry. Arc is of viral origin and its activity (like transposons) are most likely regulated by epigenetic

mechanisms such as methylation. Temporary (while recalling anxiety eliciting memory) or long term downregulation (chronic and severe anxiety disorder cases) of Arc expression would be a mechanism one should consider for further investigation. Epigenetic mechanisms in general were shown to contribute to plasticity in neurons and gene expression regulation of memory related genes such as *PP1*²³⁻²⁵, which argues for epigenetic drug exploration as anxiolytic and chronic pain treatment agents. In fact, recent research started to utilize epigenetic drugs from cancer treatments for mental health disorders in animals, and their results seem to be very promising²⁶⁻²⁸. The following part of the paper discusses possible epigenetic mechanism targets one should investigate further for future treatment development:

HDAC has been shown to enhance memory and synaptic plasticity via CREB:CBP-dependent signalling²⁹. A recent study reported that suppression of HDAC3 function in the dorsolateral striatum accelerates habit formation whereas HDAC3 overexpression prevents it³⁰. HDAC3 is a class I HDAC, which is highly expressed in the brain and believed to be enriched at learning-related and CREB-regulated gene promoters^{31,32}. Contrarily, CREB phosphorylation by *PP1* was shown to decrease CREB activity³³. Data on DNMT and HDAC inhibition and *PP1* activity proposes the opposite effects of DNA demethylation and histone acetylation on the CREB:CBP-dependent pathway, which is known to be involved in neuroplasticity. *PP1* and CREB:CBP may represent a key nexus of epigenetic regulation of neuroplasticity^{23-25,34,35}.

Both DNMT and TET enzymes are constantly expressed in adult neurons, and maintain an balanced level of methylation (dynamic steady state), that is hypothesized to be required to maintain synaptic functions³⁶. Interruption of a methylation steady state through interference with either DNMT (methylation) or TET (hydroxymethylation and demethylation) has been recorded to induce the opposite mechanism by probably activating neuroplasticity/synaptic changes via CREB:CBP-dependent pathway³⁷. Further studies targeting either specific DNMTs enzymes (via more specific inhibitors) or the expression of their genes (e.g. using miRNAs) in the context of anxiety and chronic pain may help clarify the involvement of specific DNMT subtypes and suggest improves therapeutic targets.

Prior studies of TET activity in adult neurons suggest that the loss of methylation following DNMT inhibition is likely produced by an increase in hydroxymethylation by TET1 and/or TET3, subtypes recorded to play a role in adult neuroplasticity. Future research involving dynamic overexpression or repression of specific TET enzymes would elucidate its role and therapeutic potential in anxiety and chronic pain.

Modification of the mTOR pathway via epigenetic mechanisms implicating DNA methylation and histone modifications was pointed out to be involved in memory reconsolidation³⁸. Research showed that memory retrieval was associated with transient suppression of mTOR pathway (activation of autophagy) while the subsequent reconsolidation was linked to mTOR pathway activation (suppression of autophagy), involving epigenetic regulatory changes³⁹. Epigenetically influenced fluctuations in synaptic strength during memory retrieval and consolidation may have a role in anxiety disorders and chronic pain.

DNA methylation changes in anxiety disorders and chronic pain are likely to engage methyl-CpG-binding protein 2 (MeCP2) as one of the regulatory mediators. MeCP2 is a regulatory factor that binds to common DNA methylation site CpG, that in conversly affects the expression of multiple genes. MeCP2 play an essential role in maintenance of mature adult neurons and modulates both synaptic development and function. Loss-of-function mutations of MeCP2 lead to Rett Syndrome, a neurodevelopmental disorder in humans, and impaired memory/learning and low synaptic density in mammals⁴⁰⁻⁴⁴.

MeCP2 seems to modify the inducibility of immediate early genes (IEG) related to synaptic plasticity, that was recorded in the striatum for *junb*⁴⁵. The effects of methylation and MeCP2 on Arc expression are specially important, because Arc was shown to be involved in complex regulation of various forms of neuroplasticity, including LTP, LTD and synaptic scaling^{46,47}. Recent work showed the capacity of Arc protein to transport different payloads, such as RNA, between neighboring dendrites and neurons⁴⁸. Arc proteins assemble into carrier particles analogous to a viral capsid which can be transported in and out of neurons via endo/exocytosis. The characteristics of the cargo, co-factor binding and other incompletely understood factors seem to determine the Arc's role in different types of plasticity. The inter-neural transport mechanism facilitated by Arc

is consistent with Hebbian cooperativity characteristic of both LTP and LTD. Epigenetic control of Arc-dependent pathways appears to be a promising avenue of future research.

An alternative avenue one should consider is immune response, which has been suggested to be a contributing factor to mental health disorders ⁴⁹. However, only recently a study showed a direct mechanisms of meningeal immunity influencing behavior, such as spatial learning and memory ⁵⁰. Furthermore, the authors analyzed brain and cellular transcriptomes in response to T-cell-derived cytokines, which suggested an interaction between social behavior and interferon- γ (IFN- γ)-driven responses. IFN- γ increased GABAergic (γ -aminobutyric-acid) currents in projection neurons, proposing that IFN- γ is a molecular link between meningeal immunity and neural circuits activity involved in social behavior. Aberrant immune responses lead to a self-inflicted isolation mechanism, which is a common symptom in mental health disorders such as anxiety, depression, which influences chronic pain development probability and its impact levels ⁵¹. Another contributing factor seem to be zinc levels, which were observed to influence IFN- γ production and therefore may affect cell-mediated immunity adversely. Furthermore, zinc has been shown to act as an anti-oxidant and to be involved in membrane stabilization suggesting a role in prevention of free radical-induced damaging especially during inflammatory processes ⁵². Additionally, it is known that many environmental stressors such as pollution can lead to a chronic immune response dysregulation especially affected during development ⁵³⁻⁵⁷. Underlying affected mechanisms induced by the described factors could be DNA methylation, which control key aspects of immune responses regulating peripheral immune system effector cell development, in which especially DNMT1 and DNMT3b seem to play a major role specially during development ⁵⁸. These mechanisms could explain an important part of the observed increase of mental health disorders in developed industrial countries ⁵⁹⁻⁶¹. We therefore suggest, that regulation of immune response such as expression levels of IFN- γ should be further studied.

Future studies should examine epimutations and identify epigenetic biomarkers for psychiatric diseases. Biotechnologies analyzing genome-wide detection of disorder related patterns of DNA methylation and histone modifications should be used to detect further targets for treatment development. Our technology development will allow us to

perform faster, more accurate, and affordable study of epigenetic mechanisms. These studies will enable us to develop more disorder specific, effective therapies affecting targeted the brain that can help restore function damaged connectivity.

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