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On the Cover

This year's cover features the indoor bamboo garden in the Terrence Donnelly Centre for Cellular and Biomolecular Research at the University of Toronto. The Donnelly Centre attracts scientists from all over the world to pursue biomedical research using genomic technologies in a collaborative, interdisciplinary, and open-concept environment.

Since opening its doors in 2005, the Donnelly Centre has been the home of many groundbreaking scientific achievements, including determining the genetic landscape of a cell, producing the first splicing map, and decoding how genes are turned on and off.

Staff List

Letter *from the* Editors

Dear Reader,

It is with great pleasure that we present to you the 12th issue of the Journal of Undergraduate Life Sciences (JULS). Once again, we are proud to publish outstanding research conducted by bright and dedicated undergraduate students in the life sciences. JULS exists to inspire and encourage students to pursue research, and we sincerely hope that you enjoy reading this issue.

This year, our cover features the indoor bamboo garden in the Terrence Donnelly Centre at the University of Toronto. Amid innovative and groundbreaking research, the lush green foliage that surrounds the garden benches welcomes its visitors, offering them peace and tranquility. Here, students enjoy a meditative moment with their morning coffee, a quick lunch in between classes and labs, and a respite from a busy city and an even busier school life. Ultimately, the garden energizes us to continue learning and pushing the boundaries of human knowledge.

In this issue, we have featured an interview with Emily Nicolas Angl, who works in the field of patient-oriented health research (POHR). POHR is an important initiative within our health care system that aims to engage "patients as partners" in the research process, from the conception of a research question to the dissemination and integration of the results. By engaging patients as active members, research is often more credible, transparent, applicable, and easily disseminated. Canada is a pioneer in POHR, and many countries around the world are adopting similar strategies. With this interview, we hope that our readers will recognize and be encouraged by the many unique and exciting career paths that exist in research.

Like scientific study, the production of this issue was a group effort. As we conclude our time as editors-in-chief, we would like to extend our utmost gratitude and appreciation for our staff, faculty reviewers, sponsors, and undergraduate researchers for their help in the production of this issue. Thank you, this journal would not be possible without your continued support.

Sincerely,

Iva Avramov and Linwen Huang
Co-Editors-in-Chief, 2017-2018

Improving medication adherence using KARIE, an electronic medication delivery device

Stella Bing Xin Song[1], Hanatu Tak[1]

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Canadians spend over $30 billion every year on prescription drugs for treating, managing, and preventing disease (1). Many people have multiple prescriptions for several chronic diseases, and adhering to their medication schedules often becomes difficult to manage (1). Medical adherence in long-term therapy is the extent to which a person's behavior—taking medication, following a diet, and executing lifestyle changes, for example—corresponds with agreed recommendations from a health care provider (2). Patients are considered "adherent" if their medical adherence percentage is greater than 80 per cent (3). Regarding prescription drugs, non-adherence is a serious issue because it can lead to the mismanagement of chronic disease; an increased risk of adverse drug reactions; higher rates of physician consultations, hospitalizations, and emergency room visits; increased health care costs; and decreased quality of life (4-6).

A systematic review from 2014 found that electronic medication packaging (EMP) interventions, which dispense drugs and monitor consumption, were associated with an increase in medication adherence (7). Over the summer of 2017, we helped conduct a pilot study led by Dr. Pascal Tyrrell at the University of Toronto to assess the feasibility of KARIE, a medication delivery device designed and produced by AceAge, as an intervention for improving medication adherence (Figure 1).

In this randomized, cross-over study using omega-3 supplements, medication adherence data was collected from ten students at the University of Toronto for six weeks. There was a three-week intervention period using KARIE and a three-week control period using the original bottle packaging. During the intervention period, the device rang and flashed to remind participants to take the supplements, and it also sent an email reminder 30 minutes after a missed dose. During the control period, participants took the supplements from their original packaging. Medication adherence was recorded by KARIE's electronic log of dispensed doses during the intervention period and the participants' weekly self-report recall questionnaires during the control period.

Figure 1: The KARIE automatic drug dispenser, an electronic medication delivery device.

The results showed that with an 80 per cent threshold for successful adherence, only two out of ten participants were considered to have been adherent during the control phase. During the intervention phase, however, eight out of ten participants were adherent. This amounted to a 300 per cent increase in adherence (Figure 2). Indeed, most of the participants found the device easy to use and effective in reminding them to take their supplements.

In the fall of 2017, the Centre for Aging and Brain Health Innovation (CABHI) invested $8.3 million in senior care improvement projects, which included AceAge's KARIE under its Industry Innovation Partnership Program (I2P2). In the spring of 2018, KARIE will undergo a randomized control study with 300 elderly participants at two sites: Westpark Healthcare Centre in Toronto, Ontario and Capital Care Group in Edmonton, Alberta. This study aims to determine factors affecting the usability of KARIE, its perceived usefulness by the participants, and whether it is associated with improved medication adherence (8).

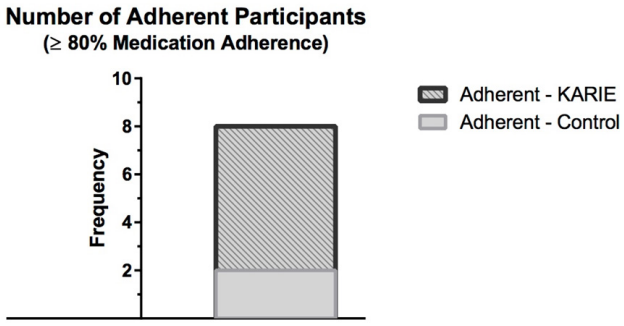

Figure 2: Number of adherent patient. Two out of ten participants in the control phase had ≥80 per cent medication adherence, while eight out of ten participants during the KARIE phase had ≥80 per cent medication adherence.

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Changing seasons and circadian rhythms: e“miR”ging roles of miR-132/212

Samuel Fung[1], Zobia Anwer[1], Nick Lowe[1]

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The human body is not designed to forgo sleep. The record for continued wakefulness is 11 days and 25 minutes—the equivalent of binge-watching the Titanic 81 times! Luckily, we have evolved intrinsic time-keeping mechanisms that anticipate day-night cycles. Multiple transcription-translation feedback loops (TTFLs) function ubiquitously to regulate circadian rhythms (CRs) in the 20,000 neurons of the suprachiasmatic nucleus (SCN), a central mammalian brain structure that is the central CR oscillator [1]. The SCN receives daily optic light inputs, adjusts individual neuronal oscillation phases, and orchestrates CRs globally by sending temporal information to peripheral tissues and organs [1,2].

Seasonal variations in the length of days, known as the photoperiod, can profoundly affect animal behaviour and physiology. However, the mechanisms behind SCN seasonal adaptations remain to be explored [3]. Recently, Dr. Hai-Ying (Mary) Cheng's laboratory at the University of Toronto discovered that day length variations change the SCN's timing mechanisms and neuronal morphology [3].

Dr. Cheng's laboratory discovered that the conditional knockout (cKO) of *miR-132/212*, a non-coding microRNA gene cluster that regulates post-transcriptional gene expression, impacts SCN dendrite spine morphology and proteomic landscape [3]. They also found that cKO mice adapt faster to shorter "winter" days and adapt slower to longer "summer" days, compared to wild-type mice expressing *miR-132/212* [3]. SCN expression of P*ERIOD2*, a key CR regulator, is noticeably enhanced in cKO mice during shorter days and remains tightly synchronized during longer days, unlike wild-type mice [3]. The authors suggest that deletion of *miR-132/212* changes CR regulation when manipulating day length [3].

SCN responses to environmental changes, such as crossing time zones, depend on neuronal network properties rather than individual neuron behaviour [3]. With new experiences, neuronal networks exhibit functional and structural plasticity [3]. Quantitative mass spectrometry revealed an attenuated time of day-dependent protein expression within the SCN of cKO mice [3]. Deletion of *miR-132/212* affected a group of proteins regulating cytoskeletal organization, which suggested abnormal neuronal morphology in the SCN [3]. Using morphometric analysis, the authors demonstrated that dendrite spine density in the SCN was significantly reduced in cKO mice [3]. They confirmed the link between SCN seasonal adaptation and *miR-132/212* expression using Syrian hamsters [3]. Here, *miR-132/212* levels and dendritic spine density varied according to the photoperiod [3]. Compared to longer days, shorter days elicited a decrease in *miR-132/212* levels and dendritic spine density [3].

Surprisingly, the authors found that knocking out another gene, *MeCP2*, reversed the effects of *miR-132/212* ablation by rescuing cKO dendrite spine density to levels observed in wild-type neurons [3]. The authors suggested that *miR-132/212* affected dendritic morphology by regulating *MeCP2* expression [3]. *MeCP2* is a genetic factor for autism and the causative gene for Rett syndrome, a neurodevelopmental disorder that affects females [3]. Interestingly, both disorders are characterized by strong circadian disruptions [3]. How the *miR-132/212-MeCP2* pathway contributes to circadian disturbances in these diseases remains unknown [3].

Dr. Cheng's laboratory elucidated the novel role of *miR-132/212* encoding day length information by modulating SCN neuronal architecture [3]. The findings of this research raise the possibility that structural neuronal changes strongly impact SCN plasticity across seasons [3]. This discovery sheds light on the roles of specific genes in SCN function, which may further illuminate potential therapeutics for sleep-related disorders [3].

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Ethical and moral concerns regarding artificial intelligence in law and medicine

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[1]University of Toronto, Canada

The seminar AI in Medicine in Context: Hopes? Nightmares? was held at the Centre for Ethics at the University of Toronto on October 17th, 2017, with special guest Dr. Sunit Das. Dr. Das is a scientist at the Keenan Research Centre for Biomedical Science, a neurosurgeon at St. Michael's Hospital, and an assistant professor in the Department of Surgery at the University of Toronto. Dr. Das discussed ethical questions and concerns regarding the impact of artificial intelligence (AI) in law and medicine. An especially interesting topic that he discussed was how AI may fail to make the most ethical decision, which may result in an undesirable outcome that negatively affects a person's life.

In the seminar, Dr. Das discussed an interesting case from the United States, where AI was used in the legal system with harmful results. AI was used in a court case to determine the length of an accused person's sentence, and recommended that the accused serve more time in prison. A lawyer challenged this decision and conducted his own investigation into the AI. He discovered that the AI recommended sentences that depended on the accused's race, which was prejudicial and racist. This is a serious issue: how can the legal system recognize when AI is using racial and socio-economic factors in sentencing decisions, rather than past criminal involvements and activities? As we have seen in this case, the AI made an unethical decision that would have kept the accused person incarcerated for additional years of his life because of his race.

Seeing as AI made the wrong decision in the legal field, it is not far-fetched to imagine it making unethical decisions in a medical context as well. Indeed, Dr. Das argued that such decisions would increase current racial and gender disparities in our health care system. If AI analyzed biased data, which exist in scientific research, then it could potentially make an unethical decision because it would not be able to distinguish wrong from right.

To prevent such issues from occurring in legal and medical fields, ethical consideration and regulation of AI is needed. AI should be developed and implemented gradually to ensure that mistakes such as analyzing biased data are avoided. Dr. Das concluded that even if AI is more accurate in empirical decision-making, it is not always the case that AI is the ethical and moral way forward. For this reason, it must be carefully used.

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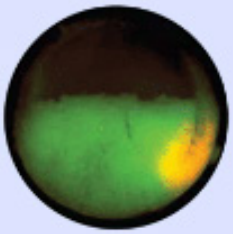

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JULS RESEARCH

Investigating perceptual grouping by common region through a repetition discrimination task

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[1]University of Toronto, Canada

Abstract

Visual perception is critical in helping people understand and infer states of the world in which they live. The Gestalt law of common region describes how elements belonging in the same region tend to be perceptually grouped together pre-attentively. An important feat in vision is the ability to organize an unstructured world into more coherent groups of objects. Recent evidence suggests that perceptual grouping occurs not only from visual similarity, but also from associative similarity. More specifically, visually dissimilar shapes are perceptually grouped if they have a learned association.

This study investigated how effectively perceptual grouping can form through visual statistical learning. Participants performed a repetition discrimination task (RDT), a type of visual task, on the computer and responded with keyboard presses.

Standard perceptual grouping was found in the training phase as participants had significantly faster discrimination times when the target shapes in the visual search row were grouped by common region (within-group condition) compared to shapes from an equidistant but different region (between-group condition). The perceptual grouping effect did not replicate in a transfer phase, where common region grouping was removed but shape associations remained. Finally, there were no significant differences in discrimination time between within-group and between-group conditions. This study showed that grouping of objects through visual cues did not lead to strong learning of associative similarity.

Introduction

A seemingly simple task such as searching for lost keys requires the coordination of multiple attentional processes that work together simultaneously without conscious awareness. Research into visual cognition has begun to elucidate how prioritizing one's attention to a specific location or to groups of features that share a common region affect our perception of the objects in the visual environment. Broadly, visual processes can be categorized as either bottom-up or top-down. Bottom-up processes refer to the automatic capture of attention by salient information in the visual environment. Top-down processes refer to how attention is preferentially guided to certain visual information based on prior experiences, goals, and knowledge.

Gestalt psychologists tried to explain how we process visual information by proposing that only bottom-up processing matters in perception. They elaborated that our perception of objects and scenes is governed by the inherent visual properties of the scenes themselves, such as the enhanced attention to objects in common region [1]. According to them, perception of objects was mostly independent of our previous learning experience (top-down processing) with our visual environment. However, accumulating evidence suggests that prior non-visual experience with visual structures affect our perception of objects, which is not explained by Gestalt laws. Indeed, non-visual factors such as hand position can influence perceptual grouping [2].

Studies by Beck and Palmer (2002) showed that perception of groups is influenced by top-down cognitive states [3]. They demonstrated this by varying the probability that the target pair appeared in a common region within an oval (within-group) [3]. After indicating the likelihood of the target appearing in a grouped region before each condition, participants became progressively faster at target discrimination in within-group conditions as the probability of the target appearing within common region increased [3]. The degree to which perceptual grouping and object-based attention occurred was influenced by top down processes instead of solely bottom-up features of Gestalt visual elements [3].

Similarly, studies by Vickery and Jiang (2009) provided more evidence that perception can be influenced by prior experience and statistical visual learning [4]. A repetition discrimination task (RDT) was used to demonstrate that participants can unintentionally group distinct shape pairs together due to their history of having appeared in a common region, even when this associative grouping is not helpful for the main task of locating a color repetition [4]. In a new context (transfer phase) without the common-region grouping of the same shape pairs, participants had a within-group advantage presumably based solely on the co-occurrence of shape

pairs [4]. The RDT was previously used by Palmer and Beck (2007) and Vickery (2008), where they successfully showed within-group advantage due to common region and maintenance of the pair associations in the absence of Gestalt features [5,6]. Interestingly, young infants did not show transfer of grouping effect due to common region [7]. The authors predicted this was because grouping through common region is a weak cue for association. However, the presence of associative learning through common region in older adults in other studies indicates that perceptual grouping is not solely due to the intrinsic properties of objects, but is also learned through experience.

More recent studies suggest that it is possible to learn associations between novel shapes through passive visual statistical learning without using common region cues to help with associations in the training phase. Zhao et al. (2014) demonstrated that the co-occurrence of novel shapes, learned only through passive viewing of those shape scenes, was enough to induce perceptual grouping [8]. This indicates that Gestalt features are not required even for initial learning of associations between objects. Together, these studies show that cognitive factors, like associative memory and top-down sets, can determine what constitutes a perceptual group.

While a number of studies have hinted at the possibility of association-based perceptual grouping, the critical factors controlling this ability remain poorly understood. This study investigated how prior experience of Gestalt grouping and statistical co-occurrence alters perceptual grouping. The strength of the learned perceptual grouping, if any, was tested in a new scene without common region, similar to the study by Vickery and Jiang (2009) [4]. Participants were exposed to pairs of shapes, some of which were always grouped by common region marked by rectangular outlines. They performed an RDT to detect the repetition of a feature that was different from the grouping features. Unlike the task of locating a single-color repetition overlaid in a scene with shape pairs in Vickery and Jiang (2009), this study used letter repetition as search targets while exposing participants to shape pairs using common region. Successful replication of perceptual grouping effects in a scene without grouping cues would further reveal that consistent and strong associations can produce grouping in the absence of Gestalt cues. This strengthens the conclusion that distinct shapes are perceptually treated as one whole object due to previous experience.

Methods

Experiment 1

Participants completed this experiment on a computer in a darkened room. The monitor was located 20in away from the chin rest and displayed the experiment trials on a light grey background. The experiment was programmed using MATLAB. Participants responded using a keyboard for all trials.

Twenty students from the University of Toronto volunteered to complete this experiment for a course credit. All participants reported normal or corrected to normal vision and were naïve to the study. Participants were briefed prior to the experiment on the key selection corresponding to each response.

This experiment had two phases, each with 360 trials. In the first phase, or the training phase, eight black shapes were displayed in one row on the screen from a pool of six different novel shapes. Shape pairs were bordered with rectangular groups that defined the border for common region. All eight shapes were evenly distributed on the screen. In each trial, there were either three or four pairs displayed. The pair number displayed was evenly counterbalanced. In the trial with three pairs, two shapes at both ends were unpaired and were not contained in a rectangle (Figure 1). The trial with four pairs had all eight shapes contained in four rectangles, with two shapes in each rectangle. For instance, if each letter represented a distinct shape, and the closed bracket representing rectangle groups, the pairs would be arranged similar to the following sequence: [A B][C D][E F]. Pairs were selected randomly with the constraint that the same pair could not appear twice in a row (e.g., [A B][A B] was not possible). In the second phase, or the transfer phase, the display and the task was identical to the training phase but without the rectangles bordering the pairs of shapes. The shape pairs from the training phase still co-occurred in the transfer phase: A B C D E F (Figure 2).

In both phases the shapes contained the letter "X" or the letter "O" at the center of the figures. In a given trial, there was one instance where

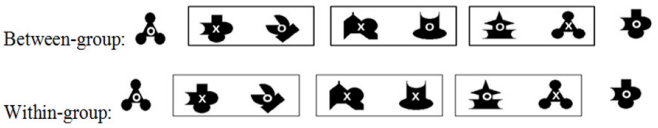

Figure 1: Example learning (Training) trials in Experiment 1 with seven shapes and three pairs.

Figure 2: Example test (Transfer) trials in Experiement 1 with seven shapes and three pairs.

two adjacent shapes had the same labeled letter. The single letter repetition could occur on the pair inside the rectangle (within-group condition), or between rectangle groups (between-group condition). The repeated letter could either be "X" or "O" appearing side by side on any position in the row. The identity of the target letter as well as the type of grouping (within- or between-group condition), were counterbalanced.

The participant's task was to select the "z" key if the adjacent repeating letter in a given trial was "X", or the "/" key if the repeating letter was "O". The display screen stayed on until the participants made their selection for each trial. The monitor displayed the message "Correct" if participants made the right selection, and "Incorrect" if they did not. A new trial began after the message. The MATLAB program recorded the position of the repetition, participants' accuracy and their reaction times.

Experiment 2

In this experiment, the same set-up and apparatus was used. This experiment replicated the main procedures of the first one, using eleven shapes on the screen instead of eight shapes, out of the same pool of six different shapes. There were always five pairs of rectangle groups in each trial, and one shape on the left or the right end was outside a rectangle without a pair. In a given trial, only four different shapes (two possible pairs) were used. The pairs alternated in position such that every other rectangle contained the same shape pairs. For instance, if each of the following letters were distinct shapes and the closed brackets were rectangles the display was arranged this order: D [A B][C D][A B][C D][A B] (Figure 3). Similar to the first experiment, the transfer phase was identical to the training phase with the exception of rectangular groups. The shape pairs

from the training phase still co-occurred in the transfer phase: D A B C D A B C D A B. The task and the key selection were identical to the first experiment.

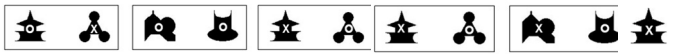

Figure 3: Example within-group trial of the training phase in Experiment 2.

Results

In the training phase of the first experiment, when discriminating and responding to the correct repeating letter, the participants had significantly faster mean reaction times ($t(19)=2.962$, $p<0.008$) when the repetition occurred in shapes within the rectangle group pairs compared to when the repetition was between groups (Figure 4A). However, in the transfer phase, the within-group advantage disappeared ($t(19)=0.898$, $p=0.38$) and reaction times to repetitions within groups were not significantly different from the reaction times to repetitions between groups. Therefore, even though the letter repetition occurred on shapes that were previously grouped in a common rectangular region during training, the participants did not show perceptual grouping of those shapes.

In the training phase of the second experiment, when discriminating and responding to the correct repeating letter, the participants had significantly faster mean reaction times ($t(19)=3.273$, $p<0.004$) when the repetition occurred in shapes within the rectangle group pairs compared to when the repetition was between groups (Figure 4B). Similar to the first experiment, in the transfer phase the within group advantage disappeared ($t(19)=1.786$, $p=0.09$) and reaction times to repetitions within groups were not significantly different from the reaction times to repetitions between groups.

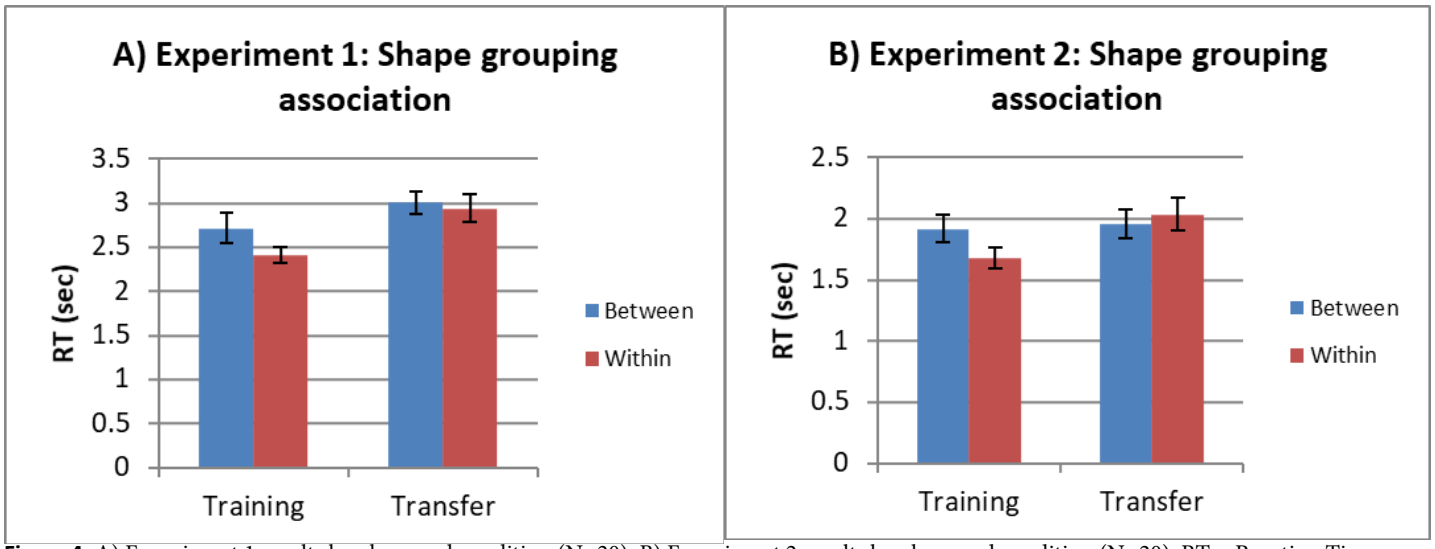

Figure 4. A) Experiment 1 results by phase and condition (N=20). B) Experiment 2 results by phase and condition (N=20). RT = Reaction Time.

Discussion

In order to understand how we perceive the world, it is critical to understand what influences automatic identification of patterns in a scene and how these perceptual associations can help interpret future scenes. Several studies challenge the assumptions made by Gestalt laws stating that our perception depends on the pre-existing intrinsic characteristics of our visual scenes [4]. However, the results from this study suggest that bottom-up features of objects during grouping induce a stronger effect in the training phase than top-down features in the transfer phase.

The first experiment did not show learned perceptual grouping after participants were passively exposed to shape pairs through common region. As expected in the training phase, the letter repetition was detected faster when they were on the shapes within the rectangle. However, the purpose of the experiment was to investigate whether this effect can be transferred and replicated without needing visual grouping cues. Since the participants did not discriminate the within-group shapes differently from the between-group shapes in the absence of visual cues, the distinct objects were not associated together as strongly. This might be due to the fact that learning to group by common region is a relatively weak visual cue for association compared to other Gestalt grouping features, such as grouping through similarity [7].

However, this experiment was also different from Vickery and Jiang (2009) in several other ways [4]. For example, they had 16 shapes on display, and used only two shape pairs on any given trial, potentially making the associations more salient. The second experiment was adjusted to better match Vickery and Jiang (2009) along these lines [4].

The second experiment investigated the effect of increasing the set size and the regularity of grouping patterns on the ability to make associations between shapes. Similar to Vickery and Jiang (2009), the grouped pairs alternated position and there were only two different pairs in a trial at a time [4]. It was predicted that due to these changes, the co-occurrence of shape pairs would be more apparent than it was in the first experiment. However, these changes to the second experiment did not appear to produce learned grouping by association. The difference in reaction time between within- and between-groups in the training phase was also unexpectedly trending towards a between-group advantage, as shown in Figure 3. Therefore, having a predictable display organization along with larger set size did not produce learned grouping.

The lack of grouping by common region in the transfer phase could be due to the type of task irrelevant stimuli presented during RDT. Task irrelevant stimuli are features of objects that are

distractors or are unnecessary to complete the task during target identification. Vickery and Jiang (2009) used color repetition of the shapes as a target feature, while this study used "X" and "O" letter repetition placed inside of the shapes. It has been suggested that processing multiple components of visual objects can be limited based on how relevant they are [9]. If the letter and the shape were treated as separate objects, the participants might not have attended to the shape during the discrimination of the letters, hindering the learning of shape features critical to grouping association.

Moreover, the sample size was also larger than Vickery and Jiang (2009), such that any strong effect of grouping would be more easily seen [4]. Their monitor display was wider than the computer used for this study, which helped them fit in fifteen shapes during training and eleven shapes during transfer phase. Using four more shapes during the training trial could have affected the participants' ability to detect the repetition of the same pairs and thus consolidate information about the pairs more easily. Changing the set size to remove four shapes during transfer, thereby adjusting the display to be less cognitively demanding, could have affected their capacity to detect grouping more easily.

However, it can also be argued that since this study used the same number of shapes for both training and transfer trials, the effect observed in training would hypothetically be more likely to be transferred in the transfer phase, since the participants would not need to adjust to a new display. This suggests that very specific conditions need to be replicated from Vickery (2008), so that learning of Gestalt features are maintained using a repetition discrimination task [6]. It is possible that grouping by common region is not strong as a visual cue for associative learning if it does not have at least fifteen shapes displayed in a wider monitor. If it was a strong effect, the grouping could have been replicated using the same task in this study.

Some features of this study would theoretically make learned grouping more apparent. Vickery (2008) used color repetition targets, which by itself is a strong and salient feature of an object that could override learning of form pairings [6]. This study used letter repetition, a type of shape feature that should not impede on the learning of shape pairs. However, if features of objects are not separated depending on what is attended to, but rather analyzed more holistically, this also poses a challenge to learning of groups in this study. If a certain triangle shape with "O" is treated as a whole object, then a triangle with "X" might be perceptually seen as an entirely different object. This could lead to a harder time in trying to learn about different shape pairings, as the pattern of letters are not consistent with the shape pairs. Thus, learning only of shape patterns might be inhibited by unconscious processing of the letter pattern, in conjunction with the shape pattern.

Future studies will be necessary to investigate ideal conditions for learned perceptual grouping. It is important to investigate the conditions in the environment that help reveal the automatic perceptual processes we often take for granted. Understanding how we perceive the world can help us navigate our environment more effectively, so that the next time someone is searching for those lost keys, they can use a faster and more efficient way to locate them.

Acknowledgements

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JULS RESEARCH

Stereoinversion of chiral carbon centers via oxidation by protonated hypochlorous acid

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Abstract

Chiral carbon centers are present in a wide array of biological molecules, and the loss or inversion of their stereochemistry leads to abnormal structure and function. One way that carbon centers reverse their stereochemistry is through racemization reactions by oxidative or reductive species. Protonated hypochlorous acid ($ClOH_2^+$), formed spontaneously *in vivo* as a consequence of reactive oxidative species, can oxidize and abstract a hydride from its chiral carbon center, which produces a planar prochiral carbocation. This planar prochiral carbocation is susceptible to hydrogenation on either of its faces, allowing for inversion of its stereochemistry.

This study investigated the thermodynamics of the racemization of carbon stereocenter analogues methane, propane, and alanine, by hydride abstraction with $ClOH_2^+$ and hydrogenation from another stereocenter. Using *ab initio ca*lculations in Gaussian09 at the B3LYP/6-31G(d) level of theory, it was determined that the reaction is exergonic and characterized by a favourable energy of activation in vacuum. Racemization completed using an additional stereocenter as a hydride donor resulted in the exergonic formation of another prochiral carbon center in addition to the racemized stereocenter. This implied that biologically active chiral carbon centers may be spontaneously oxidized by $ClOH_2^+$ to form a reactive prochiral carbon, which may react with other monomers to form aberrant racemization products. This racemization of carbon stereocenters disrupts the structure and function of biological molecules, contributing to neurodegeneration, cardiovascular disease, and several age-related diseases.

Keywords: *Amino acids, computational chemistry, racemization, protonated hypochlorous acid*

Introduction

A vast number of biological molecules, including nearly all amino acids and proteins, are stereospecific. Their specificity is crucial for normal functioning, and racemization may result in protein dysfunction that results in a host of human diseases [1]. The majority of amino acids in the human body are L-amino acids, with a small percentage of D-amino acids (D-AAs). While these D-AAs have selectively important physiological roles, their presence in high concentration can lead to disease in the central nervous system [2], vascular system [3], and musculoskeletal system [4]. Although degradative pathways for D-AAs do exist, they have not been extensively studied. If the rate of D-AA production is too high, these degradative processes may not be able to maintain normal levels of them [5]. Therefore, it is vitally important that the body maintain a low rate of D-AA formation.

In the past decade, it has become increasingly clear that natural oxidative processes important for biological functioning also contribute to an increase in D-AAs [6]. Generation of superoxide (O_2^-) occurs as a by-product of many of these processes, which may be reduced to form a variety of reactive oxygen species (ROS) [7]. Superoxide spontaneously reacts with water to produce hydrogen peroxide (H_2O_2), which may be enzymatically converted to hypochlorous acid (HOCl) [8].

$$Cl^- + H_2O_2 \rightarrow HOCl + HO^+$$

Cellular compartments specialized for degrading biomolecules, such as lysosomes and autophagosomes, have an acidic environment where the pH drops below 5.5 [9]. This allows localized and nearby chlorine species to be protonated and form protonated hypochlorous acid ($ClOH_2^+$) [10]. Although it is known that there are enzymatic pathways for the synthesis of D-AAs, their spontaneous formation by oxidation and racemization of L-AAs by ROS such as $ClOH_2^+$ has not been studied [5]. It is known that HOCl may oxidize certain amino acid groups, but not the backbone of a peptide [11]. This study proposes that a more reactive form, $ClOH_2^+$, may be potent enough to oxidize the alpha carbon of an amino acid, therefore catalyzing the formation of a prochiral carbocation intermediate and its racemization.

Computational chemistry approaches can be used to evaluate the viability of oxidative racemization of amino acids. Starting with simple carbon models such as methane and propane, then more complex protein residue structures such as alanine and alanine diamide, reactions involving $ClOH_2^+$ and the proton-alpha carbon moiety ($H\text{-}C_\alpha$) of the models can be studied, and the thermodynamic values associated with hydride abstraction reactions can be deter-

mined. Furthermore, these approaches can be extended to evaluate a mechanism for the propagation of this racemization reaction involving a hydride transfer between two different alpha carbons.

Investigating a mechanism for the spontaneous ROS-mediated racemization of an amino acid model can illuminate a potential process through which D-AAs are generated in the cell. Although they are traditionally thought to arise from natural biosynthetic pathways, this study suggests that there may be a considerable amount of D-AAs derived from the racemization of L-amino acids by reaction with ROS. Further work on evaluating the viability of the propagation of this racemization by hydride transfer between the planar carbocation intermediate and other C_α to amplify the total amount of racemized alpha carbons in a chain-like reaction can prove useful as well. Analysis of the thermodynamics of these reactions will aid in evaluating the viability of ROS-promoted racemization.

Materials and methods

Thermodynamic analysis of total (rovibrational + electrical) energy (ΔE_{total}), enthalpy (ΔH), and Gibbs free energy (ΔG) of the reactants, transition states, intermediates, and products for the reactions in Figure 1 were determined through *ab initio* calculations using density function theory (DFT) at the B3LYP level of theory [12, 13] with a 6-31G(d) basis set [14] with an ultrafine grid integration. While the transition states of the reactions required the building of a unique Z-matrix to ensure appropriately-defined bond and angle parameters, the reactants and products were designed using GaussView 5 software [15] and the calculations were conducted using Gaussian09 software [16].

The reactants and products were optimized at a standard 297K *in vacuo*, which were then used to perform a frequency calculation in the same conditions to yield the thermodynamic values ΔE, ΔH, and ΔG. These values were then converted from Hartrees to kJ/mol, where 1 Hartree = 2625.5 kJ/mol in standard conditions. Using the optimized values of the bond lengths, bond angles, and dihedral angles from the calculations conducted on the reactants, the unique Z-matrices were designed to preserve these values as the initial points of the transition states and the intrinsic reaction coordinate (IRC) calculations. These were calculated with an initial force constant calculation, which was optimized to a transition state minimum.

Results

The calculations outlined in the previous section yielded values of ΔE_{total}, ΔH, and ΔG for the reactants, transition states, intermediates, and products in (Figure 1). From these values, various vital thermodynamic values such as forward and reverse activation energy ($\Delta G^{\ddagger}_f$, $\Delta G^{\ddagger}_r$) and overall change in Gibbs free energy ($\Delta G_{reaction}$) were calculated. In interpreting the viability of the reactions from a thermodynamic standpoint, $\Delta G^{\ddagger}_f$ and $\Delta G_{reaction}$ are key indicators, and are shown in Figure 2 and Figure 3.

As a general measure of the relative stability of a given chemical species, the evolution of the Gibbs free energy as a function of the reaction coordinate plays a key role in understanding the oxidation reactions. These relationships are shown in Figure 4, Figure 5, and Figure 6.

Discussion

Thermodynamics and reaction conditions

The thermodynamic values of the oxidation of H-C_α moieties by $ClOH_2^+$ in this exploratory study offer insight into the viability of spontaneously generated D-AAs from oxidation of L-amino

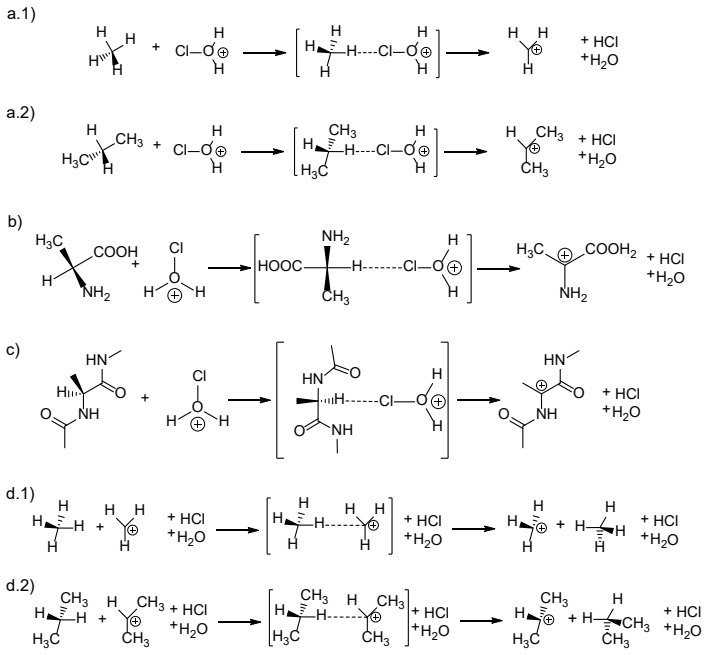

Figure 1: The reactions studied. The oxidation of methane, propane, alanine, and alanine diamide by $ClOH_2^+$, and the dimerization-hydride transfer involving oxidized methane and propane.

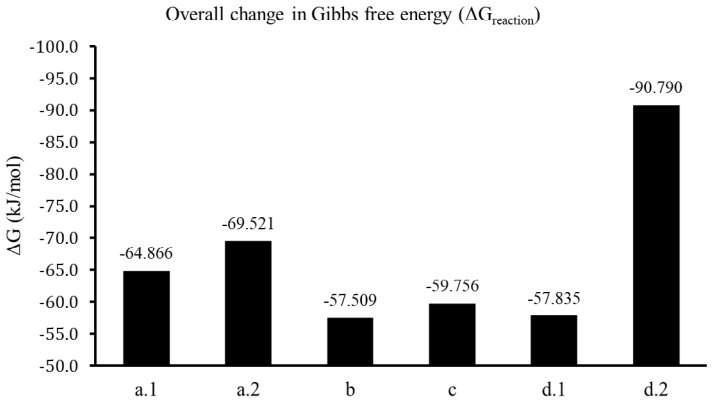

Figure 2: The overall thermodynamic change of Gibbs free energy over the course of a reaction ($\Delta G_{reaction}$) was negative for all reactions studied. The reactions were exergonic and occurred spontaneously.

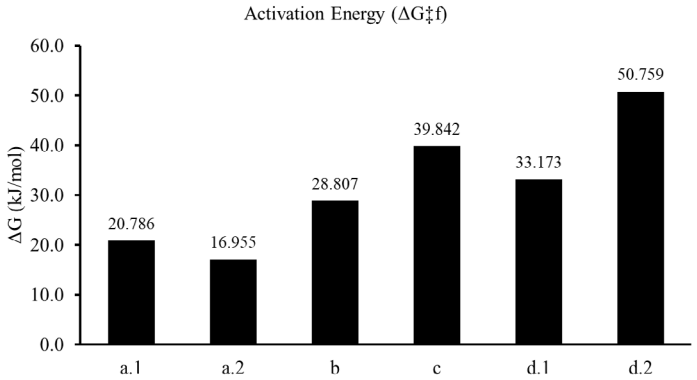

Figure 3: The activation energy is given by the forward reaction Gibbs free energy ($\Delta G^{\ddagger}_f$). This represents the minimum energetic requirement that the system must supply for a reaction to proceed.

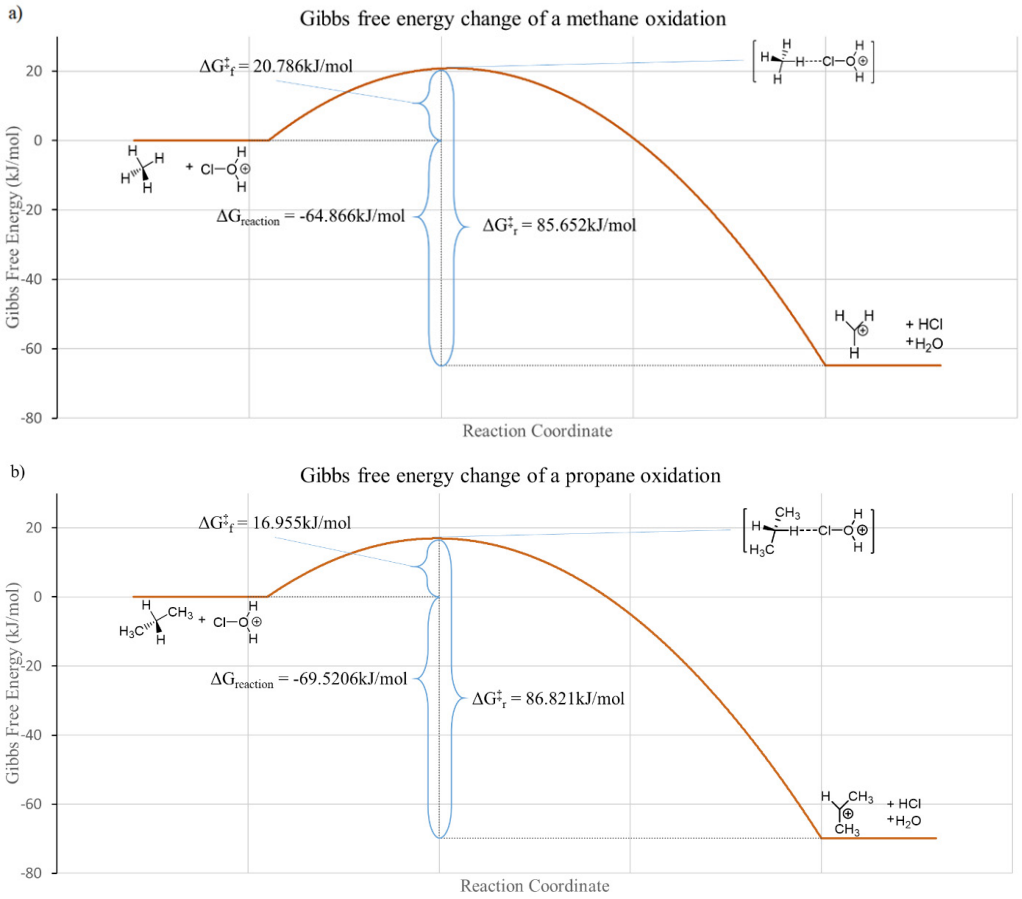

Figure 4: Methane and propane oxidation with $ClOH_2$ spontaneously produced a prochiral carbocation. a) The thermodynamic change (ΔG) as a function of the reaction coordinate during methane carbon oxidation from Figure 1.a.1. b) The thermodynamic change (ΔG) as a function of the reaction coordinate during the propane carbon oxidation shown in Figure 1.a.2.

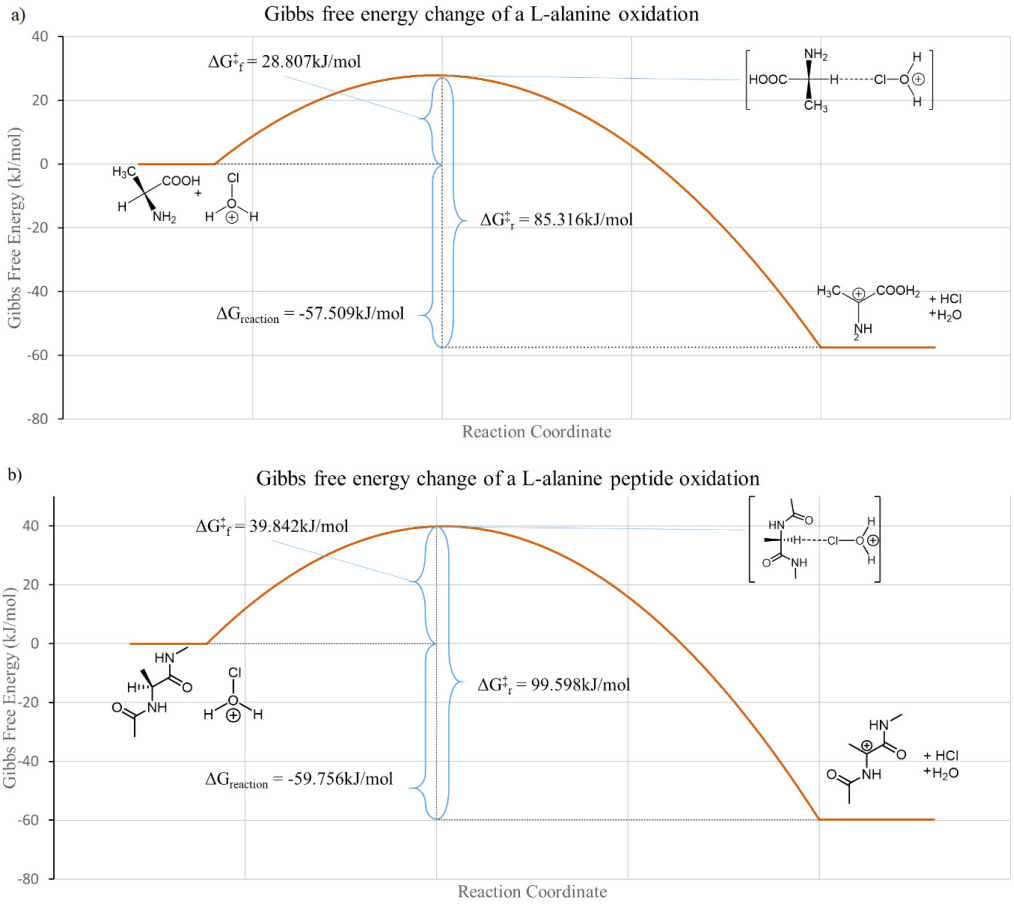

Figure 5: L-alanine and L-alanine peptide oxidation with $ClOH_2$ spontaneously produced a prochiral carbocation. a) The thermodynamic change (ΔG) as a function of the reaction coordinate during L-alanine carbon oxidation from Figure 2.b. b) The thermodynamic change (ΔG) as a function of the reaction coordinate during the L-alanine peptide carbon oxidation shown Figure 2.c.

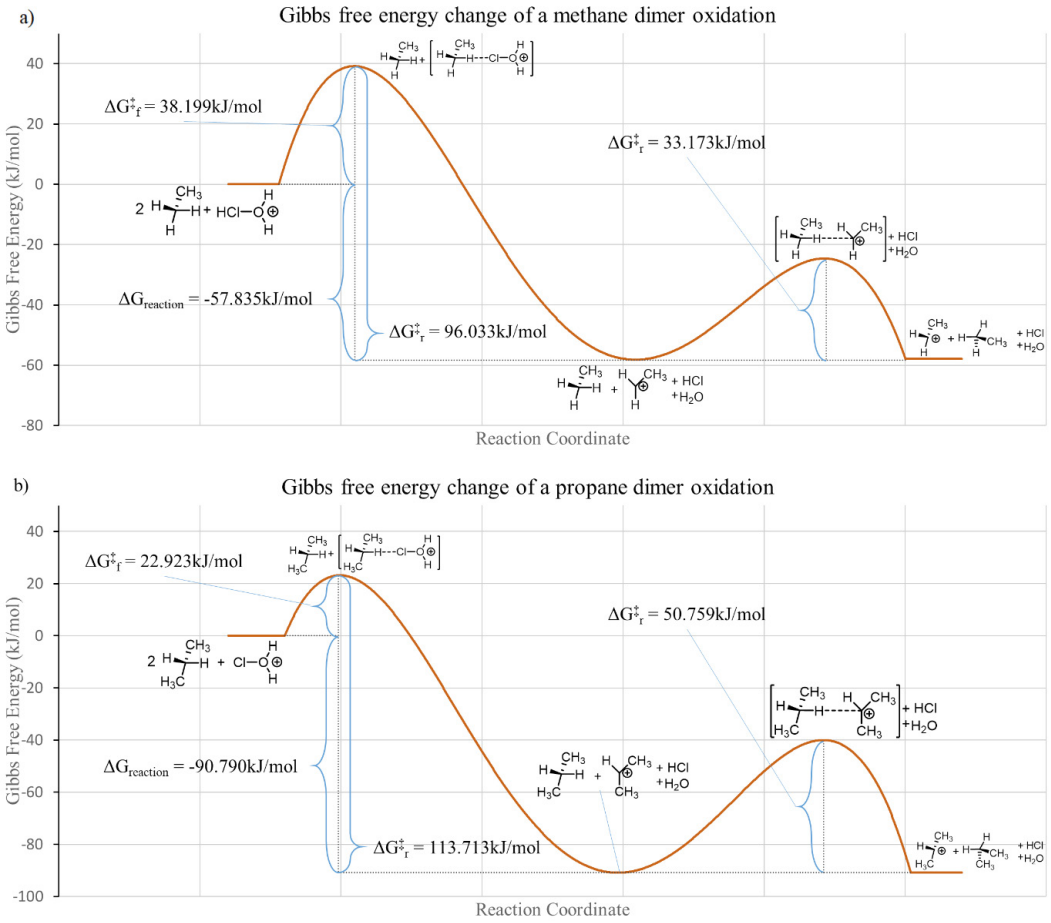

Figure 6: Methane and propane dimers were spontaneously oxidized by $ClOH_2$ and underwent a hydride transfer reaction, which produced a racemized product. a) The thermodynamic change (ΔG) as a function of the reaction coordinate during methane reaction from Figure 1.d.1. b) The thermodynamic change (ΔG) as a function of the reaction coordinate during the propane reaction from Figure 1.d.2.

acids. The calculations for every reaction showed negative values of $\Delta G_{reaction}$, indicating that the reactions occurred spontaneously. The products of the reactions were species classically known to be exceptionally stable, as most of the reactions yielded HCl and H_2O as side products, which contributed to the stability of the products compared to reactants. The reactions yielded three product molecules from two reactant molecules, which corresponded to an overall larger change in entropy and further contributed to a more negative (and therefore, more favourable) change in $\Delta G_{reaction}$.

The forward activation energies ($\Delta G^{\ddagger}_{f}$) varied from 16.996kJ/mol to 50.759kJ/mol. There are multiple reasons for the variation in $\Delta G^{\ddagger}_{f}$ between similar species, such as the oxidation of methane compared to propane. These differences are largely due to electronic effects, which refer to the ability of the structure to stabilize theorized transition states and final products. In the transition state, the H-C_α moiety is weakened and a positive charge is formed on the C_α, which develops into a formal positive charge in the final product. The two -CH_3 groups present in propane are excellent cationic charge stabilizers when compared to -H groups, due to the multiple σ-bonds present in the group. These bonds can stabilize carbocation charge by hyperconjugation, allowing for the dispersion of the charge over their own surface.

When comparing alanine and alanine diamide, potential steric effects can come into play on top of the positive contribution from the electron donating groups' electronic effects, as the large amide groups on the molecule represent major steric bulk. Given that residues exist *in vivo* as parts of much larger peptides, steric bulk will be an increasingly important consideration for future study. If these values of $\Delta G^{\ddagger}_{f}$ can be supplied by *in vivo* systems, oxidative molecules including ROS such as $ClOH_2^+$ may abstract a hydride from the C_α of an amino acid, break the H-C_α moiety, and form a prochiral carbocation intermediate. This planar prochiral carbon intermediate may be protonated on either side to form the L- or D-AA, representing an important mechanism for the generation of D-AA using ROS produced during cellular stress may to mediate the stereoinversion.

Given the exploratory nature of the study, the *in silico* conditions were not selected to match *in vivo* conditions due to computational limitations associated with simulations that mimic solvation in a variety of media. The differences in the *in vacuo* conditions used when compared to the variety of solvents, free ions, cellular interactions (including the ionic strength in the cell), and metal cofactors available in biological systems imply that the results of the study may be widely affected by these conditions [17]. Metal cofactors and high ionic stress can contribute to an increased possibility of potentially stabilizing electronic interactions between charged ions and transition states or final carbocationic products. Exploring these effects, along with the potential steric limitations presented by highly complex and well-organized protein structures that could limit the access of $ClOH_2^+$ to C_α sites, will prove vital in evaluating the potential viability of the reaction *in vivo*.

Biological implications

D-AAs are elevated in a variety of pathological conditions, most notably in the central nervous system [2], vascular system [3], and musculoskeletal disease [4]. In the human aorta, D-aspartate

accumulates in elastin, but not in other structural proteins, due to the spontaneous racemization of L-aspartate [3]. Collagen and other peptides do not display this D-isomer accumulation due to their rapid turnover [3]. In CNS disorders, there may be aggregations of peptides that are not recycled or degraded similar to elastin in the aorta. The b-amyloid (Ab) peptide affiliated with Alzheimer disease forms toxic insoluble aggregates in the brain when b-amyloid degrading proteases are disrupted [18].

Not only are these insoluble peptides more prone to accumulating D-AAs due to their low turnover [3], the elevated D-serine also overstimulates excitatory neural NMDA receptors, leading to cellular exhaustion and death [19]. Racemized forms of amino acids also accumulate in the musculoskeletal system and their elevated concentration is an indicator of disease [4]. Not only are D-AAs relevant in disease, but their concentration may also be used to determine the age of a protein and tissue [4]. In the aorta, the first-order rate constant of this racemization was calculated to be $1.14(x10)^{3}$ [3], but this constant may vary in different environments. In all, this evidence suggests that there is a spontaneous mechanism for endogenous amino acid racemization which may cause build-up of D-AAs in proteins not rapidly being turned over and ultimately contribute to disease.

Looking forward

Our findings are an initial evaluation on the possibility of amino acid racemization by ROS, but further study on this mechanism may provide a more accurate understanding. The next step is to re-evaluate the energetics of this reaction using a more rigorous computational theory, since B3LYP/DFT may not yield accurate calculations for long-distance, non-covalent van der Waals interactions in the transition state. In addition, this reaction should be verified under *in vivo* conditions and with the consideration of larger protein structures, including possible metal cofactors, to ensure that the reaction is favourable. However, we predict that the increased ionic strength of the environment will accelerate the reaction. *In vivo* research should also focus on quantifying, with a high degree of accuracy, the generation of $ClOH_2^+$ and its lifespan in tissues.

Conclusion

All racemization reactions between $ClOH_2^+$ and alpha carbon models were found to be spontaneous and exergonic in vacuum at standard conditions. The planar carbocation intermediate propagated this racemization by a hydride transfer mechanism. This suggests that there may be a similar mechanism leading to spontaneous generation of D-AAs from L-amino acids *in vivo*, which is a topic for further research. This racemization of amino acids in peptides leads to disruption of protein structure, enzymatic function, and metabolic pathways when these peptides are not degraded. Interestingly, an increase in D-AA concentration is also implicated in many diseases, and the potential application of computational chemistry in evaluating the viability of reactions in given conditions is a topic with much promise.

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JULS RESEARCH

Quantum chemical analysis of the theoretical formation mechanism of glycine in dense molecular clouds

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Abstract

More than ten years ago, the simplest amino acid, glycine (NH_2CH_2COOH), was found in cometary samples by the Stardust project of NASA. Glycine is one of the 20 proteinogenic amino acids that serve as building blocks for proteins. It has been theorized that some biomolecules, including amino acids, originated in space and were brought to Earth by meteorites, therefore playing an important role in the origin of life. The detection of glycine by Stardust may support this theory.

This study proposes a new reaction pathway for the formation of glycine in gas phase under dense cloud conditions of temperature 15 K and pressure 0 atm. The reaction starts with small and abundantly found molecules in interstellar medium (ISM), namely hydrogen cyanide, carbon monoxide, hydrogen gas, and water. Since the temperature and pressure in dense clouds are extremely low, exothermic reactions with low energy barriers are considered feasible.

B3LYP/6-31G(d) level of theory was used to optimize geometries and calculate thermodynamical properties through vibrational frequency calculations under dense cloud conditions. Single point calculations were also performed using MP2/6-31G(d) level of theory to confirm the obtained thermodynamic data. The respective stabilities were examined using the thermodynamic data calculated for the proposed reactants, transition states, and product. Since the proposed reaction is calculated to be exothermic with low energy barriers under dense cloud conditions, theoretically, the criteria for the reaction to occur in ISM have been fulfilled.

Keywords: *Astrobiology, astrochemistry, dense clouds, reaction mechanism, quantum chemical calculations*

Introduction

The origin of life has always been a great subject of research, with many theorizing that the "molecules of life"—nucleic acids, proteins, carbohydrates, and lipids—were introduced to pre-biotic Earth by a comet or meteorite that struck its surface [1]. Early or pre-biotic Earth defines a period about 4.5 billion years ago, before life existed due to the extreme environment on the planet [2]. Proteins are made up of amino acids, which are organic compounds generally characterized by a carboxylic acid group (COOH), an amine group (NH_2), and a specific "R" group, as shown in Figure 1A. While many exist, only 20 of them serve as the building blocks of proteins in organisms. Synthesis of amino acids requires the presence of certain molecules, such as H_2O, NH_3, CH_4, and HCN, which have been found in interstellar medium (ISM) [3].

Interstellar medium is the matter and radiation that exists in galaxies between star systems. ISM is organized into molecular clouds, which are areas dense in chemical species (especially hydrogen and helium), where star formation reactions could occur [4]. Molecular clouds are further divided into various types, depending on conditions such as temperature and density. One such type, dense clouds, are characterized by a temperature of 15–20 K and a molecular density of 10^3-10^6 cm^{-3} [5]. While the temperatures are low, energy sources such as cosmic rays and soft X-rays have been detected [6].

Considering amino acids are of great biological importance, there has been great interest in verifying their existence, as there is a possibility that they have extraterrestrial origins [8,9]. Indeed, studies have shown that some amino acids can be generated in conditions matching those of interstellar dense clouds [7]. Since glycine is the simplest of all amino acids (shown in Figure 1B), much research has been done to verify its presence in ISM.

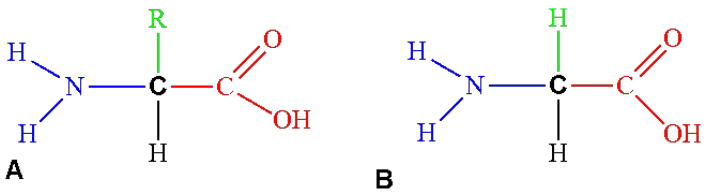

Figure 1: The structure of amino acids. A) The generalized structure of an amino acid, highlighting the functional groups. B) Glycine, the simplest of the 20 proteinogenic amino acids (R=H).

There have been many attempts to verify glycine's presence in interstellar clouds in recent years, the most famous being glycine's "detection" in 2003, based on the observation of 27 spectral lines associated with glycine from three different molecular clouds [8-13]. However, the results of this study were disputed after analysis of the data showed that the spectral data could have belonged to a number of different molecules that were structurally similar to glycine, such as formic acid and acetic acid [9]. Furthermore, all of the spectral lines from the three clouds only had three lines in common, which further weakened the evidence for glycine's detection presented in the study [9]. Therefore, glycine's presence in ISM has yet to be confirmed. However, it must be acknowledged that there are difficulties associated with glycine's detection, specifically regarding its weak lines in rotational spectrums [8].

Over a decade ago, NASA probe Stardust obtained cometary samples showing promising data [14]. Analysis of these samples showed organic matter rich in oxygen and nitrogen, while presence of deuterium and N-15 suggested that at least some of the matter had interstellar origins, most likely of dense clouds and/or protostellar nebulae. Additionally, a statistically significant amount of glycine, relatively higher than those in controls, was found in the comet sample returned by Stardust [14]. Further analysis of glycine present in the comet confirmed its detection and interstellar origin based on the stable carbon isotopic ratio. The study also ruled out the possibility of contamination of glycine since it was found on the aerogel (comet-exposed) side of the sample [15]. Overall, the presence of glycine in the comet not only confirms glycine's existence in ISM, but also illustrates the possibility of safe delivery of glycine to Earth, additionally strengthening the theory of interstellar origin of life.

Although glycine and various other molecules have been detected in comets, the mechanism for the formation of gas-phase glycine in dense clouds is unclear, although attempts have been made in numerous studies [16-22]. There is a considerable amount of research in the mechanism for glycine's formation and in precursors that could be of interest for its synthesis. A five-membered heterocyclic compound, hydantoin (glycolylurea), as shown in Figure 2, has been proposed to be a possible precursor. It is known to occur from prebiotic molecules (such as urea and glycolic acid) under ISM conditions and was also detected in carbonaceous chrondites [23]. However, hydantoin has not yet been detected in ISM [19,24].

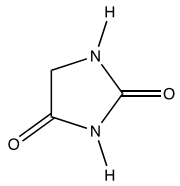

Figure 2: Hydantoin (glycolylurea), a five-membered heterocyclic molecule that is proposed to be a precursor for the formation of glycine in ISM.

Although numerous studies have been conducted to find and verify interstellar glycine, its possible precursors and formation mechanism in various ISM media remain unknown. This study proposes a new reaction for the formation of interstellar glycine in gas phase under dense cloud conditions, based on the reaction mechanism shown in Figure 3. The proposed reaction uses small molecules (HCN, CO, H_2O, and $H_{2)}$ known to be abundant in ISM [4,25], and is theoretically calculated to be exothermic in ISM, which suggests the possible feasibility of this reaction occurring in dense clouds.

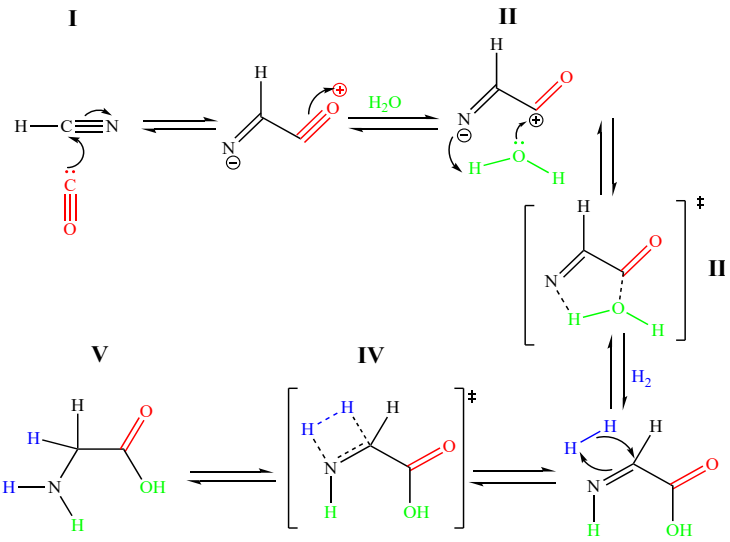

Figure 3: The proposed reaction to form glycine in dense molecular clouds in gas phase starting with small and readily present molecules HCN, H_2O, H_2, and CO.

Methods

The molecules involved in the reaction mechanism were optimized and their thermodynamic calculations were obtained. To optimize the studied species, the B3LYP/6-31G(d) method was used as it is implemented in the Gaussian 09 program package [26]. Geometry optimization and frequencies were calculated with the temperature set as 15 K and the pressure set as 0 atm. Single point calculations were performed using MP2/6-31G(d) level of theory to confirm obtained thermodynamic properties.

Results and discussion

The results of the computational data calculated in conditions that mimic Earth and dense molecular clouds are summarized in Table 1.

For the purposes of this study, changes in Gibbs free energy and entropy are the primary focus for analyzing the stability of the molecules involved in the reaction. As seen in Figures 4 and 5 and Table 1, the overall changes in Gibbs free energy ($\Delta G_{reaction}$) for gly-

	Earth				Dense Molecular Clouds			
	ΔE (kJ/mol)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (cal/mol-K)	ΔE (kJ/mol)	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS (cal/mol-K)
Reactants	220.97	107.77	228.41	171.61	210.12	207.71	210.50	84.99
1st TS	710.46	635.43	715.42	139.03	702.72	701.23	702.97	68.28
Intermediate	105.64	87.41	105.64	89.52	99.42	99.30	99.42	42.53
2nd TS	1066.71	1073.95	1066.71	69.10	1069.83	1069.84	1069.83	40.43
Product	0	0	0	74.91	0	0	0	40.57

Table 1: The relative thermodynamic data of Glycine synthesis under conditions mimicking Earth and dense molecular clouds.

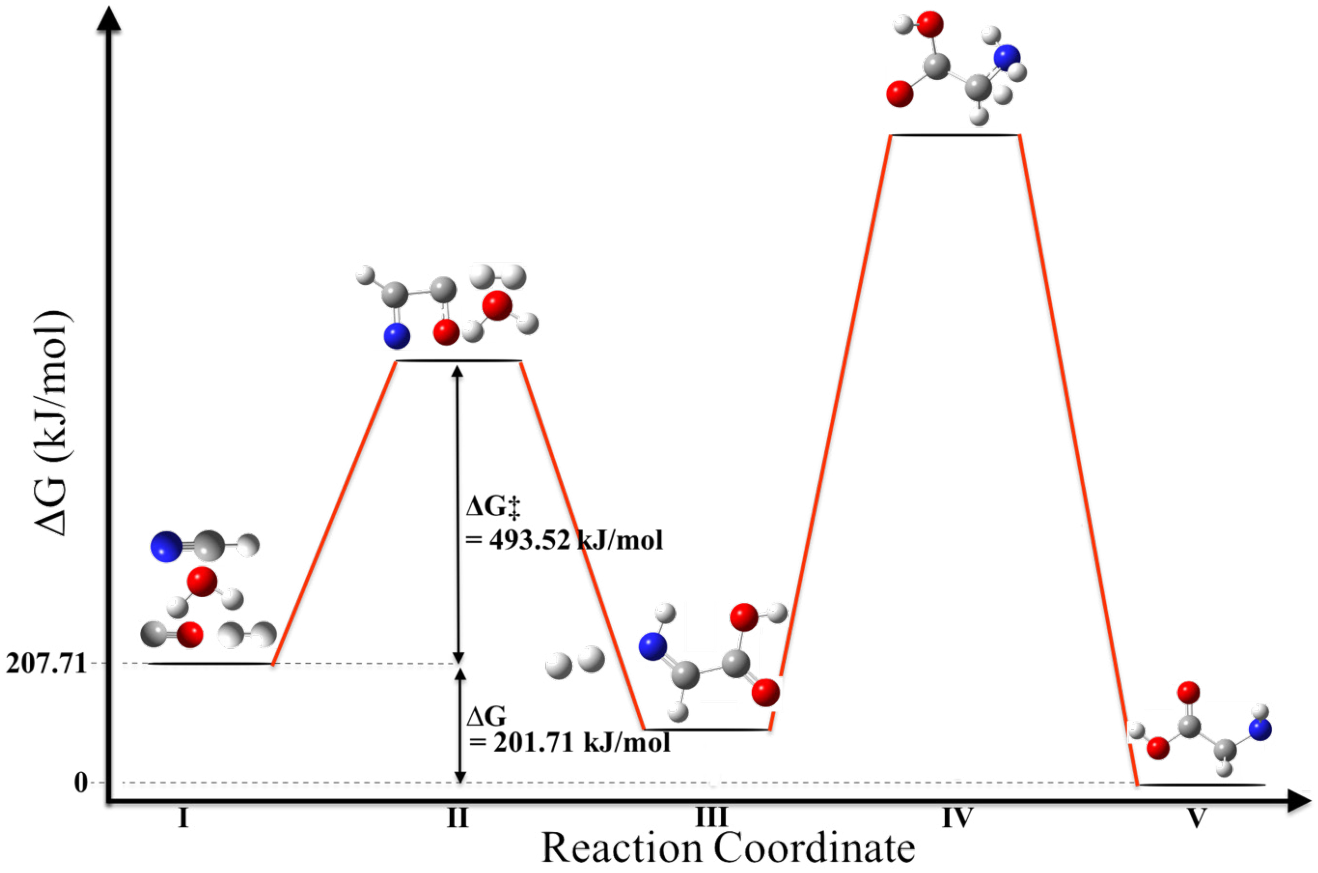

Figure 4: The relative change in Gibbs free energy as a function of reaction coordinate during the formation of glycine. The reaction is exergonic as indicated by ΔG and occurs between H_2, HCN, H_2O, and CO under dense cloud conditions (15 K, 0 atm).

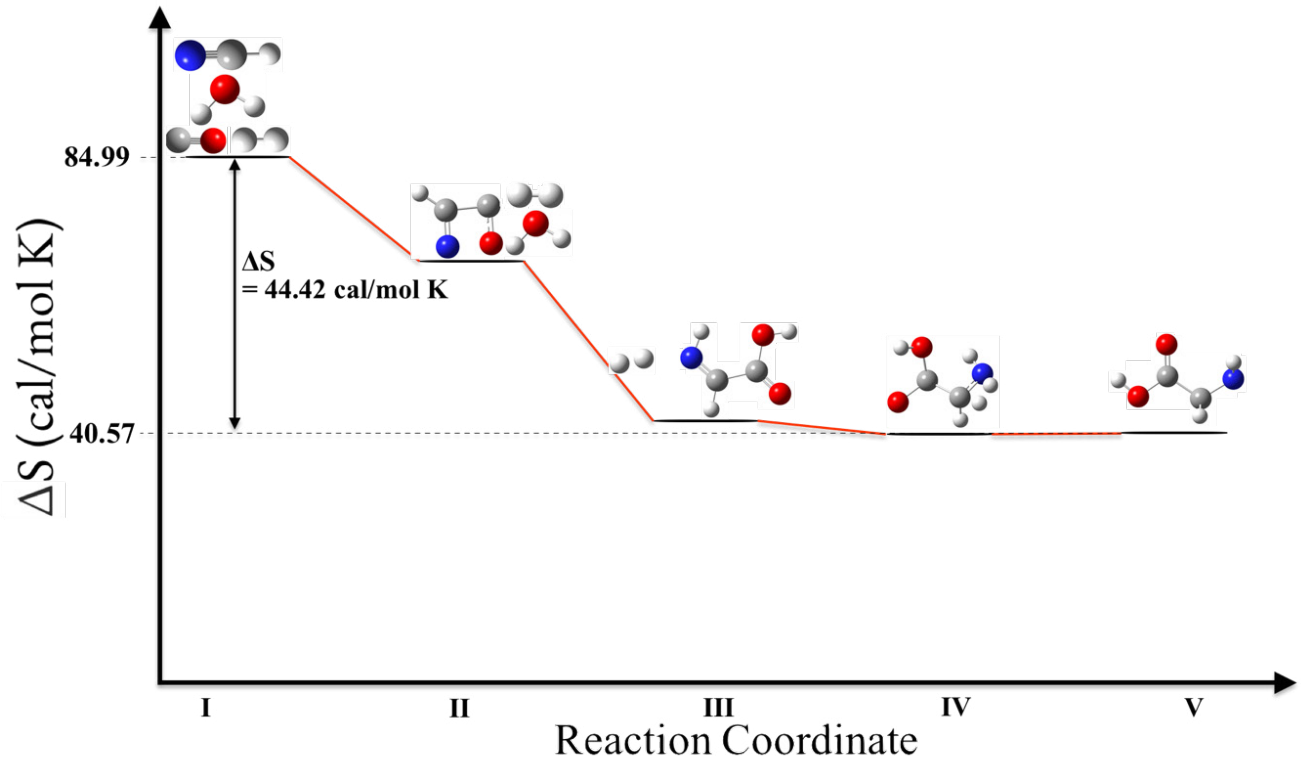

Figure 5: The relative change in entropy as a function of reaction coordinate during the formation of glycine. ΔS indicates a loss in entropy, implying that the final product is much more structured and stable. The reaction occurs between H_2, HCN, H_2O, and CO under dense cloud conditions (15 K, 0 atm).

cine formation were calculated to be -107.77 kJ/mol under Earth conditions and -207.71 kJ/mol under dense cloud conditions, respectively. The activation energies ($\Delta G^{\ddagger}$) were calculated to be 527.66 kJ/mol and 493.52 kJ/mol, and the changes in entropy (ΔS) were calculated to be -96.70 cal/mol-K and -44.42 cal/mol-K under Earth conditions and dense cloud conditions, respectively.

Since $\Delta G_{reaction}$ is negative for the proposed reaction under both Earth and dense cloud conditions, the reaction is exergonic, which indicates a release of energy that is considered favourable, especially in ISM. The activation energies ($\Delta G^{\ddagger}$), however, were found to be quite high (493-527 kJ/mol). In order to overcome this energy barrier, an energy source is required. This energy could be provided by cosmic rays and soft X-rays present in dense clouds that, when striking molecules, can provide enough energy to start the reaction. The mechanism could possibly involve tunneling as well [28].

In terms of entropy, since ΔS of glycine (the product) is lower than that of the reactants under both Earth and ISM conditions, this indicates that glycine is more structured and stable. However, due to the extremely low temperatures in ISM, the gain in entropy would not have a significant impact on the spontaneity of the reaction, based on the equation: $\Delta G=\Delta H-T\Delta S$. The low temperature would also imply that the T component could easily be overcome by the component, indicating that the driving force behind this reaction is the release of heat from differences in chemical potential and not due to changes in entropy.

Overall, ΔS under ISM conditions is less than half of ΔS under Earth conditions, which is consistent with the low temperatures of dense clouds (15 K).

Conclusion

The thermodynamic analysis, done *in silico*, have theoretically demonstrated the thermodynamic feasibility of the proposed reaction in ISM. This reaction is very important to astrobiology given the significance of glycine, the smallest proteinogenic amino acid, as it could underline the possibility of extraterrestrial origins of life. With more research into this field, findings in this paper could indicate prebiotic material, including glycine, to originate from space, confirming the exogenous nature of prebiotic material that could not be produced on Earth.

Furthermore, using insights into the thermodynamic properties analyzed, many more complex amino acids could also be investigated. Additionally, further *in silico* studies using higher levels of theory could be applied to investigate the mechanism. Since the activation energy ($\Delta G^{\ddagger}$) was quite high, and while cosmic rays and soft X-rays are possible sources of energy that are present in dense clouds, further investigation could be pursued to find a mechanism for the reaction to proceed.

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JULS RESEARCH

Serotonin mediates *Caenorhabditis elegans* associative learning by indicating presence or absence of food

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Abstract

What does it mean to learn? The full molecular mechanisms underlying the formation and storage of a memory are unknown in even the simplest model organisms. The nematode worm, *Caenorhabditis elegans*, despite having only 302 neurons, is able to learn and can undergo classical (Pavlovian) conditioning. For example, when worms are given an attractive odorant (such as benzaldehyde, Bnz) during a period of starvation, they learn to find this stimulus aversive. Previous research indicates that serotonin signaling in worms acts as an endogenous food signal. When given exogenously, serotonin blocks the formation of this Bnz-starvation association.

This study hypothesized that the Bnz-starvation association is negatively regulated by serotonergic signalling. The absence of this satiety signal was considered the unconditioned stimulus in the associative learning paradigm. Since Bnz represents the conditioned stimulus, understanding the nature of the unconditioned stimulus signal will help explain stimuli integration and, consequently, memory formation.

Serotonin synthesis and receptor mutants were screened in the Bnz-starvation associative learning paradigm. Worms were given Bnz during a period of starvation, and then tested for their approach to a point source of Bnz. It was found that worms missing a single serotonin receptor, SER-4, were able to form a starvation-odorant memory even in the presence of exogenous serotonin.

This study implicates SER-4 as the crucial molecular component necessary for receiving the serotonin/satiety signal and, consequently, the regulation of the associative memory. Therefore, the structural simplicity and facile genetics of *C. elegans* was used to understand the nature of the unconditioned stimulus and gain insight into a fundamental question: what exactly is a memory?

Introduction

The struggle to define "learning" and "memory" is a fundamental problem addressed by a multitude of behavioural and neuroanatomy studies. With the relative neural complexity of popular model organisms (135 000 neurons in *Drosophila melanogaster*, 4 000 000 neurons in mice), it remains a challenge to understand learning on anything more than the level of neuronal wiring and regional firing, much less to isolate the biochemical representation of a memory.

The nematode *Caenorhabditis elegans*, despite having only 302 neurons, can undergo classical (Pavlovian) conditioning, and can therefore be tested in an associative learning paradigm. When worms are given an odourant (such as benzaldehyde, Bnz) during a period of starvation, their natural preference for this smell switches from attractive to aversive [1]. In this way, worms are able to integrate internal and external cues in order to behaviourally adapt to their environment. This presents a simple model organism with which to study the molecular mechanisms of learning, something that is neither well understood nor easily characterized in a more complex animal.

Classical conditioning is defined as the association of a conditioned stimulus (CS) and an unconditioned stimulus (US) to produce a conditioned response. In the Bnz-starvation paradigm, the US is the innately aversive physiological state of starvation. The CS is Bnz, which untrained worms find naively attractive. Worms can be trained by exposure to the CS in the presence of the US, represented in the lab by the presence of Bnz in absence of food. Learning occurs when an association between Bnz and starvation is formed such that worms actively avoid a point source of the Bnz CS. Since this learning paradigm involves starvation, it is clear that the presence or absence of food represents an important external signal to worms. This regulation of "satiety-state" in worms has been shown to be mediated by serotonergic signaling [2,3].

Serotonin is a biogenic amine neurotransmitter that mediates multiple food-related processes in worms. The effects of applying exogenous serotonin to worms were first characterized in Horvitz et al. (1982), which described three main behavioural responses: depressed locomotion, stimulated pharyngeal pumping, and increased rate of egg laying [4]. These phenomena correlated with those observed in earlier studies describing food-mediated

behavioural responses in worms [5]. Furthermore, later studies clarified the causal role of endogenous serotonin signaling in these phenomena. The modulation of the satiety state was linked to serotonin by showing that exogenous serotonin reversed starvation-responsive behaviour [6]. Likewise, the "depressed locomotion" was a result of the role of endogenous serotonin signaling in mediating an "enhanced slowing" response of fasting worms [2]. Increased pharyngeal pumping was found to also result from the presence of food in a worm's environment, and multiple serotonin-binding receptors were implicated as necessary for modulating this response [7]. In addition, increased rate of egg-laying was also found to be mediated by serotonin-binding to different serotonin receptors [7].

These data implicate serotonergic signaling as a key regulator of satiety status in worms. Serotonin signaling has also been implicated in the aforementioned starvation-odourant associative learning paradigm. This was accomplished by demonstrating that the presence of food during the training period could block the Bnz-starvation association, and that this phenomenon could be recapitulated with the application of exogenous serotonin. These two training paradigms described a "food block" and a "serotonin block", respectively [3]. This research also showed that worms deficient in serotonin signaling (mutant for the genes tph-1 or cat-4, involved in serotonin synthesis) were not able to be food blocked, and learned to associate Bnz with starvation in the presence of food. This raises the question of whether serotonin signaling is not just important as a satiety signal, but whether the lack of serotonin signaling is the nature of the starvation US. Therefore, previous research indicates that the presence or absence of serotonin signaling appears to mediate the US arm of the associative learning paradigm by regulating a worm's satiety state.

C. elegans has five canonical serotonin receptors, and four receptors with strong serotonin receptor homology. Of the canonical receptors, four (ser-1, ser-4, ser-5, and ser-7) are G-protein coupled receptors and one (mod-1) is a ligand-gated ion channel [7]. Different receptors are involved in mediating different aspects of the food response, including pharyngeal pumping, locomotion, and increased egg-laying [8-12].

This study shows that worms mutant for the five canonical serotonin receptors ("quintuple mutants") are defective in their ability to be serotonin blocked. The receptors necessary for this phenomenon were investigated, and it was found that the loss of ser-4 alone replicated the minimal serotonin blocking seen in quintuple mutants, implicating the SER-4 receptor in mediating the US arm of the Bnz-starvation classical conditioning paradigm.

The goal of this study is to explore the mechanisms of Bnz-starvation associative learning in *C. elegans* in order to better understand the nature of memory formation and storage on a molecular level. Worms are a good model organism with which to investigate these pathways because of their structural simplicity and facile genetics. In addition, many serotonin receptors in worms are homologues of human serotonin receptors [7]. The mechanisms involved in their associative learning may comprise a simplification of the mechanisms involved in mammalian associative learning. Based on the prior research, this study hypothesizes that serotonin signaling is involved in mediating the starvation/food signal in *C. elegans*, which, upon integration with Bnz, results in associative learning of the Bnz-starvation association.

Materials and methods

Strains: N2 Bristol (wild-type), GR1321 tph-1(mg280) II, UT1310 ser-1(ok345) ser-7(tm1325) X ser-3(ad1774) I, ser-1(ok345) ser-7(tm1325) X ser-4(ok512) III ser-5(tm2654)I mod-1(ok103) V, MT9668 mod-1(ok103) V, RB745 ser-4(ok512) III.

In the standard classical conditioning paradigm, N2 (wild type) worms were trained for one hour by suspension in 1 mL M9 buffer + 0.005% Triton X, with or without 0.006% benzaldehyde [3]. Worms were then transferred to the center of an NGM agar plate, where 1uL of 1% Bnz (diluted in 100% ethanol, EtOH) was spotted on the testing side, and 1uL of 100% EtOH was spotted on the control side. NaN3 (sodium azide) was spotted at either end to preserve the worms' first chemotactic choice. Worms were allowed to move freely for one hour, the duration of the testing period. The chemotactic index (CI) was calculated as number of worms on the test spot minus number of worms on the control spot, divided by the total number of worms on the plate. A negative chemotaxis value indicated movement away from Bnz, or learning, and a positive value indicated chemotaxis towards Bnz. Food blocking was achieved by either training worms on NGM agar plates seeded with OP50 *Escherichia coli*, or on a plate with 100uL of culture diluted to an OD600 of 0.5 [3]. Serotonin blocking was achieved by the addition of 10 or 40 mM serotonin to the training tube, as per Nuttley et al. (2002) [3].

Results

Tph-1 can be food blocked at high concentrations of food.

This study sought to replicate the results of the food blocking experiment as described by Nuttley et al. (2002) [3]. The gene tph-1 encodes tryptophan hydroxylase, an enzyme responsible for the last step of the serotonin synthesis pathway. Therefore, tph-1-null mutants have no endogenous serotonin signaling. This study was unable to food block tph-1(mg280) mutants at a 6X concentration of *E. coli* OP50 (Figure 1A).

However, when the food block condition was performed on *E. coli* OP50 seeded plates (containing a much higher concentration of food), tph-1 mutants were able to be food blocked (Figure 1B). This may indicate the presence of a serotonin-independent mechanism of satiety signaling at high versus low concentrations of food in the environment. The overall lower chemotaxis observed in tph-1 may be attributed to its known developmental defects hindering the assay, such as slowed development, and small brood size [13].

Learning in quintuple mutants can be blocked at a 40mM, but not a 10mM, concentration of serotonin.

This experiment involved a serotonin blocking experiment as per Nuttley et al. (2002) [3]. As seen before, N2 (wild type) worms

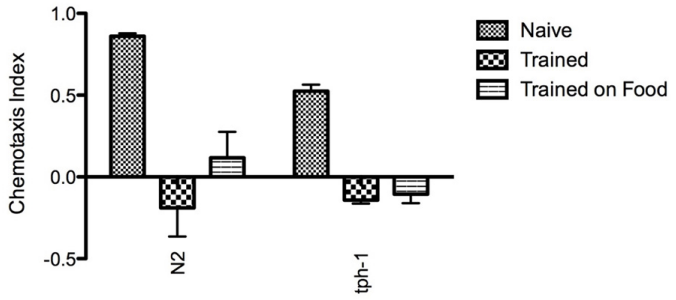

Figure 1A: Food blocking paradigm performed on the tph-1 mutant: Bnz given during training on a 6X concentration of OP50. A partial food block was observed in N2, but no food block was observed in tph-1.

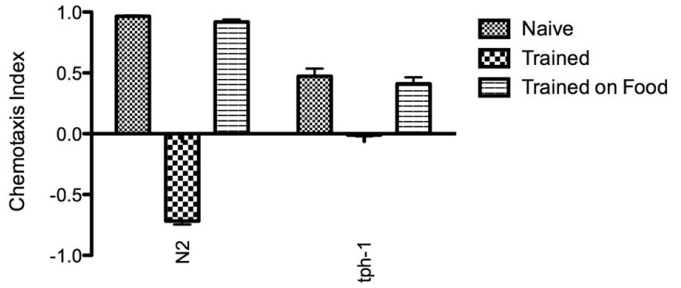

Figure 1B: Food blocking paradigm performed on the tph-1 mutant: Bnz given during training on seeded OP50 plates. A total food block was observed in both N2 and tph-1.

showed a strong serotonin blocking phenotype when trained to Bnz in a 40mM serotonin solution (Figure 2A). A quintuple serotonin receptor mutant, missing the five canonical serotonin receptors (ser-1, ser-4, ser-5, ser-7, and mod-1), was also able to be serotonin blocked at a 40mM concentration of serotonin.

It was then tested whether 40mM was the minimum concentration required to achieve a block of learning. A serotonin blocking dose response curve with N2 and quintuple mutant worms was constructed, testing separately at a 5mM, 10mM, and 40mM concentration of serotonin (Figure 2A, 2B, 2C). N2 worms did not show serotonin blocking at the 5mM concentration (Figure 2C), but were equally unable to form a Bnz-starvation memory in both the 10 and 40mM concentrations. However, relative to N2, quintuple mutant worms showed a greatly attenuated serotonin block at the 10mM concentration (Figure 2B). Therefore, at 40mM, there may be non-specific, low-affinity serotonin binding occurring to

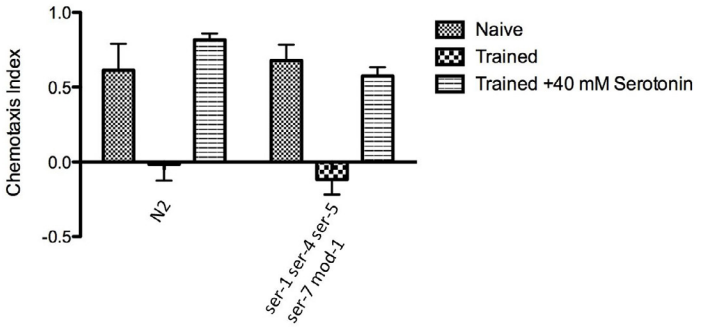

Figure 2A: Serotonin blocking paradigm performed on N2 and the quintuple serotonin receptor mutant (ser-1ser-4ser-5ser-7mod-1), with a 40mM concentration of exogenous serotonin administered during training. Both the N2 and the quintuple mutant exhibited a total serotonin block at 40mM.

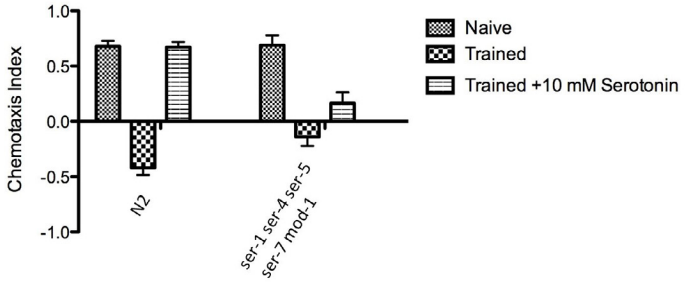

Figure 2B: Serotonin blocking paradigm performed on the quintuple serotonin receptor mutant, with a 10mM concentration of exogenous serotonin administered during training. The quintuple mutant exhibited a greatly attenuated serotonin block, as well as a diminished trained response, in comparison to N2.

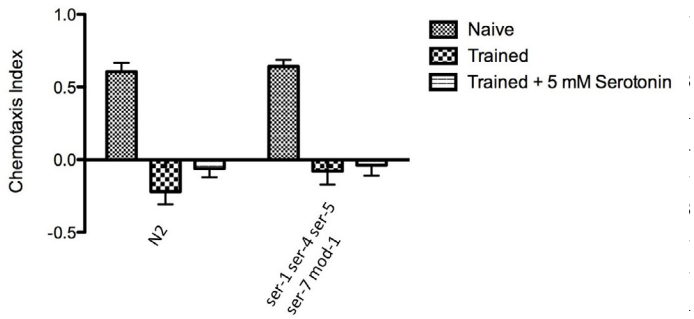

Figure 2C: Serotonin blocking paradigm performed on the quintuple serotonin receptor mutant, with a 5mM concentration of exogenous serotonin administered during training. N2 is unable to be serotonin blocked at a 5mM concentration.

associative memory, too, implicating serotonin signaling in a dual role of satiety state regulation.

Ser-4 recapitulates the attenuated serotonin blocking of the quintuple mutant at a 10 mM concentration of serotonin.

In order to determine which of the five genes in question (ser-1, ser-4, ser-5, ser-7, and mod-1) are necessary for the serotonin block, single or multi-receptor mutants in the serotonin blocking paradigm at a 10mM concentration of serotonin were tested. UT1310, a ser-1ser-3ser-7 triple mutant, was able to be serotonin blocked, indicating that the combination of SER-1 and SER-7 receptors was not the exclusive set necessary for propagating the food- serotonin signal (Figure 3). A mod-1(ok103) single receptor mutant was also serotonin blocked, indicating that MOD-1, too, was not necessary for this satiety stimulus (Figure 4).

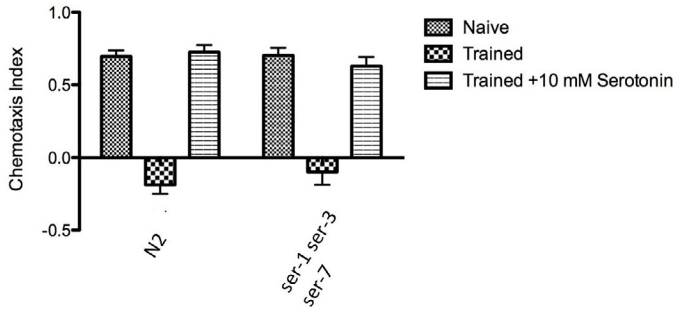

Figure 3: Serotonin blocking paradigm performed on the UT1310 (ser-1ser-3ser-7) triple serotonin receptor mutant, with a 10mM concentration of exogenous serotonin administered during training. Both N2 and UT1310 exhibited a full block of learning.

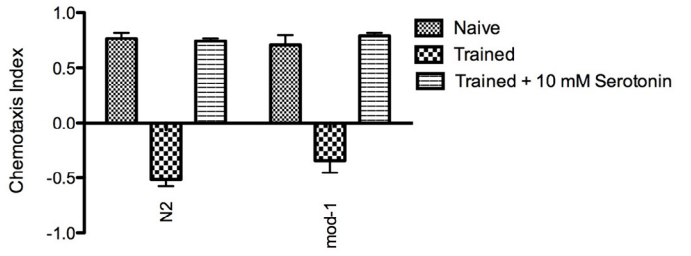

Figure 4: Serotonin blocking paradigm performed on the mod-1 mutant, with a 10mM concentration of exogenous serotonin administered during training. Both N2 and mod-1 exhibited a full block of learning.

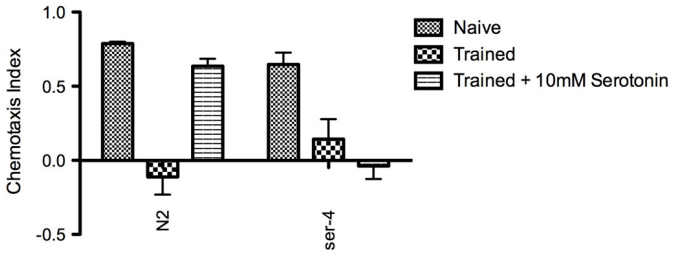

Figure 5: Serotonin blocking paradigm performed on the ser-4 mutant, with a 10mM concentration of exogenous serotonin administered during training. Ser-4 exhibited a greatly attenuated food block, as well as a diminished trained response, in comparison to N2.

However, a ser-4(ok512) single receptor mutant was unable to be serotonin blocked at a 10mM concentration (Figure 5). This data indicates that SER-4 is a necessary receptor for propagating the serotonin-satiety signal representing the absence of the starvation US, as without SER-4 worms are able to form an associative memory between Bnz and starvation in the presence of serotonin. Ser-4 mutant worms also recapitulated the weak Bnz-starvation learning phenotype, first observed in quintuple mutants. Therefore, SER-4 also appears to regulate the association of Bnz with starvation in the absence of serotonin, demonstrating the dual role of serotonin in regulating the starvation signal and mediating associative learning.

Discussion

The full molecular mechanisms underlying the formation and storage of a memory are unknown in even the simplest model organisms. The ability of *C. elegans* to be classically conditioned not only raises questions about the evolution of learning and memory, but also presents a solution to the difficulty of studying the molecular components of learning in more complex animals. With only 302 neurons in the adult hermaphrodite, worms are ideal model organisms in which to study the properties of associative learning, and to understand the nature of memory formation. This paper presented two key findings. First, the serotonin-dependent satiety signal only regulates learning at low concentrations of food; at high concentrations of food, worms have a serotonin-independent satiety signal. Second, the SER-4 receptor is responsible for propagating this serotonin-satiety signal, which negatively regulates the Bnz-starvation associative memory by blocking the formation of the starvation US.

Evidence that serotonin is the primary endogenous food signal in worms came from the observation that the serotonin-synthesis deficient mutant tph-1 is able to associate Bnz and starvation even in the presence of food [3]. However, this study's finding that tph-1 can be food blocked at higher concentrations of food demonstrates the presence of a serotonin-independent pathway mediating satiety state. In fact, while tph-1 mutants require exogenous serotonin to initiate pharyngeal pumping (a serotonin-dependent response to food), a general bacterial extract is sufficient to induce mouth-opening in tph-1, suggesting an unknown serotonin-independent mechanism for feeding stimulation [14].

A dose-response food block with tph-1 would provide more insight into the relative concentrations of food that stimulate each pathway. Due to the metabolic defects of tph-1 affecting its growth and reproductive rates [13] and, subsequently, its performance in the Bnz-starvation assay, it would be interesting to perform the same experiments on cat-4 mutants, which are also serotonin-synthesis deficient but perform better in our associative learning paradigm [3].

This study found that the loss of ser-4 alone recapitulated the attenuated serotonin block and the weak learning phenotype of the quintuple mutant. This is indicative of a dual role of SER-4, in both propagating the serotonin-based satiety signal and in forming the Bnz-starvation associative memory. Exogenous serotonin, or food that stimulates a certain neuron to release endogenous serotonin, signals though SER-4 to inhibit the starvation signal, which subsequently inhibits the association of Bnz with starvation. Therefore, the loss of SER-4 represents the loss of this inhibitory satiety signal, resulting in ser-4 (and quintuple mutant) worms that can associate Bnz with starvation even in the presence of exogenous serotonin.

The loss of SER-4 also results in the formation of a weaker Bnz-starvation association in the absence of serotonin, indicating SER-4 may play a role in memory formation. However, since SER-4 also appears necessary for a satiety signal, it is possible that ser-4 mutants exist in a semi-starved state, resulting in worms exhibiting a weaker aversion to Bnz when paired with starvation. In addition, ser-4 shows a more attenuated serotonin block than the quintuple mutant. This study speculates that the loss of additional serotonin receptors in the quintuple mutant that might inhibit the satiety signal may be responsible for this observation.

Finally, the sole receptor of the quintuple knockout that has not yet been tested is a ser-5 single mutant. Therefore, it is necessary to also test ser-5 in the serotonin blocking paradigm to understand if SER-5 performs alongside SER-4 in regulating the starvation signal.

As of yet, the full molecular mechanisms for the stimulation, secretion, and propagation of the serotonin-satiety signal are unknown. Several neurons are known to secrete serotonin, most of which have been implicated in the regulation of satiety status, including the ADFs, NSMs, and HSNs. While the enhanced slowing response appears to be mediated by mechanosensory stimulation of NSM, in general the upstream mechanisms of food-induced serotonin synthesis are still unknown [2]. To understand which neurons secrete the serotonin involved in the associative learning pathway, the food-blocking paradigm will be used to test single-neuron knockouts of tph-1. Worms unable to synthesize serotonin from a neuron involved in mediating the US-food signal should be resistant to food blocking.

Bnz sensation in *C. elegans* occurs in the AWC neuron, and odourant-starvation integration must therefore occur in either AWC or a downstream neuron [15]. In order to understand where the starvation-US is integrated with the Bnz-CS, ser-4 will be rescued in individual neurons downstream of AWC in a ser-4 mutant background. A restoration of the serotonin blocking phenotype in any of these rescues would implicate the neuron in question as a downstream recipient of the serotonin-satiety signal, specifically though the SER-4 receptor. The consideration of how this neuron connects to AWC would provide insight into where the two stimuli are integrated, and where the associative memory is formed.

Conclusion

This study used the Bnz-starvation classical conditioning paradigm to explore the molecular components of this CS-US association. The data outlined a pathway in which the presence of food

activates a neuron to synthesize and release serotonin onto a SER-4 receptor, blocking the starvation signal, and thereby inhibiting the Bnz-starvation association. By delineating the role of serotonin and serotonin receptors in regulating the starvation signal, as well as understanding which neurons are necessary for this pathway, insight can be gained into the nature of stimuli integration and memory storage. This may be applicable to higher-level organisms on a broader scale to investigate a fundamental question: what is a memory?

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JULS RESEARCH

Sugars in space: *quantum chemical* study on the formation of glycerone from formaldehyde and glycolaldehyde on interstellar medium dust grains

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Abstract

Among many theories on the origin of life, the interstellar medium (ISM) is hypothesized to provide prebiotic material on Earth. The ISM refers to regions between star systems in a galaxy. Simple sugars, including glycerone, are confirmed to exist in ISM and can be intermediates in the formose reaction.

In the studied segment of the formose reaction, glycolaldehyde and tautomers of formaldehyde were hypothesized to form glycerone. The theorised mechanism was validated through quantum chemical calculations. An exothermic and exergonic pathway favourable in ISM conditions was found, giving a possible mechanism for glycerone formation.

The products in question participate in energy production (the phosphorylated form of glycerone, DHA-P, participates in glycolysis) and storage (glycerone is the source of the glycerine backbone in lipids). The studied reaction is a segment of the formose reaction—further polymerization can lead to pentose (ribose) and hexose, which take part in the formation of RNA and DNA. Hence, this research investigates the hypothesis of exogenous production and delivery of prebiotic material to Earth, building up to the origin of rudimentary lifeforms. Such a process could apply to extra-terrestrial planets as well—indeed, prebiotic material on ISM dust grains could give provide life supporting conditions on other favourable planets.

Keywords: *Astrobiology, astrochemistry, interstellar medium, reaction mechanism, quantum chemical calculations, glycerone*

Introduction

Since the 1950s, researchers have been investigating the origins of life and the formation of prebiotic material. Specifically, the Urey-Miller experiment famously illustrated that given enough energy input into a system, it is possible to form rudimentary prebiotic material from four simple species: H_2O, H_2, CH_4, and NH_3 [1].

For the synthesis of prebiotic material, the conditions in interstellar medium (ISM) are more promising than on early Earth. In ISM, matter aggregates on "silicate/carbonaceous" dust, which range from 0.1-10μm in size and contain heavier elements that could have catalytic properties, such as Fe, Ni, and Zn [2]. Organic species, which account for 75 percent of material in interstellar space, aggregate on these dust surfaces and form "ice mantles" [2,3]. Experimental findings suggest that these surfaces may catalyze the formation of prebiotic material with varying complexities, by using elemental and small substrates found in ISM (i.e. N_2, CO, H_2) to form simple sugars (CH_2COCH_2) and organic acids ([C_6H_6]COOH) [4]. Moreover, the hypothesis that prebiotic material could be formed in the above manner is supported by studies comparing mass spectroscopic data on interstellar dust and computer simulations of the Urey-Miller experiment [5]. Investigations into data from the mass spectrometer on board the Giotto spaceship did not rule out the possibility that processes similar to those of Urey-Miller could take place on ISM dust [5].

The formose reaction mechanism, theorized in the 1850s and predating the Urey-Miller experiment, probed into the question of prebiotic material in space. The reaction describes a mechanism through which formaldehyde polymerises into increasingly complex sugars, such as: triose, tetrose, pentose, and hexose sugars [1,6]. Initially, the formose reaction was determined experimentally under basic conditions [1].

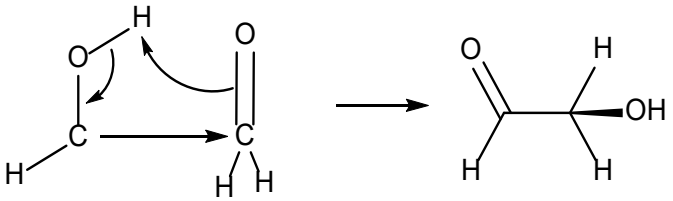

Figure 1: The first step of the formose reaction: Trans-hydroxy methylene reacts with formaldehyde to form glycolaldehyde [7].

After the first step of the formose reaction (Figure 1), the glycolaldehyde can further react with formaldehyde to form longer carbon chains, adding formaldehyde onto the glycolaldehyde.

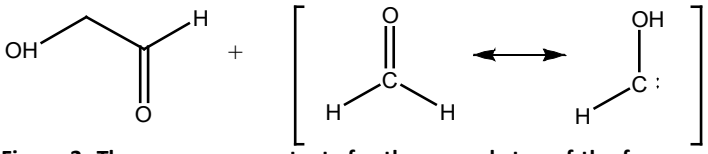

Figure 2: The necessary reactants for the second step of the formose reaction in ISM: formaldehyde, formed from the tautomerization of methanone to hydroxy carbene, and glycolaldehyde (2 hydroxy ethan-1-one).

The reactants, formaldehyde, and glycolaldehyde of the second step (Figure 2) are present in sufficient concentration in ISM [8-10]. Materials of biological significance can start to emerge in the second step of the process, beginning with the formation of glycerine, which is shown in Figure 3.

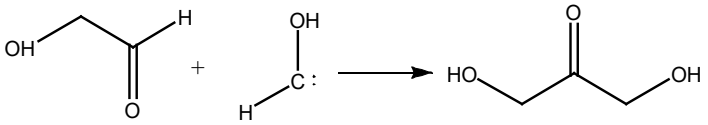

Figure 3: The product of the second step of the formose reaction, glycerone (1,3 dihydroxy propan-2-one or dihydroxy acetone).

The product, glycerone, is biologically significant due to its involvement in metabolic functions in biological systems. The phosphorylated form of glycerone (DHA-P), an integral part of metabolic pathways, participates in the production of ATP through glycolysis (energy storage). Furthermore, DHA-P can be a source of the glycerol backbone in fatty acids.

Glycerone is a simple sugar required for the formation of simple life forms—without it, bacteria and related microorganisms could not exist. Moreover, these products can further react with formaldehyde and other similar species to polymerize into more complex compounds, such as sugars and amino acids, in ISM [7]. With further formose polymerization, pentose monosaccharides (ribose) appear, which could further polymerize into five or six membered rings, approaching crucial carbohydrates found in metabolic pathways. Among its five isomers, ribose could combine with phosphate groups to form DNA and RNA (Figure 4) [11].

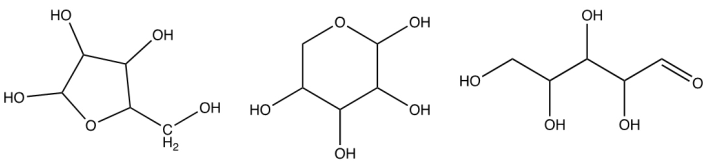

Figure 4: Chemical structures of three biologically significant isomers of ribose, including the five-membered ring that gives rise to the backbone of RNA and DNA [11].

If the Urey-Miller experiment and formose reaction were to happen in ISM, it would imply the possibility of prebiotic material or even simple biology in space. However, this does not empirically conclude that life can occur in ISM.

In ISM, the temperature is very low (10K), which could halt the procession of reactions [12]. Matter is also sparsely scattered in ISM [12]. The combination of low reagent concentrations and low temperatures imply a low reaction rate in ISM. Moreover, the formose reaction might not be applicable due to variations in experimental conditions on Earth and those in ISM. Notably, the *in vitro* formose reaction uses highly concentrated formaldehyde and is aided by alkaline catalysts. Other doubts exist, as biologically significant sugars could only be produced if the reaction "ceases after a brief reaction time" [1].

The solution to most of the above issues can be seen in the following equations:

Eq. 1 $$r = k[\mathrm{A}]^x[\mathrm{B}]^y$$

Eq. 2 $$k = Ae^{-E_a/(RT)}$$

Low concentrations and temperatures, which imply low rates of reactions, can be counteracted with the abundance of time in ISM. Another consequence of the above equation is that reactions with a low E_a would be heavily favoured over those without an E_a. The solution to this may be alternative energy sources. Previous studies have suggested that in ISM environments, the electric sparks in Urey-Miller's setup may be substituted by "ion bombardment and UV radiation" [4]. The photocatalysis of reactions are proven to be possible in simulated environments of ISM, as evidenced by the formation of glycerol and glycolic acid in one study [13]. Additionally, the continuous polymerization of sugars (over-polymerizing useful sugars) does not rule out the formation of prebiotic material. This would depend on the kinetics of each step in the formose reaction and those of other side reactions in the complex ISM environment.

Hence, if the formose reaction is applicable in space, it would be possible for prebiotic material to accumulate in sufficient abundance to form simple lifeforms in ISM. Such hypothetical lifeforms in space would have an extremely low metabolic rate due to the lack of free energy and material around them.

If material of biological significance existed in ISM dusts grains, the second implication would be the origin of life on Earth. Every year, 3×10^7 kg of extraterrestrial material impacts and enters Earth [14]. The exogenous delivery of interstellar dust grains could provide sufficient amounts of prebiotic material, eventually leading to simple lifeforms [14]. This would shed light on the seminal question of the origin of life on Earth [14].

Studies also demonstrate that simple organic material, such as glycolaldehyde, can survive simulated conditions of meteorological impact by increasing in complexity during impact. The synthesis of prebiotic material during entry into Earth is another theory regarding the origin of life supporting conditions on Earth [14].

Scope

This paper uses quantum chemical calculations to determine the feasibility of the second step of the formose reaction, the forma-

tion of glycerone from glycolaldehyde and formaldehyde, in space. During the reaction, formaldehyde can exist in various forms as it has three constitutional isomers (Figure 6).

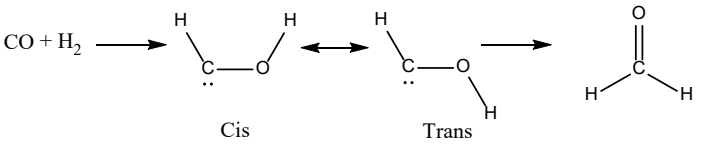

Figure 6: The formation and tautomerization reactions of formaldehyde [15].

The cis isomer is theorized to be ideal for forming the reaction complex that yields glycerone (Figure 7). The nucleophilic carbene can attack the electrophilic carbon on the glycolaldehyde and form glycerone.

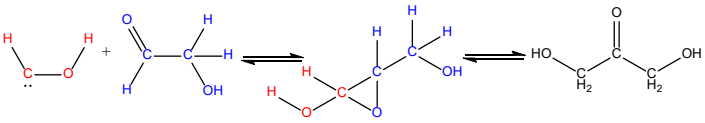

Figure 7: The hypothetical mechanism to form glycerone from formaldehyde and glycolaldehyde.

In this study, the thermodynamics and properties of the reactions were determined *in silico*. Through the thermodynamic properties of the reaction, its kinetics and, consequently, its plausibility in ISM, could be approximated.

A similar computational study was carried out by Jalbout (2008) on the formation of triose and tetrose in ISM and is used as literature in the results of this study [3]. An important difference is the molecules that were studied: Jalbout (2008) studied three- and four-membered carbon chains without any carbonyl group bearing hydroxy group on each atom [3]. However, due to other similarities, the thermodynamic properties found in this study are expected to be similar. Jalbout (2008) found that the formation of trioses from diose and formaldehyde was associated with a difference in energy of -20kJ/mol and an activation energy of approximately 55kJ/mol.

Slight differences are expected to be found in this paper. Specifically, the carbonyl group studied in the suggested reaction provides an electron deficient carbon atom to initiate the reaction, potentially lowering the activation energy for synthesizing glycerone from formaldehyde and glycolaldehyde. Hence, based on the findings of Jalbout (2008), an exothermic reaction is expected to be found, along with a positive activation energy.

Methods

The quantum chemical calculations for the hypothesized reactions were calculated using Gaussian 09 [16,17]. The molecules were optimized using the density functional theory (DFT) method at the B3LYP level of theory and the 6-31G(d) basis set [18,19]. The total energy (ΔE_{total}), Gibbs free energy (ΔG), and enthalpy (ΔH) of the reaction were analyzed. The precision of the calculations was further refined with single point calculations using the second order Møller-Plesset perturbation theory (MP2) with 6-31G(d) basis set [20]. The pressure and temperature were set according to the conditions in ISM, at 0atm and 15K, respectively.

Thermodynamic properties of each of the studied molecules were calculated relative to the reactants (the formaldehyde and glycolaldehyde mixture). Knowing the quantified properties of the reaction between formaldehyde and glycolaldehyde, the viability of the reaction in question was fully examined.

Results and discussion

The results focused on the changes in Gibbs free energy, since the reaction was conducted in an open and constant pressure environment. The results were compiled into an energy profile, shown in Figure 9.

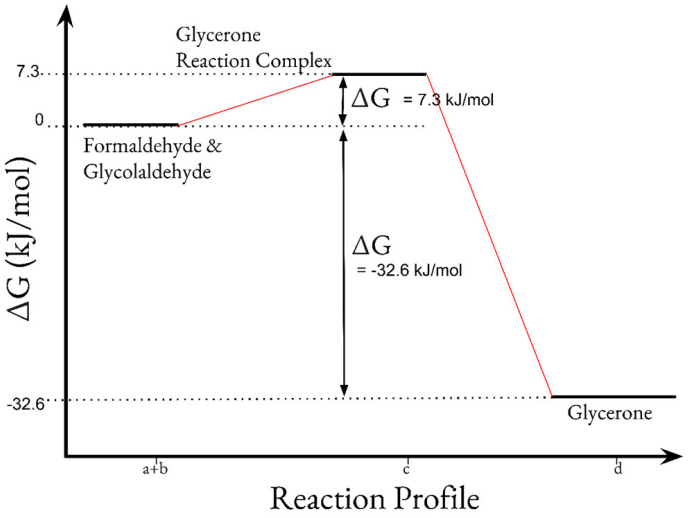

Figure 9: Changes in Gibbs free energy as the reaction progressed.

As previously hypothesized, an exergonic reaction was found, giving a ΔG of -32.6kJ/mol during the synthesis of glycerone. The reaction was not free of barriers and the formation of the reaction complex required an activation energy ($\Delta G^{\ddagger}$) of 7.3kJ/mol. Throughout the process, the hypothesized mechanisms were confirmed and glycerone was obtained.

Although the reaction had barriers, the activation energy was small enough that it could potentially be supplied from the environment in ISM. One possible source could be the photocatalysis of the reaction, whereby electromagnetic radiation from nearby stars excites the reaction mixture. Another possible solution to the small but required activation energy could be the catalytic properties of the transitional metal core of ISM dusts, which was not factored into quantum chemical calculations.

The entropy of the entire reaction followed an entirely different trend. There was a decrease in entropy in the reaction from initiation to conclusion, indicating that glycerone was a more structured

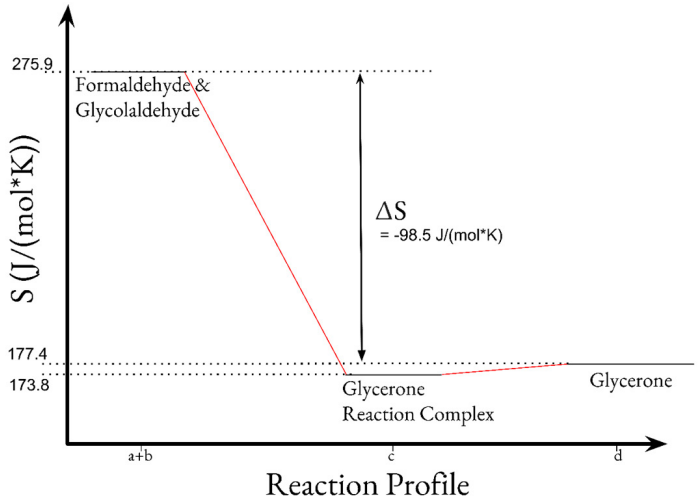

Figure 10: Changes in entropy as the reaction progressed.

arrangement. However, due to the extremely low temperatures in ISM, the decrease in entropy would not have a significant impact on the spontaneity of the reaction, since $\Delta G=\Delta H-T\Delta S$. The low temperature would also imply that the $T\Delta S$ component could have been easily overcome by the ΔH component, indicating that the driving force behind this reaction was the release of heat from differences in chemical potential and not from changes in entropy.

The above findings did not contradict the hypothesized results that were estimated from Jalbout (2008), and indeed followed similar patterns [3].

Significance of findings

The exergonic nature of the reaction and the low activation energy that was needed underlines the viability of the theorised reaction in ISM and provides a possible chemical explanation for the presence of glycerone in space. If the formose reaction proceeds through a similar mechanism, pentoses and hexoses vital to life on Earth could be synthesized in ISM. If confirmed, the exogenous production and delivery of simple sugars could shed light on the origin of life supporting conditions. Specifically, it would provide one explanation of the origins of prebiotic material, those that can be produced in a reducing environment.

Conclusion

Through *in silico* calculations on the possible interactions between formaldehyde and glycolaldehyde, the formation mechanism of glycerone is suggested. This study has outlined the thermodynamic possibility of this mechanism proceeding in ISM conditions. Moreover, through understanding the thermodynamics of each step, this paper has outlined the kinetics behind the second step of the formose reaction.

Combined with further research into this field, findings in this paper could indicate that prebiotic material, including glycerone, could be synthesized and delivered from ISM, confirming the exogenous nature of prebiotic material that could not be produced on Earth. Finally, the above method of investigating the viability of reactions could be applied to other biologically significant species, such as amino acids and DNA structures, to further investigate the viability of synthesizing prebiotic material in ISM.

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Switching sides in Alzheimer's disease: amyloid beta protein as a protector of the brain

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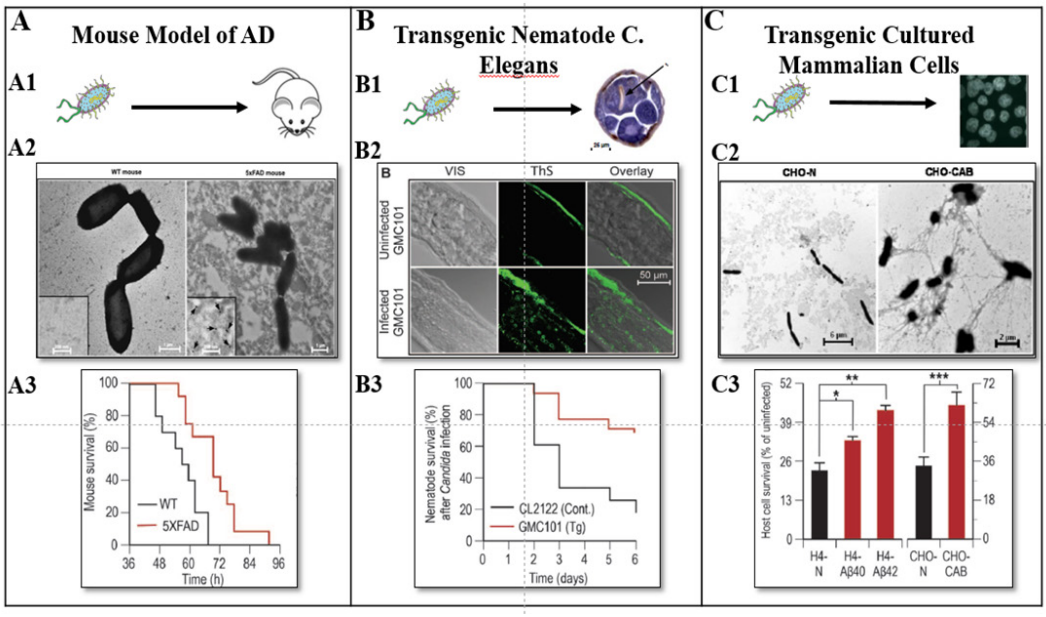
Abstract

For decades, the primary focus of Alzheimer's disease (AD) research has been amyloid beta (Aβ) proteins, due to their toxic effects on neurons and contributions to disease pathogenesis. Aβ is characterized as a catabolic by-product and is involved in protein trafficking pathways as a ligand. While the physiological function of Aβ in the brain remains unclear, some of its biological properties such as oligomerization and fibrilization are a common characteristic of antimicrobial peptides (AMPs). Latter activities of AMPs are important in the innate immune system because they act as broad-spectrum antibiotics that target bacteria, viruses, and cancerous cells. The similarity between these two peptides raises the intriguing question of whether Aβ's involvement in AD is a protective response to a neural infection. This question was recently tested on transgenic mice, *C. elegans,* and cultured cells that overexpressed Aβ. Bacterial infection caused accelerated Aβ deposition in the brain of the AD mouse model. Moreover, Aβ fibrils prevented pathogens from binding to host cell wall, which led to entrapment of unattached microbes in all transgenic models. These findings also suggest that Aβ oligomerization, which has been accepted as a pathological event, may be the innate immunity's response to AD pathology. This review focuses on this novel protective-damaging dual role of Aβ in AD progression that was recently proposed by Kumar et al. (2016). Better understanding of this complex pathology of AD requires a broader view of the disease progression and new perspectives. These findings have important implications for understanding the pathogenesis of amyloidosis in AD. Excessive Aβ deposition may arise not from an intrinsically abnormal propensity of Aβ to aggregate, but rather may be mediated by dysregulation of the brain's innate immune system. For example, this may include the consequence of an immune response mounted to microbial or sterile inflammatory stimuli. Importantly, this study is congruent with the amyloid hypothesis and the importance of Aβ in the neurodegenerative cascade of AD. However, the pathophysiology of Aβ may not only arise from abnormal stochastic behavior, but rather from dysregulated antimicrobial activities.



Visual Abstract. Amyloid beta inhibits pathogenic infections in Alzheimer's model of transgenic mouse, *C. elegans*, and cultured mammalian cells. A1), B1), C1) Transgenic mice, *C. elegans*, and cell cultures that overexpress amyloid beta were infected with *S. typhimurium*, pathogenic *C. albicans* (Candida), and *S. typhimurium*, respectively. A2) Infection induced amyloid beta fibrilization associated with insoluble deposits in transgenic mice (5XFAD) that is labelled with black arrows. B2) Infected transgenic *C. elegans* (GMC101) showed enhanced Thioflavin S fluorescence, amyloid beta plaque formation. C2) Amyloid fibrils bound to *S. typhimurium* cells in CHO-CAB bacterial aggregates. A3), B3), C3) Increased survival chance of Aβ-overexpressing transgenic 5XFAD mice, GMC101 *C. elegans*, and cells lines (CHO-CAB) were observed after the bacterial infection. Adapted from Kumar et al. (2016) [4].

Introduction

Alzheimer's disease (AD) is a common neurodegenerative disease and one of the leading causes of death in elderly people [1]. The disease pathology is recognized by amyloid beta plaques and neurofibrillary tangles, and there is currently no cure or treatment for this lethal disease [2]. Amyloid beta (Aβ) proteins have been the focus on AD research, since they lead to disruption in neural communication and eventually neural loss [2]. However, the function of Aβ in a healthy brain remains unclear [3].

A recent study by Kumar et al. (2016) suggests that Aβ can be categorized as antimicrobial peptides (AMP), since they share similar biological properties [4]. Moreover, they propose a novel, dual role for Aβ—as a protector and damaging agent in AD [4]. In this review, the suggested role of Aβ as a protector of microbial infection in AD pathogenesis will be discussed and compared with its well-studied characteristics in AD. This review will emphasize the findings that accelerated Aβ deposition may have corollaries for amyloidpathies beyond AD. Moreover, this review will discuss how Aβ may be physiologically similar to antimicrobial peptides. Although the findings are suggested to be congruent with amyloid cascade hypothesis, further research is needed to understand the role of normal antimicrobial activity of Aβ in AD pathology. For this reason, this review will end with a set of proposed experiments for future studies to test the validity of these findings.

Misfolded and aggregated proteins are common in neurodegenerative diseases

Most neurodegenerative diseases in humans are a result of intra or extracellular misfolded, aggregated, or ubiquitinated proteins causing neuron loss [5]. Diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, and many others share this common pathological characteristic, regardless of whether they are chronic or progressive [6]. Aβ are common proteins that show aggregation [7,8]. Although they exist in healthy individuals, their abnormal folding and deposition on the extracellular side causes amyloidosis diseases [7]. These depositions can also occur in other parts of the body, such as the kidneys and heart [9]. For instance, their damage to nerves and the brain leads to cognitive, motor, and sensory deficits [7,8]. Despite the fact that Aβ proteins are the hallmarks of AD, their function in non-pathological states remain unclear [3].

Alzheimer's disease is a type of amyloidosis disease

AD is one of the most common types of neurodegenerative diseases, accounting for 60-80 per cent of dementia cases [10]. The neurodegeneration seen in AD is a result of extracellular Aβ plaque formation and intracellular accumulation of neurofibrillary tangles [2]. Aβ is a catabolic by-product of amyloid beta precursor protein (APP), a transmembrane protein [2]. The most commonly accepted pathological model of AD is the amyloid cascade hypothesis, which posits that Aβ aggregation due to mutations initiates plaque formation and neuronal death [11]. Although this theory can accommodate new findings to some extent, it has been heavily criticized for failing to explain the complex relationship between spatial and temporal properties of AD pathology [12]. Additionally, head trauma and environmental factors such as smoking have been suggested to increase the risk of AD, but the exact mechanisms and risk factors affecting accelerated amyloid plaque formation are not well-understood [13,14].

Aβ is common in infectious diseases and Alzheimer's disease

Aβ deposition and aggregation is not specific to AD—it is also observed in prion protein diseases such as Creutzfeldt-Jakob disease and chronic infections such as tuberculosis [15,16]. The similar pattern of Aβ aggregation in these diseases suggests that the amyloid plaques trigger neurodegeneration in several diseases in the same manner [17]. Therefore, it is important to determine what AD has in common with these infectious diseases besides being categorized as amyloidosis.

This question highlights the necessity of identifying the function of Aβ in healthy individuals, because then their behaviour in pathological stages would be clearer. Animal studies show that Aβ is actually necessary for a healthy life. While the consequences of Aβ production depend on where and how Aβ are cleaved from APP, knocking out Aβ or APP may worsen cognitive deficits and even be lethal [18]. Moreover, several infectious agents such as herpes simplex virus type 1 (HSV-1), *Helicobacter pylori*, and *Borrelia burgdorferi*, are suggested to be involved in AD, since they are detected in older brains and cause cognitive impairments [19,20]. This necessitates research into the relationship between Aβ and such infectious pathogens.

Is Aβ accumulation a defence mechanism?

These speculations have led research to consider the possibility that Aβ aggregation in AD may be a defense mechanism against an infectious pathogen. Aβ was shown to be involved in many different biological pathways and exhibit pro-inflammatory activities [21,22]. Again, its physiological role remains unclear [23]. Previous studies have shown that AMPs, specifically LL-37, exhibit similar biological features and abnormal activities in pathological states as Aβ [24]. AMPs are a part of the innate immune system and defend the body from pathogens, and aggregate into amyloid plaques in diseases such as human amyloidopathies [25]. Since Aβ exhibits similar antimicrobial activities, it has been suggested to be a part of the innate immunity [24].

To validate the protective role of Aβ in vivo, Kumar et al. (2016) tested this idea in transgenic mouse (5XFAD), cultured cell, and *C. elegans* (GMC101) models of AD [4]. These models were specifically overexpressing Aβ in the brain. To create infection, Salmonella was injected in the mouse model and *C. albicans* and *S. typhimurium* were injected in the cell culture and *C. elegans* models. Their findings showed that the Aβ deposition increased the survival rate of all three models and increased their resistance to infections [4]. Moreover, they proposed a protective activity of Aβ in an infectious state: when Aβ binds to the carbohydrate wall of a pathogen, microbial adhesion is disrupted and unattached pathogens start agglutinating [4]. Although most research has focused on amyloid plaques as the mediator of AD, Kumar et al. (2016) propose a novel dual protective/damaging role of Aβ [4]. Although further research is certainly needed, their findings may suggest a new perspective on AD research and different treatment options.

Findings

Aβ increased the survival rate and resistance against infections in Alzheimer's disease models

The survival rate and resistance to infection of transgenic mice increased significantly after infection with *S. typhimurium* (Figure 1A and 1B). There was no upregulation of immune activation in

the mice, which indicated that inflammation was not naturally protecting them against infections. Similarly, transgenic *C. elegans* cells overexpressing Aβ in the gut and muscles, human brain neuroglioma (H4) cells, and Chinese hamster ovary (CHO) cells transferred with Aβ secreting cells all showed increased survival and resistance to infections (Figure 1C, 1D, 1E, and 1F). These results are consistent with the previous research by Soscia et al. (2010), which showed that Aβ was activated against several pathogens [24]. However, the correlation between Aβ levels and the chance of survival during an infection is novel and has not been observed before [4].

Aβ oligomerization and fibrilization induced antimicrobial activity and bacterial agglutination, respectively

Aβ and LL-37 exhibit similar microbial activities [24]. First, LL-37 causes the unattachment of pathogens from host cells [4]. Unattached cells then agglutinate and prevent microbes from binding the host cells [4]. These inhibition and agglutination behav-

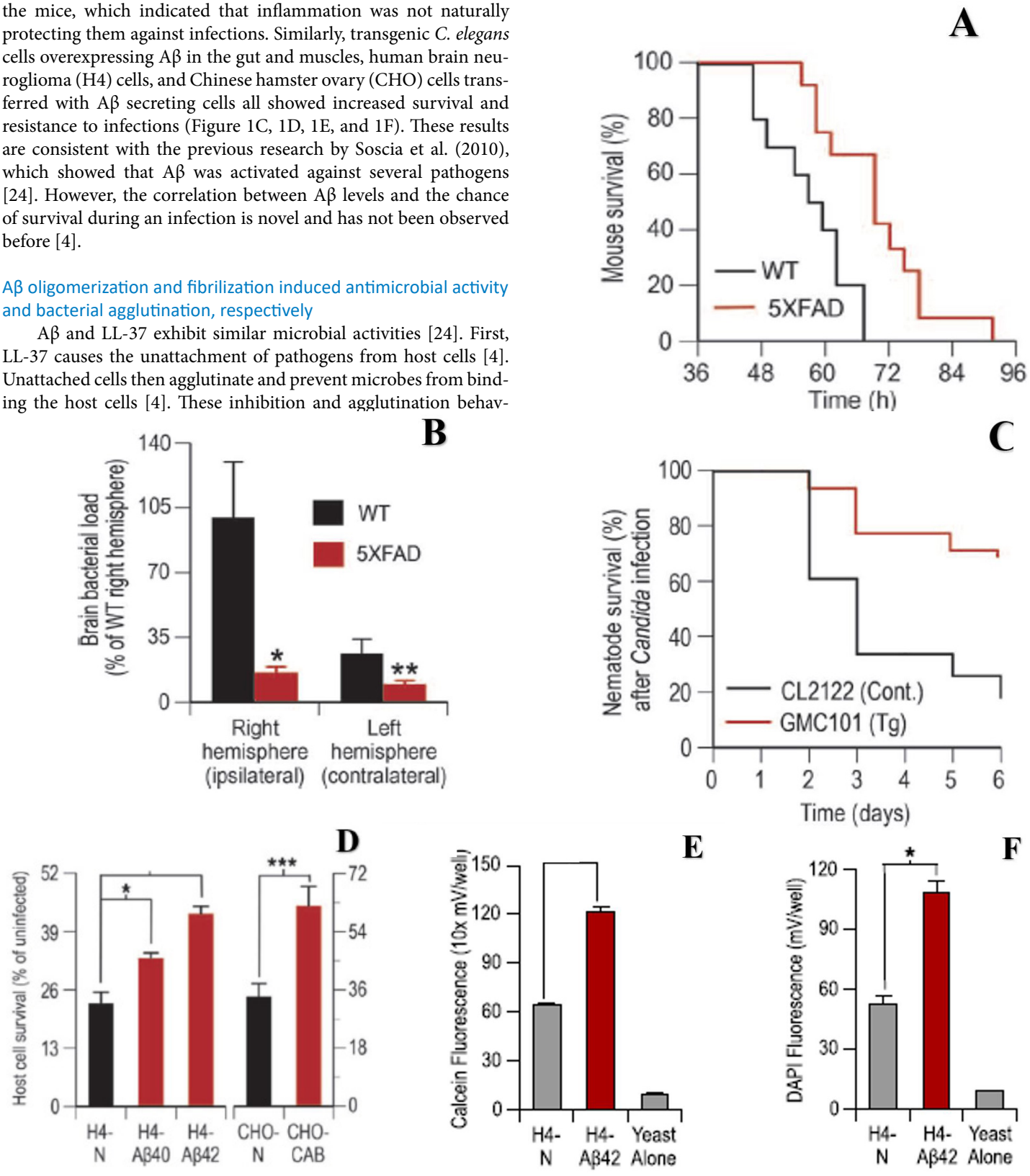


Figure 1. Increased survival rate of transgenic mice, cultured cells, and C. elegans following a bacterial infection. A) survival chance of AD mouse model was higher than the wild type, and APP knock-out that does not produce any Aβ had the lowest survival chance. B) Brain bacterial load was significantly higher in the wild type mouse that did not express Aβ depositions indicating that Aβ deposition, in fact, decreased bacterial infection. C) Following the infection, survival chance of transgenic mice (GMC101) was higher than that of the control group (CL2122). D) H4 and CHO, the cell cultures overexpressing Aβ, showed increased survival rate. E), F) To detect intact cells, two different staining, namely Calcein and DAPI, were used. H4-AB42 cells that overexpressed Aβ showed higher number viable cells than the wild type host cells (H4-N). Reprinted from Kumar et al. (2016) [4].

iours were confirmed for Aβ, where transformed cells had fewer *C. albicans* attached to their monolayer (Figure 2A). Agglutination activity of yeast cells was also observed in the transformed cells (Figure 2B). Moreover, fibrilization of AMPs is protective against pathogens [4]. Based on the findings from Kumar et al. (2016), the fibrilization of Aβ may provide a similar outcome [4]. Visualizing microbial cells with transmission electron microscopy (TEM) revealed that microbial cells were involved in the Aβ fibril structures, since *C. albicans* cannot generate fibrils (Figure 2C, 2D).

Aβ targets and binds yeast cells in transgenic mice and C. elegans models

It was previously suggested that bacterial-induced Aβ forms Aβ plaques in AD [26]. Similarly, Kumar et al. (2016) showed that transgenic *C. elegans* (GMC101) had increased amounts of Aβ fibrilization in the gut and young transgenic mice, which normally lack Aβ plaques, had increased amounts of Aβ deposition (Figure 3)[4]. The colocalization of Aβ and bacterial cells may indicate that Aβ formation was induced by bacterial infection. This idea of bacterial-induced Aβ formations has been tested in other animal studies [27]. For example, the injection of *H. pylori* into mice increased the concentration of Aβ and led to cognitive deficits [27].

Critical analysis

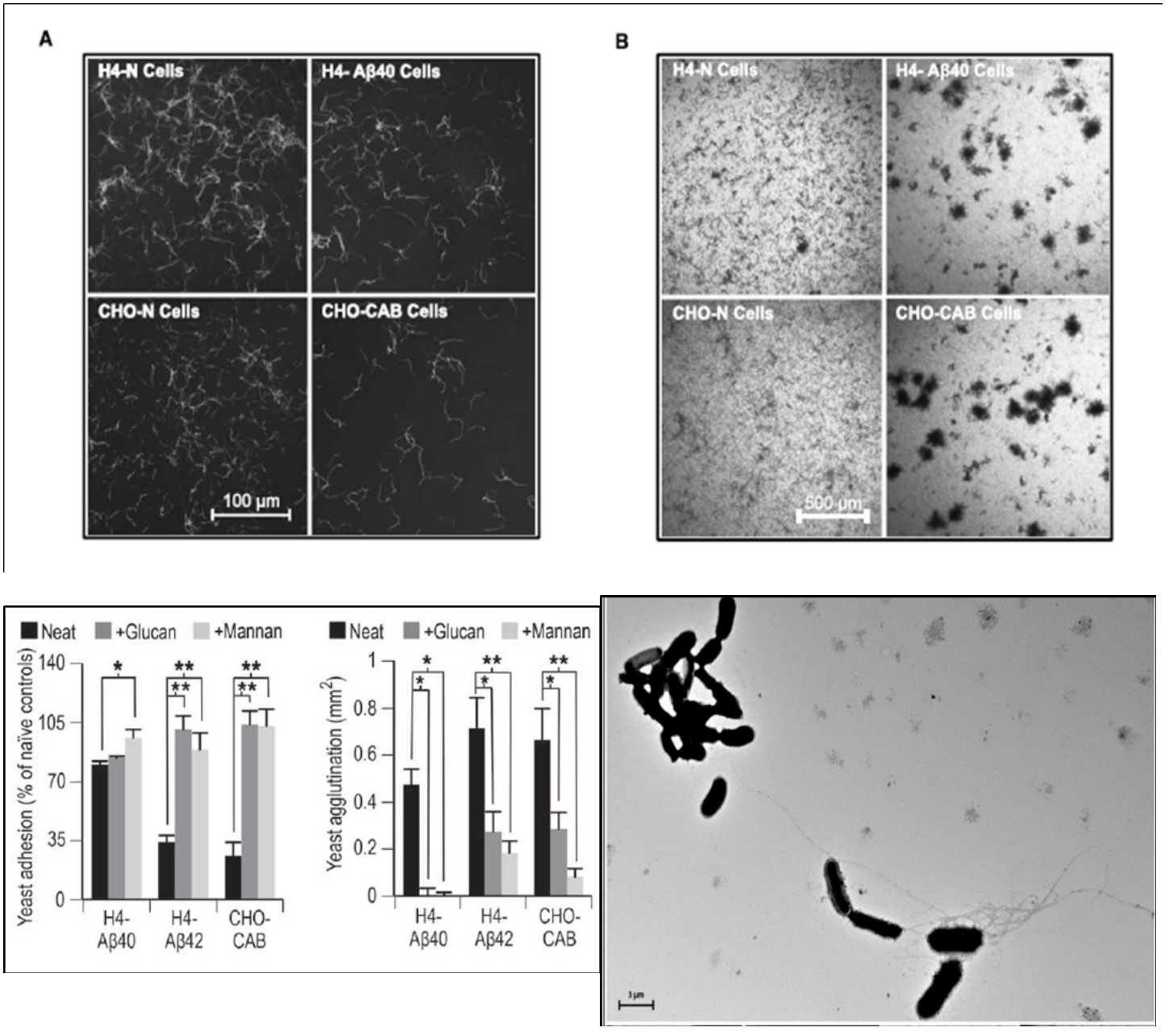


Figure 2: Adhesion inhibition and agglutination activities of Aβ oligomers and fibrils against *C. albicans* in cell cultures. A) *C. albicans* adhesion was shown through Calcofluor-white. Untransformed cells, H4-N and CHO-N, exhibited more yeast adhesion compared to the transformed cells that expressed Aβ. B) Transformed cells, H4-AB40 and CHO-CAB, showed agglutination of *C. albicans.* C) Carbohydrate inhibited Aβ binding to cell wall, which led to higher levels of yeast adhesion and thus lower levels of agglutination. D) Agglutination of yeast associated with Aβ fibrils. Reprinted from Kumar et al. (2016) [4].

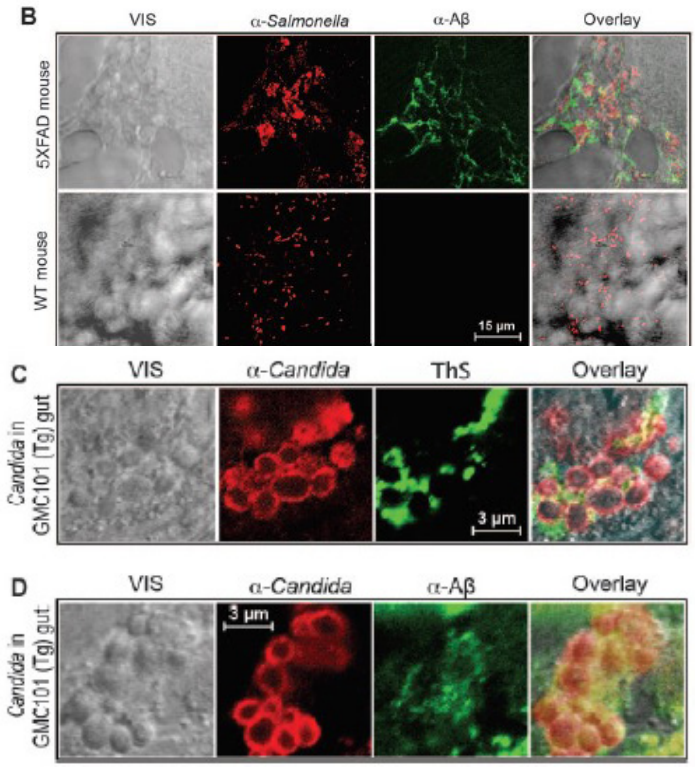


Figure 3: Bacterial infection induced Aβ fibrilization in transgenic mice brains and *C. elegans* guts. B) Immunoreactivity of alpha-Salmonella and the fluorescence staining of Aβ seem to colocalize in the transgenic mouse brain, implying that the Aβ deposits are induced by the infection. Reprinted from Kumar et al. (2016) [4].

Kumar et al. (2016) systematically tested the immune activity of Aβ in response to infections in three AD models [4]. Moreover, the mechanism of antimicrobial activity of Aβ was investigated in detail. The study outlined the protective activity of Aβ as three steps in the following manner: Aβ binds to carbohydrates in the cell wall of pathogens, and the growing Aβ fibrils prevent microbial adhesion and eventually trap unattached microbial cells [4]. These results are consistent with previous research that demonstrated that antimicrobial proteins, including Aβ, are attracted to the the cell walls of pathogens and bind and attack these pathogens [28,29].

Kumar et al. (2016) showed that Aβ increased pathogen resistance in the AD models [4]. However, the immune response of APP was not taken into consideration [4]. APP plays a role in the immune system of the central nervous system [30]. For example, APP responds to head injuries, and overexpression of APP activates microglia and cytokines [31,32]. Kumar et al. (2016) found increased survival rates and resistance to infections in their AD models [4]. However, knock-out cells, such as APP-KO mice, exhibited Aβ-mediated loss of pathogen resistance [4]. Therefore, they concluded that the decreased pathogen resistance in the APP-KO mice may be due to the absence of Aβ [4]. However, they could not explain whether the decreased pathogen resistance in the knock-outs was due to the absence of APP and its immune response or the lack of the protective activity of Aβ. BACE1 knock-out models, which lack beta-secretases that are involved in APP cleavage, could address this issue. Comparing the survival rate and pathogen resistance of BACE1-KO to APP-KO can clarify whether the antimicrobial activity of Aβ in infections is mediated by APP activity or completely based on Aβ.

Although Kumar et al. (2016) showed that Aβ formation inhibits pathogens, there is no clear link between Aβ formation and the most common infections reported in AD [4]. It was previously shown that the infectious pathogens such as HSV-1 and *H. pylori* are involved in AD [19,20]. HSV-1 alters the processing of APP and thus increases Aβ accumulation in the brain, leading to AD pathology [33]. Moreover, Kumar et al. (2016) demonstrated that Aβ fibrilization was associated with bacterial infections [4]. Aβ fibrilization was seen on the surface of the cells that infected transgenic *C. elegans*, which may be the main reason for infection resistance [4]. However, the bacterial infections utilized were simple and have not been shown to be directly involved in AD pathology [4]. Thus, it cannot be concluded that the protective activity of Aβ shown in the study is what would be observed in AD pathology [4]. Future research should consider more complex infections, such as HSV-1 and *H. pylori*, for more accurate results.

Conclusion

Aβ proteins are produced in healthy human brains, but can lead to neurodegeneration and AD in misfolded and excessive conditions [34,35]. However, recent novel findings by Kumar et al. (2016) suggest that Aβ deposition is not necessarily the cause of AD pathology, but rather a consequence of neural infection or a type of inflammatory stimuli [4]. Kumar et al. (2016) propose a protective/damaging duality as the new role of Aβ in AD pathology [4]. The protective role of Aβ, the formation of amyloidogenic fibrils that trap pathogens, is similar to AMPs, such as LL-37. These findings present a new perspective in the search for effective treatments for AD.

Currently, pharmacological research in AD aims to reduce Aβ deposition in the brain or decrease beta secretase activity, which is the protease that cleaves APP to form Aβ by-products [10,36,37]. However, as Kumar et al. (2016) propose, if Aβ does have a protective activity, then existing treatments, such as reducing the amount of Aβ, may not be the best way to approach AD [4].

The formation of amyloidogenic fibrils against pathogens by AMPs and Aβ has been compared in several studies to investigate if Aβ fibrilization in AD is also due to its antimicrobial activity. Cell attachment is an important step in the invasion of many pathogens and infections [38]. AMPs contain heparin-binding domains, which lead to the attachment of oligomers to the microbial surface [38]. This causes adhesion inhibition and agglutination of the pathogens [38,39]. Aβ has been shown to contain the same heparin domain [40]. Kumar et al. (2016) showed that Aβ deposition protected the cultured cells by disrupting *C. albicans* adhesion to the host cell [4]. Moreover, Aβ binded directly to *C. albicans* and S. typhimurium and disabled their adhesion to host cells [4]. These findings suggest that Aβ oligomerization enhances bacterial detachment and leads to agglutination by binding to the cell wall itself (Figure 2D, 2E). Thus, the agglutinated pathogens can be trapped easily [4]. This finding is consistent with previous research where elevated carbohydrate binding activity was observed during Aβ oligomerization [40]. Another study showed that protegrin-1, an AMP, also forms amyloidogenic fibrils in infection conditions and exhibits structural properties similar to Aβ [41].

The infectious AD hypothesis is consistent with the risk factors of AD [4]. However, there is no clear evidence of a single infectious agent triggering AD pathology by itself [4]. Based on the findings from Kumar et al. (2016), Aβ appears to have both beneficial and adverse effects [4]. This literature review supporting Aβ as an infection protector can bring a new perspective into pharmacological research on AD treatment, which currently focuses on the removal of Aβ plaques from the brain.

Future experiments

Kumar et al. (2016) identified the function of Aβ in infection conditions [4]. However, the different types of infections that may trigger Aβ deposition in the brain remain unclear. Future studies may systematically investigate the effects of different microbial pathogens on Aβ plaque formation. Kumar et al. (2016) used bacterial infections in their experiments, however, as mentioned before, many infections that are involved in AD are more related to the central nervous system [4]. Some of these pathogens include HSV-1 and *H. pylori*, for example, and may be useful in future research [20]. If Aβ shows antimicrobial activity against these pathogens, then HSV-1 or *H. pylori* may mediate Aβ upregulation. These infected AD models may be used to investigate different mechanisms of Aβ. The findings from these studies would be more applicable since these pathogens are shown to be involved in AD pathology. These results would also contribute to the search for the infection that triggers AD pathology.

Kumar et al. (2016) suggest that Aβ formation in AD is a type of antimicrobial activity against some infections [4]. However, the authors only focused on the activity of Aβ and did not identify the mechanism underlying this immune response [4]. Moreover, they did not investigate the role of APP and other antimicrobial proteins in infection conditions [4]. This inflammatory response may partially or completely depend on APP activities in the brain, since it was previously shown to be involved in the immune activity. For example, future studies may investigate knock-out beta secretase in the brain. Beta secretase is the main protease that cleaves APP from the cell membrane and leads to the formation of Aβ [42]. By using the same methodology as Kumar et al. (2016), BACE1, APP knockouts, and wild type mice can be infected with HSV-1 or *H. pylori* to explore whether the antimicrobial activity of Aβ is mediated by the immune response of APP [4].

Finally, behavioral tests, such as the Barnes maze, may also be conducted before and after infection to validate that the mice do not only exhibit AD-like pathology, but also reflect AD symptoms such as cognitive impairment.

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JULS REVIEWS

The role of uncontrolled neuroinflammation in epilepsy: considerations for novel targets in the search for antiepileptic medication

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Abstract

Epilepsy is a neurological condition that is characterized by recurrent seizures and is addressed by antiepileptic medication and surgery. Although there are 25 antiepileptic medications available, 29 per cent of patients who have epilepsy tend to experience adverse side effects. Also, about 30 per cent of patients do not respond to medication, and only a small subset of that group qualifies for surgical intervention. Therefore, this gap must be addressed when attempting to identify new antiepileptogenic treatments.

Animal models are commonly used for testing and developing new antiepileptic medication. However, even with an abundance of animal models of epilepsy, there has been considerable difficulty in authenticating the likelihood of antiepileptogenic effects in humans, as potential medications appear to have low efficacy on a particular subset of people affected by epilepsy. Consequently, it is essential to explore new targets and therapeutic techniques for pharmacoresistant patients. Emerging evidence now suggests that there might be a relationship between epilepsy and inflammation. Several immune system mediators released by the peripheral and central nervous system (CNS) might play a role in epilepsy.

Seizures result in a combination of chemical, molecular, and anatomical changes, and emerging evidence suggests that there may be a bidirectional link between uncontrolled inflammation and seizures. Models of epilepsy tend to show increased levels of cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-α (TNFα). Therefore, an in-depth critical analysis of current epilepsy models targeting these three cytokines will be reviewed. Targeting cytokines and their downstream pathways in epilepsy models could be a potential strategy in the search for new antiepileptic medication and possibly be a treatment for other neurological disorders.

Keywords: *Epilepsy, antiepileptic drugs, inflammation, cytokines, novel targets, IL-1ß, IL-6, TNFα*

Introduction

Epilepsy is a neurological disorder characterized by recurrent seizures, which are the result of abnormal excessive activity in the brain [1]. Treatment options can vary between antiepileptic medication, dietary restrictions, surgery, and vagal nerve stimulation [2-6]. However, cognitive and intellectual impairments tend to be comorbid in pharmacoresistant patients, which increases resistance to treatment [7]. Other neurobehavioral comorbidities that are associated with epilepsy include depression and anxiety [8,9]. Although there has been research into effective antiepileptic medications, current treatments for epilepsy are ineffective for one third of the population [10,11]. Therefore, it is necessary to explore other mechanisms that could be involved in epilepsy. In light of emerging evidence, several studies suggest a positive feedback relationship between epilepsy and neuroinflammation in the brain [12]. Therefore, a potential medication treatment could involve targeting downstream pathways of brain inflammation in response to seizures [13].

How do seizures manifest?

During the onset of a seizure, several chemical, molecular, and anatomical changes occur, which cause neurons to become excitable and lead to an imbalance of excitatory and inhibitory neurons. Seizures do not necessarily mean too much neural activity, as they can also involve too little activity [1]. Knowing the symptoms or conditions observed during a seizure could be used as an indicator to decide upon the treatment [14]. However, there are forty different types of seizures that are broadly classified into three categories: focal onset, generalized onset, and unknown onset seizures, which makes it difficult to classify the type [15]. Therefore, it could be

helpful to investigate any commonalities among pharmacoresistant patients for a better outlook.

Several factors can lead to the occurrence of seizures, such as single gene disorders, hippocampal sclerosis, meningitis, exposure to toxins, bacterial infection, abnormal cortical migration during development, stroke and other brain injuries, and metabolic disorders [16-21]. The time at which these factors occur plays a crucial role in the manifestation of seizures. For example, thyroid hormone plays a crucial role in the development of the human brain, and both hyper and hypothyroidism can lead to changes in synaptic transmission and structure of the brain [22]. Disruption of maternal thyroid hormone during the first trimester can cause the fetus to become more susceptible to seizures due to an imbalance of neural activity [23]. Similarly, activation of a neural response during development due to maternal stress or infection can also lead to a higher susceptibility to seizures in the fetus [24,25]. Maternal infection can also activate a neuroinflammatory response that can enter the fetus [26]. When inflammatory markers enter the fetus, they can cross the blood-brain barrier and can elicit an immune response in the fetus [25,26]. This premature response can lead to exacerbated activity in the microglia, which can cause exacerbated neurological responses and increase the possibility of developing seizures [25,26].

There are also several neurodegenerative diseases associated with seizures. Approximately 10 per cent of Alzheimer's disease (AD) patients present with symptoms of seizures [27]. Epilepsy can sometimes be observed in patients suffering from neurodegenerative dementia syndrome and in the juvenile form of Huntington's disease (HD) [28,29]. While patients with Parkinson's disease (PD) were previously thought to be without seizures, recent evidence indicates that PD patients also present clinical manifestations of epilepsy [28,29].

The role of the immune system and inflammation

Our immune system plays a protective role in response to foreign substances, pathogens, and injury. When an infection or some brain insult occurs, the immune system releases cytokines, which are soluble effector proteins that initiate, mediate, and terminate immune responses such as inflammation [12]. Recently, it has been suggested that inflammation might play a role in the pathology of epilepsy based on experimental and clinical evidence. For example, pharmacoresistant patients with medial temporal lobe epilepsy seem to show an exacerbated innate and adaptive immune response with overexpressed levels of inflammatory mediators [12,30-32]. Genetic models of epilepsy have also shown overactive glial cell activation and increased numbers of macrophages and neutrophils during absence and spontaneous seizures [33-38]. High activation of macrophages can result in the release of cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-α (TNFα) [30]. Additionally, damage or leakage in the blood-brain barrier is often associated with high levels of inflammation. It is unclear how the blood-brain barrier becomes compromised, but studies have shown that high levels of autoantibodies have been detected in patients who presented symptoms of seizures [39-41].

It is unclear whether enhanced immune responses alone lead to exacerbated seizures, or whether recurrent seizures themselves cause the uncontrolled inflammation. Recurrent seizures tend to show increased responsiveness of astrocytes and microglia [30]. The overactive microglia and astrocytes can cause a greater or prolonged release of pro-inflammatory mediators, which could further lead to a higher susceptibility of seizures [42, 43]. Experimental evidence has shown that disturbing cytokine signaling in transgenic rodents can result in altered seizure susceptibility and increase the levels of pro-inflammatory cytokines, which can also lead to a reduced threshold of seizures in animal models [31,32]. Evidence from clinical trials have also shown that delivering anti-inflammatory medication could successfully reduce seizures in pharmacoresistant models of epilepsy [44-46]. However, it is inconclusive whether these related changes are causative or correlational, as it is difficult to determine whether anti-inflammatory treatments can successfully reduce seizure susceptibility. Studies have linked increasing levels of complement system COX-2, which is an enzyme responsible for inflammation and pain in epilepsy models [47]. Using pro-inflammatory antagonists like COX-2 inhibitors, however, showed no effect when trying to reduce seizures [48]. One explanation as to why there was no effect was because blocking inflammatory processes is a very global approach. Therefore, it could be more beneficial to target a pathway more downstream of the inflammatory mediators, or target pro-inflammatory cytokines. Figure 1 illustrates the possible interplay between seizures and inflammation.

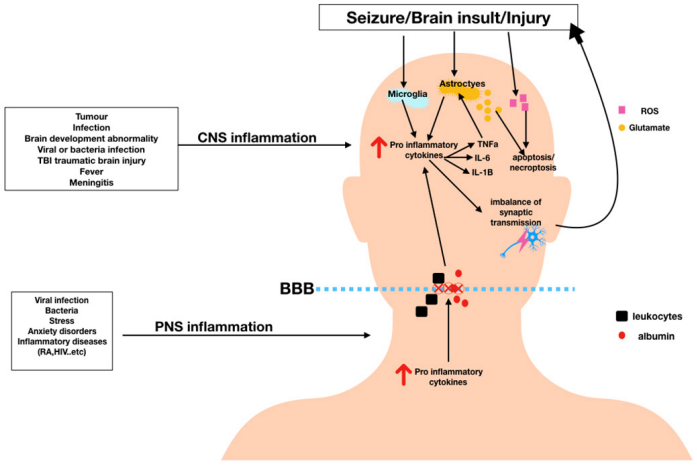

Figure 1: Several conditions can contribute to inflammation in the central nervous system (CNS) or the peripheral nervous system (PNS). For example, viral infections, stress, anxiety disorders, inflammatory diseases such as rheumatoid arthritis and HIV, and several other disorders have shown to increase inflammatory markers in the PNS. The prolonged release of inflammatory cytokines could potentially lead to a loss of integrity in the blood-brain barrier. This, in turn, can allow the entry of molecules like albumin and leukocytes into the CNS, which can trigger the release of more cytokines and induce inflammation in the brain. Following brain injury or seizures in the CNS, microglia, astrocytes and reactive oxidative species (ROS) can become activated. With activated microglia, several pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-α (TNFα) can be released. A downstream effect of these cytokines is an imbalance of neuronal activity and greater release of glutamate in the brain, which can result in necroptosis. The prolonged imbalance of synaptic transmission could potentially lead to exacerbated or recurrent seizures.

Cytokines

Following a brain insult or seizure, microglia release pro-inflammatory cytokines to mediate the immune response, and, when the stimulus from the injury is no longer present, the microglia release anti-inflammatory cytokines to return to the normal state [30]. Through unknown mechanisms, the occurrence of seizures can lead to an exacerbated response in the microglia, resulting in prolonged inflammation and excess release of cytokines [30]. Studies showed that pro-inflammatory cytokines IL-1β, IL-6, and TNFα were increased the most in pharmacoresistant epilepsy patients [32]. When present in high amounts, cytokines have the potential to modulate neuronal activity in the PNS and CNS [49,50]. Although it is not extensively researched, some studies, as mentioned in Table 1, have shown that cytokines can modulate voltage-gated channels and synaptic transmission [31,46,51-56]. Cytokines released from the microglia tend to modulate neurotransmission and also attract macrophages, neutrophils, and lymphocytes, which recruit other cytokines [57]. Although the release of these cytokines is critical during an immune response, their prolonged release can have harmful effects.

The blood-brain barrier can also become compromised due to continued release of cytokines [59-61]. Evidence from epilepsy models have demonstrated that high levels of cytokines tend to be associated with a loss of integrity or greater permeability in the blood-brain barrier [59-61]. Destruction of tight junctions in the blood-brain barrier have also been associated with increased levels of cytokines [62]. There are several consequences of the breakage of blood-brain barrier. For instance, damage to the blood-brain barrier could potentially allow albumin and leukocytes to enter the CNS, further triggering the release of more cytokines [62,63].

Interleukin 1 beta (IL-1β)

IL-1β is an important cytokine known to mediate the inflammatory response [64]. When a seizure occurs, levels of IL-1β and its target receptor, IL-1R1, are increased. Following the activation of the IL-1R1 receptor, various inflammatory genes are upregulated [33]. Higher levels of IL-1β in epilepsy models have been shown to lower the threshold for seizure occurrence by acting on classical neurotransmitters [30,65]. When IL-1R1 is overexpressed, it results in the phosphorylation of the NR2B subunit of the NMDA receptor, leading to enhanced calcium conductance and excitatory glutamatergic transmission [66]. IL-1β is overexpressed by microglia when a seizure occurs, and studies suggest that IL-1β can remain elevated in rodents for two months following a seizure [67,68]. The prolonged release of IL-1B potentially correlates with dysfunction of the blood-brain barrier, and, in turn, potentially leads to upregulation of more inflammatory mediators [61,62]. One study looked at this pathway in alleviating seizures and found that blocking the enzyme that produces IL-1β or blocking the IL-1β receptor reduced the prevalence of seizures in rodent models [69].

Interleukin 6 (IL-6)

IL-6 is another cytokine whose levels increase in response to models of epilepsy [70]. Increased levels of IL-6 can decrease the activity of inhibitory GABAergic neurons and cause an imbalance between excitatory and inhibitory neurons. High levels of maternal IL-6 have also been associated with altered structures in the hippocampus and the prefrontal cortex of a fetus [26,71]. Theiler's murine encephalomyelitis virus (TMEV) has been associated with epilepsy [9]. In one study, IL-6 receptor knockout rodents showed a drastic reduction in seizures, which suggests that inflammatory cytokines may play an important role [9,72]. Some rodent models of epilepsy also showed altered excitability in neurons of the hippocampus due to enhanced expression of IL-6 in the CNS [73]. Vagal nerve stimulation is a beneficial therapy for treating epilepsy disorders [4,55]. Vagal stimulation can activate the HPA axis that releases corticosteroids from the adrenal glands, which has anti-inflammatory properties [4,74]. Further studies showed that vagal nerve stimulation in rodents with synaptic hyper-excitability and seizure-like symptoms reduced the symptoms and reduced levels of IL-6 [55]. High concentrations of IL-6 along with TNFα in the cerebral spinal fluid have also been observed in dogs with seizures [75]. Unfortunately, not many drugs have been developed to observe the anti-seizure effects of IL-6, further research is needed to understand the complex relationship between IL-6 and inflammation [70].

Tumour necrosis factor alpha (TNFα)

Tumour necrosis factor alpha (TNFα) plays an important role in regulating synaptic plasticity and interactions between neurons and glia. High levels of TNFα follow from microglia activation in the brain. Inflammatory cell death, also known as necroptosis, is initiated by TNFα, reactive oxidative species (ROS), and other death receptor activators [76]. A downstream effect of TNFα is its

Cytokine	Study	Type	Function	Outcome
IL-1β	Plata-Salaman et al. (1994), Vezzani et al. (2000), Wang et al. (2000), Vezzani et al. (2005), Balosso et al. (2008)	Pro-inflammatory	Inhibits calcium currents through protein kinase C Inhibits $GABA_A$ receptor	Neuron hyper-excitability Less inhibition
IL-6	Garcia-Oscos et al. (2012), Gruol D.L et al. (2014)	Pro-inflammatory	Inhibits GABAergic neurons	Excitation/inhibition imbalance
TNFα	Ogoshi et al. (2005), Stellwagen et al. (2005), Leonoudakis et al. (2008)	Pro-inflammatory	Promotes recruitment of AMPA receptors to the postsynaptic membrane Endocytosis of $GABA_A$ receptors from the surface of the membrane	Increased EPSP Lowered inhibitory synaptic strength

Table 1: The effect of cytokines on synaptic transmission based on experimental evidence.

ability to act on astrocytes and stimulate the release of glutamate, which causes hyper-excitability and recruits AMPA receptors to the post-synaptic membrane [50,51]. Therefore, excessive release of glutamate in the CNS can also induce apoptosis. In one study, TNFα was exogenously given to tadpoles during development and led to an enhancement of excitatory synaptic transmission and increased AMPA and NMDA receptor activity [77]. Furthermore, in another study, prolonged exposure to TNFα led to a chronic inflammatory state, predisposing the brain to seizures [30].

Conclusion and future directions

Treating epilepsy is extremely difficult because of the varied causes of the condition, which include brain injury, fever, infections, stress, chemical imbalances, and even a lack of sleep [20,23,26,30,78,79]. Although inflammation alone cannot explain the complete pathology of epilepsy, it can suggest potential pathways that are targeted. Aside from following a ketogenic diet, which is beneficial to treating inflammation, omega-3 fatty acid supplementation has also been widely implicated as a highly valuable method of treating inflammation [80,81]. Omega-3 supplements are known for their anti-inflammatory properties, and, since they have been beneficial for pharmacoresistant patients, it warrants further research into the downstream targets of inflammatory mediators [80,82].

Although this further suggests that inflammation plays a role in seizures, it is necessary to determine whether all clinical and experimental models of seizures have exacerbated immune responses or whether it is seen only in pharmacoresistant patients. Nevertheless, information about cytokines may still be used to develop new methods and targets for antiepileptic medication and may provide future directions for studying the pathogenesis of some forms of epilepsy. Currently, most studies have observed the effects of targeting one or two cytokines, but future directions may include targeting a combination of cytokine pathways to determine whether they work in concert or whether they act as antagonists.

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Behavioural and neural correlates of emotional dysregulation in core symptoms of borderline personality disorder

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Abstract

Borderline personality disorder (BPD) is a mental disorder characterized by impulsivity, unstable relationships, and a chronic feeling of emptiness. One of the defining features of BPD is extreme emo-tional reactivity, which leads to engaging in self-destructive behaviours and impulsive actions. The ma-jority of studies on emotional dysregulation in BPD examine the behavioural manifestation of affective symptoms, with the focus on obtaining data through the administration of behavioural tasks and various psychometric tests. Several neuroimaging studies, however, have shown functional abnormalities in such brain regions as the amygdala and anterior cingulate cortex (ACC). Despite existing evidence, neural mechanisms regulating the affect of patients with BPD are poorly understood.

This paper examines the difficulties underlying the research on symptoms of BPD caused by such phenomena as emotional dysregulation. By analyzing theories of emotional dysregulation and em-pirical evidence collected in BPD studies, this review explains why it is difficult for neuroscientists to conduct research on BPD. Successful treatment of BPD is hypothesized to depend on correlational anal-ysis between behavioural and neural data instead of relying on each type of data individually when deriv-ing conclusions.

Keywords*: Borderline personality disorder, emotional dysregulation, theories of psychopathology, cor-relational analysis, psychometrics, neuroimaging, anterior cingulate cortex, amygdala*

Introduction

Borderline personality disorder (BPD) is a mental disorder characterized by impulsivity, affective instability, and interpersonal difficulties, and is often accompanied by suicidal ideation and self-mutilating behaviours [1]. Emotional dysregulation, or the inability to effectively modulate one's emo-tional response, is one of the primary characteristics of BPD. Emotional dysregulation drives the mani-festation of core BPD symptoms located along four major domains, which are assessed by the Zanarini rating scale for borderline personality disorder (ZAN-BPD) [2, 3]. These domains are affect, cognition, impulsivity, and interpersonal relationships (Figure 1). The concept of emotional dysregulation does not simply account for the affective component of BPD symptomatology, but rather constitutes a diathesis along all four domains, which dictate the formation of one's personality [4]. According to the diathesis-stress model, borderline personality tends to develop in young individuals as a result of interactions be-tween underlying biological predispositions and environmental factors influencing personality formation [5]. However, the exact mechanisms of that interaction remain largely unknown. Today, this theory di-rects the methodologies of the majority of BPD studies, which rely primarily on the administration of psychometric clinical scales or various neuroimaging techniques, but not necessarily on both [6].

The primary challenge that current BPD studies face is the heterogeneity of the disorder, which is difficult to control in the research environment, and the subjectivity underlying the diagnosis of the con-dition. DSM-IV classifies BPD as an Axis II disorder, thereby implying the prevalence of ego-syntonic person-

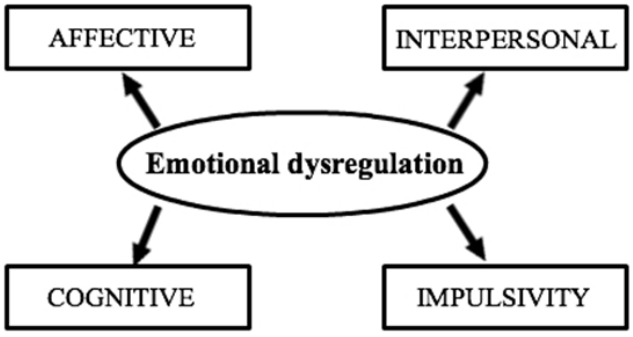

Figure 1: Emotion regulation domains of borderline personality disorder according to the Zanarini rat-ing scale for borderline personality disorder (ZAN-BPD) [2,3].

ality features and the pervasive nature of its symptomatology [1,7]. However, due to the high comorbidity with affective and anxiety disorders and the potential symptomatic linkage with impulse control disorders, some evidence suggests reconsidering the diagnostic criteria of BPD and classifying it as an Axis I disorder [8,9].

Theories of emotional dysregulation in BPD psychopathology

The biosocial developmental model of BPD

The biosocial developmental model of BPD was proposed by Linehan (1993) and remains one of the most studied etiological theories regarding BPD symptomatology [10]. According to Linehan, bor-derline personality forms as a result of the interaction between emotional dysregulation, which serves to be a diathesis, and an invalidating environment [10,11]. Emotional dysregulation makes the individual vulnerable by affecting the entire emotional construct, which incorporates affective, cognitive, and be-havioural emotion-linked processes, as well as the biochemistry and physiology of emotion (Figure 2). As a result, such individuals have high emotional sensitivity and reactivity, and a slow return to baseline after the emotional reaction takes place. Although such vulnerability primarily manifests itself behav-iourally, various neurobiological substrates for emotional dysregulation have been proposed. These in-clude the abnormal activity in the anterior cingulate cortex (ACC) and the amygdala, and the disrupted functional connectivity between the ACC and the insula [12,13].

The invalidating developmental context accounting for the development of borderline personality is primarily characterized by parental intolerance towards child's emotional expression and the neglect of emotional experiences [10,11]. An invalidating environment communicates to the child that emotional responses should be managed internally without parental support and should not be mentioned in inter-personal interactions. As a result, the child does not learn how to label and accept their emotional experi-ences and how to communicate them to primary caregivers. This leads to the inability of the child to regulate emotions in response to situational contexts and to solve problems based on the principles of decision-making and cognitive-behavioural processing.

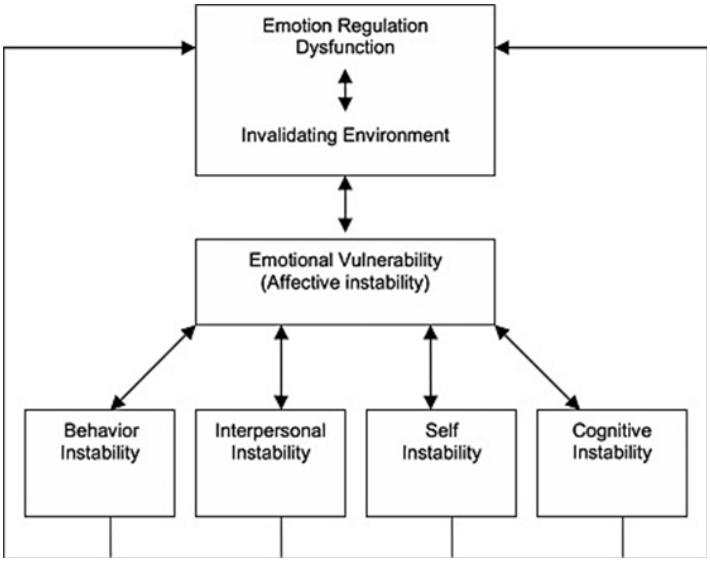

Figure 2: The biosocial developmental model of emotional dysregulation in BPD. Adapted from Linehan (1993) [10].

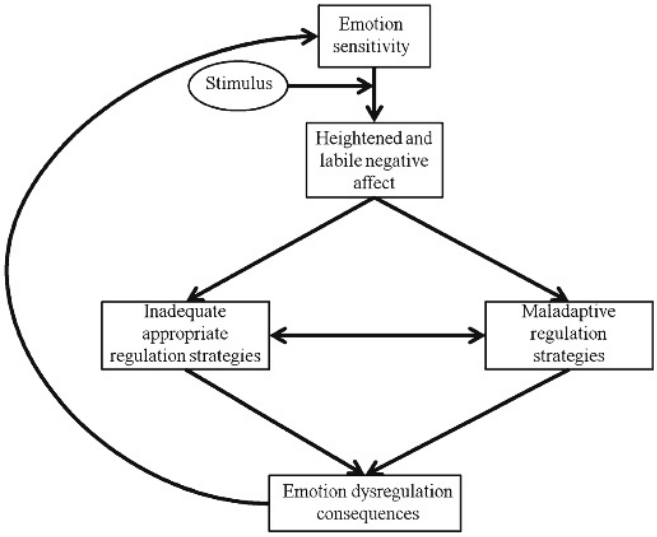

Figure 3: The multi-component model of emotional dysregulation in BPD. Adapted from Carpenter and Trull (2013) [14].

The multi-component model of BPD

Carpenter and Trull (2013) further elaborated Linehan's bio-social model and examined the com-plexity of emotional dysregulation in BPD within the developmental context [14]. According to their theory, emotional dysregulation is not viewed simply as a broad construct, which constitutes a diathesis for BPD pathology, but rather as a multifactorial variable consisting of four psychological components interacting with each other during childhood psychological development. These components are emo-tional sensitivity, negative affect, deficits in appropriate regulation strategies, and the presence of maladaptive regulation strategies.

From birth, a child is biologically predisposed to emotional sensitivity, which induces heightened emo-tional responses and negative interpretations of emotion-evocating environmental stimuli (Figure 3). These interactions between the diathesis and the environment result in negative affect being highly un-stable and unresponsive to the situational context. Such volatility is characterized by rapid intensification of negative moods without obvious environmental triggers. Because negative affect significantly influ-ences a child's worldview and perception of various stimuli, such emotional experiences prevent a child from learning adaptive emotion regulation strategies. This results in the inability of the individual to choose appropriate emotional responses. According to the emotional cascade model, undeveloped emo-tion regulation strategies are replaced by maladaptive responses, which lead to the immediate short-term resolution of negative affect but have negative long-term consequences [15]. Each constituent of the multi-component model is empirically supported, which emphasizes the importance of Linehan's bioso-cial model in BPD diagnostics.

The process model of BPD

According to the modal model of emotion regulation proposed by Gross and Thompson (2007), any emotional response is generated in response to person-situation transactions, which act as cues and directs one's attention to the situation (Figure 4) [16,17]. Appraisal of the event that occurs within the presented context generates an emotional response, which leads to the after-

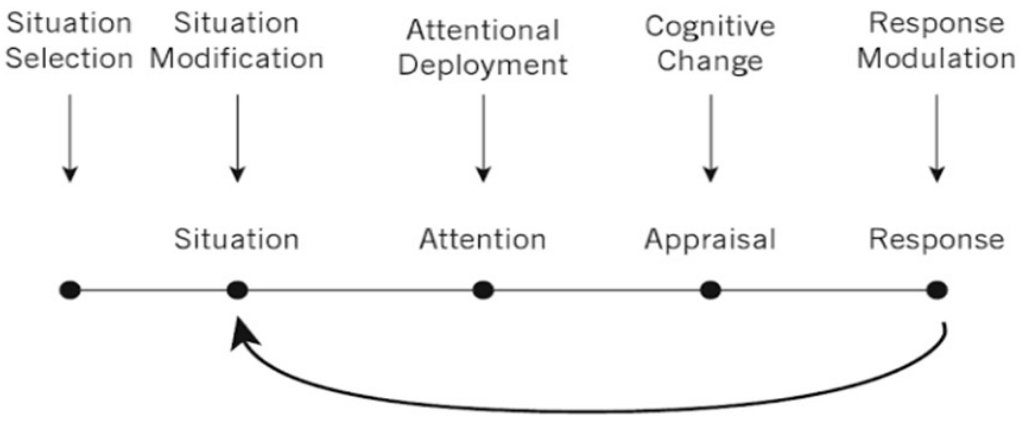

Figure 4: The process model of emotional dysregulation in BPD. The formation of emotional response occurs in four steps in response to a specific situational context with which it forms a feedback loop. In-dividuals with BPD use maladaptive version of regulatory strategies at each step of emotion formation. Adapted from Fairholme et al. (2010) [17].

effects being manifested be-haviourally and physiologically [18]. Because the emotional response is capable of altering the context in which the emotional reaction takes place, the interaction between the response and the situation forms a feedback loop.

The process model is based on the modal model and implies that each step within the emotion genera-tion process can be regulated by five strategies: situation selection, situation modification, attentional deployment, cognitive change, and response modulation. In BPD, each of these five strategies has a mal-adaptive version, which alters the generation of adaptive emotions for a particular situation [19]. For ex-ample, the maladaptive version of situation selection used by individuals with BPD is complete behav-ioural avoidance of the situation, which allows them to avoid negative affect short-term but does not lead to the adaptive emotional response.

As a result, theories of emotional dysregulation in BPD psychopathology are based on the diath-esis-stress model, which overall aims to integrate the biological and psychosocial environmental varia-bles into BPD diagnostics. The theoretical approach chosen by researchers in their study is highly im-portant, as it directs the way in which dependent variables are formulated. Moreover, it will dictate how changes in a particular cluster of symptoms will be approached and investigated during treatment ma-nipulation.

Research and emotional dysregulation in BPD

Challenges in the multifactorial analysis of BPD symptomatology

In modern psychiatry, BPD is considered "a disorder of the emotion regulation system" [10]. In-deed, the majority of symptoms outlined in the DSM-IV-TR, such as avoidance of real or imagined abandonment, engagement in unstable relationships, suicidality, and difficulty in controlling anger are manifested primarily as a cause of affective dysregulation [1,20]. However, the emotional regulation sys-tem is a difficult concept to define. As it has been shown by multiple theories of emotional regulation, such a system can exist on multiple levels, including the psychological, behavioural, and biological do-mains [10,14,16]. Objective data that would allow mental health researchers to derive conclusions re-garding the nature of emotional regulation system have yet to be collected [21].

Since researchers aim to study both the affective and behavioural components in emotional dysregulation in BPD, it would be accurate to define emotional dysregulation as the combination of "affective and maladaptive emotion regulation strategies" [19]. A multimodal assessment of emotional regulation was performed by Bornovalova et al. (2008), which showed that both the self-reported affective instability and the frequency of maladaptive behaviours should be used as a single variable in BPD studies [22]. The research emphasized the importance of a multifactorial approach to the concept of emotional dysregulation by taking into account multiple varia-bles and co-variables that influence emotional dysfunction in BPD. However, different studies continue to use unifactorial definitions of the emotional regulation system, which significantly affect the method-ological approaches of BPD research and, hence, the obtained results and derived conclusions. Because emotional dysregulation is a complex definition that implies both the psychological and the biological components, it is particularly challenging for neurobiologists to examine emotional dysregulation clini-cally and neurologically, and draw correlations between two data sets.

Another challenge that almost all personality disorder (PD) studies face is the use of self-report in measuring affective instability and maladaptive behaviours [23]. BPD caused by emotional dysregula-tion is difficult to assess clinically, as is research due to an absence of a definitive biological marker that would predict symptoms of the disorder [24]. Nevertheless, various research groups have attempted to examine emotional dysregulation on the neural level with the potential to critically evaluate changes in the brain function of BPD patients associated with the manifestation of core BPD symptoms. Performing neuroimaging procedures during the experiment reduces the subjectivity of ambiguous self-report results and provides insight into neurophysiology and neuroanatomy underlying PDs [25]. The problem with using neuroimaging in the investigation of PDs is highlighted by the fact that PD diagnostics is shifting from a categorical approach to a dimensional one, which reflects an attempt of psychiatrists to place PD psychopathology on the continuum [26]. Neuroimaging data cannot explain dimensional differences re-sponsible for the heterogeneity of BPD and the variability in symptom severity reported by patients.

Psychological and behavioural studies

Studies examining the psychological or behavioural variables in emotional dysregulation in BPD focus primarily on the assessment of BPD symptoms using the administration of psychometric clinical scales or behavioural tasks. Taking into account the

heterogeneity of the disorder, such as the differences in symptom manifestation between males and females and the dichotomous diagnosis requiring only five out of nine DSM symptoms to be present, psychological and behavioural research is not able to eliminate confounding variables, such as personality traits, reactivity to environmental stimuli and social situ-ations, and interpersonal life situations, which may direct the results of applied treatment manipulation [27,28]. Moreover, research focused on the assessment of the efficacy of psychotherapeutic treatment modalities is highly influenced by variables such as therapeutic alliance, real relationships, and therapist and client effects [29]. As a result, the literature on the psychological and behavioural assessment of BPD faces significant discrepancies in findings.

In order to support this argument, a pair of psychological studies with three variable methodolo-gies were assessed for the consistency of findings. Koenigsberg et al. (2014) used the affective lability scale (ALS) and Hamilton depression rating scale (HAM-D) to assess symptoms of affective instability in BPD patients based on self-reports [30]. Participants viewed a set of pictures for 27 minutes, with one-third of pictures being presented only once (novel), and two-thirds being repeated 5.3 minutes after the first presentation (repeat). Participants were required to indicate their emotional response to each pic-ture on the scale from one to five. It was shown that BPD participants failed to habituate to negative pic-tures, and high emotional reactivity was shown to constitute one of the core characteristics of BPD. Harned et al. (2015) used the same methodology and conducted a study on female participants with comorbid BPD and post-traumatic stress disorder (PTSD) [31]. Based on the ASL questionnaire data, Harned et al. (2015) showed that BPD participants did show the emotional habituation to negative pic-tures, which contradicted the findings of Koenigsberg et al. (2014).

Similarly, Gratz et al. (2008) reported no interaction between early childhood maltreatment and the intensity of negative affect in BPD participants [32]. Almost a decade later, however, Kurdziel et al. (2017) showed that not only did childhood maltreatment influence the affective instability in BPD ado-lescents, but also that specific dimensions of maltreatment, such as severity and chronicity, were associ-ated with borderline features [33]. Other examples are the studies examining the efficacy of cognitive-behavioural therapy (CBT) on BPD patients. It has been consistently reported that CBT tends to be inef-fective for BPD due to alliance ruptures and poor treatment adherence [34,35]. Some research groups, however, have shown that CBT was efficacious in managing BPD symptoms related to emotional dysregulation, which imposes a significant clinical progress in the area of psychiatry [36,37].

Since no consensus in the literature has been established, behavioural data remains highly subjec-tive and unreliable when investigated alone. In order to increase validity and reliability, such findings should be correlated with some other objective measure of data, which would allow researchers to ensure the replicability of results.

Neuroimaging and functional connectivity studies

The majority of functional magnetic resonance imaging (fMRI) and functional connectivity stud-ies aiming to elucidate the neural correlates of emotional dysregulation in BPD investigate the resting-state and task-based activity of brain regions involved in emotion processing and regulation, with the primary focus on amygdala, insula, and dorsal anterior cingulate cortex (dACC) [38]. Over the past dec-ade, literature has provided consistent evidence showing abnormalities in gray matter, fMRI-BOLD re-sponse, and functional connectivity in the above-mentioned brain areas (Figure 5) [39]. It has been shown that BPD patients tend to have increased activity in the left amygdala, decreased activity in dACC, and abnormal functional connectivity between the amygdala and the insula [30,40]. Such con-sistent findings were obtained by multiple trials that used similar neuroimaging methodologies without making any correlational analysis to psychometric or clinical data [40-42]. Donegan et al. (2003), Min-zenberg et al. (2007), and Buccheim et al. (2016) presented emotion-evoking picture sets to BPD partici-pants and performed a task-based fMRI procedure in which they examined the neural response [40-42]. Hyperactive amygdala and hypoactive dACC were reported by all three groups as statistically significant results specific to BPD participants [40-42].

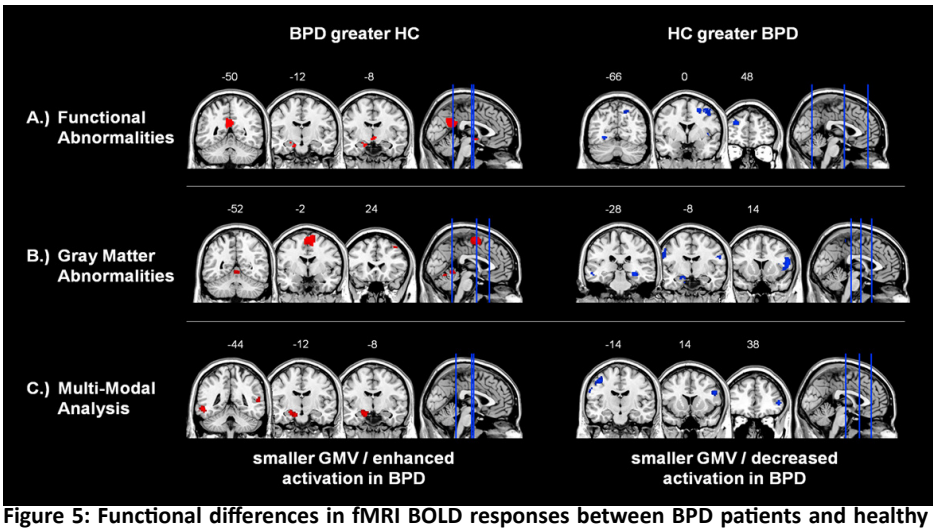

Figure 5: Functional differences in fMRI BOLD responses between BPD patients and healthy controls (HC) during emotion processing A). Structural differences in gray matter volume (GMV) between BPD and HC groups are also shown B). Red represents increased activity or greater GMV; blue represents de-creased activity or smaller GMV. Meta-analysis of neuroimaging results shows significant differences in amygdala and dorsal anterior cingulate cortex (dACC) between BPD and HC groups. Overlapping func-tional and structural abnormalities (multi-modal analysis) between BPD and HC are shown C). Adapted from Schulze et al. (2016) [39].

Neuroscientific approaches to psychiatry direct mental health clinical practice towards the estab-lished medical model, which has been prevalent in the field since the early nineteenth century [43,44]. Since psychiatry is still classified as a branch of medical rather than psychological science, it has tradi-tionally implied the presence of biological correlates for mental disorders and diseases. Therefore, the identification of brain regions underlying the core symptoms for BPD is essential for di-agnostic purposes and treatment improvement for BPD populations [45]. The consistency of neuroscien-tific findings provides a potential for reexamining the core theories of BPD psychopathology and emo-tional dysregulation. The overall purpose is integrating the

psychologically-based theory of the disorder with the neurological mechanisms, which would increase the physician's diagnostic perspective on BPD and other cluster BPDs [1]. Moreover, these findings improve the overall understanding of mechanisms of emotional dysregulation in BPD patients on the neurophysiological level. This directs research in neu-ropsychopharmacology to test the efficacy and of various agents that would be capable of targeting the abnormal neurological functional response in emotional dysregulation.

In psychiatric research, however, such an approach is not grounded enough to fully direct the methodologies of randomized control trials (RCTs.) This is due to the complex nature of BPD and the substantial psychosocial component proposed by BPD etiological theories, which has been supported empirically [10,14,16,46]. Neural data alone cannot explain dimensional differences observed in BPD symptoms across populations, which dictate the symptom's severity and individualistic components forming one's personality. Moreover, neural data largely does not provide any causal relationships ob-served in psychometric clinical scores in response to various modalities of psychiatric treatment ap-proaches, including pharmacology, psychotherapy, and brain stimulation.

For example, Koenigsberg et al. (2014) conducted a study in which they compared the neural re-sponse of BPD participants to healthy controls (HC) and psychopathological controls that comprised participants with avoidant personality disorder (APD) [30]. The fMRI procedure was performed while participants viewed a set of pictures for 27 minutes, with five consecutive runs of 16 neutral pictures and 16 negative pictures. Two-thirds of the pictures were presented twice. Blood-oxygen-level-dependent (BOLD) images were acquired during the task using the dorsal anterior cingulare cortex (dACC) as a re-gion of interest (ROI). Both BPD and APD groups showed decreased activation of the dACC (neural habituation) relative to healthy controls when viewing repeated negative images (Figure 6). Since both psychopathological groups showed similar underlying biological strata, neural data alone cannot explain differences accounting the symptomatologies of BPD and APD.

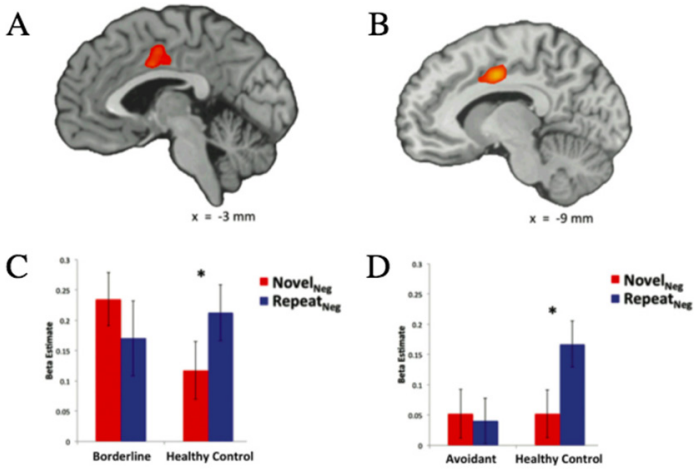

Figure 6: Activity of the dorsal anterior cingulate cortex (dACC) during the image viewing task. The brain map shows a 156 voxel dACC cluster representing a significant difference between A) BPD and healthy controls, and between B) APD and healthy controls for RepeatNeg vs. NovelNeg BOLD response (p<.05, k=150, family-wise error-corrected). Histograms show C) extracted beta-weights for BPD vs. healthy controls, and D) APD vs. healthy controls group comparisons. Adapted from Koenigsberg et al. (2014) [30].

Moreover, some studies suggest that amygdala and dACC dysfunctional activity is not simply related to the symptoms of emotional dysregulation, but that recruitment of these regions underlies the processing and integration of much more complex emotional processes which potentially interact with each other. Cullen et al. (2016) found increased amygdala activation in BPD patients only when they were viewing the pictures of overt fearful content but not covert fearful content [47]. In another fMRI study, Krause-Utz et al. (2014) reported abnormal functional connectivity between the amygdala and the dACC during the working memory task in BPD patients with interpersonal trauma history [48]. Based on provided results, it is biased to conclude that emotional dysregulation in BPD serves as the primary cause of ob-served neural correlates. Co-variables such as working memory task and interpersonal trauma history cannot be excluded from analysis.

The consistency of findings among studies shows that neuroimaging and functional connectivity data is reliable and can be used as a successful tool in the investigation of a biological marker for emo-tional dysregulation in BPD. However, due to the complexity and the heterogeneity of the disorder, neu-ral data cannot be used to establish causal relationships between symptoms of emotional dysregulation and observed neural correlates. A multivariant approach should be taken by research on BPD, which would aim to target all the possible domains of the disorder and to take into account individual differ-ences in symptom manifestation, psychometric scores, behavioural responses, and interpersonal reac-tions.

Correlational analysis studies

Today, BPD continues to be classified as a PD, which highlights the pervasiveness of the disor-der and the multifactorial nature of symptoms. This directs clinicians to look at BPD cases with an ap-proach different from diagnostic tools used for episodic Axis I disorders [49]. In a clinical setting, the categorical model of classification is used as it allows physicians to identify discrete features of BPD as they are manifested on the psychopathological continuum in patients without such cases being over-looked as the presence of multiple comorbid Axis I disorders. ZAN-BPD is a questionnaire that identi-fies the symptomatic domains of BPD and ensures that symptoms are assessed along with all four do-mains. Therefore, behavioural studies relying on psychometrics tend to account for the individualistic component of clinical cases. Obtained results serve a descriptive function and support the accuracy of a proposed diagnosis. However, because of the inferential nature of behaviour and the subjectivity of re-sults, such evidence does not support the majority of proposed etiological theories. This emphasizes the psychological component of the disorder. Affective and behavioural symptoms, however, are clearly ob-servable and are filtered by physicians' perceptual prisms. This limits the objectivity of such symptoms since it relies on the labelling of patient's behaviour based on psychological interpretation [50]. Neurological data largely cannot explain such interpretations and does not provide any causal relationship be-tween abnormally activated brain regions and observable behavioural characteristics.

In an attempt to show that neural correlates are capable of explaining psychologically-based symptoms, numerous fMRI studies have conducted correlational analyses of the psychometric data and fMRI neural data (Table 1). A random sample of 12 studies,

which shared a common result of reported increased fMRI BOLD response in the amygdala for BPD patients, was assessed for the versatility of clinical scales that were used as a behavioural variable in the correlational analysis. Along with the fMRI technique, investigated research groups used multiple numbers of questionnaires in their studies as-sessing different domains of BPD symptom severity (M=3.83, SD=1.59). In the sample, a total of 23 dif-ferent questionnaires were used. These findings highlight the fact that research groups recognize the het-erogeneity of the disorder and inconsistent day-to-day symptom manifestation. By performing a correla-tional analysis between the clinical scales data and the fMRI data, researchers aim to elucidate the correl-ative linkage between amygdala hyperactivity and the individualistic manifestation of BPD sympto-matology.

A variety and complexity of psychometric tests used in research and clinical setting are capable of assessing BPD symptomatology by implementing a dimensional approach. By breaking down total scores into discrete behavioural and psychological variables corresponding to a particular symptom or a cluster of symptoms within one of the major domains of BPD, researchers can test the correlation be-tween neuroimaging findings and changes in psychometric scores within those variables. Conducting these analyses is necessary to derive conclusions about the causal relationship between the activation of a particular brain region and the manifestation of symptoms. These analyses will improve the understand-ing of the importance of fMRI and functional connectivity results in the clinical mechanism. This may enable the identification of biomarkers for a particular symptom and the elucidation of a neural mecha-nism that causes particular affective states and behavioural manifestation in BPD populations.

Significance and translational aspect

Here, a novel approach in BPD research that would allow scientists to correlate both the physio-logical and the psychosocial factors of the disorder is proposed. Establishing such correlations is particu-larly important for symptoms of affective instability and behaviours that result from impulsivity and un-stable affect—self-harm, suicidality, aggressiveness, and physical assault. Since increased amygdala ac-tivity, decreased dACC activity, and abnormal amygdala-insula functional connectivity are observed in symptoms of emotional dysregulation in BPD, future studies will elucidate the biological marker for every BPD symptom outlined in DSM-IV-TR and DSM-5 with the potential development of pharmaco-logical agents which would target brain regions involved in the emotion regulation system.

This review also contributes to the field of psychiatry of PDs. It is particularly challenging for psychiatrists to accurately diagnose BPD based on descriptive self-report measures. Due to the high comorbidity of BPD with other psychiatric disorders and the absence of a definitive biological marker for any affective disorders, BPD still remains highly misdiagnosed and untreated [63,64]. Relying on scientific evidence demonstrating the correlation between the physiological and psychological processes will allow psychiatrists to make more accurate diagnoses. This, in turn, will improve treatment outcomes and will decrease the rate of hospitalization due to BPD.

Study	Total number of psychometric clinical measures	Psychometric clinical measures
Koenigsberg et al. 2009 [51]	3	ALS, SCID, SIDP-IV
Niedtfeld et al. 2010 [52]	2	BSL, ERQ
Beblo et al. 2006 [53]	4	SCID, BDI, SCL-90-R, DES
Herpertz et al. 2001 [54]	4	IPDE, HAM-D, STAI, DTAXI
Hazlett et al. 2012 [55]	6	BPAQ-40, BIS-11, ALS, DES, AIM, CTQ
Schulze et al. 2011 [56]	4	CTQ, BSL, IPDE, SCID
Driessen et al. 2004 [57]	6	BSL, SCID, SCL-90-R, DES, IES-R, WMS-R
Kamphausen et al. 2013 [58]	2	BSL, BPDSI
Guirart-Masip et al. 2009 [59]	4	SCID, DIB-R, CGI-S, ZKPQ
Radaelli et al. 2012 [60]	3	SCID-I, SCID-II, HAM-D
Frick et al. 2012 [61]	5	SCID-I, SCID-II, ALS, BDI, EHI
Mier et al. 2013 [62]	1	BDI
	M = 3.83 SD = 1.59	

AIM - Affective Intensity Measure; ALS - Affective Lability Scale; BDI - Beck Depression Inventory; BIS-11 - Baratt Impulsiveness Scale; BPAQ-40 - Buss-Perry Aggression Questionnaire; BPDSI - Borderline Personality Disorder Severity Index; BSL - Borderline Symptom List; CGI-S - Clinical Global Impression of Severity; CTQ - Chidhood Trauma Questionnaire; DES - Dissociative Experiences Scale; DIB-R - Revised Diagnostic Review for Borderlines; EHI - Edunburgh Handedness Inventory; ERQ - Emotion Regulation Questionnaire; HAM-D - Hamilton Depression Rating Scale; IES-R - Impact of Event Scale; IPDE - International Personality Disorder Examination; SCID (I/II) - Structured Clinical Interview for DSM-IV (I/II), SCL-90-R - Symptom Checklist; SIDP-IV - The Schedule for Interviewing DSM-IV; STAI - State-Trait Anxiety Scale; STAXI- State-Trait Anger Expression Scale; WMS-R - Wchsler Memory Scale; ZKPQ - Zuckerman-Kuhlman Personality Questionnaire

Table 1: Psychometric variables of BPD amygdals hyperactivity studies used for correlatioinal analysis (n = 12)

Conclusions and future directions

The aim of this review was to present scientific evidence that used integrative theories of emo-tional dysregulation in BPD and to provide guidelines for revising methodological and data analysis ap-proaches of BPD studies. There is a potential translational aspect to psychiatric assessment, diagnosis, and protocol design for future RCTs that test the efficacy of pharmacological, psychotherapeutic, and brain stimulation treatments for BPD. Based on these findings, behavioural data tends to remain highly inconsistent among studies. This is, in part, due to the highly variable manifestation of BPD symptoms among patients, inconsistency between symptoms and self-report measures, and high comorbidity be-tween BPD and Axis I disorders. Empirically-obtained neuroimaging and functional connectivity results tend to be reliable and consistent among multiple studies, but do not provide a general insight into the nature of the disorder's psychopathology. Therefore, such findings

remain largely untranslatable to the psychiatric clinical practice and diagnostics of BPD. In order to increase the generalizability and ap-plicability of neuroimaging findings to the real-world BPD population, studies should aim to perform correlational analyses between the neural and psychological data to take into consideration all the poten-tial aspects influencing the mental state of a BPD patient during the research process.

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Emily Nicholas Angl

The Institute of Health Policy, Management, and Evaluation at the University of Toronto held a seminar on patient-oriented health research (POHR). The objective of this event was to explore how patients can take an active role and be engaged in health research.

Emily Nicholas Angl, an advocate for patient engagement, helped plan and facilitate the event. Using her personal experience as a patient, she has been helping bring the patient voice to health care for over ten years. She previously sat on the board at Patients Canada and worked with them as a patient advisor. She has been involved in workshops, conferences, and various projects with hospitals, government agencies, and other organizations across the country. Today, she is the director of health engagement and communications at Reframe Health Lab in Toronto, Ontario, and is also an independent consultant.

Interview conducted by Yasamin Sadeghi

"Time is often limited for research teams, but the reality is that meaningful engagement is only possible with concerted efforts and conscientiousness."

YS: What inspired you to pursue this non-traditional career path to serve patients and improve their experiences within the health care system?

ENA: I have always been interested in health and medicine, and, from an early age, I had wanted to be a doctor or a researcher. However, my education was interrupted when I suffered a stress fracture to my hip. The fracture was misdiagnosed and resulted in a severe break with complications. This led to four surgeries in ten years, including a total hip replacement. It felt like my life was a constant series of waiting for appointments and treatments.

The resulting depression took me into the world of mental health care—both private and public. I was surprised by the different and varied approaches to healing; it wasn't just psychotherapy, but also nutrition, daily coping skills, and exercise. It was the first time that I felt like a whole person and not just "Emily the Leg". I began to reflect on the difference between theory and practice—one reflects a lot when in multiple therapy groups or when staring at a wall from a hospital bed!

As a patient, I had a very different and important perspective on health care, which went beyond my classes in the life sciences. During my patient journey, I learned about Patients Canada (formerly The Patients Association of Canada), founded and led by Dr. Sholom Glouberman. At the time, this organization explored how patients and their family members can be involved in health care governance, service design, research, and education. The more I learned about the association, the more I became interested and excited about patient engagement in health care.

YS: What is SPOR and how does it intend to make the patient experience better?

ENA: SPOR stands for the Strategy for Patient-Oriented Research. The Canadian Institutes for Health Research (CIHR) started SPOR to support research that, in their words, "engages patients as partners, focusses on patient-identified priorities, and improves patient outcomes". SPOR adheres to the following principles, which are listed on the CIHR website:

•Patients need to be involved in all aspects of the research to ensure questions and results are relevant;

•Decision-makers and clinicians need to be involved throughout the entire research process to ensure integration into policy and practice;

•Funding under SPOR is based on a 1:1 matching formula with non-federal government partners to ensure relevance and applicability;

•Effective patient-oriented research requires a multi-disciplinary approach; and

•SPOR is outcome-driven and incorporates performance measurement and evaluation as integral components of the initiative.

YS: How do you think education systems, such as health research institutions, can help remove barriers to patient engagement?

ENA: It is interesting to see the changes that have occurred since I started working in the field of patient engagement. When I started ten years ago, we were struggling to have health care organizations, academic institutions, and funding agencies even recognize the concept of involving patients in health care and research. Now we are more likely to ask ourselves "how do we do it well?" rather than "why do we even do it?"

However, there are still institutional and systemic barriers to equitable patient engagement. For example, recruiting and training patient partners is often the responsibility of the individual research team, so there can be variation in goals and standards. In addition, this means that supporting patient partners is most often dealt with at this level. For many research teams, engaging patients in research is a new practice, and often not enough time and effort is put into planning it.

In the process of carefully considering who needs to be involved in research, the steps to engaging patients become clear. For example, once you have decided which patient populations you want to partner with, you can reach out to community organizations, colleagues, and other allied health professionals to assist you. It also becomes clearer what reimbursement and accessibility considerations might be needed. I understand that time is often limited for research teams, but the reality is that meaningful engagement is only possible with concerted efforts and conscientiousness.

Other significant barriers are funding and resources. Although patient engagement is now explicitly stated in many grant applications, by the time all other components are considered, there is often little room left in the budget to support it. In grant applications, you are expected to know your definitive plan in advance, but patient engagement requires listening and reacting. This fluidity can present a challenge to grant proposals and research ethics board approvals (which is a whole topic regarding barriers to patient engagement). Considering these issues, it is not surprising that SPOR is also trying to gauge how the entire institution of research might need to adapt to make patient engagement possible.

YS: What is a common misconception regarding patient engagement?

ENA: There is often the misconception that patients can speak for entire communities and patient groups. In reality, we will never have patient partners who represent every type of patient, and this should not be the goal either. The old model of patient involvement—as subjects rather than as team members—is hard to shake off, and will likely take time and effort to change this dynamic.

When we engage patients, we are not looking for a representative sample to tell us what all patients think and want. Instead, we want patients to shape research in new ways, to offer different expert perspectives, and to share experiences that give fresh insight into problems. This new lens can profoundly impact the direction of research.

YS: You were planning on pursuing a traditional career path to medicine but ended up in a different profession. What is something you would not have been able to do had you become a physician?

ENA: When I started studying the life sciences at the University of Toronto, it felt as though every other student around me wanted to become a doctor. There were hundreds of students interested in health and medicine who felt there was a single choice for their careers. I am grateful that I turned off the auto-pilot track and moved towards a different goal. My experience as a patient, which disrupted my "big plan", insisted on a new set of priorities and goals. I realized that I did not have to wait for the day I became a doctor/lawyer/mother/Jeopardy champion to be satisfied with my life. My life was just as real and important every day, including today.

This change in thinking led me to pursue new interests that I would have previously considered a waste of time. This didn't come as an epiphany, or as a flight of fancy, but out of serious consideration and trial-and-error. It included settling into a more comfortable relationship with the cards I have been dealt with in my life, going from there, and, when possible, enjoying the process.

Don't get me wrong though—in another situation I would have loved to pursue medicine. Instead, I work with physicians and other interesting health care professionals, I enjoy a bit of vicarious living, and then I go home at 5:00pm to make dinner and watch Jeopardy.

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