

Research Article

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The Phylogenetic Background of Neurotransmitters in the Unicellular Organism *Tetrahymena Pyriformis*

Tziakouri A, Lajkó E and Kőhidai L*

Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, Hungary

***Correspondence:** László Kőhidai, Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest, 1089, Hungary, Tel: +36-30-4743803; Fax: +36-1-3036968; E-mail: kohlasz2@gmail.com

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Abstract

The Human Central Nervous System (CNS) is governed by electrochemical networks forming a delicate interplay between the different regions of the brain. The objective of the present experiment is to investigate the phylogenetic background of this electrochemical network by creating a comparable binary and ternary interplay of interactions between different neurotransmitters (noradrenaline, histamine, serotonin, acetylcholine, glutamate, and dopamine) in the unicellular eukaryote *Tetrahymena pyriformis*. *Tetrahymena* – as a protozoon – has no nervous system; however, it has been shown that it has not only the ability to store, synthesize and secrete biogenic amines but it also bears binding sites for the corresponding receptors of some of these molecules. The chemotactic responsiveness elicited by the neurotransmitters was examined in *Tetrahymena* cells, using a modified version of Leick's two-chamber capillary chemotaxis assay with 20-minute incubation times. The concentration course of each neurotransmitter was determined and the concentration eliciting the strongest effect was further used to examine the chemotactic response of the neurotransmitters when used in pairs and in groups of three. Adequate cellular responses (chemoattractant and chemorepellent) were detected in both cases when the neurotransmitters were used alone and in combinations. A pattern detected in these responses was related to the neurotransmitters' physicochemical characteristics (XlogP, TPSA). These provide evidence that the chief regulatory molecules of the CNS can be identified even in lower, eukaryotic unicellular levels of phylogeny and possibly alter the basic functions of these organisms. In summary, our results support the theory that any evolved nervous system-like interplay could stem from a common origin. Therefore, identifying the "ancient" function of a molecule or its receptor effect can open new windows in the advancement even of therapeutic interventions.

Keywords: Phylogeny, Neurotransmitter, *Tetrahymena*, Chemotaxis

Abbreviations: Ach: Acetylcholine; DA: Dopamine; Glu: Glutamic Acid; His: Histamine; NA: Noradrenalin; NT: Neurotransmitter; Ser: Serotonin; TPSA: Topological Surface Area; XlogP: Partition Coefficient.

Introduction

The key function of the brain is communication. Neurons form networks that connect with each other allowing a delicate interplay between the different regions of the brain. The homeostasis of this system is essential for the physiological function of the brain and hence the

psychological and emotional stability of the mind. As a result, it comes with no surprise that any imbalance in the brain's chemical composition is associated with many psychiatric, neurologic and neurodegenerative diseases. Understanding the unique interactions between the different neurotransmitters (NTs) will aid in the advancement of therapeutic interventions while

determining the origin of these interplay may provide a new perspective on the phylogenetic development of the central nervous system and its specific cellular activities prior to differentiation.

Evolution of signal molecules: Evolutionary studies have proved that the presence of NTs, their receptors and their biosynthetic and degradative machinery, dates back to several hundred million years [1]. In an article regarding chemical evolution, Danielli raised the question of whether the patterns and tissue competencies observed among species exist as a result of a change in the synthetic abilities of the organism or if the synthetic competence for proteins, receptors and other signaling molecules are to some extent always present within an organism. He introduced the hypothesis that evolution of biological systems is not a result of the formation of new molecules but the ability of the already existing receptor and effector proteins to acquire novel and improved functions [2].

A permanent symbiotic relationship between prokaryotes resulted in the production of eukaryotic organisms whose intracellular organelles are individual prokaryotes (chimaeras). The subsequent evolution of organisms leads to the creation of multicellular species such as animals, fungi, and plants [3]. Notably, the substances we now call neurotransmitters, have been shown not only in many organisms lacking a nervous system but also in unicellular organisms. For this reason, Roshchina V.V (2010) has proposed the term 'biomediators' rather than neurotransmitters since as they explain, these molecules are the basic building blocks of transmission of information within and between cells in the form of chemical signals [4].

A signal is nothing more than a trait that has evolved to serve as an advantage for the signaler by altering the behavior of the receiver [5]. Living organisms are largely influenced by their environment; they are constantly observing features and behaviors of other living organisms (both of their own species and of other species) that could be advantageous for them [5],[6]. The 'signal selection' theory claims that the effectiveness of a trait aids in its dissemination among signalers serving as an indication of their present or future activity. The purpose of these traits is not to alter the receiver's response but rather to test how beneficial they are to their bearers (autocrine regulation); an aspect that will enable them to become part of the signaling system later on [6].

The theory of signal adaptation is supported by the fact that NTs and their enzymes precede their respective receptor proteins [1]. Epinephrine and acetylcholine (ACh) were shown to exist in unicellular organisms but the appearance of their receptors became more evident in higher-ranked, multicellular organisms. A similar study proved that although dopamine (DA) is seen in almost all animals, DA receptors seem to have emerged only during the early vertebrates [6].

Conservation of both structure and function of receptors has been observed over the years. For example, gene cloning of beta-adrenergic receptors and muscarinic cholinergic receptors showed striking similarities in their structure [1]. GABA receptors of invertebrates expressed a high degree of homology with mammalian GABA-A receptors; however, lacking the benzodiazepine modulatory site [3]. In the case of DA, a principal modulator of the central nervous system, it was established that the two main receptor families (D1 and D2) emerged independently; however, the main receptor subclasses are preserved in most vertebrates. This is thought to be due to the acquisition of some specific properties such as a higher affinity for DA, intrinsic receptor activity, and agonist-induced desensitization [7]. We, therefore, conclude that conservation of structure and function seems to be the most genetically cost-effective way of the nervous system of vertebrate species to adapt to various environments and behaviors and this idea leads all the way up to the constitution of the concept of neurology. Nonetheless, it's worth noting that receptors executing functions that are unnecessary or decreased in humans or whose disappearance would potentially be beneficial can also be withdrawn during evolution. An example of such receptor is the 5-HT_{5B} whose disappearance may be related to decreased demand of pheromone perception in humans or it may have occurred as an aid of social interactions by reducing aggressiveness in more social species [8].

The monoamine theory: The system of behavioral control in humans is a rather complex one and it is thought to be highly dependent on monoamine neurotransmitter interactions (Figure 1). Monoamines are molecules derived from the aromatic amino acids in small, distinct areas of the brain. DA is produced in the ventral tegmental area and substantia nigra while noradrenaline (NA) and serotonin (Ser) are produced in the locus ceruleus and raphe nuclei, respectively. The importance of these molecules in the control of human emotions and behavior has been illustrated over the

years not only through several psychiatric diseases, but also through the effects of psychotropic and psychedelic drugs (e.g., antidepressants, antipsychotics and drugs of abuse) which by altering the brain's chemistry interfere with the person's mood and performance [9][10][11]. However, it is important to note that by no means, individual NTs can be designated to distinct emotions. The brain's homeostasis is preserved through reciprocal control and complex networks of feedbacks within and in between the monoamine systems with the numerous receptor subtypes also creating various subsystems [9],[12],[13]. Moreover, even though ACh, glutamic acid (Glu) and histamine (His) are not considered monoamines, their contribution to the dynamics of the system cannot be disregarded as they have also been proven to promote alertness, attention, learning memory and overeating inhibition [14][15][16][17].

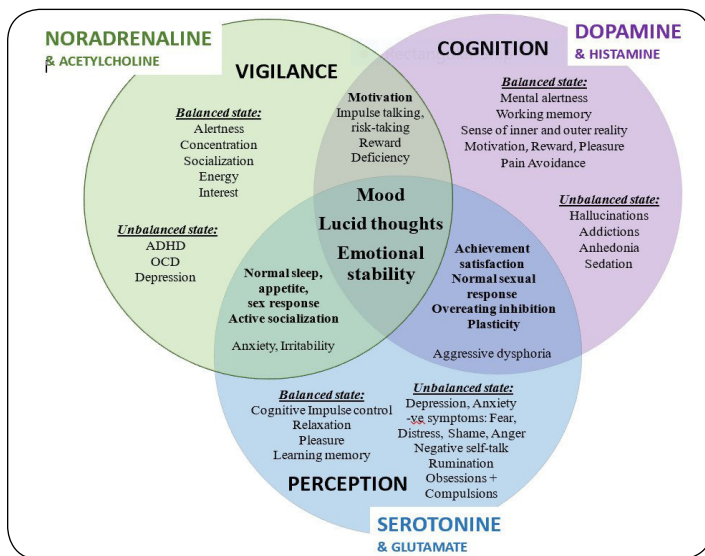


Figure 1: Network interactions in the homeostasis of mental stability in humans.

Constructed from: [11][16][17][18][19][20].

Unicellular model cell: The hypothesis that NTs are found in all eukaryotes independently of their position on the evolutionary tree has been extensively studied and confirmed. Their presence was even illustrated in some species of prokaryotes; with His being the most widely characterized [21],[22]. Adrenaline was the first NT to be isolated and described by Napoleon Cybulski (1895) and John J. Abel (1899) from animal pituitary gland extracts [5],[23]. Interestingly, the concentration levels of the NTs are very similar in all kingdoms with exceptions being some specialized cells or organs of higher ranked species. In some cases, the levels of the molecules are even found to be higher in microorganisms than in humans

[4]. *Tetrahymena pyriformis* is a unicellular ciliate proved to possess all the components of a complete signaling system, very similar to that of a well-developed mammal. The presence of various vertebrate-like hormones including Ser, melatonin, His, adrenocorticotropin (ACTH), thyrotropic hormone (TSH), gonadotropic hormones (FSH and LH), relaxin, insulin, somatostatin, endothelin, prostaglandin (PGF2) and even endocannabinoids have been demonstrated in this protozoon along with various cell membrane receptors [24][25][26][27][28][29]. The importance of *Tetrahymena pyriformis* as a model for studying the evolution of signal transduction mechanisms stems from the interconnections existing between these components. Utilizing also intracellular second messenger pathways like cAMP, cGMP, Ca²⁺-calmodulin and inositol trisphosphate (IP3), *Tetrahymena* exhibits a complete neuro-endocrine regulatory system, highly homologous to vertebrates [25]. A striking example of this, is the regulation of triiodothyronine (T3) synthesis after exogenous administration of TSH, mimicking the effect of the hypophyseal regulation of higher ranked animals [29].

According to Lenhoff, unicellular organisms possess simple binding sites on their surface membranes which aid primarily in the cell's nourishment by recognizing different amino acids or other simple organic compounds before their ingestion [30],[31]. Over time, numerous binding sites develop an inevitable interaction with their appropriate ligand establishing a signal that orders the execution of a specific cellular behavior, such as chemotaxis [32]. Chemotaxis is one of the cell's most basic and primordial functions making it an ideal parameter for determining behavioral changes in a cell. To analyze the effect of the chief regulatory molecules of human behavior on the behavior of a lower ranked species we evaluated the chemotactic response of the eukaryote *Tetrahymena pyriformis* to each of the following NTs: noradrenaline, histamine, serotonin, acetylcholine, glutamic acid and dopamine. We also reproduced interplay of interactions between the NTs reflecting the human brain's complexity by evaluating the chemotactic response of *Tetrahymena* when these molecules were used in pairs and in groups of three.

As a result, the hypothesis arises that the hormonal networks of *Tetrahymena* may provide the basic building blocks for a multicellular organism's more sophisticated systems. In this study, we want to focus mainly on the existence of neurotransmitter systems and their effect on the behavior of organisms lacking a complex nervous

system.

Objectives

The objectives of this study were first, to examine the effect of different NTs on *Tetrahymena Pyriformis* by evaluating the chemotactic response of the protozoon to different NT concentrations; and secondly, to establish the presence of a NT system in *Tetrahymena* using various combinations of NTs selected from the already accepted network of mood regulation in humans (Figure 1).

Materials and Methods

Chemicals: In the present study the chemotactic ability of the following NTs was investigated as cell physiological responses elicited by significant members of the NT-network of the unicellular eukaryotic ciliate model, *Tetrahymena pyriformis*: Acetylcholine (Fluka, Buchs, Switzerland); Dopamine (Sigma-Aldrich, St. Louis, MO, USA); Glutamic acid (Reanal, Budapest, Hungary); Histamine (Sigma-Aldrich, St. Louis, MO, USA); Noradrenalin (Sigma-Aldrich, St. Louis, MO, USA); Serotonin (Sigma-Aldrich, St. Louis, MO, USA).

Cell culture: Axenic cultures of *Tetrahymena pyriformis* GL were grown at 28° C, in medium containing 1% Bacto-tryptone (Difco) + 0.1 yeast extract. Logarithmic phase (24 h) cultures were used in the experiments.

Chemotaxis assays

Single neurotransmitter response experiments:

In the first set of experiments we tested the response of *Tetrahymena* to each of the NTs. A stock solution of 10^{-2} M was made in distilled water for each NT which was diluted 7 times in complete cell culture medium to obtain a range of concentrations (10^{-12} , 10^{-11} , 10^{-10} , 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} M). One test substance did not contain any NT serving as the control of the experiment. The chemotactic ability of *Tetrahymena* cells was determined by a modified version of Leick's two-chamber capillary chemotaxis assay [33]. In this assay, the pipette tips of an eight-channel micro-pipette filled with 100 μ l of the test substances were used as the upper chamber. Wells of a microtitration plate filled with 420 μ l of cell cultures served as lower chambers. The incubation time was 20 min. as it was proven by previous experiments to be an optimal time maintaining the concentration gradient in the chamber [34]. After incubation, the samples - positive responder cells of the upper chamber - were fixed with 4% formaldehyde

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dissolved in phosphate-buffer saline (pH = 7.2). The number of cells was determined oculometrically by Neubauer hemocytometer in light microscope Zeiss AXIO Observer A1 (Carl Zeiss Microscopy GmbH, München, Germany) and all experiments were repeated four times. Samples (collected from the upper chamber) containing a significantly higher number of *Tetrahymena* cells compared to the control were labeled chemoattractants while those containing significantly lower number of cells than the control were labeled chemorepellants.

Binary and ternary interplay of neurotransmitters: The most significant concentration of each NT (attractant or repellent) was determined and used for the subsequent experiments which were prepared to investigate the response of *Tetrahymena* to different combinations (groups of two and three) of NTs. The only exception was ACh for which the least chemorepellent concentration was used as the overall response of *Tetrahymena* to ACh was repellent. The choice of NTs for the binary interplay was based on the already established interactions in the literature (Figure 1) while the tertiary interactions were chosen in a way as to utilize the highest number of possible interactions between NTs. The dilutions were prepared in the same manner as in the previous experiments and as a last step the different concentrations were mixed to get the combination of significant concentrations in complete cell culture medium. As a result, the test substances of the upper chamber of the capillary assay contained the significant concentrations of each NT, the combined solution and the control. The incubation time, fixation of samples and the determination of cells were done according to the description of the single NT assay.

Statistical analysis

Chemotactic responses were analyzed using the inbuilt statistical routines of Origin Pro 8.0 (OriginLab Corporation, Northampton, MA, USA). The evaluated value was normalized to the control and was given as 'Chemotaxis Index' (Chtx. id. = number of cells assayed with test substance / number of cells assayed with control) in percent. Significance of chemotactic responses was assessed by one-way ANOVA and represented in figures as *: $P < 0.05$; **: $P < 0.01$; and ***: $P < 0.001$.

Results

Single neurotransmitter response: The chemotactic response of *Tetrahymena pyriformis* to each NT was examined and the most significant concentration was determined using statistical evaluation. The results are shown in figure 2. DA demonstrated an overall,

moderate chemorepellant effect with a significant chemoattractant peak ($P < 0.01$) at 10^{-6} M. NA and Glu showed a broad chemoattractant response with maximum positivity ($P < 0.01$) at 10^{-11} M. His exhibited mostly a chemoattractant effect with the highest number of cells in the capillary at 10^{-10} M ($P < 0.01$) while Ser showed a chemoattractant character at $10^{-10} - 10^{-6}$ M concentration range with a very strong response at 10^{-10} M ($P < 0.001$). ACh was the only neurotransmitter to display an overall chemorepellant response with 10^{-6} M being the most significant concentration ($P < 0.001$).

Binary and ternary interplay of neurotransmitters: In this set of experiments, the chemotactic response of *Tetrahymena pyriformis* was tested against different groups of NTs. The pairs of NTs were chosen to reflect the interactions maintaining the preliminary functions of the human mind; vigilance, perception and cognition. The groups comprising of three NTs were chosen to represent the more complex interactions responsible for the long-term stability of mood and prosperity of the human mind (Figure 1). For each experiment, we evaluated the response of *Tetrahymena* to the most attractant concentration of the chosen molecules, the combination solutions and the control.

The results of the binary and ternary interplay are seen in figure 3. In the binary interplay, combination solutions demonstrated a cumulative response when compared to the control. The combination of DA + His resulted in a cumulative chemorepellant response ($P < 0.001$) whereas the combination of Ser + Glu showed a cumulative chemoattractant response ($P < 0.001$) when compared to the control. The combination of NA + ACh showed a chemoattractant response ($P < 0.01$) when compared to the control and an additive activity when compared to NA and ACh ($P < 0.01$ and $P < 0.05$, respectively). The previously observable chemorepellant effect of ACh was not detectable when this NT was used inside a network.

In the ternary interplay, the majority of combination solutions displayed a negative response when compared to the single NT effect and to the control. This tendency was most visible in case on the group of NA + Glu + DA. When the mixture of Ser + His + NA was tested, a slight, but not significant decrease could be detected by comparing to the individual NTs. The negative effect of Glu + DA + ACh group was able to be observed, but only in relation with Glu; DA was significantly more repellent alone than in the ternary combination.

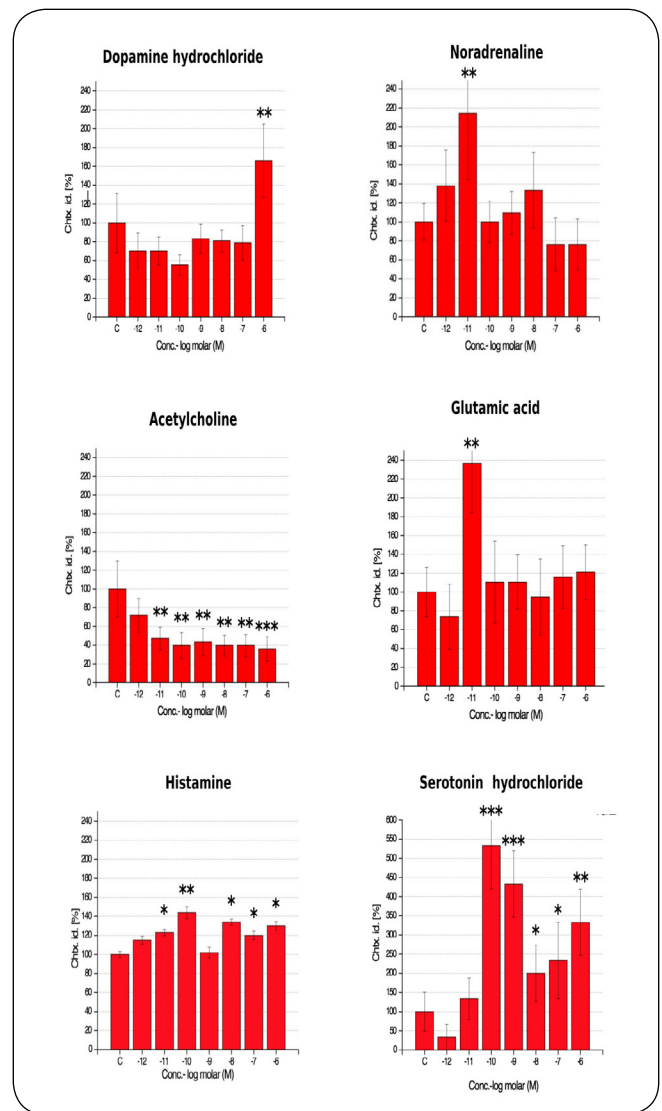


Figure 2: Single neurotransmitter effects.

The graphs represent the chemotactic response of *Tetrahymena pyriformis* to each neurotransmitter. Chtx. id. (chemotaxis index) is expressed as a percentage of the control. DA showed a chemoattractant response at 10^{-6} M. His and Ser displayed a maximum chemoattractant effect at 10^{-10} M while NA and Glu displayed a maximum chemoattractant response at 10^{-11} M. ACh showed an overall chemorepellant response with the most negative concentration being at 10^{-6} M. (* - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$).

The graphs represent the chemotactic response of *Tetrahymena* to the most attractant concentration of the chosen NTs, the binary/ternary combination solutions and the control. Neurotransmitters in the binary interplay displayed a cumulative response; chemorepellant for DA-His and chemoattractant for Ser + Glu and NA + ACh. NA + ACh showed also an additive effect when compared to NA and ACh individually. The previously observable

chemorepellant effect of ACh was not detectable inside the network. In the ternary interplay, the combination solutions displayed a negative response when compared to the control. Data shown in the figure represent mathematical averages of four parallels and \pm S.D. values. The levels of significance are * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$; * - Group vs. Control; * Group vs. Group.

of Chtx. id. = compound X/(compound X + compound Y) \times 100%.

Table 1: Ratios of mean.

Combination	Neuro transmitter	Ratio of mean (comp x/comp x + comp y) x 100%
Binary interplay		
DA+His	DA	131%
	His	150%
NA+ACh	NA	43%
	ACh	57%
Ser+Glu	Ser	24%
	Glu	96%
Ternary interplay		
NA+Glu+DA	NA	264%
	Glu	244%
	DA	152%
Ser+Hi+NA	Ser	112%
	His	141%
	NA	149%
Glu+DA+ACh	Glu	144%
	DA	56%
	ACh	88%

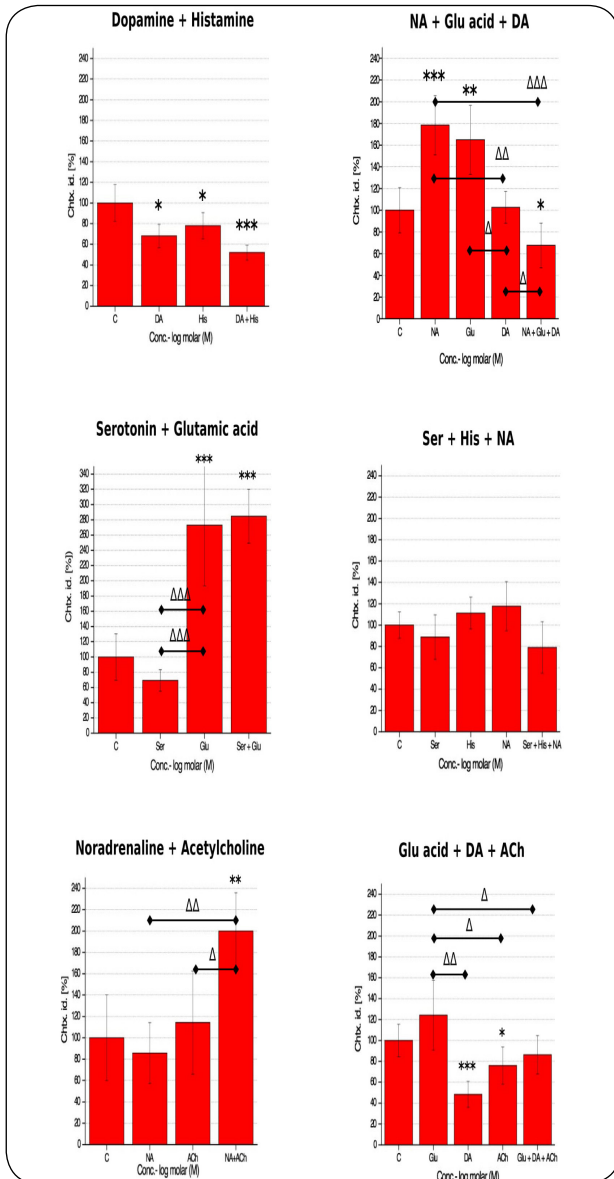


Figure 3: Effects of binary and ternary interplay of neurotransmitters on chemotaxis.

Interpretation of results using physicochemical parameters of neurotransmitters:

These chemotaxis results suggest that most of the NTs are more effective when they act on Tetrahymena cells as individual entities rather than inside a network. In order to confirm that assumption, the ratio of Chtx. id. was calculated for each NT as shown in table 1 where Ratio

In the binary interplay, DA and His were found to be more effective when acting on Tetrahymena cells as individual entities, whereas NA, ACh and Ser exhibited a stronger response when they were found inside a combination. NA and ACh showed about half the effectiveness when compared to the combined solution (43% and 57% respectively) while Ser was only 24% effective when it acted on the cells alone. Glu did not show any significant difference between the combination and its own response (96% effectiveness).

In the ternary interplay, the majority of the molecules were more effective when acting on Tetrahymena cells alone rather than when being part of a combination. The least effective combination was that of NA + Glu + DA with their ratios of Chtx. id. being 264%, 244% and 152% respectively showing that they all exhibit a much higher response as individual entities. In the combination of Ser + His + NA, Ser did not show any significant change in its response when being part of the network but His and NA proved to be weaker in the combined solution. It's worth noting that the response of NA as part of this combination was not as weak as in the previous interplay.

The combination of Glu + DA + ACh proved to be the most effective as only Glu was found to have a stronger effect on its own (ratio of Chtx. id.: 144%). Unlike its response in the first interplay with NA and Glu, DA exhibited a stronger response as part of this combination.

The results were then summarized in table 2 where “I” represents the test substances that are *Ineffective* alone, manifesting stronger response when found inside a network; “E” represents the NTs that are more *Effective* as unique entities as opposed to being part of an interplay and “n” describes the *neutral* molecules that did not show any significant difference when utilized on their own or inside a combination.

The molecular weight and structure of each NT are also included in table 2 along with some physicochemical characteristics that are thought to influence the potency of the molecules and thus the behavior of Tetrahymena cells. The topological polar surface area (TPSA (Å²)) can be used to predict the capacity of a molecule to cross the cell membrane and the partition coefficient (XLogP) is a measure of how hydrophilic a compound is.

The values of TPSA and XlogP were obtained from <http://pubchem.ncbi.nlm.nih.gov/>. There was no real correlation between the molecular weight and the responsiveness of Tetrahymena cells to the molecules; however, we could observe that high TPSA (Table 3) and low XLogP (Table 4) values rendered the NTs more effective alone.

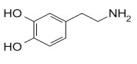
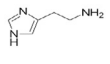
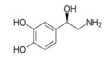
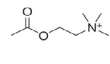
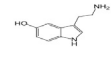
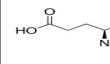
Discussion

In the present work the chemotactic response of the

unicellular organism *Tetrahymena pyriformis* against NTs was extensively examined for the first time. All the substances tested exhibited a significant chemoattractant or chemorepellent response in a concentration-dependent manner. This proves that the molecules regulating human behavior can also influence the behavior of lower-ranked species. A complete mammalian neurotransmitter function is not expected by these molecules; however, triggering chemotaxis implies a possible survival role for the ciliate [30]. The only hormone recognized so far for its justified life-saving function in Tetrahymena is insulin but this does not exclude the utilization of other molecules for the same purpose [29],[35]. It is worth noting that the only three monoamines referenced in the literature for their chemotactic effects on Tetrahymena were His, Glu and Ser [32],[35]. Our results were concurrent with that of His and Glu but Ser was previously found to be chemorepellent. This indicates a possible cell cycle dependency for Tetrahymena cells which can be confirmed with synchronized cell-cycle phase cultures. Nonetheless, the set-up of the experiments was chosen to reflect the real conditions found in a multicellular organism where cells exist in different cell-cycle phases.

In the binary interplay experiments, the pairs of test substances were chosen according to figure 1 which represents a simplified NT network regulating mood and behavior in humans. Our results show that these interactions can function even on a unicellular level as the chemotactic response of Tetrahymena in all cases was a cumulative one (positive or negative) when compared to the control. What was interesting it was the additive response of NA and ACh when compared to their individual responses indicating that they potentiated each other's effects. A possible explanation is given by

Table 2: Summary and comparison of the chemotactic effects induced by individual NTs or their combination solution with the physicochemical characteristics of neurotransmitters.

Molecular structure							
MW (g/mol)	153.181	111.148	169.18	146.21	176.219	147.13	
TPSA (Å ²)	66.5	54.7	86.7	26.3	62	101	
XlogP	-1	-0.7	-1.2	0.2	0.2	-3.7	
Binary interplay	DA	His	NA	ACh	Ser	Glu	DA+His
	E	E					NA+ACh
Ternary interplay	E		E			E	Ser+Glu
		E	E		n		NA+Glu+DA
	I			n		E	Ser+His+NA
						E	Glu+DA+ACh

the interhormone relationship theory which states that hormones produced by *Tetrahymena* alter each other's expression [30]. For example, Ser decreases His level while Ser and His enhances EGF production [36],[37]. Another theory stems from the fact that the cholinergic and adrenergic systems are the oldest; therefore, their signal transduction mechanisms are likely to be more developed at this stage of phylogeny. The opposite can also be true as their receptor structures are known to be highly homologous thus preventing the differentiation between these NTs in a less evolved organism [1].

Table 3: Neurotransmitters in order of ascending topological polar surface area (TPSA) values. Effectiveness increases with decreasing surface membrane penetration.

TPSA (Å ²)	Neurotransmitters	Effect
26.3	Ach	I, n
54.7	His	E, E
62	Ser	I, n
66.5	DA	E, E, I
86.7	NA	E, E, I
101	Glu	E, E, n

Table 4: Neurotransmitters in order of descending partition coefficient (XLogP) values. Effectiveness increases with increasing hydrophilicity.

XLogP	Neurotransmitters	Effect
0.2	Ach	E, n
0.2	Ser	E, n
-0.7	His	E, E
-1	DA	E, E, I
-1.2	NA	E, E, I
-3.7	Glu	E, E, n

In the ternary interplay, most of the molecules were more effective when acting on *Tetrahymena* cells alone rather than when being part of a combination. It is probable that many NTs still exert their functions through the same or functionally homologous receptor at this stage of phylogeny (while the huge variety of receptors appeared later through hormonal imprinting [38]); thus binding of one NT can interfere with the binding of the others. It was found that His, Ser and adrenaline increase cAMP allowing for the assumption that the interference between the signals might occur even on the second messenger level [30].

After comparing the physicochemical characteristics of the NTs (Table 2) a pattern was observed between *Annal Behav Neurosci*, 1(1): 108-118 (2018)

the effectiveness of the molecules and a poor surface membrane penetration along with increasing hydrophilicity. This pattern is represented in tables 3 and 4 where the molecules are shown in order of descending partition coefficient (XLogP) and ascending topological polar surface area (TPSA) values. The opposite correlation was established in a previous study done by our lab which evaluated the chemotactic response of *Tetrahymena* to mono- and disaccharides and determined a cut-off point for ligand penetration below 140 Å² and higher than -3.0 for TPSA and XLogP, respectively [39]. This means that molecules have a higher capacity of crossing the surface membrane when they are small (low TPSA) and more lipophilic (high XLogP). These findings along with the fact that some biogenic amine receptors such as that of adrenaline and His have been described on the plasma membrane of the ciliate [30] give rise to the hypothesis that the chemotactic response induced by NTs is carried out by surface-mediated receptor systems and not by mechanisms depending on ligand internalization [39]. A proposed schema for the mechanisms inducing chemotactic responses in *Tetrahymena* and possibly other eukaryotes is described in figure 4.

Rec: Receptor; Lig: Ligand; TPSA (Å²): Topological Polar Surface Area in Ångstroms square; XlogP: Partition Coefficient (P) Molecules with low XLogP and high TPSA values act through surface mediated receptor mechanism. Molecules with high XLogP and low TPSA values act on intracellular receptors or metabolic processes.

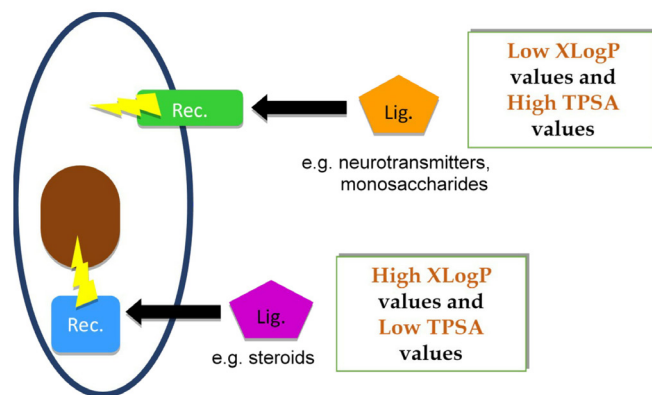


Figure 4: Hypothetical schema representing the chemotactic response induced by different molecules depending on their physicochemical parameters.

Conclusion

A complete hormonal network has been described in the eukaryote *Tetrahymena pyriformis* raising the question

of its contribution to the evolution of multicellular organisms [29]. The substantial homology of the components of the signaling system to that of mammals and the preservation of function of many molecules, allow for the speculation that the hormonal network of unicellular organisms served as the basic model for the evolution of the more sophisticated hormonal system in higher-ranked species. The findings of our study expand this notion by providing evidence of NT systems in *Tetrahymena pyriformis* which may function as the building blocks of the human CNS. We propose that mammalian NTs induce chemotactic responses in the unicellular ciliate by mechanisms dependent on surface-mediated receptor signaling.

Conflicts of Interest

Andria Tziakouri declares she has no conflict of interest. Eszter Lajkó declares she has no conflict of interest. László Kőhidai declares he has no conflict of interest.

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