Glycine as a signaling molecule and chemoattractant in *Trichoplax* (Placozoa): insights into the early evolution of neurotransmitters

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The origin and early evolution of neurotransmitter signaling in animals are unclear due to limited comparative information, primarily about prebilaterian animals. Here, we performed the comparative survey of signal molecules in placozoans – the simplest known free-living animals without canonical synapses, but with complex behaviors. First, using capillary electrophoresis with laser-induced fluorescence detection, we performed microchemical analyses of transmitter candidates in *Trichoplax adhaerens* – the classical reference species in comparative biology. We showed that the endogenous level of glycine (about 3 mM) was significantly higher than for other candidates such as L-glutamate, L-aspartate, or gamma-aminobutyric acid. Neither serotonin nor dopamine were detected. The absolute glycine concentrations in *Trichoplax* were even higher than we measured in ctenophores (*Beroe*) and cnidarians (*Aequorea*). We found that at millimolar concentrations of glycine (similar to the endogenous level), induced muscle-like contractions in free behaving animals. But after long incubation (24 h), 10^{-3} M of glycine could induce cytotoxicity and cell dissociation. In contrast, micromolar concentrations (10^{-5}–10^{-4} M) increased *Trichoplax* ciliated locomotion, suggesting that glycine might act as an endogenous signal molecule. However, we showed than glycine (10^{-6} M) can also be a chemoattractant (a guiding factor for food sources), and therefore, act as the exogenous signal. These findings provide an evolutionary base for the origin of transmitters as a result of the interplay between exogenous and endogenous signaling systems early in animal evolution. *NeuroReport* 31: 490–497 Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

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Introduction

Understanding the fundamental principles of neural organization and deciphering the signaling mechanisms in the brain is a substantial challenge [1], which also makes the reconstruction of the early evolution of neurotransmitter systems very difficult [2,3]. It is well established that nerve cells in bilaterian animals communicate at chemical synapses by releasing specific molecules into the cleft between them. Various low molecular weight transmitters, among them amino acids such as glutamate, aspartate or glycine, are being used. These molecules bind to specific receptors on the surface of the receiving cell and thus transmit the signal. It is unclear when in the evolution of animals these endogenous metabolites started to be used for signaling. This shifts attention to nonbilaterian animals such as cnidarians (polyps and jellyfishes), poriferans (sponges), ctenophores (comb jellies), and placozoans as key reference species in this endeavor [1,4]. However, little is known about the (neuro) transmitter organization in these groups of animals.

Cnidarians do have the complement of neurotransmitters, which is generally similar to low-molecular-weight chemical neurotransmitters in bilaterians [5,6]. In contrast, ctenophores lack most of the canonical neurotransmitter candidates (except glutamate), and independent origins of neurons and synapses might explain a remarkably different neural organization and signaling in this lineage [7,8]. The complement of endogenous intercellular signal molecules in nerveless sponges is ambiguous [9], mostly unknown, but pharmacological data show that many bilaterian neuroactive molecules can change sponge behaviors [10,11]. Thus, low-molecular-weight transmitters might be recruited for neuronal functions in the common ancestor of Cnidaria and Bilateria [7,12].

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Placozoa, and *Trichoplax* in particular, is likely the most prominent reference species and critical comparative neurobiology model [13], sister to the clade of Cnidaria+Bilateria [14–16]. These flat, disk-shaped organisms have the simplest known organization among all free-living animals, with only three cell layers and six major cell types [17]. However, *Trichoplax* shows quite complex behaviors [18–20], including social interactions [21]. It was suggested that peptides are prominent transmitter candidates in early metazoans [2], and an array of peptides as putative signal molecules have been predicted [22] from the sequenced genome [23]. Some of these endogenous peptides are indeed prominent regulators of *Trichoplax* behaviors and can induce three main locomotory patterns [24], which are different modes of gliding via ventral cilia.

Nevertheless, we know nothing about low-molecular-weight transmitters in these animals. Here, using a combination of direct microchemical analyses and behavioral assays, we show that glycine is the most abundant endogenous molecule, which can act in a concentration-dependent way to control contractility, activate locomotion and be a potential chemical cue for food.

Material and methods

Animal culture

We used three different species of Placozoa: *Trichoplax adhaerens*, H1; *Trichoplax* sp., H2 [13]; *Hoilungia hongkongensis*, H13 [25]. We cultured animals in large (15–20 cm) glass dishes with 35 ppm artificial seawater (artificial seawater [ASW], pH 8.0) at 24 ± 2°C using rice grains as a food source, and the detailed protocol has been described elsewhere [26]. For experiments, animals (up to 1 mm) were transferred to sterile Petri dishes and washed with ASW three times. For control comparative microchemical tests, we collected ctenophore, *Beroe abyssicola*, and the hydrozoan jellyfish, *Aequorea victoria*, from plankton at Friday Harbor Laboratories (University of Washington). These animals were maintained in running seawater at ambient temperature before experiments.

Amino acids microanalysis using capillary electrophoresis with laser-induced fluorescence detection

All protocols were similar to our previous assays [7,27,28]. Briefly, the capillary electrophoresis, coupled with the ZETALIF detector (Picometrics, France), was used for the assay of amino acids. In this work, a helium-cadmium laser (325 nm) from Melles Griot, Inc. (Omnichrome Series56, Carlsbad, California, USA) was used as the excitation source. All solutions were prepared with ultrapure Milli-Q water (Milli-Q filtration system, Millipore, Bedford, Massachusetts, USA) to minimize the presence of impurities. Borate buffer (30 mM, pH 9.5) was used for sample preparation. A 75 mM o-Phthalaldehyde (OPA)/β-mercaptoethanol stock solution was prepared by dissolving 10 mg of OPA in 100 μL of methanol and mixing with 1 mL of 30 mM borate and 10 μL of β-mercaptoethanol. Stock solutions (10 mM) of amino acids were prepared by dissolving each compound in the borate buffer. All experiments were conducted using a 75 cm length of 50 μm inner diameter × 360 μm outer diameter fused silica capillary (Polymicro Technologies, Arizona, USA). A 30 mM borate/30 mM sodium dodecyl sulphate electrolyte (adjusted to pH 10.0 with NaOH) was used as a separation buffer for amino acid analysis. The precolumn derivatization method was used. One microliter of OPA was added to a 0.5-mL PCR tube. The total volume of the sample, OPA, and internal standard inside the tube was 20 μL. For separation steps, the capillary inner wall was successively washed with 1 M NaOH, Milli Q water, and separation buffer by applying pressure (1900 mbar) to the inlet vial. Then, the sample was loaded using electrokinetic injection (8 kV for 12 s). The separation was performed under a stable 20 kV voltage at 20°C. In all capillary electrophoresis tests, once an electropherogram was acquired, peaks were assigned based on the electrophoretic mobility of each analyte, and the assignments were confirmed by spiking corresponding standards into the sample [27,28]. Five-point calibration curves (peak area vs. concentration) of analytes were constructed for quantification using standard solutions. All chemicals for buffers were obtained from Sigma-Aldrich, and standard amino acids were purchased from Fluka.

Pharmacological testing and imaging

For experiments, we used animals ranging from 200 to 800 μm in size. Before pharmacological tests (unless it is specified separately), animals were maintained for 2–5 h in sterile Petri dishes with ASW and washed two to three times to remove any remains of food traces. Final concentrations of glycine (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} M) were prepared in 35 ppm ASW (pH 8.0), and animals were imaged using either stereo or inverted Nikon microscopes. The testing of potential chemosensation to glycine was performed as described [26]. About 130 individuals were tested, and each set of video images was analyzed using ImageJ (NIH), calculating for velocity, animal area, and perimeter with at least 3–6 replicates.

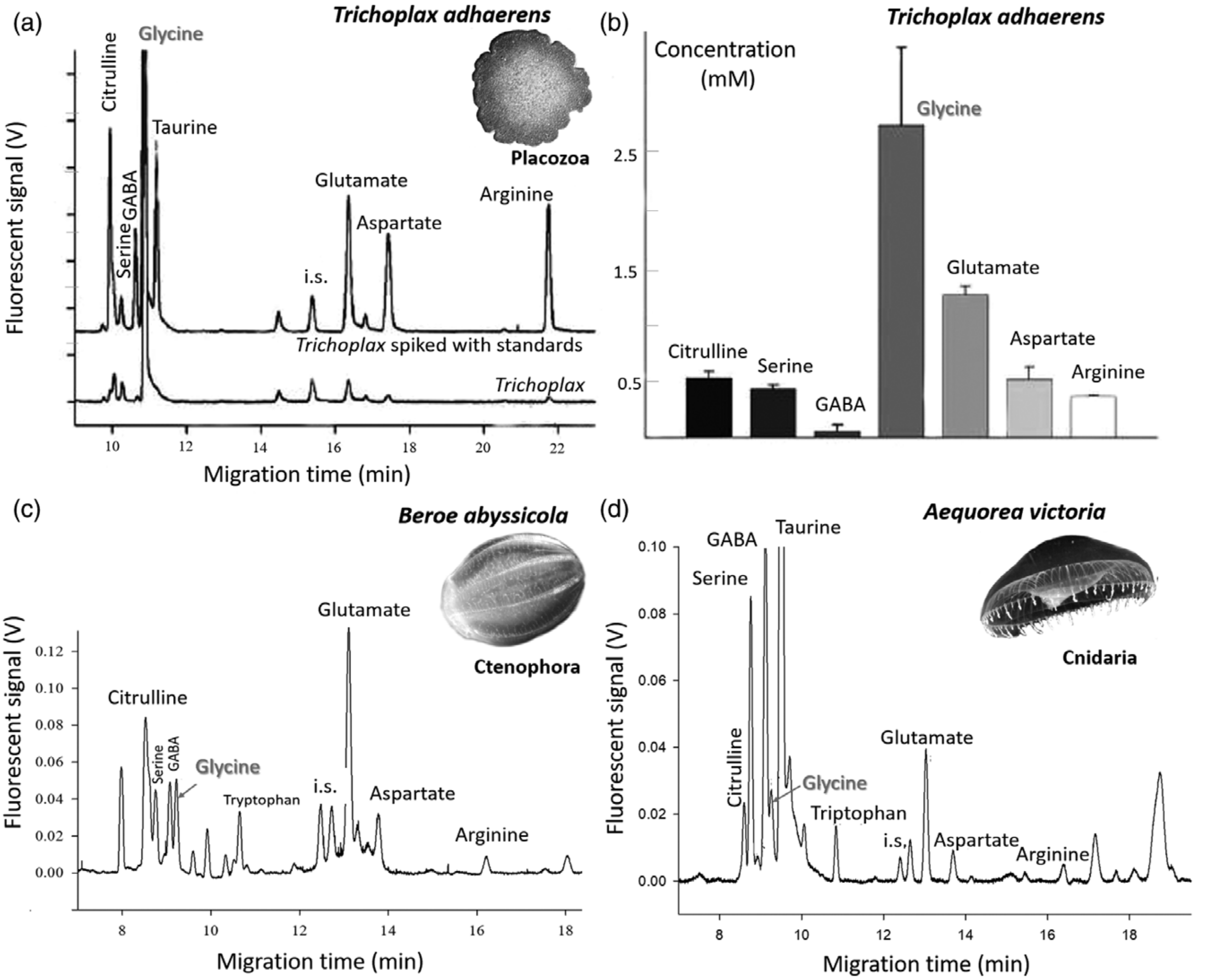
Results

Endogenous glycine present at micromolar concentrations in *Trichoplax*

In order to search for potential low molecular weight neurotransmitters in *T. adhaerens*, we performed microchemical analyses of amino acids and other compounds by sampling individual animals (200–600 μm in size, Fig. 1a). We used capillary electrophoresis, as it is the most direct and sensitive technique to quantify endogenous analytes with attomole detection limits [29].

Surprisingly, we found that the endogenous level of glycine was higher (2.6–3.1 mM) than any other amino acid

Fig. 1



Electropherograms and endogenous concentration profiling of *Trichoplax adhaerens*. (a) Electropherograms of *Trichoplax* and *Trichoplax* spiked with standards. (b) Concentration profile of *Trichoplax* ($n=5$). Electropherograms of the ctenophore *Beroe* (c) and the hydrozoan jellyfish *Aequorea* (d) showing different patterns of endogenous molecules detected under the same experimental conditions. All samples were loaded using electrokinetic injection (8 kV for 12 s) and then analyzed under a stable 20 kV voltage at 20°C in 50 μm ID and 360 μm OD capillary with 30 mM borate/30 mM SDS, pH 10.0. Identified peaks are marked with relevant analytes. GABA, gamma-aminobutyric acid; ID, inner diameter; i.s., internal standard in all cases; OD, outer diameter. See the text for details.

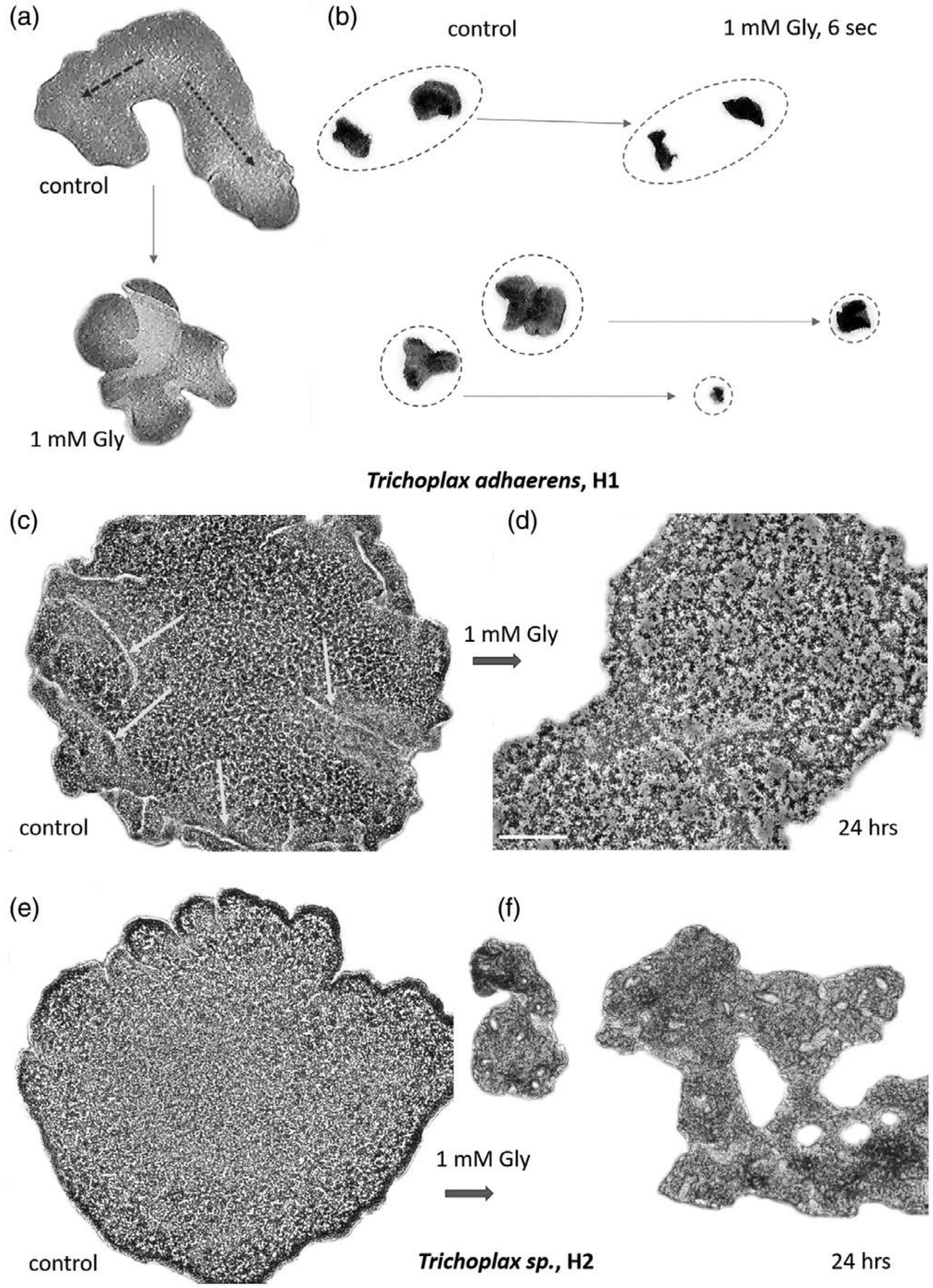
measured in *T. adhaerens* (Fig. 1a and b). We also detected gamma-aminobutyric acid (GABA, 50–60 μM) and several other metabolites, but no serotonin, dopamine, or octopamine. Both relative and absolute glycine concentrations in *Trichoplax* are even higher than those we measured in representatives of two other nonbilaterian lineages with nervous systems (Fig. 1a and d): ctenophores (*B. abyssicola*, Lobata) and cnidarians (*A. victoria*, Cnidaria). The unusually high concentration of glycine suggests that this molecule might not only be an intermediate metabolite but also serve as an intercellular messenger/transmitter in placozoans.

Glycine induced contractions and activation of ciliated locomotion in *Trichoplax*

Ultrasmall sizes of most of the cells in *Trichoplax* (usually less than 5 μm) prevents direct single-cell measurements, and therefore, behavioral assays that track the movements of the animals are the predominant pharmacological tests for placozoans [24]. Under control conditions without food, animals usually maintain steady exploratory locomotor activity by gliding over the substrate, which is significantly reduced in the presence of food such as algae or grains of rice, in agreement with previous observations [18,21,24,26]. Because the endogenous glycine concentration in *Trichoplax* is in the millimolar range, we applied in a pilot experiment 1 mM glycine (10^{-3} M) into the ASW medium of Petri dishes, where 16 animals of H1 and nine animals of H2 haplotypes were kept.

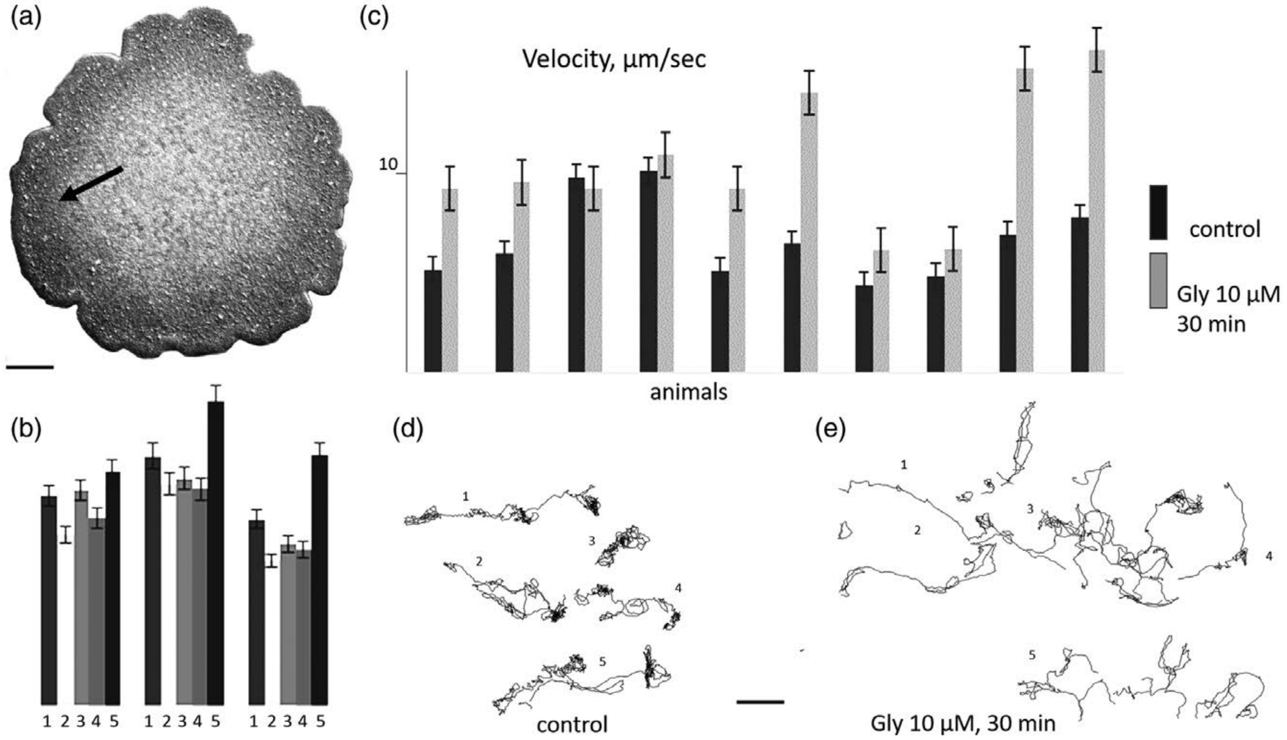
In all cases, local application of 1 mM glycine induced contractions of the animals immediately after the application (Fig. 2a and b); animals reduced the surface area of their disk-like body for several minutes and returned to their flat shapes and adhered to the substrate in the presence of glycine. In further experiments, we noted that long-term incubation (overnight or 24 h) of animals with 1 mM glycine induced the dissociation of animals into smaller fragments or even individual cells (Fig. 2c and d). The state might resemble a severe injury. The

Fig. 2



Glycine (1 mM) induces short-term (several seconds) contractions and folding of *Trichoplax* (a and b) with subsequent behavioral recovery. (c-f) Long-term (24) incubation of animals in the presence of 1 mM glycine leads to fragmentation of animals and cell dissociation. Scale: 100 μm.

Fig. 3



(a) A view of partially contracted *Trichoplax* (induced by 100 µM of glycine) with dynamic changes of cell density; the arrow shows a higher density of cells at the perimeter of the animal. (b) Relative changes of the surface areas of *Trichoplax* following application of 100 µM of glycine. 1 – control; 2 – first contraction (within 10 s); 3 – relaxation (60–100 s); 4 – second contraction (2 mins) with subsequent relaxation and flattering during locomotion (5). (c) Glycine (10 µM) activate locomotion ($n=10$); (d) the locomotory tracks under control conditions in Petri dishes without food; (e) in the presence of 10 mM of glycine.

process was irreversible after the washing. It seems that prolonged millimolar concentration of glycine can be cytotoxic.

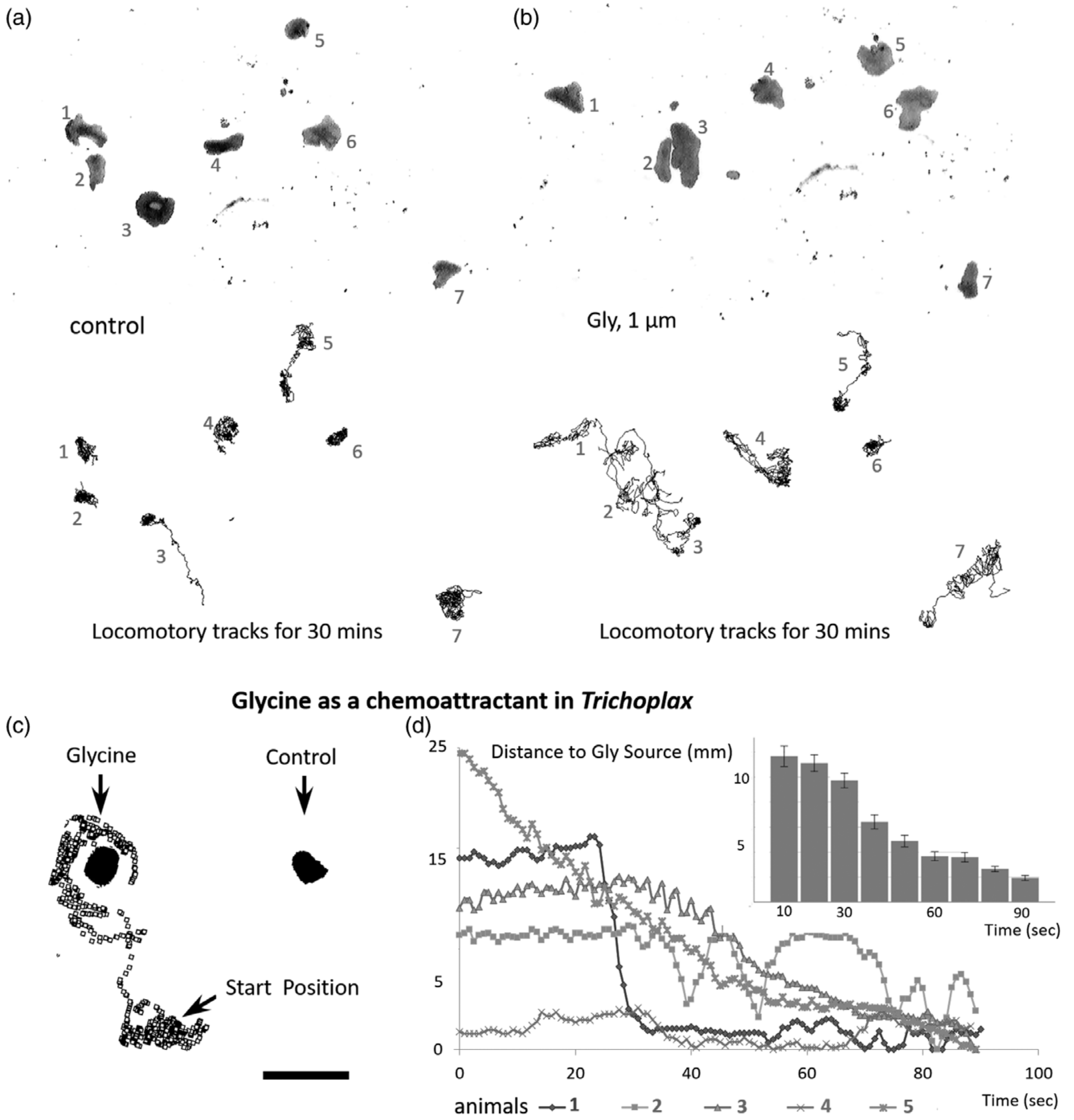
In the next set of experiments, we applied lower concentrations of glycine to the medium. Micromolar concentrations of glycine induced either one (10^{-5} M) or two (10^{-4} M) contractions of *Trichoplax*. The contractions usually occurred at the peripheral areas of the animals (Fig. 3a) in a ring-like manner, after which animals flattened (Fig. 3b), increased their locomotion activity and the speed of gliding (Fig. 3c and f). All placozoan haplotypes (H1, $n=9$; H2, $n=64$; and H13, $n=9$) showed the same initial contractive responses and long-term activation of gliding.

Glycine as a chemoattractant in *Trichoplax*

In the presence of food (rice grains or algae), animals reduce their movements [18,21], while active locomotion is more prominent in animals deprived of food (as in Fig. 3), and it is significantly increased after one day of starvation. Active locomotion is perceptible as stochastic-like movements without any particular direction [26] and can be described as an exploratory behavior. Fig. 4a and b shows that 10^{-6} M of glycine induced exploratory-like behavior and activate locomotion ($n=33$, H2). In contrast, to control animals, which are predominantly moving close to their algal/food substrate with frequent clockwise or anticlockwise rotation (sometimes for more than 30–60 mins at the same place), animals significantly reduced their rotation patterns and can glide at greater distances with different and changeable vectors of their movements in the presence of 1 µM glycine.

These observations suggest that *Trichoplax* might sense glycine as an extracellular cue in their microenvironment. To test directly that glycine can act as a chemoattractant, we performed experiments with a localized source of glycine and compared it with the control (Fig. 4c and d). Specifically, we prepared agar embedded with 1 mM glycine and placed a small block into the experimental arena; the control treatment was agar made with seawater (Fig. 4a). We then placed individual animals (one by one) *Trichoplax* in the arena and filmed its movement over 30 mins using time-lapse photography and quantified their trajectories (Fig. 4). In all trials, *Trichoplax* moved toward the glycine source (Fig. 4a and d). This suggests the role of glycine as a specific chemosensory

Fig. 4



Glycine activates locomotion and exploratory behavior in *Trichoplax*. (a) Control locomotory tracts of seven animals (indicated by numbers; animal positions are at the top). In the presence of algae (*Tetraselmis marina*, pink dots), animals are closely associated with the food source and have a slower speed, dominant rotation patterns, and shorter gliding trajectories. (b) Administration of glycine (1 µM) induces exploratory-like behaviors with reduced localized rotation patterns, higher velocity (as in Fig. 3), and longer gliding trajectories around food particles. (c) A representative example of the directional movement toward the source of glycine (agar block with 1 mM glycine is shown as a black spot at the left vs. a control block of agar; see text for details). (d) The distance to the glycine source (mm on Y axes) is shown as a function of time for all five individuals tested. Of note, one of the animals (number 4) was originally positioned closer to the source and was mostly stayed at this location during the experiment. The paths were quantified by calculating the distance between the glycine source and the individual (d1) relative to the distance between the control source and the individual (d2). Data were normalized by calculating the difference between d1 and d2. Insert shows the average distance to the Gly source at 10 mins intervals (n=5). Scale bar: 2 mm.

molecule capable to induce coordinated and directed locomotion in the animal.

Discussion

Using capillary electrophoresis microchemical assays, we identified glycine as the most abundant amino acid in *Trichoplax*. This situation is not typical for some other nonbilaterian animals. Indeed, by performing the same assays on selected ctenophores (*Beroe*) and cnidarians (*Aequarea*), we found that both relative amount and the endogenous concentrations of glycine were lower than in *Trichoplax*. Of note, the concentration of glycine in *Trichoplax* resembles that of that in rat astrocytes (3–6 mM glycine) [30]. Moreover, the concentration of glycine in the synaptic cleft is estimated to be around 1 mM [31]. Thus, the observed glycine concentrations in *Trichoplax* are in a range observed for cells that are known to communicate using glycine as a transmitter.

L-glutamate was the most dominant metabolite in *Beroe*, consistent with the proposed role of glutamate as a neuromuscular transmitter in ctenophores [7]. In the jellyfish *Aequorea*, both taurine and GABA were dominant analytes; it also consistent with the proposed transmitter role of these two molecules in Cnidaria and the role of taurine as a major osmolyte [32–34]. Our behavioral and pharmacological tests suggested that glycine can be an endogenous signal molecule in placozoans, involved in the control of locomotion, exploratory-like, and feeding behaviors.

We noted that glycine could act as a chemoattractant [26], and therefore serves as an exogenous signal and environmental cue in search of food. These findings provide interesting insights into the origin of transmitter systems as a result of the interplay between exogenous and endogenous signaling systems early in animal evolution and cross-kingdom signaling similarly as described during the development of marine larva and metamorphosis [35]. For example, the early ecologically relevant development of receptors for glycine can be viewed as a preadaptation for subsequent recruitment of glycine as an endogenous signaling molecule and neurotransmitter.

Our screening of the *Trichoplax* genome [23] and available transcriptomes [9] does not reveal any conventional ionotropic glycine receptors. Thus, the observed relatively high sensitivity of contractive (more likely fiber) cells and locomotory ciliated cells in *Trichoplax* might be explained either by the presence of orphan G-protein-coupled receptors, which are abundant in the genome, and noncanonical ionotropic receptors. We identified 12 ionotropic glutamate receptors (iGluR) encoded in the *Trichoplax* genome, and these receptors possess potential glycine-binding sites as it was reported for N-methyl-D-aspartate-like receptors across species [36]. Moreover, it was also shown that iGluRs in ctenophores are gated by glycine [37]. Thus, their placozoan homologs should be expressed in oocytes and studied as potential candidates for endogenous glycine-gated receptors. We might also hypothesize that initial recruitment of iGluRs toward glycine sensing might contribute to evolutionary development glycine (neuro)transmitter functions and, eventually, specific ionotropic glycine receptors in the course of evolution of bilaterian neural systems. In conclusion, *Trichoplax* and related placozoans are attractive and perspective model systems to decipher early origins of neural signaling in Metazoa and mechanisms of recruitments of low-molecular-weight transmitters in particular.

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Conflicts of interest

There are no conflicts of interest.

References

1 Striedter GF, Belgard TG, Chen CC, Davis FP, Finlay BL, Güntürkün O, *et al*. NSF workshop report: discovering general principles of nervous system organization by comparing brain maps across species. *J Comp Neurol* 2014; **522**:1445–1453.

2 Moroz LL. On the independent origins of complex brains and neurons. *Brain Behav Evol* 2009; **74**:177–190.

3 Moroz LL. Phylogenomics meets neuroscience: how many times might complex brains have evolved? *Acta Biol Hung* 2012; **63** (Suppl 2):3–19.

4 Moroz LL. Neurosystematics and periodic system of neurons: model vs reference species at single-cell resolution. *ACS Chem Neurosci* 2018; **9**:1884–1903.

5 Kass-Simon G, Pierobon P. Cnidarian chemical neurotransmission, an updated overview. *Comp Biochem Physiol A Mol Integr Physiol* 2007; **146**:9–25.

6 Walker RJ, Brooks HL, Holden-Dye L. Evolution and overview of classical transmitter molecules and their receptors. *Parasitology* 1996; **113** (Suppl):S3–33.

7 Moroz LL, Kocot KM, Citarella MR, Dosung S, Norekian TP, Povolotskaya IS, *et al*. The ctenophore genome and the evolutionary origins of neural systems. *Nature* 2014; **510**:109–114.

8 Moroz LL, Kohn AB. Independent origins of neurons and synapses: insights from ctenophores. *Philos Trans R Soc Lond B Biol Sci* 2016; **371**:20150041.

9 Moroz LL, Kohn AB. Unbiased view of synaptic and neuronal gene complement in ctenophores: are there pan-neuronal and pan-synaptic genes across metazoa? *Integr Comp Biol* 2015; **55**:1028–1049.

10 Nickel M, Scheer C, Hammel JU, Herzen J, Beckmann F. The contractile sponge epithelium sensu lato–body contraction of the demosponge tethya wilhelma is mediated by the pinacoderm. *J Exp Biol* 2011; **214**:1692–1698.

11 Leys SP. Elements of a 'nervous system' in sponges. *J Exp Biol* 2015; **218**:581–591.

12 Moroz LL. The genealogy of genealogy of neurons. *Commun Integr Biol* 2014; **7**:e993269.

13 Schierwater B, DeSalle R. Placozoa. *Curr Biol* 2018; **28**:R97–R98.

14 Laumer CE, Fernández R, Lemer S, Combosch D, Kocot KM, Riesgo A, *et al*. Revisiting metazoan phylogeny with genomic sampling of all phyla. *Proc Biol Sci* 2019; **286**:20190831.

15 Whelan NV, Kocot KM, Moroz LL, Halanych KM. Error, signal, and the placement of ctenophora sister to all other animals. *Proc Natl Acad Sci U S A* 2015; **112**:5773–5778.
16 Whelan NV, Kocot KM, Moroz TP, Mukherjee K, Williams P, Paulay G, *et al*. Ctenophore relationships and their placement as the sister group to all other animals. *Nat Ecol Evol* 2017; **1**:1737–1746.
17 Smith CL, Varoqueaux F, Kittelmann M, Azzam RN, Cooper B, Winters CA, *et al*. Novel cell types, neurosecretory cells, and body plan of the early-diverging metazoan trichoplax adhaerens. *Curr Biol* 2014; **24**:1565–1572.
18 Smith CL, Pivovarova N, Reese TS. Coordinated feeding behavior in trichoplax, an animal without synapses. *PLoS One* 2015; **10**:e0136098.
19 Smith CL, Reese TS, Govezensky T, Barrio RA. Coherent directed movement toward food modeled in trichoplax, a ciliated animal lacking a nervous system. *Proc Natl Acad Sci U S A* 2019; **116**:8901–8908.
20 Armon S, Bull MS, Aranda-Diaz A, Prakash M. Ultrafast epithelial contractions provide insights into contraction speed limits and tissue integrity. *Proc Natl Acad Sci U S A* 2018; **115**:E10333–E10341.
21 Fortunato A, Aktipis A. Social feeding behavior of *Trichoplax adhaerens*. *Front Ecol Evol* 2019; **7**:19.
22 Nikitin M. Bioinformatic prediction of trichoplax adhaerens regulatory peptides. *Gen Comp Endocrinol* 2015; **212**:145–155.
23 Srivastava M, Begovic E, Chapman J, Putnam NH, Hellsten U, Kawashima T, *et al*. The trichoplax genome and the nature of placozoans. *Nature* 2008; **454**:955–960.
24 Varoqueaux F, Williams EA, Grandemange S, Truscello L, Kamm K, Schierwater B, *et al*. High cell diversity and complex peptidergic signaling underlie placozoan behavior. *Curr Biol* 2018; **28**:3495–3501.e2.
25 Eitel M, Francis WR, Varoqueaux F, Daraspe J, Osigus HJ, Krebs S, *et al*. Comparative genomics and the nature of placozoan species. *Plos Biol* 2018; **16**:e2005359.
26 Heyland A, Croll R, Goodall S, Kranyak J, Wyeth R. Trichoplax adhaerens, an enigmatic basal metazoan with potential. *Methods Mol Biol* 2014; **1128**:45–61.
27 Floyd PD, Moroz LL, Gillette R, Sweedler JV. Capillary electrophoresis analysis of nitric oxide synthase related metabolites in single identified neurons. *Anal Chem* 1998; **70**:2243–2247.
28 Moroz LL, Dahlgren RL, Boudko D, Sweedler JV, Lovell P. Direct single cell determination of nitric oxide synthase related metabolites in identified nitrergic neurons. *J Inorg Biochem* 2005; **99**:929–939.
29 Fuller RR, Moroz LL, Gillette R, Sweedler JV. Single neuron analysis by capillary electrophoresis with fluorescence spectroscopy. *Neuron* 1998; **20**:173–181.
30 Verleysdonk S, Martin H, Willker W, Leibfritz D, Hamprecht B. Rapid uptake and degradation of glycine by astroglial cells in culture: synthesis and release of serine and lactate. *Glia* 1999; **27**:239–248.
31 Harsing LG Jr, Matyus P. Mechanisms of glycine release, which build up synaptic and extrasynaptic glycine levels: the role of synaptic and non-synaptic glycine transporters. *Brain Res Bull* 2013; **93**:110–119.
32 Anderson PA, Trapido-Rosenthal HG. Physiological and chemical analysis of neurotransmitter candidates at a fast excitatory synapse in the jellyfish *Cyanea capillata* (Cnidaria, Scyphozoa). *Invert Neurosci* 2009; **9**:167–173.
33 Walther M. Taurine in the marine hydrozoan hydractinia echinata: stabilizer of the larval state? *Comp Biochem Physiol A Mol Integr Physiol* 2002; **133**:179–190.
34 Anctil M, Minh CN. Neuronal and nonneuronal taurine-like immunoreactivity in the sea pansy, *Renilla koellikeri* (Cnidaria, Anthozoa). *Cell Tissue Res* 1997; **288**:127–134.
35 Heyland A, Moroz LL. Cross-kingdom hormonal signaling: an insight from thyroid hormone functions in marine larvae. *J Exp Biol* 2005; **208**:4355–4361.
36 Yu A, Alberstein R, Thomas A, Zimmet A, Grey R, Mayer ML, Lau AY. Molecular lock regulates binding of glycine to a primitive NMDA receptor. *Proc Natl Acad Sci U S A* 2016; **113**:E6786–E6795.
37 Alberstein R, Grey R, Zimmet A, Simmons DK, Mayer ML. Glycine activated ion channel subunits encoded by ctenophore glutamate receptor genes. *Proc Natl Acad Sci U S A* 2015; **112**:E6048–E6057.