

GABA and glutamate specifically induce contractions in the sponge *Tethya wilhelma*

Kornelia Ellwanger · Andre Eich · Michael Nickel

Received: 30 January 2006 / Revised: 17 August 2006 / Accepted: 18 August 2006 / Published online: 5 October 2006
© Springer-Verlag 2006

Abstract Sponges (Porifera) are nerve- and muscleless. Nevertheless, they react to external stimuli in a coordinated way, by body contraction, oscule closure or stopping pumping activity. The underlying mechanisms are still unknown, but evidence has been found for chemical messenger-based systems. We used the sponge *Tethya wilhelma* to test the effect of γ -aminobutyric acid (GABA) and glutamate (L-Glu) on its contraction behaviour. Minimal activating concentrations were found to be 0.5 μ M (GABA) and 50 μ M (L-Glu), respectively. Taking maximum relative contraction speed and minimal relative projected body area as a measure of the sponge's response, a comparison of the dose–response curves indicated a higher sensitivity of the contractile tissue for GABA than for L-Glu. The concentrations eliciting the same contractile response differ by about 100-fold more than the entire concentration range tested. In addition, desensitising effects and spasm-like reactions were observed. Presumably, a GABA/L-Glu metabotropic receptor-based system is involved in the regulation of contraction in *T. wilhelma*.

Electronic supplementary material Supplementary material is available in the online version of this article at <http://dx.doi.org/10.1007/s00359-006-0165-y> and is accessible for authorized users.

K. Ellwanger and A. Eich contributed equally and designed and performed experiments, analysed data and revised the paper, M. Nickel designed the study and experiments, analysed data, prepared the figures, wrote and revised the paper.

K. Ellwanger · A. Eich · M. Nickel (✉)
Department of Zoology, Biological Institute,
University of Stuttgart, 70550 Stuttgart, Germany
e-mail: michael.nickel@bio.uni-stuttgart.de

We discuss a coordination system for sponges based on hypothetical chemical messenger pathways.

Keywords *Tethya wilhelma* (Porifera) · Induced contraction · Coordination · γ -Aminobutyric acid · L-Glutamate

Abbreviations

ANOVA	Analysis of variance
$A_{r \max}$	Maximum relative projected area
$A_{r \min}$	Minimal relative projected area
A_r	Relative projected area
ΔA_r	Change of relative projected area
c_s	Substance concentration
GABA	γ -Aminobutyric acid
GABA _A R, GABA _C R	Ionotropic GABA receptors
GABA _B R	Metabotropic GABA receptor
iGluR	Ionotropic L-Glu receptors
L-Glu	L-Glutamic acid
LSD	Least significant difference
mGluR	Metabotropic L-Glu receptor
P_C	Contracted phase
P_E	Expanded phase
v	Contraction speed
v_{\max}^C	Maximum relative contraction speed
v_{\max}^E	Maximum relative speed of expansion
v_{\max}^i	Maximum induced relative contraction speed
ΔpH	pH-shift

Introduction

Aristotle (384–322 BC) first stated the sensitivity and contractility of marine sponges (Aristotle 1498). The observations that members of the Porifera react to external stimuli (McNair 1923; Emson 1966; Pavans de Ceccatty 1979; Leys and Mackie 1997; Nickel and Brümmer 2004), contract (Lieberkühn 1859; Schmidt 1866; Weissenfels 1990; Nickel 2004), move (McNair 1923; Jones 1962; Kilian 1967; Fishelson 1981; Bond and Harris 1988; Bond 1992; Sarà et al. 2001; Nickel and Brümmer 2004; Nickel 2006) and even display diurnal rhythms (Reiswig 1971; Nickel et al. 2002; Nickel 2004) are the base to ask whether a nervous system exists in sponges or which alternative coordination mechanism may have evolved in sponges (Parker 1919; Pantin 1952; Jones 1962; Lentz 1968; Pavans de Ceccatty 1974, 1979; Mackie 1979, 1990; Perovic et al. 1999; Weyrer et al. 1999; Ellwanger and Nickel 2006). However, neither a nervous system nor muscles have been found, which could explain the described behaviour. Nevertheless, the molecular as well as the physiological characterisation of a neuronal-like metabotropic glutamate/GABA-like receptor in the sponge *Geodia cydonium* calls the attention to chemical messenger systems (Perovic et al. 1999).

Both, GABA and glutamate are among the most important neurotransmitters in invertebrate and vertebrate nervous systems and act upon a variety of metabotropic and ionotropic receptors (Fagg and Foster 1983; von Bohlen und Halbach and Dermietzel 2002). These receptors are not specific to organisms with a nervous system. GABA and L-Glu-based messenger systems have evolved well before the multicellular animals. They are found in a wide variety of organisms: GABAergic systems have been demonstrated in plants (Bouche et al. 2003), ciliates (Ramoino et al. 2003, 2005), nematodes (Schuske et al. 2004), molluscs (Ito et al. 2003), arthropods (Mezler et al. 2001; Panek et al. 2003) and all vertebrate classes (Xue 1998). The same applies for glutamatergic systems, which has been described for plants (Davenport 2002), many invertebrates (Xue 1998; Panek and Torkkeli 2005) and vertebrates (Xue 1998). The wide distribution of both systems underlines their early evolution and their biological significance (Chiu et al. 1999). Most likely these systems also play a role in sponges, as suggested by Perovic et al. (1999).

Recently, we demonstrated the value and model character of the contractile sponge *Tethya wilhelma* (Fig. 1a, b) for research on signalling and coordination in sponges (Nickel 2004; Ellwanger and Nickel 2006). Its contraction can be addressed quantitatively and

manipulated within experimentation chambers. In the present study, we tested the effect of the agonists GABA and L-Glu on the contraction of *T. wilhelma* by establishing dose–response curves and characterising concentration-dependent effects in general.

Materials and methods

Sponges

Specimens of the sponge *T. wilhelma* (Sarà et al. 2001; Tethyidae, Hadromerida, Demospongiae) were obtained from the type location in the aquarium of the zoological–botanical garden ‘Wilhelma’ in Stuttgart. This small sponge species was originally described from a tropical aquarium habitat (Sarà et al. 2001) and can be maintained and bred easily in aquariums due to its asexual propagation, based on budding (Nickel 2001; Nickel and Brümmer 2004). For the experiments the sponges were kept in a 180 l aquarium at 26°C, using artificial seawater (Nickel et al. 2001), at a light–dark cycle of 12/12 h. The sponges were fed four to five times a week using suspended Artificial Plankton (Aquakultur Genzel, Fellbach, Germany, <http://www.aquakultur-genzel.de>). Seawater was exchanged about monthly at a rate of around 10% of the total aquarium volume.

Experimentation chamber

All experimental manipulations were carried out in a 250 ml closed experimentation chamber. It consisted of an aerated chamber, designed on the principles of air-lift reactors, connected to a temperature regulation unit (F25, Julabo, Seelbach, Germany). Oxygen level and temperature were monitored using a multi-sensor system (P4, WTW, Weilheim, Germany), controlled by a computer-software (MultiLab Pilot 3.0, WTW). An optical glass plate on the front side allowed proper imaging.

Digital time-lapse imaging

Digital images of the sponges were taken at a resolution of 2,048 × 1,536 pixels at regular intervals of 180 s (pre- and post-experimental monitoring) and 30 s (monitoring of induced contraction), resulting in an image-data accumulation rate of 60 and 360 mb/h, respectively (uncompressed 8-bit image data). A Nikon Coolpix 990E digital camera connected to a Nikon SB 24 flash unit was used to acquire greyscale images. The camera was controlled by a PC, using the

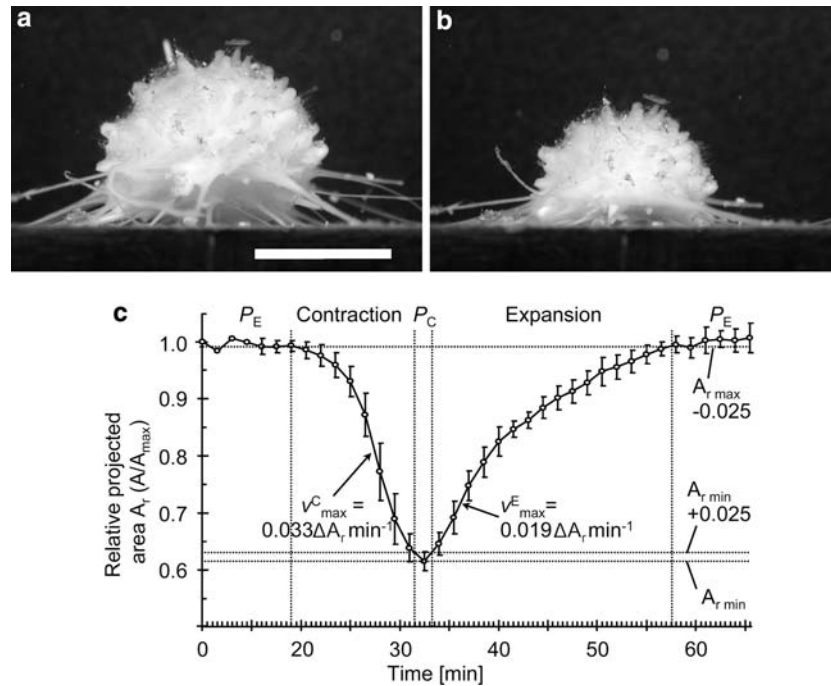


Fig. 1 **a, b** A specimen of *T. wilhelma* in non-contracted (**a**) and contracted state (**b**); scale bar represents 5 mm. **c** Contraction kinetics based on a dataset of 13 subsequent endogenous body contractions of *T. wilhelma*, displaying changes of the relative projected area over time. The cycle consists of four major phases: expanded phase (P_E), contracted phase (P_C) with *Contraction* and *Expansion* in-between. To divide the cycle into these phases,

the following limits are used: maximum and minimum relative projected areas ($A_{r\max}$ and $A_{r\min}$), as well as 2.5% -deviations of these ($A_{r\max} - 0.025$ and $A_{r\min} + 0.025$). The maximum contraction and expansion speeds can be derived from this diagram (v_{\max}^C and v_{\max}^E). For further explanations refer to the text and a previous publication (Nickel 2004)

software bundle DC_RemoteShutter V 2.3.0/DC_TimeTrigger V. 1.0 (Madson 2003). Images were automatically saved on the PC and erased on the CF-card of the camera instantly after being taken. A reference image including a scale bar placed next to the sponge was taken for each experimental series. In all cases, a black background maximised contrast.

Image analysis and statistics software

For image analysis, ImageJ 1.30 and 1.31 (NIH, Washington, USA) was used (Rasband 1997–2005). Excel 2000 (Microsoft, Redmond, USA) was used to prepare activity diagrams and the Excel add-on WinSTAT for statistical tests (Fitch 2005).

Projected area

The measurement of projected areas was based on the contrast between sponge (whitish) and background (black). The size of all images was scaled using the reference image. A greyscale threshold value between 50 and 90 was applied to the 8-bit images, and the projected area of the sponge was measured using ImageJ's built-in measurement tool. Each time-lapse series was

loaded as an image stack into ImageJ. A macro was programmed to measure semi-automatically. Results were written to a text file and evaluated using Excel 2000.

Test substance application

We tested and characterised the contractile response of *T. wilhelma* upon glutamate (L-Glu; L-glutamate acid sodium salt hydrate; Sigma-Aldrich G1626) and GABA (γ -aminobutyric acid; Sigma-Aldrich A2129). Diluted stock solutions were injected into the experimental reactor to reach final concentrations between 10 μ M and 10 mM for L-Glu and 0.1 μ M and 1 mM for GABA. Care was taken not to inject the solutions directly into the sponge specimen, but to allow mixing in the circulating water current of the system. The mixing may have varied slightly from experiment to experiment, slightly affecting the time between injection and onset of the reaction of the sponge. Consequently, the point in time just before the onset of the reaction was defined as experimental time $t_0 = 0$ min and not the injection of the substance. For each experiment, substances were only applied during a phase of expansion in-between two subsequent endogenous rhythmic

contractions, but the relative time within the contraction cycle varied among the experiments. After each experiment, the reactor was perfused by fresh, aerated artificial seawater at 26°C. The same specimens were eventually used for another experiment after retaining a normal contraction rhythm for at least several hours.

Control experiments and control measurements

Prior to the experiment, each specimen used was allowed to acclimatise to the experimental reactor for several hours. Experiments were only started after the sponge specimens displayed typical regular contraction patterns as described previously (Nickel 2004). During all of the experiments, temperature and oxygen-level were monitored and recorded.

Changes in pH due to application of L-Glu and GABA were measured. Control experiments were carried out to test the sponge's reaction to pH-changes.

Average contraction cycles

For the calculation of the average non-induced contraction cycle, values of 13 subsequent cycles of a time-lapse series were analysed. For the calculation of the dose dependent average induced contraction cycles, each agonist concentration was tested three times on different specimens. For induced kinetics, 57 experiments were performed.

For each cycle, relative contraction values were calculated, by setting the starting non-contracted state (maximum area at t_0) of each cycle or substance test to 1. The relative projected area values of each cycle were calculated in relation to the antecedent maximum. In this way, the influence of changes in the body extension on the projected area was minimised. The average contraction including standard deviation was calculated for each relative time point. Contraction kinetic diagrams were plotted for each independent dataset.

The maximum speed of contraction (v_{\max}^C) was calculated for each dataset, as well as the maximum speed of expansion for the datasets of the non-induced contraction kinetic measurement (v_{\max}^E). An ANOVA based on least significant difference (LSD) was performed to compare the differences in v_{\max}^C between non-induced and induced contractions.

Dose–response curves

The dose–response curves for L-Glu and GABA were plotted using the average maximum contraction speed (v_{\max}^C) and the minimum relative projected body area (A_{\min}) for both substances over the whole

concentration range tested. A correlation between v_{\max}^C and A_{\min} for all concentrations of both substances was plotted and we tested the correlation using a linear regression analysis.

Results

Kinetics of endogenous contractions

In the present study, we analysed 13 consecutive rhythmic endogenous contractions of a specimen settled in the experimentation chamber. The average contraction cycle curve followed the kinetics shown for the aquarium habitat (Fig. 1, compare Nickel 2004). Calculated from this graph, the maximum contraction speed $v_{\max}^C = 0.033 \Delta A_r \text{ min}^{-1}$ (from $t = 540 \text{ s}$ to $t = 720 \text{ s}$ prior to maximum contraction) and the maximum expansion speed $v_{\max}^E = 0.019 \Delta A_r \text{ min}^{-1}$ (from $t = 350 \text{ s}$ to $t = 540 \text{ s}$ after maximum contraction) represent the absolute value of the maximum changes of relative projected sponge area (A_r) per time. The average maximum contraction speed calculated from the maxima of each independent dataset is $v_{\max}^C = 0.039 \pm 0.007 \Delta A_r \text{ min}^{-1}$ at $t = 665 \pm 135 \text{ s}$ prior to maximum contraction. The average maximum expansion speed calculated from the maxima of each independent dataset is $v_{\max}^E = 0.021 \pm 0.003 \Delta A_r \text{ min}^{-1}$ at $t = 512 \pm 100 \text{ s}$ after maximum contraction.

Four distinct values of A_r were set to define the phases of the contraction cycle: Maximal relative projected area ($A_{r \max} = 1.0$, per definition), minimal relative projected area ($A_{r \min} = 0.615$, specific experimental value for dataset of Fig. 1), $A_{r \max}$ minus 2.5% ($A_{r \max} - 0.025$) and $A_{r \min}$ plus 2.5% ($A_{r \min} + 0.025$). The transition from $A_r = A_{r \max} - 0.025$ to $A_r = A_{r \min} + 0.025$ represents the *contraction* and lasted 12.5 min in the experiment. The period with $A_r < A_{r \min} + 0.025$ represents the *contracted phase* P_C and lasted 1.75 min. The transition from $A_r = A_{r \max} - 0.025$ to $A_r = A_{r \min} + 0.025$ represents expansion and lasted 24.25 min. The period with $A_r > A_{r \max} - 0.025$ represents the *expanded phase* P_E . The contraction cycle kinetics for *T. wilhelma* in the experimental reactor showed no major difference to the kinetics in the aquarium habitat, with v_{\max}^C and v_{\max}^E in the expected range.

Control experiments

All experiments were performed at a seawater temperature of $25.5 \pm 0.25^\circ\text{C}$. Temperature changes within this range did not alter the contraction cycle patterns. Due to permanent aeration of the experimentation

chamber, the O_2 concentration never dropped below 98% of saturation. The slight changes in O_2 concentration did not affect the contraction cycles.

The pH-value of the seawater was determined to pH 8.15 ± 0.04 . Application of L-Glu and GABA in final concentrations of 10 and 0.97 mM, respectively, resulted in minor pH changes: $\Delta pH_{\text{Glutamat}} = -0.50 \pm 0.05$ and $\Delta pH_{\text{GABA}} = -0.01 \pm 0.01$. The higher pH-shift $\Delta pH_i = -0.50$ was imitated by applying an appropriate HCl concentration to the seawater in the experimental reactor, which did not result in any contraction induction.

Reactions upon L-Glu and GABA

Glutamate induces full contractions and sub-contractions (as defined in Nickel 2004), depending on the agonist concentration (Fig. 2). All sponges displayed a regular endogenous contraction pattern prior to stimulation and after washout of the substances. Consequently, the experiments did not affect their viability. A direct comparison between a 10 mM L-Glu induced full contraction and a preceding endogenous full contraction reveals corresponding and differing aspects of both types of contractions (Fig. 2a). Both contractions have similar durations, but the induced contraction is slightly stronger and *contraction* (as defined in Fig. 1) is shorter, resulting in a higher maximal contraction speed. In addition, *expansion* is prolonged, which may be an effect of the remaining L-Glu in the experimental reactor. In several experiments, the sponge remained in a prolonged contracted phase (P_C , Fig. 2b). However, after expansion and several hours in the expanded phase (P_E) a second contraction could be induced, without previous washing, by applying the same quantity of L-Glu again, virtually doubling the concentration inside the experimental reactor (Fig. 2b).

Sub-contractions, which are weaker than full contractions, are generally induced by lower concentrations (Fig. 2c). The lowest L-Glu concentrations inducing sub-contraction in most experiments were found to be 200 and 400 μM . Lower concentrations were tolerated by the sponges without reaction in many experiments. In addition, *T. wilhelma* displays an increased tolerance for L-Glu within the first hour after an induced contraction and early subsequent washout of the initial L-Glu (Fig. 2c). The L-Glu contraction inside the experimental reactor had to be raised stepwise to 1.6 mM to induce a second sub-contraction.

GABA induces full contractions and sub-contractions in dependence to concentration, too (Fig. 3). Nevertheless, the actual concentrations are lower. The

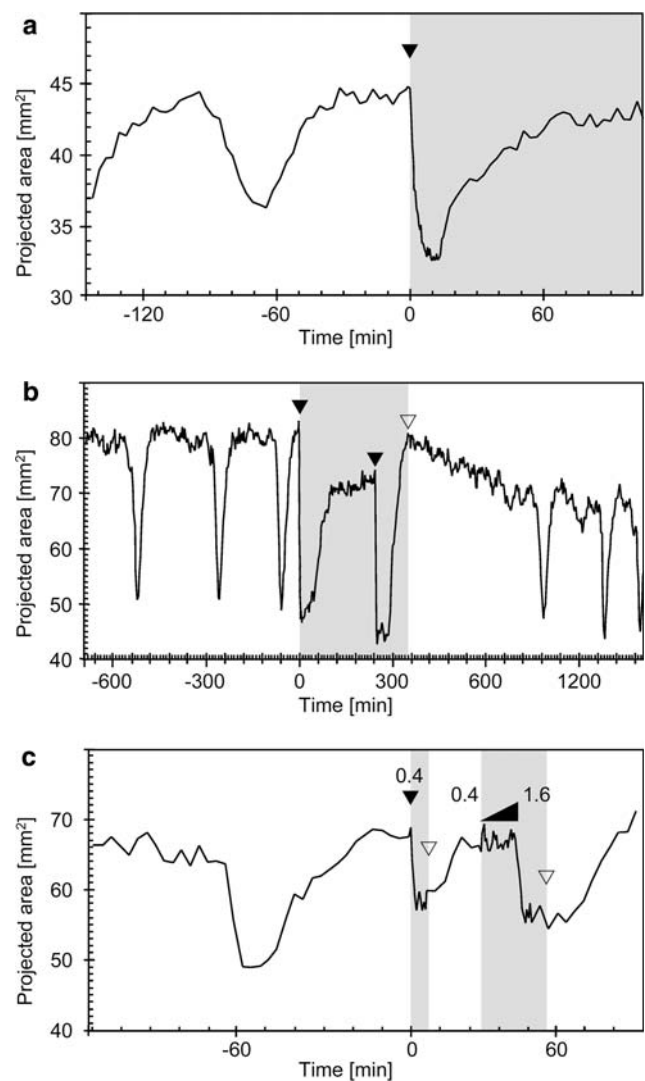


Fig. 2 a–c Time course of contraction of *T. wilhelma* treated with L-Glu. Response given as absolute projected area over time. Periods of agonist exposure to the sponge are displayed in grey. Agonist application is indicated by solid arrowheads, washout of the agonist from the system is indicated by open arrowheads. **a** Endogenous contraction prior to a 10 mM L-Glu-induced contraction, displaying the higher contraction speed. **b** Double induction by 10 mM L-Glu within a period of 4 h, with no washout in-between; the agonist seems to be attenuated, after the sponge expands again to a level 10–15% lower than prior to the contraction, but reaches full expanded state again after the second induction. The rhythm is disturbed after washout. **c** Double stimulation with L-Glu within 1 h, with a washout directly after the first induction, displaying desensitisation after the first stimulation. First induction at 0.4 mM results in a sub-contraction. After expansion the sponge is stimulated again with increasing concentrations of L-Glu starting at 0.4 mM up to 1.6 mM, at which the sponge recontracts

lowest GABA concentration reliably resulting in sub-contractions was found to be around 0.5 and 1.0 μM (Fig. 3c). GABA concentrations of higher than 10 μM reliably induced full contractions. A direct comparison

between a 97 μM GABA induced full contraction and a preceding endogenous full contraction reveals corresponding and differing aspects of both types of contractions (Fig. 3a), similar to those found in L-Glu induction: a slightly stronger contraction, a higher maximum contraction speed, a prolonged P_C and lower expansion rate. Occasionally *T. wilhelma* displays a spasm-like pattern of local contractions and expansions during P_C , resulting in a characteristic serrated graph (Fig. 3b, Movie S1).

Both, after L-Glu and GABA application the long-term rhythm of contraction in *T. wilhelma* seems to be disturbed. We found a tendency for lower frequencies of the endogenous contraction cycles (data not shown). However, since regular complete water exchanges were necessary from time to time to maintain the system, and each of these water exchanges usually prolonged the sponges P_E , we were not able to characterise this long-term effect of GABA and L-Glu.

Dose–response curves

Using three independent replicas of induced contractions for each concentration of L-Glu and GABA, we were able to quantify the concentration-dependent effects for both substances. We revealed eight concentration-dependent contraction kinetic graphs ranging from 10 μM to 10 mM for L-Glu, based on 24 measurements totally (Fig. 4) and eleven concentration-dependent contraction kinetic graphs ranging from 0.1 μM to 1 mM for GABA, based on 33 measurements totally (Fig. 5). In comparison to kinetics of the endogenous contraction, all induced contraction patterns differ in the contraction phase as well as in the expansion phase. In the single measurements as well as in the average kinetic graphs, the maximum contraction speed v_{max}^C is a multiple of v_{max}^C in endogenous contractions: between 2.6-fold higher for 2 μM GABA, 5.2-fold higher for 500 μM GABA and 4.3-fold higher for 5 mM L-Glu, respectively. For each concentration, the average maximum relative contraction speed v_{max}^C and the minimal relative projected area A/A_{max} (Fig. 6), the later representing the degree of maximum contraction, are given in Table 1. The ANOVA revealed significant differences ($P \leq 0.01$) in v_{max}^C between the non-induced control dataset and all concentrations of L-Glu and GABA used for induction, except for three GABA concentrations (2, 10 and 1,000 μM) and one L-Glu concentration (200 μM). The values of A/A_{max} and v_{max}^C correlate linearly (Fig. S1) for L-Glu and for GABA, with coefficients of correlation $R^2 = 0.7398$ and $R^2 = 0.7665$, respectively. Following Nickel (2004), we regarded a contraction event as

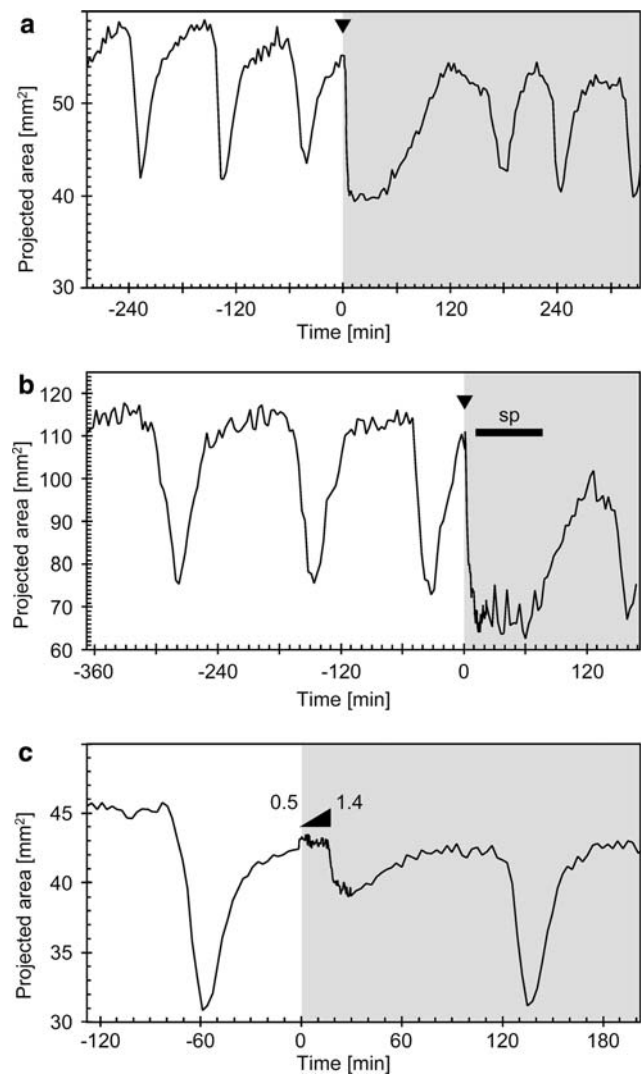


Fig. 3 a–c Time course of contraction of *T. wilhelma* treated with GABA. Response given as absolute projected area over time. Periods of agonist exposure to the sponge are displayed in grey. Agonist application is indicated by solid arrowheads. **a** Induction by 97 μM GABA after several endogenous contractions, displaying a faster contraction and significantly slower expansion; The onset of regular rhythmic contraction without washout within around 120 min points towards an agonist attenuation mechanism. **b** Induction by 97 μM GABA after several endogenous contractions, displaying a spasm-like reaction (sp) for 50 min and the onset of a second contraction within 120 min after contraction. This sequence is shown in time-lapse in Movie S1. **c** Determination of the minimal concentration of GABA resulting in a sub-contraction, by stepwise increasing the concentration from 0.5 to 1.4 μM

sub-contractions, if $A/A_{\text{max}} > 0.85$, which means a reduction of the projected area of less than 15%. Such sub-contractions occur at low concentrations (c_s) in both cases: for L-Glu at $c_s < 800 \mu\text{M}$ (Fig. 4), for GABA at $c_s < 10 \mu\text{M}$ (Fig. 5). In concordance to sub-contractions, we found a higher divergence in the expansion phase after induced contractions at lower

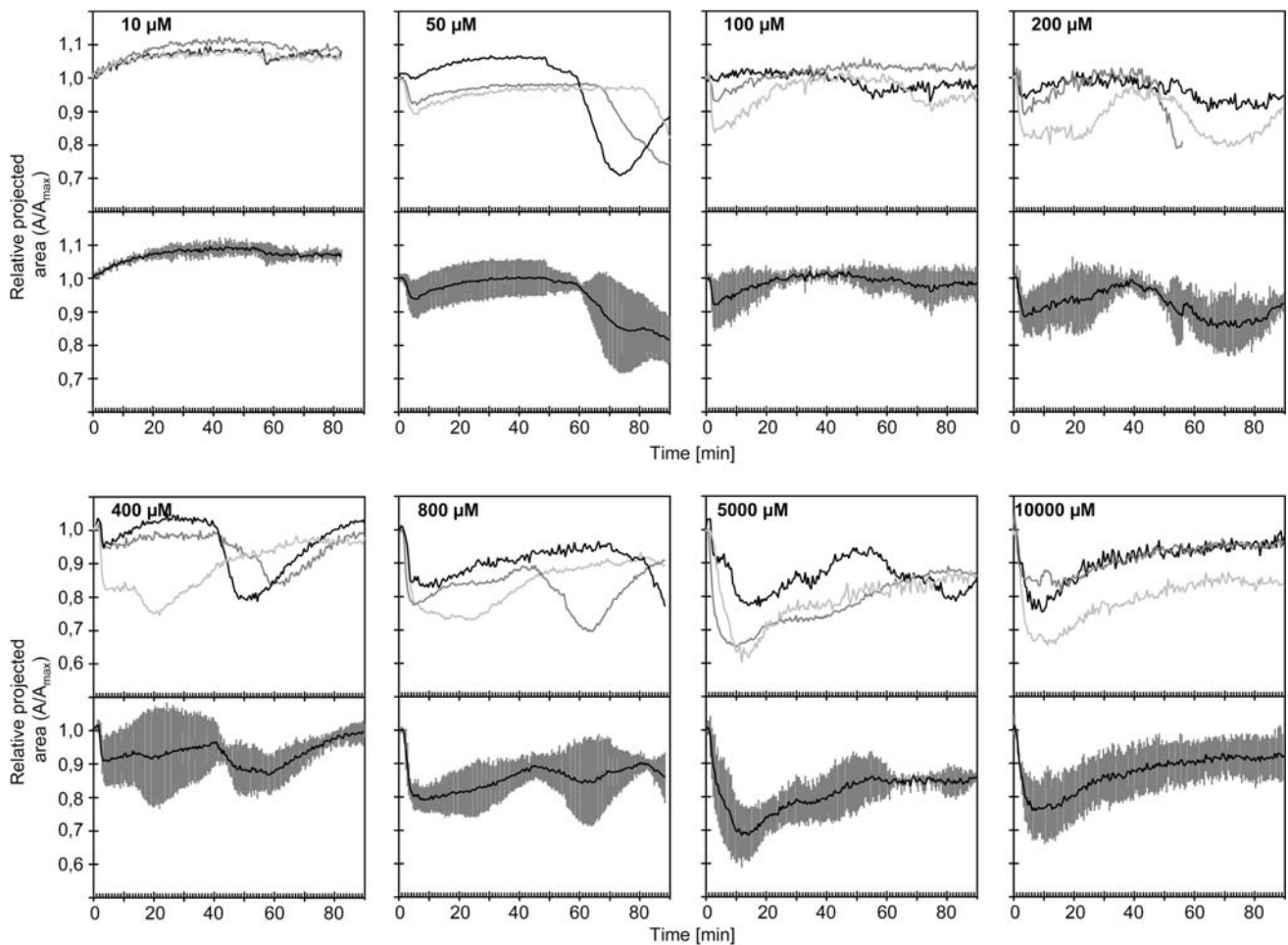


Fig. 4 Time course of contraction of *T. wilhelma* treated with L-Glu. Response given as relative projected area over time. For each concentration, three independent experiments are shown

(upper part of the graphs) as well as the averaged curves with standard deviation (lower part of the graphs)

concentrations. In some cases a subsequent full contraction followed within 90 min after the induced (sub-) contraction. In general, at higher concentrations, the expansion phase is extended. The long-term reactions of the single individual sponges in the experiments vary stronger with increasing time after induction, for biological system-inherent reasons, e.g. due to differences in endogenous rhythms and varying time of induction in relation to the endogenous contraction cycle. Therefore, only an experimental time of 90 min is analysed. Consequently, in case of the high concentrations, most sponges did not expand to the maximum again within this time.

Affinity for GABA and L-Glu

To compare the specific concentration ranges of GABA and L-Glu, which induce body contractions, the dose–response for the maximum relative contraction

speed v_{\max}^C and the minimum relative projected body area (A/A_{\max}) was compared, which correlates to the maximum contraction of the specimens (Table 1, Fig. 6). The dose–response curves of the maximum relative contraction speed (Fig. 6b) resemble saturation kinetics. However, due to the relatively high standard deviation of our measurements, no mathematical kinetics model could be found to fit well. The large standard deviations for all relative contraction speeds and minimum relative projected areas are a consequence of the experimental system, which is discussed subsequently.

We obtained similar results for the dose–response curves of the minimal relative projected area (Fig. 6b). Nevertheless, these curves show a linear correlation over a wide concentration range. GABA induces the strongest contractions. For both substances, over-dose effects seem to apply at concentrations higher than 200 μM (GABA) and 5 mM (Glutamate). However, these concentrations are

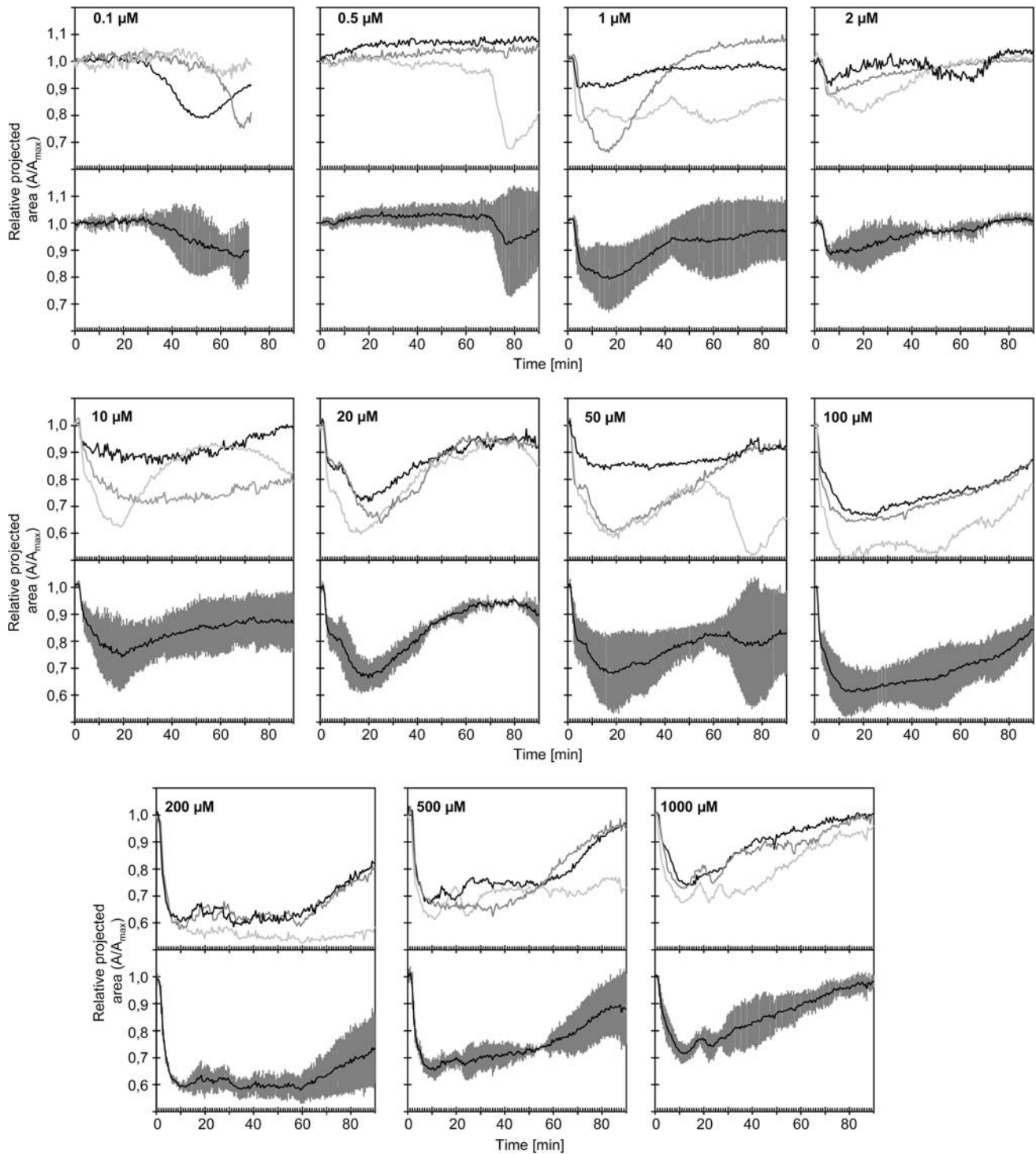


Fig. 5 Time course of contraction of *T. wilhelma* treated with GABA. Response given as relative projected area over time. For each concentration, three independent experiments are shown

(upper part of the graphs) as well as the averaged curves with standard deviation (lower part of the graphs)

above physiological levels. Taken together, GABA induces contractions at tenfold to 100-fold lower concentrations than L-Glu. Hence, the specificity of induction is higher for GABA.

Discussion

GABA and L-Glu are major neurotransmitters in vertebrate and invertebrate nervous systems (Fagg and

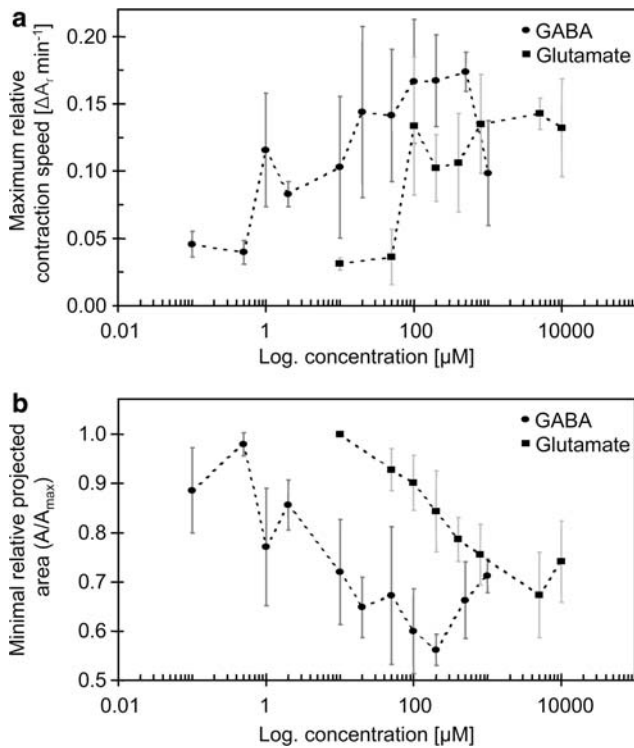


Fig. 6 Dose–response relationships for the agonists GABA and L-Glu, the response given as maximum relative contraction speed (a) and minimum relative projected area (b) (experiments shown in Figs. 4 and 5)

Foster 1983; von Bohlen und Halbach and Dermietzel 2002). Both are known to act via two different receptor types: (1) the metabotropic, G protein coupled receptors, e.g. the metabotropic L-Glu receptors (mGluRs) and the metabotropic GABA receptors (GABA_BR);

(2) the ionotropic, channel-forming receptors, e.g. the ionotropic L-Glu receptors (iGluRs) and the ionotropic GABA receptors (GABA_AR and GABA_CR) (Chebib and Johnston 1999).

A glutamate/GABA-like receptor with sequence similarities to GABA_B receptors and metabotropic glutamate receptors was cloned from the sponge *Geodia cydonium* and it was demonstrated that L-Glu induces a [Ca²⁺]_i response in dissociated cells of this sponge (Perovic et al. 1999). In the present study, we demonstrate the functional role of GABA and L-Glu as messenger substances in the behaviour of a sponge: *T. wilhelma* responded with body contraction immediately after external application of both substances, within a wide range of concentrations.

Non-induced, endogenous contractions of *T. wilhelma* in the aquarium habitat were characterised previously (Nickel 2004). In comparison, non-induced contraction kinetics of sponges inside the experimentation chamber displayed no major differences. However, there are differences between endogenous (Fig. 1c) and induced contractions (Figs. 4a, b, 5a–c). The onset of the later is more rapid and characterised by a higher speed of contraction during the whole contraction phase. This is supported by the ANOVA test, which confirmed the significant difference between v_{max}^C in the endogenous and most of the induced contractions.

Functionally, this difference is a consequence of simultaneous induction of all contractile elements, in contrast to local contraction waves in non-induced sponges (Movie S1). The later implies cells triggering local contractions. GABA and L-Glu are possible candidate

Table 1 Concentration-dependent effects of glutamate and GABA on the average maximum contraction speed (v_{max}^C) and the minimal average projected area (A/A_{max}) in *T. wilhelma*

	Concentration (μM)	Average v_{max}^C (N = 3) ($\Delta A_r \text{ min}^{-1}$)	Minimal average projected area A/A_{max} (N = 3)
Glutamate	10	0.031 ± 0.004	1.000 ± 0.000
	50	0.036 ± 0.020	0.928 ± 0.056
	100	0.105 ± 0.074	0.901 ± 0.056
	200	0.102 ± 0.025	0.843 ± 0.082
	400	0.106 ± 0.037	0.787 ± 0.044
	800	0.135 ± 0.037	0.756 ± 0.0616
	5,000	0.143 ± 0.012	0.6736 ± 0.086
	10,000	0.132 ± 0.036	0.7416 ± 0.082
GABA	0.1	0.050 ± 0.010	0.886 ± 0.087
	0.5	0.040 ± 0.009	0.980 ± 0.024
	1	0.116 ± 0.042	0.771 ± 0.120
	2	0.083 ± 0.009	0.856 ± 0.051
	10	0.103 ± 0.053	0.720 ± 0.107
	20	0.144 ± 0.064	0.648 ± 0.061
	50	0.141 ± 0.049	0.672 ± 0.140
	100	0.167 ± 0.046	0.600 ± 0.086
	200	0.167 ± 0.034	0.562 ± 0.032
	500	0.174 ± 0.015	0.663 ± 0.078
	1,000	0.098 ± 0.039	0.713 ± 0.034

signal substances involved in this process. It is most likely that sponge pinacocytes are the main effectors of contraction (Wilson 1910; Bagby 1970; Pavans de Ceccatty 1986; Nickel 2004; Nickel et al. 2006). Consequently, a putative simple signal spreading mechanism is the release of a messenger into the aquiferous system or the mesohyle.

The expansion phase after induced contractions may be disturbed or prolonged, most likely due to remaining stimulating substance inside the chamber. Additionally, spasm-like contraction patterns may be found, resulting from a sequence of local extensions and re-contractions (Fig. 3b and Movie S1). This is the first report of such behaviour in a nerve- and muscle-less organism. The cause of this spasm-like reaction is not an overdose-effect, because the same or even higher concentrations did not result in spasms in other experiments.

The dose–response curves for both substances, testing maximum relative contraction speed and minimal relative projected area, are similar. However, *T. wilhelma* shows a higher affinity for GABA, which induces contraction at lower concentrations than L-Glu.

For the first time dose–response curves have been examined for reactions upon applied stimuli in sponges. Increasing concentrations of L-Glu and GABA lead to an increase of v_{\max}^C and $A_{r\min}$. However, the inducing concentration ranges found here just represent affinity ranges for the complete tissue, not for single cells. Because of non-synchronised reactions of a large population of contractile cells, the tissue represents a biological statistical filter. Consequently, the spatial and temporal resolution is limited (compare Fig. 6). The method used does not allow finding the minimum concentrations for single contractile cells. For the same reason, the maximum relative contraction speed v_{\max}^C is affected at very low concentrations. Unfortunately, this is a system inherent limitation of our integrated approach. Nevertheless, the measurement of induced body contraction of *T. wilhelma* is up to date the only sponge model at all, to assess quantitative data of integrated behaviours.

Nevertheless, these concentration ranges are useful for the evaluation of putative paracrine messenger substances in *T. wilhelma*.

Our results indicate that body contractions in sponges are highly regulated. In conjunction with previous findings of a sponge metabotropic glutamate/GABA-like receptor (Perovic et al. 1999), our results suggest that a GABA_BR and/or an mGluR is involved in the regulation. In *G. cydonium*, only 20% of dissociated cells did react upon L-Glu (Perovic et al. 1999),

indicating that a limited set of effector-cell-types are equipped with specific receptor systems. We anticipate the existence of a molecular mechanism, which triggers the rhythmic pattern of contractions in *T. wilhelma* (Nickel 2004; Ellwanger and Nickel 2006). Interestingly, activating GABA_B systems are involved in rhythmical processes in other invertebrates (Richmond et al. 1994).

Paracrine chemical messenger pathways represent a straightforward concept to explain problems of sponge biology due to the “missing” integration system in sponges. However, further details will be discovered with the ongoing work on physiological, molecular and cell biological phenomena, related to coordination in this basal nerveless metazoan group.

Acknowledgments We are grateful to Hans-Dieter Görtz and Franz Brümmer for their support of our research, Isabel Koch and Kai-Uwe Genzel for providing and maintaining sponges in the zoological Garden Wilhelma, Wolfgang Hauber for discussion on experimental design, Birgit Nickel and Markus Götz for discussion of results, Isabel Heim and Carsten Wolf for technical assistance with the aquarium. Our work was partly supported by the German Federal Ministry of Education and Research (BMBF) through the project Center of Excellence BIOTECmarin (F 0345D), by the Ministry of Science, Research and the Arts of the State of Baden-Württemberg and the University of Stuttgart. The experiments conducted herein comply with the current laws of Germany where they were performed.

References

- Aristotle (1498) De natura animalium libri novem; de partibus animalium libri quatuor; de generatione animalium libri quinque. Electronic facsimile: Bibliothéque Nationale de France: <http://www.gallica.bnf.fr/Catalogue/NoticesInd/FRBNF37256929.html>, Paris, pp 89
- Bagby RM (1970) The fine structure of pinacocytes in the marine sponge *Microciona prolifera*. Z Zellforsch Mikroskop Anat 105:579–594
- Bond C (1992) Continuous cell movements rearrange anatomical structures in intact sponges. J Exp Zool 263:284–302
- Bond C, Harris AK (1988) Locomotion of sponges and its physical mechanism. J Exp Zool 246:271–284
- Bouche N, Lacombe B, Fromm H (2003) GABA signaling: a conserved and ubiquitous mechanism. Trends Cell Biol 13:607–610
- Chebib M, Johnston GAR (1999) The ‘ABC’ of GABA receptors: a brief review. Clin Exp Pharmacol Physiol 26:937–940
- Chiu J, DeSalle R, Lam HM, Meisel L, Coruzzi G (1999) Molecular evolution of glutamate receptors: a primitive signalling mechanism that existed before plants and animals diverged. Mol Biol Evol 16:826–838
- Davenport R (2002) Glutamate receptors in plants. Ann Bot 90:549–557
- Ellwanger K, Nickel M (2006) Neuroactive substances specifically modulate rhythmic body contractions in the nerveless metazoan *Tethya wilhelma* (Demospongiae, Porifera). Front Zool 3:7
- Emson RH (1966) The reactions of the sponge *Cliona celata* to applied stimuli. Comp Biochem Physiol 18:805–827

- Fagg GE, Foster AC (1983) Amino acid neurotransmitters and their pathways in the mammalian central nervous system. *Neuroscience* 9:701–719
- Fishelson L (1981) Observations on the moving colonies of the genus *Tethya* (Demospongia, Porifera): 1. behavior and cytology. *Zoomorphology* 98:89–100
- Fitch R (2005) WinSTAT—the statistics add-in for Microsoft Excel release 2005.1, Staufen, Germany
- Ito I, Watanabe S, Kimura T, Kirino Y, Ito E (2003) Distributions of gamma-aminobutyric acid immunoreactive and acetylcholinesterase-containing cells in the primary olfactory system in the terrestrial slug *Limax marginatus*. *Zoolog Sci* 20:1337–1346
- Jones WC (1962) Is there a nervous system in sponges? *Biol Rev* 37:1–50
- Kilian EF (1967) Ortsveränderungen von Süßwasserschwämmen unter dem Einfluß von Licht. *Verh Dtsch Zool Ges* 31(Suppl):395–401
- Lentz TL (1968) Primitive nervous systems. Yale University Press, New Haven
- Leys SP, Mackie GO (1997) Electrical recording from a glass sponge. *Nature* 387:29–30
- Lieberkühn N (1859) Neue Beiträge zur Anatomie der Spongien. *Arch Anat Physiol*:353–358, 515–529
- Mackie GO (1979) Is there a conduction system in sponges? In: Lévi C, Boury-Esnault N (eds) *Biologie des Spongiaires*. Editions du C.N.R.S., vol 291. C.N.R.S., Paris, pp 145–152
- Mackie GO (1990) The elementary nervous system revisited. *Am Zool* 30:907–920
- Madson P (2003) Digital camera remote shutter and digital camera time trigger. <http://www.digital-camera.dk/>
- McNair GT (1923) Motor reactions of the fresh-water sponge *Ephydatia fluviatilis*. *Biol Bull* 44:153–166
- Mezler M, Muller T, Raming K (2001) Cloning and functional expression of GABA_B receptors from *Drosophila*. *Eur J Neurosci* 13:477–486
- Nickel M (2001) Cell biology and biotechnology of marine invertebrates—Sponges (Porifera) as model organisms. *Arb Mitteil Biol Inst Uni Stuttgart* 32:1–157
- Nickel M (2004) Kinetics and rhythm of body contractions in the sponge *Tethya wilhelma* (Porifera: Demospongiae). *J Exp Biol* 207:4515–4524
- Nickel M (2006) Like a ‘rolling stone’: quantitative analysis of the body movement and skeletal dynamics of the sponge *Tethya wilhelma*. *J Exp Biol* 209:2839–2846
- Nickel M, Brümmer F (2004) Body extension types of *Tethya wilhelma*: cellular organisation and their function in movement. *Boll Mus Ist Biol Univ Genova* 68:483–489
- Nickel M, Leininger S, Proll G, Brümmer F (2001) Comparative studies on two potential methods for the biotechnological production of sponge biomass. *J Biotechnol* 92:169–178
- Nickel M, Vitello M, Brümmer F (2002) Dynamics and cellular movements in the locomotion of the sponge *Tethya wilhelma*. *Integr Comp Biol* 42:1285
- Nickel M, Donath T, Schweikert M, Beckmann F (2006) Functional morphology of *Tethya* (Porifera): 1. quantitative 3D-analysis of *T. wilhelma* by synchrotron radiation based x-ray microtomography. *Zoomorphol* 1–15 (online first)
- Panek I, Torkkeli PH (2005) Inhibitory glutamate receptors in spider peripheral mechanosensory neurons. *Eur J Neurosci* 22:636–646
- Panek I, Meisner S, Torkkeli PH (2003) Distribution and function of GABA_B receptors in spider peripheral mechanosensilla. *J Neurophysiol* 90:2571–2580
- Pantin CFA (1952) The elementary nervous system. *Proc R Soc Lond B Biol Sci* 140:147–168
- Parker GH (1919) The elementary nervous system. Lippincot, Philadelphia
- Pavans de Ceccatty M (1974) Coordination in sponges—the foundations of integration. *Am Zool* 14:895–903
- Pavans de Ceccatty M (1979) Cell correlations and integrations in sponges. *Biologie des Spongiaires* 291:123–135
- Pavans de Ceccatty M (1986) Cytoskeletal organization and tissue patterns of epithelia in the sponge *Ephydatia mülleri*. *J Morphol* 189:45–66
- Perovic S, Krasko A, Prokic I, Mueller IM, Mueller WEG (1999) Origin of neuronal-like receptors in Metazoa: cloning of a metabotropic glutamate/GABA-like receptor from the marine sponge *Geodia cydonium*. *Cell Tissue Res* 296:395–404
- Ramoino P, Fronte P, Beltrame F, Diaspro A, Fato M, Raiteri L, Stigliani S, Usai C (2003) Swimming behaviour regulation by GABA_B receptors in *Paramecium*. *Exp Cell Res* 291:398–405
- Ramoino P, Usai C, Beltrame F, Fato M, Gallus L, Tagliaferro G, Magrassi R, Diaspro A (2005) GABA_B receptor intracellular trafficking after internalization in *Paramecium*. *Microsc Res Tech* 68:290–295
- Rasband WS (1997–2005) ImageJ. National Institutes of Health, Bethesda, Maryland, USA, <http://www.rsby.info.nih.gov/ij/>
- Reiswig HM (1971) *In-situ* pumping activities of tropical Demospongiae. *Mar Biol* 9:38–50
- Richmond JE, Murphy AD, Lukowiak K, Bulloch AGM (1994) GABA regulates the buccal motor output of *Helisoma* by two pharmacologically distinct actions. *J Comp Physiol A* 174:593–600
- Sarà M, Sarà A, Nickel M, Brümmer F (2001) Three new species of *Tethya* (Porifera: Demospongiae) from German aquaria. *Stuttgarter Beitr Naturk Ser A* 631:1–15
- Schmidt O (1866) Zweites Supplement der Spongien des Adriatischen Meeres enthaltend die Vergleichung der Adriatischen und Britischen Spongiengattungen. Verlag von Wilhelm Engelmann, Leipzig
- Schuske K, Beg AA, Jorgensen EM (2004) The GABA nervous system in *C. elegans*. *Trends Neurosci* 27:407–414
- von Bohlen and Halbach O, Dermietzel R (2002) Neurotransmitters and neuromodulators. Wiley-VCH, Weinheim
- Weissenfels N (1990) Condensation rhythm of fresh-water sponges (Spongillidae, Porifera). *Eur J Cell Biol* 53:373–383
- Weyrer S, Rutzler K, Rieger R (1999) Serotonin in Porifera? Evidence from developing *Tedania ignis*, the Caribbean fire sponge (Demospongiae). *Mem Queensland Mus* 44:659–665
- Wilson HV (1910) A study of some epitheloid membranes in monaxonid sponges. *J Exp Zool* 9:536–571
- Xue H (1998) Identification of major phylogenetic branches of inhibitory ligand-gated channel receptors. *J Mol Evol* 47:323–333