Analysis of Taurine as Modulator of Neurotransmitter in *Caenorhabditis elegans*

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Abbreviations

GABA	γ-Aminobutyric acid
GAD	Glutamic acid decarboxylase
NGM	Nematode growth media
SR	Success ratio

1 Introduction

Taurine is synthesized from methionine throughout the human body, and from cysteine in the liver in the presence of vitamin B6. As it is stored in various types of cells, taurine plays an important role in maintaining homeostasis by sustaining an osmotic balance within animal cells. It helps cells recover from damage and prevents physiological stress by preserving osmolarity. In neuronal cells, taurine is involved in intercellular ion trafficking via functional regulation of the ion channels. Taurine plays an important role in the development, survival, and neural growth of vertebrate neurons. Along with caffeine, taurine is one of the most utilized psychoactive drugs and serves as a psychoactive agent that can adjust perception, mood, consciousness, or behavior. Since taurine is commonly applied in combination with

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caffeine, the psychoactive effect of taurine alone is difficult to evaluate Whirley and Einat (2008) have even negated taurine's psychoactive effect. They claim that taurine's psychoactive attributes are misleading since their animal study failed to confirm any specific improvement using taurine.

When treated with taurine, the mouse brain responds with significant changes in the γ-aminobutyric acid-producing (or GABAergic) neurons (El Idrissi 2008). Taurine induces hyperexcitability by lowering the levels of $GABA_A\beta$ subunits and augmenting the expression of glutamic acid decarboxylase (GAD) and GABA, most likely due to the sustained interaction of taurine with $GABA_A$ receptors. This can be considered a potential mechanism for coping with taurine administration, and it can affect motor behavior (Niebur and Erdos 1993; Santora et al. 2013). Taurine's effects may stem from its structural similarity to GABA. Still, taurine's function as a psychopharmaceutical or psychotropic agent is highly controversial among taurine researchers. Although its psychostimulative effect has been attested in numerous human studies, Giles and colleagues (2012) state that mental enhancement by taurine can be achieved only in the presence of caffeine.

The act of learning typically occurs in the early stages of development in most animal species. It is a sophisticated process of adaptation or classical habituation, rather than a simple gathering of actual and practical information (Vallotton and Ayoub 2010; Wicks and Rankin 1997). Among the various types of learning, associative learning refers to having a new response attached to a particular stimulus in animal behavior (D'Adamo et al. 2004; Wen et al. 1997). This term covers almost all types of learning, except simple acclimatization. Despite lacking a central nervous system, recent reports indicate that *Caenorhabditis elegans* exhibits a basic form of associative learning (Amano and Maruyama 2011; Pandey et al. 2011; Qin and Wheeler 2007). *C. elegans* has short-term and long-term memory and behavioral flexibility related to learning, both associative and non-associative (Amano and Maruyama 2011; Ardiel and Rankin 2010).

C. elegans is practical for studying various biological functions despite its simple structure. It has become a prized animal system for furnishing an integrated view of organismal responses to numerous forms of environmental stimulation (Croll 2009). In combination with the comprehensive genetics and neuroanatomy of *C. elegans*, some of its mutants show deficiencies in associative learning, thus providing the foundation for an actual characterization of the cellular and molecular aspects of associative learning.

C. elegans has a nervous system consisting of 302 neuronal cells. This number is remarkable considering a wild-type adult body consists of 959 somatic cells (Albeg et al. 2011). In the nematode, neuronal synapses are found mostly in the nerve ring, ventral nerve cord, and dorsal nerve cord (Lee et al. 2012). The nerve ring is the brain of the worm and the complex behaviors of the worm are coordinated in this area. Despite having only 5,000 synapses among the 302 neurons, *C. elegans* displays complex forms of behavior including habituation, sensitization, and conditioning in response to stimuli such as drugs and neurotransmitters. *C. elegans* has a particular set of proteins to synthesize, transport, or utilize GABA, and these proteins

are required for its neurotransmission; the proteins include a biosynthetic enzyme, vesicular transporter, transcription factor for shaping GABA neuron identity, and different receptors for inhibition and excitation (Schuske et al. 2004). This makes it a functional system for understanding behavioral plasticity in terms of its neurochemistry, the neurobiology of potential neurotransmitters, and evolutionally conserved molecules such as insulin, monoamines, and neuropeptides (Lorimer et al. 1996; Rankin et al. 1990; Sasakura and Mori 2013). When compared to mammalian neurons, the neurons of *C. elegans* are remarkably similar in terms of their essential functions and connectivity, in spite of its simplistic nervous system.

To characterize the potential effect of taurine on associative learning, groups of *C. elegans* were treated with taurine or caffeine in the present study. Caffeine is a highly used psychoactive drug that is often used with taurine as a co-stimulant in many types of energy drinks (Zeratsky 2008). Although caffeine extends the life span of *C. elegans*, its effect on learning has not been studied in the nematode (Sutphin et al. 2012). In vertebrates, behavioral plasticity that leads to learning is assessed using mazes, and recent studies have shown that a similar approach can be applied to nematodes using microfluidic channel techniques (Chronis 2010; Crane et al. 2010; Lockery et al. 2008). However, without interdisciplinary efforts, a microfluidic maze is hard to prepare (Park et al. 2008; Rohde et al. 2007).

In this study, a new type of maze was invented by placing coverslips on an agar gel. Gaps can be easily generated and worms can move along the path created by the gaps. The effect of taurine was studied by monitoring the difference in the success ratio (SR) of foraging between the taurine-treated and taurine-free groups. The effect of taurine on movement was also studied by comparing the distance traveled between the taurine-treated nematodes and their non-treated counterparts. The aim of the present study was to address taurine's effect on the nematodes' initial foraging abilities and the enhancement of learning attached to foraging, which refers to looking or searching for food.

2 Methods

2.1 Strain and Culture Conditions

The N2 strain of *C. elegans* was maintained under typical conditions (Stiernagle 2006). When treated with taurine or caffeine, *C. elegans* grew in S medium having concentrated *Escherichia coli* OP50 and the respective treatment agents. Taurine was added into the OP50 mixture to the final concentrations of 5 and 10 mg/mL in the S media. For the control worms, no taurine supplementation was provided when treated in the S media. In addition to taurine, worms were also treated with caffeine at 0 or 5 mg/mL as the final concentration. Therefore, worms were treated with six different combinations of taurine-caffeine concentrations: 0-0, 0-5, 5-0, 5-5, 10-0, and 10-5 (mg/mL).

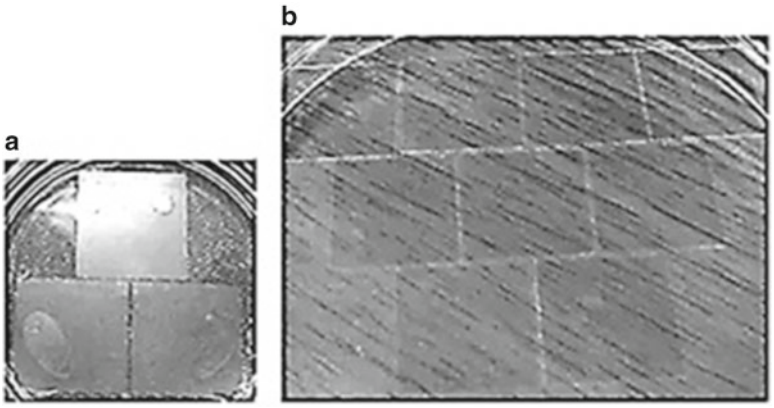

Fig. 1 Simple and complex mazes. The mazes were assembled by placing microscopic coverslips on the surface of agarose gel. Gaps were made between the coverslips, and their width was approximately 1 mm. The height was 0.5 mm and the worms were unable to climb up the edge. The simple maze (**a**) consisted of three coverslips, while the complex maze (**b**) was assembled using nine coverslips

2.2 Maze Construction

After overnight growth with taurine or caffeine in OP50, worms were thoroughly washed in M9 media. They were then transferred to the simple maze, which was made by utilizing gaps between pieces of microscopic coverslips, as shown in Fig. 1. Two types of mazes were used: simple (T-shaped) and complex (branched). The edges of the coverslips were mildly coated with petroleum jelly (Vaseline); otherwise, worms would stick to the edge by extending their bodies. The coverslips were arranged so that they were in close contact with the surface of the nematode growth media (NGM) gel, without air bubbles or gaps between the coverslips and the gel. Once worms were placed at the start inlet, the area was sealed using Vaseline ointment to prevent the worms from moving out of the maze.

2.3 Calculation of Success Ratio and Comparison

The worms were allowed to move along the maze to forage until they reached the end of each maze. In the simple maze, one end had an area of OP50, with the other area being free of OP50. In the case of the complex maze, only one outlet had the food, while the other two ends did not have food. The worms were subjected to the maze experiments for 3 days before the data were analyzed. Each foraging success was compared among the six different taurine-caffeine concentrations. At each end, worms were counted and the success ratio (SR) was calculated as the

percentage of worms found at the food area per the total number of participating worms. The SRs were compared between the taurine and taurine-free treatments. The SR was calculated by the following formula: SR (%) = number of successful worms/number of total participating worms × 100. Comparisons were made in terms of the SR among the different treatments according to the analysis of variance (*ANOVA*) or Student's *t*-test.

2.4 Movement Comparison Assay

Another group of worms was placed on the complex maze and the SR was calculated using the same procedures as those described above for the simple maze. In addition to the SR, taurine-treated and taurine-free worms were compared in terms of the distance traveled on the gel. Ten worms were permitted to move on the gel for 24 h, and the total distance traveled was visually quantified and compared. The distance traveled by the worms was divided into five groups and each grade of length was scored as in the number of plus (+) symbols.

3 Results

Using the *C. elegans* system, this study focused on whether the worms' foraging abilities could be accelerated following treatment with taurine. The effects of taurine on learning were assayed alone or in combination with caffeine. Taurine treatment alone significantly augmented the ability of the worms to find food. Worms that underwent taurine treatment appeared to have an accelerated learning ability compared to the control worms. In combination with caffeine, taurine helped worms forage successfully in the case of the complex maze. Worms showed significant improvements in reaching the area with food when treated with both taurine and caffeine.

3.1 Taurine Increases Foraging Success Ratios for the Simple Maze

Worms were treated with taurine or caffeine in the liquid S media containing each treatment chemical. Worms were subjected to the maze experiments for 3 days to analyze their foraging performance after taurine treatment. When taurine-treated worms were placed in a simple maze and allowed to move freely to find the area with food, differences were apparent between the treatment groups. The taurine-treated worms showed notable success on the simple maze in terms of their foraging performance when the SR was compared among the six different combinations of

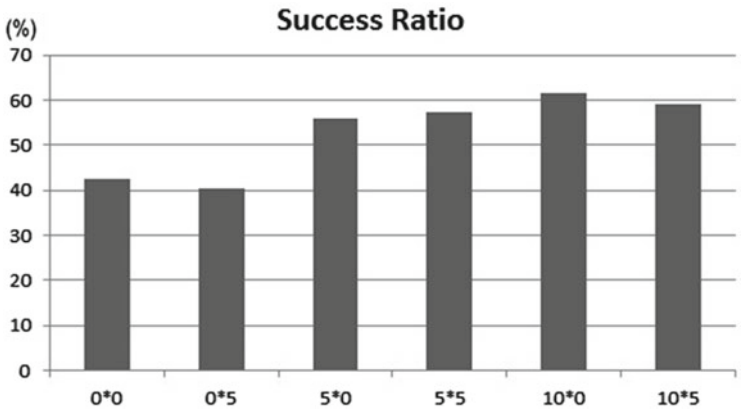

Fig. 2 Success ratios (SRs) for the simple maze

taurine and caffeine following the treatments (Fig. 2). More taurine-treated worms appeared to reach the OP50 area compared to the taurine-free control group. No significant differences were apparent between the taurine only and taurine-caffeine co-treated groups. When the worms were treated only with caffeine (5 mg/mL) and subjected to the simple maze, their SRs were significantly lower than the SRs of the taurine-only treatment group. This shows that caffeine alone exerted a minimal effect in the case of the simple maze experiment.

Groups of *C. elegans* were treated with taurine or caffeine (six different concentrations) and placed into the simple maze. After 3 days, the majority of worms reached either end of the T-shaped maze ($n = 30$). Taurine-treated worms show a higher ratio of success in reaching the arm with the OP50 ($p < 0.05$). As for the different taurine concentrations, worms appeared to increase the foraging SR. The X-axis refers to the concentrations of taurine (t) and caffeine (c) in terms of mg/ mL.

3.2 Foraging Success Increases in the Presence of Caffeine for the Complex Maze

To examine the effects taurine on foraging success in the complex maze, worms were pre-treated with taurine and subjected to the complex maze experiments. When the SRs were measured after 3 days, the taurine-treated group was more successful at reaching the OP50 area compared to the taurine-free control group (Fig. 3). This result is consistent with the outcome from the simple maze study. When treated with caffeine in addition to taurine, however, worms appeared to show increased SRs, which was different from the results of the simple maze experiment.

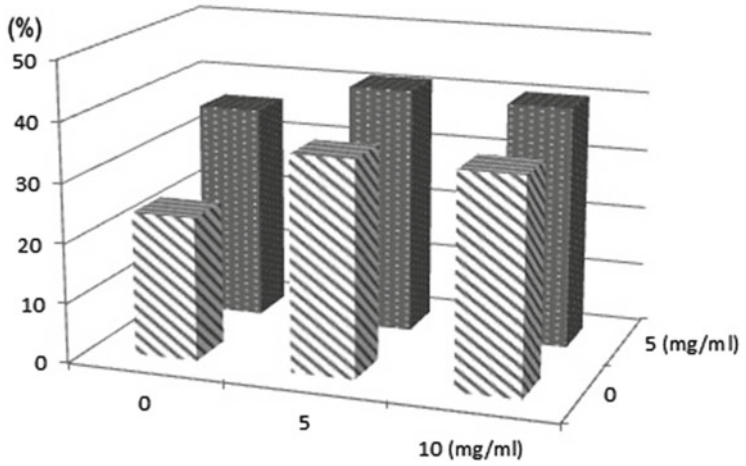

Fig. 3 Success ratios (SRs) for the complex maze

The effect of taurine on foraging performance was assayed using the complex maze. Worms were pre-treated with taurine and subjected to the complex maze experiments. The SRs were compared after 3 days between the three different treatment groups. When treated with caffeine in addition to taurine, worms show increased SRs, which is different from the results of the simple maze experiment ($p < 0.05$). The concentrations at front and side belong to taurine and caffeine, respectively. The SRs are lower than 50 % since the complex maze has three outlets, different from the case of the simple maze that has no more than two outlets.

3.3 Repeated Application of Taurine Augments Foraging Success for the Simple Maze

Worms that demonstrated successful foraging were collected and divided into two groups. One group of worms was briefly treated with taurine and the other was not. Both groups were subjected to the simple maze experiment and their performances were compared. A difference was evident between the two groups (Fig. 4). The taurine-treated group showed a higher SR in comparison to the taurine-free group. A similar experiment was performed using the complex maze. No differences were shown among the taurine-treated and taurine-free groups (data not shown).

Successful worms were treated further with taurine and caffeine on media. The control group was not treated with taurine. Then, the worms were subjected to the simple maze. In terms of the SR, the taurine-treated group showed a higher SR compared to the taurine-free group ($p < 0.05$). The X-axis refers to the concentrations of taurine (t) and caffeine (c). The light and dark bars represent non-treated

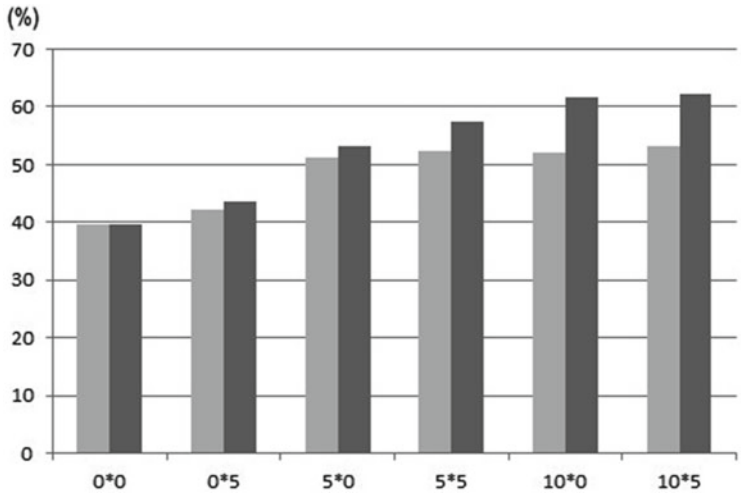

Fig. 4 Simple maze experiment on worms with successful foraging

and treated groups, respectively, except for [0*0]. Using the complex maze, a similar experiment was performed to compare the SRs between the taurine and taurine-free treatments. There were no apparent differences between the taurine-treated and taurine-free groups (data not shown).

3.4 Combination of Taurine and Caffeine Promotes Motility of C. elegans

The potential effects of taurine and caffeine on *C. elegans* were also assayed in terms of motor activity. Along with the increased foraging success, the distance traveled by the worms increased when treated with taurine. The total movement of the worms was visually compared between the taurine-treated and taurine-free groups. When treated with taurine, worms appeared to travel farther than their non-treated counterparts (Table 1). Comparisons were also made between the caffeine and caffeine-free taurine treatments. The distances traveled by the worms increased when treated with caffeine. Increased movement was evident for the worms treated with caffeine in addition to taurine. This strongly indicates that taurine has a positive effect on the worms' motor activity.

The length of the path that worms took on the gel surface was visually compared after each treatment. The length was quantified into five levels. The taurine-treated group showed an increase in the distance traveled compared to the taurine-free controls. The length of the movement paths appeared to increase in a concentration-dependent manner.

Table 1 Visual comparison of the distances traveled

Taurine (mg/ml) Caffein (mg/ml)	0	5	10
0	+	+++	++++
5	++	+++	+++++

4 Discussion

In the present study, *C. elegans* showed a significant level of success in searching for food when treated with taurine. Taurine clearly helped the worms find the area with food or to successfully forage for food. When treated with caffeine in addition to taurine, however, worms showed better responses via increased SRs, especially in the complex maze study. Taurine produced more positive effects when combined with caffeine.

In vertebrates, GABA serves as a vital neurotransmitter that works at synapses in the central nervous system. In *C. elegans*, however, GABA works mainly at neuromuscular synapses to ease the body muscles during movement and foraging. Because taurine resembles GABA in structure, taurine is expected to work similarly in the muscles. One possible explanation for the increased foraging SRs may be related to a taurine-mediated increase in the total distance traveled, that is, the increased motor activity and distance traveled may result in the rates of successful foraging on a trial and error basis.

Foraging has been perceived as the primary subject of associative learning. Trial and error is essential for the development of foraging since the worms learn how to cope with their environment. For *C. elegans*, vigorous locomotive movement may help them find their food source when they are treated with taurine or caffeine. After treatment with taurine, *C. elegans* showed increased travelling distances and flexibility; thus, augmented locomotive activity improves the chance for successful foraging.

When successful worms were subjected to the simple maze experiment, they showed a higher rate of success under a repeated application of taurine. Despite the brief application of taurine, the worms showed significant SRs for foraging. In the taurine-free group, the level of success was lower. This strongly indicates that taurine may help worms improve their foraging. In the case of the complex maze, however, no difference was evident between the taurine and taurine-free groups. This result indicates that taurine may not work sufficiently toward associative learning due to the simplicity of the worm's nervous system.

Taurine is known to shorten the reaction time in working memory tasks, although it extends decision-making time for humans. Caffeine, however, helps improve executive control and working memory, and reduces simple reaction and decision-making times (Giles et al. 2012). In this study, caffeine was highly functional in the complex maze study. This result might come from the fact that caffeine helps reduce the length of time required for decision-making. In the simple maze, worms experienced once to be successful in foraging; however, they had to encounter at least

twice to finalize the appropriate foraging. For this doubled case of decision-making, caffeine might have provided a "correct" decision twice for successful foraging, and thus its effect became noticeable in this study. Decision-making usually involves the ability to assess the significance of obtainable choices from experience. Taurine might have played a role in associative learning. Furthermore, taurine might have helped the worms adjust their behaviors for an optimal foraging task as an associative learning model.

This study suggests that taurine may work alone to exert a convincing effect on associative learning in the absence of caffeine. This strongly indicates that taurine affects worms in a positive way during the course of associative learning. Caffeine provides a synergistic effect on associative learning when combined with taurine. Future studies may be necessary to describe the degree of synergy between taurine and caffeine for enhancing the level of associative learning. Additionally, a future study should employ locomotion to verify the results of the present study.

5 Conclusion

This study focused on whether the foraging abilities of the *C. elegans* could be promoted upon treatment with taurine alone or in combination with caffeine. Taurine alone considerably increased the capability of the worms to find food and promoted learning ability compared to the control worms. This observation proposes that taurine may work alone to exert a definite effect on associative learning in the absence of caffeine. In combination with caffeine, worms showed substantial progresses in reaching the area of food. This strongly indicates that taurine alone affects worms in a positive way during the course of associative learning and that caffeine provides a synergistic effect on associative learning when combined with taurine.

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