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Enhanced Efficacy of the Commissural Transmission between Lateral Giants in the Sensitization of Crayfish Escape Behavior

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=ABSTRACT=

Lateral giant (LG)-mediated escape response of crayfish is sensitized by natural traumatic events. Such sensitization has previously been shown to be associated with increased transmission between primary afferents and sensory interneurons at the cholinergic synapse of LG escape reflex circuit. In the present study, it was firstly investigated as to whether transmission is also altered at other synapses of the LG-escape reflex circuit by traumatic shock-induced sensitization. Evidence that traumatic shock also directly affects the excitability of lateral giants is now provided by the finding that traumatic shock produces a significant reduction of the time needed for LG to recruit its contralateral homologue, which is defined as commissural delay. Octopamine, a naturally occurring neuromodulator in the crayfish nerve cord, has also been shown to enhance transmission at the cholinergic synapse between primary afferents and sensory interneurons, and has been conjectured to mediate sensitization. Like traumatic shock, octopamine ($10^{-5}-5\times10^{-4}$ M) also enhanced the efficacy of commissural transmission between lateral giants, as indicated by a significant reduction of commissural delay. This effect was blocked by an octopamine antagonist phentolamine, suggesting a specific action of octopamine on the octopamine receptor present on LGs. These observations suggest that both traumatic shocks and octopamine may cause a rather broad alteration in the excitability of the crayfish nervous system that contributes to the sensitization of the LG escape response.

Key Words: Sensitization, Octopamine, Commissural transmission, Crayfish escape behavior, Lateral giants

INTRODUCTION

Sensitization refers to nonassociative enhancement of responsiveness in an animal's behavior following a traumatic event (Domjan & Burkhardt, 1984). Extensive studies on the cellular and molecular mechanisms mediating sensitization have been performed in a number of vertebrate and invertebrate species. These have provided evidence that simpler forms of nonassociative learning, habituation and sensitization are important building blocks for the expression of the more complex forms of associative learning (Kandel & Schwartz, 1982). The best described analysis of sensitization is for the gill-and siphon-withdrawal reflex of the marine mollusk, *Aplysia Californica* (Hawkins et al, 1987). In this animal, the location of the neural change responsible for sensitization seems to be largely at the presynap-

tic side of synapses between relevant sensory neurons and their follower neurons. The plastic change in this synapse is believed to be induced by an input to the terminal from a presynaptic "facilitator" neuron that releases either a serotonin-like molecule or a peptide when sensitizing stimuli are presented (Glanzman, et al, 1989). The presynaptic facilitatory input increases the amount of transmitter released by sensory neuron spikes by virtue of producing a persistent decrease in the K^+ currents that repolarize the terminal after a spike (Kandel & Schwartz, 1982).

A system that holds particular promise for probing the generality of the findings from Aplysia is the lateral giant (LG) mediated escape response of the crayfish. LG escape is a reaction to an abrupt mechanical stimulation of the abdomen that serves to remove the abdomen from the stimuli. Most of the elements in the LG escape circuit are well identified (Krasne & Wine, 1987 for review), thereby allowing a parallel investigation of the plastic properties of the various synapses of the network (Krasne & Lee, 1988). Previous work by Krasne & Glanzman (1986) established that strong electrical shocks to the animal's head enhance the LG escape response of crayfish. This sensitization effect is at least in part the result of increased efficacy of the cholinergic synapse (Miller et al, 1992) of the reflex arc between primary afferents and sensory interneurons. Such sensitization was shown to be induced by a descending modulatory ("heterosynaptic facilitatory") pathway whose own excitation occurs rostral to the abdominally located reflex mediating circuitry (Krasne & Glanzman, 1986).

Exogenously applied octopamine, a naturally occurring monoamine in crayfish (Livingston et al, 1981), has been shown to enhance the excitability of LG neurons in crayfish escape response (Glanzman & Krasne, 1983). This octopamine has been shown to be operating at the cholinergic synapse (Bustamente & Krasne, 1991) between the primary afferents and sensory interneurons, and has been conjectured to be a possible mediator of the sensitization of the LG escape response. Consistent with the physiological results, an electron microscopic study by Lee & Krasne (1993) has confirmed the presence of the presumed octopaminergic input synapses on the dye-filled primary afferent terminals in the neutrophils of crayfish abdominal ganglion.

Recent work by Vu & Krasne (1993) showed that tonic inhibition, another form of modulation of crayfish LG escape reflex, affected the efficacy of the commissural transmission between lateral giants. This transmission is believed to be electrical synapses. The present study was aimed at determining whether the above commissural transmission is also altered by traumatic shock induced sensitization, or similarly by octopamine. Using chronic animals, the first experiment examined the effect of traumatic shock to the head on the commissural transmission between LGs. The next experiment determined the effects of exogenously applied octopamine on the efficacy of the same commissural transmission, using preparations of the isolated abdomen. A preliminary result of the octopamine effect on commissural transmission between LGs in crayfish has been presented in abstract form (Lee & Krasne, 1991).

MATERIALS AND METHODS

Subjects

Crayfishes *(Procambarus clarkii)* of both sexes measuring 8~10 cm rostrum-telson were obtained from California Golden Egg (P.O. Box 681 W. Sacramento, California, 95691, USA). They were maintained individually in well aerated aquaria.

Anatomical background for LG escape reaction circuit

Lateral giants (LGs), for which the escape reaction is named, are bilaterally paired and are

actually chains of segmental neurons joined end to end by very efficient electrical "septal" junctions (Johnson, 1926). Mechanosensitive primary afferents in each abdominal segment excite the LGs, both monosynaptically via electrical synapses, and disynaptically via cholinergic excitation of a group of sensory interneurons that make electrical synapses on the LG (Krasne, 1969; Zucker, 1972a; Miller et al, 1992). Once it fires, the LG recruits its contralateral homologue via an electrical "commissural" synapse across the midline in each ganglion (Watanabe & Grundfest, 1961). The two LGs recruit a variety of premotor and motor neurons that cause flexion of the abdomen, which then results in an escape response to remove the abdomen from the source of stimuli that caused the response. A single firing of the LG is all that is needed to produce a vigorous escape response (Wiersma, 1947).

Animal preparations

Intact chronic animals: Freely moving intact animals with chronically implanted stimulating and recording electrodes (Fig. 1A) were used to monitor changes in commissural transmission between the two LGs, following the presentation of the traumatic shock. LG axons are large enough for their firing to be readily detected not only in the isolated nerve cord but also in freely behaving animals with suitably located chronic electrodes, as described in detail by Krasne & Lee (1988). Briefly, stimulating and recording electrodes, referred to as skewed electrodes, were stainless steel insect pins (00 size). These pins were insulated except for a small bare gap halfway along their lengths. To stimulate the LG axonally, the narrow gap in the insulation of the skewer electrodes was positioned over a single lateral giant axon at the 5~6 segments of the nerve cord (Fig. 1A). To record electrical responses, a pair of electrodes were thrust through the animal at 2~3 and 3~4 segments of the abdomen, such that the insulation-free gap was positioned over the dorsal surface of the nerve cord (Fig. 1A). These electrodes recorded two large spikes, followed by volume-conducted flexor muscle potentials, when the action potentials of LGs were produced (Fig. 1B). During the experiments, electrode leads were run to a float that carried connections for attachment to electronics.

Physiological preparations: Isolated abdominal

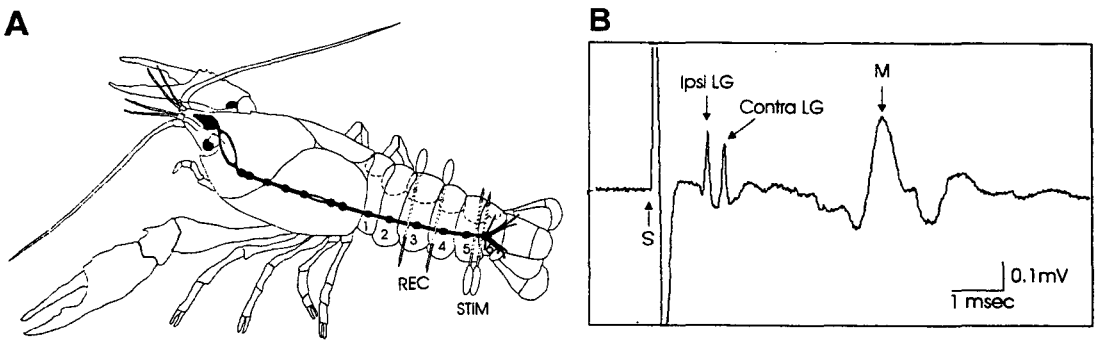

Fig. 1. A: Locations of electrodes in chronically prepared intact crayfish. Recording (REC) electrodes, at the 2 -3 and 3-4 segments, and stimulating (STIM) electrodes, at 5-6 segments are shown in the abdominal portion of the crayfish. B: Typical extracellulary recorded action potentials of LGs. The trace shows a response to axonal stimulation of one LG from a chronic preparation when the LGs fired. The first upward spike is produced by an action potential in the ipsiliateral (ipsi) LG that was directly stimulated axonally and the second by an action potential generated in the contralateral (contra) LG by the commissural synapse. The volume-conducted flexor muscle potentials is marked M and a start of stimulus pulse by S.

cords pinned to Sylgard, or isolated abdomens with the cord left in situ, were used to test effects of the perfused octopamine on the commissural transmission of LGs. Dissections were done in cold ringer and the ganglion in which commissural transmission was to be examined was desheathed to aid in the penetration of pharmacological agents. Bipolar platinum hook electrodes were used to stimulate the LG axon, and also to record the electrical activity of the dorsal nerve cord. Preparations were housed in a 30 ml plexy glass dish, and physiological saline (22℃) was superfused at 20 ml/min. The physiological media used was van Harreveld solution (Van Harreveld, 1936) with bicarbonate replaced by 15 mM HEPE's, adjusted to PH 7.2. Octopamine (at 3×10^{-4} M) or phentolamine (a competitive blocker of octopamine receptors) at 10^{-4} M were added as required for the experiment.

Stimulation and recording

In an experiment, the test stimuli on the single LG axon were 0.2 msec cathodal current pulses from a pulse generator (WPI 310 Accupulser; World Precision Instrument., Saratoga, Fl, USA), which were photo-isolated from ground (WPI A365). Traumatic shock (TS) was induced by a 35 V AC shocks, 10 s in duration, which are delivered by a pair of platinum wires with 2 mm uninsulated at their ends. The platinum wires were glued to the rostrum of crayfish. Extracellularly recorded electrophysiological signals were amplified by high gain, high-input-impedance differential amplifiers and displayed on a digital oscilloscope. A computer with a laboratory interface (CED 1401 Plus) was used to run the experiments and analyze the electrophysiological responses.

Experimental procedures

Test trials with supra-threshold stimuli for firing of the LG action potentials were delivered every 30 s and 90 s in acute and chronic experiments, respectively. The efficacy of the commissural transmission was determined by measuring the latency of crossover from one LG to its contralateral homologue, defined as commissural delay (CD). Quantitative data on post-TS (or octopamine) changes are expressed as a percentage of the pre-TS (or octopamine) values. A statistical analysis was performed by comparing the values of CD measured before the TS (or octopamine) with the minimal values observed after the TS (or octopamine). Five consecutive responses were averaged. Values given in the text and in the figures are means ± SEM of percentage changes. Effect of TS on the conduction speed along the LG axons was also determined in chronic experiments by calculating the percentage changes in conduction time (CT), which is measured between the start of a test stimulus and a peak of the ipsilateral LG action potential (see Fig. 1B).

RESULTS

Effects of traumatic shock-produced sensitization on commissural transmission

In a previous study, Krasne & Glanzman (1986) have shown that TS can sensitize LG escape reactions in the crayfish LG escape circuit partly due to the augmented transmission of the cholinergic synapses between the primary afferents and sensory interneurons (e.g. interneuron A). To ensure that traumatic shock (35 V AC currents) to the animal's head was capable of sensitizing LG escape, in a few experiments we tested the ability of a test stimulus to sensory roots innervating the tail fan to elicit an action potential of LG. The results confirmed that TS increased the excitability of LG. The data is not shown as it had already been presented (Krasne & Glanzman, 1986). The effect of TS on the electrical commissural transmission between the two LGs was then examined.

Fig. 2A illustrates the results of a typical experiment, using an intact animal with implanted chronic

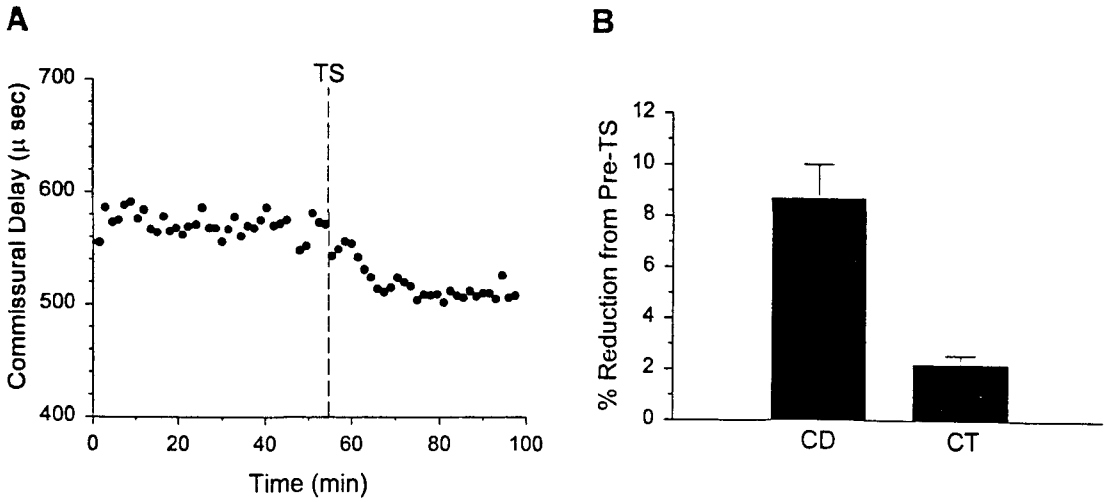

Fig. 2. Effects of traumatic shock (TS) on the commissural transmission between LGs in chronic animals. A. Example of an experimental session. Each trial is portrayed as a point whose abscissa is the time of axonal stimulation of one LG every 90 s and whose ordinate is the commissural delay (CD) from each response. TS indicates the time of traumatic stimulation. B: Mean percent reductions in CD and conduction time (CT) following a presentation of TS.

electrodes. CD for each test trial was measured for about 50 min. After obtaining a baseline of CD a traumatic shock was then presented at midway between test trials. A persistant reduction in CD (tested at least 20 min after TS) was observed following the TS. It was, however, noted that the TS effect on the commissural transmission appeard to develope fairly gradually such that the CD value often did not reach its lowest level until several trials after the presentation of TS (Fig. 2A). Results of TS effects on the commissural transmission between two LGs are summarized in Fig. 2B. A significant percentage reduction of CD (8.7 ± 1.3 %; $p<0.01$, n = 8, Wilcoxon signed-ranks test, two tailed) indicates the enhancement of the commissural transmission by the presentation of TS. The effect of TS on the conduction speed along the LG axons was also estimated in each animal by measuring conduction time (CT). Although the effect was very small (Fig. 2B), the conduction speed along the LG axon also appeared to be slightly increased, as estimated from a statistically significant reduction in CT (1.8 ± 0.35 %; $P<0.01$, two tailed Wilcoxon signed-ranks test).

Effects of octopamine on commissural transmission

The effects of bath-applied octopamine on the commissural transmission between the bilaterally located LGs were investigated. The results of a typical drug experiment are depicted graphically in Figure 3A. Octopamine was applied three times, but phentolamine was superfused during second application. Figure 3B shows the distribution of percentage CD reductions following the application of octopamine in 19 animals. The results show a significant reduction of CDs by 8.33 ± 1.0% ($p<0.01$, student t-test). In deteriorated preparations, in which commissural transmission had failed, octopamine often restored the commissural transmission. The effect of repeated octopamine exposure and its blocking effect by phentolamine are summarized in Fig. 3C. Phentolamine diminished the octopamine effect by 51 ± 7.1 % ($p<0.01$, student t-test), implying that the effect was not due to some nonspecific action of the octopamine superfusion. However, octopamine alone

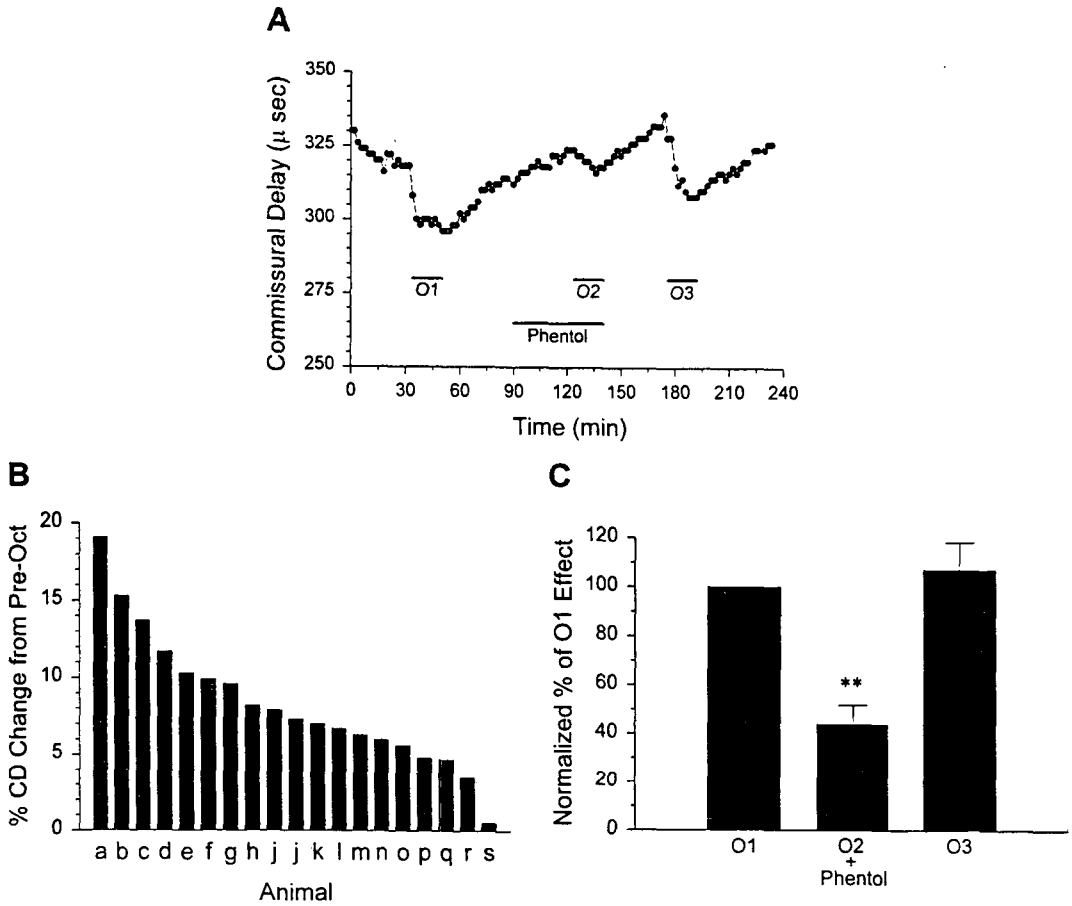

*Fig. 3. Effects of octopamine, in an acute preparation, on the commissural transmission between the bilateral LGs. A: A typical experimental session with octopamine application. In this experiment, test stimuli were given once every 30 s. Octopamine was applied to the bath three times (indicated as O1, O2 and O3). But the second application was done during superfusion of phentolamine (phentol). B: Distribution of effects of octopamine on commissural delay (CD) in all preparations examined. The letters of the alphabet denotes the individual animal. C: Summary of the effects of repeated octopamine exposures and effect of phentolamine. The first octopamine effect was normalized to 100 % in each preparation. **($p < 0.01$; student t-test).*

could be applied and washed out several times with consistent effect, as shown by comparable percentage CD changes between the first and the third octopamine applications (see Fig. 3C).

DISCUSSION

Effects of traumatic shock-produced sensitization on commissural transmission

Results from the present experiments demonstrated that in chronic crayfish preparations the time needed for the lateral giant to recruit its contralateral homologue via electrical synapses was significantly reduced by TS_S to the head. The enhanced efficacy of commissural transmission between the LGs may contribute to the sensitization of the LG escape response. This sensitizing effect has previously been shown to be a result of augmented transmission at the cholinergic synapses of the reflex (Krasne & Glanzman, 1986; Miller et al, 1992; unpublished

personal observations). Therefore, the traumatic shocks may cause a rather broad alteration in the excitability of the nervous system, of which augmented transmission at the cholinergic synapses of the LG circuit is only a small part.

Consistent with the present conclusion, there are growing evidences that many cells within a behavioural circuit can be modified as a result of sensitization training (Frost et al, 1988; Walter et al, 1991) and/or several cellular changes can occur within a given neuron (Hochner et al, 1986; Boulis & Shaley, 1988). Therefore, resultant behavioral outcomes could be determined by the interaction and balance of all of these changes. Recently, Trudeau & Castellucci (1993) reported that a noxious stimulus can have multiple effects on the short-term sensitization of the gill and siphon withdrawal (GSW) reflex. These effects may be largely attributable to parallel modifications at four different loci within the GSW reflex neuronal network. Studies of long-term potentiation (LTP) in the hippocampus, for example, have focused on changes in transmission at the principal excitatory junctions of the circuit (Madison et al, 1991; Bear & Malenka, 1994 for review). It is, however, also possible that parallel modifications at other sites such as inhibitory synapses could also play a role in LTP in hippocampus (Tomasulo & Ramirez, 1993).

Effects of octopamine on commissural transmission

This present study found that octopamine significantly reduces the CD between LGs. This finding provides an evidence that octopamine, a naturally occurring neuromodulator in crayfish (Livingston et al, 1981) and an analogue of adrenaline (Evans, 1985), also enhances the commissural transmission between crayfish LGs. The presence of an octopamine receptor in the LG would greatly facilitate analysis of octopamine's mechanism of action in this system. The fact that sensitization effects on the commissural transmission developed fairly gradually and lasted well beyond 1 hr following TS (Fig. 2A) may suggest an involvement of a second messenger system in the sensitization of LG escape responses.

Research in a number of systems has shown that neuromodulators can alter the output from neural networks by two major mechanisms: 1) changing the intrinsic properties of the component neuron and 2) changing the synaptic efficacy in the circuit (Harris-Warrick & Marder, 1991). A simple explanation for the direct changes in the excitability of the LGs, reported here, is that octopamine increases the input impedances of LG neurons. This increase in the input impedance might affect conduction velocity generally or might specifically affect the electrical commissural synapses. This hypothesis will be evaluated in future intracellular experiments.

Octopamine perfused into the isolated abdominal nerve cord appears to exert modulatory effects on at least 3 different loci in the crayfish LG escape circuit: 1) cholinergic sensory afferent synapse (Glanzamn & Krasne, 1983; Bustamante & Krasne, 1991), 2) electrical commissural transmission of LGs, and 3) mechanoreceptors (Bustamente & Krasne, 1991). Thus, it is possible that octopamine has multiple sites of action in the neural circuit for the LG escape response in crayfish. It has also been observed to elicit an arousal-like state of continuous jittery leg movement and increased eye movement in crayfish (Arnsen & Olivo, 1988).

The physiological actions of octopamine within the nervous system in isolated abdomen do not provide a direct evidence that octopamine actually participates in the production of the behavioral sensitization in LG escape response. Therefore, unless octopamine can be shown to be released during sensitization in vivo, the physiological action of octopamine is little more than a pharmacological curiosity. An examination of the effects on sensitization of pharmacological agents that block the action of octopamine would be the next step in this study. Such an experiment should help to determine whether an octopaminergic facilitating

system in crayfish plays the same sort of role that has been ascribed to a serotonergic facilitatory system in *Aplysia* (Glanzman et al, 1989). Phentolamine is the best blocker of octopamine. By itself, phentolamine had an effect on crayfish behavior so that it cannot be used to determine whether it prevents sensitization (unpublished personal observation). Reserpine has been used successfully to deplete monoamines in both vertebrates and invertebrates (O'Gara et al, 1991). Experiments to test whether monoamine depletion affects the ability to induce sensitization are in progress in our laboratory.

In conclusion, the present study has demonstrated that TS_S to an animal's head or exogenous application of octopamine in isolated nerve cord enhance the efficacy of commissural transmission between crayfish LG_S that normally operate electrically. Taken together, the present observation and previous studies suggest that both TS_S and octopamine may cause a rather broad alteration in the condition of the crayfish nervous system, which then contributes to the sensitization of the LG escape response. Further studies are needed to determine whether octopamine is the specific neuromodulator responsible for the sensitization of crayfish LG escape produced by TS_S.

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