The Effects of Tachykinin on Olfactory Reception in *Periplaneta americana*

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KEYWORDS. Tachykinin, tachykinin-related peptide, olfaction, electroantennogram, *Periplaneta americana*

BRIEF. A neuropeptide found in invertebrates, the tachykinin-related peptide, caused an overall decrease in olfactory sensitivity for the American cockroach.

ABSTRACT. Neurotransmitters are responsible for sending information throughout the nervous system, yet some of their behavioral functions in organisms are unknown. One specific neurotransmitter, tachykinin, is a part of a common family of neurotransmitters, whose behavioral functions in invertebrates are unknown. In a recent study, the invertebrate form of tachykinin, the tachykinin-related peptide (TKRP), was shown to reduce sensitivity in odor perception in the fruit fly. To better understand their function, 15 variants of tachykinin were tested in American cockroaches to determine its effects on odor sensitivity. It required an electroantennogram, which measures the initial odor response from the cockroach. Injecting a small amount of the neurotransmitter into the head of the cockroach and observing the responses to periodical odor pulses in the following hour determined the effect of a variant. Many variants caused the odor sensory sensitivity to decrease, suggesting that TKRP plays a role in regulating odor sensitivity in the cockroach. Additionally, the cockroaches' responses to TKRP were affected by the time of day, signaling an influence of their biological clocks.

INTRODUCTION.

Proteins perform a wide variety of functions, and some types of proteins are neurotransmitters that are responsible for sending information throughout the body's entire nervous system. This type of protein is called a neuropeptide. Neuropeptides, the most chemically diverse and numerous of the neurotransmitters, are not well understood in invertebrates, especially concerning their roles in their central nervous systems [1]. To better understand this opaque field, we researched the neuropeptide family of tachykinins as they relate to invertebrate behavior. By better understanding the functionality of this specific type of neurotransmitter, the scientific community can gain more understanding of the brain, central nervous system, and peptide interactions, and also the functionality of neuropeptides and the behavioral roles they play.

Tachykinins comprise the largest family of neuropeptides, which has been conserved through evolution [1]. A subgroup of tachykinins commonly found in invertebrates, the tachykinin-related peptides (TKRP) are structurally related to the mammalian tachykinins, specifically substance P and neurokinins A and B [1, 2, 3]. While some progress in knowledge has been made in understanding tachykinin, tachykinin's neural function in invertebrates is largely unknown, especially with regards to its effects on invertebrate behavior, despite its diverse distribution [1, 2, 3]. In a previous study, genetic manipulation of *Drosophila*, the fruit fly, tested the inhibition of tachykinin production and resulted in a reduction of sensitivity in odor perception [1], making the olfactory system a point of interest for TKRPs.

Periplaneta americana, the American cockroach, proves to be the best model organism for studying olfactory reception in relation to TKRPs. There has been a significant amount of work on both the physiology and behavior of olfaction in cockroaches [3]. It has 15 variants of TKRP, the most of any invertebrate species [2], which provides a broader range of scope in the role of TKRP. This broad range of variants also provides specificity to the importance of the order of amino acids in TKRPs, since there are very slight differences between each variant [2].

To better understand the functional role of tachykinin in the invertebrate olfactory system, the 15 TKRP variants in the American cockroach were surveyed to identify any that had an effect on the antenna's response to odors. By understanding these relations, this will not only further understanding of neuropeptides, it will also target specific variations of tachykinin that display a statistically significant effect on cockroaches. Based on the results from *Drosophila*, one could predict that when TKRPs are injected into cockroaches, there will be an increase in sensitivity in olfaction.

MATERIALS AND METHODS.

Electroantennogram.

The electroantennogram (EAG) measures an electrical potential change in the antennae that accompanies the response of the olfactory receptors. This potential change is recorded as EAG amplitude, measured in millivolts (mV), and is an indirect measure of the olfactory sensitivity.

EAGs were recorded from restrained cockroaches, which were briefly anesthetized with carbon dioxide. The procedure followed the method of Page and Koelling (2003) [4]. The diagram and description of the cockroach setup in the EAG is seen in Figure S1. Contact between the antenna and the electrode was made with the electrocardiogram (EKG) electrode cream. The signal produced by the antenna was led to an amplifier (Gain: X100, Bandpass: 0.1-200 Hz), then to an oscillographic recorder and computerized data acquisition system (Spike2).

Performing Injections.

Injections occurred during the EAG testing and were only performed after five consistent EAG amplitude responses from the cockroach, which was determined subjectively. The experiments used 15 variants of the tachykinin-related peptide (TKRP). Sixty micrograms (~60 nanomoles) dissolved in 3 microliters of physiological saline was injected in each cockroach; for all of the cockroach tests, the molar amount of tachykinin was relatively constant. The TKRPs were received from Hyung-Wook Kwon, Seoul National University. Injections occurred at the soft cuticle underneath its right antenna. Saline injections served as the control. A micromanipulator (Brinkman) was used to adjust the needle to a proper position prior to and after the injection. After the injection, the cockroach was monitored through the EAG for the following hour.

Supplemental Information.

Refer to the supplemental information for information regarding the model organism and methods pertaining to stimulus delivery and data analysis.

RESULTS.

Saline injections produce no significant effect on cockroach EAGs.

As a control group, saline injections were performed on cockroaches, as seen in Figure S3. Each of the EAG amplitudes that is a response to an odorant pulse corresponds to one point on the graph. After five consistent responses from the cockroach, an injection of three microliters of saline was performed on the cockroach. Saline injections did not have any significant effect on EAG amplitude ($P=0.982$, ANOVA).

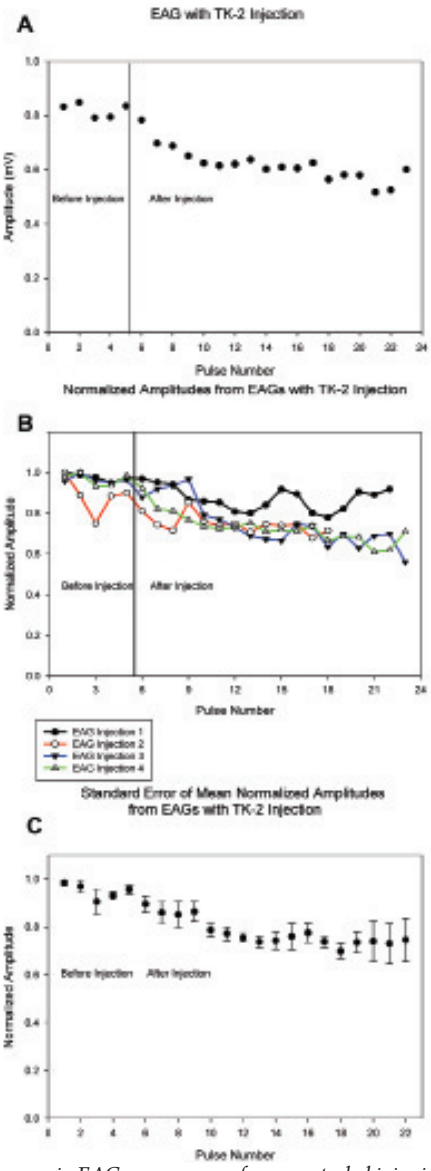

Figure 1. These graphs show the EAG responses as a function of pulse number for the effects of TK-2 injections. (**A**) displays amplitudes of EAGs recorded before and after injection for one of the four cockroaches. (**B**) shows normalized EAG amplitudes for all four cockroaches. (**C**) displays the mean (± standard error of the mean) normalized EAG amplitude. TK-2 had a significant effect on EAG amplitude ($P<0.001$, ANOVA). Between each pulse was a three minute waiting period.

Decreases in EAG response seen for many tachykinins indicate decreased sensitivity.

The injections of each of the 15 tachykinin variants were performed following the same protocol as the saline injections. Each TKRP variant was tested on at least four cockroaches in order to survey the overall effect of TKRP and identify those with substantial effects for further study. The EAG response is an indirect indicator of olfactory sensitivity; a higher response indicates a higher sensitivity, while a decreased response indicates a decreased sensitivity. A chosen example to demonstrate a change in sensitivity is seen in Figure 1.

Table 1: Effects of TKRP on EAG Response

Injection	p-Value from ANOVA †	Averages of the Ratios of Pre-Injection to Post-Injection	Number of Animals Tested	Effect of Sensitivity: Increase, Decrease, or No Change
Saline	0.982	0	4	No Change
TK-1	0.081	1.0026575	4	Increase
TK-2	<0.001*	0.7038973	4	Decrease
TK-3	0.244	0.8734591	4	Decrease
TK-4	0.893	0.9390949	4	Decrease
TK-5	0.414	0.8785413	4	Decrease
TK-6	0.433	1.02156	6	Decrease
TK-7	0.054	0.8174478	4	Decrease
TK-8	0.986	0.8750605	4	Decrease
TK-9	0.403	1.085529	4	Increase
TK-10	0.892	0.8740333	4	Decrease
TK-11	0.521	0.901947	4	Decrease
TK-12	0.003*	0.7406023	4	Decrease
TK-13	0.797	0.9542075	4	Decrease
TK-14	0.027*	0.819551	4	Decrease
TK-15	0.043*	0.9101849	4	Decrease

†ANOVA was run on the normalized data before and after the injection.

*Statistically significant decrease in amplitude, according to ANOVA.

In order to compare the proportional change for each of the tachykinin responses, a subjective test was created. For each individual cockroach, the EAG amplitude 30 minutes after the injection was divided by the EAG amplitude immediately before the injection, seen in Figure 2. Overall, there was a general decrease in the EAG responses to the tachykinin injections. The summary of the EAG responses to the TKRPs is seen in Table 1. The impression is that TKRPs decrease amplitude. Some individual TKRPs seem to have little or no effect. The effect of other TKRPs appears to be more robust, especially TK-2, -12, -14, and -15. In the table, the mean value for all of the values from this subjective test for all of the cockroaches is shown.

The response from the TKRP injection depended on the time of day.

After analyzing the data more closely, we found that the effect of TKRP appeared to vary with the time of day. In order to better determine time's role in the responses, the EAG responses were divided into morning injections (8 AM – 11 AM) and afternoon injections (11:01 AM – 5 PM). The same subjective test was performed again, and those two results are seen in Figure 2. Overall, the morning injections had a higher decrease in olfactory sensitivity, whereas the injections performed in the afternoon varied across the board. Because of the small experimental size for each of the tachykinins (n=4), statistical tests were not performed on this data.

It is important to mention that the time of day is not the only factor of analysis. While it is true that EAGs performed in the morning tend to cause a greater decrease in response, there were a number of TKRPs that had no effect on cockroaches in the morning, or else any effect at all. Therefore, doing a single-variable analysis on a multivariable concept would misinterpret the data.

DISCUSSION.

The results obtained suggest that tachykinin may play a role in regulating olfactory sensitivity in the cockroach.

Unlike what was predicted based on behavioral effects of tachykinin "knock-down" in the fruit fly, several of the tachykinins appeared to cause a significant decrease in EAG amplitude, signifying a decrease in sensitivity. This is somewhat surprising, since *Drosophila* and *P. americana* are similar in terms of olfactory responses and structures. However, these two experiments look at very different processes in olfactory reception; while the experiments reported in this study examined the effect of TKRP on primary olfactory reception, the *Drosophila* experiment focused on the behavior in response to olfactory reception. Between the primary olfactory reception and the generation of behavior, there is a substantial amount of processing that occurs in the nervous system. Therefore, it would be beneficial to better understand the relationship between *Drosophila* and *P. americana* and their olfactory responses in comparison to this experiment. In addition, one could also compare how knocking-out TKRP-

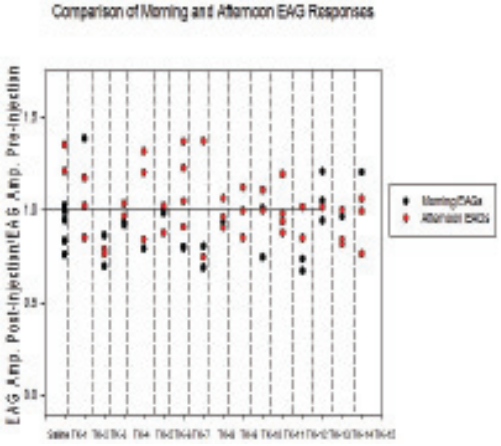

Figure 2 displays an overview of the EAG responses to all of the variants the variants of TKRP and saline. The colors, black for morning and red for afternoon, provide a comparison of the morning and afternoon EAG responses.

producing genes in *P. americana* compares to that of *Drosophila* in terms of physiological and behavioral olfactory sensitivity.

The results obtained serve as an overview for the overall responses to the 15 variants of TKRP in *P. americana*. Therefore, having 4-8 animals per variant is reasonable for a surveying experiment. However, more detail needs to be added to this experiment. For those TKRPs that yielded the largest effect on EAG amplitude, additional cockroaches should be tested to verify the validity of the results obtained. Additionally, the results that seemed to vary from morning to afternoon should be verified by adding more cockroach tests at various times of day. Further experiments are needed for TK-1, -4, -6, -7, -9, as their results were inconsistent and need further attention.

As seen in the results, the time of day may affect the olfactory sensitivity and response of cockroaches to these neuropeptides, signaling that the biological clock may play a pivotal role in the response to tachykinin, just as it does on other olfactory responses [3]. EAGs performed in the morning repeatedly showed lowered sensitivity in olfactory response, whereas EAGs performed in the afternoon proved to be more variable. This time difference may be the key to not only understanding peptide relations, but also those peptide relations in concordance with biological clocks. Therefore, future experiments should include analyses of the overall, daily and nocturnal modulation of olfactory response curves in response to TKRPs to determine the role of the biological clock in neuropeptide effects.

The tachykinin-related peptide is only one of many neuropeptides, yet understanding the functions of one peptide can give insights to the functionality of those peptides that are structurally related. The mammalian tachykinin, substance P, is one such example, being structurally similar to TKRPs [1]. Substance P has been shown to modulate pain sensitivity throughout the central nervous system in various mammals [6]. In our study, we had found that the TKRPs in cockroaches may regulate olfactory sensitivity. Because both of these tachykinins are responsible for regulating sensory sensitivity, one could predict that this trend may continue to a variety of other tachykinins of similar structures to propose that tachykinins are responsible for sensory reception. However, like TKRP, few studies have been conducted to determine how these reception processes function. By understanding an invertebrate model, to an extent, one may then be able to extend this model to other tachykinins in mammalian systems.

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SUPPORTING INFORMATION.
Figure S1. Experimental set-up for an electroantennogram
Figure S2. Dilution response curve of *Periplaneta americana* for ethyl acetate
Figure S3. EAG responses as a function pulse number to show the effects of injections of saline
Supplementary methods section

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