Gain Control of Synaptic Transfer from Second- to Third-order Neurons of Cockroach Ocelli

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Abstract Synaptic transmission from second- to third-order neurons of cockroach ocelli occurs in an exponentially rising part of the overall sigmoidal characteristic curve relating pre- and postsynaptic voltage. Because of the nonlinear nature of the synapse, linear responses of second-order neurons to changes in light intensity are half-wave rectified, i.e., the response to a decrement in light is amplified whereas that to an increment in light is compressed. Here I report that the gain of synaptic transmission from second- to third-order neurons changes by ambient light levels and by wind stimulation applied to the cerci. Transfer characteristics of the synapse were studied by simultaneous intracellular recordings of second- and third-order neurons. Potential changes were evoked in second-order neurons by a sinusoidally modulated light with various mean luminances. With a decrease in the mean luminance (a) the mean membrane potential of second-order neurons was depolarized, (b) the synapse between the second- and third-order neurons operated in a steeper range of the exponential characteristic curve, where the gain to transmit modulatory signals was higher, and (c) the gain of third-order neurons to detect a decrement in light increased. Second-order neurons were depolarized when a wind or tactile stimulus was applied to various parts of the body including the cerci. During a wind-evoked depolarization, the synapse operated in a steeper range of the characteristic curve, which resulted in an increased gain of third-order neurons to detect light decrements. I conclude that the nonlinear nature of the synapse between the second- and third-order neurons provides an opportunity for an adjustment of gain to transmit signals of intensity change. The possibility that a similar gain control occurs in other visual systems and underlies a more advanced visual function, i.e., detection of motion, is discussed.

Introduction

The visual environs animals encounter in nature are a modulation of intensity around a mean, and visual systems must have evolved to process such stimuli. Studies using sinusoidally or white-noise-modulated light show that the peripheral visual systems of many vertebrates and invertebrates share common features for processing such modulated signals. The response of photoreceptors is linearly related to the depth of changes in intensity for at least a moderate range of intensity change in vertebrate retinas (Baylor and Hodgkin, 1974; Chappell et al., 1985), the compound eyes of Limulus (Fortes and Hodgkin, 1964), and insect compound eyes (Pinter, 1974; Juusola et al., 1994). The linear voltage modulation of photoreceptors produces a linear response in second-order neurons, where a dynamic component of the response is amplified while a steady component is compressed in vertebrate retinas (Naka et al., 1979; Tranchina et al., 1983), the compound eyes of Limulus (Knight et al., 1970), and insect compound eyes (Laughlin et al., 1987; Weckström et al., 1992) and insect ocelli (Mizunami et al., 1986). A nonlinear response first appears in third-order neurons: half-wave or full-wave rectified responses are observed in some third-order neurons of vertebrate retinas (Spekreijse, 1969; Toyoda, 1974; Sakai and Naka, 1987a, b), insect compound eyes (Osorio, 1987, 1991; Jansonius and van Hateren, 1993), insect ocelli (Mizunami, 1990a), and barnacle ocelli (Stuart and Oertel, 1978). Although the visual processing in photoreceptors and second-order neurons has been the subject of extensive studies (see the references cited above), less is known of neural mechanisms involved in signal conversion between second- and third-order neurons.
Insects possess two or three ocelli in addition to a pair of compound eyes. Because of its simplicity and the accessibility for intracellular microelectrodes, the insect ocellar system has been used as a model for examining the basic mechanisms of visual processing (Chappell and Dowling, 1972; Dowling and Chappell, 1972; Mizunami et al., 1986). The insect ocellus does not form images nor does it sense anything other than changes in illumination integrated over the large receptive field (Chappell and Dowling, 1972; Wilson, 1978), but it does play important roles in the insect’s behavior, because it is superior to the compound eye in terms of photic sensitivity and speed of signal transmission (Wilson, 1978; Stange, 1981; Mizunami, 1994). In the cockroach ocellar retina, there are ~10,000 photoreceptors that converge onto only four second-order neurons (Weber and Renner, 1976; Mizunami et al., 1982). Second-order neurons exit the ocellar retina and project into the ocellar tract neuropil of the protocerebrum, where they form synapses onto a number of third-order neurons (see Fig. 1 A; Toh and Hara, 1984; Mizunami, 1995). Third-order neurons exit the ocellar tract and project into various neuropil areas of the brain (Mizunami, 1995).

Transfer characteristics of the synapse between the second- and third-order neurons of the cockroach ocellus have been examined in detail using simultaneous microelectrode penetrations (Mizunami and Tateda, 1988; Mizunami, 1990a). The major findings were (a) a continuous release of excitatory transmitters is maintained during steady illumination; (b) the synapse operates at an exponentially rising part of the overall sigmoidal characteristic curve relating pre- and postsynaptic voltage (see Fig. 1 B); (c) the transmission is static, i.e., the characteristic curve remains unchanged over a wide range of frequencies; and (d) because of the exponential nature of the characteristic curve, the linear response of second-order neurons is converted into a nonlinear, half-wave rectified response in third-order neurons; the depolarizing response to light decrements is amplified whereas the hyperpolarizing response to light increments is compressed (see Fig. 1 C; Mizunami, 1990a). The synapse between the second- and third-order neurons of locust ocelli have much the same properties (Simmons, 1993).

The study reported here was an attempt to gain further knowledge about signal processing at the synapse between second- and third-order neurons of cockroach ocelli. The membrane potential of second-order neurons is more depolarized at a dimmer illumination (Mizunami and Tateda, 1988), and I addressed the question of whether the change in the membrane potential of second-order neurons would affect the signal transmission from second- to third-order neurons. Ohyama and Toh (1986, 1990) reported that second-order neurons of cockroach ocelli are depolarized in response to wind and tactile stimuli applied to various parts of the body including the cerci, illumination of the compound eyes, and active and passive movements of the wing, and I examined the effects of the wind-evoked depolarization on the signal transmission from second- to third-order neurons. I found that (a) there is a slight increase (40–75%) in the gain of synaptic transmission for a 3.6-log decrease in the mean luminance; (b) wind stimulation leads to an increase in the gain of synaptic transmission of up to 2–2.5-fold, and (c) the increased gain is due to a positive shift of the operating range of the synaptic transmission over a positively accelerated characteristic curve. Thus, a nonlinear synaptic transfer from second- to third-order neurons of cockroach ocelli allows for an adjustment of gain to transmit signals of intensity change, in accordance with behavioral situations and ambient light levels.

MATERIALS AND METHODS

Biological

Experiments were done on adult male cockroaches, Periplaneta americana, raised in our colony. Each cockroach was anesthetized by cooling it with ice, and then mounted, dorsal side up, on a Lucite stage and fixed with beeswax. The compound eyes and one of two ocelli were shielded from light with beeswax mixed with carbon black. The dorsal surface of the head capsule was removed and the dorsal surface of the brain exposed. The esophagus was excised, and the brain was mechanically stabilized by inserting a glass rod into the esophageal foramen. Saline containing a digestive enzyme, 1% Actinase (Type E, Kaken Seiyaku, Tokyo, Japan) was applied to the brain for 1 min and then washed off to facilitate insertion of microelectrodes.

Two microelectrodes were inserted into the ocellar tract of the protocerebrum where second-order ocellar neurons synapse onto a number of third-order neurons (Fig. 1 A; Mizunami, 1990a). The electrodes were filled with either (a) 5% lucifer yellow in 0.2 M lithium chloride, (b) 0.5 M cobalt lysine (Gorcs et al., 1976) mixed with 2 M potassium chloride, or (c) 2 M potassium acetate. The recorded neurons were identified as either second- or third-order neurons from their response: the wave-form of the response of second-order neurons to sinusoidal light was almost sinusoidal, whereas that of third-order neurons exhibited a characteristic deviation from a sinusoid (Fig. 1 c; Mizunami, 1990a). The recorded third-order neurons were filled with lucifer yellow or cobalt to determine morphologic type.

The electrodes were connected to a preamplifier (MEZ-8201, Nihon Kohden, Tokyo, Japan) equipped so that a constant current could be passed through an active bridge circuit. A small piece of platinum placed in the bathing solution served as an indifferent electrode. The amount of stimulus current was continuously monitored. Measurements indicative of electrical coupling between the two electrodes were not considered for this study.

A light-emitting diode, LED (Sharp Corp., Tokyo, Japan), was used as a light source. The LED had a spectral peak at 560 nm. The LED was driven by a sinusoidal current provided by a function generator (ET1101, NF Design Block, Tokyo, Japan). The lu-
minance of the stimulus depended linearly on the magnitude of the driving current. The stimulus light was monitored by a photodiode (TFA1001W, Siemens-Allis, Inc., Cherry Hill, NJ). A series of calibrated neutral-density filters attenuated the light beam. The light stimulus and cellular response were observed on an oscilloscope and stored on analogue tape. For data analysis, the stored signals were digitized and averaged with an averager (DAT1101, Nihon Kohden, Tokyo, Japan) and observed on a chart recorder or x-y recorder.

Air puffs to the cerci were used to induce a depolarization in second-order neurons. Air flow from an air compressor was directed toward the cerci through a delivery tube. The air speed was 1 m/s and the stimulus period was controlled by an electromagnetic valve. All experiments were done at room temperature (20–24°C).

Analytical

The sinusoidal light stimulus consisted of two components, a steady mean, I0, and a dynamic component, I(t). The maximal and minimal I0 applied were 20 (0 log) and 0.005 μW/cm² (~3.6 log), respectively. The dynamic component was defined by the modulation frequency (Hertz) and the depth of modulation, (I max − I min)/(I max + I min), where I max is the maximum luminance and I min is the minimum luminance. The depth of modulation represents the “contrast” between the stimulus and the adapting light. The resulting response recorded from a second- or a third-order neuron consisted of a steady potential, V0, and a dynamic component, V(t). The former relates to I0 and the latter relates to I(t), respectively. The ocellus was first allowed to adapt to the dimmest illumination (~3.6 log) for 5 min. When increasing or decreasing the mean luminance, the ocellus was allowed to adapt to a new luminance for 1–2 min before recording was started.

Two methods were used to characterize the input-output relationship of the synaptic transmission. First, the frequency transfer function between the potential of a second-order neuron, V prep(t), and that of a third-order neuron, V post(t), was measured. The gain of synaptic transmission was defined as a peak-to-peak amplitude of a modulatory response of a second-order neuron, A prep per unit of amplitude of response of a second-order neuron, A post. In the actual experiments, the stimulus frequency was fixed and the depth of modulation was adjusted for the 1-mV measurement to produce a 0.5–1 mV and 1–1.5 mV change of potential in the second-order neuron. This sequence was performed over a frequency range of 0.3–20 Hz, and the recorded responses were averaged for 30–100 cycles. A prep at 1 mV of A prep was estimated by linear interpolation between the two measurements. The phase relationship between V prep(t) and V post(t) was measured at the peak of the modulatory responses. Second, the modulation frequency was fixed and the potential of the third-order neuron, V post(t), was plotted against that of the second-order neuron, V prep(t), which I refer to as an input-output voltage relationship or a characteristic curve of synaptic transmission. Because the input-output voltage relationship is independent of the modulation frequency for at least a range of 0.3–20 Hz (Mizunami, 1990a), measurements at a fixed frequency were sufficient to determine the input-output voltage relationship. In actual experiments, a sinusoidal light of 0.5 Hz was applied to an ocellus, the response was averaged for 20–50 cycles, and V post(t) was plotted against V prep(t) on an x-y recorder. The trajectory of the plot, which I refer to as a phase-plane plot, formed a loop when there was a phase lag between V prep(t) and V post(t). At a frequency of 0.5 Hz, where the phase lag is very small, the loop approaches a single curve, which represents the characteristic curve of synaptic transmission. In experiments where low-frequency stimulus could not be used (see Fig. 8), a sinusoidally modulated light of 4 Hz with various modulation depths was applied to an ocellus, the resulting response was averaged for 20–50 cycles, and the potentials at the peak and bottom of the modulatory response of the third-order neuron were plotted against those of the second-order neuron.

The light-evoked responses of second- and third-order neurons were characterized by measuring the frequency transfer functions between the light stimulus and the resulting responses. A sinusoidal light of a depth of 0.3, 0.5, and 0.7 and a frequency of 0.3–30 Hz was applied to an ocellus, and the resulting voltage responses were averaged for 20–100 cycles. The gain was measured as a peak-to-peak amplitude of response per unit of depth of a stimulus. The phase relationship was measured at the peak of light and voltage modulation.

In some experiments, a steady (DC) or noise current was injected into a second-order neuron while an ocellus was stimulated by a sinusoidal light. For the injection of noise current, the potentials of a second-order neuron during a steady illumination of 0.005 μW/cm², where there was a large membrane fluctuation (voltage noise), were stored on tape. The potentials were passed through an active high-pass filter to remove the DC component and were used to drive a current-passing circuit. The magnitude of voltage change induced by an extrinsic current was estimated on the basis of input resistance data reported previously (Mizunami and Tateda, 1988; Mizunami, 1990a).

RESULTS

Simultaneous intracellular recordings from a second- and a third-order ocellar neuron of the American cockroach were made at the ocellar tract, where four second-order neurons make synaptic connections with at least 12 morphologic types of third-order neurons (Fig. 1 A; Mizunami and Tateda, 1986; Mizunami, 1995). The four second-order neurons exhibited similar photic responses (Mizunami et al., 1982), and the properties of their output synapses onto third-order neurons were similar (Mizunami, 1990a, 1995). Among third-order neurons, the most stable recordings were obtained from OT-PS1 (Fig. 1 A) and OT-OL1 neurons (Mizunami and Tateda, 1986). The results presented here are based on observations of 12 pairs of a second-order and an OT-PS1 neuron, and eight pairs of a second-order and an OT-OL1 neuron, from which stable intracellular recordings were attained for more than 20 min. Experiments were done after 10−5 g/ml tetrodotoxin (TTX) was added to the saline solution to suppress spike activity and for a quantitative measurement of the synaptic potential.

In an early stage of this experiment, a sinusoidal current was used to induce a potential change in a second-order neuron. An application of current for >3–5 min often induced a small but irreversible change in the
membrane potential, and this affected the synaptic transmission from second- to third-order neurons. Thus, in all the experiments presented here, a sinusoidal light was used to evoke potential changes in second-order neurons, where the evoked potential change in third-order neurons was the sum of the synaptic inputs from the four second-order neurons. Because effects of feedback from third- to second-order neurons and synaptic interactions among both the second-order neurons and the third-order neurons are small or absent (Mizunami, 1990a), the relationship between the recorded potentials of the second- and third-order neurons represents the input-output voltage relationship of the synapse between these neurons.

Steady Membrane Potential under Steady Illumination

Fig. 2 A shows responses of a second- and a third-order neuron to prolonged illumination. The second-order neuron exhibited a hyperpolarization in response to a light stimulus given in the dark, which in turn evoked a hyperpolarization in the third-order neuron because of a reduction in excitatory transmission (see Fig. 1 B; Mizunami, 1990a). A prolonged step stimulation produced an initial peak hyperpolarization in both neurons, followed by a gradual recovery to reach a steady level within 30–40 s. The steady state potentials of both second- and third-order neurons were more positive and the variance of membrane fluctuations (voltage noise) was larger in case of a dimmer illumination. In Fig. 2 B, membrane potentials were repeatedly measured at four different mean luminances in a pair of second- and third-order neurons, and the averages and standard deviations were plotted against the log of luminance. For a 3.6-log decrease in the mean luminance, second- and third-order neurons were depolarized for ~2 and 1.5 mV, respectively.

Effects of Mean Luminance on Synaptic Transfer Function

Fig. 3 shows gain (A) and phase (B) portions of the transfer function of synaptic transmission from second- to third-order neurons, measured using a sinusoidally modulated light with various mean luminances. In Fig. 3 A, the peak-to-peak amplitude of the modulatory response of a third-order neuron at a 1 mV peak-to-peak response of a second-order neuron was plotted against the modulation frequency. The synaptic transmission had low-pass filter characteristics with a cutoff frequency (~3 dB) at 25–30 Hz. The gain at the dimmest mean luminance (~3.6 log units) was ~3.5 dB (50%) higher than that at the brightest mean luminance (0 log unit). The increase in the gain for a 3.6-log decrease of mean luminance measured in seven pairs of second- and third-order neurons was 57% ± 19%. Figure 3 B shows that the phase lagged at higher frequencies, thereby reflecting a synaptic delay. The phase characteristic remained unchanged over a 3.6-log range of mean luminance. Measurements of transfer function at a 3 mV of presynaptic voltage modulation confirmed the higher gain seen with a dimmer mean luminance.
Figures 2 and 3 show transfer functions from light input to the resulting response of a second- and a third-order (OT-OL1) neuron to prolonged illuminations. The light intensities are indicated as log₁₀ attenuation (0 log = 20 μW/cm²). (B) The magnitude of steady state hyperpolarization, measured from the dark potential, is plotted against the log of luminance. The ocellus was first dark adapted for 5 min. Then a very dim illumination (0.005 μW/cm²) was applied and the potential at the steady state was measured. Luminance was increased sequentially, and, after the steady state potential at the brightest illumination (20 μW/cm²) was measured, the sequence was reversed. This series was repeated two times, and the averages and standard deviations are shown. Potentials of second- and third-order neurons were recorded simultaneously in the ocellar tract in this and in all subsequent figures.

Fig. 4 shows transfer functions from light input to the resulting response of a second- (A and B) and a third-order neuron (C and D) measured using a sinusoidally modulated light with various mean luminances. In Fig. 4, A and C, the peak-to-peak response of the second- and third-order neurons at a depth of modulation of 0.5 was plotted against frequency. Second-order neurons exhibited a bandpass filter property with optimal frequencies of 1–5 Hz. Third-order neurons had similar optimal frequencies, but the decay of gain at low and high frequencies was sharper than that of second-order neurons. This is explained by the positively accelerated nature of the input-output voltage relationship of synaptic transmission between these neurons (Mizunami, 1990a). The gain of second-order neurons was roughly constant over a 3.6-log range of mean luminance, or slightly higher at a brighter mean luminance, whereas the gain of third-order neurons was higher at a dimmer mean luminance (Fig. 4 C). The phase characteristic was unchanged over a 3.6-log range of mean luminance (Fig. 4 D). Similar results were attained for transfer functions at a modulation depth of 0.3 and 0.7.

Effects of Mean Luminance on the Characteristic Curve

In Fig. 5, a sinusoidally modulated light of 0.5 Hz with a mean luminance of 20 or 0.02 μW/cm² was applied to an ocellus, and the potential during the modulatory response of a third-order neuron was plotted against that of a second-order neuron. Responses were recorded for 20 cycles (40 s) or 50 cycles (100 s) at a mean illumination of 20 or 0.02 μW/cm², respectively, and averaged for each two cycles (4 s). Fig. 5 A shows that (a) the input–output voltage relationship of the synaptic transmission is positively accelerated, in agreement with my previous observations (Mizunami, 1990a), and (b) the characteristic curve at a mean illumination of 0.02 μW/cm² deviated from that at 20 μW/cm². For a quantitative analysis, the potentials of second- and third-order neurons were measured with an interval of 250 ms (45 degrees of the modulatory cycle), and the logarithm of the postsynaptic potential was plotted against the presynaptic potential. The postsynaptic po-
tential is represented as $V_{\text{post}} + V_0$, where $V_0$ is the postsynaptic potential maintained during a steady illumination of 20 $\mu$W/cm$^2$ and $V_{\text{post}}$ is the postsynaptic potential measured from a mean potential at 20 $\mu$W/cm$^2$. $V_0$ was determined from the peak hyperpolarizing potential to a stepwise illumination where the transmission is almost shut off (see legend of Fig. 1 C). The transsynaptic voltage relationship at a mean illumination of 20 or 0.02 $\mu$W/cm$^2$ fitted to a single line, which has a form

$$V_{\text{post}} + V_0 = V_0 \exp \left( \frac{V_{\text{pre}}}{k} \right),$$

where $k$ (slope of the regression line) indicates that an e-fold change in the postsynaptic voltage is attained by a $k$ mV change in the presynaptic neuron. Constant $k$ was 2.55 mV at a mean illumination of 0.02 $\mu$W/cm$^2$, which was similar or slightly smaller than that at a mean illumination of 20 $\mu$W/cm$^2$ (2.68 mV). $V_0$ (the point where the regression line crosses the y axis) at a mean illumination of 0.02 $\mu$W/cm$^2$ (1.24 mV) was smaller than that at a mean illumination of 20 $\mu$W/cm$^2$ (1.40 mV), thereby indicating a shift of the characteristic curve of synaptic transmission from second- to third-order neurons.

In Fig. 6, the effects of mean luminance on the transfer function are shown in relation to effects on the characteristic curve, where the peak-to-peak amplitude of postsynaptic voltage modulation, $A_{\text{post}}$, at 1 mV of presynaptic voltage modulation, $A_{\text{pre}}$, was graphically calculated on the basis of the regression curves of Fig. 5 B. Assume that the mean potential of second-order neurons is suddenly depolarized from 0 mV (mean potential level at a mean luminance of 0 log unit) to 1.4 mV (mean potential level at −3 log units). The gain of the transfer function before the potential shift, $A_{\text{post}}(0)$, is 0.55. Immediately after the potential shift, the gain, $A_{\text{post}}(0)/A_{\text{pre}}(-3)$, increases to 0.97, which is 76% higher than that before the shift of the presynaptic potential. If the presynaptic potential remains at the new level, the postsynaptic potential decreases slightly, then reaches a steady level. At this new steady state, the transsynaptic voltage relation is negatively shifted and the gain, $A_{\text{post}}(-3)/A_{\text{pre}}(-3)$, is reduced to 0.80, which is still 45% higher than that before the positive shift of the presynaptic potential.

**Current Injection Experiments**

The observations in Fig. 2 show that a decrease in the mean luminance accompanied (a) a positive shift in the mean membrane potential, and (b) an increase in noise variance in second-order neurons. The observations shown in Figs. 5 and 6 suggest that an increase in the gain of synaptic transmission with a decrease in the mean luminance is due to the first effect, that of steady depolarization. To confirm this, current injection experiments were done. In Fig. 7, a second-order neuron was depolarized for 2 mV by applying a steady (DC) current of 2 nA (see Methods) during the sinusoidal light stimulation at a mean illumination of 20 $\mu$W/cm$^2$. The peak-to-peak amplitude of modulatory response of the second-order neuron remained almost unchanged during the current-evoked depolarization, whereas that of the third-order neuron increased by ~20%, confirming that the gain of synaptic transmission increases with depolarization of the presynaptic neuron. Next, a noise current was injected into a second-order neuron during a steady illumination of 20 $\mu$W/cm$^2$. The magnitude of the current was adjusted so that variance of the resulting voltage fluctuation was similar to that observed at 0.005 $\mu$W/cm$^2$ (see Methods). No measurable
change in the gain of synaptic transmission was observed during the noise current injection (not shown).

Effects of Wind Stimulation on the Synaptic Transmission

When wind was applied to the cerci (a pair of wind-receptive organs located at the terminal of abdomen), both second- and third-order ocellar neurons exhibited a transient depolarization (Fig. 8 A). The depolarization of second-order neurons is due to excitatory synaptic inputs from multimodal neurons (Ohyama and Toh, 1986, 1990; see insects in Fig. 8 A). Because the recordings were made without application of TTX to not suppress spike activity of the multimodal neurons, the second-order neuron sometimes exhibited solitary spikes at the peak of depolarization, which induced a large transient depolarization in the third-order neuron. The recordings where the second-order neuron generated spikes were ignored when measuring the synaptic potential of the third-order neuron. Third-order neurons did not exhibit spikes at the ocellar tract, i.e., their synaptic region, whereas a majority do exhibit spikes when recordings are made from their axons (Mizunami and Tateda, 1986).

In Fig. 8 B, wind was applied to the cerci while the ocellus was illuminated by a sinusoidally modulated...
When wind is applied to the cerci during a steady ocellar illumination of 20 μW/cm², second- and third-order neurons exhibit depolarization. Inset is a diagram of the synaptic connections between the photoreceptors (PR), second-order neurons (2nd), third-order neurons (3rd), and multimodal neurons that respond to mechanical stimulation of the cerci (Multimodal), based on reports by Ohyama and Toh (1986, 1990), Lin et al. (1990), and Mizunami (1990a). Solid and open triangles indicate that the reversal potential of the synapse is negative or positive to the resting potential, respectively. (B) Wind is applied to the cerci while a sinusoidal light of 4 Hz, a depth of 0.6, and a mean illumination of 20 μW/cm² is applied to an ocellus. The third trace is the light stimulus monitored by a photodiode. (C) The relationship between the potential of the third-order neuron, V₃, and that of the second-order neuron, V₂, during wind stimulation (triangles), during sinusoidal light stimulation (open circles), and during a combination of light and wind stimulations (solid circles). To measure the transsynaptic voltage relation during wind-evoked transmission, the potentials for the first 1 s of wind-evoked depolarization were measured at intervals of 125 ms. Wind stimulations were repeated eight times at 1-min intervals and the potentials were averaged. To measure the light-evoked transsynaptic transmission, a sinusoidally modulated light of 4 Hz with various depths and a mean illumination of 20 μW/cm² was applied, and the peak and bottom potentials of the modulatory response were averaged for 20–40 cycles. To measure synaptic transmission during a combination of light and wind stimulations, the peak and bottom potentials of the modulatory response during the first 1 s of wind-evoked depolarization were measured, and averaged for 8 wind stimuli applied at 1-min intervals. The regression curve is for the transsynaptic voltage relation during light-evoked transmission.

**DISCUSSION**

**Gain Adjustment at the Second Synapse of Cockroach Ocelli**

Much knowledge has been accumulated on signal transmission at the synapse from photoreceptors to second-order neurons (which I refer to as the first synapse) in a variety of visual systems, but less is known about transmission at the synapse from second- to third-order neurons (the second synapse). In a previous paper (Mizunami, 1990a), I reported that (a) signal transmission at the second synapse of cockroach ocelli occurs at an exponentially rising part of the overall sigmoidal characteristic curve, and (b) because of the nonlinearity of synaptic transmission, the linear response of presynaptic neurons is converted into a half-wave rectified response, i.e., the depolarizing response to light decrements is amplified whereas the hyperpolarizing response to light increments is compressed. Similar observations have been made for second synapses of locust ocelli (Simmons, 1993). These findings fundamentally differed from those described for first synapses of visual systems, including vertebrate retinas (Normann and Perlman, 1979), barnacle ocelli (Hayashi et al., 1985), insect compound eyes (Laughlin et
larize in response to (a) wind stimuli applied to various parts of the body including the antennae and cerci, (b) light stimuli applied to the compound eyes, and (c) active and passive movement of the wing. Functional significance of the multimodal input has not been established (Ohyama and Toh, 1990). The present finding that the gain of the second synapse increases during wind stimulation suggests that detection of a change in intensity by the ocelli is facilitated when the cockroach is mechanically or visually stimulated and during active locomotion.

The present study dealt only with the graded (synaptic) component of the response, and the spike response of third-order neurons needs to be given attention in future studies in order to fully understand information processing in the cockroach ocellar system. This system contains at least 12 types of third-order neurons that project into various neuropil areas of the brain (Mizunami, 1995), the majority of which transmit signals by way of action potentials (Mizunami and Tateda, 1986; Mizunami, 1995). Processes that convert graded potential into spikes in third-order neurons are the subject of an ongoing study.

Implications for Other Visual Systems

The accumulated evidence shows that the response to changes in intensity of photoreceptors and second-order neurons of most visual systems is essentially linear. This is the case for vertebrate retinas (Baylor and Hodgkin, 1974; Naka et al., 1979; Tranchina et al., 1983), Limulus compound eyes (Fuortes and Hodgkin, 1964; Knight et al., 1970), insect compound eyes (Pinter, 1974; Juusola et al., 1994), and barnacle ocelli (Stuart and Oertel, 1978). A nonlinear response first appears at third-order neurons: some third-order neurons of vertebrate retinas (Spekreijse, 1969; Sakai and Naka, 1987, a, b), insect compound eyes (Osorio, 1987, 1991; Jansonius and van Hateren, 1993), and barnacle ocelli (Stuart and Oertel, 1978) exhibit half-wave or full-wave rectified, on-off responses, thereby suggesting that some of second synapses are rectifying, as is the case in cockroach ocelli. Thus, the present findings that the second synapse offers opportunity for adjustment of response sensitivity may be applicable to other visual systems. A more notable possibility is that a gain control at the second synapse may underlie more advanced visual functions, namely motion detection. In the fly compound eye, Mimura (1972) noted that some medulla neurons, possibly third-order neurons, exhibit directionally selective responses to motion. Hassenstein and Reichardt (1956) showed that motion detection in insect compound eyes is represented by a correlation-type algorithm where directional selectivity is formed by a nonlinear, multiplicative spatial interaction, and Franceschini et al. (1989) demonstrated that a gain control or a threshold control mechanism should be the basis of nonlinear spatial interaction. More recently, I proposed a neural model that is mathematically equivalent to the correlation-type movement detector, where the multiplicative spatial interaction is
formed by linear spatial interaction followed by a rectifying synaptic transmission (Mizunami, 1990b). The possibility that a gain control at the second synapse underlies motion detection deserves attention.

I thank M. Ohara for helpful comments.

Supported by grants from the Ministry of Education of Japan.

Original version received 30 March 1995 and accepted version received 9 August 1995.
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