LONG AND SHORT TERM EFFECTS OF GLUTAMATE ON SPIDER MECHANOSENSORY NEURONS DURING RANDOM STIMULATION

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1. Introduction

Mechanosensory neurons innervating the VS-3 slit sensilla in the spider (*Cupiennius salei*) patella receive extensive efferent innervation. The efferent fibers contain GABA, glutamate, octopamine and acetylcholine, and the mechanosensory neurons have receptors for these transmitters. Activation of the structurally related ionotropic $GABA_A$ and glutamate receptors opens Cl^- channels. However, while $GABA_AR$ agonists depolarized VS-3 neurons by ~20 mV, agonists of inhibitory glutamate receptors (IGluR) only caused ~5 mV depolarization (Panek and Torkkeli 2005).

When VS-3 neurons were made to fire action potentials using electrical step stimulation, application of IGlu or $GABA_A$ receptor agonists inhibited most neurons. However, when mechanical step stimulation was used, only IGluR agonists inhibited these neurons, suggesting that IGluRs may be present in all regions of the neurons including the dendrites while $GABA_A$ receptors may only be present in the axo-somatic region (Panek and Torkkeli 2005). However, we demonstrated recently that $GABA_AR$ activation during mechanical or electrical pseudorandom white noise stimulation caused triphasic responses, consisting of a brief initial excitation, followed by short duration inhibition and ending in prolonged excitation (Pfeiffer et al. 2009). These results suggest that $GABA_AR$ activation may inhibit repetitive signals appearing at low frequencies but enhance inputs that change rapidly, carrying higher frequencies. This could be an effective way of prioritizing input information, and similar systems could be used by the other transmitters that modulate VS-3 and other mechanosensitive neurons.

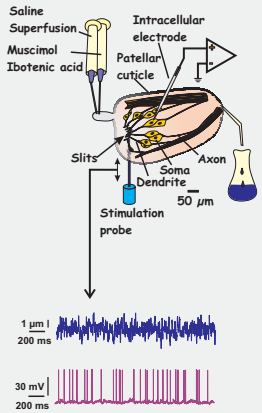
Here, we applied the IGluR agonists, glutamate and ibotenic acid, during similar random noise mechanical stimulation and observed their effects on firing rate and frequency response properties of the VS-3 neurons. The results of these experiments were compared to those with the $GABA_AR$ agonist muscimol.

Cupiennius salei

Lyriform slit sensilla

The lyriform slit sensilla on the patella of the leg of the tropical wandering spider (*Cupiennius salei*) were used in these experiments. Each of the cuticular slits is innervated by a pair of bipolar sensory neurons.

2. Methods



A small piece of patellar cuticle containing the VS-3 slit sense organ was dissected and placed on a plexiglass holder where the slits could be stimulated mechanically. Preparations were superfused continuously with spider saline (in mM: 223 NaCl, 6.8 KCl, 8 $CaCl_2$, 5.1 $MgCl_2$, 17 glucose, and 10 HEPES, pH 7.8).

Intracellular recordings were performed using a SEC-10LX amplifier (NPI Electronic) in discontinuous single-electrode current-clamp mode. Recording electrodes were filled with 3 M KCl and their resistances were 40-80 MΩ in solution. Muscimol, glutamate and ibotenic acid were applied through a small tube that drained ~2 mm from the VS-3 neuron somata. Mechanical stimulation was performed using a piezoelectric stimulator (P-841.10 translator and a LVPZT controller - PI, Physik Instrumente) that pushed a glass probe against the slits.

Raw data showing pseudorandom Gaussian white noise displacement stimulation that was provided via the probe, and the resulting action potentials.

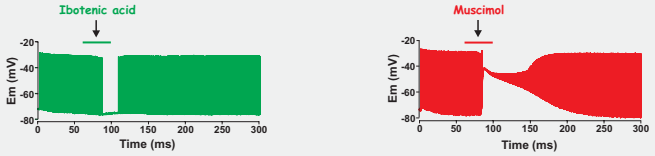
References

Panek I, Torkkeli PH (2005). Inhibitory glutamate receptors in spider peripheral mechanosensory neurons. Eur. J.Neurosci. 22: 636-646.

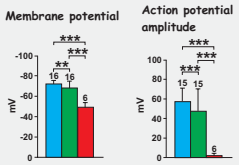
Pfeiffer K, Panek I, Höger U, French AS, Torkkeli PH (2008). Random stimulation of spider mechanosensory neurons reveals long-lasting excitation by GABA and muscimol. J. Neurophys. 101: 54-66.

Shannon CE, Weaver W (1949). The mathematical theory of communication. (Urbana, Chicago, London: University of Illinois Press), pp. 1-117.

3. Agonist effects on membrane potential and action potential amplitude

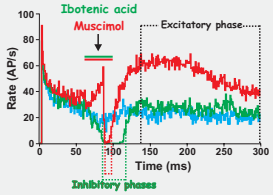


Original recordings showing effects of 100 µM ibotenic acid and 100 µM muscimol on a VS-3 neuron that was stimulated with pseudorandom noise displacement signals. Muscimol induced a 30 mV depolarization while the membrane potential of this cell did not change in response to ibotenic acid. In both cases, the cell stopped firing action potentials after agonist application, but in the experiment with muscimol complete inhibition was only observed at the peak of the depolarization. Small spikes appeared ~10 s after the inhibitory period.

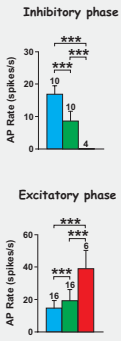


Bar graph shows the mean (± sd) values of membrane potentials and action potential (AP) amplitudes in several experiments under control conditions, after ibotenic acid or glutamate application and after muscimol application. Activation of IGluRs led to ~4 mV depolarization while $GABA_AR$ activation resulted to a significantly larger depolarization of ~23 mV. Changes in AP amplitudes were also statistically significant in response to both agonists, but muscimol caused a significantly larger change.

4. Agonist effects on action potential firing rate



To investigate action potential firing rate more closely, original recordings (Box 3) were converted to action potentials/s using 1 s wide bins. Control shows a typical decrease in firing rate during the first 60 s of the recording followed by a plateau lasting until the end of the 5 min recording period. Inhibitory period in response to application of 100 µM ibotenic acid lasted ~40 s and was followed by a long lasting excitatory period. Application of 100 µM muscimol induced a triphasic response with ~20 s excitation, followed by ~20 s inhibition and a long lasting large excitation.



Bar graphs showing the mean (± sd) firing rates during the inhibitory and excitatory phases after agonist application and during the corresponding time period under control conditions. Significant short term decrease and long term increase in mean firing rates occurred after all agonists. However, agonists of IGluRs did not cause as dramatic changes as $GABA_AR$ agonists. However, in 2 of the 6 experiments muscimol did not inhibit the neurons, but had only an excitatory effect.

5. Frequency response analysis

Frequency response analysis was performed on the data during the inhibitory (left) and excitatory (right) periods following application of IGlu and $GABA_A$ receptor agonists or the corresponding section of the control recording. Frequency response functions were fitted by the power law function:

$$G(f) = A\, f^k$$

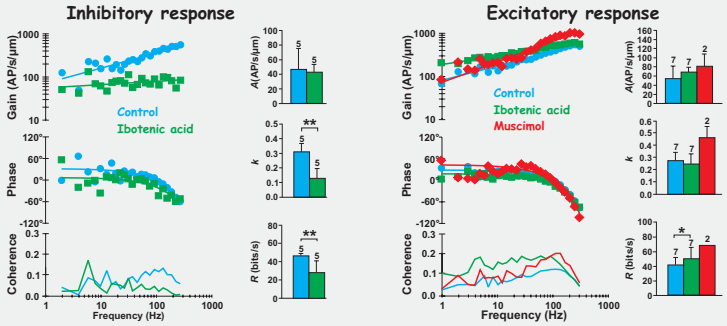
where G(f) is the gain and f is frequency. A is a fitted parameter describing the sensitivity, and k describes the rate of adaptation or the relative sensitivity to higher frequencies. The phase relationship was fitted by:

$$P(f) = k\ 90° - \Delta t\ f\ 360°$$

where P(f) is the phase as a function of frequency and Δt is a time delay.

The coherence function, $\gamma^2(f)$, a measure of linear correlation between input and output signals, allowed us to estimate the linear information capacity R, using the Shannon formula (Shannon and Weaver 1949):

$$R = \int \log_2\{1/(1 - \gamma^2(f))\}df$$



6. Summary and Conclusions

IGlu and structurally related $GABA_A$ receptors are both present in the spider VS-3 mechanosensory neurons. Here, we investigated the short and long term changes in cellular responses to pseudorandom white noise signals produced by IGlu and $GABA_A$ receptor agonists:

- $GABA_AR$ activation caused ~23 mV depolarization and a large reduction in the action potential amplitude while IGluR activation depolarized the neurons only by ~4 mV and caused a significantly smaller reduction in the spike size.

- Activation of IGluRs resulted in initial inhibition followed by a long-lasting excitatory period. Inhibitory and excitatory periods were also seen when the $GABA_AR$ agonist was used, but the inhibitory period was shorter and sometimes missing while the excitatory period was more substantial.

- IGluR activation did not induce statistically significant changes in the sensitivity parameter A, but the fractional exponent k was smaller than control during the inhibitory period. Information capacity (R) decreased during the inhibitory period, but increased during the excitatory period. During the excitatory period $GABA_AR$ activation increased information capacity more significantly and it also increased the fractional exponent k.

These results indicate that the VS-3 neuron frequency sensitivity can be modulated differently by activation of the two receptor types: In the short term, IGluR activation decreases the sensitivity, especially to high frequency signals, while $GABA_AR$ activation may completely inhibit firing. In the long term, $GABA_AR$ activation leads to a significant increase in sensitivity to high frequency signals while IGluR activation increases the sensitivity less but over a wider frequency range.

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