GLUTAMATE ACTS ON INHIBITORY RECEPTORS ON SPIDER PERIPHERAL MECHANORECEPTORS

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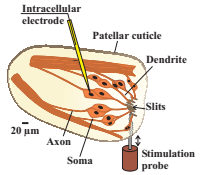
Introduction

Peripheral mechanosensilla of the spider, *Cupiennius salei*, receive a complex efferent innervation onto their sensory neurons. Most of the efferent fibers contain GABA, and the sensory neurons were inhibited by agonists of ionotropic GABA receptors. Some efferents contain octopamine, and the sensory neuron sensitivity increased in response to octopamine. Glutamate immunoreactivity was also present in some efferent fibers. Here, we examined the effects of glutamate on sensory neurons of lyriform VS-3 slit sensilla in the spider patella. These sensilla detect vibrations induced by mates, prey and predators, as well as strains caused by the spider's own muscular activity.

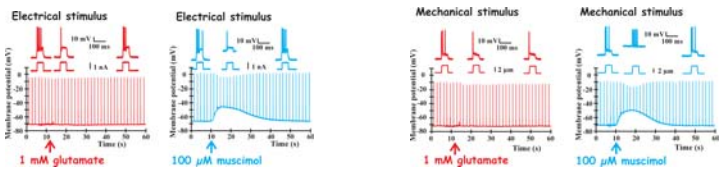
Invertebrates have excitatory and inhibitory glutamate receptors. The inhibitory glutamate receptors (IGluRs) are Cl^--channels (GluCl-channels), phylogenetically related to ionotropic GABA receptors, and to vertebrate glycine receptors. In the present study, we show that the spider VS-3 neurons have an inhibitory response to glutamate and its analog ibotenic acid, and to ivermectin, an antiparasitic drug that paralyzes its target animals. These agonists have previously been shown to act on IGluRs. Using specific antibodies against subunits of GluCl-channels we also investigated the distribution of these receptors on spider leg mechanosensilla.

Preparation



A cuticular preparation of the spider lyriform slit sensillum VS-3. Dendrites of 7 pairs of sensory neurons are attached to the cuticular slits. A sensory neuron was impaled with an intracellular electrode and single electrode current- or voltage-clamp experiments were performed using an SEC-10 L amplifier (NPI Electronic, Germany). Neurons were stimulated electrically, using current pulses via the recording electrode, or mechanically using a piezoelectric stimulator (PZT-Servo controller and P-841.10 Actuator, Physik Instrumente, Germany) that pushed a glass probe against the slits from below. The preparation was continuously superfused (~1 ml/min) with spider saline. Agonists were injected close to the preparation and antagonists were added to the superfusion solution.

Glutamate effects on dendritic excitability

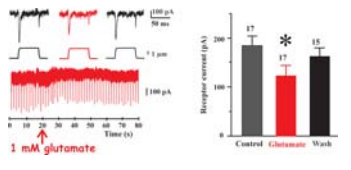


Type of stimulation	Percentage of neurons inhibited	
	500 µM - 1 mM glutamate	25 µM – 1 mM muscimol
Electrical	37% (n = 24)	80% (n = 71)
Mechanical	49% (n = 39)	28% (n = 74)

Glutamate and muscimol effects on an electrically vs. mechanically stimulated Type B neuron. Left: Glutamate application did not change the membrane potential, but the number of action potentials in response to current steps reversibly reduced from 4 to 1. Similar change in firing in response to electrical stimulation was observed when muscimol was applied, but the membrane also depolarized by 21 mV. Right: When the same neuron was stimulated mechanically and glutamate applied, the firing reduced from 3 to 1 action potentials per pulse and this effect lasted for more than 200 s. When muscimol was applied to the mechanically stimulated neuron, it depolarized by 22 mV and the cell fired more action potentials at the crest of depolarization. These results suggest that glutamate inhibits the action potentials initiated in the dendrite as effectively as axonal action potentials. However, muscimol inhibits axonal action potentials, but not those initiated in the dendrites.

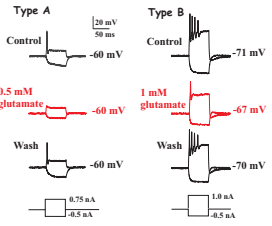
Table 3: Percentages of VS-3 neurons that stopped firing after glutamate or muscimol application when neurons were stimulated electrically vs. mechanically. Recordings were performed at -70 mV.

Glutamate effect on receptor current



Action potential activity was blocked with 1 µM TTX and recordings were performed under voltage-clamp at -70 mV. Left: Lowest trace shows a recording during which the slits were stimulated with mechanical displacements. Responses to single displacement stimuli are shown before, during and after glutamate effect. When glutamate was applied, a small outward current was elicited and the mechanically activated currents became smaller than before glutamate application. Left: Bar graphs showing the mean (±s.e.m.) amplitudes of the receptor current under control conditions, immediately after glutamate application, and ~200 s after glutamate application. The receptor current amplitude was significantly smaller immediately after glutamate application than under control conditions (p = 0.0107, paired t-test).

Glutamate effects on VS-3 neurons

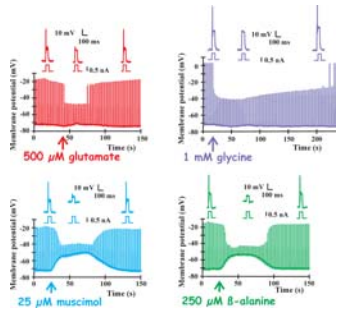


Left: When superfused by normal spider saline a rapidly adapting Type A VS-3 neuron responded to current pulses with one action potential. Glutamate application increased the threshold for action potentials.
Right: Under control conditions this Type B neuron produced a brief burst of action potentials in response to 1 nA stimulus. When glutamate was applied, the neuron depolarized, and the 1 nA stimulus elicited only one action potential.

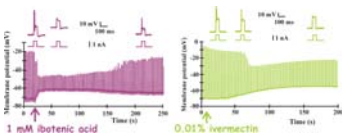
Table 1. Effects of glutamate (0.5-1 mM) on Type A and Type B VS-3 neurons. All neurons were current-clamped at -70 mV. "Inhibition" indicates the percentage of electrically stimulated neurons within each group that stopped firing action potentials in response to glutamate application. ΔV is the membrane potential change in response to glutamate application (mean ± s.d.). Inhibition was not dependent on the cell type (Pearson's Chi square test) and there were no statistical differences in the ΔV values between the two neuron types (Mann-Whitney rank sum test).

Cell Type	Glutamate Effect	
	Inhibition	ΔV (mV)
Type A	73% (n = 33)	+5.2 ± 6.7 (n = 46)
Type B	60% (n = 60)	+4.2 ± 7.6 (n = 101)
p (Type A vs. Type B)	0.2198	0.241

Glutamate and GABA receptor agonist and antagonist effects on VS-3 neurons



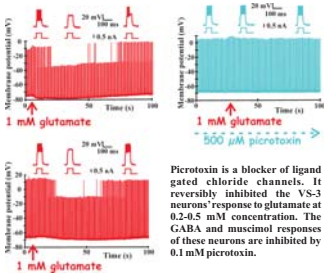
At the resting membrane potential (~-70 mV), both glutamate and glycine inhibited action potentials but had very small effects on membrane potential. When muscimol or β-alanine were applied, the neurons depolarized ~20 mV, membrane resistance decreased and the neurons stopped firing.



The glutamate analog ibotenic acid inhibited this neuron and induced a 10 mV depolarization. Both effects were reversible. Ivermectin, an antiparasitic drug, slowly depolarized the electrically stimulated neuron by 16 mV and the neuron stopped firing. Ivermectin effects did not reverse even after more than 15 min wash in normal spider saline.

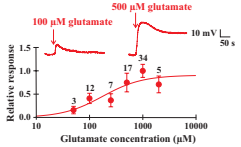
Table 2: Agonist effects on VS-3 neurons. Neurons were current-clamped at -70mV. ΔV indicates the change in membrane potential in response to each agonist (mean ± s.d.). "Inhibition" indicates the percentage of electrically stimulated neurons that stopped firing action potentials in response to each agonist. One-factor factorial variance analysis was used to test whether the inhibitory effects by glutamate or muscimol were correlated with the change in membrane potential. Data from Type A and Type B neurons is pooled in this table.

Agonist (mM)	Effect		
	ΔV (mV)	Inhibition	p
Glutamate (0.5 - 1)	5.1 ± 8.7 (n = 108)	74% (n = 89)	0.9966
Ibotenic acid (1)	6.3 ± 4.9 (n = 13)	69% (n = 13)	
Glycine (1 - 5)	4.5 ± 9.9 (n = 19)	42% (n = 19)	
GABA (0.2 - 1)	23.4 ± 12.6 (n = 17)	94% (n = 17)	
Muscimol (0.025 - 1)	20.6 ± 9.6 (n = 46)	78% (n = 23)	0.0001
β-Alanine (0.1 - 1)	19.8 ± 11.1 (n = 12)	75% (n = 12)	
NMDA (1- 5)	0 ± 0 (n = 6)	0% (n = 6)	
AMPA (0.1 - 1)	0.8 ± 2.8 (n = 20)	0% (n = 19)	



Picrotoxin is a blocker of ligand gated chloride channels. It reversibly inhibited the VS-3 neurons' response to glutamate at 0.2-0.5 mM concentration. The GABA and muscimol responses of these neurons are inhibited by 0.1 mM picrotoxin.

Dose-response relationship

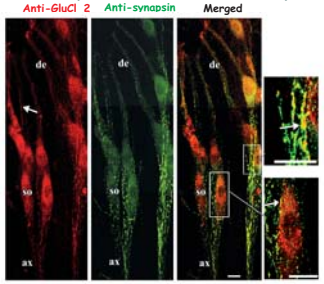


Membrane potential changes in response to glutamate were recorded when cells were current-clamped to -90 mV. The peak responses were normalized and plotted against glutamate concentration (mean ± s.e.m.). The data were fitted with the logistic Hill equation:

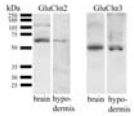
$$(Y = Y_{max}\,[C]^n / ([C]^n + [EC50]^n)$$

where Y is the response, Y_{max} is maximal response, $[C]$ is agonist concentration, $[EC50]$ is the half maximal effective concentration and n is the Hill coefficient. The $[EC50]$ was 176 µM and the Hill coefficient 1.2.

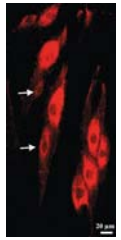
Expression of DmGluClα receptor subunits in spider tissue



Confocal images of two dimensional projections of a whole-mount preparation of VS-3 organ double labeled with antibodies against GluClα2 and synapsin. Immunoreactive clusters against GluClα2 were present mainly in the somata (so) of the sensory neurons. Some clusters were also present in the axons (ax) and dendrites (de, arrow). The anti-synapsin antibody has been shown to label the synaptic vesicles in the efferent fibers. It was used here to see if GluClα2 immunoreactivity was also present in the efferent neurons. Merged image and the two insets demonstrate that GluClα2 and synapsin labeled some of the same structures (arrows in the insets). Therefore, the GluCl-channels are present in all parts of the sensory neurons and also in some of the efferent fibers surrounding the sensory neurons. Scale bars in all images 20 µm.



Western blots of spider brain and hypodermis tissue extracts with antibodies against two peptide sequences of *Drosophila* GluClα channel subunit. The α2 antibody recognized bands at about 60 kDa and the α3 identified bands at 50 kDa.



Confocal image of a two-dimesional projection of a whole-mount preparation of VS-3 organ labeled with an antibody against GluClα3 peptide. While most somata were strongly labeled with this antibody, some had significantly less labeling (arrows). Similar differences in intensity between different cells were detected randomly in most preparations with both GluClα antibodies.

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Summary

- Glutamate inhibited the VS-3 neurons without the strong depolarization that occurred when GABA or muscimol was applied, suggesting that these agonists act on a different group of receptors.
- Glutamate inhibited both the axonal action potentials elicited by electrical stimulation, and dendritic action potentials produced by mechanical stimulation, while muscimol only inhibited the axonal action potentials.
- Antibodies against two different GluClα subunits labeled clusters in all parts of the sensory neurons. Some labeling was also detected in the efferent fibers.
- The inhibitory glutamate receptors in the VS-3 sensilla are distinct and differently distributed than the GABA receptors, providing subtle control of the neurons' sensitivity in varying behavioral situations.