Uptake of Nanoparticles in the Olfactory System and Transport to the Brain in Locust

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Abstract and Introduction:

The olfactory nerve, which projects directly from the nasal epithelium to the brain, is the shortest and most direct route to the brain. It has been suggested that nanoparticles deposited in the nasal epithelium are capable of being uptaken into the neurons and transported to the central nervous system [1]. This olfactory route is of particular concern as it completely bypasses the blood-brain barrier, responsible for protecting the brain from foreign materials. The growing use of nanoparticles in consumer and industrial products also underscores the importance of understanding how nanoparticles may gain access to the central nervous system.

We expect nanoparticle transport to the brain to begin with the uptake of nanoparticles by either receptor mediated or non-receptor mediated endocytosis. While the former requires interaction with surface proteins, the latter occurs randomly as the cell samples material from its extracellular environment. Once inside the cell, nanoparticles bind to "motor" proteins, which move along a microtubule system spanning the entire neuron [2]. While this is normally responsible for transporting important cellular proteins and organelles, it could also be used by nanoparticles to quickly travel to the brain.

It is unknown what impact nanoparticles may have on neuron activity once they reach the brain. To characterize this, we used electrophysiology tests to measure what effect a nanoparticle injection in a sample's brain had on its ability to perceive odors. We also used transmission electron microscopy (TEM) to determine the cellular localization of nanoparticles.

Experimental Procedure:

Due to its relatively simple nervous system and established role in olfaction studies [3], locust (*Schistocerca americana*) was chosen as the experimental organism. It was prepared for a nanoparticle injection by exposing the brain and carefully removing excess tissue. A controlled air/hexanol mixture was passed over one of the locust's antennae and its nervous response was measured by a gold-plated recording electrode placed in its antenna lobe as shown in Figure 1.

A gold nanoparticle (5 nm) suspension previously produced by the reduction of $HAuCl_4$ with $NaBH_4$ (Figure 2) was injected into the antenna lobe by a glass injection pipette. This was controlled by a pneumatic picopump to ensure consistent delivery of the odorant and injection of the nanoparticle suspension. A total of five trials were run before the nanoparticle injection, two were taken during the injection and five more were taken after the injection.

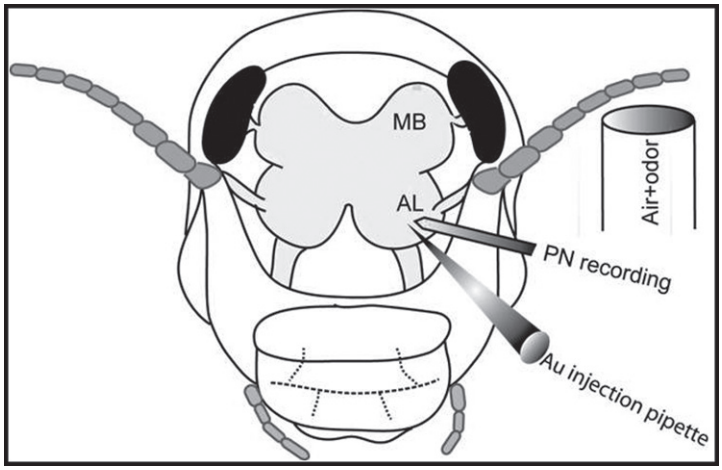

Figure 1: Experimental setup.

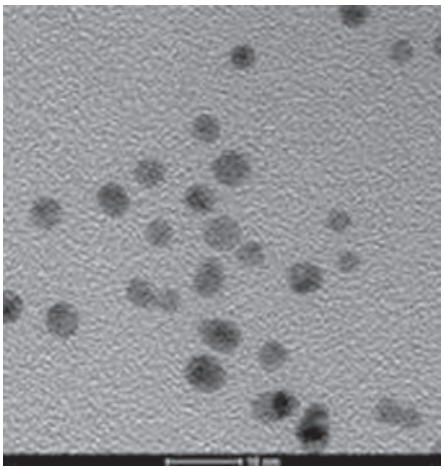

Figure 2: TEM of gold nanoparticles.

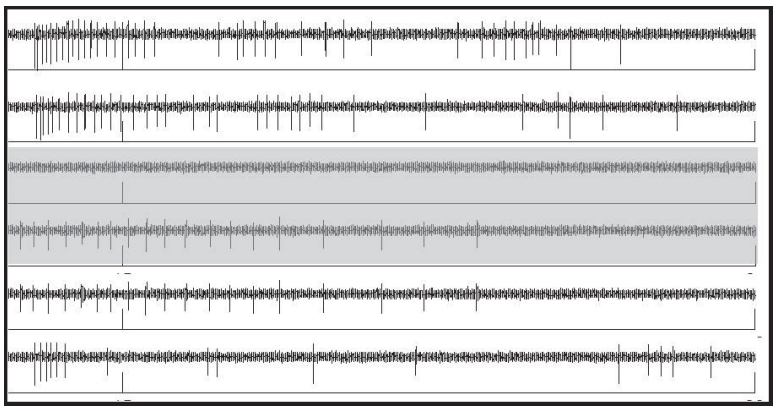

Figure 3, above: Electrophysiology results.

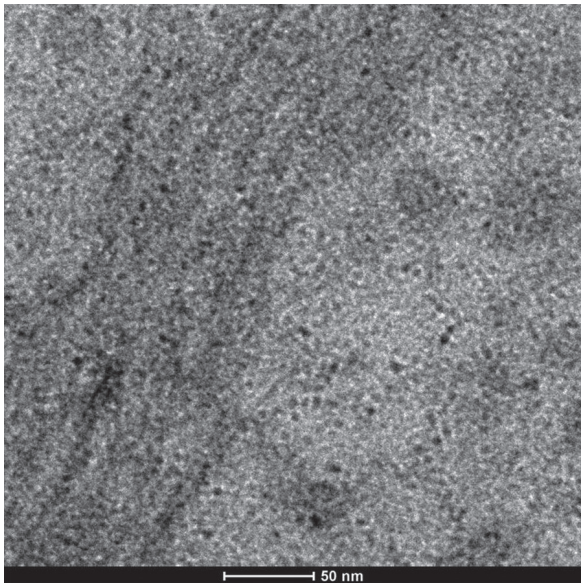

Figure 4, right: TEM of locust brain.

At the conclusion of the electrophysiology experiment, the brain was recovered and immediately fixed using glutaraldehyde to preserve the sample. A secondary fixative of osmium tetroxide was used to prevent lipid degradation as well as to add contrast to TEM images. After drying with ethanol and propylene oxide, the sample was embedded using Eponate 12. Thin (75 nm) sections were then cut using a diamond knife to prepare the sample for TEM.

Results and Conclusions:

Simplified results for the electrophysiology experiment are presented in Figure 3. Two pre-exposure trials, two injection trials (highlighted) and two post-exposure trials are shown after the odorant was discontinued. Each is a function of voltage versus time, with spikes corresponding to neurons firing. Interestingly, some gold nanoparticles themselves appeared to cause a nervous response as spikes can be seen after their injection. There was also a clear drop in neuron activity after these injections, as the frequency of neuron firing was diminished. Pending a control experiment with a saline injection, this supports our claim that nanoparticles can affect neuron activity.

Figure 4 shows highly contrasted, spherical particles of diameter ~ 5 nm, which is consistent with the characteristics of our gold nanoparticles. TEM appears to have verified the presence of gold nanoparticles inside brain cells and suggests that they are capable of crossing the synapse, shown running from the bottom left to the middle top of the image. Since this is where electrical signals are converted to chemical signals, this may be where interference is produced.

Future Work:

Our results suggest that nanoparticles are able to interfere with normal neuron firing when injected into the brain. However, this is an unlikely exposure route. To better model realistic exposure methods, this experiment should be repeated with nanoparticles delivered via an aerosol route. This could be accomplished by using an electrospray system to deliver nanoparticles to the locust's antennae.

Since surface charge is primarily responsible for nanoparticle interaction with proteins, repeating these trials with varying charged nanoparticles would allow for better characterization of relevant factors for nanoparticle interference with neuron activity. Other relevant properties worth studying include size and crystal phase.

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References:

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[2] Brown, A.; "Axonal Transport of Membranous and Nonmembranous Cargoes: A Unified Perspective"; The Journal of Cell Biology, Vol. 160 (6); pp. 817-821 (2003).

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