Neurobehavioral Effect of Nanosilver in Adult Male Offsprings

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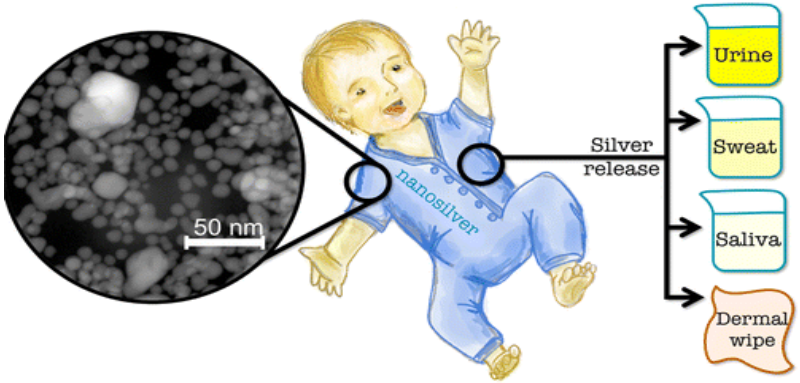
Why Silver?

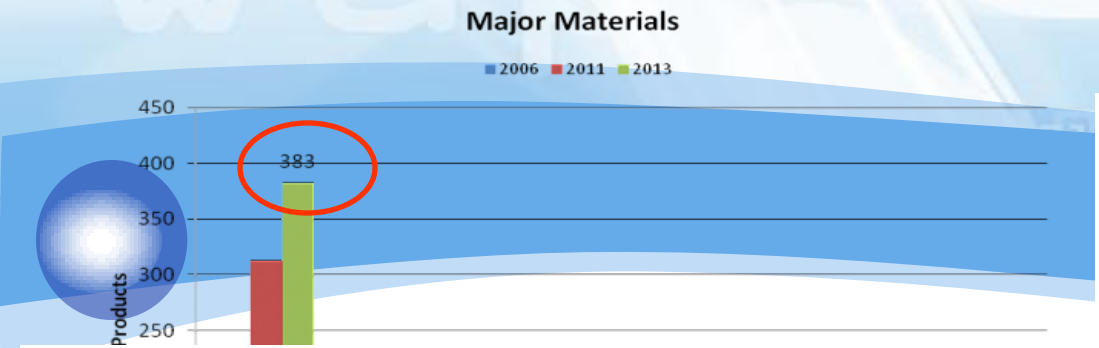
- Effectiveness in killing a wide range of bacteria
  - Including some of the strains that have proven resistant to modern antibiotics
- Can be readily incorporated into plastics, fabrics and onto surfaces
- Delivers toxic silver ions (not safe silver metal) in large doses directly to sites where they most effectively attack microbes

- Cloths (Underwear, Socks)
- Tooth pastes
- Washing machines
- Cosmetic materials

(nanosilver coated dressings)

- Baby bottles





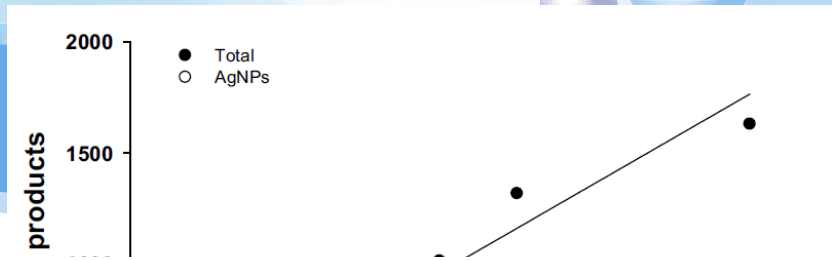
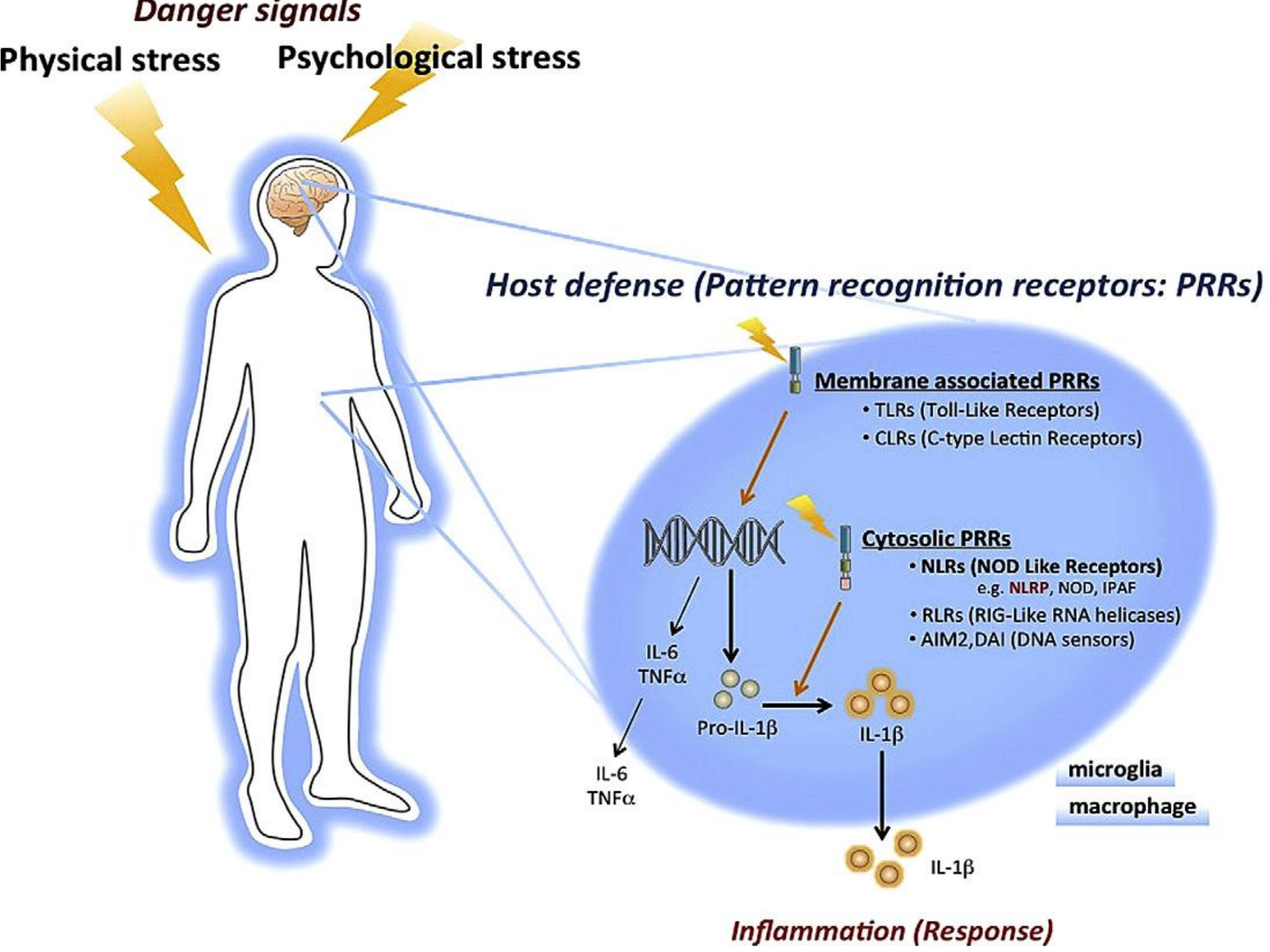


Table 1 – Predicted environmental concentrations (PECs) of AgNPs. The PECs in surface water, sediment, and sludge-treated soil are presented by year based on the increase in AgNP-containing consumer products and AgNP production. See footnotes as well as Sections 2 and 3 for more details.

Year	Number of products[a]	AgNP production worldwide (t/a)[b]	PEC surface water (ng/L)[c]	PEC sediment (μg/kg/a)[c]	PEC surface water (ng/L)[d]	PEC sediment (mg/kg/a)[d]	Sludge-treated soil (μg/kg/a)[c]
2006	**25**	0.4–46	0.012–0.35	0.020–1.35	7–59	0.4–2.6	0.070–0.32
2007	84	1.3–133	0.039–1.17	0.068–4.53	25–197	1.2–8.6	0.234–1.06
2008	136	2.2–216	0.064–1.90	0.111–7.37	**40–320**	**2.0–14.0**	0.381–1.72
2009	188	3.0–300	**0.088–2.63**	**0.153–10.18**	55–443	2.8–19.4	**0.526–2.38**
2010	241	3.8–383	0.113–3.37	0.196–13.04	71–567	3.5–24.8	0.674–3.05
2011	**313**	5.0–498	0.147–4.38	0.255–16.95	92–736	4.6–32.2	0.876–3.96
2012	346	**5.5–550**	0.162–4.84	0.281–18.71	102–813	5.1–35.6	0.967–4.38
2013	**383**	6.1–609	0.179–5.36	0.312–20.74	113–901	5.6–39.4	1.072–4.85
2014	450	7.2–716	0.211–6.30	0.367–24.39	132–1060	6.6–46.4	1.260–5.70
2015	503	8.0–799	0.235–7.03	0.409–27.22	148–1183	7.4–51.8	1.407–6.36
2016	555	8.8–882	0.260–7.77	0.452–30.06	163–1306	8.2–57.1	1.553–7.03
2017	608	9.7–966	0.284–8.50	0.452–32.90	179–1430	8.9–62.5	1.700–7.69
2018	660	10.5–1049	0.309–9.23	0.537–35.73	194–1553	9.7–67.9	1.846–8.35
2019	712	11.3–1132	0.333–9.96	0.580–38.57	210–1676	10.5–73.3	1.993–9.02
2020	765	12.2–1216	0.358–10.70	0.622–41.41	225–1799	11.2–78.7	2.140–9.68
Sum[e]	na	94.9–9488	na	4.857–323.16	na	87.8–614.4	16.698–75.55


Danger signals
Physical stress
Psychological stress
Host defense (Pattern recognition receptors: PRRs)
Membrane associated PRRs
• TLRs (Toll-Like Receptors)
• CLRs (C-type Lectin Receptors)
Cytosolic PRRs
• NLRs (NOD Like Receptors)
e.g. NLRP, NOD, IPAF
• RLRs (RIG-Like RNA helicases)
• AIM2,DAI (DNA sensors)
IL-6
TNFα
Pro-IL-1β
IL-1β
IL-6
TNFα
microglia
macrophage
IL-1β
Inflammation (Response)

Prenatal → Early life → Adulthood

P1

Mitochondrial

Gene expression

Ag $^{+}$detection

P60

Behavioral

Pathology

Different Experiments and Different Mice Life Cycle

- Gestation period (19-22 days)
- Birth (P1)
- Adulthood (P60)

- Prenatal **exposure** to Ag-NPs (G1 to Birth)
- Orally (oral gavage)
- Two size: 10 and 30 nm

P0 (newly-born**) - bright red**

P1 (one day old**)– pink**

Mitochondrial Function and Gene Expression

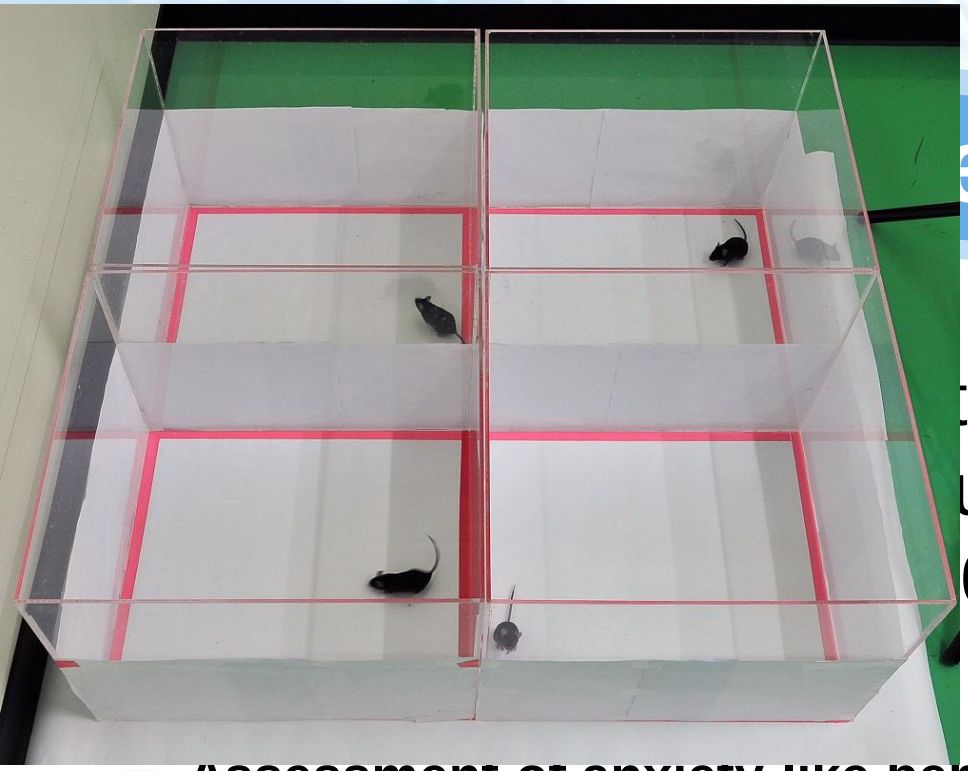
- ROS formation
- MDA levels
- GSH levels
- ATP levels
- Nlrp3
- Toll-like receptors (2,4)
- MYD88
- IL-1β
- IL-6
- TNf-α
- BDNF

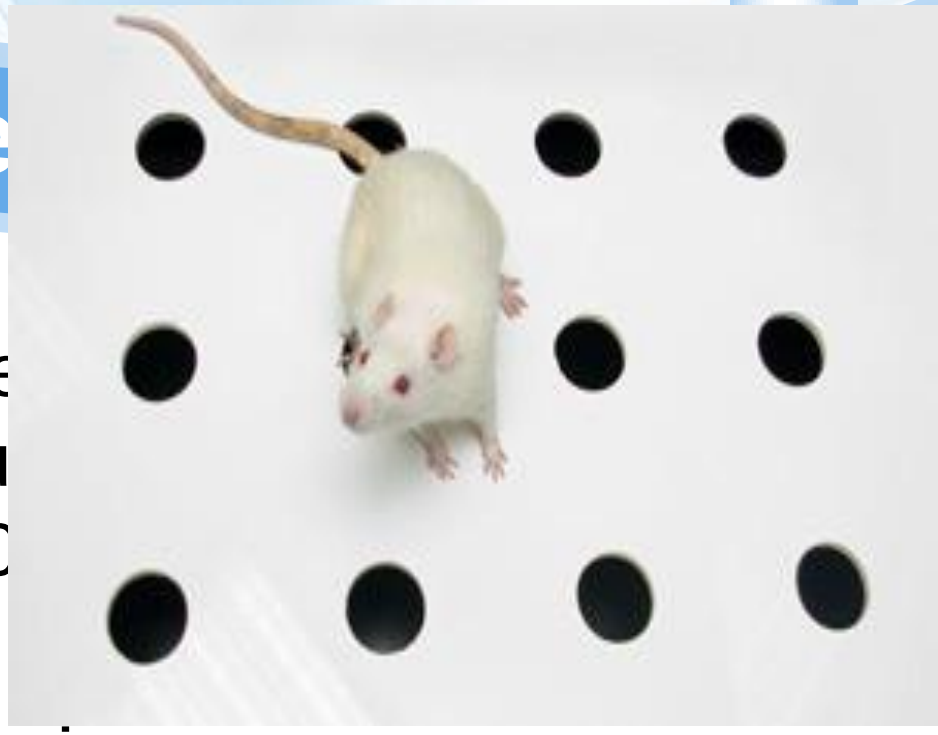
P60 (adulthood)

- Pathology Assessment
- Behavioral tests
  - Depressive-like behavioral
  - Anxiety-like behavioral
  - Locomotion
- Seizure susceptibility
- Memory

Pathology Assessment

- Macroscopic and microscopic study of brain
- Macroscopic (size, color and etc.)
- Microscopic(morphology)

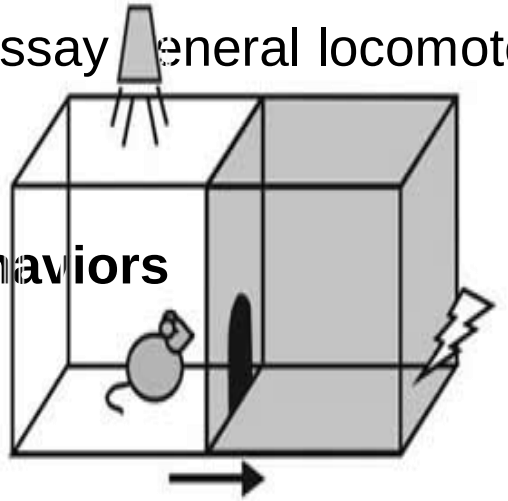




- Assessment of anxiety-like behaviors:

ssay eneral locomotor

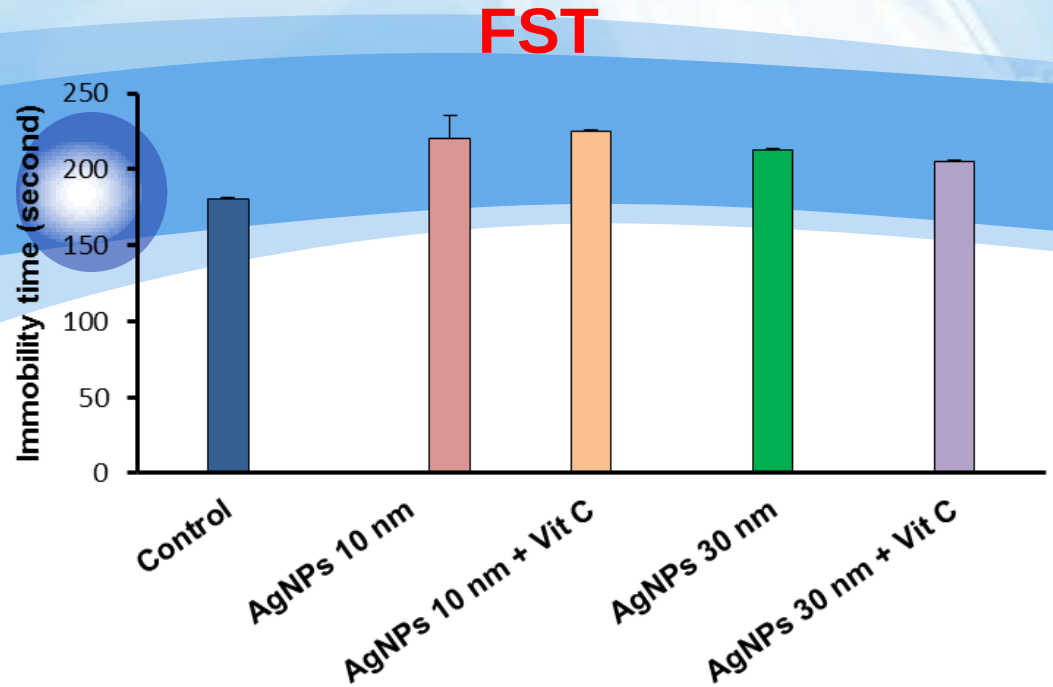
haviors

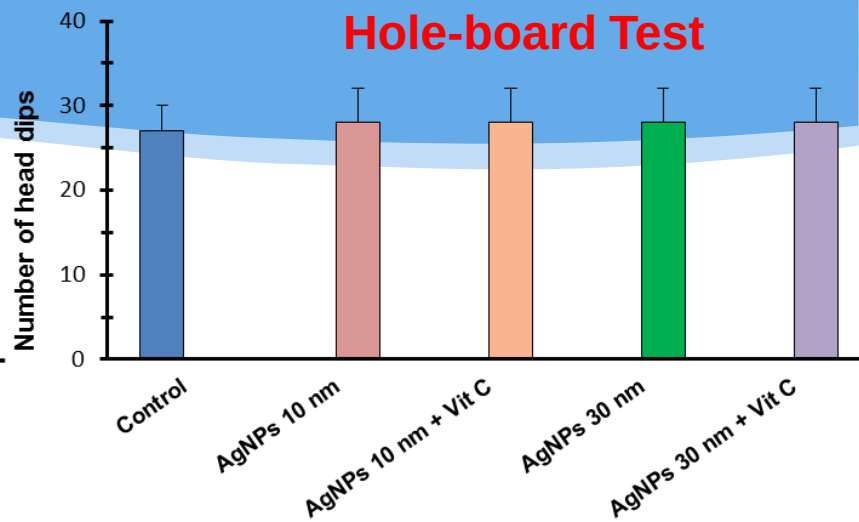


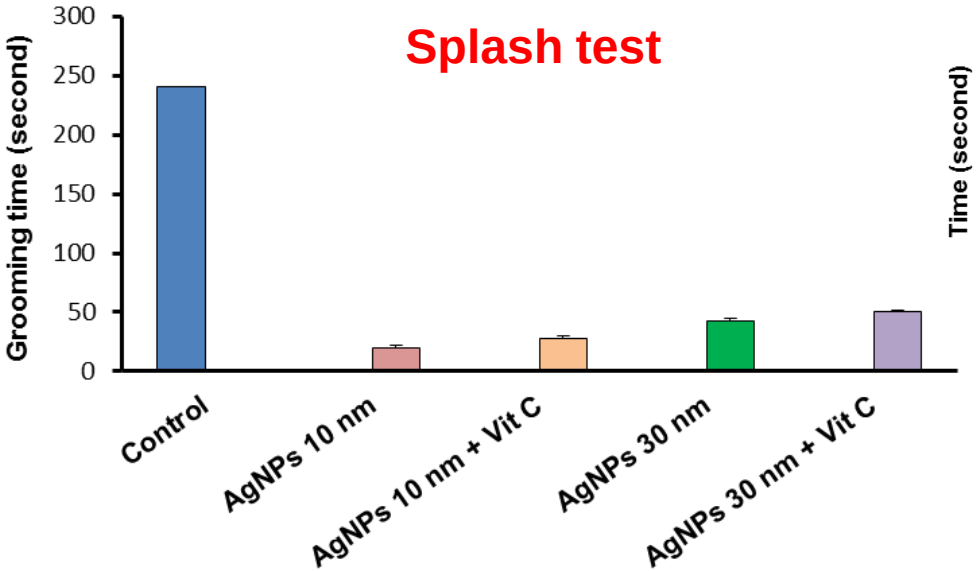
Passive Avoidance

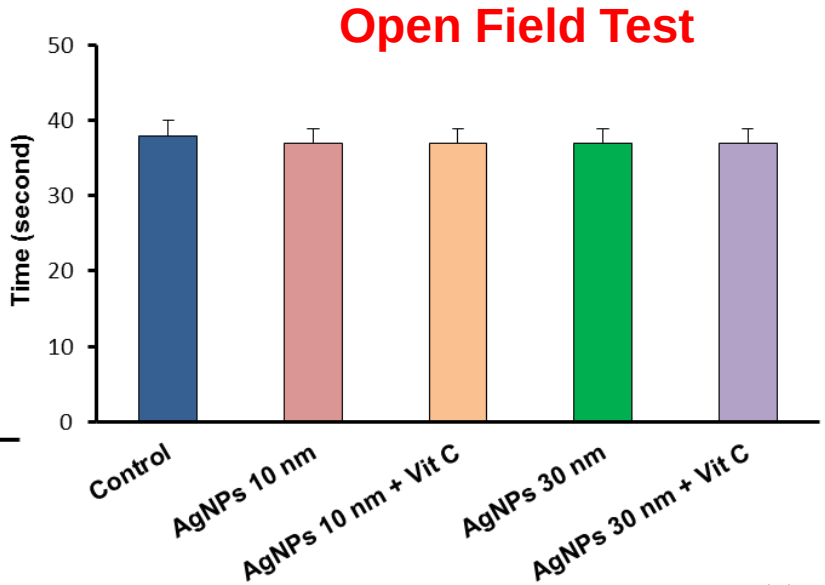
Exploits a natural tendency of mice to enter dark environments.

Unidirectional: mouse goes from light to dark chamber.

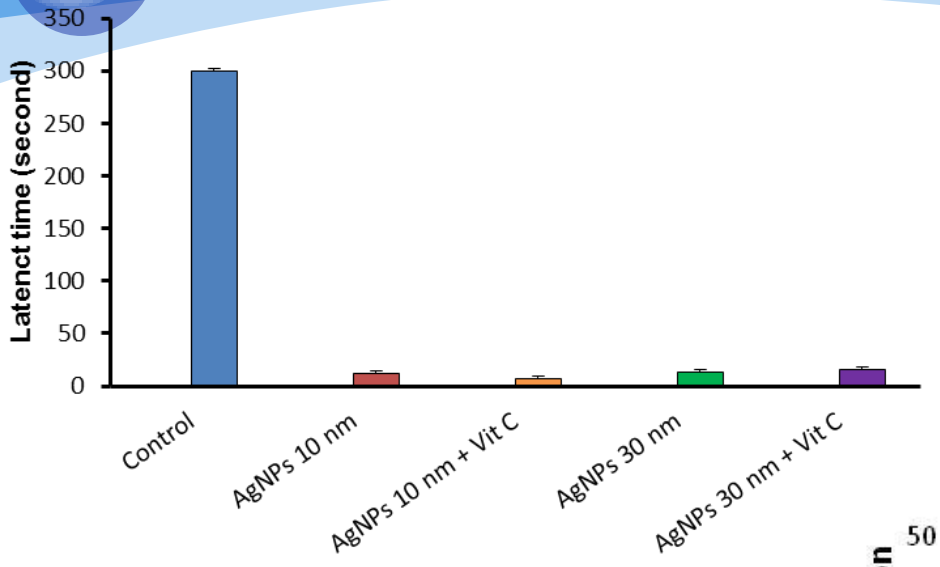

FST
Immobility time (second)
250
200
150
100
50
0
Control
AgNPs 10 nm
AgNPs 10 nm + Vit C
AgNPs 30 nm
AgNPs 30 nm + Vit C


Hole-board Test
Number of head dips
40
30
20
10
0
Control
AgNPs 10 nm
AgNPs 10 nm + Vit C
AgNPs 30 nm
AgNPs 30 nm + Vit C

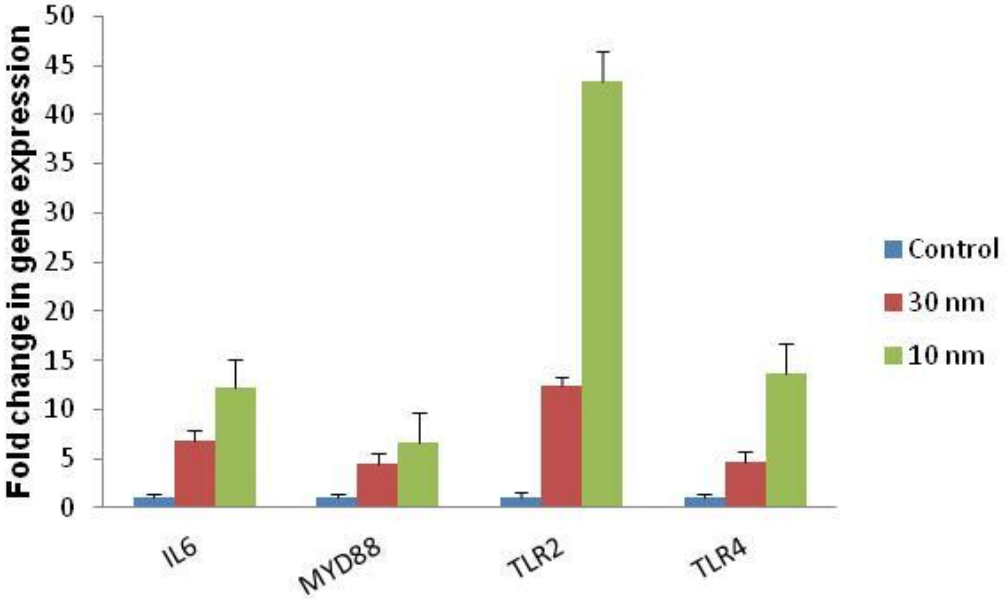

Splash test
Grooming time (second)
300
250
200
150
100
50
0
Control
AgNPs 10 nm
AgNPs 10 nm + Vit C
AgNPs 30 nm
AgNPs 30 nm + Vit C


Open Field Test
Time (second)
50
40
30
20
10
0
Control
AgNPs 10 nm
AgNPs 10 nm + Vit C
AgNPs 30 nm
AgNPs 30 nm + Vit C

Memory



Gene expression



Ag-NPs Detection & Pathology

ICP-MS analysis

Sample	Ag (ppb)	SD (ppb)
30 nm	27	0.09
10 nm	120	0.03

- Dark neurons ↑
- Neuronal density ↓
- Pyramidal layers (thicknesses) ↓
- Morphological changes
  - plasma membrane integrity, cell shrinkage, nuclei were not in the center of the eosinophilic cells

Pathology

- Dark neurons ↑
- Neuronal density ↓
-
- pyramidal layers (thicknesses) ↓
- Morphological changes
  - plasma membrane integrity, cell shrinkage, nuclei were not in the center of the eosinophilic cells

Conclusion

- Prenatal exposure to AgNPs can induce depression like behaviors in the offspring and ascorbic acid can not reverse its toxicity.
- Toxicity of AgNPs are size dependent.
- Warning of pregnant women exposure to AgNPs!!!

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Thank You