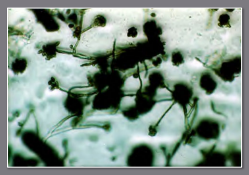

Exposure to environmental mold causes hippocampal inflammation and memory loss

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INTRODUCTION

Evidence is mounting that exposure to mold causes a broad range of health problems, including serious cognitive deficits.

Many American buildings are moldy:
- The common estimate is 40%.
- 34% of homes sampled across the USA contained measurable levels of the toxic black mold *Stachybotrys*.

Individuals who live or work in moldy buildings have multiple cognitive problems:
- 80% of mold-exposed individuals reported CNS-related symptoms, including headaches, difficulty concentrating, confusion, memory loss.
- Mold-exposed patients have difficulties with cognitive tasks of the type mediated by the hippocampus.
- Neurologists could not differentiate between individuals exposed to mold and patients suffering from mild traumatic brain injury.

GOALS:
- Develop a mouse model of the effects of mold exposure on neural and cognitive function.
- Determine what causes the problems—is it a reaction to the spore skeleton or to the toxic chemicals inside the mold spores?

To determine if cognitive problems primarily result from exposure to toxins, proteinases, and other fungal components or to fungal cell wall elements (including beta glucan and mannose), we compared the effects of 1) intact spores, 2) spores which had had their toxins removed and proteins denatured, and 3) treatment with vehicle alone.

HYPOTHESIS

Mold inhalation causes cytokine release by the lungs. This is communicated to the brain and ultimately activates hippocampal microglia, resulting in deficits in spatial and contextual memory that depend on hippocampal function.

METHODS

ANIMALS AND MOLD TREATMENT: Male C57Bl/6 mice were purchased from Jackson Labs (Bar Harbor, ME). Characterized JS 58-17 *Stachybotrys* toxin-producing spores originally isolated from a house in Ohio were generously provided by Dr. Dorr Dearborn, Case Western University. *Stachybotrys* is probably the best studied of the damp building molds.

Acclimation to the laboratory.

↓

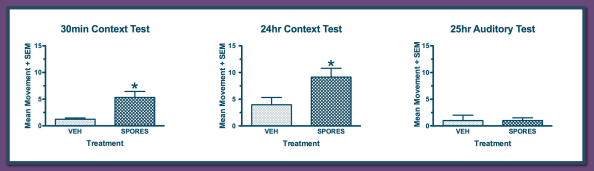
Mice anesthetized with isofluorane and nasally instilled three times per week with either freshly-prepared:

Non-pyrogenic saline vehicle (VEH) | **Extracted *Stachybotrys* spores (EX)*** | **Intact *Stachybotrys* spores (IN)**

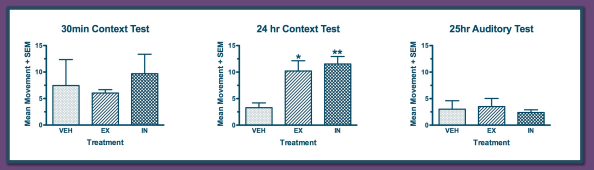
*Extracted spores were treated twice with alcohol to remove toxins and denature proteins.

RESULTS

Mold spores cause deficits in contextual, but not auditory memory

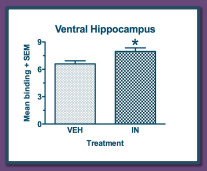


Low spore dose. After three weeks of treatment (9 instillations with 4,000 spores/g body weight), males treated with intact and extracted spores (two groups combined) moved significantly more than vehicle-treated males during both the 30min and 24hr tests of contextual memory ($P = 0.025, 0.042$ respectively). However, spore-treated males showed no deficits in auditory memory when tested 25hr after training. They froze in response to the tone, just like the vehicle-treated males.



Moderate spore dose. After three weeks of treatment (9 instillations with 15,000 spores/g body weight), both males treated with extracted spores (EX) and males treated with intact spores (IN) moved significantly more than vehicle-treated males (VEH) during the 24hr test of contextual memory ($P < 0.05, 0.01$ respectively). However, spore-treated males showed no deficits in auditory memory when tested 25hr after training. They froze in response to the tone, just like the vehicle-treated males.

Mold exposure increases activated microglia in the hippocampus



Activated Microglia were assessed in brain tissue from mice treated for 5 weeks (15 instillations) with saline vehicle or the low dose of intact spores using standard *in vitro* autoradiographic techniques. Spore-treated mice had significantly higher levels of specific binding (nCi/mg, derived from tritium standards) to [3H] PK11195, an established marker of microgliosis and neuroinflammation, ($P < 0.05$).

BEHAVIORAL TESTING

We utilized a conditioned fear task (Kinder Scientific, San Diego, CA) that quantifies effects on hippocampal (contextual) and non-hippocampal (auditory) memory. Mice were tested individually, and the apparatus was thoroughly cleaned with Conflikt Disinfectant, alcohol, and water to prevent cross contamination.

Training
A distinctive wallpaper was placed on the apparatus. A mouse was placed in the box for 2min. A tone then sounded for 30sec. During the last 2sec, the mouse was shocked. The mouse's movements were recorded for an additional 1min.

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Context Test (30 minutes)
Mice were placed back in the apparatus with the same wallpaper but no tone, no shock and their movements quantified for 5min.

↓

Context Test (24 hours)
Mice were placed back in the apparatus with the same wallpaper but no tone, no shock and their movements quantified for 5min.

↓

Auditory Test (25 hours)
The wallpaper was changed, the grid floor covered with plastic. This test mirrored the training trial, except that no shock was given during the tone.

DISCUSSION

As predicted, mold-exposed mice showed significant deficits in hippocampal-dependent contextual memory compared to vehicle-treated controls, but no deficits in auditory memory. Treatment with the low dose of intact *Stachybotrys* spores increased microglial activation in the hippocampus. Thus, the effects of mold inhalation appear to parallel the effects of bacterial infection. The hippocampus appears particularly vulnerable, and hippocampal-dependent memory is impaired in mold-exposed animals.

We expected intact spores to cause greater cognitive deficits than extracted spores. However, in all experiments we have run, extracted spores caused the same deficits as intact spores. This is probably attributable to the relatively low doses. The fact that spore skeletons can cause significant cognitive deficits suggests that exposure to many other molds, not just the particularly toxic strains, may prove problematic.

Approximately 25% of Americans have MHC genes that make them susceptible to long-term health problems following mold exposure. Unlike most people, these individuals show persistent and sometimes worsening health/cognitive problems after mold exposure ends. They are susceptible to autoimmune problems following mold exposure, including antibodies to myelin basic protein, cardiolipin, and gliadin. Many individuals with autoimmune problems may be suffering from undiagnosed mold susceptibility.

Understanding the neural mechanisms mediating mold's effects on neural function and cognition will allow the development of more rational treatments for these serious problems.

MOLD-SUSCEPTIBLE MHC HAPLOTYPES

	DRB1	DQ	DRB3	DRB4	DRB5
MULTI*	4	3		53	
	11/12	3	52B		
	14	5	52B		
MOLD	7	2/3		53	
	13	6	52A,B,C		
	17	2	52A		
	18	4	52A		

Ritchie Shoemaker, Surviving Mold, Otter Bay Books, Baltimore, MD 2010

*Multi-susceptible – mold, chronic Lyme disease, chronic chemical sensitivity

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