An alternative treatment of diabetes with Ganoderma lucidum: a case report

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Caging wild foraging rats decreases marker proteins of synaptogenesis in the brain
or
Confining wild forage rats decreases marker proteins of synaptogenesis in the brain

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Abstract

Foraging, the search strategy for food, is considered a learning process in animals because it involves neurobehavioral plasticity. Confining foraging rats in home cages limits their navigational ability, which is mainly based on spatial memory formed in the hippocampus. To investigate whether restraining foraging rats decreases their synaptogenesis-related proteins in the brain, we trapped and housed wild rats (F1, first generation) in our laboratory cages. On day 0 and day 60 after cage housing, some of the male F1 rats were sacrificed and their corticohippocampal brain tissues cryopreserved. In addition, some F1 male-female pairs were cohabited to breed a 2nd generation (F2). The F2 offspring were sacrificed at 20 weeks of age. SNAP-25, TrKB, PSD-95, VAChAT, C-fos, BDNF, TGF levels were determined by standard ELISA methods. Levels of these marker proteins were significantly reduced from day 0 to day 60. Also, the levels of these proteins in the corticohippocampal tissues of F2 rats decreased significantly compared to those from day 0 to day 60 of F1 rats. Our result clearly demonstrates that homing of foraging animals in cages reduces synaptogenesis-related proteins in the brain that are crucial for better neural network and neurobehavioural plasticity, and thus may inhibit their navigational/spatial memory.

Key words: Homing, Caging, Synaptogenesis, C-fos,VAChT, TGF, TrKB, BDNF, SNAP-25, PSDN-95, Foraging, Wild rats

Introduction

The background of this investigation is very interesting and funny. We, faculty members begun lunch in a room in our department with food prepared by a cooker. So we don't have to go out for our own lunch. This saved our time and left us more time for research and professional activities. It is worth noting that the ancestors of today's humans once had to navigate(travel) constantly, wasting all of the day's time only in search of food sources. The ancient people's ability to grow crops and vegetables and domesticate goats, pigs, sheep and cattle transformed their lives and led them to live in families at home. Interestingly, at present day, the people whose occupations/activities are more related to navigation than most other people have shown a larger spatial/navigational memory and larger hippocampal size, for example, London city taxi drivers (*). Larger hippocampal volumes are positively correlated with higher verbal learning and memory scores in aged population (), (*), children and adoloscents (*). Rats displayed a performance deficits on hippocampus-dependent tasks due to hippocampal volume reduction (*).Also, animals with larger brain sizes are expected to learn better.[5] A higher ability to innovate has been linked to larger forebrain sizes in North American and British Isle birds [6]

As the natural food sources in their fields are dwindling day by, alien rodents such as wild rats invade human communities: one such event happened in the dining room of our department. The

presence of some pellets of rat feces on the dining room floor was claimed that our experimental rats came out of the animal house in search of food and defecated here. It is notoriously true that the life science researchers/scientists, those who are not specifically using rats or mice, hate these animals the most because they doubt that the lab animals can carry pathogenic bacteria, fleas, ticks, and other parasites that can be harmful to humans. Finally, we had to remove our animal facility from the department and took them to a far away room. Despite this, the rat droppings in the dining room were not stopped. In order to search for the truth, we installed rodent traps and started catching the culprits. In daylight we discovered that they were feral rats that normally make a living from their spatial cognition and smell function, evidently from the surrounding jungle as no nests were available in or near the dining room. At one hand, this relieved us of illegal jurisdiction over the nocturnal presence of our own lab rats in the dining room, on the other hand, this led us to believe that these feral rats frequently foraged to the fourth floor of our department in search of food, and that the rats' spatial/navigational memory might have been stabilized. Otherwise, the rats could have not travelled (foraged) to ~400 feet distances and reach our dining room. Since scientific inquiry is a process of discovering new information and answering how and why, we thought that if wild rats were confined to home cages, protein levels involved in spatial memory, such as SNAP-25, PSD- 95, TrKB and others are changed?

The foraging annovation of the rats also depnend on the smelling capability, which in turn depends on olfactory memory. Of note, larger volumes of hippocampus and associted structures including, amaygdala and parahippocampal cortex, which are conducive to higher synaptogenesis and neuronal activity, are correlated with better olfactory functions (*).

When young rats wean and start to eat solid foods, they forage where the adult rats forage (Galef 1971, 1981; Galef and Clark 1971a, b) or where adults have previously scent-marked (Galef and Beck 1985, Galef and Heiber 1976, Laland and Plotkin 1991, 1993). Their food choices are influenced by social interactions. The follower rats smell foods on the fur, whiskers and especially the breath of other rats and strongly prefer the foods those rats had previously eaten. The relevant chemical cue may be carbon disulfide (CS2) of the rotten food residuals, which is present in the adults rats' breath. Rats strongly prefer the food swabbed with CS2 (Galef, Mason, Preti and Bean 1988). These characteristics of the rats might have significant roles in the continous foraging and subsequent dropping of feces in our dining room despite we removed our own lab rats to a far distant room and allowed us to capture more foraged rats for the purpose of our experiments. Therefore, we further bred a 2nd generation (F2) wild colony by mating the male/female F1 wild rats and allowed us to compare the levels of synaptogenesis-related marker proteins of F2 rats to compare to those of their parental F1 rats. Eventually, while the experiment was fun, the findings swere consistent with the notion that confining animals reduces synaptogenesis-related proteins and thus their spatial memory.

METHODS AND MATERIALS

Animals

After capture, six of the male rats (F1, 1st generation) were sacrificed immediately on laboratory day 0 (caging day 0). To compare whether protein levels decrease due to home cage confinement, six male F1 rats were sacrificed on day 60 after cage housing. Also, six male and six female wild rats (F1) were weight-matched and caged in pairs and allowed to mate to

produce 2nd generation (F2) wild rats. After birth, the F2 rats were allowed to grow up in the cage for 4 months. After that they were sacrificed. All brain hippocampal and cortical tissues were dissected and stored at -20 (C) until use. All F1 and F2 rats were housed at 25°C ± 2°C under 12 hour dark-light cycles in standard steel cages (bedding was wood chips). All rats were allowed free access to basal food and tap water. The rats in this study were cared for and sacrificed in accordance with ethical standards approved by the Bangladesh Association for Laboratory Animal Science.

Fig 1. Pictures of the department.

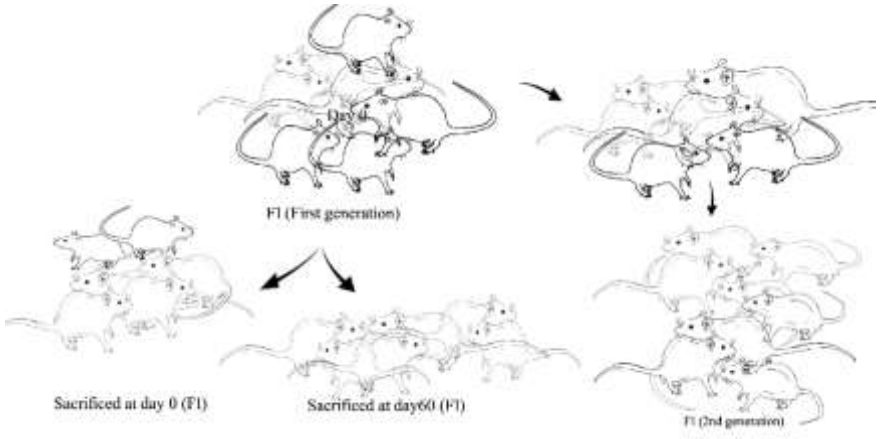

Fig 2. Experimental design

Experimental animal and maintenance

Sixteen ~22 weeks old in-bred Long Evan rats (180-210g) were used in the present experiment. The rats were housed in plastic cages under controlled conditions of 12-h dark-light cycle (light: 8 am - 8 pm; dark: 8 pm - 8 am) and maintained by balanced diet. The rats were divided into two groups: control group and test group. Test group were orally administered hot water extract of Pentaplus at a dose of 10 ml (20% w/v)/ kg body weight daily whereas control group rats received normal water orally. All the rats in this study were cared for and sacrificed in accordance with the ethical norms approved by Bangladesh Association for Laboratory Animal Science.

Behavioral activities of the rats

A field test was not possible for either the F1 or the F2 rat. They were very afraid of the experimenters. The F2 rats showed the same behavior. Even when they watched the people, they just jumped into the cages at random.

Separation of the brain samples

After overnight fast, the rats were sacrificed under light anaesthetia (100 mg kitamin/kg body weight) and blood was collected into a heparinized tubes. The brains were perfused with ice-cold saline. Afterwards, the hippocampus and the cortex were separated and stored at -20 °C until uses. On the day of ELISA assay, the brain tissues were homogenized (10 mg/ml) in phosphate buffer (50 mM, pH 7.4) using Dounce glass homogenizer and centrifuged at 500 × g to remove unruptured tissues. The resulting supernatants were assigned as whole homogenates. The whole homogemnates were again centrifuged at 12000 × g for 30 min to separate the supernatants (cytosolic fractions). Samples were immediately subjected to the assays and/or stored at −20 ºC.

ELISA assays

ELISA was performed as described previously (Hashimoto et al., 2008) with minor modifications. In brief, ELISA microplates (Costar® 3590, Corning incorporated, NY, USA) were coated with samples (40 μg of protein) in 0.1 M carbonate buffer (pH 9.6) and then blocked with 3% bovine serum albumin in PBS. The primary antibody used was in this study rabbit anti-PSD-95 (Life Technologies, NY, USA). After blocking, primary antibody was added at a dilution of 1:1000 and incubated for 1 h at 37 ºC. HRP-conjugated anti-rabbit or mouse IgG antibodies (Biosource International, Inc., Camarillo, CA, USA) was used as secondary antibody and was incubated for 2 h at room temperature before the addition of tetramethylbenzidine (Life Technologies, NY, USA) substrate. The reaction was stopped by the addition of 0.1N HCl after incubation for 30 min at room temperature. Wells coated with 0.1 M carbonate buffer (pH 9.6) alone were used as blanks. The plates were analyzed with a multiwall plate reader (DTX 880 multimode detector, Beckman Coulter, Inc., USA) at 450 nm.

Statistical analysis

The results are expressed as mean ± SEM (standard error of mean). All parameters for inter-group differences were analyzed by one-way ANOVA, followed by Fisher's protected least square differences (PLSD) for post hoc comparisons. Correlation was evaluated by simple linear regression analysis. The statistical programs used were StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA) and GRAPHPAD PRISM® (version 5.00; GraphPad Software Inc., San Diego, CA, USA). A level of $P < 0.05$ was considered as statistically significant.

Yet to be finished