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# An Experiment Design for Investigations on Possible Mutations for Autistic Savant Syndrome

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## Abstract

**Autism spectrum disorder can be diagnosed by a series of genetic mutations and it is possible that Savant syndrome, a neurodevelopmental disorder, is also triggered by certain mutations. Among those mutations, specific ones may be related to a particular prodigious talent. Specific mutations may be identified via DNA sequencing from individuals who are separated into groups by talents. Similar mutations may exist showing certain ones contribute to the talents. To certify the identified mutations, those genes are induced into mice and tested in three behavioral contrast experiments covering visual, memory, acoustic, and spatial performances. Results of both wild typed and mutated mice will be compared and analyzed to identify relationships between certain mutations and prodigious talents of savant individuals.**

## Keywords

**Autism Spectrum Disorder, Savant syndrome, CRISPR, Mutation, Behavioral test.**

## 1. Introduction

Autism spectrum disorder (ASD) is defined by a range of various mental disorders, which includes autism and Asperger syndrome. Individuals who are diagnosed with ASD usually experience problems with social communication and interaction [1]. Savant syndrome is a special type of autism, under which condition individuals with significant mental disabilities demonstrate certain abilities far beyond average. These abilities include art, memory, arithmetic, musical and spatial skills. It is estimated that one in ten autistic individuals shows some level of savant skills, ranging from splinter-skill savant, talented savants, to prodigious savants. Nonetheless, no matter which type of skills one may possess, special skills are always companied by outstanding memory [2].

However, existing studies have not yet determined a detailed mechanism for the acquirement of the savant syndrome. Though scholars have launched an investigation in 2003, aiming to identify a gene that may correlate to savant syndrome, the result from this study is still not adequate. In this study, Dr. Erica Nurmi and her team used Autism Diagnostic Interview (ADI) to derive six clusters of variables: language, social, milestones, savant skills, rigid, and sensory aversion. Then, a genetic analysis was performed on the autistic candidate region on chromosome 15q11-13, where they found that when a Collaborative Linkage Study of autism sample was divided based on savant skill cluster. The heterogeneity logarithm of the odds (HLOD) score of the GABRB3 gene increased from 0.6 to 2.6 in the family whose probands had greater savant skills. Thus, they have concluded that savant abilities and autism may be genetically related, and the loci on 15q11-13 possibly contribute to autism may also be relevant to savants who are not autistic [3].

Given all the information above, it is likely that savant syndrome has a genetic basis. Thus, we hypothesize that a mutation of a neural gene or multiple ones is directly related to savant syndrome.

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To further specify and locate the genes, we plan to collect data on possible savant syndrome mutations by sequencing autistic savant individuals and further analyze their genetic characteristics. If there is a specific type of mutation discovered, a similar one will be induced via homologous recombination in animal models, i.e. laboratory mice, to be proved. A series of behavior test is designed to evaluate and quantify their art, music, memory, and spatial performances. By analyzing the data that we will be collecting, it is possible to pinpoint the mutation that may correlate to autistic savant syndrome.

## 2. Methods and Materials

### 2.1 Data collecting

In order to locate possible mutations, it is necessary to collect genetic data from autistic savant individuals. An Autistic Diagnostic Interview (ADI) is carried out to confirm the autistic status of participants [3]. Since there has not yet been a formal diagnostic method for savant syndrome, a self-report is usually completed. One of the questionnaires used by researchers is known as Sussex Savant Questionnaire [4]. Created by Hughes and his team, this questionnaire first asks the individual whether they are formally diagnosed with any form of Autism Spectrum Disorder (ASD) such as Autism, Asperger Syndrome, or Pervasive developmental disorder not otherwise specified. Then, a definition of prodigious savant syndrome is given to the participants to ask whether they think they possess any skills or abilities that are far beyond average. Participants who answered with a positive response are provided with a list of nine categories of savant skills to be corresponding to according to their own understanding. They will be able to choose from the checkbox and a choice of 'other' is also provided. For the purpose of accuracy, the other two control groups are added: individuals are neither autistic nor savanistic, and individuals with autism but not savant syndrome.

### 2.2 Sequencing

Saliva samples of all participants are collected and sequenced by high throughput sequencing method. Around a thousand cells will be extracted from each participant to be sequenced and common mutations will be determined so technological errors can be eliminated. Since different talents may possess different mutations, all participants are categorized into groups. By analyzing the DNA sequences of all autistic savant participants, it is possible to pinpoint some mutation similarities among autistic savants, especially at 15q11-13 region [3]. Savant families will be especially focused to reveal common mutations, since if savant syndrome is familial, it is probably half-dominant. Furthermore, if several non-related savants possessing same ability, the similar mutations they possessed are potential savant mutations. If the common mutations we discovered are not related to the savant ability, such as a mutation in skin cells, we can eliminate that possibility and further analyze the data. It is possible that several mutations are required at different locations to provoke a single particular savant skill.

### 2.3 Induce Mutation

The same type of mutation discovered in human beings will be introduced into the laboratory mouse genome by homologous gene recombination [5]. Gene constructs containing antibiotic markers flanked by two Lok sites are developed with its sides homologous to the gene of interest followed by its transfer into the cell by electroporation. Cells possessing the mutation, as determine by DNA sequencing, are then selected and transferred to a blastocyst-staged embryo to establish the transgenic mouse line. This mutant-cell containing blastocyst is then transplant into a surrogate mother that has Cre recombinase gene inserted in her genome. In this way, antibiotic marker can be effectively discarded since we want to make sure the lowest level of disruption of the genome of the transgenic mouse line. The skills of these mice will be later evaluated by several behavioral tests to confirm whether the mutation is correlated to the acquirement of savant skills. Another line of the transgenic mouse is also established with common autistic mutations such as shank 3 to serve as a control group.

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## 2.4 Behavioral Tests

### 2.4.1 Visual Ability

In autistic savants, outstanding artistic skills is usually accompanied by a much higher visual acuity than the general population. Similarly, the mouse that possess the mutation may have a higher visual acuity. Visual acuity can be distributed into two visual tasks: the resolution of detail (minimum separable) and the detection of detail (minimum visible) [6]. The two tasks can be evaluated by testing the ability to perceive multiple small dots or alternating black and white stripes with the same width while they are moving closer together, and the ability to see a single line or dot on a contrasting background. However, it seems to be challenging to teach mice to demonstrate what they perceive, so we plan to take a similar path that will lead to the same result, optokinetic, whose response is called optokinetic nystagmus. During the experiment, the mouse will be placed on a stable platform with an MRI detector on its head, tail taped and covered by a half-cylinder of lead to restricting its disruptive movements. A cylindrical drum (see from figure 1) with a white background and black strips centered on the y-axis is placed in front of the mouse. The cylinder is able to turn at variable speed and reversibly. Instinctively, the mouse will be motivated to follow one of the strips till the end of their visual angle. The optokinetic response will be videotaped and the telephoto lens on the camera will be able to calculate their visual angle. The rotational speed of the drum will also increase over time in order to test if mice are able to follow the stripe. The maximum speed is recorded and a limit data is calculated. Two control groups of mice will be experimented with the same rotating speed, and the same source of luminance to ensure data accuracy. The MRI results between groups will also be compared to reveal mutation effects since parts of the brain to be used or areas of regions may vary between groups. Mice carrying mutations may have a larger visual angle than wild-type ones to enhance visual capacity. The ability to follow faster-moving objects may suggest savants possess a better ability to perceive fast-moving scenes to help them depict a live scene in paintings.

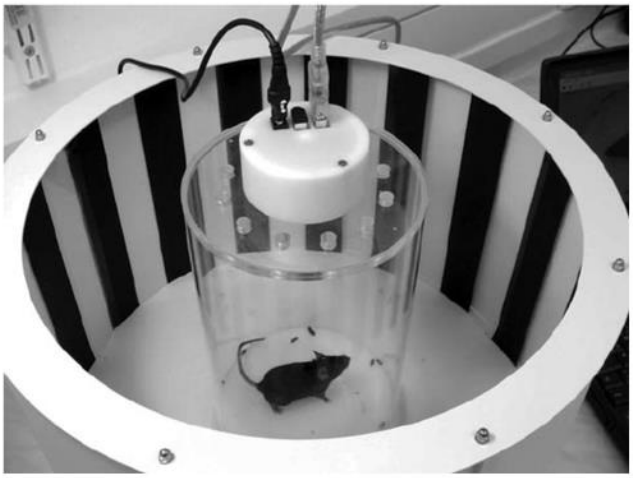

Figure 1. A mouse performing vision test in the optokinetic drum [11].

### 2.4.2 Memory Skill

To evaluate the memory skill of the mouse, we plan to use the Novel Object Recognition (NOR) task (see from figure 2). This is a task used frequently by the researchers to assess cognition, especially recognition memory in rodent models [7]. Since the mouse has the instinctive tendency to explore the unfamiliar object more than the previously known one, we can use this tendency as a fuel to carry out the task. Typically, an empty area is served as the experiment arena for the mouse. In the habituation phase, mice from each group are selected separately and put into the arena for twenty-four hours. During the habituation, mice can freely explore the space and no objects will be presented in the box. Twenty-four hours later, mice are transferred into a similar space and the training phase starts. The

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training phase is another twenty-four hours, however this time, there are two identical objects presented in the space. The last phase of the task is known as the testing phase, mice are transferred into a box with two objects: one is identical to the objects from the training day, the 'familiar object', and another one is the 'novel object', which never seen by the mouse before. A camera is anchored at the top of the arena, and the movements made by the mouse are recorded. The time that the mouse spends on exploring the novel object is recorded and the discrimination index is calculated.

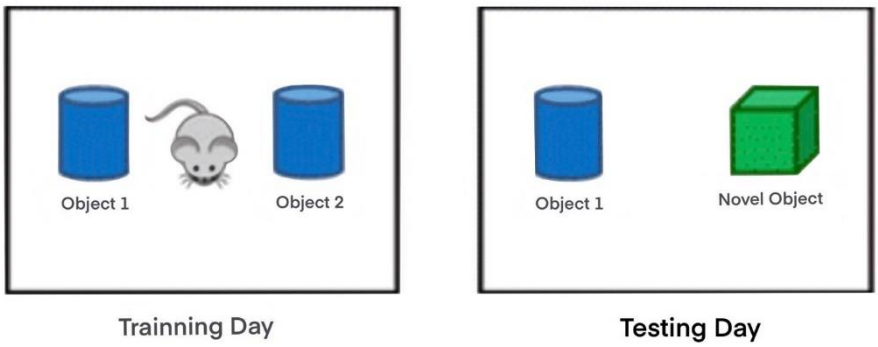


Figure 2. NOR task schematic diagram [12]

### 2.4.3 Spatial Skill

In order to test whether mice subjects are able to navigate themselves in a three-dimensional space, we adapted a three-dimensional maze known as Radiolarian maze and its two-dimensional analog known as hexagon maze developed by Wilson and his coworkers (see from figure 3) [8]. Radiolarian maze is a three-dimensional version of the classic radial arm maze: a central sphere with 30 cm diameter as well as 13 arms that are 14 cm long and 3.5 in diameter. On the other hand, the 12-armed hexagon maze is comprised of a hexagonal ring, with arms extended inward and outward at corners of the ring. 8 cohorts (mutant and control) of mice are habituated in each maze for 5 days before the experiments start. In the first two days, no arms are baited and mice are allowed to freely explore the maze for 15 minutes. In the last 3 days, however, mice are introduced to the maze from the arms and removed from the maze once the mouse reached the center section of the maze.

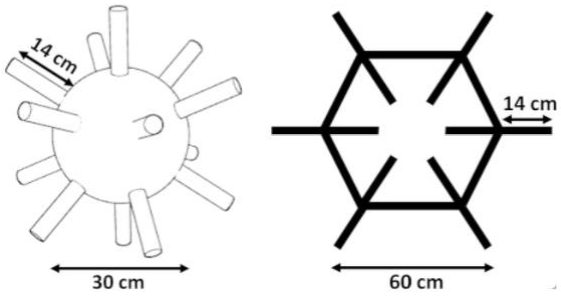


Figure 3. Radiolarian maze (left) and its 2-dimensional analog hexagon maze (right) [8].

This process will be repeating until the mouse is able to navigate itself from each arm to the center with a minute. Once habituation is complete, the experiment phase begins. 4 pairs of mice will be performing their task in Radiolarian maze and the other 4 pairs will be performing their task in the hexagon maze. In this experiment, six arms are baited with condensed milk, and the baited position is stipulated in allocentric coronations so that mouse is not able to locate themselves by extra-maze cues. Once enter the maze, mice are required to retrieve their prizes. Each trial lasts for 5 minutes or until all six rewards are successfully collected by the mouse and the maze are rotated 180 degrees horizontally to prevent mouse utilizes tactile or olfactory cues. During each trial, re-entry errors

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(repeated visits to depleted arm), omission errors (number of unvisited arms), working memory errors (visits to already-visited arms) and reference memory errors (visits to never-baited arms) are recorded and later analyzed. The experiment is performed in a frequency of 2 trials per for 25 consecutive days. In order to rule out the possibility of using olfactory sense to locate the reward, two 5-minute probe trials are performed after the completion of the experiment with no arms that are baited. Similarly, mazes are rotated 180 degrees horizontally after each trail and all the errors are scored.

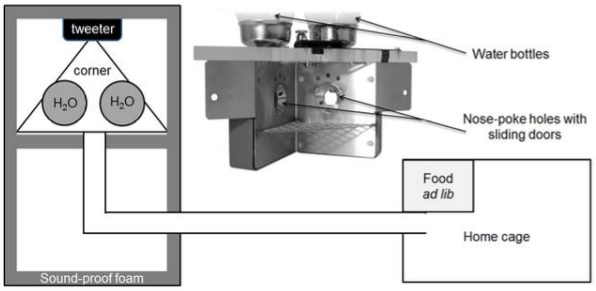


Figure 4. The inner structure of the Audiobox [9].

### 2.4.4 Acoustic Ability

To determine whether the mutation can lead to an enhanced acoustic ability, here we intend to use an apparatus known as Audiobox (see from figure 4) [9]. Audiobox is a commercially available tool that serves both as a living cage and experiment area for mice. The Audiobox is constituted by two parts and connected by a tunnel: home cage, a living space for the mouse with food at the corner and food can be accessed by the mouse at the lid, and across the tunnel is a sound-attenuated box where water is provided known as the corner. In order to access the water, the mouse needs to stick their nose into a port then the water will be dispensed. 5 cohorts of mice are lightly anesthetized then a transponder is implanted in the back region. After recovered from the anesthesia, subjects are transferred into the home cage of Audiobox. The implantation of the transponder is to monitor the activity of the mouse as well as so that the apparatus knows what frequencies of sound it should emit. Entering into the corner, a 'visit', is monitored by the antenna at the entrance of the corner. If the transponder is detected by the antenna at the same time with the activation of a heat sensor in the corner, the visit starts. Similarly, if both signals disappear, the visit stops. A speaker is positioned behind the corner in order to deliver the stimuli. In this experiment, the tones are varied between 6 to 15kH as this range tends to be the natural background used by the mouse to acquire information and environmental cues. During the experiment, there is a 'safe' frequency: when this frequency is presented during a visit, the door to the port is open, and the mouse can access the port to drink water. However, a 'conditioned' sound is associated with a negative outcome – an air puff. If the conditioned frequency is present, the visit of a mouse is associated with an aggressive air puff delivered by the air tube that is attached to the corner, and the door to the port will not open. At the beginning of the experiment, the difference between safe tone and conditioned tone is one octave apart (df = 100%) First 7 days is the habituation phase: during this time, the mouse to freely explore the two parts of the Audiobox and the door to the port will always be open. Once the mouse knows how to access the water, 4 days of safe-only phase starts: in this period of time, door to water remains closed unless the mouse performs a nose stick, and during every visit, the safe frequency is presented. In phase three of the experiment, conditioned frequencies are introduced at a 5% of the visits for three days: if the mouse performs a nose stick into the port when the conditioned frequency is present, the air puff is administered, and the door to water remains close. Later, the probability of presenting a conditioned tone from 15% for three to five days and 17% for another 3 days. Once the discrimination among the mouse reaches stabilization at df = 100%, we start to lower the frequency of the conditioned sound so that it becomes more similar to the safe sound (see from figure 5). Every new conditioned sound will be present for two to three days until the just-noticeable differences are found.

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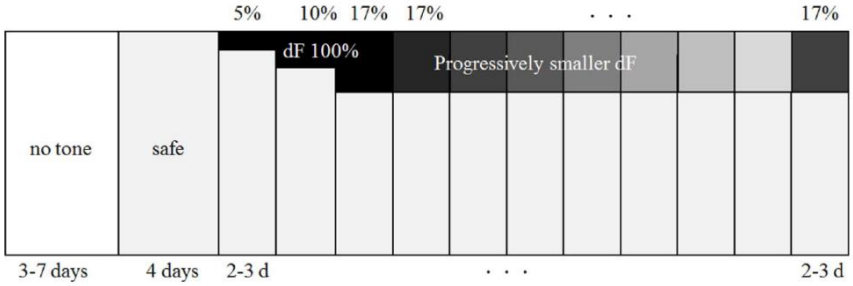


Figure 5. A demonstration of how the study will be processed [9].

## 3. Conclusion

By analyzing the experiment data, it is possible to determine whether we have successfully induced savanistic mouse and whether the mutation we found in the human has a correlation with the acquirement of the savant abilities. According to our behavioral tests, if the savant mouse has the ability to: (1) follow faster-moving cylindrical drum, (2) spend more time on the novel object, (3) score better on the performance in the maze, (4) differentiate more similar tones, then we are able to conclude that the mutation has a correlation to the gaining of savanistic abilities. One source of error is that High Throughput DNA sequencing contains a certain degree of false output that may be recognized as mutation. In order to minimize this effect, multiple cells from the same individual can be extracted and sequenced to detect the same frequent mutations. However, if the study is performed without mistakes but the savant mice do not show any of the above, it means that there is still a gap between the inducement of the mutation to the acquirement of the ability and more possibilities need to be explored. It is also possible that mutations are not the essential cause of autistic savant syndrome.

## 4. Future Direction

Since it is well established that the abundance of the spine on neurons does not experience a decrease within the brain in ASD patients [10]. They usually have a weakened effect on refinement during the critical period and thus, the plasticity of the neurons is not decreased. Even if the data does not imply a correlation between the mutation and acquirement of the ability, we cannot simply deny the possibility that the excessive spines and plasticity are somehow related to the savant syndrome. Further studies can be done on observing activities of various regions of the brain instead of the genetic aspect of the pittances. What if savant individuals have increased plasticity not only in the hippocampal region but in the entire brain that makes them into a giant storage factory? It makes sense since savants usually possess extraordinary memory abilities. Someone can propose that those autistic savants can utilize other parts of their brain to store memory or compute calendrical problems, though normally the hippocampus is the part responsible for memorization. If there, however, is a correlation between the mutation and the abilities, further studies can be done on the protein products of these genes and how these proteins are related to the development of savant syndrome. Hopefully, we can have a better understanding of brain function and its mechanism by completing this study.

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