Analysing Movement and Behavioural Patterns of Laboratory Mice in a Semi Natural Environment based on Data collected via RFID-Technology

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Abstract. In this paper we present a continuous 24 hour data collection and a semi automated data analysis of laboratory mice in a spacious indoor environment. The data is collected via an RFID tracking solution, a scale and an optical tracking system. The visualisation and the premilary analysis of the data provide information about behavioural and movement patterns of laboratory mice under semi-naturalistic conditions.

1 Introduction

In biomedical research mice play a dominant role as an animal model for deciphering gene functions in vivo. Especially in the investigation of hereditary human diseases numerous gene targeted mice were created. A detailed behavioural characterization of these mice aims to find differences between the genetically manipulated (transgenic; TG) mice and their wild-type conspecifics. Most commonly mice are tested in standardized but highly artificial situations that allow to analyze defined behavioural domains in detail but sometimes fail to bring about a thorough, externally valid behavioural phenotype [7]. Here we report on a semi-naturalistic setup where TG mice who carry a genetic predisposition to develop Alzheimer's disease like symptoms are constantly monitored 24h-7d by means of RFID technology.
Aim of this project is to support the direct behavioural observations of the mice that is carried out by humans. The population under surveillance consists of up to 40 TG and wild-type mice that are living in a semi natural environment (SNE). The SNE is realized as a large indoor cage measuring 1.75 x 1.75 x 2.1 m (L x W x H) comprising several floors which are connected by Plexiglas tubes. Mice are individually marked with RFID-chips and positional data is obtained continuously from various antennas placed in the SNE. The automated tracking solution is established to collect behavioural and movement data of the mice 24h-7d. A GIS module is developed to analyze the gathered data. The project focuses on the automated detection of potential differences in behavioural and movement patterns of the TG and wildtype mice.

2 Related work

To obtain behavioural information from different sensors is even used by testing of humans. The combination of different sensors to collect data and subsequently obtain behavioural information was applied in various species including cows [4; 5] and even humans. For humans one well described testing environment was an everyday office and it was demonstrated that simple sensors support models which are able to estimate human interruptibility [1]. Furthermore in a museum environment information about visitors were collected. In use are defined visiting styles to assign the museum visitors to different classes [8]. Also machine learning techniques have been applied also to detect and classify common motion patterns, to support users with dementia in their daily routines [6]

3 Scenario setup

For the collection of behavioural and movement data the SNE had to be structured in a way that only defined passages were accessible. A schematic view of the cage design and RFID based tracking solution is shown in figure 1.

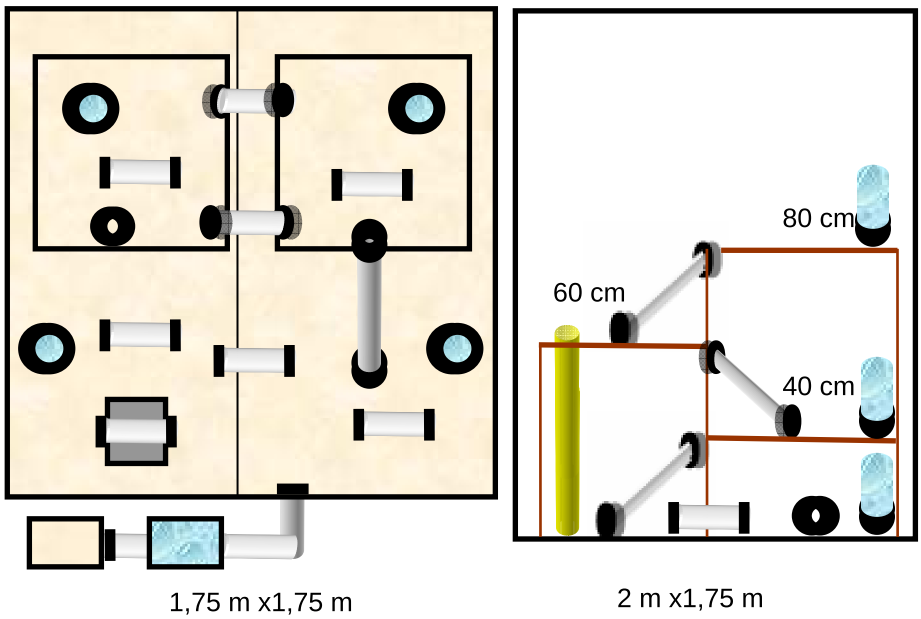

Figure 1: Schematic view of the SNE. Left: top-view, right: side view. Black circles represent coil antennas that are connected to Plexiglas tubes (grey) or water bottles (blue).

Cage design

In the setup following information about the mice is of interest: changing floors, movement on the floors, the direction of movement, the home range of individuals, drinking and emigration behaviour.
The design of the SNE has to warrant that movement on and between different floors can be detected. Therefore several constraints for the freedom of movement must be realized. The SNE comprises five floors, two on the ground and three in different levels above the ground. These floors are connected by Plexiglas tubes and / or rope. Outside the SNE an emigration cage is provided that can be accessed from the ground floor (i.e. to give shelter to low-ranking animals within the group hierarchy) via a tube and crossing a water basin (see figure 1).

Data collection via RFID

The RFID antennas are placed on points where the mice must cross. On every Plexiglas tube two antennas at both ends are attached. This allows to detect when a mouse changes floors, in which direction and at what speed mice cross the tubes. Each floor contains an antenna beneath the drinking bottle to get data about the drinking behaviour and to establish a warning system when a mouse does not drink. Furthermore on every floor is a tube supplied with two antennas which enables to collect data about the movement on the floors. Two antennas are used to identify mice using a scale. In the SNE are at least 29 antennas integrated in the SNE (see table 1).

Table 1: Distribution of the 29 antennas; in the left column: number of antennas, right column: position of the antennas

Number of antennas	Positioning
10 antennas	Floors
6 antennas	Floor connection
2 antennas	Connection floor 0
5 antennas	One drinking bottle per floor
2 antennas	Rope
2 antennas	Exit SNE → Emigration cage
2 antennas	scale

Data collection via Jerry TS

To collect data of the mice we use the RFID technology. The RFID-System (Trovan Electronic Identification Systems) consists of reader (LID 665 Miniature OEM Board), ring antennas (air-core coil antenna for LID 665) and animal glass transponders (ID 100). All mice wear a passive integrated transponder (PIT) that is injected subcutaneously between the scapulas. The transponder ID is read while a mouse traverse the electromagnetic field which is established by the ring antennas, e.g. when passing through tubes or visiting drinking places. The minimum distance between two antennas is 20 cm. The ID of the transponders is read within a distance of 0.5 cm. The readers are able to read several transponders at the same time at a maximum rate of 26 Hertz.

We wrote a Java based software component (JerryTS) to configure the RFID reader and to store the read data in a database. If the transponder ID is read (a mouse gets into the electromagnetic field of a ring antenna), a data set is created which consist of date, time, milliseconds, antenna ID and transponder ID. This data is stored online in a relational data base [2].

Data collection via scale

The described setting was extended by a scale (Kern & Sohn GmbH: Typ 440-33N) that continuously allows to measure the weight of individual animals. The scale was protected against dirt and damage by a plastic body (see figure 2). The transponder ID is read by the RFID antennas placed at the sides of the scale by entering or leaving the scale. The modified scale was integrated in the SNE on the left ground area.

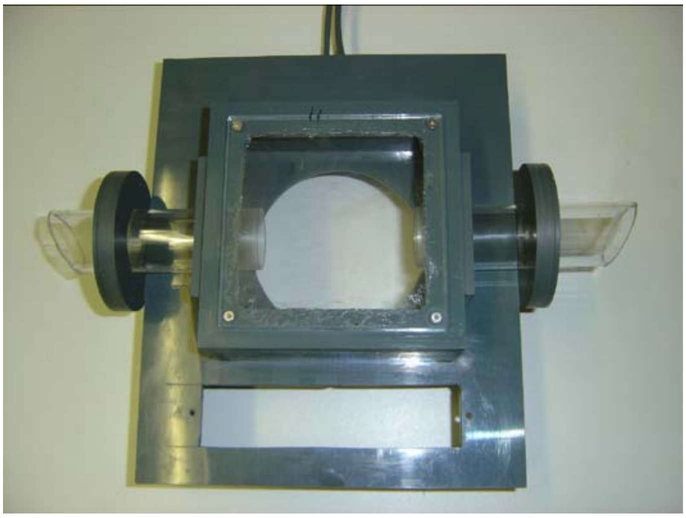

Figure 2: Plastic body of the scale with two RFID antennas

Data collection via optical tracking

Optical camera tracking was considered for tracking a single mouse to get continuous positional data compared to point data derived from the antennas. The camera (Logitech Quickcam Pro 5000) was placed above the highest floor in the SNE (see figure 3). This level can be accessed by just one tube thus a mouse that enters this level is recognized by the antenna. Additionally the antennas on the level allow to reassure the identity of optically tracked mouse in those cases when more than one subject is on the level.

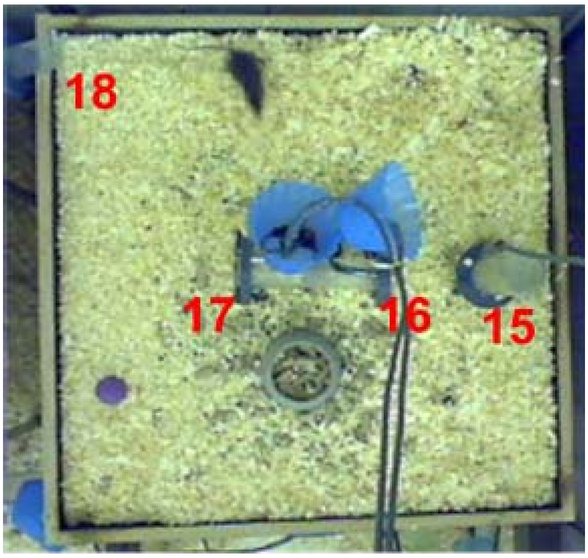

Figure 3: Camera tracking on the highest level, antennas 15 to 18 are labelled in red. Antennas on the level confirm the ID of the animals

4 Data analysis

The different data sources enable different analysis opportunities.

Anlaysis of the RFID data

For the post processing of the data a second software component (TOM) was written in the programming language C#. TOM is an ArcGIS extension and allows a visualization and analysis of the collected data. The attribute data for a mouse can be queried and viewed in a table.
The visualization module of TOM shows the position of the mice in the SNE at a certain point of time. To start the visualization of the movement a time interval has to

be chosen. In this interval the mice move from antenna to antenna. It is possible to select different display speeds and different play back rates. The spatial component is displayed in three dimensions, the temporal component is realized by the clock in the GUI [3]. Through the proposed solution in milliseconds an accurate representation of the data is possible: if during one second, a signal of the same mouse is registered at two antennas—when antennas are directly connected through a tube—a linear movement is displayed.

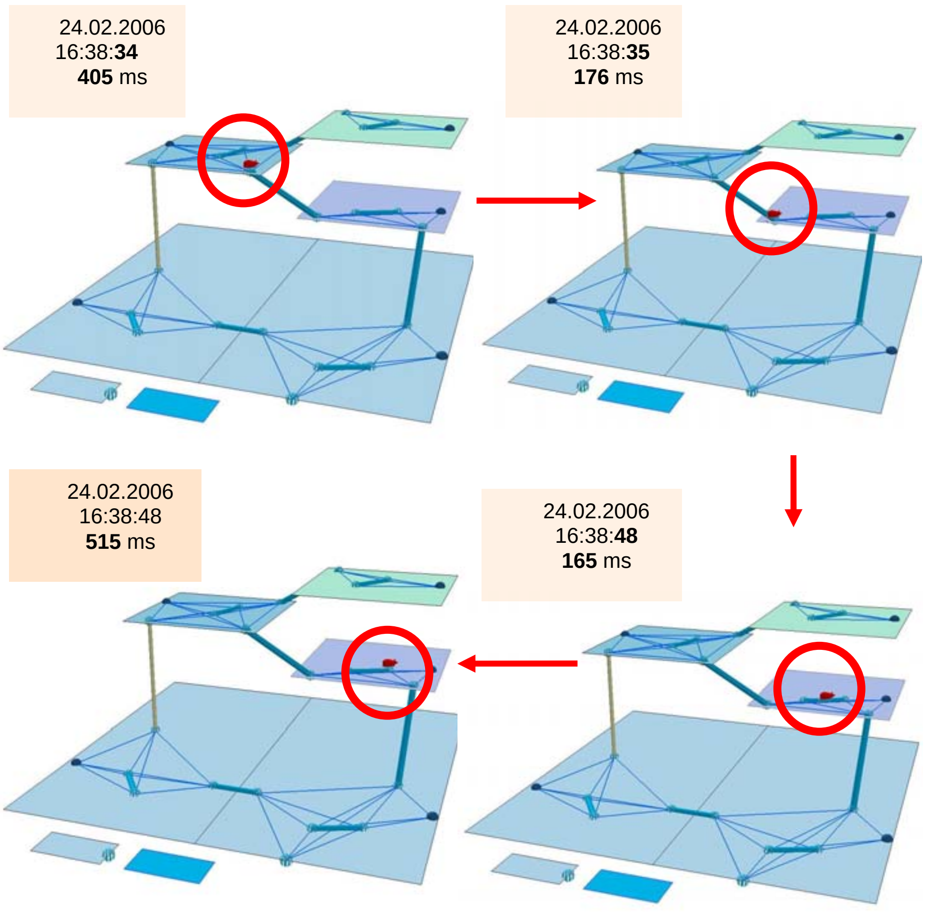

Figure 4 Sequence of mouse movement in time

In figure 4 the movement in time is shown. The advantage of this representation of the data is the movement of the mice can be observed for 24 hours. Especially the movement in the night phase – mice are nocturnal animals – can be observed. By observing the mice different patterns can be figured out. For example: Mice that trigger antennas only on a defined level can be considered as territorial. By analysing the movement patterns of all the individuals on that level dominance hierarchies might be obtained. This can be realized by analysing interaction patterns when two animals enter the tubes from different ends causing the subordinate to leave the tube backwards while the dominant one triggers both antennas. Additionally dominant mice are expected to trigger more antennas by patrolling their territory.

Besides the optical detection of patterns automated analysis of the data is prototypically implemented. On the one hand statistics can be calculated for the analysis per day, one the other hand statistics are available for the analysis per level (see table 2).

Table 2 Analysis functions

Analysis per day	Analysis per level
Time of last drinking	Number of antenna contacts
Time of last weighting	Duration of stay
Number of antenna contacts	Stay with other mice
Number of used levels	

Information about analysis per day and the number of antenna contacts per level are queried as SQL statements out of the database. Below the idea and functionality of the algorithm which calculates the duration of stay of one mouse per level is outlined (see listing 1):

The method “contactToOtherMiceOnLevel”, which returns an array with Boolean values has two parameters: the selected mouse and the date for which the information is queried (line 1). In line 2 to 5 necessary attributes are declared and initialized: the array that will be returned as a result is initialized with the Boolean value ‘false’ for each level (line 2). The clustered data for one day (contacted antennas und timestamps) of the other unselected mice are stored in an array “miceWithoutSelected” (line 3). The number of the antenna where the selected mouse has the first contact on a particular date and the time of this contact are recognized (line 4 and 5). First the changes of levels for the selected mouse must be detected (lines 6-9). Therefore the antennas were associated with the corresponding levels before. Now the list “antennaTimeArray” (antenna and time data) of the selected mouse is scanned until an antenna is found that is not derived from the same level as “antennaStart“ (line 8). If a change of level is found, we know the current level (line 9) and the time interval when the mouse was on this level (line 10). In line 13 it is verified whether an antenna entry of an unselected mouse for this time interval exists:

- If an antenna entry of an unselected mouse exists, all entries in the list “miceWithoutSelected” are checked if the antennas are from the same level as antennaStart (line 14):
    - If the antennas are from the same level, the corresponding Boolean attribute for the level is set to true (line 15). This means the selected mouse had contact with another mouse on this date on this level.

- o If the antennas are not from the same level, then the loop starts again: antennaStart gets the first antenna of the next level as new value and the next change of level will be detected (go to line 6).

- If no antenna entry of an unselected mouse within the time interval exists in line 13, the loop starts again, the start antenna gets the first antenna of the next level as new value and the next change of level will be detected (go to line 6).

The algorithm ends when the list “antennaTimeArray” is completely traversed and all possible level changes are detected.

```
// returns an array with Boolean values, which shows
whether a mouse had contact to other mice per level

1 contactToOtherMiceOnLevel bool[] (String mice,
                                     String currentDate){
2 bool[]   levelsMeet=
                {false,false,false,false,false,false};
3 String[] miceWithoutSelected;   //array with all
                                      unselected mice
4 String   antennaStart;          //number of start
                                      antenna
5 Date     timeStart              //time of contact
                                      with antennaStart
6     for(i = 1; i < antennaTimeArray; i++){
7           String antenna = antennaTimeArray[i][0];
8           if(antennaStart and antenna not on the
                                           same level){
9                 findLevelWhereMouseIs();
10                DateTime time =
11                antennaTimeArray[i-1][1];
                                 //last time on level
12                 if(two mice on one level){
13                      getTimeFromDB&Antennas-
                        ForUnselectedMice;
14                      if(unselectedMiceOnLevel-
                        WithMice){
15                            set levelsMeet
                              corresponding of true;
16                            timeStart = time;
17                            antennaStart = antenna;
18                      }
19                }
20          }
21          return levelsMeet;
22    }
23 }
```

Listing 1: Pseudo code of the method “contactToOtherMiceOnLevel”

Analysis of weight data

The analysis of weight data has to be scaled to the age of the animals in order to compare weight development of two or more individuals. Therefore the weights are ordered by the age of individuals (see figure 5).

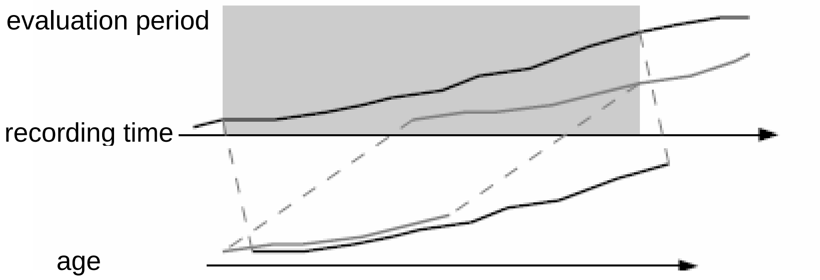

Figure 5: The weight data are independent of the recording time

Additionally movement patterns within the scale can be differentiated by means of the high frequency the scale sends data. The behaviour (see figure 6) of the mice can be summarized as follows:

1. A mouse moves fast by constant speed through the scale.
2. A mouse moves on the scale, remains there for a while and leaves the scale.
3. A mouse moves to the access of the scale and enters it step by step:
    - The mouse enters the scale completely.
    - The mouse returns and leaves the scale without entered it completely.

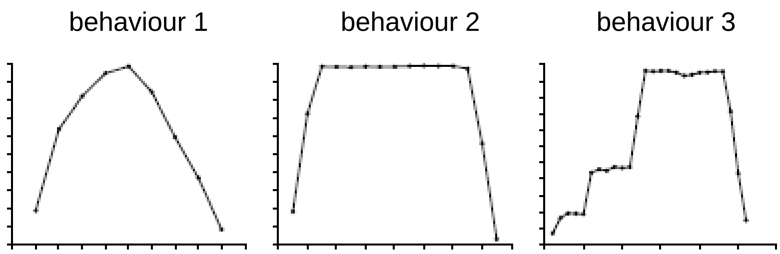

Figure 6: characteristic results of weight data, a mouse crosses the scale fast (behaviour 1), slow (behaviour 2) or step by step (behaviour 3)

Analysis of camera data

With the help of RFID data it is possible to identify each single mouse on the observed level. The positional data and the movement of the mice on the floor can be visualised as shown in figure 7.

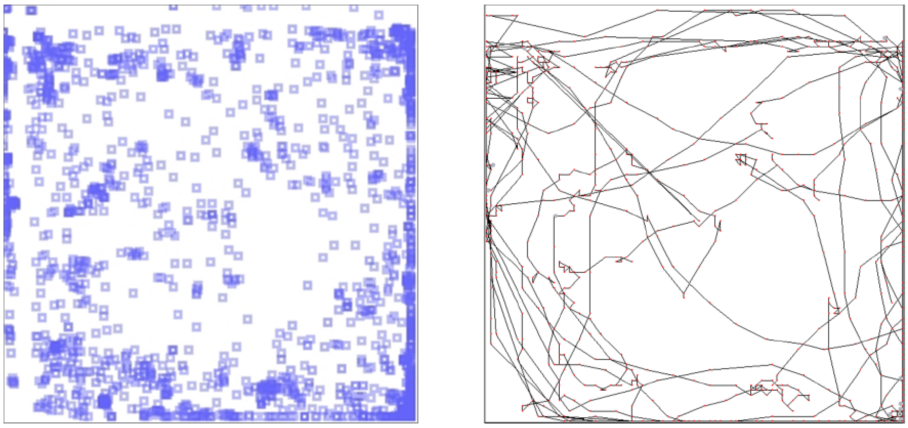

Figure 7: Representation on the positional data of a single mouse. Left: collected positional data as x-y-coordinates, right: tractorial representation

5 Further plans

We hold a huge amount of data and domain specific knowledge in this project. So we plan for our future work the use of data-mining methods for further analysis.
With these methods an automated classification of the mice between wildtype and TG should be derived. The bases for the decision are the different sensor sources in the SNE which provide data about behaviour and movement. To identify a mouse and to obtain behaviour partly a combination of the sensors is necessary (e.g. the RFID data is necessary to know which mice are filmed). We differentiate between simple behaviour and complex behaviour. Whereas complex behavior is combined of the simple behaviour of a mouse and might be related to behaviour of other mice (e.g. to obtain information about dominance behaviour the interaction of two or more mice must be observed). The analysis of the detected behaviour can then guide with a certain likelihood to the decision whether it is a wildtype or TG genotype (see figure 8). Most time will be spend on finding and defining a lot of simple and complex behaviours and movement patterns which guide us to the features. The qualitative premium features must be selected to build a reliable classificatory.
We consider for example the use of unsupervised and supervised classification to group the mice into TG and wildtype animals. For the unsupervised classification the cluster analysis will be used to group objects because of similarity. For a supervised

classification we consider to use naive Bayes classification, Bayses network or a decision tree which represents successive hierarchical decisions.

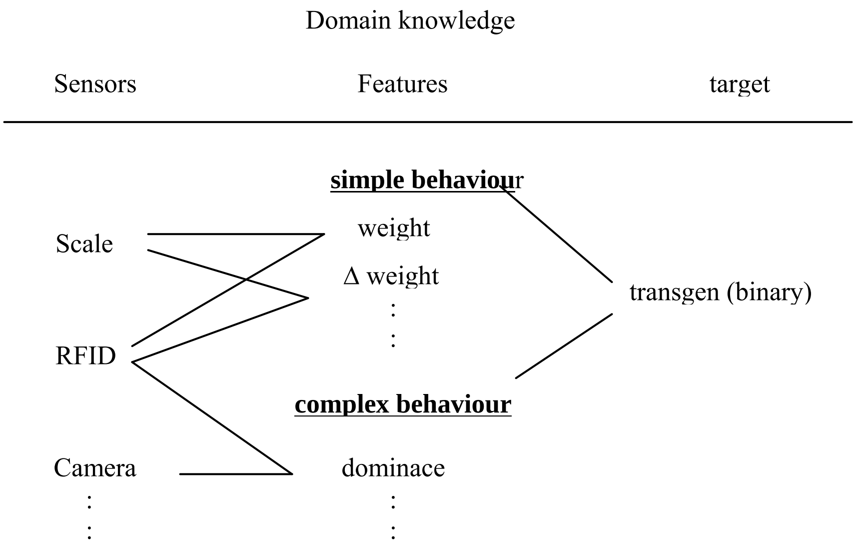

Figure 8: Features must be selected manually which can be measured by the sensors. We differentiate between simple behaviour or states (e.g. weight) and complex behaviour (e.g. dominance, combination of territorial and covered distance) which is combined by two or more simple behaviours

6 Conclusion

In this paper a system for collection and analysis of behavioural and movement data of laboratory mice is presented. The data were collected continuously with an indoor RFID tracking solution for laboratory mice, a scale and an optical tracking system in a SNE. Twenty-four hour per day, seven days a week observation is possible without disturbing the animals. Social interaction and the outward appearance are not influenced by this technology. The data stored in a relational database is analysed by an extended GIS framework. The movement of mice is visualized in a model of the SNE. Furthermore analysis functions, which offer information about the behaviour and movement of the mice per day and per level are implemented. We present initial analysis functions, which show that the collected data can support a continuous observation of the mice in a SNE.

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