

**THE RAT, THE LIGHT AND THE HEIGHT:  
A MODEL OF FREE-WILLING MOVEMENTS OF THE RAT IN THREE-  
DIMENSIONAL SPACE**

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## **ABSTRACT**

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Hippocampal place cells are specialized neurons that react to certain characters of given environment, serving as our key in remembering places and routes. Place cells, studied mainly with rats, have been on the focus of extensive academic interest for decades, but some aspects of place cell functioning have nevertheless remained ambiguous. The question concerning the sensitivity of place cells' to three-dimensionality of space, especially whether the place cells encode the vertical dimension of given environment, has not yet got a convincing answer. This is partly because studies focusing on this question are relative few thus far, and partly because the research frames of these existing studies could be considered as too complicated. Also, what we think is not sufficiently acknowledged in previous place cell studies executed in 3D-environments are the aspects concerning rats' locomotion in vertical dimension and motivational factors attached to it. The target of this study was to create a simple model for rats' voluntary movements in three-dimensional space, without using invasive methods or physical punishment as stimulators of desired locomotion. In order to motivate the rats to explore the vertical dimension, we used moderate food deprivation; selectively positioned food rewards; and localized, selective light stimulus, which has not been utilized in place cell studies prior to this study. We propose that application of this kind of behavioral model in place cell studies would help to reveal the 3D-coding of hippocampal place cells more clearly.

Keywords: place cells, three-dimensionality, behaving rat, maze, navigation, light stimulus

## TIIVISTELMÄ

Rotta, valo ja korkeus: Malli rotan vapaaehtoiselle liikkumiselle kolmiulotteisessa ympäristössä

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Hippokampuksessa sijaitsevat paikkasolut ovat erikoistuneita hermosoluja, joita tarvitaan eri ympäristöjen tunnistamiseen ja niissä suunnistamiseen. Paikkasoluja on tutkittu pääasiassa rotilla, ja vaikka tutkimusta on tehty runsaasti jo vuosikymmenten ajan, kaikkia paikkasolujen toiminnan yksityiskohtia ei edelleenkään tunneta tarkasti. Eräs merkittävä ja toistaiseksi selvittämätön kysymys on, missä määrin paikkasolut reagoivat ympäristön kolmiulotteisuuteen. Erityisesti paikkasolujen osallistumista tilan syvyysulottuvuuden (vertikaaliulottuvuuden) koodaamiseen on toistaiseksi tutkittu varsin vähän, eivätkä tähänastiset tutkimukset ole onnistuneet antamaan asiasta vakuuttavaa käsitystä. Olemassa olevien kolmiulotteisessa tilassa toteutettujen paikkasolututkimusten ongelmana ovat paitsi monimutkaiset tutkimusasetelmat, myös puutteet rotan vertikaaliliikkumiseen ja sen motivaatiotekijöihin liittyvissä oletuksissa. Tämän tutkimuksen tavoitteena oli luoda malli rotan omaehtoiselle liikkumiselle yksinkertaistetussa kolmiulotteisessa ympäristössä ilman kajoavien menetelmien, kuten mielihyvakeskuksen stimulaation tai fyysisen kipurangaistuksen, käyttöä. Liikkumisen motivaatiotekijöinä käytettiin mittauksia edeltävää paastoa, vertikaaliulottuvuuteen sijoitettuja ruokapalkkioita sekä valikoidusti sijoitettuja ja käytettyjä, paikallisia LED-valoärsykeitä. Viimeiseksi mainittua menetelmää ei ole aikaisemmin hyödynnetty paikkasolututkimuksessa. Ehdotamme, että kehittämämme käyttäytymismalli yhdistettynä paikkasolututkimuksen menetelmiin voisi tulevaisuudessa tuottaa uutta ja aiempaa luotettavampaa tietoa paikkasolujen toiminnasta kolmiulotteisessa ympäristössä.

Avainsanat: paikkasolut, kolmiulotteisuus, rotan käyttäytyminen, labyrintti, navigointi, valoärsyke

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# 1. INTRODUCTION

The hippocampus, which lies bilaterally in medial temporal lobes, was first observed to be involved in learning and memory processes, when neurosurgical removal of this structure caused amnesia in human patients (Scoville & Milner, 1957). In spatial cognition, the salient role of hippocampus was revealed in 1970's, when O'Keefe and Dostrovsky (1971) discovered that complex-spiking neurons in rat hippocampus fired at high rates in specific places within an environment. Further on, O'Keefe and Nadel (1978) proposed that these place cells are the basic elements of a distributed, non-centered representation, a cognitive map, which provides the animal with a dynamic, continuously updated representation of allocentric space (Moser, Kropff, & Moser, 2008). Inspired by these early findings and theorizations, a number of subsequent studies, mainly based on electrophysiological approaches in rats, have specified and extended our understanding of spatial learning and navigation in mammals (Eichenbaum, Dudchenko, Wood, Shapiro & Tanila, 1999).

Nowadays it is known that in addition to hippocampal place cells, mammalian brains contain also other structures and cell types that are crucial for effective spatial cognition and performances. Together with head direction cells of the thalamus and presubiculum (Taube, Muller & Ranck, 1990; Taube, Muller & Ranck, 1990a), and grid cells of the medial entorhinal cortex (Fyhn, Molden, Witter, Moser & Moser, 2004; Hafting, Fyhn, Molden, Moser & Moser, 2005), place cells are perceived to be part of a boarder circuit for dynamic representation of self-location (Moser et al., 2008). Compared to other cells that are relevant for spatial cognition, the characteristic that makes place cells such a special focus of interest is that they encode unique locations in unique environments, while for example grid cells are active in every given environment (Derdikman & Moser, 2010). Yet, despite the 40 years of intensive research, place cells are believed to embed still unsolved features, one of which is sensitivity to three-dimensionality of the environment.

Place cells are pyramidal neurons typically observed by monitoring extracellularly recorded single units from the two major cytoarchitectonic fields of the hippocampus: CA1 and CA3 (Fox & Ranck, 1981). When animal arrives in restricted location of space, a selective network of place cells fires in a pattern that presents the animal's prevailing location in that environment, the place field, while remaining cells stay virtually silent (for example O'Keefe & Convey, 1978; O'Keefe & Nadel, 1978; Thompson & Best, 1989; Wilson & McNaughton, 1993). Place fields vary in size and shape, they are partly overlapping, and if a sufficiently large population of place cells is recorded at the same time, the place fields of place cells extend into every part of given environment (for

example O'Keefe & Nadel, 1978; Muller, Kubie & Ranck, 1987; Eichenbaum, Wiener, Shapiro & Cohen, 1989; Wilson & McNaughton, 1993; Derdikman & Moser, 2010).

Firing of place cells is controlled primarily by the configuration of extrinsic, distal visual landmarks, while individual proximal landmarks are also believed to have an influence on firing (Muller & Kubie, 1987; Gothard, Skaggs, Moore & McNaughton, 1996; Shapiro, Tanila & Eichenbaum, 1997; Tanila, Shapiro & Eichenbaum, 1997). Although place selective firing is considered robust, it is also perceived that place cells frequently alter their firing patterns in response to apparently minor changes in sensory or cognitive and motivational inputs (Colgin, Moser, & Moser, 2008). Appearing, disappearing or moving of place fields, as well as changes in firing rates, are considered as remapping of the place cell representation of space (Cressant, Muller & Poucet, 2002; Colgin et al, 2008).

Place cells' capacity to encode aspects of space and modify their firing patterns due to environmental changes have been studied mainly with rats exploring two-dimensional environments. Disadvantage in these kinds of research frames is that they do not comprehend the fact that for majority of moving creatures, the real world appears as three-dimensional. The question of whether the place cells encode locations and environmental changes which take place at vertical dimension (also often referred as z-dimension) of given environment, is then essential but yet unsolved. One reason to this is that studies concerning three-dimensional (3D) properties of place cells are relatively few thus far. Observations made about rats' behavior in 3D environments suggest that the priority is given to horizontal dimension of space (Grobéty & Schenk, 1992; Jovalekic et al, 2011).

Also hippocampal single-cell recordings (which are electrophysiological research methods for functioning of individual place cells) with rats exploring 3D space have proposed that neuronal encoding is less precise at vertical than at horizontal plane (Knierim & McNaughton, 2001; Hayman, Verriotis, Jovalekic, Fenton & Jeffery, 2011). Studies of single-cell activity have, however, used quite complex research frames, which might attenuate the reliability of acquired results. For example, in their study, Knierim and McNaughton (2001) used a track that was tilted and rotated to different three-dimensional orientations, while place cells were analyzed to determine whether their firing was bound to the external, three-dimensional cues of the environment; to the two-dimensional rectangular surface; or to some combination of these cues. As for Hayman et al (2011), they observed the firing fields of both place cells and grid cells, when rats explored two different kinds of apparatus: a climbing wall (the pegboard) and a helix. If the focus is to strictly

explore the 3D properties of place cells as reliable as possible, it seems that these existing models for rat's behavior and movement in vertical dimension might be too intricate, and the application of more simple research frame would be reasonable.

Besides the quite complex research frames of previous 3D studies with rats, aspects that also should be taken into account when evaluating these studies are factors affecting to rats' spontaneous locomotion and motivational issues in situations where moving is expected to occur in vertical dimension. Rats are highly exploratory animals and their locomotion behavior is considered as neophilic: they tend to approach what is unfamiliar (Barnett, 2005). Although rats are considered as relatively good climbers which naturally live in complex burrow-systems including three dimensional elements, they are dominantly surface-travelling and also highly cautious animals which prefer to be in contact with vertical surface via their fur and vibrissae when moving around (Calhoun, 1962; Barnett, 2005). These are facts that should be acknowledged when planning models for studies including exploration of new environment, and they also indicate that gravitation to high situated places might need training as well as profusely motivating elements.

Motivational factors are generally considered as either appetitive (directed towards positive hedonic goals) or aversive (directed away from unpleasant condition) (Bozarth, 1994). For rats in laboratory conditions, appetitive motivation is most often generated with sugary or fatty food rewards (Burn, 2008) or with invasive electrical brain stimulation of medial forebrain bundle (MFB), which is known to produce the most robust rewarding effect (Bozarth, 1994). As instigator of aversive motivation, infliction of pain via electric shocks (towards for example foot) has been used in some studies, but it is also perceived that this kind of procedures might cause undesirably powerful stress for animal (Dagyte et al, 2009; Telensky et al, 2011). With food rewards, deprivation from food or water is often applied in order to enhance the animal's motivation to perform the complex behavioral tasks, but it also might undesirably affect behavior, physiology, neurochemistry, and drug efficacy of the animal (Mumby, 2005; Slawecki & Roth, 2005). In existing 3D studies of place cells, mainly food deprivation, food rewarding and electrical stimulation of medial forebrain bundle have been used as motivators (Knierim & McNaughton, 2001; Jovalekic et al., 2011). In invasive, electrical methods like stimulating MFB, there is a possibility that the function of place cells is unintentionally interfered. What comes to the deprivation and food rewarding arrangements, these traditional motivators might not be effective enough to get the animal to reach for vertical dimensions, if the rats' innate bias towards horizontal surfaces is acknowledged, as for example Jovalekic et al (2011) suggest.

One ecological fact that has not yet been utilized in place cell studies is that rats are nocturnal animals which show an aversion to light also in laboratory conditions (Burn, 2008). Although it has been claimed that illumination would not have an effect on rats' behavior (Becker & Grecksch, 1996), many studies have quite firmly demonstrated that rats either move to areas of lower illumination, or move more if the illumination is low, which is why research rooms are often equipped with dim lightning (for example Campell & Messing, 1969; Cardenas, Lamprea & Morato, 2001; Garcia, Cardenas & Morato, 2005; Burn, 2008). However, the effects of *selective lightning* on rats' behavior in exploratory tasks have not yet studied at all. In selective lightning, the idea is that localized and selective light sources are turned on or off depending on observed activity and positions of the animal. Usage of selective lightning might offer an effective way to motivate the locomotion of the rat and persuade the animal to avoid certain (lighted) areas and strive for others (to darkness). Besides that this method is high with ecological relevance, it is also non-invasive.

In this study, we have combined aspects of the above mentioned motivational factors with the lack of 3D place cell data, and created a simple model that enables to study behaving rats in real 3D environment. With or without 12 hours food deprivation (fasting), the rats explored an arena equipped with construction of three ascending levels, where food pellets were scattered. Each rat explored the arena in six separate trials, each lasting approximately 15 minutes, and all trials were video recorded for subsequent analysis. In half of the cases, base level of the arena was equipped with selectively used light-emitting diodes (LED stimulus). The levels situated on the vertical dimension of the arena were kept dark in all trials. It was observed that selectively used LED stimulus was the factor which motivated the rats' to move relatively the most and also explore the vertical dimension of environment most likely. Combination of motivational factors (fasting, food rewarding and LED stimulus) also had its benefits by diminishing the differences in proportional amounts of movement between animals. We suggest that in future, if this kind of behavioral approach is combined with electrophysiological studies of place cells, new and more specific information about 3D-coding of place cells could be achieved.



## 2. METHODS

### 2.1 Rats

Five Sprague Dawley male rats obtained from Harlan Netherlands at ~1,5 months of age were used as subjects. They were ~6 months old at the time of measurements and weighing 400 to 500 grams. The rats were housed in groups of two and three animals in polypropylene cages in the research animal laboratory of University of Jyväskylä. Food and water was provided ad libidum (except 12h before four out of five measurements, when access to food was restricted) and room temperature and humidity were controlled. Rats were maintained under a 12:12-hour dark and light cycle (lightning was generated with 40w bulb which was turned on at 07:00 am), measurements were conducted during the light portion of the cycle. All the experimental procedures were implemented in accordance with the European Communities Council Directive (86/609/EEC) on care and use of animals for research purposes.

### 2.2. Arena and the level construction

The arena was set up in a rectangular plywood box (111 x 51 x 40 cm) that was open from the top and covered with black rubber inside, in order to ease cleaning. With four metallic legs, the base of the box stood 40 cm above the floor of the research room. Inside the box was a staircase-like construction made of dark plywood, Perspex® and wood, and it consisted of three levels and 16 small steps that enabled transitions between the levels (pictures 1A, 1B and 2). The levels of the level construction consisted of three boards of dark plywood that were in ascending relation to each other. With supportive structures of plywood, the levels formed a three-step-staircase. The long sides and one ends of levels were flanked with transparent Perspex® and other ends where left open. This way the progression from one level to another could happen only via small steps situated next to open ends of levels.

The construction of levels was situated in the arena box in a way that the rats could not go behind it, and they had an access only to that side of formation which was equipped with small steps that led to levels. Other side of formation was faced to the wall of the plywood box and also the back of the construction was placed against the wall of the box (picture 1A).

### *2.2.1 Lightning of the research room and LEDs*

There was one lamp with a traditional 40W light bulb situated outside the arena box (it leant against one of the plywood walls of the box) so that there was no straight light illuminating the arena. The room had no other lightning. Some amount of illumination was necessary for the video recordings, but it was important to keep the arena as dark as possible in order to get the animals to move around.

LED-system consisted of five small LEDs that were attached to each other with a lead. The lead was attached to a control box allowing individual LEDs to be separately switched on and off. LEDs were situated at the base level of the arena box (picture 2).

### *2.2.2 Recording equipment*

Sessions were recorded for further observations with a standard web camera (Logitech, Newark, CA, USA) that was attached about 50 cm straight above the arena box, so that the angle of view of the camera captured the whole arena.

## *2.3 Experimental protocol*

The recordings took place between 5.3.2012 and 29.3.2012. There were all together six trials (T1-T6) executed in separate days. Each day, all five rats (R1-R5) were observed, when the total number of sessions was 30. Estimated 12 hours before four trials the animals were deprived from food, access to water remained free. Two trials were carried out without preceding food deprivation (fasting). Sweet food rewards were present on fixed points of the arena in all trials. Each observation lasted for 15 minutes (900 seconds), with the exception of cases where the rat visited all three levels of the level construction in less than 15 minutes. In these cases, the trial was terminated as soon as the rat had visited all levels and returned to the floor of the arena box. Three of the rats always started the trials at a random spot of the floor of the arena box. Two of the rats always started the trials from the highest level of the level construction (level number three, see picture 2). In each trial, the collocation of the rats was random. For all rats, first three trials were performed without the usage of the LED stimulus (LEDs) and during last three trials, the LEDs were employed. In certain trials, both LEDs and fasting were applied at the same time. In trials with LEDs, the LEDs were used only if the rat was motionless or did not approach the level construction, otherwise the LEDs were not used. Illustration of how different conditions of LEDs and fasting were distributed between trials, and numbers of cases (trials \* rats) under certain motivational

conditions, are displayed in table 1A. Table 1B illustrates the different starting points of trials between rats, and total numbers of cases with different starting points (rats \* trials).

Before each observation, the arena was swept with cleaner, in order to eliminate odor of preceding rat. After cleaning, sweet food pellets which served as food rewards were placed to fixed points of level construction as follows: There were four pellets at each of levels at approximately 15 cm distance from each other, and four pellets at small steps that led to levels (picture 2).

*Table 1A. Demonstration of different conditions of LEDs and fasting in different trials; total amounts of trials with certain conditions; and total amount of cases (trials\*rats) where these conditions occurred.*

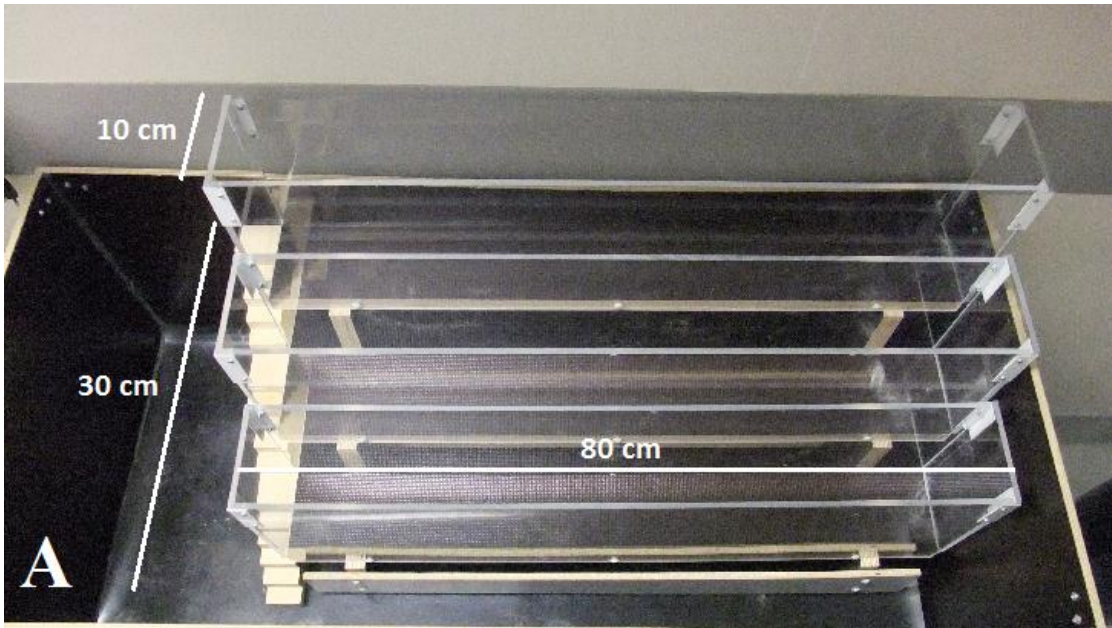
Trial	LEDs		Fasting		LEDs and fasting			
	on	off	yes	no	both present	both absent	only LEDs	only fasting
T1		x		x		x		
T2		x	x					x
T3		x	x					x
T4	x		x		x			
T5	x		x		x			
T6	x			x			x	
Total amount of trials per condition	3	3	4	2	2	1	1	2
Total amount of cases per condition (trials * rats)	15	15	20	10	10	5	5	10

*Table 1B. Demonstration of different starting points between rats; total amounts of rats with different starting points and total amounts of cases (rats\*trials) with different starting points.*

Rat	Starting point	
	up	down
R1		x
R2		x
R3		x
R4	x	
R5	x	
Total amount of rats	2	3
Total amount of cases (rats * trials)	10	15

## 2.4 Coding and variables

Before statistical analysis, the behavior of the rats in all together 30 video-recorded sessions was coded into numeric variables. As the target of the study was to get information concerning rats' movements on 3D arena, the coding was executed by taking into account two distinct circumstances in parallel: 1) the rats' vertical position in the arena; and 2) the status of movement (whether the rat was on move or not). Both were measured as amount of time (seconds) spent on certain vertical position committed either to movement or stillness. Vertical positions were determined on the basis of on which zone of the arena the rat was, possibilities varying from the floor of the arena box (from now on referred as 'base level') to one of three levels of the level construction (from now on referred as 'levels'). On every level, the rat could either be on move (*m*) or in situ (*s*). Status of movement was coded as *m* if rat clearly seemed to move (walked, ran or climbed) or was oriented towards its environment (was at one place but reached for places, sniffed or moved its head around). Rat's status of movement was coded as *s* if it did not move or did not seem to be orientated towards its environment (if it was for example grooming or quietly sitting still). After initial coding, variables representing time spent on move on any given zone of the arena were added up as a variable of total movement, which was normalized because the length of the different trials varied. Normalization was executed by dividing the time dedicated to movement on certain trial by the total duration of that trial, and this new variable was named as 'proportional movement'.



*PICTURE 1. A) The arena box with level construction inside photographed from above. Levels of the construction are walled in with transparent Perspex® in order to direct the transitions between levels to take place only via connective steps (situated on the left of the construction from this angle), and also in order to offer the rats a safety promoting experience of vertical surface when they search the levels. B) The construction of levels taken out from the arena box and photographed from the side equipped with connective steps. Here it is also demonstrated that those steps that offer the best access to the levels are wider than other connective steps in order to ease the entrance to level. These certain steps served also as fixed points for sweet food rewards during trials. The measurements (cm) of different parts of the level construction are illustrated in both pictures.*

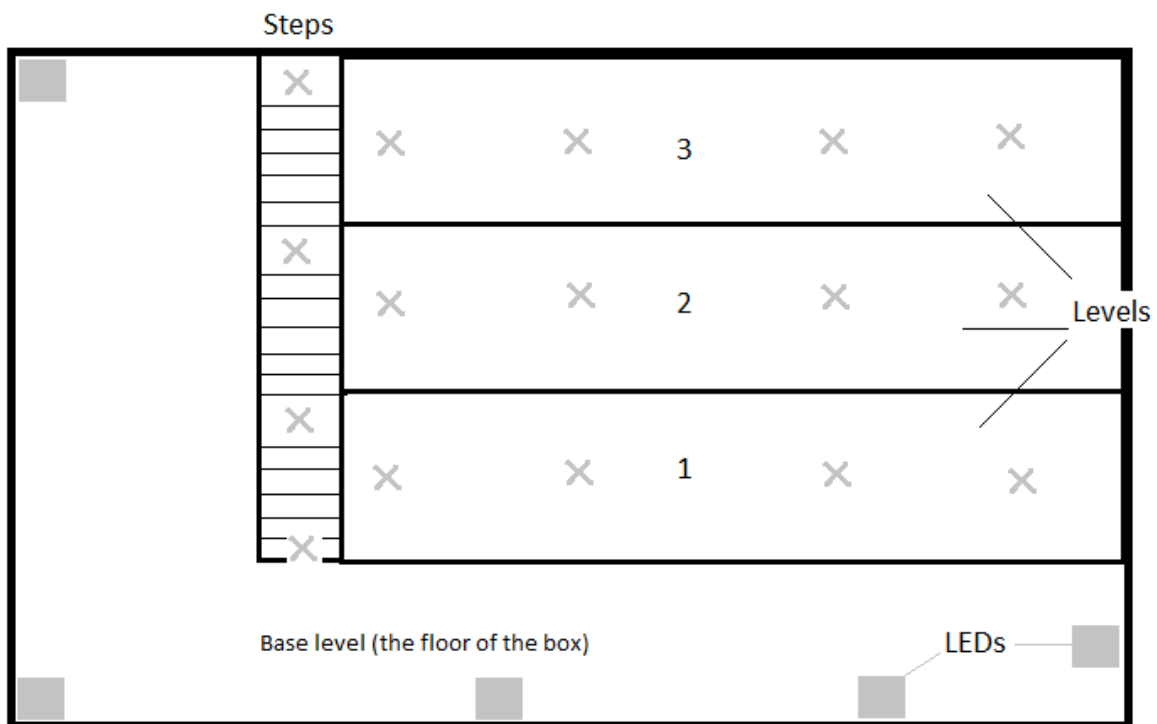
Rats' willingness to explore the vertical dimension of the arena during trials was measured in two ways: first, by counting how many individual visits rats made to levels in different trials; and second, by counting the time (as seconds) that rats spent on levels during these visits. One visit was defined as entrance to level and exit from it, and variable representing the total amount of visits per trial was named as 'number of visits to levels'. Time spent on levels during these visits could be either passive or active; here the status of movement was not relevant. Time spent on levels was also normalized because of the different duration of trials, and it was therefore named as 'proportional time spent on levels'. Different options for starting points of trials (up from the level number three / down from the base level) were taken into account as follows: if the rat started the trial from the base level, all visits to levels were counted as visits, and all time dedicated to residency on levels was counted as time spent on levels. In cases where the rat started the trial from up, this starting position was not counted as visit nor was the time spent on this level counted in as level time, unless the rat left the level and returned to it later during that same trial.

Proportional movement, proportional time on levels and number of visits to levels were considered as dependent variables which might vary in relation to interpretative variables: to starting point (up / down), condition of LEDs (off / on), and condition of fasting (no / yes). Because conditions of LEDs and fasting were partly overlapping (see table 1A), dependent variables were also examined in relation to interactions of LEDs and fasting which consisted of four distinct classes (LEDs on, no fasting; LEDs on, fasting; LEDs off, no fasting; LEDs off, fasting).

## *2.5 Statistical analyses*

All analyses were performed with SPSS 11.5 for Windows (Armonk, NY, USA). An alpha-level of .05 was used in all statistical testing primarily, but test results were also considered relevant when *p*-value fell below .1. As continuous variables, dependent variables were tested for normality with Shapiro-Wilk ( $\lambda=0.91$ ,  $p=0.015$  for proportional movement;  $\lambda=0.74$ ,  $p=0.000$  for proportional time spent on levels; and  $\lambda=0.751$ ,  $p=0.000$  for number of visits to levels). The main effects of the LEDs and fasting on dependent variables were inspected with Related Samples Wilcoxon Signed Ranks Test (a nonparametric test equivalent to the dependent t-test) because same rats were used in all conditions of LEDs and fasting and samples were therefore related. The main effect of the starting point on dependent variables was inspected with Independent Samples Mann-Whitney U test (a nonparametric equivalent to the independent t-test), because here the groups with different starting points were independent. Interactions of LEDs and fasting comprised four different conditions which were formed on the basis of two factors (LEDs and fasting) which both had two levels of

treatment (off/on and no/yes). However, the application of 2-way ANOVA here was challenging because of small sample size, nonparametric nature of the variables on focus, and also because the measurements were related. Therefore, conditions of LEDs and fasting were considered as four distinct treatments given to same sample and comparison between measurements were then executed with Friedman Test (a nonparametric tests equivalent to the repeated measures analysis of variance). Subsequent to analysis of variance, pair-wise comparisons for four conditions were executed with Related Samples Wilcoxon Signed Ranks Test. Pair-wise comparisons were executed independent from the results of Friedman Test, because the detection of the differences between four related measurements with varying sample sizes and considerable dispersions was assumed as challenging, and pair-wise comparisons were believed to perceive differences otherwise left hidden.



*PICTURE 2: Arena box with level construction seen from above illustrated as chart. Locations of five LEDs positioned on the base level of the arena in trials 4-6 are marked in chart with grey squares. Sweet food pellets which served as food rewards in all six trials were positioned on three levels of level construction and connective steps. Fixed points of pellets are marked in the picture with grey crosses.*

### 3. RESULTS

In this study we wanted to create a model for testing rats' behavior in 3D environment without using invasive methods. In order to validate our model, we tested whether our hypothesized motivators of locomotion actually facilitate the moving of rats and especially whether they promote the rats' movements in vertical dimension.

#### *3.1 Main effects of LEDs, fasting and starting point*

When proportional movement was explored in relation to two conditions of LEDs (no/off), the descriptive statistics indicated that with LEDs, the rats moved relatively more on average ( $82\% \pm 11\%$ ) than without LEDs ( $70\% \pm 18\%$ ). Related Samples Wilcoxon Signed Ranks Test confirmed this difference in proportional movement between conditions of LEDs as statistically significant ( $Z=-2.215$ ,  $p = 0.027$ ). As differences in proportional movement between conditions of LEDs were examined between rats, it was perceived that within almost every rat, the mean proportional movement increased when LEDs were on compared to condition where LEDs were off, and this increase seemed to be greatest (over 10%) within those rats whose proportional movement was under 70% without LEDs. Only exception here was one rat whose movement remained stable in both conditions of LEDs. Among all other rats, the increase in proportional movement with LEDs compared to condition with no LEDs off varied between 9% - 22% (figure 1 A). Examination of proportional time on levels in relation to conditions of LEDs indicated that more time was spent on levels when LEDs were on ( $30\% \pm 32\%$ ) compared to condition where the LEDs were off ( $14\% \pm 30\%$ ) (figure 2 A). With Related Samples Wilcoxon Signed Ranks Test, the difference in proportional time spent on levels between conditions of LEDs approached statistically significant ( $Z = -1.784$ ,  $p=0.074$ ). As the number of visits to levels was explored in relation to condition of LEDs, it was seen that when LEDs were off, on average  $1,4 \pm 2,6$  visits to levels were made, whereas when LEDs were on, corresponding amount of visits was  $4,3 \pm 4,6$ . With Wilcoxon Signed Ranks Test, this difference in number of visits to levels between conditions of LEDs was perceived as statistically significant ( $Z=-2.135$ ,  $p=0.033$ ).

When proportional movement was explored in relation to two different conditions of fasting (yes/no), the mean proportional movement was  $78\% \pm 16\%$  with fasting and  $71\% \pm 17\%$  without fasting. With Related Samples Wilcoxon Signed Ranks Test, this difference was not statistically significant ( $Z=-0.561$ ,  $p=0.575$ ). Examination within subjects demonstrated that the proportional movement of almost every rat was slightly greater in condition with fasting compared to condition



with no fasting. These increases varied from 0% to 18% and the greatest increase was seen within rat whose proportional movement was least (under 50%) without fasting (figure 1 B). In examinations of proportional time spent on levels in relation to conditions of fasting, it emerged that less time was spent on levels with fasting ( $19\% \pm 27\%$ ) than without fasting ( $28\% \pm 38\%$ ) (figure 2 B). With Related Samples Wilcoxon Signed Ranks Test, this difference was found as not statistically significant ( $Z=-0.169$ ,  $p=0.866$ ). The number of visits to levels between conditions of fasting indicated that on average, slightly more visits to levels were made without fasting ( $3,2 \pm 4,8$ ) than with fasting ( $2,7 \pm 3,4$ ). With Related Samples Wilcoxon Signed Ranks Test, difference between these values was perceived as not statistically insignificant ( $Z=-0.352$ ,  $p=0.725$ ).

As differences in proportional movement and proportional time spent on levels were explored between two different conditions of starting point (for means and standard deviations see table 2) with Independent Samples Mann-Whitney U test, perceived differences did not reach the statistical significance (for proportional movement:  $U=101.0$ ,  $p=0.767$ ; for proportional time spent on levels:  $U=106.0$ ,  $p=0.925$ ). As for visits to levels, descriptive statistics indicated that in group where trials were started from up, on average  $3,5 \pm 4,9$  visits to levels were made, and in group where trials were started from down, the corresponding mean was  $2,4 \pm 3,1$ . As these groups were compared with Independent Samples Mann-Whitney U test, no statistically significant difference was found ( $U=92.0$   $p=0.468$ ).

*TABLE 2. Means and standard deviations of proportional movement, proportional time spent on levels and number of visited levels in different conditions of starting point (up / down).*

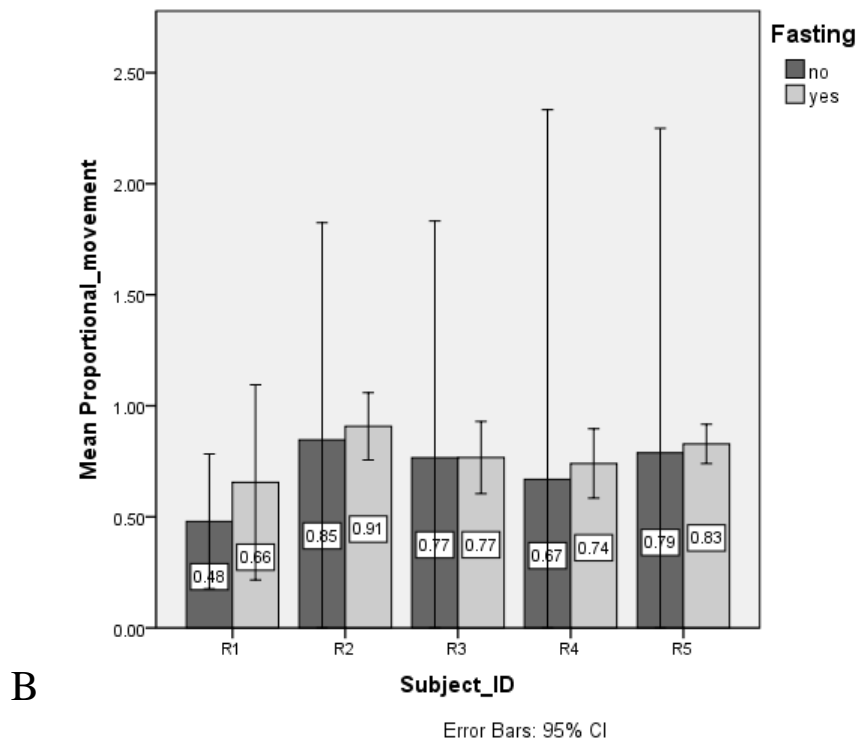
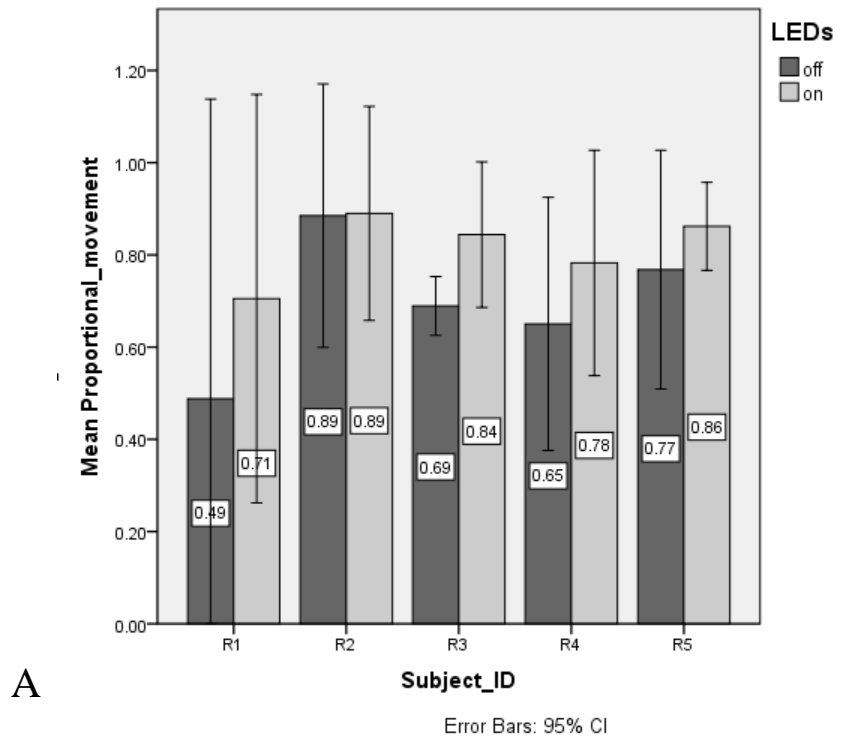
Dependent variable	Starting point	
	Up (mean / std.)	Down (mean / std.)
Proportional movement	77 % / 11 %	75 % / 19 %
Proportional time spent on levels	21 % / 31%	22% / 32%

### *3.2 Interactions of LEDs and fasting*

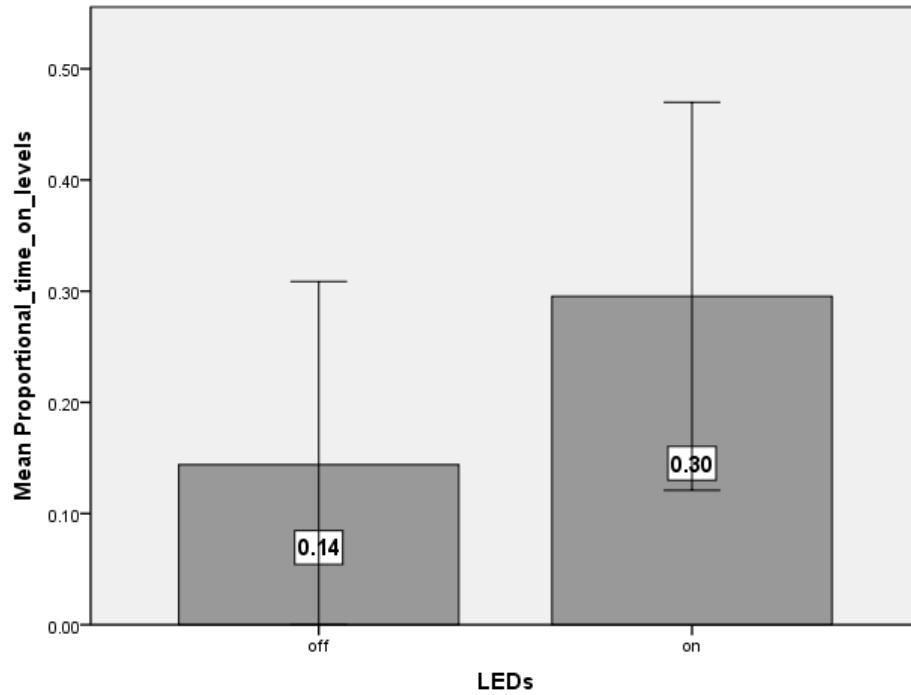
When four compound conditions of LEDs and fasting were compared in relation to proportional movement, according to descriptive statistics and graphics (figure 3) the rats moved proportionally the most in condition where LEDs were combined with fasting ( $83\% \pm 8\%$ ) and least when both of these conditions were absent ( $62\% \pm 13\%$ ). With only LEDs (no fasting), the mean proportional movement was  $80\% \pm 17\%$ , and with only fasting (no LEDs), the corresponding value was  $73\% \pm$

20%. As these four conditions were considered as repeated measures and examined with Friedman Test, no statistically significant differences in proportional movement between different measurements were found ( $\chi^2(3)=6.120$ ,  $p=0.106$ ). When all conditions were compared pair-wise with Related Samples Wilcoxon Signed Ranks Test, a statistically significant difference in proportional movement was found between conditions of ‘LEDs off, no fasting’ and ‘LEDs on, no fasting’ ( $Z=-2.023$ ,  $p=0.043$ ). When means of proportional time spent on levels were compared between different compound conditions of LEDs and fasting, the descriptive statistics indicate that rats spent most time on levels in condition with LEDs and no fasting ( $34\% \pm 38\%$ ) and least time on levels was spent in condition with LEDs off and fasting ( $10\% \pm 23\%$ ). In condition where both LEDs and fasting were present, the mean proportional time spent on levels was  $27\% \pm 30\%$ , and in condition with both LEDs and fasting were absent, the mean was  $22\% \pm 43\%$ , respectively. With Friedman Test, no statistically significant differences in proportional time spent on levels between four repeated measurements were found ( $\chi^2(3)=2.854$ ,  $p=0.415$ ). In comparisons of groups in twos with Related Samples Wilcoxon Signed Ranks Test, a statistically significant difference was found between groups of ‘LEDs on, fasting’ and ‘LEDs off, fasting’ ( $Z=-2.201$ ,  $p=0.028$ ). As number of visits to levels was compared between different conditions of LEDs and fasting, it was seen that most visits were executed during condition where only LEDs were used ( $5,4 \pm 6,3$ ), as condition where both motivators were absent comprehend least visits ( $1,00 \pm 1,00$ ). When only fasting was executed, mean of visits was  $1,6 \pm 3,1$ , and when both motivating conditions were present, mean was  $3,8 \pm 3,5$  (see also figure 3). With Friedman Test, these differences in number of visits to levels between measurements were not statistically significant ( $\chi^2(3)=2.455$ ,  $p=0.484$ ). Pair-wise comparisons with Wilcoxon Signed Ranks Test indicated that difference between conditions of ‘LEDs off, no fasting’ and ‘LEDs on, fasting’ was the only difference approaching statistically significant ( $Z=-1.761$ ,  $p=0.078$ ).

The differences in proportional movement between four different conditions of LEDs and fasting were also examined graphically between rats, as displayed in figure 4. Figure indicates that in condition where both LEDs and fasting were present (‘LEDs on, fasting’), the dispersion of the proportional movement between rats was the most modest.

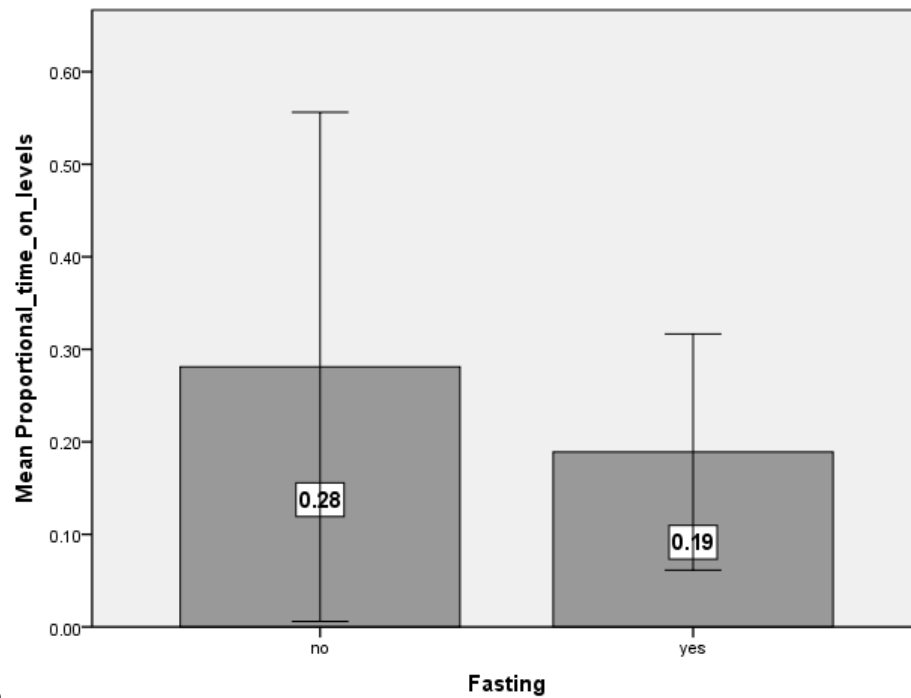


*FIGURE 1. Mean proportional movement in different conditions of A) LEDs (off / on), B) fasting (no / yes) within individual rats demonstrated as bar charts with error bars indicating 95% confidence intervals (CI).*



A

Error Bars: 95% CI



B

Error Bars: 95% CI

*FIGURE 2. Mean proportional time spent on levels in different conditions of A) LEDs (off / on) and B) fasting (no / yes) within whole sample demonstrated as bar charts with error bars indicating 95% confidence intervals (CI).*

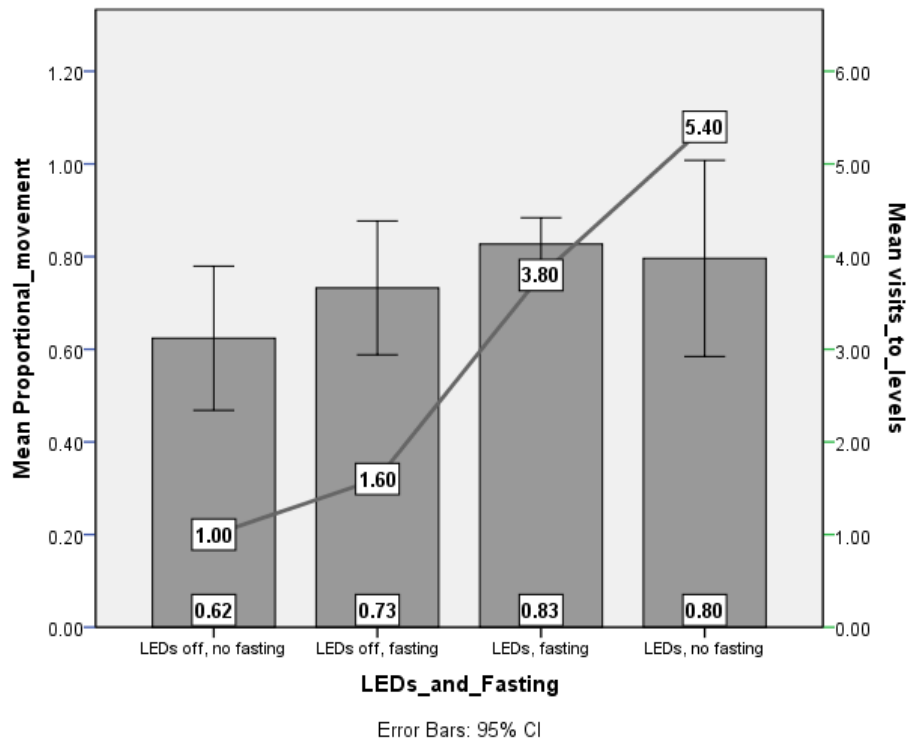


FIGURE 3. Group means of proportional movement illustrated as bar charts with error bars indicating 95% confidence intervals (CI) and group means of number of visits to levels illustrated as line chart in different conditions of LEDs and fasting.

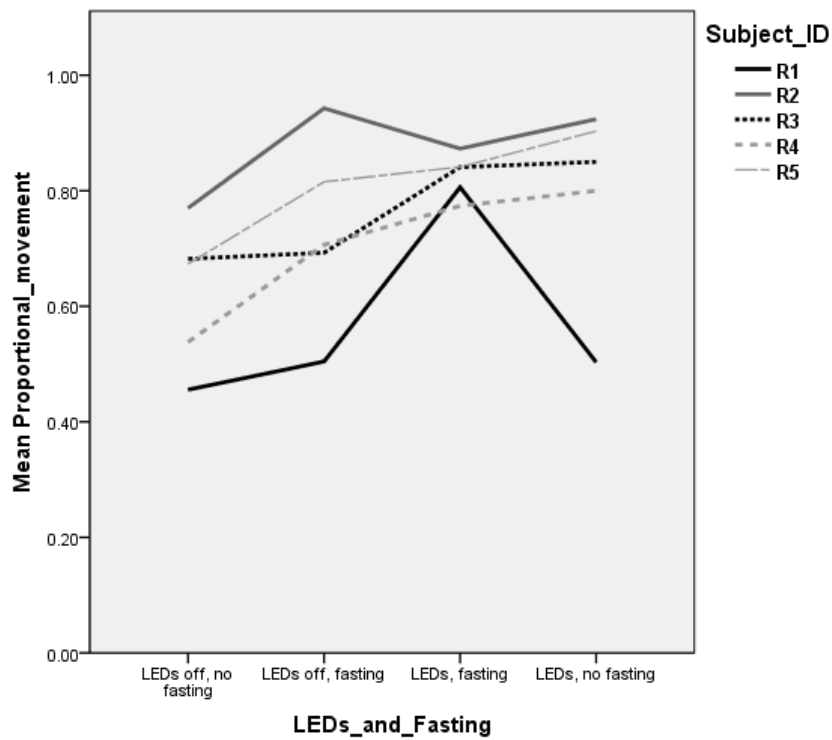


FIGURE 4. Mean proportional movements of individual rats between different conditions of LEDs and fasting illustrated as line charts.

### *3.3 Summary of results*

The presence of LEDs seemed to have a statistically significant effect on proportional movement and number of visits to levels. In proportional time spent on levels, the effect of LEDs was not statistically significant, but it approached it. Fasting seemed to cause gradual differences in dependent variables, but these failed to reach the statistical significance in all cases. When four conditions of interactions of LEDs and fasting were considered as four repeated measurements, different conditions did not cause statistically significant differences in dependent variables, but when these conditions were compared pair-wise in relation to dependent variables, statistically significant differences were found. Also, one of the combinations of LEDs and fasting clearly seemed to diminish the dispersion of proportional movement between rats. Starting point did not have a statistically significant effect on any of the dependent variables. In all examinations, the differences in activity between individual rats were taken into account with normalization.

## 4. DISCUSSION

The results of this study indicate that in 3D environment, with localized and selective used LED stimulus, the rats can be stimulated to move proportionally more, and also to use more the vertical dimension of the environment. Fasting, which, based on previous literature, is widely used and approved as a motivator in tasks where rats are supposed to search the given environment, did not seem to have as notable effect on proportional movement or on variables that measured the gravitation towards vertical dimension of the arena, as expected. When LED stimulus and fasting were employed together, this interaction seemed to diminish the dispersion of the proportional movement between rats. What was also observed was that both proportional movement and willingness to gravitate towards the vertical positions differed at some rate between rats in all conditions, which indicates endogenous differences in activity levels between animals. Despite these innate and individual biases might generally be out of reach of comprehensive manipulation, in this study the usage of LEDs seemed to have a locomotion-stimulating effect especially on more passive animals, while rats that were highly active already without the light stimulus did not react to LEDs in great manner.

### *4.1 Main effects*

The application of LEDs increased the proportional movement of the animals compared to situation where the LEDs were not used in a statistically significant manner. This increase in movement induced with LED stimulus is against the predominant argument which claims that as nocturnal animals rats tend to move more in darkness (for example Calhoun, 1962; Cardenas et al., 2001; Prusky & Douglas, 2005), but in harmony with the idea that they show aversion to light in laboratory conditions (Campell & Messing, 1969; Garcia, Cardenas & Morato, 2005) and most importantly, that this aversion might come out as striving from lightened areas to darker areas of given environment (Burn, 2008). The determining factor in our study might have been the selectivity and locality of the used lightning: in all trials, the darkness was the dominating condition, and the LEDs were used selectively in certain conditions (if the rat was passive) and locations (only on the base level of the arena); the whole arena was never illuminated, and the rats could avoid the LEDs by moving away from the light source and especially moving to upper areas of the arena. By using the LEDs on a case-specific consideration and offering the animals a chance to escape the light in all situations, we also took into account the fact that albino rats' eyes are very sensitive to light, and forced exposure to bright and intense lightning with no possibility to hide,

might cause harmful stress for animals and in some cases even damage their vision (Rao, 1991; Castelhana-Carlos & Baumans, 2009; Burn, 2008).

One major feature of our light stimulus generated with LEDs was that it seemed to have the most powerful stimulating effect on those rats that were more passive in trials with no LEDs, that is, on rats that presumably are less active and interested of their environment by nature. Naturally active rats, whose proportional movement was perceived as high already without LEDs, increased their moving only moderate or not any with LEDs, while animals with low activity without LEDs increased their moving substantially more when LEDs were added to model. This way, the LEDs seemed to effectively increase the degree of movement within quite moderate sample by homogenizing the activity levels of different rats.

What comes to rats' willingness to gravitate towards the vertical dimension and explore it, it was seen that rats spent almost twice as much time on upper levels in condition where LEDs were used, compared to condition where LEDs were off. This difference between conditions approached statistically significant but did not quite reach it, which is probably due to small sample size and notable variability between observations. However, the other variable measuring the gravitation towards the vertical dimension, the number of visits to levels, did differ between conditions of LEDs with adequate statistical significance. This implies that more visits to levels were made with LEDs compared to condition with no LEDs, although the time dedicated to these visits did not increase consistently.

Based on these findings it seems to be evident that for majority of the rats, the usage of the LEDs increased the willingness to gravitate towards the vertical dimension of the arena and explore it. This transmits the impression that from two species-specific, endogenous tendencies of a rat, the urge to maintain on the horizontal plane (as demonstrated by Grobéty & Schenk, 1992; Jovalekic et al, 2011); and the urge to avoid lightened areas (for example Campell & Messing, 1969; Burn, 2008), the latter is more dominant and could be applied as an effective motivator in future studies of place cells where the locomotion of the rats is crucial.

In examinations of the main effects of the motivators, an unexpected finding was that food deprivation did not have a statistically significant main effect on any of the dependent variables, and in some cases the effect of fasting was perceived as contrary to what was expected based on previous literature. Fasting did seem to slightly increase the proportional movement of almost all rats, but these increases were remote (especially if compared to corresponding increases generated



with LEDs) and statistically insignificant. The most striking aspects were revealed when conditions of fasting were compared in relation to variables measuring the gravitation towards vertical dimension of the arena (proportional time spent on levels and number of visits to levels): instead of promoting rats' visits to vertical dimension or residency on this dimension, fasting actually seemed to have a reducing effect on these variables, although the differences between conditions were not statistically significant. Nevertheless, it is evident that these observations express at least fair inconstancy of fasting as a generator of locomotion and gravitation towards vertical dimension, and they challenge the dominating status of food deprivation as a widely approved and one of the major motivators for rats in cognitive tasks. Also the methods of previous place cell studies with rats in 3D environment, where food rewards and preceding food deprivation have been used as main motivators (Hayman et al, 2011) or in addition with MFB stimulus (Knierim & McNaughton, 2001) become slightly questioned. What also should be noticed is that in relation to fasting, the dispersions of observations of dependent variables were salient, which implies that the capacity of fasting and food rewarding to generate highly homogenous behavior among limited and fixed sample of rats might be constricted. These results as a whole give the impression that if only one motivator ought to be selected for experiments where rats are wanted to actively move in 3D space, more powerful motivational state can be generated with LED stimulus than with fasting and food rewarding.

The significance of starting point for the degree of movement or for rats' willingness to approach the vertical dimension of the arena was discovered as insignificant in this study. As a general observation it can be pointed out that when LEDs were situated only on the base level of the arena, those rats that started the trials from up were out of reach of effective LED manipulation until they descend the levels. Then again, if the LEDs would have been situated also on levels of the levels construction, the idea of rats striving from light to darkness would have suffered, because the area with no illumination would have then diminished.

#### *4.2 Interactions*

When differences among dependent variables were examined between four related treatments of LEDs and fasting, no differences between conditions were found, but when all conditions were compared pair-wise as two related samples at a time, statistically significant differences between certain conditions were found. The failure in finding differences between four repeated measurements was probably due to small and uneven sample sizes which might have allowed the statistical differences to be hidden under dispersion. In proportional movement, against the

presupposition originating from the descriptive figures which suggested that major difference in values of this variable was between the condition where both motivational factors were present and the condition where both of these motivators were absent, the statistically significant difference was found between the condition with only LEDs (with no fasting), and the condition with both motivators absent. This statistical finding implies that the LEDs were the dominating condition which mode defined the level of proportional movement, while the condition of fasting did not vary and, hence, seemed to be insignificant.

When the mean values of proportional time spent on levels were compared between different conditions of LEDs and fasting, differences were again found only in pair-wise comparisons, as comparison between four repeated measures did not indicate any statistically significant differences and, again, the statistically significant difference found was not the one presupposed on the basis of the descriptive figures. Conditions where means of proportional time spent on levels differed from each other in statistically significant manner were the condition with LEDs and fasting, and the condition with only fasting (no LEDs). Also here, the condition of LEDs seemed to have the determining effect on dependent variable, while the mode of fasting is the same between groups, appearing as insignificant factor. In relation to proportional movement, fasting was absent in conditions that differed from each other in a statistically significant manner; and here in relation to proportional time spent on levels, fasting is present in conditions that differ from each other in a statistically significant way. These findings seem to indicate that fasting does not have an essential and consistent capacity for stimulating the rats to move in given environment in desired way. This also points towards the same possibility debated already in the section of main effects, that fasting does not seem to motivate the rats to strive for vertical dimension of the environment, whereas selective lightning seems to have its benefits in this respect.

Fasting, however, comprehends some degree of utility when combined with the usage of LEDs. When proportional movements of individual rats were compared to each other in relation to conditions of LEDs and fasting, we perceived that the dispersion of proportional movement between rats was the most modest in condition where both LEDs and fasting were present. That is, this condition with two motivators executed at the same time seemed to make the movement of the animals more homogenous. This way, the combination of fasting and LEDs brings about what is wanted from the model: quite regular results with moderate sample of rats and with moderate amount of trials.

### *4.3 Methodical considerations*

Possible disadvantage that slightly hinders the evaluation of the stimulating role of the LEDs is that the trials with LED stimulus were not randomized, as the first half of the trials were executed without the LED stimulus and last half with LED stimulus. It is possible that naturally occurring habituation to new environment might explain some proportion of increases seen in dependent variables throughout trials. Also what moderates the criticism demonstrated towards fasting in this study is that the number of cases with fasting and no fasting were not equal, which might cause that differences between conditions of fasting are not fully expressed, and real efficiency of fasting as locomotive motivator is at least slightly greater than perceived here.

One thing that might need critical evaluation in this research frame is the race of the rats used as subjects: some authorities think that albino rats should not be used in cognitive tests which dependent on vision because in albinos, this sense is observed to be impaired (Prusky & Douglas, 2005). In our study, however, we did not observe that albino rats would have had problems sensing the LED stimulus used. Also, what should be mentioned as deficiency of this study is quite small sample size which impedes the executions of statistical procedures.

## **5. CONCLUSION**

In this study we perceived that the localized and selectively used light stimulus created with five light-emitting diodes (LEDs) clearly motivated the rats to move proportionally more in 3D arena. Similar, with usage of LEDs, the rats spent more time on upper dimension of the arena and conduct more visits to levels situated on vertical dimension, compared to the situation were LEDs were not used. The effect of the LED stimulus on both locomotion and gravitation to vertical dimension was found as superior when compared to moderate food deprivation, which capacity as a motivator was vague. In conclusion we suggest that in future, if this new model for rats' free-willing movements in three-dimensional space is combined with electrophysiological studies of place cells, new and more specific information about 3D-coding of place cells could be achieved.

## 6. REFERENCES

- Barnett, S.A. (2005). Ecology. In I.Q. Whishaw & B. Kolb (Eds.), *The behavior of the laboratory rat* (pp. 15-24). New York: Oxford University Press.
- Becker, A. & Grecksch, G. (1996). Illumination has no effect on rats' behavior in the elevated plus-maze. *Physiology & Behavior*, 59 (6), 1175-1177.
- Bozarth, M.A. (1994). Pleasure systems in the brain. In D.M. Warburton (ed.), *Pleasure: The politics and the reality*. New York: John Wiley & Sons.
- Burn, C.C. (2008). What is it like to be a rat? Rat sensory perception and its implications for experimental design and rat welfare. *Applied Animal Behaviour Science*, 112, 1-32.
- Campbell, B.A. & Messing, R.B. (1969). Aversion thresholds and aversion difference limens for white light in albino and hooded rats. *Journal of Experimental Psychology*, 82 (2), 353-359.
- Calhoun, J.B. (1962). *The ecology and sociology of the norway rat*. Maryland: U.S. Department of health, education, and welfare.
- Cardenas, F., Lamprea, M.R., & Morato, S. (2001). Vibrissal sense is not the main sensory modality in rat exploratory behavior in the elevated plus-maze. *Behavioral Brain Research*, 122, 169-174.
- Castelhano-Carlos, M.J. & Baumans, V. (2009). The impact of light, noise, cage cleaning and in-house transport on welfare and stress of laboratory rats. *Laboratory Animals*, 43, 311-327.
- Colgin, L.L., Moser, E.I., & Moser, M.-B. (2008). Understanding memory through hippocampal remapping. *Trends in Neurosciences*, 31 (9), 469-477.
- Cressant, A., Muller, R.U., & Poucet, B. (2002). Remapping of place cell firing patterns after maze rotations. *Experimental Brain Research*, 143, 470-479.
- Dagyte, G., Van Der Zee, E.A., Postema, F., Luiten, P.G.M., Den Boer, J.A., Trentani, A., & Meerlo, P. (2009). Chronic but not acute foot-shock stress leads to temporary suppression of cell proliferation in rat hippocampus. *Neuroscience*, 162, 904-913.
- Derdikman, D. & Moser, E.I. (2010). A manifold of spatial maps in the brain. *Trends in Cognitive Sciences*, 14 (12), 561-569.

- Eichenbaum, H., Dudchenko, P., Wood, E., Shapiro, M., & Tanila, H. (1999). The hippocampus, memory, and place cells: Is it spatial memory or a memory space? *Neuron*, 23, 209-226.
- Eichenbaum, H., Wiener, S.I., Shapiro, M.L., & Cohen, N.J. (1989). The organization of spatial coding in the hippocampus: A study of neural ensemble activity. *The Journal of Neuroscience*, 9 (8), 2764-2775.
- Fox, S.E. & Ranck, J.B. Jr. (1981). Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. *Experimental Brain Research*, 41, 399-410.
- Fyhn, M., Molden, S., Witter, M. P., Moser, E. I., & Moser, M.-B. (2004). Spatial representation in the entorhinal cortex. *Science*, 305, 1258-1264.
- Garcia, A.M.B, Cardenas, F.P., & Morato, S. (2005). Effect of different illumination levels on rat behavior in the elevated plus-maze. *Physiology & Behavior*, 85, 265-270.
- Gothard, K.M., Skaggs, W.E., Moore, K.M., & McNaughton, B.L. (1996). Binding of hippocampal CA1 neural activity to multiple reference frames in a landmark-based navigation task. *The Journal of Neuroscience*, 16 (2), 823-835.
- Grobéty, M.-C. & Schenk, F. (1992). Spatial learning in a three-dimensional maze. *Animal Behaviour*, 43, 1101-1120.
- Hafting, T., Fyhn, M., Molden, S., Moser, M.-B., & Moser, E. I. (2005). Microstructure of a spatial map in the entorhinal cortex. *Nature*, 436, 801–806.
- Hayman, R., Verriotis, M.A., Jovalekic, A., Fenton, A.A., & Jeffery, K.J. (2011). Anisotropic encoding of three-dimensional space by place cells and grid cells. *Nature Neuroscience*, 14 (9), 1182-1188.
- Jovalekic, A., Hayman, R., Becares, N., Reid, H., Thomas, G., Wilson, J., & Jeffery, K. (2011). Horizontal biases in rats' use of three-dimensional space. *Behavioural Brain Research*, 222, 279-288.
- Knierim, J.J. & McNaughton, B.L. (2001). Hippocampal place-cell firing during movement in three-dimensional space. *Journal of Neurophysiology*, 85, 105–116.
- Moser, E.I., Kropff, E., & Moser, M.-B. (2008). Place cells, grid cells, and the brain's spatial representation system. *Annual Review of Neuroscience*, 31, 69–89.

- Muller, R.U. & Kubie, J.L. (1987). The effects of changes in the environment on the spatial firing of hippocampal complex-spike cells. *The Journal of Neuroscience*, 7 (7), 1951-1968.
- Muller, R.U., Kubie, J.L., & Ranck, J.B. (1987). Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. *The Journal of Neuroscience*. 7 (7), 1935-1950.
- Mumby, D.G. (2005). Object recognition. In I.Q. Whishaw & B. Kolb (Eds.), *The behavior of the laboratory rat* (pp. 15-24). New York: Oxford University Press.
- O'Keefe, J. & Conway, D.H. (1978). Hippocampal place units in the freely moving rat: why they fire where they fire. *Experimental Brain Research*, 31, 573–90.
- O'Keefe, J. & Dostrovsky, J. (1971). The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. *Brain Research*, 34, 171–75.
- O'Keefe, J. & Nadel, L. (1978). *The Hippocampus as a cognitive map*. Oxford: Clarendon Press.
- Prusky, G.T. & Douglas, R.M. (2005). Vision. In I.Q. Whishaw & B. Kolb (Eds.), *The Behavior of the Laboratory Rat* (pp.49 -59). New York: Oxford University Press.
- Rao, G.N. (1991). Light intensity-associated eye lesions of Fischer 344 rats in long-term studies. *Toxicological Pathology*, 19 (2), 148-155.
- Scoville, W.B. & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery & Psychiatry*, 20, 11-21.
- Shapiro, M.L., Tanila, H., & Eichenbaum, H. (1997). Cues that hippocampal place cells encode: Dynamic and hierarchical representation of local and distal stimuli. *Hippocampus*, 7, 624-642.
- Slawecki, C.J & Roth, J. (2005). Assessment of sustained attention in ad libitum fed Wistar rats: Effects of MK-801. *Physiology & Behavior*, 85, 346-353.
- Tanila, H., Shapiro, M.L., & Eichenbaum, H. (1997). Discordance of spatial representation in ensembles of hippocampal place cells. *Hippocampus*, 7, 613-623.
- Taube, J.S., Muller, R.U., & Ranck, J.B. Jr. (1990). Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. *The Journal of Neuroscience*, 10 (2), 420-435.

Taube, J.S., Muller, R.U., & Ranck, J.B. Jr. (1990a). Head-direction cells recorded from the postsubiculum in freely moving rats. II. Effects of environmental manipulations. *The Journal of Neuroscience*, 10 (2), 436-447.

Telensky, P., Svoboda, J., Blahna, K., Bureš, J., Kubik, S., & Stuchlik, A. (2011). Functional inactivation of the rat hippocampus disrupts avoidance of a moving object. *Proceedings of the National Academy of Sciences*, 108 (13), 5414-5418.

Thompson, L.T. & Best, P.J. (1989). Place cells and silent cells in the hippocampus of freely-behaving rats. *The Journal of Neuroscience*, 9 (7), 2382-2390.

Wilson, M.A. & McNaughton, B.L. (1993). Dynamics of the hippocampal ensemble code for space. *Science*, 261, 1055-1058.