Memory and motor function in wild type Wistar rats -

A pilot study to evaluate a series of behavioural tests

Facilitating future research on Huntington's disease

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Degree project, programme in medicine

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Gothenburg, Sweden 2020

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Abstract

Huntington's disease (HD) is a debilitating neurodegenerative disorder. The core symptoms associated with the disease are psychiatric symptoms, impaired cognition and loss of motor function. Recent biotechnological advancements have yielded tools for targeting the pathological expansion of the Huntingtin-gene and are thus offering new, potential treatment strategies. Our research group is currently involved in molecular and cell-based research aiming to study one of these techniques in a genetic rat model for Huntington's disease, the BACHD rat. The main aim of this project was to evaluate test protocols and practical aspects of four behavioural tests assessing memory and motor function in adult wild type Wistar rats. Object recognition and object location tests were used for assessment of episodic-like memory. Rota-rod and Montoya staircase test were used for assessment of gross- and fine motor function respectively. The animals were unable to discriminate between familiar and novel objects or locations when presented to LEGO® constructions in novel object recognition and location tests. However, a significant novel object preference was seen when using more fundamentally different objects. The rats showed a significant increase in sugar pellet consumption and success rate in Montoya staircase test. No significant improvement in latency to fall was seen in the Rota-rod test as a majority of the rats learned to escape the device. All tests evaluated in the present study have potential for future use in assessing memory and motor function the BACHD rat, but methodological improvements are needed before the tests are implemented in future research on experimental treatment strategies for Huntington's disease.

Key words: BACHD rat, object recognition, object location, Montoya staircase, Rota-rod

1. Introduction

1.1 Huntington's disease

Huntington's disease (HD) is a debilitating condition with a prevalence of approximately 10.6-13.7 per 100'000 in a Western-European population (1). HD is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion (polyglutamine repeat) in the HTT-gene on chromosome four encoding the protein Huntingtin. The physiological role of Huntingtin is not completely clear, it is expressed in cells of the nervous system as well as in various cell types throughout the body and appears to be is involved in multiple fundamental cellular processes (1). It has been shown to interact with a wide array of proteins and has been described as a scaffolding protein vital to intracellular vesicle trafficking and selective macroautophagy (a protective cellular mechanism to cope with stressors) (2, 3). The CAG polyglutamine expansion seen in HD leads to the expression of a defect protein, mutant huntingtin (mHTT), prone to fragmentation and intracellular aggregation promoting cellular dysfunction through several mechanisms which ultimately leads to cellular death (1). The penetrance and age of onset of HD is dependent on the number of CAG repeats present. Less than 27 repeats do not cause the disease, 34-39 repeats show variable penetrance while more than 39 repeats have full penetrance (4). More repeats have been demonstrated to cause earlier onset (5). The disease commonly debuts in mid-life and progresses inexorably with severe consequences for patients and relatives (4). The consequences of mHTT are most prominent in medium spiny neurons (MSN) of the putamen and caudate nucleus, together forming a part of the basal nuclei of the brain known as the dorsal striatum. As the disease progresses it affects other parts of the brain including thalamus, hippocampus and cortical white

matter (4). The core symptoms associated with the disease are psychiatric symptoms, impaired cognition and loss of motor function. Common psychiatric symptoms are apathy and affective symptoms such as depression, anxiety and irritability. Cognitive symptoms are often presented as impairment of executive functions, information processing and memory. The disease is well known for the choreatic "dance-like" movements associated with the initial hyperkinesia seen in Huntington's disease. The hyperkinesia gradually progresses to hypokinesia with symptoms such as gait disturbances, dystonia and rigidity (1, 4). Despite being a monogenetic disease in which the pathogenesis is well understood no cure is currently available and the treatment options are limited (6).

1.2 Gene therapy in Huntington's disease?

Recent biotechnological advancements have yielded tools for targeting the pathological expansion of the HTT-gene and are thus offering new, potential treatment strategies. One such strategy is genetic editing using the CRISPR/Cas9 system, a molecular machinery that originates from the immune systems of prokaryotic organisms. CRISPR is an RNA sequence that acts as a guide for the protein Cas9 is which is capable of acting as a nuclease ("genetic scissor") targeting specific DNA sequences complementary to CRISPR (7). The sequence in CRISPR can be modified to target a desired genetic sequence in order to silence it. This method could therefore be used to silence the pathogenic polyglutamine repeats seen in HD, demonstrated in a recent study by Shin and colleagues (8). However, many challenges remain before the technique could be implemented in clinical research. Our research group is currently involved in molecular and cell-based research aiming to study the CRISPR/Cas9 technique in animal models for Huntington's disease.

1.3 Behavioural assessment

In vivo testing in animal disease models for HD is essential to investigate the CRISPR/Cas9 based treatment strategy described in the section above. Behavioural neuroscience is scientific field where the behaviour of an organism is studied to draw conclusions about functions of the central nervous system as a whole. It is one of a few ways to study complex brain functions that rely on intact neuronal networks. Psychiatric status, memory and motor function are examples of such functions which are all affected in Huntington's disease. Preclinically, behavioural studies are conducted in animal models. The rat (*Rattus Norvegicus*) is an intelligent animal capable of complex behaviours which enables researchers to study the core symptoms of HD. We have previously investigated behavioural tests regarding psychiatric symptoms in a rat model for depression presented in a previous study by our research group (9). In addition to this, we now aim to evaluate tests of memory and motor function. Numerous behavioural tests assessing memory and motor-function in rodents are described in the literature but there are variations in the results obtained when applying them on genetic rat models (or more commonly mouse models) of Huntington's disease. A challenge faced regarding behavioural assessment is that small discrepancies in testing protocols, equipment, animal handling and housing can affect the results. Therefore, the testing procedures needs to be evaluated in wild type rats under the conditions present in our laboratory before applying the tests on disease models. The long-term goal is to construct a series of behavioural tests to assess all the core symptoms of Huntington's disease. The behavioural tests chosen for this study will be presented in the following section.

1.3.1 Object recognition and object location test

Novel object test (NOR), also called object recognition test (ORT), was originally described by Ennaceur and Delacour in 1988 (10). It is a simple test to assess episodic-like memory in rodents (11). An innate "bias towards novelty" appears to be a well-preserved trait in rodents (12). A rat with intact memory function, when presented to one familiar and one novel object simultaneously, will spend significantly more time exploring the novel object. This behaviour is instinctive which offers the advantage that no training or food rewards are needed. A variation of this test called object location test (OLT) has been developed to add a spatial component to episodic-like memory testing (13). The rat is presented to two identical objects and then one object is moved to a novel location. Analogous to ORT the rat will spend significantly more time investigating an object at a novel location. For a rat to exhibit novel object or location preference, it must successfully encode the memory of an object (or its spatial orientation) during a familiarization phase (trial one, T1). The memory must remain intact for a period of time called retention interval (Ri) and the rat must be able to retrieve the memory when presented to a familiar and novel object (or novel location) during the testing phase (trial two, T2). Sufficient object exploration time in T1 is a prerequisite for memory formation and thus to identify an object or position as novel in T2. Therefore, an inclusion criterion of at least 20-30 seconds is often set as the minimum exploration needed during the familiarization phase (11). The length of the retention interval is important since it has been shown to affect the performance in T2. A longer retention interval provides a greater challenge for rats to differentiate between familiar and novel objects (10). The time applied varies significantly between studies, retention intervals of seconds, minutes, hours and days have been used (11). Different lengths of retention intervals have been discussed to address different aspects of

memory function. A short retention interval may test short term memory while novel object identification after longer intervals is more dependent on long-term memory (11). Furthermore, the objects used for ORT needs to be chosen carefully. A rat must be able to differentiate between the objects, but it must also find the objects equally interesting in order to avoid object preference at baseline.

1.3.2 Montoya staircase test

The Montoya staircase test was originally described by Montoya and colleagues in 1991 (14). The test was constructed to assess fine motor skills in the forelimbs of rats. Rats are placed in a small box with a narrow compartment where they can reach for sugar pellets placed in wells on different levels in a stair-like construction, the rats can reach the pellets with their tongue at the highest levels of the stair but at lower levels they must learn to collect the pellets with their paws which requires dexterity and fine motor skills. Rats are trained on repeated sessions, the number of sugar pellets consumed, and the number of sugar pellets dropped is counted after each session. The test includes critical steps such as food deprivation to promote food-seeking behaviour, and the rats must be habituated to sugar pellets before testing since they can show neophobia towards new diets. The time needed for wild type rats to learn the procedure seems to vary between strains, but the Wistar rat has been demonstrated to learn and improve in collecting sugar pellets over a period of at least 10 days, reaching an average of approximately 15 sugar pellets on the last day of testing (15). Another study has defined learning in Wistar rats as collecting at least twelve sugar pellets in a single session (16).

1.3.3 Rota-rod

Rota-rod is a classical and widely used behavioural test to assess gross motor function, originally described by Dunham and Miya in 1957 (17). Rodent such as rats are trained to run

on an elevated, rotating rod and the latency to fall is recorded. There are two main protocols described, one protocol where the rats run at a constant speed which is the ramped up in discreet steps, and one where the rotating speed is gradually increased over a few minutes, thus increasing the level of difficulty (18). The accelerating protocol is perhaps the most common, rats usually learn to balance on the rod and reach a stable performance over a few days with an increase in the latency to fall (15). The test does not rely on food reward or extensive training since the height of fall is, supposedly, sufficient to motivate the rats to stay on the rotating rod.

1.4 The BACHD rat.

The most recent rat disease model for HD is the transgenic BACHD rat, developed in 2012 by Yu-Taeger and colleagues (19). BAC is the acronym for Bacterial Artificial Chromosome which is a genetic element developed from the *E-coli* F-factor, a plasmid that enables cloning of genes and transferring of genes between organisms (20). The BAC of the HD disease model contains the human Huntingtin gene with 97 CAG repeats, encoding the full-length mutant Huntingtin protein (fl-mhtt) (19). Several studies have been conducted to characterize the BACHD rat, most of them by the group that developed the model. The BACHD rat has been shown to have earlier onset and faster disease progression compared to the earlier genetic models. Huntingtin aggregates have been demonstrated at twelve months of age, primarily in the neocortex and limbic structures, but only to a lesser extent in the dorsal striatum. The BACHD rat exhibit several behavioural deficits that can be related to the core symptoms of HD. Gross motor deficits have been demonstrated from one month of age in the Rota-rod device. An abnormal gait pattern with shorter, wider steps has been demonstrated from 14 months of age. Furthermore, it has been showed to exhibit less avoidance of open areas

(normally disliked by healthy rats) (19). Further research on the model have demonstrated less endurance and grip strength, less overall motor activity, altered circadian activity and impairment in cognitive tasks regarding spatial, associative and short-term memory (21-23). However, tests regarding fine motor skills have not shown signs of impairment in the BACHD rat model (21). Apart from the behavioural deficits, the BACHD rat has been demonstrated to have an altered metabolic profile with more adipose tissue, less muscle-mass, and morphological characteristics. Measurements of leptin-levels in blood has showed elevated levels in BACHD rats, they consume less food and seem less motivated to food intake even after food-deprivation compared to WT rats (24, 25). However, it remains unclear whether the decrease in food intake is caused by motoric, metabolic or psychiatric abnormalities.

1.5 Aim

The main aim of this project is to evaluate test protocols and practical aspects of four behavioural tests assessing memory and motor function in adult wild type Wistar rats. The long-term goal is to construct a series of behavioural tests to assess the core symptoms of Huntington's disease in the BACHD rat disease model, facilitating future studies of experimental treatment strategies.

2. Materials and methods

2.1 Ethical considerations

This project is based on research using live animals since evaluation of complex brain functions such as memory and motor function is best assessed in vivo. All means have been taken to minimize stress in animals, vital to maintain the quality of the experiments at high standards. The number of live animals used was reduced to a minimum. The principle of "Replacement, Reduction and Refinement" has been applied. The experiments were approved by the Gothenburg ethical committee for animal research. Object recognition- and object location tests (Regionala etikprövningsnämnden Göteborg, ethical permit 31-2016). Rota-rod and Montoya staircase test (Regionala etikprövningsnämnden Göteborg, ethical permit 101-2016) has been performed in collaboration with the Department of Pharmacology.

2.2 Animals

A total of twenty-eight, adult, wild-type Wistar rats from own breeding was used for behavioural experiments (twenty-two males and six females). The age of the rats was 56 to 63 postnatal days at start of testing (p56-p63). The age was chosen to match the age we aim to use for future behavioural assessment in the BACHD rat. All animals were healthy and untreated. Rats were housed under standard conditions in the Centre for Experimental Biomedicine in Gothenburg (three or four per cage, twelve-hour light/dark cycle, ad libitum access to food and water, basic enrichment). The rats were handled daily (picked up, tickled on the back and marked on the tail with a pen) for a total of ten days prior start of experiments, habituating the rats to being handled in order to minimize stress during the experimental procedures. Object recognition- and location tests were performed in the room in which they were housed.

However, for the Montoya staircase test and Rota-rod testing, rats were carefully transported to a separate testing room. They were allowed a minimum of fifteen minutes for acclimation in the testing room prior to the start of the experiments.

2.3 Experimental design

2.3.1 Memory

Seven males and six females (one litter) were assigned for memory testing in two main sessions of object recognition test (ORT1 and ORT2) using different retention intervals, four and one-hours respectively. These intervals were chosen to assess long-term memory at different levels of difficulty. The same animals were assigned to one session of object location test (OLT) using a retention interval of one hour. An additional four males were assigned to a third session of ORT (ORT3), using a one-hour retention interval and more fundamentally different objects. The age of the rats was p56 at start of testing.

2.3.2 Motor function

Fifteen males (two litters) were assigned for assessment of motor function in the Montoya staircase test and Rota-rod test. Litter one (n=6) and litter two (n=9) was p63 and p56 respectively at the start of Montoya staircase test. Montoya staircase test was performed in the morning and Rota-rod was performed in the afternoon. The experiments were designed so that all animals would be the same age when starting the Rota-rod test. The rats were allowed a minimum of three hours in their home-cages between the experiments. A total of three animals

were taken out of testing in the Rota-rod experiment since they systematically escaped the device before testing began.

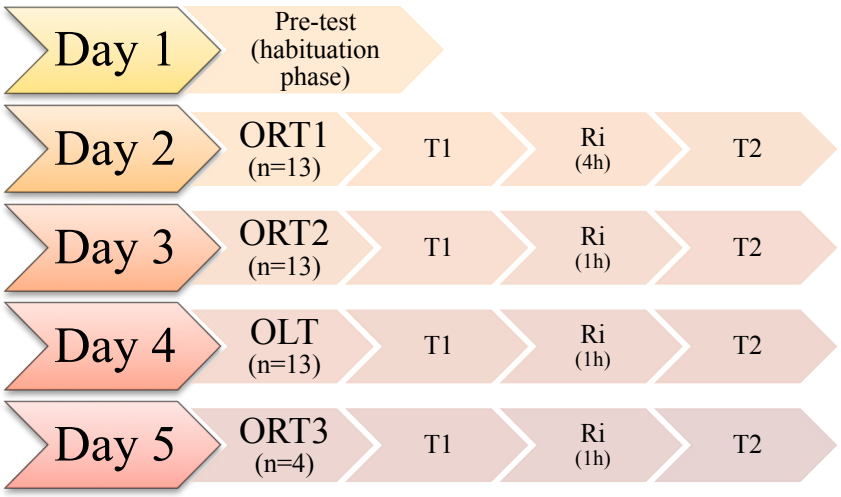


Figure 1. *Experimental design of object recognition test (ORT) and object location test (OLT). Testing was conducted on five consecutive days. A pre-test was conducted on day one to minimize stress during subsequent testing. ORT was comprised of three sessions (ORT1, ORT2, and ORT3). The familiarization phase (T1) was separated from the test phase (T2) by a delay called the retention interval (Ri) of one- or four hours.*

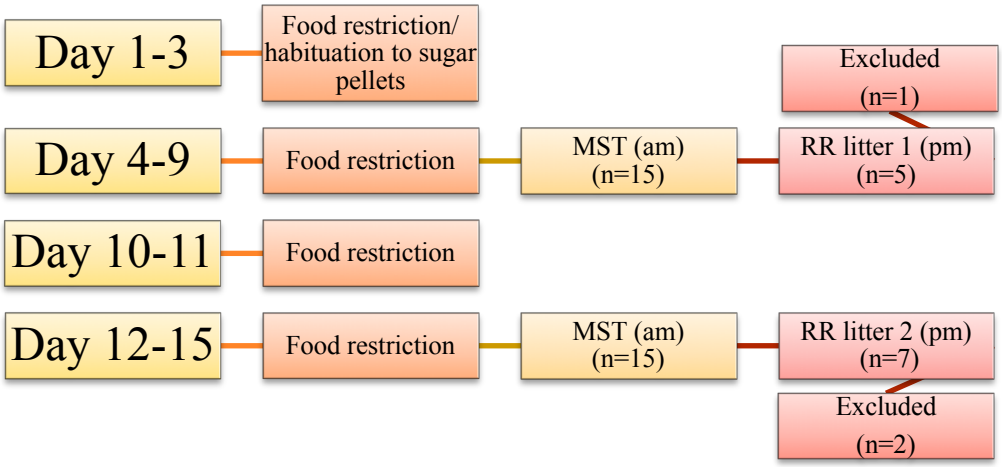


Figure 2*. Experimental design of Montoya staircase test (MST) and Rota-rod (RR). The same animals were used for the experiments which were run in parallel. MST was performed in the morning (am) and RR in the afternoon (pm). The two litters were tested separately in RR. A total of three animals were excluded from RR since they systematically escaped the device before testing began.*

2.4 Object recognition and object location test

The methods used was similar to the procedure originally described by Ennaceur and Delacour (10). ORT and OLT was carried out in a transparent Plexiglas square-shaped arena (70x70 cm). The inside of the arena was covered with a black, matte film in order to minimize light reflections and prevent potential visual distractions. A square-shaped centre-area (35x35cm) was marked out with red tape. The objects were placed in the corners of the centre-area (fig. 4). All objects used were constructed of LEGO® blocks of different shapes and colours (fig. 3). The objects were designed to be similar in colour intensity and size. The objects did not have any value for the rats and were not associated with food rewards.

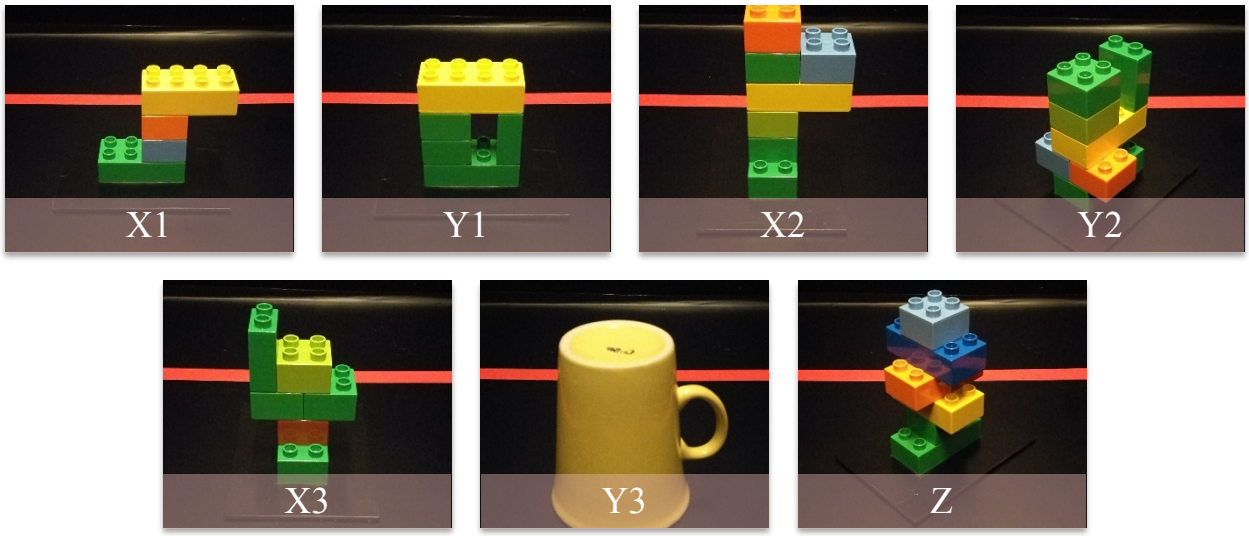


Figure 3. *Objects used for object recognition test (ORT) and object localization test (OLT). X1 and Y1 was used for the first session of ORT (ORT1), X2 and Y2 for the second (ORT2), X3 and Y3 for the third (ORT3). Z was used for object location test (OLT).*

A total of seven different objects were used for memory assessment, two pairs of different objects (X and Y) for each session of ORT and one pair of identical objects for OLT (Z). For half of the rats, object X were presented as the familiar object in T1 and object Y was the novel

object in T2. For the other half the objects were switched. This was done in order to control for baseline difference in object preference in ORT1 and ORT2, but not in ORT3. Furthermore, the side on which the novel object was presented was alternated to control for side preference. The same principle of side switching was applied in OLT. The LEGO® constructions were mounted on black plastic plates which were fixated to the floor of the arena with black duct tape. This was done to prevent the objects from being displaced by the rats. The test was set up in relatively low light conditions (40 +/- 5 lux in the arena). The arena and the objects were thoroughly cleaned with 70% ethanol solution between every rat tested to minimize olfactory stimuli.

On day one, the rats were placed individually in the arena without objects for a habituation phase where they were allowed to explore the arena for five minutes. On day two, the rats were returned individually to the arena for ORT1. During the five-minute familiarization phase (T1) they rats were presented to two identical objects (a1 and a2). The rats were returned to their home cages for a retention interval of four hours. They were returned to the arena for a three-minute testing phase (T2) with one of the familiar objects (a3) and a novel object (b). ORT2 was performed on day three, the same procedure was applied but with a new set of objects and the retention interval was shortened to one hour. On day four, the rats were again presented to a new pair of identical objects in T1 of OLT. The rats were then returned to their home cages for a one-hour retention interval. When the rats were returned for the test phase (T2), the same objects were present but the location of one of them had been changed. On day five, ORT3 was performed in four of the male animals used in previous sessions applying a one-hour retention interval. One of the LEGO® constructions was exchanged for a yellow coffee cup (novel object)

in T2 in order to investigate object preference when presented to a more fundamentally different object. Males and females were tested separately in all sessions.

The rats were filmed from above using a Logitech webcam and the open-source software OBS studio. The video footage was used to measure object exploration, exploration was defined as having the nose pointed towards the object at a distance of two centimetres or closer as described in the original study (10). The video-footage was analysed manually, the time spent exploring each object in T1 and T2 was measured using a stopwatch. The total object exploration time in each T1 and T2 (defined as e1 and e2 respectively) was calculated. The object exploration time of each T1 was clocked one time. The video footage of each T2 was clocked two times at different occasions and the results of the measurements averaged. If the measurements of exploration time differed two seconds or more in a single trial, the video footage of that trial was analysed a third time. A discrimination index (d1) was calculated as the difference in time spent exploring the novel object and the familiar object in T2. A discrimination ratio (d2) was calculated as the discrimination index (d1) divided by the total exploration time in T2 (e2) using the formula: $d2 = \frac{(\mathrm{b}-\mathrm{a3})}{e2} = \frac{\mathrm{d1}}{e2}$.

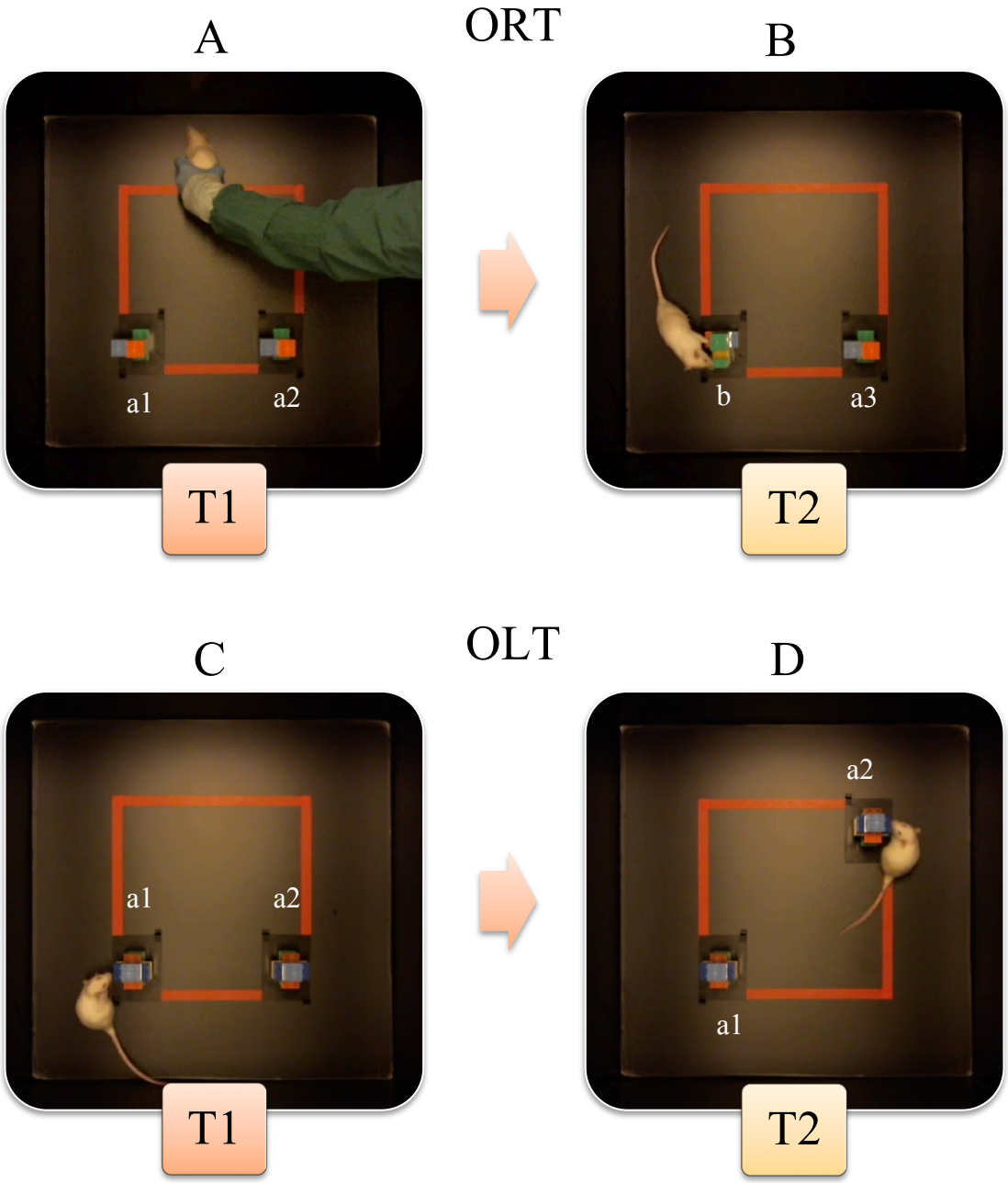


Figure 4. *The objects and their placements in the arena during the familiarization phase (T1) and test phase (T2) in object recognition test (ORT) and object location test (OLT) respectively. The familiar objects are marked "a", the novel object is marked "b". The rats were always placed in the same spot facing the wall, shown in the upper left picture (**A**).*

2.5 Montoya staircase test

The rats were put on an overnight food restriction schedule starting two days before testing to stimulate food-seeking behaviour. The food restriction started by removing all food from the cages in the afternoon at 17:00. The morning after, the rats were weighed, and received 8 % of their body weight in food. The rats continued to receive 8% of their body weight in food every day during the testing period. The weight curve of each rat was followed. The rats were acclimated to sugar pellets (45mg, BioServ, Frenchtown, NJ, U.S.A.) three days before testing. On day one, the rats were fed 2-3 sugar pellets each by hand in their home cage. On day two, the rats picked sugar pellets from a bowl held in front of them. On day three, a bowl with sugar pellets was put in the front left corner of each cage to allow consumption without human interaction.

The testing was performed in a Montoya staircase apparatus (Campden Instruments Ltd, Loughborough, UK) dimensioned for rats. The large compartment measured approximately 20.5 cm in length, 12.5 cm in height and 12.0 cm in width. The small compartment measured 16.5 cm, 12.5 cm and 6.5 cm correspondingly. The apparatus was placed in a sound isolated, well-ventilated cupboard. The apparatus was covered with blankets to minimize sensory distractions. A small portion of the narrow section was left uncovered enabling the experimenter to observe the behaviour of the rats during testing. The rats were placed individually in the apparatus and were left to explore and collect sugar pellets for one session à fifteen minutes per day for ten days. If a rat only consumed one sugar pellet or less during a session, the stair with sugar pellets was lifted manually to help the rat to reach a few sugar pellets in order to maintain motivation. The pellets picked after the testing session with help from the experimenter was not

included in the analysis. The rats only received help on the first five days of testing to enhance the learning process and promote motivation. All rats were tested between 08:00 – 12:00 and received 8 % of body-weight in food directly when testing was completed. The rats that were assigned to Rota-rod testing in the afternoon was tested first in the morning. The number of pellets eaten (pe), and the number of pellets dropped (pd) during each session was counted. The mean number of sugar pellets consumed each day was calculated for each day of testing. The mean success rate for each day of testing was calculated using the formula:

$$Success\ rate = \frac{\text{pe}}{(\text{pe+pd})}$$

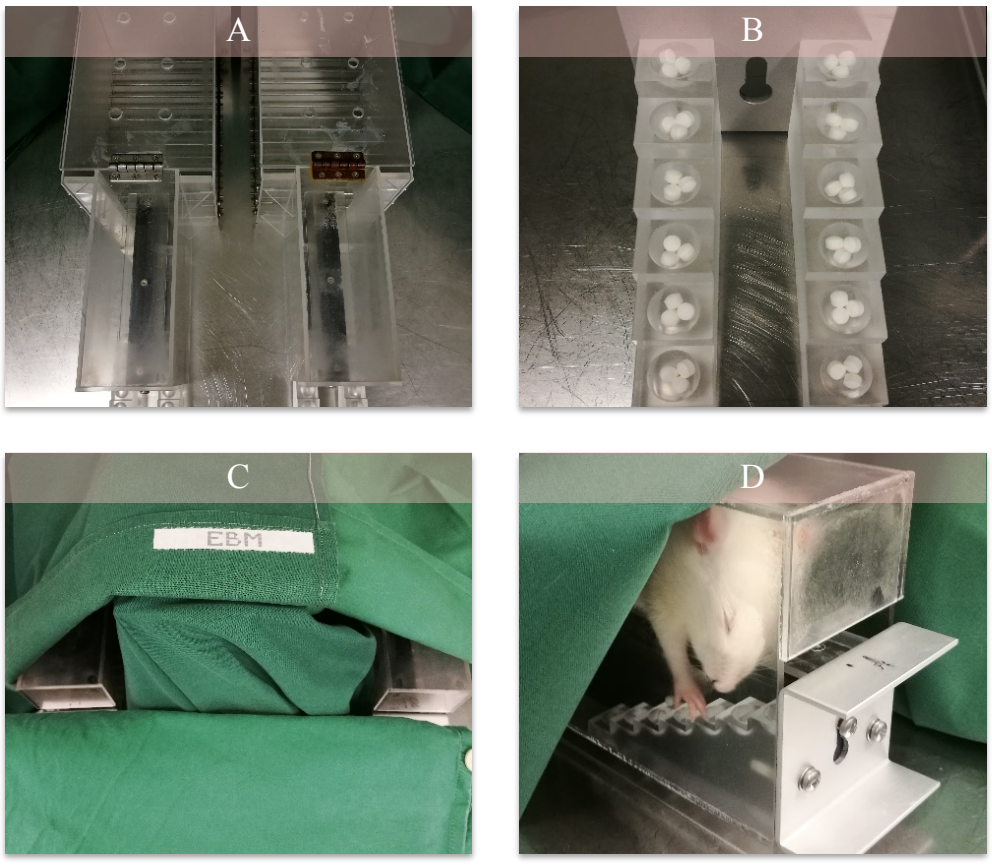


Figure 5. A*: Two identical Montoya staircase devices. One rat is placed in the larger compartment (top) and can enter the narrow compartment (bottom) to reach for sugar pellets.* ***B:*** *A staircase loaded with three sugar pellets per well.* ***C:*** *The apparatuses were covered with blankets to minimize distractions. A small opening allowed observation during testing.* ***D:*** *Rat reaching for sugar pellets in the Montoya staircase device.*

Figure 6. A: *Rats running on the Rota-rod, one rat has fallen down.* ***B:*** *One rat learned to escape the device elegantly by jumping up on the top section.*

2.6 Rota-rod

The Rota-rod device (LE-8500, Panlab, S.L.U. Spain) was placed in the same ventilated, sound attenuating cupboard as described above. A rotating cylinder with a diameter of 6 cm was used. The drop from the bottom of the cylinder was approximately 14 cm. A removable top section separating the rats from each other was used to prevent the rats from distracting each other. Two or three rats were tested at the same time. Care was taken not to disturb the rats while running. An accelerating protocol was applied, the apparatus was set to accelerate the cylinder automatically from 4 to 40 rotations per minute (rpm) over five minutes. The latency to fall and rpm at fall was recorded when a rat fell down. Rats that fell down within 20 seconds from start were put back on the rod several times if needed on the first day of testing. The following days, the rats were only put back once if they fell within 20 seconds. All rats underwent four trials per day with a minimum of 20 minutes between trials. Rota-rod consisted of five days of testing, the mean latency to fall was calculated for each day of testing. Rats that figured out how to

escape the Rota-rod immediately after being placed on the cylinder had to be taken out of testing (fig.6-B).

2.7 Statistical analysis

All tests measured quantitative, continuous variables in the ratio scale. The standard deviation of the sampling distribution for the mean was calculated (standard error of the mean, SEM). The data was analysed in Excel and GraphPad Prism 8. Normal distribution was assumed (and tested where applicable), a paired Student's t-test was used for comparisons within groups and an unpaired t-test for comparison between groups. A Wilcoxon signed rank test was used for significance testing when the data was not normally distributed. To compensate for multiple comparisons, in order to avoid type 1 errors, the threshold for statistical significance was corrected using a modified Holm-Bonferroni correction.

3. Results

3.1 Object recognition and object location test

One litter consisting of thirteen rats (seven males, six females) were tested in the object recognition test (ORT) and the object location test (OLT) assessing different aspects of episodic-like memory. Object recognition test was performed in two main sessions (ORT1 and ORT2) applying a four- and one-hour retention interval respectively. Four males were tested in a third session of object recognition test (ORT3) applying a one-hour retention interval and more fundamentally different objects. Object location test (OLT) was performed in one session using a one-hour retention interval. The rats displayed similar total exploratory behaviour (e1)

during the familiarization phase (T1) in all sessions of ORT and OLT (table 1). Female rats spent more time exploring the objects compared to their male counterparts in all sessions. This difference was not statistically significant in each session alone ($p>0.05$, data not shown), but since the procedure of T1 was identical in all sessions the data from each session was pooled prior to comparison. When comparing e1 of males and females in all sessions, there was a statistically statistical significance ($p = 0.03$, fig. 7). Most rats reached a total exploration time in the familiarization phase (e1) exceeding 20 seconds. Only a few animals displayed a relatively short exploration time in T1 compared to their littermates, three males and one female in ORT1, one male in ORT2 and two males in OLT explored the objects less than 20 seconds. The rats did not show side- or object preference at baseline in any of the sessions ($p > 0.05$, data not shown). Object preference at baseline was not controlled in the third session of ORT. The total exploration time (e2) in the test phase (T2) was similar in all session of ORT and OLT (table 1). Males and females showed a similar total exploration time in T2 ($p > 0.05$). Most rats explored the objects in the test phase (e2) for at least 10 seconds. Only two animals displayed a relatively short exploration time in T2 compared to their littermates, one male in ORT1 and one male in ORT2 explored the objects less than 10 seconds in T2.

Table 1. Total object exploration time in T1 and T2

	ORT1		ORT2		ORT3		OLT	
	e1	**e2**	**e1**	**e2**	**e1**	**e2**	**e1**	**e2**
Males	32.5 (7.0)	22.5 (4.7)	31.2 (4.4)	22.1 (3.1)	30.6 (3.2)	34.8 (4.3)	31.7 (5.7)	28.8 (5.7)
Females	42.8 (7.1)	21.6 (1.6)	42.3 (4.3)	20.1 (1.6)			38.9 (3.7)	20.9 (2.2)

Mean total object exploration time (s) in the five-minute familiarization phases (e1) and three-minute test phases (e2) in all three sessions of object recognition test (ORT1-ORT3) and object location test (OLT), data for males (n=7) and females (n=6) shown separately. ORT3 only included males (n=4). The standard deviation of the sampling distribution is shown within parentheses as standard error of the mean (SEM).

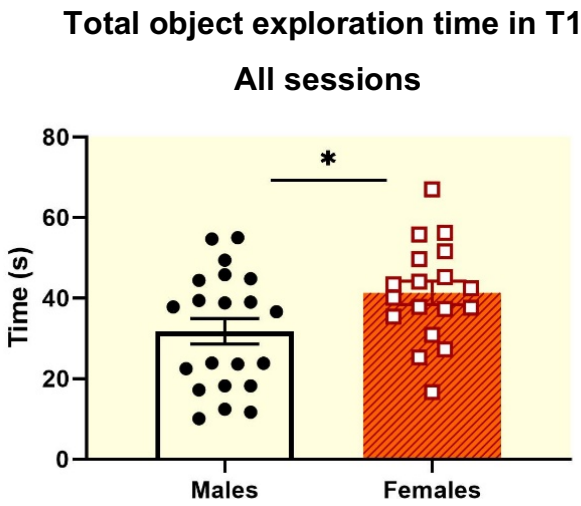


***Figure 7**. A comparison of gender differences in mean total object exploration time in all familiarization phases of object recognition/location tests. Females spent significantly more time exploring the objects (p=0.03) over a five-minute period. Error bars shows the standard deviation of the sampling distribution as standard error of the mean (SEM).*

There was no significant difference in exploration time comparing the familiar object and the novel object when applying a four-hour retention interval in ORT1 ($p>0.05$, fig.8-A). When the retention interval was shortened to one hour in ORT2, there was a subtle tendency towards preference for the novel object although slightly below the threshold for statistical significance ($p=0.06$, fig.8-B). When exchanging one of the LEGO® figures to a fundamentally different novel object (yellow cup) in ORT3, the rats spent significantly more time exploring the novel object ($p=0.005$, fig.8-C). There was no significant difference in exploration time in OLT comparing the familiar and novel position ($p>0.05$, fig.8-D). The ability of the rats to discriminate between the objects in all sessions is shown as a discrimination ratio. When comparing the discrimination ratio of ORT1 and ORT2, the discrimination ratio was significantly higher when using a one-hour retention interval compared to a four-hour retention interval ($p=0.02$, fig.9-A). When comparing the discrimination ratio of ORT2 and ORT3, the discrimination ratio was significantly higher when using a cup as the novel object compared a

different construction of LEGO® (p=0.002, fig.9-B). Females achieved a slightly higher discrimination ratio in all sessions of ORT and OLT compared to males, but the difference was not statistically significant (p>0.05, data not shown). The results described above were not altered if rats with low total exploration time in T1 and T2 (less than twenty and ten seconds respectively) were excluded (data not shown).

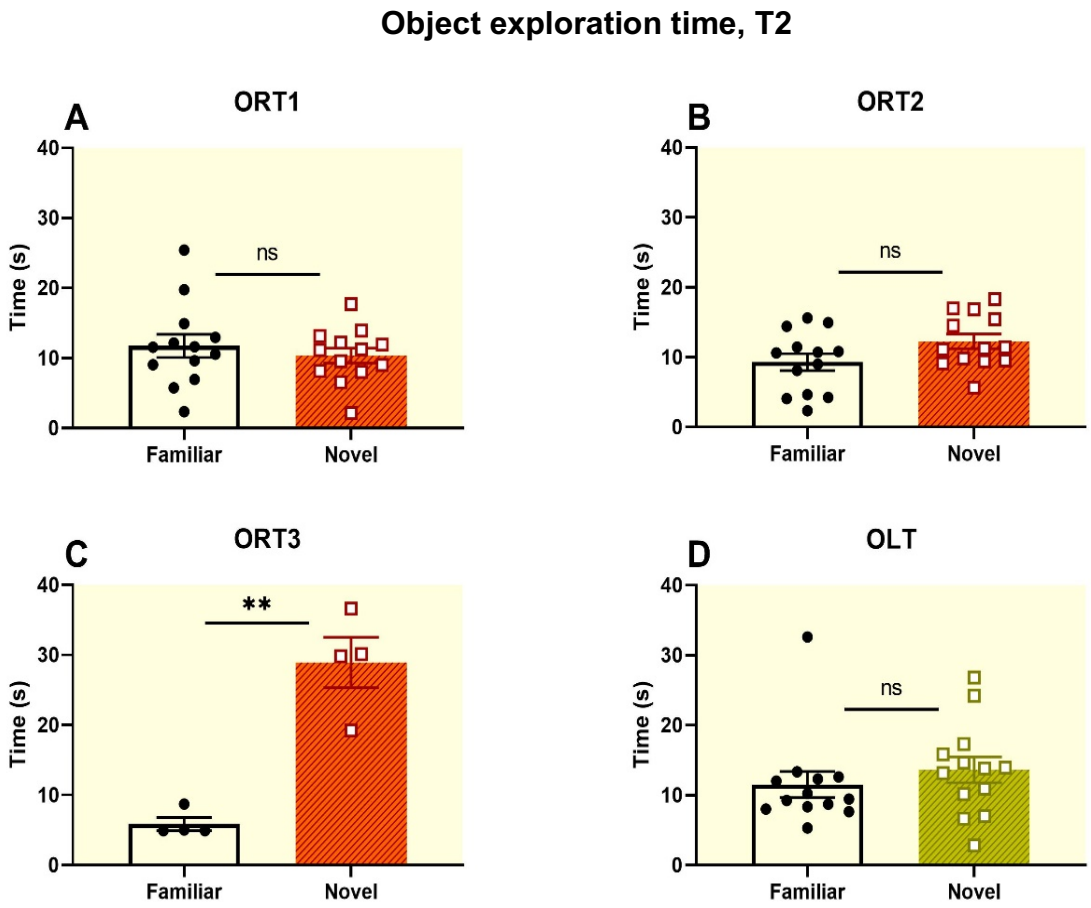


Figure 8. ***A****: The exploration time of familiar and novel objects in the test phase of the first session of object recognition test (ORT1) after a retention interval of four hours (n=13). The difference in exploration time was not statistically significant (ns).* ***B****: The exploration time of familiar and novel objects in the test phase of the second session of object recognition test (ORT2) after a retention interval of one hour (n=13). The difference in exploration time was not statistically significant (ns).* ***C****: The exploration time of familiar and novel objects in the test phase of the third session of object recognition test (ORT3) after a retention interval of one hour (n=4, only males). A cup was used as the novel object instead of LEGO®. The difference in exploration time was highly significant (p=0.005).* ***D****: The exploration time of objects in a familiar and novel positions in the test phase of object location test (n=13). A retention interval of one hour was used. The difference in exploration time was not statistically significant (ns). Error bars shows the standard deviation of the sampling distribution as the standard error of the mean (SEM).*

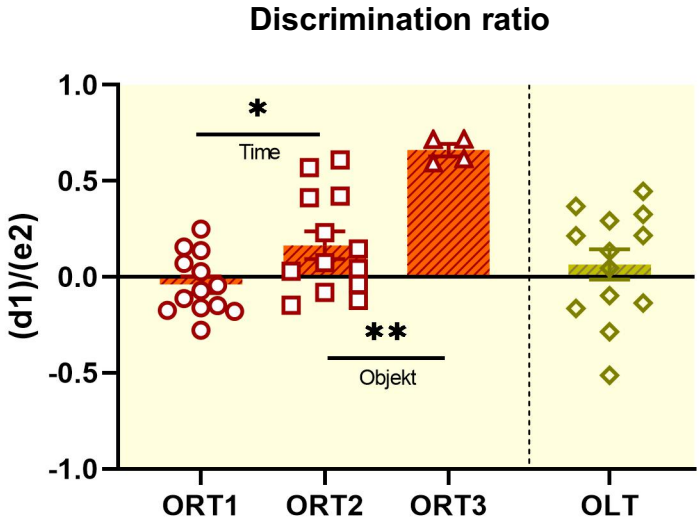


Figure 9*. Discrimination ratios shown as the difference in exploration time between novel and familiar objects (d1) divided by the total exploration time in the test phase (e2) in all three sessions of object recognition test (ORT1-ORT3). A value close to zero indicates equal preference for familiar and novel objects. Negative values indicate preference for familiar objects while positive values indicate novel object preference. In ORT1 (n=13) and ORT2 (n=13), different retention intervals were applied, four- and one hours respectively. The rats showed significantly more novel object preference in ORT2 (p=0.02). In ORT3 (n=4, only males), a one-hour retention interval was used similar to ORT2. A cup was used as the novel object instead of LEGO®. The difference in discrimination ratio when comparing ORT2 and ORT3 was highly significant (p=0.002). The discrimination ratio in object location test (OLT) after a retention interval of one hour is shown to the right in green. Error bars shows the standard deviation of the sampling distribution as the standard error of the mean (SEM).*

3.2 Montoya staircase test

Fifteen male rats (two litters) were assigned for motoric assessment in the Montoya staircase test. The rats were on food restriction throughout the testing period to stimulate food-seeking behaviour. The mean decrease in body weight after the first day of food restriction was 4.9% (+/- 0.4%, SEM). Thereafter, all rats displayed a positive weight curve with a stable increase in body weight (fig.10). All animals remained healthy during the food restriction period. The animals in litter one (p63 at start of testing, n=6) did not consume all food given from the sixth day of food restriction until end of testing. This phenomenon occurred when the rats were p66 and had a mean body weight of 331.4 g (+/- 5.2 g, SEM). The animals were housed three per

cage and rats consumed 7% (+/- 0.001%, SEM) of their body weight on average. The rats in litter two (p=56 at start of testing, n=9) consumed all food (8 % of body weight) during the food restriction period.

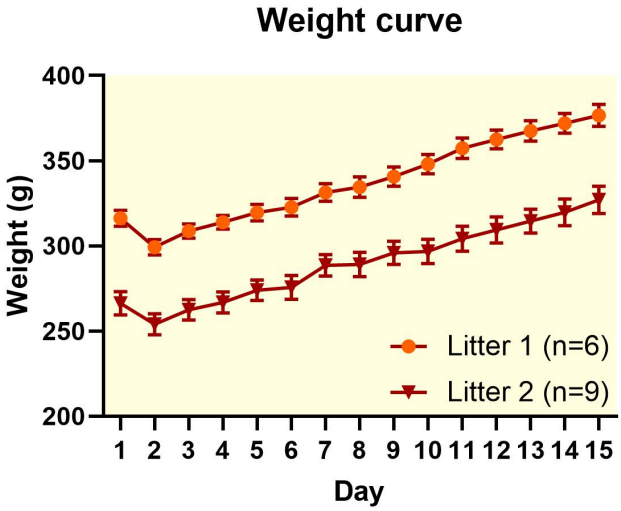


Figure 10. *Mean changes in weight day by day during food restriction. The age of litter one was 63 days and litter two 56 days at start of food restriction. Error bars shows the standard deviation of the sampling distribution as the standard error of the mean (SEM).*

The rats were habituated to sugar pellets in their home cages three days before testing. On day one, the rats would not pick or eat sugar pellets spontaneously from a bowl but would taste and eat sugar pellets when offered by hand through the metal bars in their home cages. Four animals tasted the sugar pellets but rejected them, dropping the pellets to the ground. On day two, after starting the food restriction schedule, all rats except one ate sugar pellets when presented in a bowl in front of their nose. On day three all rats consumed sugar pellets from a bowl spontaneously without human interaction

The rats were tested individually in the Montoya staircase apparatus and were allowed to reach for sugar pellets in one fifteen-minute session per day for ten days. Eleven animals (80%) of the fifteen animals tested increased in number of sugar pellets consumed per session over the

ten days of testing, consuming at least five pellets per session at the end of the testing period. Three rats never consumed more than two pellets and were observed to show less food seeking behaviour compared to their littermates. The mean number of pellets consumed the last day of testing was 9.5 (+/- 1.7, SEM), a significant increase compared to the first day of testing (p=0.0001, fig.11-A). The success rate was 56% (+/- 7%, SEM) on the last day of testing, a significant increase compared to the first day of testing (p=0.0002, fig.11-B). Seven rats learned to consume a minimum of twelve sugar pellets in a single session. No difference in performance was seen when comparing the two litters on the last day of testing (p>0.05, data not shown).

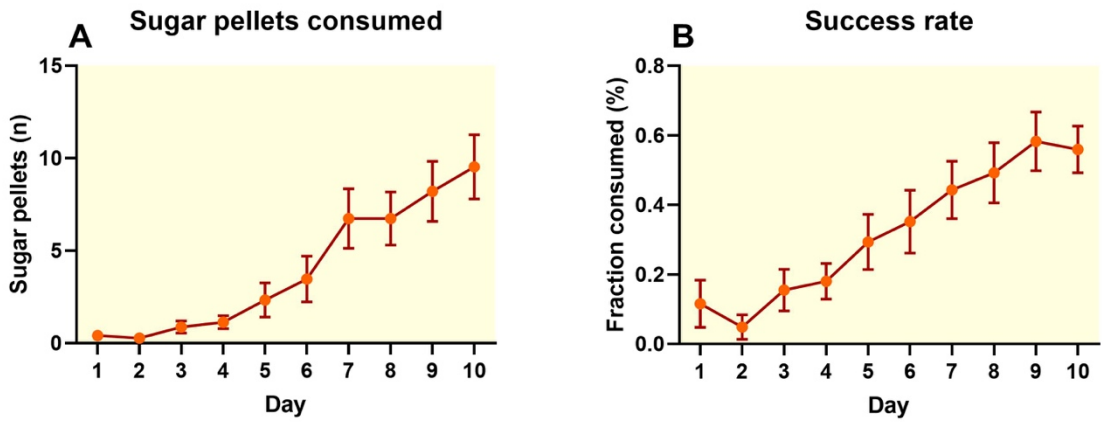


Figure 11. ***A***: *The mean amount of sugar pellets consumed each day in Montoya staircase test (n=15).* ***B***: *The mean success rate on each day of testing in Montoya staircase test (n=15). Success rate was calculated as number of sugar pellets consumed divided by the sum of sugar pellets eaten and sugar pellets dropped. Error bars illustrates the standard deviation of the sampling distribution as the standard error of the mean (SEM).*

3.3 Rota-rod

Fifteen male rats (two litters) were assigned for motoric assessment in the Rota-rod. The mean latency to fall increased steadily over the first three days of testing but decreased over the last two days. There was no statistically significant increase in latency to fall comparing day 1 and day 5 (p > 0.05, fig.12). There was no significant difference in performance on the last day of testing comparing the two litters tested (p > 0.05, data not shown).

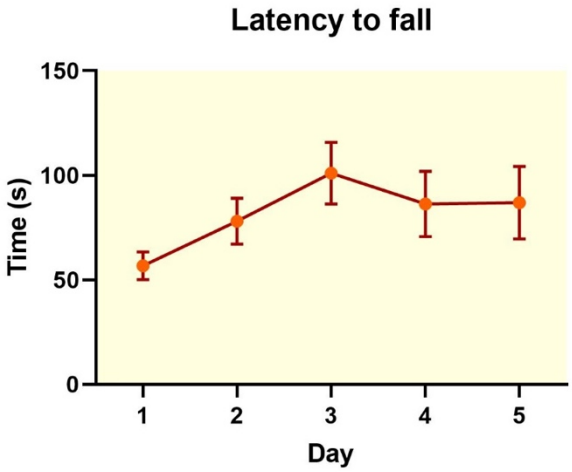


Figure 12. *The mean latency to fall in the Rota-rod test on each day of testing (n=12). Error bars shows the standard deviation of the sampling distribution as the standard error of the mean (SEM).*

4. Discussion, conclusions and future directions

4.1 Object recognition and object location test

4.1.1 Discussion

The aim of object recognition and object location test was to assess episodic-like memory through the innate bias towards novelty seen in rodents when presented to novel objects or novel object locations. The retention intervals chosen aimed to assess long term memory at different levels of difficulty. The rats appeared to be curious during all sessions of testing, a majority showed sufficient exploratory behaviour in T1 and T2 respectively with results comparable with earlier studies (10, 26). This is important since exploratory behaviour is a prerequisite for discriminating between the familiar and novel objects or positions. Furthermore, this indicates that rats can be tested repeatedly for several days without losing exploratory behaviour. Females appeared to spend significantly more time exploring the objects

in T1 compared to males. This was interesting since gender differences in novel object and location recognition have been reported (27). However, the gender differences in exploration time in T1 did not have an apparent effect on the ability to discriminate between objects in T2 although females generally achieved a slightly higher discrimination index in all sessions of ORT and OLT. The animals did not show a statistically significant difference in object exploration when comparing the familiar and the novel objects neither in ORT1 (four-hour retention interval) nor ORT2 (one- hour retention interval). It has to be noted that the difference in exploration time in favour of the novel object in ORT2 had a p value of 0.06, thus almost reaching a statistical significance level of 0.05. Nevertheless, the differences in exploration time comparing the familiar and novel objects seen in this study using both one and four-hour retention intervals were subtle. This was surprising since novel object preference in Wistar rats has been demonstrated using much longer retention intervals (28). When comparing the discrimination ratios of session one (ORT1) and two (ORT2), the discrimination ratio was significantly higher in session two. This indicates that it is easier for rats to discriminate between objects when using a shorter retention interval which is consistent with earlier work (10). The discrimination ratios are still low compared to other studies. A discrimination ratio of approximately 0.3-0.4 should be expected in wild type rats after a one-hour retention interval (10, 29) whereas the discrimination ratios barely reached half of that in this study. In the third session of object recognition test (ORT3), when one of the LEGO® constructions was replaced by a fundamentally different object (coffee cup), a clear difference in exploration time was seen in favour of the novel object. This difference in exploration time was dramatic in comparison to the earlier sessions. On average, the rats spent roughly seven times more time exploring the novel object in ORT3 compared to ORT2. Thus, the difference in discrimination ratio

comparing ORT2 and ORT3 was highly significant. This effect may have been enhanced by the fact that the rats had been presented to different LEGO® figures for several days before being presented to a cup. The results indicate that the objects used must be profoundly different in order to enable discrimination between familiar and novel. However, it has to be noted that ORT3 only included four animals and that object preference at baseline was not controlled for in that session. Therefore, there is a possibility that the rats simply found the cup more appealing. Nevertheless, it remains an interesting finding in the context of this study, indicating that the rats could discriminate between objects.

The LEGO® objects used (fig. 3) were designed to be similar in size and contrast to avoid bias at baseline. This aim was successful since no baseline preference was seen, but the aim likely resulted in too similar objects, rendering the rats unable to differentiate between them. Alternatively, the rats did recognize the objects as different but did not find the novel object different enough to care. The objects chosen for ORT is of great importance, but authors often fail to describe details about what types of objects they have used. Furthermore, little is known about what senses rats primarily use when memorizing an object. Rats have a sophisticated olfactory sense, but olfactory input was eliminated in this test by cleaning the objects with 70% ethanol solution. The visual acuity of rats is generally believed to be poor, especially in albino laboratory rats such as the Wistar rat used in this study. Studies have shown that rats use their vibrissae to receive information about their environment in a sophisticated manner (30, 31), but to which extent whisking is essential for object recognition is unknown. Perhaps tactile information of variations in texture, material and size of objects is more important than visual input.

Surprisingly, no significant difference was seen in OLT when comparing the exploration time of the objects in familiar or novel positions. Thus, the discrimination ratio was close to zero. This cannot be explained by the appearance of the objects since the same objects were used in T1 and T2. The most apparent explanation is that the rats lost orientation in the arena. The animals were always placed in the same spot in the arena with the nose facing towards the same wall to facilitate orientation. However, they often ran along the walls of the arena before exploring the objects. It is possible that the rats noticed that one object had been moved, but if they had lost orientation they would not know which one since the arena was identical in all directions. If so, the rats would possibly end up exploring the objects equally which would explain the results. One study demonstrating novel location preference in rats applied an almost identical testing protocol but used an arena that was comprised of one transparent and one grey section which may have facilitated orientation (29).

4.1.2 Conclusions and future directions

The testing procedure of object recognition test is simple, it is not dependant on motivation or food rewards and a majority of the rats showed sufficient exploratory behaviour throughout testing. Overall the rats showed a tendency towards novel object preference after a one-hour retention interval. Furthermore, the rats exposed to a fundamentally different novel object displayed a strong novel object preference. Thus, there are several findings supporting future use of the test for evaluation of memory function in the BACHD rat model for Huntington's disease. However, a number of challenges remain before the object recognition test could be implemented. The first challenge will be to find objects that are different enough for rats to easily differentiate between familiar a novel while simultaneously attracting the same attention.

One strategy would be to select pairs of objects with as different characteristics as possible, both visually and tactilely, and validate each pair in a population of rats. Pairs that receive equal interest could be used for object recognition testing in a separate group of animals. A suggestion would be to apply a retention interval of one hour or shorter to eliminate time as a confounding factor in the initial stage. Furthermore, increasing the group sizes would likely increase the chances of detecting novel object preference (decrease the risk of a type II error) since the standard deviation of the sampling distribution in the tests was fairly large. It is also worth considering the indications of gender differences found in this and other studies regarding exploration time and performance when designing future studies.

ORT has only been performed in one study of the BACHD rat, that study did not find any significant difference when comparing the disease model and wild-type rats, both groups could discriminate between familiar and novel objects (26). However, the retention interval (90 minutes) was relatively short. In theory, the longer the retention interval the bigger the chance to find a potential difference between wild type animals and disease model. Long-term memories stored in the brains of BACHD rats could be hypothesized to deteriorate more rapidly over time compared to wild type rats. On the other hand, a retention interval too long would increase the risk that not even wild type animals would be able to identify the novel object. If this test is to be used in the BACHD model, it should be determined how much the retention interval can be increased in wild type rats without losing novel object preference. If the retention interval can be successfully increased in wild type animals, this could be examined further in the BACHD model to investigate if the memory of a familiar object deteriorates at the same rate.

Regarding object location test, the rats appear to be unable to identify an object in a novel location after a one-hour retention interval when spatial cues are missing. Thus, a simple improvement would be to mark the arena with spatial cues to facilitate orientation. Object location memory has to the best of my knowledge never been evaluated in the BACHD rat and would be an interesting addition to future studies due to the spatial component. The same principles regarding retention intervals discussed in the section above could be applied to OLT. A final suggestion for improvements would be to use a tracking software for automated analysis of the video footage to facilitate the analysis and avoid potential observer bias in both ORT and OLT.

4.2 Montoya staircase test

4.2.1 Discussion

The aim of Montoya staircase test was to assess fine motor function in the forelimbs of wild type Wistar rats when reaching for sugar pellets. The rats were food deprived to stimulate food seeking behaviour. The weight changes related to food deprivation was as expected and in line with a previous study using a similar method (15). An interesting finding was that the rats in the older litter (n=6) did not consume all food given during food restriction from p66 and forward, there was always a small amount food left in their cages in the morning. The weight curve of the rats was not affected by less food consumption. This phenomenon was not seen in the one-week younger litter (n=9). However, the younger litter did not reach the age of p66 until the ninth day of testing and their mean weight never exceeded 320 grams. It is possible that the appetite of rats decreases with age or that eating 8 % of body weight in food is too much when body weight reaches a certain limit. The food left raised the concern that the rats was not

sufficiently food deprived and would be less prone to food seeking behaviour. Interestingly, there was no noticeable difference in motivation or performance in the MST comparing the two litters. Habituating the rats to sugar pellets was more complicated than expected. The original idea was to place a bowl with sugar pellets in each cage allowing the rats to pick sugar pellets freely. However, the rats would not consume pellets spontaneously. Instead, they seemed more interested in playing with the bowl, causing all pellets to fall out. After a change of strategy, the rats were fed sugar pellets by hand which were successful. All rats would pick sugar pellets spontaneously from the bowl on the last day of habituation.

The rats consumed approximately one third less sugar pellets on the last day of testing compared to the average of 15 pellets seen in the study by Soderlund and colleagues mentioned above (15). However, the success rate seen on the last day of testing was similar. Seven rats learned to consume at least twelve sugar pellets in the Montoya staircase test over ten days of testing. Twelve pellets have been described as a sufficient amount in a previous study showing that approximately 80 % of the rats reached that level on the tenth day of testing (16). Only half of the rats reached that level in my experiments. However, in the other study rats were trained on two sessions per day, thus exposing the rats to twice the amount of trials which can explain the difference. It should be noted that four rats in this study learned to successfully grasp and consume pellets relatively late in the process, they did not consume five pellets or more until the sixth to ninth day of testing. It is likely that the rats would continue to improve if the training was extended or intensified. The impression from observing the rats during testing was that the crucial step in the learning process was for the rats to realize that they can use their paws to collect pellets. Initially, most rats try to collect pellets using only their nose and tongue. Using

this technique, the rats can collect pellets placed on the highest level of the staircase device but with great difficulty. The risk is that the rats loses motivation if they do not receive sugar rewards. In this study, rats consuming one or zero pellets in a single session were assisted by the researcher to reach a few pellets on the first days of testing. By doing so, the rats would receive sugar rewards in order to maintain motivation. However, when assisting the rats in this way they mostly collected pellets using their nose and tongue. Therefore, it would likely not help the rats overcoming the crucial step of learning to use their paws for collecting pellets. If it is too easy to collect pellets by mouth, rats might not feel the need to use their paws and the purpose of this test would be lost. While only half of the rats learned to collect more than twelve sugar pellets, a majority did improve over the testing period. This indicates that motivation was maintained successfully. It appears to be a delicate balance of finding the right level of difficulty to maintain motivation and at the same time stimulate the use of forelimbs.

4.2.2 Conclusions and future directions

The methods used for food deprivation and habituation to sugar pellets appear to have been effective, even though the older litter did not consume all food offered during food restriction. Since food-seeking behaviour was high in the older litter there is no apparent need for a stricter food deprivation schedule. However, only half of the rats improved to reach a result that could be considered as sufficient and a few rats learned the crucial step of using their forelimbs quite late in the process. One area of improvement would be to prolong the initial testing phase to at least fifteen days or expose the rats to two trials per day instead of one. Furthermore, the initial helping described in the section above appear to have helped keeping motivation up but may have hindered rats from learning how to use their front paws for collecting pellets. An option

would be to refrain from helping the rats to collect pellets the first days of testing to possibly improve learning, although this might lead to a larger fraction of rats losing motivation.

A challenge when interpreting the results of Montoya staircase test is that several aspects of brain function are needed to successfully collect and consume sugar pellets. The performance in the test does not rely solely on fine motor function which the test was originally designed to assess. Motivation and the cognitive ability to learn are equally important aspects and should be controlled for in separate behavioural tests. Despite being the most complex test evaluated in this study including several critical steps such as food deprivation and habituation to sugar pellets, the rats showed a stable improvement regarding sugar pellet consumption and success rate which supports future use of the test. Montoya staircase test has, to the best of my knowledge, never been tried in the BACHD rat. However, other tests assessing fine motor function in forelimbs such as “pasta handling test” and “skilled paw reaching” have been used without results indicating that fine motor function is impaired in the model (21). Furthermore, studies indicate that BACHD rats have an altered metabolic profile, consume less food and are less motivated to food intake after food-deprivation (24, 25). Altogether, these issues make the Montoya staircase test problematic for motoric assessment in the BACHD rat and it would be worth considering additional behavioural tests in future research. A suggestion would be to test the learners repeatedly over time to evaluate changes in fine motor function with increasing age.

4.3 Rota-rod

4.3.1 Discussion

The Rota-rod test was designed to assess gross motor function, measuring how long rats can remain on an accelerating, rotating rod until falling down. Reaching a stable latency to fall of at least 100 seconds was used as a minimum inclusion criterion in the study by Soderlund and colleagues (15). Barely one third of the animals fulfilled this criterion in the present study which is remarkably low compared to the beforementioned study where a majority of wild type Wistar rats reached this level (15). The animals could be categorized into four main groups based on behaviours observed during testing. Four rats increased in latency to fall steadily over the whole testing period and ran until they fell (group one). This was the only group reaching a stable latency to fall over 100 seconds. Three rats ran until they fell but did not increase in latency to fall over the testing period (group two). Five rats learned to turn around on the Rota-rod after running for a short period of time and could escape the rota rod by following the rotation forward down to the floor (group three). Three rats figured out how to jump off the rod directly when placed on it (group four). The latter were taken out of testing and their results were not included in the analysis. Thus, the challenge faced in this study was that a majority of the fifteen male rats tested did not run until they fell down, they ran until they did not want to run anymore. They found strategies to escape the Rota-rod device in more or less elegant ways. Most rats started learning how to escape on day two to three. This explains the decrease in latency to fall seen after day three. The impression was that it was relatively easy for the rats to escape, the height of the rotating rod appeared to be low compared to the size of the rats. The fall is supposed to be unpleasant, motivating the animals to stay on the rod but it could be argued that the height was not enough to keep them motivated.

The rats were p63 at start of testing. In other studies rats are often put through initial training at younger ages and are tested repeatedly, investigating changes over time or evaluating treatment effect (15, 19). It is reasonable that younger (smaller) rats would find it more difficult to escape the Rota-rod since the height of fall would be higher compared to body-size. It is possible that early training and memories of unpleasant falls promotes running even at older ages. Another important aspect would be using a device with narrower lanes to prevent the rats from turning around while running. Furthermore, the starting speed of 4 rpm was quite slow and they did not appear to struggle remaining on the rod until much higher rotation speeds. This may have allowed the rats to focus on things other than running, such as how to escape. Lastly, the animals appeared to be easily distracted while running. Care was taken not to disturb them during testing, but since several rats were tested at the same time they may have distracted each other. The rats were running in lanes separated by plastic walls. However, if a rat escaped the device, there was a small time-window where it could interact with others still running before the researcher managed to take it away. Since rats are quick learners they may learn dysfunctional behaviours by observing their littermates.

4.3.2 Conclusions and future directions

Rota-rod is perhaps the methodologically simplest of the tests assessed in this study, yet it proved to be the most challenging. The test did not measure what it was supposed to under the conditions present. Only half of the rats until they fell, the rest escaped the Rota-rod apparatus voluntarily. No conclusions regarding the gross motor function of the rats can be drawn from the data obtained. Rota-rod training in two months old male rats, using an accelerating protocol with a starting speed of 4 rpm in the device used in this study does not provide a reliable method for assessment of gross motor function. The most likely reasons were that the rats were too

large compared to the height of the apparatus and that the lanes were too wide. A contributing factor could have been the slow starting speed of the rotating rod. A suggestion for future Rota-rod testing would be to conduct the initial Rota-rod training in younger animals using a device with narrower lanes. Another area of improvement would be to increase the initial rotating speed. Some laboratories apply a training session on constant speed before using an accelerating protocol. The training is performed during one or two days at a constant rotating speed of 12 rpm (19, 26). This may prevent the rats from learning how to escape since they will be forced to focus on running. A final area of improvement would be to minimize visual distractions. One way to achieve this would be to simply place a blanket over the top section separating the animals.

The test protocol needs major improvements before it can be used to assess gross motor function in the BACHD rat. However, if this can be achieved, the test has great potential for assessing gross motor function in the model. Rota-rod has been used in several studies of the BACHD rat, a significant decline in gross motor function compared to wild type rats from one- to two months of age have been demonstrated (19, 26, 32). However, these studies only included male rats. One study of an another HD disease model including both male and female rats showed that only transgenic males was impaired in the Rota-rod test (33). Thus, potential gender differences are worth considering when designing future studies involving assessment of motor function in the BACHD rat.

5. Final remarks

With adequate adjustments of each behavioural test discussed in the sections above, the tests evaluated in this study can be used to assess motor function and cognitive deficits in the BACHD rat, thus targeting two of the three core symptoms of Huntington's disease. The remaining core feature of HD, psychiatric symptoms, may be the most challenging to assess in a rat disease model. Psychiatric assessment is beyond the scope of this study, but we have earlier evaluated forced swim test (despair), open field test (anxiety) and sucrose preference test (anhedonia and motivation) in a rat disease model for depression (9). In summary, forced swim test is not appropriate to use in adult rats due to size and weight, adult rats struggle to remain immobile in water without sinking (unlike mice). Open field test appeared to have low sensitivity and specificity, a better choice for evaluation of anxiety in the BACHD model would be testing in the elevated plus maze since the model has been demonstrated to have an altered behaviour in that specific test. Sucrose preference test is likely a good choice for psychiatric evaluation in the BACHD rat since apathy and depression is two of the most common symptoms seen in Huntington's disease in humans. Furthermore, sucrose preference test would control for motivation and reward-seeking behaviour which would be of outmost importance if using the Montoya staircase test for assessment of fine motor function since the test is highly dependent on motivation.

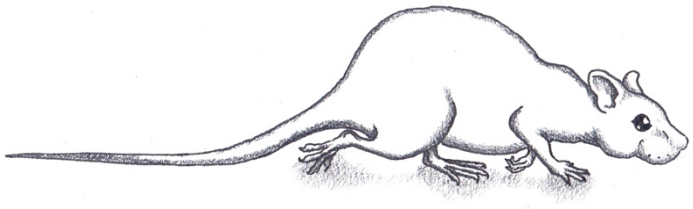
6. Acknowledgements

I would like to thank everyone who made this project possible: Jesper Vestlund for expertise and support regarding Montoya staircase test and Rota-rod. Filip Bergquist and Elisabet Jerlhag Holm for borrowing of equipment and laboratory space. Georgia Culley for expertise regarding object recognition test. Ana Costa for support regarding animals and routines at EBM. Finally, special thanks to my supervisor Henrik Seth for excellent guidance and support throughout all phases of this project.

7. Populärvetenskaplig sammanfattning

En metodstudie för att underlätta framtida forskning kring experimentella behandlingsmetoder för Huntingtons sjukdom

Huntingtons sjukdom är en ovanlig men svår, ärftlig sjukdom som drabbar hjärnan. Den orsakar ofta psykiatriska symptom, minnesproblem och försämrad rörelseförmåga med ofrivilliga, dansliknande rörelser. Sjukdomen sitter i arvsmassan, den beror på en enda defekt gen. Idag finns ingen botande behandling för sjukdomen och den leder ofta till stort lidande för drabbade och anhöriga. Nya vetenskapliga framsteg inom gentekniken, metoder för att påverka arvsmassan, har öppnat upp för möjligheten att ta fram läkemedel som angriper den gen som orsakar sjukdomen. Många utmaningar kvarstår dock innan en sådan behandling kan bli verklighet. Behandlingsmetoden måste först undersökas i studier av djur med sjukdomen. Ett sådant djur är den så kallade BACHD-råttan, en råtta med samma defekta gen som människor med sjukdomen och som visat sig ha liknande symptom. För att kunna studera behandlingseffekt av nya behandlingsmetoder i BACHD-råttan måste man ha väl utprövade metoder för att mäta symptom. Syftet med den här studien har därför varit att utvärdera praktiska aspekter av fyra beteendeförsök, tester där djurens beteende studeras för att kunna dra slutsatser om hjärnans funktion. Två tester för att mäta minnesfunktion och två för att mäta rörelseförmåga har utvärderats i friska råttor och den här studien belyser fördelar, nackdelar och svårigheter med testerna. Med vissa förbättringar har de tester som genomförts potential att kunna användas för att mäta behandlingseffekt i BACHD-råttan. På så vis bäddar den här studien för fortsatt forskning inom området och underlättar framtida studier av nya behandlingsmetoder för Huntingtons sjukdom.



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