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# The influence of strain on demand functions for water in rats (Rattus Norvegicus).

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*Introduction*

Demand functions generated by operant conditioning techniques may be used to assess animal priorities *(Dawkins 1990, Matthews & Ladewig 1994, Sherwin & Nicol 1997, Fraser & Matthews 1997, Matthews 1998)*. The animal needs to perform a number of simple responses (e.g. bar-presses), to obtain one unit of a reinforcer *(Lea 1978, Dawkins 1990, Matthews & Ladewig 1994).* This reinforcer enables the animal to perform a certain behaviour. The relationship between the workload (traditionally set by a fixed ratio (FR) reinforcement schedule) and the amount obtained of the reinforcer is described by a curve with FR-value on the horizontal axis as the independent variable and the amount consumed as the dependent variable on the vertical axis *(Hursh 1980)*. The resulting slope of the demand function will then provide a measure for the demand of the reinforcer. If the animal is highly motivated to obtain the reinforcer the animal will work at an increasing rate as the workload increases and thus keep its intake close to constant. The slope of the demand function will be close to zero, which indicates a high demand for the reinforcer. The steeper the demand function, the less important the reinforcer *(Lea 1978, Hursh 1984).* The slope of the demand function is nearly always negative and it is not influenced by e.g. the weight of the animal *(Hursh 1984, Ladewig 1997).* The size of an animal will influence the intercept of the demand function, but not the slope of the demand function. Using this method it should be possible to measure quantitatively to which extent an animal is motivated to obtain a given reinforcer and to compare demands for different reinforcers among different animals.

It is important to note that the demand function reflects the demand for the reinforcer. The demand depends on both the internal preferences of the animal but also on the decisions made by the animal of how much to obtain of the reinforcer considering the workload imposed on the animal and the availability of alternatives.

Many factors influence the slope of the demand curve; some are varied by the experimenter (e.g. FR-values and test-time), some factors might depend on the animal's gender and genetics (e.g. sensory capacities and basic level of anxiety), and some are physiological factors influencing the motivational state (e.g. thirst, hunger, aggression, or phase in oestrous cycle). Furthermore, it is well known that, at least in rats, differences exist in performance in cognitive and operant tasks between different rat strains *(Andrews et al. 1995).*

The purpose of this experiment was to determine if the method was able to detect differences in demand for water between two different strains.

For the selection of the two strains of rats, three criteria were used. First, we wanted inbred strains in order to minimize the variation between rats in the two test groups. Second, in order to avoid any confounding influence of rats having difficulties in performing the operant response-task, we needed two strains, which were known to perform well in operant systems. Third, the strains should neither be transgenic nor spontaneous animal models of human disorders *(Svendsen & Hau 1994).* The two strains chosen were pigmented inbred Long Evans rats (LE/Mol) and albino inbred Wistar-Kyoto rats (WKY/Mol).

*Materials and Methods*

*Animals*

A total of eight Long Evans inbred male rats (LE/Mol) and eight Wistar Kyoto inbred male rats (WKY/Mol), all barrier bred and health monitored according to FELASA guidelines *(Kraft et al., 1994)*, were used for the experiment. The rats were moved to the housing facilities at 4 weeks of age. After an adaptation period of 21 days, adaptation to test chambers and shaping by successive approximation was initiated. In the adaptation period gentle handling and transportation between the housing facilities and the test room was done daily. The rats all weighed 180-200 gr. at the beginning of the training period.

*Housing and feeding*

All rats were housed in the same room with temperature maintained at $20 \pm 1^{o}$ C and a relative humidity of 55-80 %. Rats are nocturnal animals, being active during night time, so to ensure that the animals were active during daytime and hence testing, the rats were housed under a reversed 12:12 hour light: dark cycle with no natural light. The light period started at 19.00 h. The same light: dark cycle was applied to the test-room. The rats were housed in environmentally enriched cages to optimise learning and development of the animals' behavioural potential *(Rosenzweig & Bennett 1996)*. The cages were modified rabbit cages, 90x50x50 cm with wire mesh front and floor and supplied with one stainless steel rat-nest with two floors, one plastic jar with two cut holes and a litter-tray. Hard wood shavings and wood wool were used for bedding (Tapvei, Finland). Each cage housed two LE rats and two WKY rats. The rats were fed standard rat chow (Altromin 1324; Brogaarden, Denmark) ad libitum. They were given free access to distilled water in the adaptation period. After adaptation they were given free access to distilled water for 30 minutes/day in periods with no training or testing. During training and testing, no water was supplied outside the test.

*Equipment*

Eight standard operant test chambers (MED Associates, Inc., operant test chamber ENV-007) were used. The test chambers were equipped with a house light (ENV-215M, 28 V, 100mA), 2 stimulus lights (ENV-221M, 28 V, 100 mA), 2 response levers (ENV-110RM), 2 water dispensers and a grid floor. The front, top and back of the chambers were clear acrylic plastic and the side panels were stainless steel. The response levers were situated in the left and right side panels, both to the right side of the water dispenser, which was placed in the central side panel (Figure 1). The operating tension of the response levers was set to 25 grams. The water dispensers were constructed by Neurosearch Ltd. and consisted of a small water cup mounted on a magnetic valve. Operation of test chambers and registration of data were done by the MED-PC software.

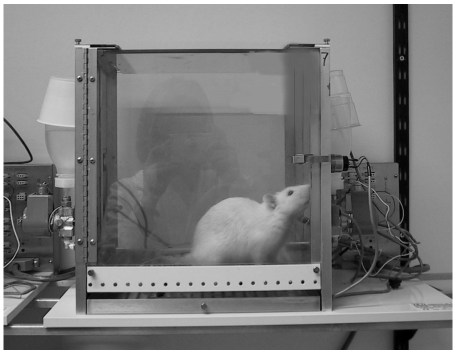

Fig. 1: Standard operant chamber with one water dispenser on each side.

At the beginning of the test the house lights were turned on in all boxes and stimulus lights were on above the activated levers. Only the left lever was operational in all boxes. After each response, the stimulus light was turned off for 3 msec and each water reinforcement (0.1 ml) was signalled by a click from the water dispenser.

*Experiments*

Three different types of water were used as reinforcers in three different experiments. The rats had been accustomed to all three types of water during the training period. Water was an essential reinforcer, since testing was done in a closed economy (i.e. no water was given outside the test situation). Distilled water was presumed to be a neutral or slightly attractive tasting stimulus, whereas saccharine sweetened water (2 mg saccharine/L) was presumed to be more attractive than distilled water. Acidified water (HCl, pH 2.5) is commonly used to prevent bacterial growth in drinking bottles, and it is often observed that serving tap water increases the ad libitum water intake compared to acidified water *(Hansen 1997)*. Acidified water was therefore considered slightly aversive.

*Training and test procedure*

The rats were first introduced to the test-chambers in pairs for two hours a day on two consecutive days. Both levers were active on an FR1-schedule. After two days the rats were placed separately in the chambers on an FR1-schedule. Only the left lever was active.

The rats were trained in a closed economy, i.e. no water was given outside the test situation. Exceptions were made on the initial FR1-schedule if the test animal obtained less than 5 ml water in two hours. These rats were given free access to water for 10 minutes right after the training session.

When the rats had learnt to press the lever to obtain the reinforcer, the schedule were gradually increased to FR2, FR3, FR5, FR7, and, finally, to FR10. When all the rats responded fast and without hesitation the schedule was increased in jumps of 10 up to FR140. Each rat was kept on the lower FR-schedule, until it responded persistently and without any pausing until reinforcement was delivered, obtaining not less than 5 ml water during the two hours.

The rats were monitored either directly by the experimenter or by video recordings both during training and testing to allow for retrospective identification of factors periodically influencing the work of the animals. No such factors were found during the tests.

All testing was done in a closed economy *(Hursh 1984)*. Had any of the rats obtained less that 4.5 ml water in two consecutive test days, they would have been removed from the experiment. However, this step never became necessary. All rats were tested on a fixed ratio schedule, which was altered every day. Three replicates were done in each experiment. The FR-schedule was ascending FR10, 30, 50, 70, 100 in the first replicate, either ascending or descending (FR100, 70, 50, 30, 10) in the second replicate, and ascending in the third replicate. Each experiment thus lasted 15 consecutive days. The two strains of test rats were randomly assigned to one of two groups, so that each group consisted of four LE rats and four WKY rats. Prior to each experiment, the rats were always given one day on FR10 to allow the rats to adjust to the operant procedure and to ensure that each experiment had the same start-point, whether the rats had had a pause in testing or not. Data from this first day was not used for the demand functions. The rats were tested from 10.00 - 14.00 h. in 15 consecutive days for each of the three types of waters. Between each experiment an adaptation period of one week allowed the animals to adjust to another kind of water, before testing began. The results of three weeks of testing were omitted because of failure of the equipment and because two of the rats broke loose one night. The rats were tested from October 1998 - January 1999.

*Statistics*

The proc mixed *(SAS, 1997)* was used for the statistical analysis. Three replicates were done on each FR-value and the mean amount consumed was calculated and used for statistical analysis. The rat thus was the unit of observation and the data for the 8 rats in each strain were used to estimate the demand function. The data consisted of repeated measures (the 5 FR-values) on each subject. The analysis used an AR (1) correlation structure to account for possible correlation between measurements within each subject over time.

First, simple regression was used to estimate the effect of workload (FR-value) and strain on water for each kind of water. Second, for each strain, the difference in water consumed at each workload was calculated.

A simple regression was performed to estimate the effect of workload on the pair wise differenced values of water consumed. The estimated average difference (factor of difference) was used to compare the reaction of rats to different kinds of water.

*Results*

Comparing the demand for distilled water in the two strains revealed a significant difference between LE rats and WKY rats. The slope of the demand function of the LE rats was significantly shallower than that of the WKY rats (Table 1).

Table **Error! Unknown switch argument.**: Slopes of the demand functions. For each of the three kinds of water,
the slopes of the two strains are compared.

| | Long Evans | Wistar Kyoto | P-value |
|---|---|---|---|
| Distilled water | -0.0557 | -0.1234 | 0.0001 |
| Saccharine water | -0.1042 | -0.1347 | 0.1281 |
| HCl water | -0.0984 | -0.1014 | 0.8602 |

Figure 2 shows the predicted demand functions for the two strains. The actual amount of consumption on each FR-value (workload) is indicated.

Table 2 shows the difference in consumption from one FR unit to the next both for LE rats and WKY rats. This factor of difference expresses the difference in water consumption between the two demand functions for each unit change in workload (FR-value). A negative value of "factor of difference" indicates that the demand functions approach each other as the FR-values increases. A positive value indicates a divergence towards the higher workloads. The bigger the divergence of the two curves, the smaller is the possibility of the slopes being the same. In LE rats the slopes for acidified water and distilled water differed significantly as did the slopes for distilled water and saccharine water (Figure 2). In both cases the demand functions approached each other, as the workload increases. The slope of the demand function for saccharine water and acidified water did not differ significantly ($p>0.05$), and neither did the intercepts of the two functions ($p>0.05$).

For the WKY rats, the slopes for saccharine and distilled water did not differ, but the intercept for saccharine water was significantly higher than the intercept for distilled water ($p< 0.05$). Furthermore the slopes for saccharine water and acidified water were significantly different. The slopes of saccharine water and acidified water started out with a relative large difference in consumption (Figure 2), which decreased by 0.033 as FR-values increased. The difference in slopes for acidified water and distilled water (Figure 2) was also significant ($P<0.05$), but in this case the two functions diverged by 0.0212 as FR-values increased.

*Discussion*

The plotting of data on log-log scales is common in economic analysis. The reason may be due to the fact that economics often focuses on proportional changes, not absolute changes *(Bickel et al., 1993)*. On these scales the slope of the demand curve equals its elasticity, and a horizontal line corresponds to an elasticity of zero *(Lea & Roper, 1977)*. Furthermore, the transformation of the data to log-log coordinates most likely reduces the variance of the data *(Christensen 1996)* and reduces or eliminates a possible curvature of the demand functions. However, no log-transformation was done in this experiment. Testing for normality showed that the raw data were normally distributed, but the logarized data

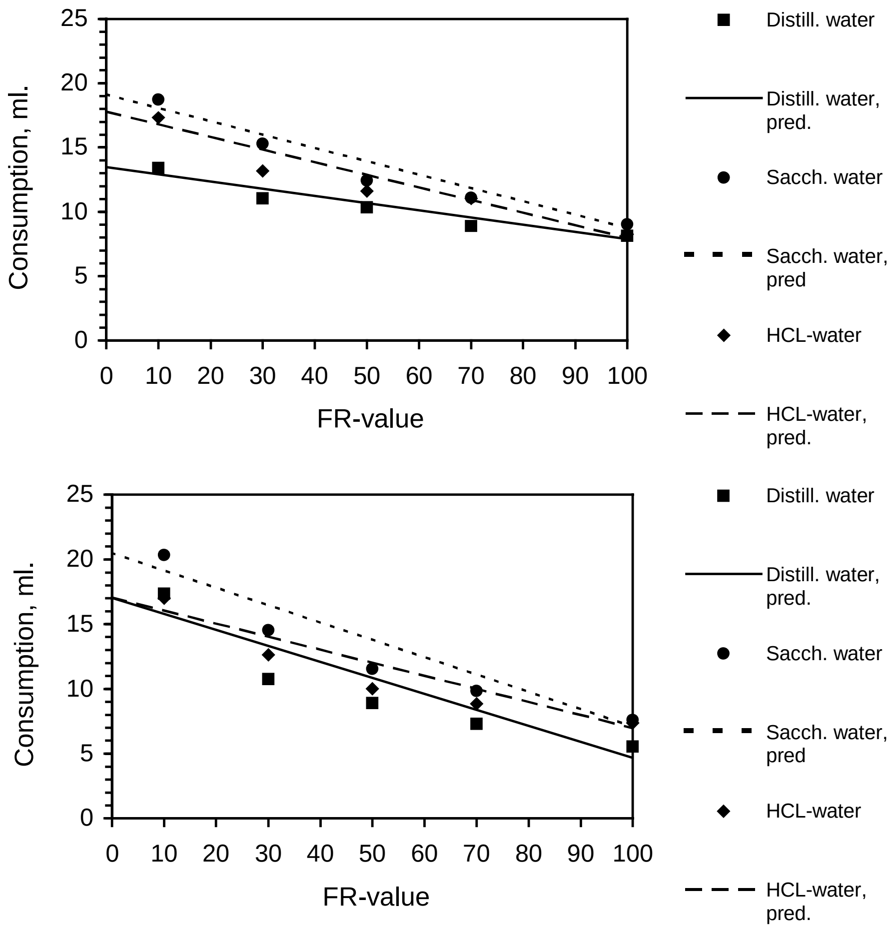


Fig. 2: Top panel shows the demand functions for Long Evans rats. Bottom panel, shows the demand functions for Wistar Kyoto rats. The mean values are shown for each FR-value and the predicted demand functions are shown.

Table **Error! Unknown switch argument.**: Comparing two demand functions by use of the factor of difference. A negative value of "factor of difference" indicates that the demand functions approach each other as the FR-values increases. A positive value indicates a divergence towards the higher workloads. The more the factor of difference deviate from zero, the smaller is the possibility of the slopes being the same.

| | Long Evans | | Wistar Kyoto | |
|---|---|---|---|---|
| | Factor of difference | P-value | Factor of difference | P-value |
| HCl/distilled water | -0.0382 | 0.0001 | 0.0212 | 0.0468 |
| Saccharine/distil. water | -0.0491 | 0.0001 | -0.0114 | 0.3037 |
| Saccharine/HCl water | -0.0113 | 0.2936 | -0.0330 | 0.0002 |

were not. Furthermore, we wanted to concentrate on absolute changes in the rats' consumption.

The behavioural demand curves showed that, as the workload increased, the water intake progressively decreased. But it is important to note that the rats actually work harder to obtain the total amount of water on high FR-values, than on low ones. E.g. 18 ml of water costs 1800 lever presses at FR10, but 18.000 presses on FR100. So when a rats' intake drop from e.g. 18 ml. on FR10 (total workload: 1800 lever presses) to 8 ml on FR100 (total workload: 8000 lever presses), it actually works harder, though its water intake decreases.

All the slopes of the behavioural demand curves were negative and close to zero (Table 1). According to e.g. Matthews & Ladewig (*1994*) a slope close to zero indicates that there is a high demand for the tested reinforcer. Since the present experiment was done in a closed economy, anything else would be surprising.

For distilled water, the LE rats showed a significantly shallower slope compared to WKY rats. Because the LE rats started out with a lower consumption compared to the WKY rats, the LE rats could not fall in consumption as much as the WKY rats. The rats have to have a certain amount of water in order to fulfil their basic metabolic need. The difference between LE rats and WKY rats in the slopes of distilled water indicated that the two strains may perceive the attractiveness of distilled water differently. The reason for this difference might be the fact that the rats were given distilled water in between experiments. Therefore, when working for distilled water, the LE rats just consumed what they needed. When a different taste was given (such as saccharine and acidified water) the variation in taste may have made them drink more. For some reason, the WKY rats did not show this preference for variation in tastes.

Another possible explanation is that the LE rats did not find the taste of the distilled water as attractive as the taste of saccharine water and acidified water.

WKY rats showed a clear preference for saccharine water. The slope was the same as for distilled water, but the absolute values of consumption were higher. This was shown by the significantly higher intercept. In LE rats, saccharine water also showed the highest absolute intake, but neither the intercept nor the slope was significantly different from that of acidified water. Both strains preferred the saccharine water, but for LE rats the acidified water held some of the same qualities. Both strains seemed to rank distilled water lower, but this could be because the taste was known and therefore not interesting.

For both strains the demand function for acidified water indicated that acidified water was not less desirable than distilled water. LE rats drank even more acidified water than distilled water, when they were working on low FR-values. This finding does not seem to be in agreement with the common opinion that rats find HCl acidified water less attractive than tap water. One explanation might be that if the rats are used to drinking acidified water, the novelty increases the ad libitum water intake, when they are presented with another kind of non-aversive water such as tap water.

Several experiments have revealed important strain differences in cognitive tasks, such as auto shaping, Morris swim maze and avoidance tasks *(e.g. Andrews 1996, Paré 1993, Paré & Redei 1993, Van der Staay & Blokland 1996).* Strains differed in e.g. intensity of exploration, accuracy, speed of choice-making and locomotor activity *(Andrews et al 1995).* All of these abilities may very well have an impact on an operant response. Also in behavioural paradigms such as the acoustic startle response, strain differences are found. The acoustic startle response test is often used to evaluate anxiety and strain dependent differences indicate that some strains are more emotional than other strains *(Andrews 1996).* It is likely that the level of emotionality also influences the slope of the demand function.

Moreover, there may be differences in both sensory capacities and locomotor capabilities. It is well known that albinism results in a deficit in the visual system as well as in the pigmentation system. This deficit influences e.g. the susceptibility for bright light and deterioration in visual acuity with age is seen in many albino strains *(Andrews 1996).* However, in operant tasks, which do not require visual discrimination, there is no reason to believe that albinism should result in poorer performance *(Andrews 1996, Didriksen 1993).* It is possible, however, to imagine some strain-dependent differences in sensory capacities, which could influence the slope of the demand function. For example, the perception of taste and smell may be important in this experiment, in which we establish the demand for three partly substitutable reinforcers. The variation among strains found in this study should be considered in animal experiments using operant conditioning.

A factor, which also calls for caution, is the lack of an alternative. In most operant demand experiments the animal is faced with only two options in regard to the reinforcer. Either the animal can respond in order to obtain the reinforcer or it can desist from responding. Although not the issue in this experiment, it must be emphasized that testing only one reinforcer implies the risk that the rats respond even though the reinforcer is less than optimal. If the reinforcer is in any way essential, the animal has to take what is being offered - even an aversive reinforcer (such as bad tasting water). Presenting two or more simultaneously available reinforcers in the same system probably would increase the sensitivity of the method, since it presents the animal with a choice.

In conclusion, the main strain difference lied in the demand for the distilled water. The fact that there was a strain difference, when it comes to an essential reinforcer such as water, indicates that the variation in demands was rather large. This study supports the notion that the strain of the rats is an important factor, which should be thoroughly considered when experiments are planned.

*Summary*

Demand functions generated by operant conditioning techniques are used to assess animal priorities. In an operant system, the animal works to obtain a certain reinforcer, which enables the animal to perform a certain behaviour. The more motivated the animal is to perform this behaviour, the harder it will work to gain access to the reinforcer. It is important, however, to realise that a large number of factors may influence the demand function. To evaluate the impact of strain differences in rats on the demand function, two inbred rat strains worked for three different kinds of water in a closed economy. In Long Evans rats it was found that the demand for saccharine sweetened water and for acidified (HCl) water was the same, whereas the slope of the demand function for distilled water was significantly different from the other two. Wistar Kyoto rats

showed the same demand for saccharine water and distilled water, although the total intake of saccharine water was significantly higher. The demand for acidified water was significantly different form the other two. The fact that there are strain differences when it comes to an essential reinforcer such as water in a closed economy, shows the necessity of considering strain differences when demand is evaluated in animals.

*Resumé*

Efterspørgselskurver konstrueret ved hjælp af operant konditionering kan bruges til at vurdere dyrs behov. I et operant system arbejder rotter for at opnå adgang til en forstærker, en ressource, som gør rotten i stand til at udføre en specific adfærd. Jo mere motiveret rotten er for at udføre den givne adfærd, jo hårdere er den villig til at arbejde for at opnå adgang til forstærkeren. Mange forskellige faktorer kan have indflydelse på efterspørgselskurven. For at vurdere om der er forskel på to rotte-stammers behov for forskellige typer vand arbejdede to indavlsstammer for tre typer vand i en lukket økonomi. Long Evans rotter viste det samme behov (ikke forskellige hældninger af efterspørgselskurverne) for saccharin vand som for syrnet (HCl) vand, mens hældningen for efterspørgselskurven for destilleret vand var signifikant forskellig fra de andre to. Wistar Kyoto stammen viste samme behov for destilleret vand og saccharin vand, selvom det samlede vandindtag var højere for saccharin vand. Behovet for syrnet vand var signifikant forskellig fra de andre to typer vand. Når det drejer sig om essentielle forstærkere som vand i en lukket økonomi, understreger den påviselig forskel på to indavlsstammer vigtigheden af at være opmærksom på stamme-forskelle, når efterspørgselskurver hos dyr evalueres.

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