

**RESPONSE TO NOVEL OBJECTS AND FORAGING TASKS BY COMMON  
MARMOSET (*CALLITHRIX JACCHUS*) FEMALE PAIRS**

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## **Abstract**

Many studies have shown that environmental enrichment can significantly improve the psychological well-being of captive primates, increasing the occurrence of explorative behavior and thus reducing boredom. The response of primates to enrichment devices may be affected by many factors such as species, sex, age, personality and social context. Environmental enrichment is particularly important for social primates living in unnatural social groupings (*i.e.* same-sex pairs or singly housed animals), who have very few, or no, benefits from the presence of social companions in addition to all the problems related to captivity (*e.g.* increased inactivity). This study analyses the effects of enrichment devices (*i.e.* novel objects and foraging tasks) on the behavior of common marmoset (*Callithrix jacchus*) female pairs, a species that usually lives in family groups. It aims to determine which aspects of an enrichment device are more likely to elicit explorative behaviors, and how aggressive and stress-related behaviors are affected by its presence. Overall, the marmosets explored foraging tasks significantly longer than novel objects. The type of object, which varied in size, shape and aural responsiveness (*i.e.* they made a noise when the monkey touched them), did not affect the response of the monkeys, but they explored objects that were placed higher in the enclosure more than those placed lower down. Younger monkeys were more attracted to the enrichment devices than the older ones. Finally, stress-related behavior (*i.e.* scratching) significantly decreased when the monkeys were presented with the objects; aggressive behavior as unaffected. This study supports the importance of environmental enrichment for captive primates and shows that in marmosets its effectiveness strongly depends upon the height of the device in the enclosure and the presence of hidden food. The findings can be explained if one considers the foraging

behavior of wild common marmosets. Broader applications for the research findings are suggested in relation to enrichment.

Key words: common marmoset, female pairs, environmental enrichment, animal welfare.

The concept of boredom in captive animals is difficult to define, but most ethologists agree that insufficient environmental stimulation can lead to boredom<sup>1</sup>. Boredom, due to the absence of new and/or adequate stimuli, is a major problem for laboratory and zoo primates<sup>2,3</sup>. Enrichment devices, such as novel objects and foraging tasks, may improve the psychological well-being of animals as these devices provide new and/or additional stimuli to the monkeys that may elicit exploration, manipulation, and attempts to gain food items from them<sup>4-6</sup>. As such, presenting animals with enrichment devices on a regular basis should become the normal routine in captive settings.

Many studies on environmental enrichment have focused their attention on common marmosets (*Callithrix jacchus*<sup>7</sup>), which are commonly kept in laboratories and zoos. Laboratories often require housing common marmosets in same-sex pairs for research purposes or practical reasons, but female pairs, as well as male pairs, are an unnatural social grouping for this species. Common marmosets usually live in family units composed of a breeding pair, the older offspring, and sometimes a few unrelated individuals, which all participate in rearing the infants<sup>8,9</sup>. In captivity the dominant/breeding female often suppresses the ovulation of subordinate females by releasing pheromones by means of scent-marking<sup>10</sup> (although Ziegler and Sousa<sup>11</sup> have recently shown that this suppression may also occur at low rates). Moreover, female-female aggression is high among captive marmosets<sup>12,13</sup>. Enriching pairs, or singly housed primates, where social stimulation is restricted is of utmost importance. However, for enrichment devices to be effective in improving the well-being of captive animals (by increasing explorative behavior and reducing abnormal behaviours), their presence should decrease, or at least not adversely affect, the rates of aggression and stress-related behavior.

Despite the number of studies on environmental enrichment, little is understood about which object properties are most effective in improving common marmoset well-being. In addition, object properties may interplay with other factors known to affect responses such as sex, social context, personality, and age<sup>14-16</sup>. This study examines the effects of age and object properties in common marmosets housed in an unnatural social group (*i.e.* female pairs). Object properties play a major role in determining responsiveness. Objects containing hidden food, and/or those that are moveable, or respond to the primates in some way (*e.g.* aurally responsive) elicit more exploration than those that do not<sup>17-21</sup>. Moreover, in some arboreal species devices placed higher in the enclosure receive greater attention<sup>22,23</sup>. Finally, the social environment may affect how the monkeys respond to environmental enrichment, although previous studies on this topic, and on how novelty affects the occurrence of stress-related behaviors, have given contrasting results. For example, cotton-top tamarins (*Saguinus oedipus*) living in unnatural social conditions react slowly to novelty and with increased signs of distress in comparison to those living in family groups<sup>24</sup> whereas the presence of a foraging board elicits a strong response in individually housed rhesus monkeys and it also reduces the occurrence of abnormal behaviors<sup>25</sup>.

Environmental enrichment may have two alternative effects on common marmoset female pairs. It may help to reduce social tension within the pair (as the attention of the monkeys is focused on the devices), and improve the psychological well-being of the monkeys due to an increase in exploratory behaviour. Alternatively, the presence of enrichment devices may have deleterious effects on female pairs due to the unnatural social grouping. If environmental enrichment elicits high rates of explorative behaviors aggression and stress-related behavior might increase as the

monkeys try to monopolize the objects. This may be particularly true for enrichment devices containing highly palatable food. Indeed, an increase in aggression when new, attractive objects are added to an enclosure has been found in some species, and especially when foraging tasks are used<sup>4, 26, 27</sup>. Moreover, Michels<sup>28</sup> has recently shown that frequency of aggression over food is higher among captive common marmoset females than among males. As such, it is important to examine whether the presence of enrichment devices (*i.e.* foraging tasks) increase the occurrence of aggression within female pairs, and avoid their use if so. Therefore, the aims of this study are to analyze how common marmoset female pairs respond to artificial enrichment devices; how their response is affected by type of enrichment device (novel objects or foraging tasks), its individual properties (*i.e.* size, shape, aural responsiveness and height in the enclosure), and by the age of the monkeys. Finally, it aims to determine if allo-grooming, aggressive and stress-related behaviors are affected by the presence of the devices.

## **Methods**

### ***Study animals and housing conditions***

The study animals were 32 common marmoset female pairs, housed at the Medical Research Council (MRC) Unit, Edinburgh, Scotland, in the upper level of two-tier cages approximately 55 cm wide, 95 cm high, 110 cm deep. Common marmoset females are peacefully kept in pairs at the MRC by housing an older with a younger, usually unrelated, monkey. Details of the method of initial pairing, and success rate of pairing common marmoset females are provided in Majolo *et al.*<sup>29</sup>. All the cages were furnished with a nest-box, ample natural branches, and a bottle of water. Temperature was maintained at 22-23°C and there was a 12 hours light on cycle per day. Monkeys were fed once a day (approximately at 13.00 hrs) and water was available *ad libitum*.

### ***Apparatus***

Three different novel objects were used in this study, chosen for their different characteristics (*e.g.* responsiveness) and not for their “naturalness”. The first enrichment device (novel object A) consisted of a cup (approximately 12 x 9 x 12 cm) filled with 10 small plastic test-tubes. The second one (B) consisted of a plastic bottle (approximately 17 x 9 x 9 cm) with a large hole on its side, filled with ten pieces of cloth. The third one (C) consisted of four film cases (approximately 9 x 6 x 9 cm) joined together by means of plastic strings, each containing a marble. Objects A and C were aurally responsive (*i.e.* they made a noise when the monkeys touched them). Object B was silent. The same novel objects, this time also containing ten raisins hidden in them, were used as foraging tasks in Experiment 1 (see below). The monkeys usually ate raisins twice a week as part of their diet.

### ***Procedure***

There were two experiments. Experiment 1 was designed to test whether the kind of enrichment device (*i.e.* novel object or foraging task) and relative age of the monkeys influenced the response of the monkeys towards the objects and, whether rates of stress-related and aggressive behaviors were affected by environmental enrichment. Experiment 2 examined the effect of object location on marmoset behavior. For Experiment 1, data were collected by BM on 20 female pairs, using a between subjects design. Novel objects were presented to ten female pairs, whilst foraging tasks were presented to the remaining ten (see Table 1). Each cage was divided into three equal parts (*i.e.* low, middle, and high) and objects located accordingly. Object A was located in the center of the floor (low), object B was attached horizontally to the middle of the

cage door (middle), and object C was hung from the ceiling by means of a string (high). Each object was presented to each pair of monkeys once, on three observation days with one-day intervals. Presentation order was counterbalanced among the pairs. The mean age of the pairs presented with novel objects did not differ to those presented with foraging tasks ( $25.8 \pm 2.2$  and  $23.1 \pm 2.1$  months  $\pm SE$  respectively,  $F(1,18) = 0.79$ ,  $NS$ ). The mean age of the older monkeys in each pair was  $31.3 \pm 2.2$  months  $\pm SE$ , and  $17.6 \pm 1.0$  months  $\pm SE$  for the younger monkeys.

All the data were collected from a hide with a one-way mirror. Observation sessions were scheduled after cleaning and before feeding. Data were collected using focal pair sampling<sup>30</sup> on check-sheets for 20 minutes. The objects were removed at the end of the observation sessions. An additional 10-minute observation session immediately preceded the presentation of each object in order to have baseline rates of allo-grooming, stress-related and aggressive behaviors. Latency to explore (defined as touching, manipulating, or playing with the novel object/foraging task), time spent exploring, and latency to eat the first food item contained in the foraging tasks were used as measures of the responsiveness of the monkeys towards the enrichment devices. Aggressive behavior (such as frown, tufts/ears flick or forward, arch bristle locomotion, tail raised present, cackle, cuff, and bite) and scratching were also recorded. Recent studies have shown that scratching is a reliable measure of stress in common marmosets<sup>31,32</sup> as it is in Old World monkeys<sup>33,34</sup>. Time spent exploring was recorded by using instantaneous sampling method (20 seconds intervals) whilst behavioral events (*i.e.* aggression and scratching) were recorded by using all occurrences sampling method<sup>35</sup>. Allo-grooming was collected using the one-zero sampling method, with 20 seconds intervals, as this behavior was rarely observed and had variable duration<sup>36</sup>.



Experiment 2 was designed to assess whether responsiveness to the enrichment devices was due to the object properties (*i.e.* size, shape or aural responsiveness) or to its location in the enclosure. The same three novel objects used in the first part of the study were presented to 12 different female pairs (see Table 2). Monkeys were divided in three groups and each group was presented with the same three objects in different locations. For example, object A was located on the floor of the cage (low) when presented to group 1, it was attached to the cage door (middle) when presented to group 2, and it was hung from the ceiling (high) when presented to group 3. The same procedure was used for objects B and C. Presentation order was counterbalanced among the pairs. Data were collected by JB, using the same methodology as in Experiment 1, except observation sessions were restricted to 10 minutes as most activity occurs immediately upon presentation of objects. The mean age of the female pairs in the three groups did not differ significantly (group 1 =  $17.9 \pm 3.6$ , group 2 =  $18.5 \pm 2.3$ , group 3 =  $19.8 \pm 2.6$  months  $\pm SE$ ; ANOVA:  $F(2,9) = 0.17$ , *NS*), nor was the mean age of the five groups used in the two experiments significantly different (ANOVA:  $F(4,27) = 1.71$ , *NS*).

### ***Data analysis***

The mean scores per hour of each pair were used in the data analysis, except when a comparison was made between the behavior of older and younger monkeys within each pair and when scratching was analyzed at the individual level. Data were analyzed using a series of mixed-design ANOVAs and repeated-measures ANOVAs. The between subjects factor was enrichment type (novel object or foraging task), and the within subjects factors were object (A, B or C), height (high, middle, low), time (before and during object presentation) and relative age (older or younger in pair).

Tukey post-hoc tests were run when a significant result was found<sup>37</sup>. Only significant interactions are presented. Presentation order was considered in the data analysis to assess whether habituation towards the objects was a factor that affected the response of the monkeys, but as no significant result was found these data are not presented for the sake of brevity. A Pearson correlation was run to relate the baseline level of scratching (*i.e.* frequency before the presentation of the objects) and its variation (*i.e.* baseline level minus frequency of scratching during the presentation of enrichment devices). All the tests were two-tailed and significance was set at  $p < 0.05$ .

## Results

A 2 x 3 way ANOVA with enrichment type and object as factors showed that the latency to explore the foraging tasks was significantly shorter ( $F(1,18) = 16.73, P < 0.01$ ) and time spent exploring them significantly longer than the novel objects ( $F(1,18) = 5.16, p < 0.05$ ). The response of the monkeys to the three enrichment devices varied significantly in relation to kind of object (latency to explore:  $F(2,36) = 19.96, P < 0.01$ , time spent exploring:  $F(2,36) = 21.60, P < 0.01$ ; see Fig 1a, b). Post-hoc tests showed that latency to explore objects B and C (*i.e.* middle and high in the enclosure, respectively) was significantly shorter than object A (*i.e.* low,  $P < 0.01$ ). Moreover, monkeys explored both objects B and C for longer than A ( $P < 0.01$ ). No significant difference was found between objects B and C.

These results show that the monkeys interacted significantly more with the foraging tasks than with the novel objects. They also suggest that other factors such as object properties or height in the enclosure affect the responses towards the enrichment devices, as the monkeys responded to them differentially. However, the experimental design did not allow assessment of the relative importance of these factors as each

object was presented in the same position. Experiment 2 was thus designed to determine which factor (*i.e.* object kind or its height in the enclosure) was primarily responsible for differences in marmoset responsiveness.

To this end, two series of repeated-measures ANOVAs were run for Experiment 2. In the first one, data were divided by object (*i.e.* A, B and C) regardless their height in the enclosure. Neither latency to explore nor time spent exploring the objects differed significantly in relation to object (latency to explore:  $F(2,22) = 0.32$ , *NS*, time spent exploring:  $F(2,22) = 2.00$ , *NS*; see Figure 2a, b). Then, data were divided according to the height of the object in the enclosure (*i.e.* low, middle and high), collapsing data across the three objects. Object height affected the latency to explore ( $F(2,22) = 25.84$ ,  $P < 0.01$ ) and time spent exploring ( $F(2,22) = 13.19$ ,  $P < 0.01$ ; see Figure 3a, b). Post hoc tests showed that the monkeys explored the objects that were placed high or in the middle of the cage more quickly than those placed low in the enclosure ( $P < 0.01$ ). Moreover, the monkeys spent more time exploring the objects when they were placed in the middle or high in the enclosure than when placed low ( $P < 0.01$ ). There was no significant difference in the latency to explore and time spent exploring between objects placed in the middle and high in the cage.

### ***The effect of relative age***

To test whether the response towards the enrichment devices varied between older and younger members of the pairs a series of repeated-measures ANOVAs were run. Data from Experiment 1 for the three novel objects/foraging tasks were pooled together. Latency to explore did not significantly differ between older and younger monkeys (older monkeys:  $371.4 \pm 65.2$  mean seconds  $\pm SE$ , younger monkeys:  $368.2 \pm 68.9$  mean seconds  $\pm SE$ ;  $F(1,19) = 0.00$ , *NS*). However, younger monkeys explored the

objects for longer than the older ones (older monkeys:  $0.12 \pm 0.02$  mean  $\pm SE$ , younger monkeys:  $0.19 \pm 0.02$  mean  $\pm SE$ ;  $F(1,19) = 14.44$ ,  $P < 0.01$ ). Finally, no significant difference was found for latency to eat between older and younger monkeys within each pair (mean older monkeys:  $474.11 \pm 94.03$  seconds  $\pm SE$ ; mean younger monkeys:  $418.87 \pm 103.01$  seconds  $\pm SE$ ;  $F(1,9) = 0.48$ ,  $NS$ ).

### ***The effect of environmental enrichment on allo-grooming, aggressive and stress-related behaviors***

Allo-grooming was never performed by female pairs whilst they were presented with the enrichment devices. However, this behavior was also rarely observed when the objects were absent ( $0.47 \pm 0.19$  mean percentage of sample bouts  $\pm SE$ ).

As the marmosets spent a considerable proportion of their time exploring the enrichment devices, one might expect them to fight in order to monopolize the objects, this effect being stronger in female pairs presented with foraging tasks. To this end, the frequency of aggression and scratching before and during the presentation of the enrichment devices was analyzed. Scratching is a reliable measure of stress in common marmosets (see Methods section). Two  $2 \times 2$  mixed-design ANOVAs were run using enrichment type and time as factors. Data were collapsed across the three novel objects/foraging tasks.

No significant difference was found in rates of aggressive behaviors before and during the presentation of enrichment devices ( $F(1,18) = 4.53$ ,  $NS$ , see Figure 4), nor was it affected by enrichment type ( $F(1,18) = 0.99$ ,  $NS$ ). The rate of scratching was significantly lower during object presentation than before it ( $F(1,18) = 20.26$ ,  $P < 0.01$ , Figure 4) and this decrease was stable throughout the 20 minute observation sessions. No significant difference was observed between female pairs presented with foraging

tasks and those presented with novel objects ( $F(1,18) = 1.26$ , *NS*), but a significant interaction was found ( $F(1,18) = 14.64$ ,  $P < 0.01$ ). The decrease in scratching was greater for foraging tasks, than for novel objects. Finally, a significant positive correlation was found between the baseline level of scratching and the reduction in the occurrence of this behavior during the presentation of the enrichment devices ( $r_s = 0.87$ ,  $N = 40$ ,  $P < 0.001$ ).

## **Discussion**

All the monkeys explored the enrichment devices but the foraging tasks were explored earlier and for longer than the novel objects were. The shorter latency to explore the foraging tasks suggests that the monkeys could smell the food hidden in the objects as soon as they approached them. Clearly, the presence of desired food in the foraging tasks accounts for the significant difference observed between foraging tasks and novel objects in time spent exploring, as this was the only factor that differed between them. Other studies have also shown that foraging tasks elicit the strongest responses in marmosets and tamarins<sup>18,38</sup>. As such, the foraging tasks are a more effective means of environmental enrichment than the novel objects in the short term for common marmosets. However, the intensive use of foraging tasks containing desirable food items (such as raisins in this study), has to be considered carefully, as captive animals are often inactive<sup>3</sup>. The presence of highly calorific food in their diet may make them fatter, more inactive, and reduce their behavioral repertoire. A solution to this problem may be to present the monkeys with foraging tasks containing food items from their daily diet. Data on rhesus macaques (*Macaca mulatta*) have shown that this practice increased the foraging effort (and thus reduced inactivity), the waste of food was lower than with the control feeder, and the weights of the monkeys did not

change<sup>39</sup>. It is likely that this practice would have similar positive effects on common marmoset female pairs. Moreover, the objects used in this study were not “natural” (as, for example, an artificial gum tree is). However, these objects elicited high levels of curiosity in the monkeys and they were easy to get and clean, cheap, and not dangerous for the monkeys. As such, another, potentially complementary approach to the previous one may be to present the monkeys with two/three objects each day and to alternate the presence, and quantity, of food items unpredictably. This practice should be easy to implement, not time consuming for laboratory staff, and the marmosets would spend a large amount of time exploring the objects to determine whether or not they contain food. This situation is more similar to the natural foraging experiences of wild marmosets and can be easily achieved even with artificial devices.

Although the three objects used in this study differed from one another in size, shape and aural responsiveness, the monkeys did not show any significant preference for any one of them. However, they explored the objects placed higher in the enclosure more than those on the ground indicating that this factor greatly affects its attractiveness in common marmosets, as already noted by Millar<sup>16</sup>. This result is not surprising as, in the wild, common marmosets occupy the lower strata of the canopy and rarely go to the ground presence of potential predators<sup>40</sup>. Therefore, exploring stimuli located in the upper part of the enclosure is a situation that resembles what wild common marmosets experience whilst foraging. However, placement of enrichment devices lower in the enclosure need not necessarily be rejected, as Reinhardt *et al.*<sup>41</sup> argue that this might encourage greater space utilization. This does not appear to be the case in common marmosets which spend the same amount of time in the upper part of their enclosure regardless of the height of food dishes<sup>42</sup>.

Neither latency to explore nor latency to eat significantly differed between older and younger monkeys. However, younger monkeys explored the enrichment devices for significantly longer than the older ones. This result indicates that the older monkeys lost interest in the objects significantly earlier than their younger social companions, as already noted in other studies<sup>43</sup>.

Environmental enrichment is only effective when it not only decreases boredom but it also does not adversely affect rates of aggressive and stress-related behaviors. In this study, rates of aggressive behaviors were unaffected by the presence of the enrichment devices and scratching significantly decreased in their presence. Scratching and exploration are not mutually exclusive categories. The reduction in the occurrence of scratching was significantly greater in female pairs presented with foraging tasks than with novel objects, but this may be due to the higher baseline level of scratching in females pairs presented with foraging tasks (Figure 5). However, although not conclusive, these data suggest that the presence of the enrichment devices may be beneficial for the psychological well-being of captive common marmosets, especially for monkeys with high baseline levels of stress.

Overall, these results support the importance of environmental enrichment for the psychological well-being of captive primates<sup>3</sup>. In particular, they are important as housing common marmoset females in same-sex pairs is an unnatural social grouping for this species, and the positive benefits of a social companion appear to be limited. Aggression among captive female marmosets is frequent (Introduction) and moreover female pairsexchange affiliative behaviors (*i.e.* allo-grooming) at extremely low rates. Therefore, enrichment devices may be extremely important for common marmoset female pairs (and probably for other captive primates living in similar unnatural social groupings), even if they are presented for relatively brief periods of time, as they reduce

boredom through increases in exploratory behaviour, decrease the occurrence of stress-related behavior, and do not affect aggression within the pair



### *Acknowledgements*

We should like to thank Keith Morris, Irene Greig, Kevin Hawes, and Mark Fiskien of the MRC Unit, for their practical help and kindness throughout the data collection, Gabriele Schino and Raffaella Ventura for valuable comments on the manuscript. BM was funded by a University “La Sapienza” of Rome studentship, and by a grant from the Universities Federation for Animal Welfare (report number 26-00) for which he is very grateful.

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Table 1: Procedure used for Experiment 1 (novel object/foraging task A = low; novel object/foraging task B = middle; novel object/foraging task C = high).

N of female pairs used	Presentation order of the three novel objects in the three observation days		
	First day	Second day	Third day
	2	A	B
2	B	C	A
2	C	B	A
2	C	A	B
1	A	C	B
1	B	A	C

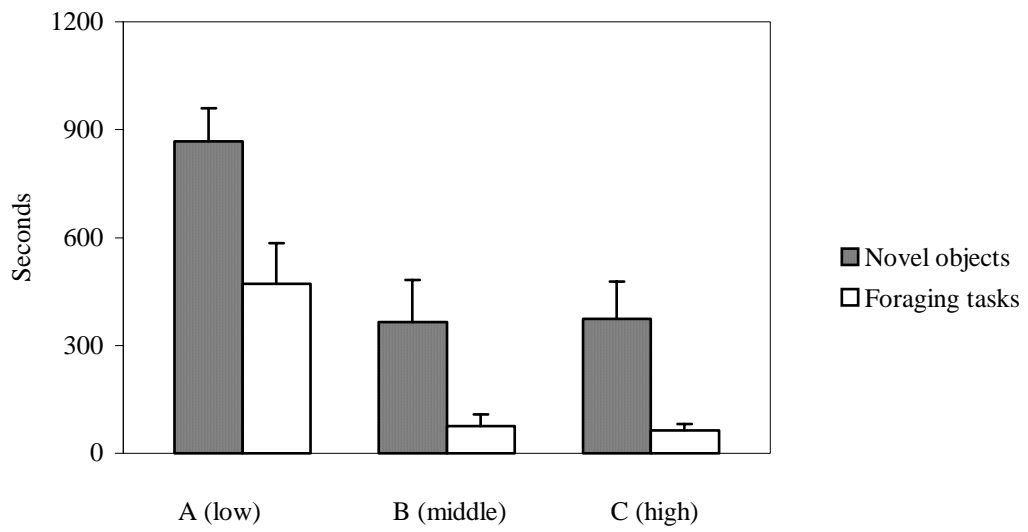
N of female pairs used	Presentation order of the three foraging tasks in the three observation days		
	First day	Second day	Third day
	1	A	B
1	B	C	A
2	C	B	A
2	C	A	B
2	A	C	B
2	B	A	C

Table 2: Procedure used for Experiment 2 using the same novel objects used for Experiment 1 but in different locations in the enclosures.

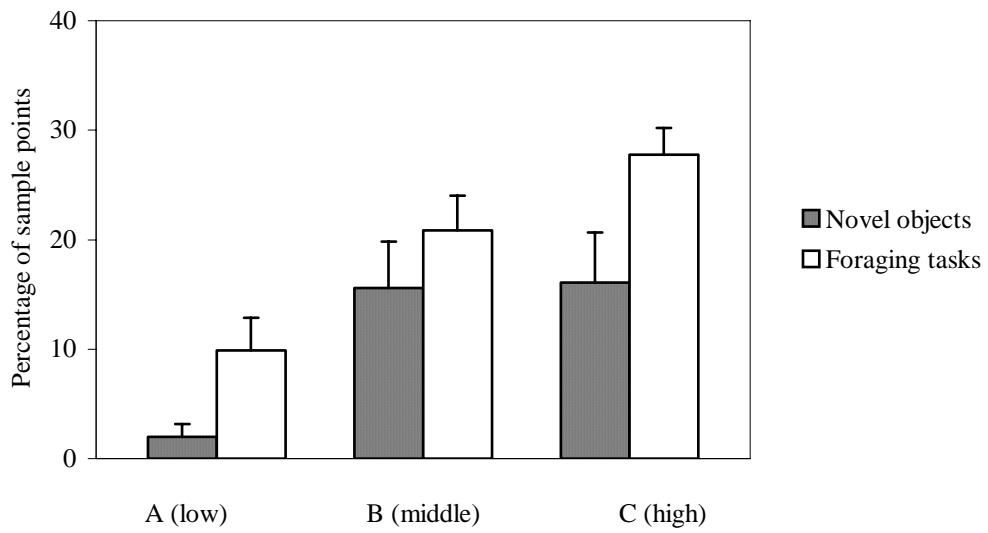
Group	N of female pairs used	Presentation order of the three novel objects in the three observation days		
		First day	Second day	Third day
Group 1	1	A = Low	B = Middle	C = High
	1	B = Middle	A = Low	C = High
	1	C = High	A = Low	B = Middle
	1	A = Low	C = High	B = Middle
Group 2	1	A = Middle	B = High	C = Low
	1	C = Low	B = High	A = Middle
	1	C = Low	A = Middle	B = High
	1	B = High	C = Low	A = Middle
Group 3	1	A = High	C = Middle	B = Low
	1	B = Low	A = High	C = Middle
	1	B = Low	C = Middle	A = High
	1	C = Middle	B = Low	A = High



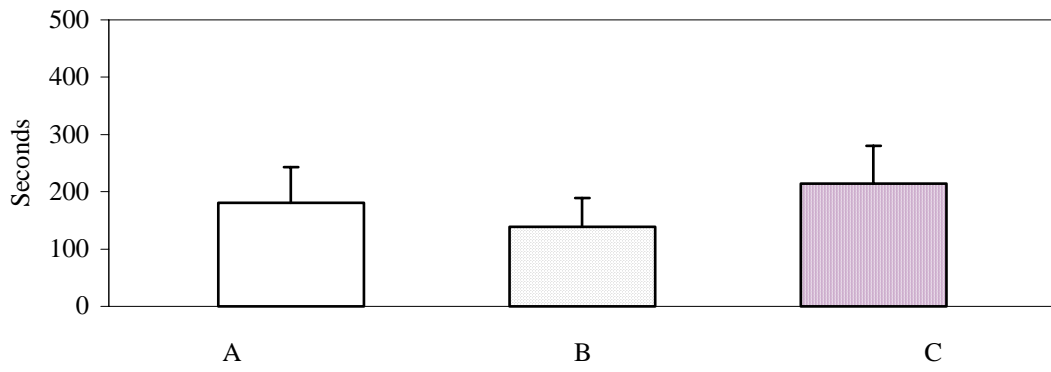
1a



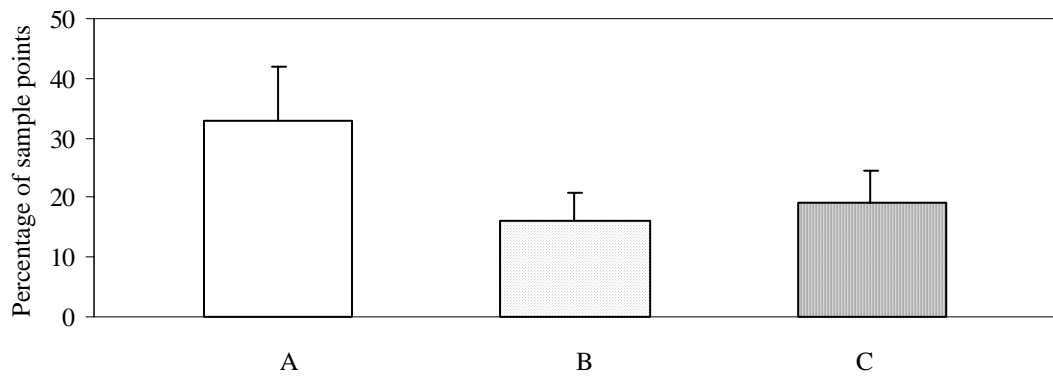
1b



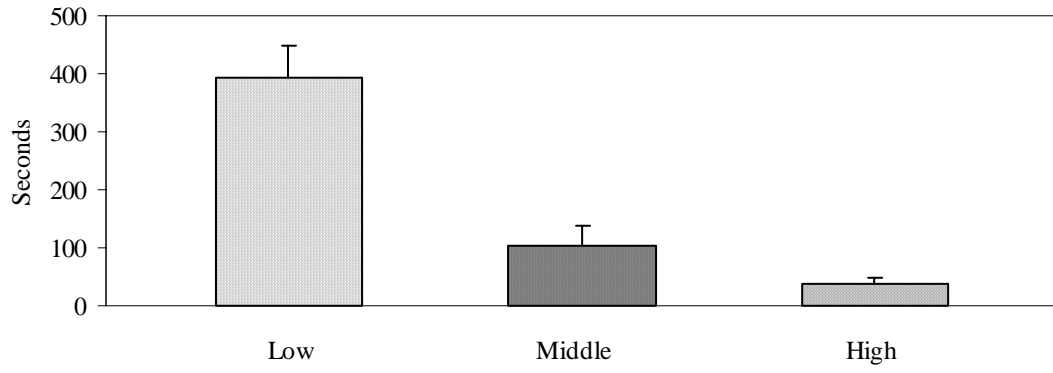
2a



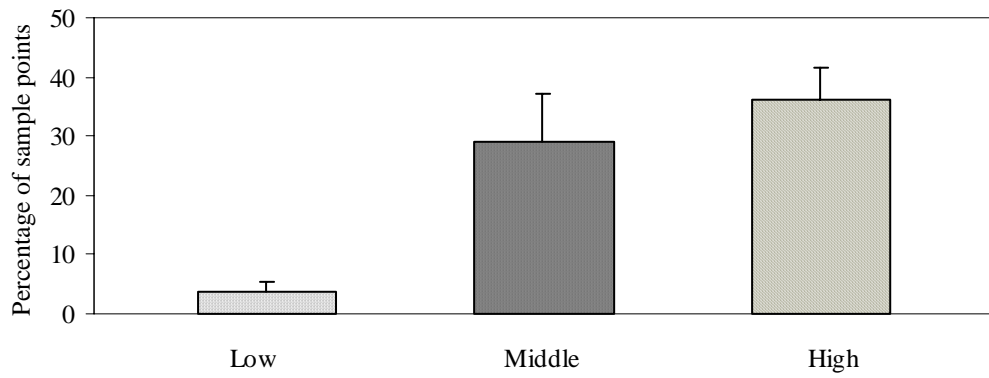
2b



3a



3b



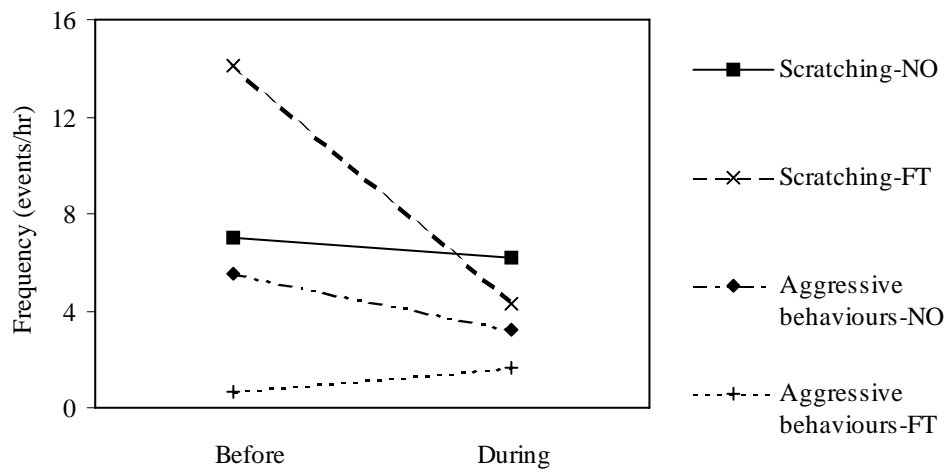


Figure 1: Latency to explore (1a) and time spent exploring (1b) the three novel objects/foraging tasks (mean  $\pm$  SE) - Experiment 1.

Figure 2: Latency to explore (2a) and time spent exploring (2b) objects A, B, and C (mean  $\pm$  SE) - Experiment 2.

Figure 3: Latency to explore (3a) and time spent exploring (3b) the objects placed low, middle, and high in the cage (mean  $\pm$  SE) - Experiment 2.

Figure 4: Mean frequencies of scratching and aggressive behaviours per hour displayed before and during the presentation of the novel objects (NO) and the foraging tasks (FT) - Experiment 1.