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RESEARCH ARTICLE

The Effects of Individual Cubicle Research on the Social Interactions and Individual Behavior of Brown Capuchin Monkeys (*Sapajus apella)*

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The effects of individual cubicle research on the social interactions and individual behavior of brown capuchin monkeys (*Sapajus apella)*

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Short title: Effects of cubicle research on capuchins

Abstract

Primates are increasingly being tested individually in purpose-built research centres within zoos. The voluntary nature of research testing indicates that participation is enriching for the primate subjects, but previous studies have generally focused only on stress-related behavior, indicating that the research does not have a negative effect. Few data are available on the effects that individual research may have on social behavior, yet given primates' complex social lives and their responses to how conspecifics are treated, it is important to determine whether individual testing impacts upon their social interactions.

The current study compared the social and individual behavior of 11 brown capuchin monkeys (*Sapajus apella)* between three conditions: (1) directly after undergoing individual testing, (2) a control and (3) upon returning to the group having voluntarily left. The results indicate that individual and stress–related behaviors were affected very little by individual research testing and that social behaviors increased. However, although affiliative interactions were enhanced, aggressive interactions were also seen to increase in the condition following individual testing. Suggestions for minimizing the negative interactions are given. Provided that these suggestions are taken into account by researchers, our results provide support for developing research centers within zoos given the important findings emerging on our closest living relatives, combined with the potentially positive effects the research has on their welfare.

Key words: Research testing, social interactions, *Cebus*, human interactions.

INTRODUCTION

Given our interest in understanding the evolution of our own behavior and cognitive abilities, it is not surprising that primates are often the focus of zoo-based research testing. There is also a growing interest on the effects that using captive primates for individual research has on the welfare of subjects themselves, with several recent topical papers investigating this [Yamanashi & Hayashi, 2011; Herrelko et al., 2012; Whitehouse et al., 2013; Fagot et al., 2014]. To assess primate welfare, measures of anxiety are commonly used, including the frequency of self-directed behavior (SDB) such as scratching, vigilance, head tilting, self-grooming and agitated locomotion. Agitated locomotion involves the monkey moving at a rapid pace, including running and pacing, but is not scored when playing. In contrast, slow locomotion is not associated with stress [e.g. Maestripieri et al., 1992; Dufour et al., 2011].

An important aspect to consider when assessing primates' welfare in general is their interactions with other members of their group. To ensure good welfare, high levels of affiliative behavior are desirable and in turn low levels of aggressive, especially injurious, interactions [Leonardi et al., 2010]. Individual research testing of group-housed primates usually involves temporary separation and the provision of food rewards for correct responses, with primates then returning to the group. It is possible that this may impact social interactions; evidence indicates that brown capuchin monkeys (*Sapajus apella*) are highly aware of differences in the way they are treated in comparison to others and have been found to comprehend inequality, showing aversion to inequality in the rewards if they have worked as hard to receive them [Brosnan & de Waal, 2014]. Chimpanzees (*Pan troglodytes*) have been found to display punitive behavior when they feel victimized, meaning they may be motivated to punish others out of anger [Jensen et al., 2006]. In the context of the present study, the captive capuchin monkeys may not be receiving unequal treatment, but they are treated differently depending on whether they take part in research. Therefore it is important to establish not only whether individuals who are involved in individual research behave any differently upon return to their group but also whether they are treated differently by others.

Whitehouse et al. [2013] found that crested macaques (*Macaca nigra*) increased association with others during testing days, compared to non-testing days, and suggested this may be due to providing the more naturalistic opportunity to separate from group mates for short periods, thus providing opportunity for reunion. Capuchins [Izar et al., 2012] unlike some baboon species [Kummer, 1968; Dunbar, 1988], chimpanzees [Aureli et al., 2008] and some macaque species [Fukuda, 1989; Menard et al., 1990] do not naturally live in a fission-fusion environment and therefore may be more sensitive to separation from the group for research purposes.

The classical approach to research testing in a laboratory setting originally involved forcibly isolating individuals, often by pole and collar, and using a restraint chair during testing [e.g. Lily, 1958. See McMillan et al., 2014 for Refinement of the approach that continues today). However, as technology improves, Automated Testing Systems (ATS) that the primates can access at the time they choose are becoming increasingly popular [e.g. Fagot & Paleressompoulle, 2009]. ATS give primates the freedom to participate voluntarily in research at their own initiation without the stress of forced separation. Social conflicts and most individual behavior do not appear to be affected by the use of ATS, at least in the case of baboons [Fagot & Bonte, 2010] and salivary cortisol and stereotypies were reduced when the ATS was accessible, compared to inaccessible [Fagot et al., 2014]. However, as with other studies on macaques following research testing [Gazes et al., 2013] the subjects were not observed directly upon leaving the research session, so critical social interactions may have been missed.

Although ATS is becoming more prominent, much individual research still requires human-primate contact [e.g. Morton et al., 2013]. This form of testing with individuals volunteering to be separated for research testing, and be in contact with humans, is the focus of this paper. There is a need to establish whether human interactions independently influence primate behavior outside of the effects that research participation alone may have. A recent study saw higher than normal levels of agonistic behavior and lower levels of pro-social behavior following chimpanzee interactions with keepers [Chelluri et al., 2013]. Analysis of multiple studies also found negative implications of human-chimpanzee interactions, with a greater than expected level of wounding on weekdays compared to weekends [Lambeth et al., 1997]. This may be due to an increase in husbandry activities on weekdays, indicating that human-chimpanzee interactions may be related to increased incidences of aggression and injury.

In contrast, exposure to humans in a research environment may be enriching for some chimpanzees who were eager to participate [Herrelko et al., 2012]. Whilst the rate of chimpanzee self-directed behavior (SDB) did not differ between research and baseline levels, during research sessions SBDs increased when visual access to humans was restricted, suggesting visual contact was important, possibly as it increased certainty of the process and reward [Herrelko et al., 2012]. Leavens et al. [2001] also found a link between uncertainty and increased SDBs, and showed with increasing cognitive challenge rates of SDBs were higher. This suggests rates of SDBs are influenced by multiple factors.

Participation in research has also been found to encourage captive chimpanzees to mimic the activity patterns of wild chimpanzees [Yamanashi & Hayashi, 2011], a factor which is considered important for improving captive primate welfare [Hosey et al., 2013]. However, in neither of these studies were data collected on how research participation directly influenced conspecific social interactions.

Another recent study compared both self-directed and social behavior on research testing and non-testing days in captive crested macaques [Whitehouse et al., 2013]. They found that the affiliative behavior “lip-smacking” was more prevalent on testing days whilst aggressive interactions decreased. Cognitive research was therefore interpreted as being useful for improving the welfare of captive primates. However, the sample size was limited (5 individuals, only 3 of which were actively involved in research) and there was likely a lack of clear boundaries between the research testing and non-testing conditions for the macaques, as the testing days included pre-test times, which may have been indistinguishable to the macaques from non-testing days.

The purpose of the current study was to provide a more thorough and comprehensive analysis of primate welfare with regards to individual cubicle research. The aim was to determine whether engaging in individual cubicle research (involving human contact) has an effect on capuchin social interactions and individual behavior. Following individual research participation, observations were made immediately upon the subjects’ return into the group in order to determine any influence. Two other conditions were compared, a non-testing control day and a “return to group” condition to determine the influence of the individual testing.

METHODS

Subjects and study site

The subjects were 11 brown capuchin monkeys (*Sapajus apella*) residing at the Living Links to Human Evolution Research Centre (LL) situated at the Royal Zoological Society of Scotland’s Edinburgh Zoo, UK. The subjects lived in one of two symmetrical enclosures in two separate mixed-species groups (named East and West), with squirrel monkeys (*Saimiri*), a species with which they naturally associate in the wild [Podolsky, 1990]. Each of the two mixed-species enclosures consisted of an indoor enclosure exclusively for use by the squirrel monkeys, an indoor capuchin enclosure for use by both species and a mixed-species outdoor enclosure (Fig. 1). Inhabitants were able to move freely between their indoor and outdoor enclosures through doors and tunnels. The research cubicles were situated in a separate area in between the two indoor enclosures, and when no research was taking place the primates could use the cubicles as an alternative route to travel between their indoor and outdoor enclosures. Further details on the primate housing, husbandry and LL can be found in Leonardi et al. [2010] and MacDonald and Whiten [2011].

All capuchins had the opportunity to participate voluntarily in cubicle research with researchers at LL, and the 11 monkeys who did so regularly were selected as the study subjects. When observations began, the West group contained 14 adults and 2 infant capuchins, with two more infants born within the observation period. The East group consisted of 15 adults. All of the capuchin monkeys were born in captivity. Of the 11 subjects, 5 were from the West group and 6 were from the East. They ranged in age from 2-15 years (mean=7 years 2 months), consisting of 3 females and 8 males. These 11 individuals were selected for observation as they participated regularly in research, permitting sufficient data to be collected on them within the study time frame. The behavior of individuals in the group other than the 11 subjects was not recorded, other than in relation to the social interactions with the focal subject.

Procedure

Observations were carried out between September - December 2013 at the Living Links to Human Evolution Research Centre, Edinburgh Zoo. There were three conditions: (1) the Post Testing condition (PT) which occurred as soon as the subject was released into the observation starting area after participating in cubicle research; (2) the Control condition (C) where subjects were required to have been in the observation starting area for at least 5 minutes before the observation began; (3) the Return to Group condition (RG) which involved no participation in research, occurring when the subject returned of their own accord to the observation starting area from another section of the enclosure; for example, returning to the indoor enclosure from the outdoor. The inclusion of this third condition made it possible to distinguish whether individual research participation has an effect on behavior, or whether merely returning to the group has an effect. For the Return to Group condition the data were collected opportunistically as it was not possible to control how long the focal individual chose to be away from the group for. The Post Testing observations took place on Monday, Tuesday, Thursday and Friday, which were the days individual research took place at LL, whist the Control and Return to Group observations were made on Wednesday, Saturday and Sunday when no researchers were working directly with the subjects in cubicles.

Data were collected across three conditions coinciding with the LL research sessions, from 11AM-1PM (morning) and 2PM-4PM (afternoon). Observations were not made for a minimum of 30 minutes after the monkeys' morning feed and afternoon scatter feed.

In total, 5 focal samples lasting 6 minutes each were completed within each of three conditions per subject. Within each condition every subject was observed for two morning and two afternoon sessions, with the fifth sample being collected at random in either session for convenience. There were 15 samples collected for each individual, with a total of 165 samples (990 minutes of data). Data were collected direct to checksheets; a Dictaphone was also used to describe more complex interactions orally for later transcription.

As the subjects could move between their indoor and outdoor enclosures, observations always began in the area of the enclosure that the majority of the group gathered for the C and RG conditions. This was the indoor enclosure for the West group for the study duration. The East group tended to remain outdoors until the weather worsened in the winter, hence observations began outside until late November at which point observations began in the indoor enclosure (see Fig. 1).

The type of cubicle research which the individuals participated in varied and included a mixture of video observations, reactions to photos and collection of saliva. Participation was rewarded with treats consisting of peanuts, raisins or sunflower seeds. There were either one or two researchers present at any time, with a total of four researchers working between both groups. In order to encourage the individuals to leave the cubicles they were given a monkey nut in its shell to take with them upon completing the session. The capuchins were separated from the group for up to approximately 15 minutes to take part in research. Capuchins volunteered themselves for testing by entering the open testing cubicles. Each monkey was tested individually and could not see, or be seen by, any other conspecifics during testing (i.e. they could not see the rewards being given during testing). The capuchins briefly had visual access to each other before the test monkey was released back into the group and could see the final and largest reward given to the test monkey at the end of the session.

Behavioral observations

Focal samples were used to identify the frequency of short-term behavior events. Point sampling every 15 seconds was used to identify the amount of time spent in longer lasting states [Martin & Bateson, 2007]. It was possible for some behaviors to be recorded concurrently, for example if the individual was foraging whilst in close proximity to another. Mutually exclusive behaviors were also recorded, for definition and further details see Tables I and II. Each observation session lasted for 6 minutes within a period of up to 9 minutes, allowing for up to 3 minutes of time that the subject might spend out of sight. The observation sample was discarded if the individual was out of sight for more than 3 minutes in total. Average sample length, including time spent out of sight, was 6 minutes 36 seconds.

Table II describes individual behavior recorded, adapted from previous papers [Dufour et al., 2011; Leonardi et al., 2010]. The social behaviors of interest were divided into three key categories consisting of aggressive, affiliative and neutral (Table II). These behaviors were based on those from Leonardi et al. [2010], modified for relevance to intra-species interactions. Details of the first social interaction in each sample were recorded, including who made the first approach (focal individual, other individual, or already in contact), who left the interaction (focal or other), its valence (affiliative, aggressive, neutral, submissive or no interaction) and finally exactly what social behavior it was.

Modifications to behavior definitions

As there were infants less than 6 months old present in the West group, interactions with infants were recorded including lip smacking, suckling and approaching the infant. One focal subject, Pedra, carried her own infant on her back the majority of the time at the beginning of the observation period. As this was a necessity rather than a choice, in her case this was not included as an affiliative behavior. However, when she chose to further interact with her infant (e.g. grooming or suckling) then this was considered an affiliative behavior. When Pedra carried other infants beside her own, this was also included as affiliative.

Data analysis

For each 6 minute sample, all behavior occurrences within each category (affiliative, aggressive and neutral) were combined to create counts for the total frequency per category, and multiplied by 10 to create estimated rate per hour for graphs. The total estimated duration of time engaged in behaviors were also calculated for each category. The total frequencies of submissive behavior were combined to make an overall frequency of submissive behavior. All data were tested using the Shapiro-Wilk test of normality. The data could not be log transformed, and therefore non-parametric tests were run. Friedman tests were followed by Wilcoxon signed-ranks test. Bonferroni corrections were used for adjusting significance levels. Chi-square analysis was used on frequency data. All tests are two tailed.

Ethical statement

This research followed the protocols of the Living Links Research Centre and was approved by the University of Stirling Psychology Ethics Committee. It adhered to the legal requirements in the UK as well as the American Society of Primatologists (ASP) Principles for the Ethical Treatment of Non-Human Primates.

RESULTS

The number of capuchins present in the enclosure area the focal was in at the beginning of each data collection sample was statistically different between conditions (Freidman, χ^2 (2) =27.078, $P \leq 0.001$). There were fewer capuchins present in the Post Testing than the Control (median 8 vs 11 Wilcoxon, Z=-5.245, $P \leq 0.001$) and the Return to Group conditions (mean 8 vs 10, Z=-3.569, $P \leq 0.001$). Having fewer capuchins present meant there was less opportunity to interact with others in the Post Testing condition in comparison to the Control and Return to Group conditions. There was no significant difference between the Return to Group and Control conditions (Z=-1.582, P = 0.114).

Individual behavior

The frequency of agitated locomotion for the focal differed significantly across conditions (χ^2 (2) =11.285, P=0.004). The frequency of agitated locomotion was higher Post Testing than in the Control condition (Z=-3.016, P=0.003) and the Return to Group condition (Z=-2.137, P=0.033). There was no significant difference in the frequency of agitated locomotion between Control and Return to Group conditions (Z=-0.406, P=0.684). No significant differences were seen between conditions for the duration of time spent in agitated locomotion (χ^2(2)=5.679, P=0.058) (Fig. 2). However the estimated time spent in slow locomotion was significantly different between conditions (χ^2(2)=11.692, P=0.003) (Fig. 3). In the Control condition, significantly less time was spent in slow locomotion compared to the Post Testing (Z=-2.942, P=0.003) and the Return to Group condition (Z=-3.033, P=0.002). There was no significant difference between the time spent in slow locomotion in the Return to Group and Post Testing conditions (Z=-0.559, P=0.576).

No significant differences were seen between conditions for any other individual behavior when the Bonferroni correction was applied; scratching (χ^2(2)=2.919, P=0.232), self-grooming (χ^2(2)=6, P=0.050), head tilting (χ^2(2)=2.526, P=0.283), urination/defecation (χ^2(2)=3.406, P=0.182), vigilance (χ^2(2)=2.455, P=0.298), foraging (χ^2(2)=2.722, P=0.256) and resting (χ^2(2)=5.093, P=0.078) (see Table III which summarises all the findings).

Social behavior: First interaction

A Chi-square analysis was used to investigate the distribution across 4 behavioral categories of first interaction; no contact, neutral, aggressive and affiliative. Only one of the first interactions was classified as submissive so this category was excluded from the analyses. The frequency of first interactions per category was significantly different between conditions ($\chi^2(8)=26.811$, $P \leq 0.001$). This was the case for neutral interactions ($\chi^2(2)=613.818$, $P \leq 0.001$) and cases of no contact ($\chi^2(2)=6.276$, $P=0.043$). Pair-wise comparisons showed significantly more neutral first interactions Post Testing compared to the Return to Group condition ($\chi^2(1)=7$, $P=0.008$) and the Control ($\chi^2(1)=9.846$, $P=0.002$), but no significant difference in the number of neutral first interactions between Return to Group and Control conditions ($\chi^2(1)=0.333$, $P=0.564$). Post Testing, there were significantly fewer incidences of no contact compared to the Control condition ($\chi^2(1)=5.556$, $P=0.018$). There were no significant differences between conditions in the frequency of aggressive first interactions ($\chi^2(1) = 1$, $P=0.317$) nor affiliative interactions ($\chi^2(2) =2.102$, $P=0.350$). Fig. 4 shows the distribution of first interactions per behavioral category across all conditions.

A Chi-square analysis was used to investigate which behaviors were seen as a first interaction, and how their frequencies varied between conditions (Fig. 5). The behavior seen as first interactions were; curious approach towards, and by focal, focal chased, focal displaced, focal displaces, allogroom, play, scalp lift, focal chased squirrel monkey, vocal exchange, already in contact and no contact. Between conditions, there was a significant difference in how often different types of behavior occurred as the first interaction ($\chi^2(22)=81.973$, $P\leq0.001$). There were few differences in how often behavior occurred between the Return to Group and Control conditions, with the only significant difference being that in the Control condition there were more cases of already in contact ($\chi^2(1)=12.8$, $P\leq0.001$). Post Testing, there were significantly fewer occurrences of curious approaches by the focal then in the Return to Group condition ($\chi^2(1)=5.261$, $P=0.022$) and significantly more incidences of the focal being displaced than the Return to Group condition ($\chi^2(1)=8.167$, $P=0.004$). When comparing the Post Testing and Control conditions, there were also significantly more incidences of the focal being displaced Post Testing ($\chi^2(1)=13.762$, $P\leq0.001$), more incidences of the focal being curiously approached by another Post Testing ($\chi^2(1)=7.1348$, $P=0.004$), and fewer cases of no interaction Post Testing ($\chi^2(1)=6.368$, $P=0.012$).

When investigating who initiated the first interaction, cases of "already in contact" and "no contact" were excluded. Analyses revealed that Post Testing, another individual (not the focal subject) was significantly more likely to make the first approach ($\chi^2(1)=20.447$, $P\leq0.001$) but there was no difference within the Control condition ($\chi^2(1)=0.391$, $P=0.532$) nor within the Return to Group condition ($\chi^2(1)=0.857$, $P=0.355$) (Fig. 6). The focal subject was significantly more likely than the other individual to end the first interaction in both the PT ($\chi^2(1) =21.353$, $P\leq0.001$) and Return to Group conditions ($\chi^2(1)=5.233$, $P=0.022$) (Fig. 7). There was no significant difference in who left the first interaction within the Control condition ($\chi^2(1)=2.778$, $P=0.096$).

Social Interactions

There was a statistically significant difference in the frequency of aggressive behavior between the three conditions ($X^2(2)=9.5$, $P=0.009$). Post-hoc tests found no statistically significant difference between the Return to Group and Control conditions ($Z=-1.069$, $P=0.285$) or the Post Testing and Control conditions ($Z=-2.486$, $P=0.272$). However there were significantly more occasions of aggressive behavior Post Testing compared to the Return to Group condition ($Z=-2.486$, $P=0.013$). These data are not plotted as all medians were zero. There were 11 cases of aggressive interactions Post Testing, 10 in the Control condition, and 1 in the Return to Group condition. Overall, 7 of these interactions were directed towards a squirrel monkey, 6 were threat displays by the focal, 3 consisted of the focal chasing another, in 4 cases the focal was chased by another and 2 were vocal exchanges between capuchins. The amount of time spent in aggressive interactions did not vary significantly between conditions ($\chi^2(2)=2$, $P=0.368$). Out of the 11 subjects, 73% (n=8) were involved in aggressive interactions. Seven of these individuals were involved in aggressive interactions involving other capuchins. One subject only engaged in aggressive interactions with squirrel monkeys, and was responsible for 7 of the 10 aggressive interactions in the Control condition.

The frequency of neutral interactions did not vary significantly between conditions ($\chi^2(2)=2.989$, $P=0.224$), nor did the time spent engaged in neutral interactions ($\chi^2(2)=4.136$, $P=0.126$). The frequency of affiliative behavior varied significantly between conditions ($\chi^2(2)=6.570$, $P=0.037$) (Fig. 8). Post-hoc tests showed significantly more affiliative interactions occurred Post Testing compared to the Control condition ($Z=-2.891$, $P=0.004$). There were no significant differences between the Post Testing and Return to Group ($Z=-1.712$, $P=0.087$) or the Control and Return to Group conditions ($Z=-0.336$, $P=0.737$). The duration of affiliative behavior did not vary significantly between the three conditions ($\chi^2(2)=2.161$, $P=0.339$). Table III summarises the statistical tests from additional analyses.

DISCUSSION

The aim of this study was to determine whether engaging in individual cubicle research (involving human contact) has an effect on capuchin social interactions and individual behaviors. Our results indicate that it affects both. Following testing, individuals engaged in an increased number of affiliative interactions. There was also an increase in aggressive interactions and agitated locomotion. We suggest that the additional factor of taking a high value reward back to the group following research influenced these behaviors.

Participating in individual research did not affect the time the Living Links capuchins spent engaged in behavior such as foraging or resting. However in the Control condition, where the monkeys had not been tested, significantly less time was spent in slow locomotion compared to the other conditions. This could have been due to the fact that this was the only condition where the individual had the potential to be stationary from the beginning of the sample as they had been in the observation area for a minimum of 5 minutes beforehand.

If research testing and/or returning to the group following testing increased anxiety, it would be expected that rates of scratching, vigilance and in the case of capuchins, head tilting, to be elevated in the condition following testing. This was not the case, bearing similarities to the findings of Herrelko et al. [2012] and Whitehouse et al. [2013] that the research did not have a negative impact. In the present study there were significantly more occurrences of agitated locomotion following testing compared to the non-testing conditions. This increase in agitated locomotion may indicate an increase in anxiety levels; however the other anxiety-related behavior were not affected, suggesting it may be more related to positive arousal. It may be a consequence that they were returning to a space with significantly fewer monkeys than in the other two conditions, and their agitated locomotion may be a result of lack of social support, or eagerness to find specific group members, rather than a direct result of cognitive testing.

Given that capuchins do not live in fission-fusion groups, unlike chimpanzee and some macaque species (see Introduction), we cannot assume that they will cope as well with temporary separation for research testing as other species which do live in fission fusion societies. Therefore it is important to investigate how their social interactions are affected by members of the group participating in individual research. Whilst the frequency of neutral interactions did not vary between conditions, affiliative behavior was more frequent following testing compared to in the Control condition, whilst aggressive behavior were more frequent Post Testing than in the Return to Group condition which did not involve testing (the two conditions which did not involve testing did not differ significantly for either behavior type). This is surprising given that overall there were significantly fewer capuchins present at the beginning of data collection in the condition that followed testing. This finding indicates that using the Living Links capuchins in individual research does affect their social interactions, at least in the short term. If merely leaving and then returning to the group instigated an increase in affiliative or aggressive interactions for the returning capuchin then it would be expected that both conditions which involved returning to the group would also show a significant increase compared to the Control condition. However this was not the case, indicating that for the Living Links capuchins an aspect of research participation particularly encourages an increase in social interactions, both affiliative and aggressive. It should be noted that none of the aggressive interactions observed in this study resulted in any injuries.

The first interaction behavior, who initiated and who ended it showed some distinct patterns between conditions. Following testing, the likelihood that subjects would be involved in some sort of interaction significantly increased compared to the Control condition. The chances of engaging in a social interaction were not significantly higher in the non-testing Return to Group condition, again indicating something different in the group's reaction towards individuals who have attended research sessions. In the condition Post Testing the first interaction was significantly more likely to be neutral than the other two conditions, and was significantly more likely to be initiated by a non-focal individual whilst the focal individual was significantly more likely to leave. The two conditions which involved returning to the group show similarities here, as in both cases when the subjects had been separated from the group they were significantly more likely to end the first interaction.

Differences in the first interaction may be partly explained by the fact subjects were given a monkey nut before leaving the research cubicles to encourage them to leave promptly. Although this did not affect the time spent foraging, it is possible that it had some effect on the first interaction. In the Post Testing condition there were significantly more displacements directed at the subject than the other two conditions and significantly more curious approaches directed towards the focal compared to the Control condition. Both of these interactions could have been motivated by an interest in the focal individual's food. Therefore it is possible that these differences in the first interaction Post Testing are influenced by the fact that subjects had a high value food. In order to test what extent this factor influences behavior, it would be necessary to conduct a similar study including a Control condition where the subjects are not given a reward to return to the group with following testing. It would also be important to identify whether agitated locomotion is still significantly higher following testing than in the other conditions, or whether it is also influenced by the possession of a high value reward. Even without confirmation of the reasons why, it is clear that out of the three conditions in this study the Post Testing condition stands apart from the other two conditions, and it appears that being given a large food reward causes subjects to be of particular interest to others when they return to their group after participating in a research session.

The current study has provided a more in-depth and comprehensive analysis on the effects that individual research has on primates' social interactions and individual behavior than previously seen. Furthermore, this is the first study directly focusing on the effect of individual testing on a species that does not live in fission-fusion societies, as opposed to baboons [Fagot et al., 2010, 2014] chimpanzees [Herrelko et al., 2012] and macaques [Whitehouse et al., 2013]. The evidence indicates that participating in research may have a positive effect on Living Links capuchins' welfare upon their return to the group, as a higher than expected frequencies of affiliative interactions were seen following testing participation. However, given that there was also an increase in aggressive and agitated locomotion behavior, it would be beneficial for the welfare of captive capuchins who participate in research to work to eliminate the higher frequency of aggressive interactions whilst sustaining the increase in affiliative interactions. As previously mentioned, it is possible that the use of a food reward upon completion of research may influence the behavior of capuchins as the focal individual was holding and eating something desirable, encouraging others to approach them. If the capuchins were given the opportunity to consume their rewards before being released from the research cubicles, the frequency of non-injurious aggressive interactions upon return to the group might reduce. The significant increase in affiliative social interactions after research is a positive finding, indicating that research may have a beneficial effect on the social interactions of capuchins.

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TABLE I: Definitions and methods of recording individual behaviors*

[x b] Rest	The monkey is either sleeping or in a state of calmness, with the body relaxed in a stationary position. Eyes may be closed or open, but not actively scanning the environment.
[x b] Forage	Searching for food, including ground digging, scanning the Environment for insects or pieces of food and eating.
[x b] Slow locomotion	Moving, usually walking, with no jumping or running.
Anxiety- related behaviors	
[x b] Self-groom	The monkey's hands and/or lips are drawn through their own coat, skin, or teeth and particles are occasionally removed.
[x a] Scratch	The monkey repeatedly moves hand or foot using nails to scrape the skin and/ or fur.
[a] Head tilt	The monkey repeatedly tilts his/her skull to one side abruptly, sometimes alternating sides. Not directed at another individual, sometime occurs during locomotion.
[x] b Vigilant	Sitting or standing, with eyes actively scanning the surroundings.
[a b] Agitated locomotion	Monkey is moving in relation to its surroundings: movements are made at a rapid pace, including running, jumping and repeatedly pacing. Not scored when playing.

* All behaviors in this table are mutually exclusive amongst themselves but could occur simultaneously with behaviors in **Table II** unless stated otherwise.

[a] All occurrences recorded to provide estimated rate/hr

[b] Recorded by point sample to provide estimated duration

[x] Identical to definition by Dufour et al. [2011]

TABLE II: Definitions and methods of recording social behaviors *

Affiliated behavior	
[abx] Play and play elicitation	Monkey plays with other or attempts to elicit play. Also includes attempts to join in play, for example moving close and engaging in similar play behavior.
[bx] Allogroom	The monkey's hand and/or lips are drawn through the coat, skin or teeth of another and particles are occasionally removed.
[abx] Food share passive	The possessor of the edible item neither solicits nor restricts the attempts of other individuals to take it. Individuals remain in close proximity to each other and do not engage in aggressive behavior.
[abx] Food beg	Monkey(s) make gestures to otherindividual holding an edibleitem,forexample,an arm extended with outstretched hand, palm facing upwards or reaching toward food item while in the other monkey's hand.

[abx] Curious approach	Monkey moves toward other(s) and does not display any aggressive behavior, but shows interest in other individual or initiates interaction (e.g. sniffing, gentle touch, or moving into >50 cm and observing).
[b] Close proximity- contact	Monkey is in physical contact with another but not engaging in any other social behavior listed- does not include brushing in passing.
[ab] Close proximity- interest	Monkey is within 50cm of another, not touching but showing an interest in the other/interacting.
[ab] Infant interaction	The monkey interacts with an infant, including playing, grooming, lipsmacking, carrying, sucklingor approaching.
Neutral behaviors	
[bx] Close proximity- no contact	Monkey(s) moves to <50cm of other individual (not simply passing to go elsewhere) but shows no interest in interacting, and does not touch.
[ab] Displace- no contact	Monkey(s) approaches another individual, causing the other individual to move from it's immediate area, but without making physical contact and without appearing aggressive.
[abx] Unclear	An interaction occursbetween two or more monkeys, but it is difficult to discern the type of interaction (e.g. many members, or many overlapping types of interaction occurring at once, making it difficult to establish overriding type).
Aggressive behaviors (all mutually exclusive)	
[abx] Chase- no contact	One or more monkeys actively pursue one or more monkeys, moving at a rapid pace but not physically touching.
[abx] Chase- contact	As above but physically touches (e.g. grabs/pinches).
[abx] Vocal exchange	Individuals face each other and call/shriek/scream, often accompanied by facial grimaces and retracted lips.
[abx] Threat display	Monkey(s) engages in non-vocal aggressive behavior toward another member(s) of the same species such as genital displays, facial grimaces (retracted lips, mouth open), branch shaking, or rapid body movements in their direction (thrusting head forward then pulling back). No physical contact is made.
Submissive behaviors	
[ab] Submissive grin	The monkey's lips are apart, and the corners of the mouth are down-turned.
[ab] Scalp lift	The monkey raises its eyebrows, directed at another individual.

* All behaviors in this table are mutually exclusive amongst themselves but could occur simultaneously with behaviors in **Table I** unless stated otherwise.

[a] All occurrences recorded to provide estimated rate/hr

[b] Recorded by point sample to provide estimated duration

[x] Based on Leonardi et al. [2010], modified for relevance to intra-species interactions.

TABLE III: Significant and non-significant results for pairwise comparisons. PT = Post testing; C = Control; RG = Return to Group condition

Individual behavior	Friedman test	Wilcoxon **PT vs C**	Wilcoxon **PT vs RG**	Wilcoxon **RG vs C**
No. capuchins present at beginning	$P \leq 0.001$	$P \leq 0.001$	$P \leq 0.001$	$P=0.114$
Freq. agitated locomotion	$P=0.004$	$P=0.003$	$P=0.033$	$P=0.684$
Time spent in agitated locomotion	$P=0.058$	NA	NA	NA
Time spent in slow locomotion	$P=0.003$	$P=0.003$	$P=0.576$	$P=0.002$
Scratching	$P=0.232$	NA	NA	NA
Self-grooming	$P=0.050$	NA	NA	NA
Head tilting	$P=0.283$	NA.	NA	NA
Urination/defecation	$P=0.182$	NA	NA	NA
Vigilance	$P=0.298$	NA	NA	NA
Foraging	$P=0.256$	NA	NA	NA

Resting	*P*=0.078	NA	NA	NA
First interaction type	**Chi-square**	**Pairwise comparisons**	**Pairwise comparisons**	**Pairwise comparisons**
		PT vs C	**PT vs RG**	**RG vs C**
Neutral	*P*≤0.001	*P*=0.002	*P*=0.008	*P*=0.564
No contact	*P*=0.043	*P*=0.018	*P=0.109*	*P*=0.317
Aggressive	*P*=0.317	NA.	NA	NA
Affiliative	*P*=0.350	NA	NA	NA
First interaction behavior		**PT vs C**	**PT vs RG**	**RG vs C**
Already in contact	*P*≤0.001	DD in PT	DD in PT	*P*≤0.001
Allogroom	NA	NA	NA	NA
Curious approach by focal	*P*=-0.036	*P*=0.593	*P*=0.022	*P*=0.072
Focal displaced	*P*≤0.001	*P*≤0.001	*P*=0.004	*P*=0.257
No interaction	*P*=0.043	*P*=0.012	*P*=0.109	*P*=0.317
Curious approach towards focal	*P*=0.018	*P*=0.004	*P*=0.289	*P*=0.059
Focal displaces another	*P*=0.867	*P*=0.655	*P*=0.999	*P*=0.655
Play	*P*=0.497	*P*=0.655	*P*=0.257	*P*=0.480
Squirrel monkey chase	NA	NA	NA	NA
Chased	NA	NA	NA	NA
Scalp lift	NA	NA	NA	NA
Vocal exchange	NA	NA	Na	NA
Social Behavior	**Chi-square**	**Pairwise comparisons**	**Pairwise comparisons**	**Pairwise comparisons**
		PT vs C	**PT vs RG**	**RG vs C**
No. Aggressive interactions	*P*=0.009	*P*=0.272	*P*=0.013	*P*=0.285
Duration aggressive interactions	*P*=0.368	NA	NA	NA
No. Neutral interactions	*P*=0.224	NA	NA	NA
Duration Neutral interactions	*P*=0.126	NA	NA	NA
No. Affiliative interactions	*P*=0.037	*P*=0.004	*P*=0.087	*P*=0.737
Duration Affiliative interactions	*P*=0.339	NA	NA	NA

NA = initial analyses not significant or data deficient so further analysis inappropriate. DD = data deficient so analysis not possible.

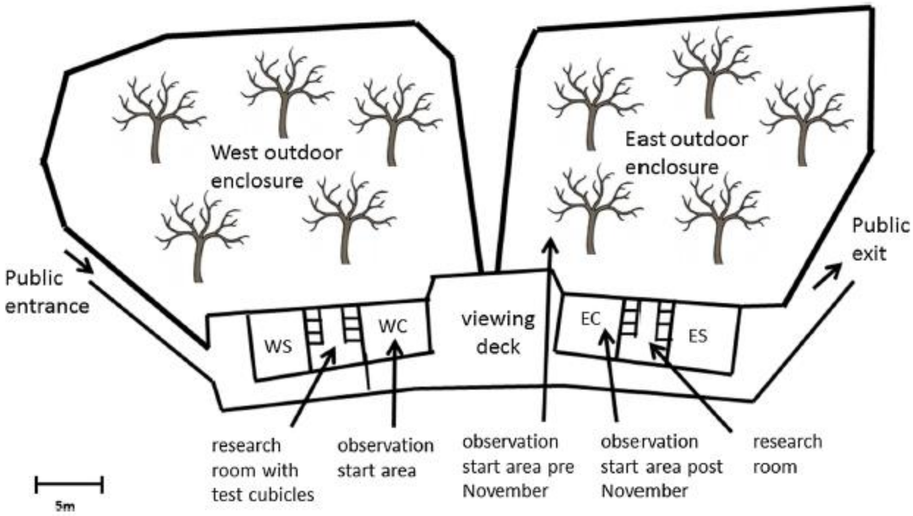

Fig. 1. Schematic diagram of the Living Links to Human Evolution Research Centre, approximately to scale (adapted from Leonardi et al., 2010). Key for indoor enclosures; WS, West squirrel monkeys; WC, West capuchins; ES and EC, East squirrel monkeys and East capuchins.

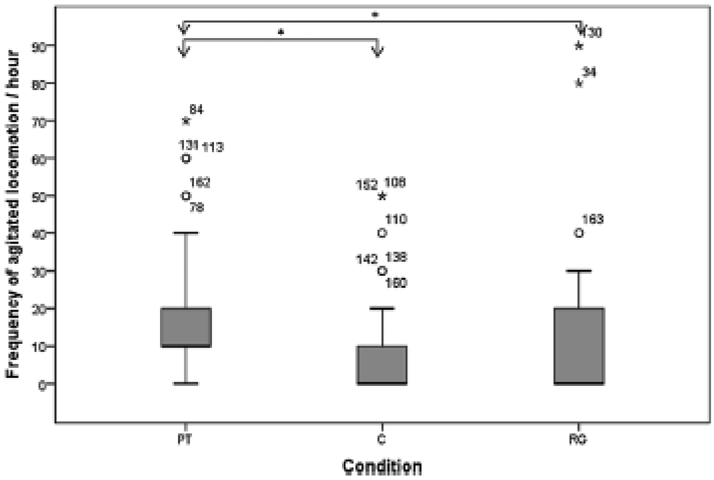

Fig. 2. Frequency of agitated locomotion/hour across all conditions; the band in the middle shows median, the bottom and top of the box show lower and upper quartiles, respectively, and whiskers indicate 1.5 interquartile range. Small circles are outliers outside this range. Arrows indicate significant difference. PT, Post-Testing; C, Control; RG, Return to Group.

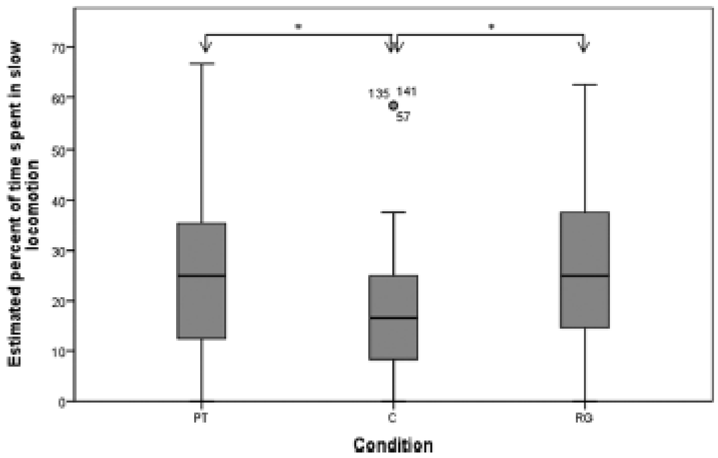

Fig. 3. Estimated percent of time spent in slow locomotion across all conditions; the band in the middle shows median, the bottom and top of the box show lower and upper quartiles, respectively, and whiskers indicate 1.5 interquartile range. Small circles are outliers outside this range. Arrows indicate significant difference. PT, Post-Testing; C, Control; RG, Return to Group.

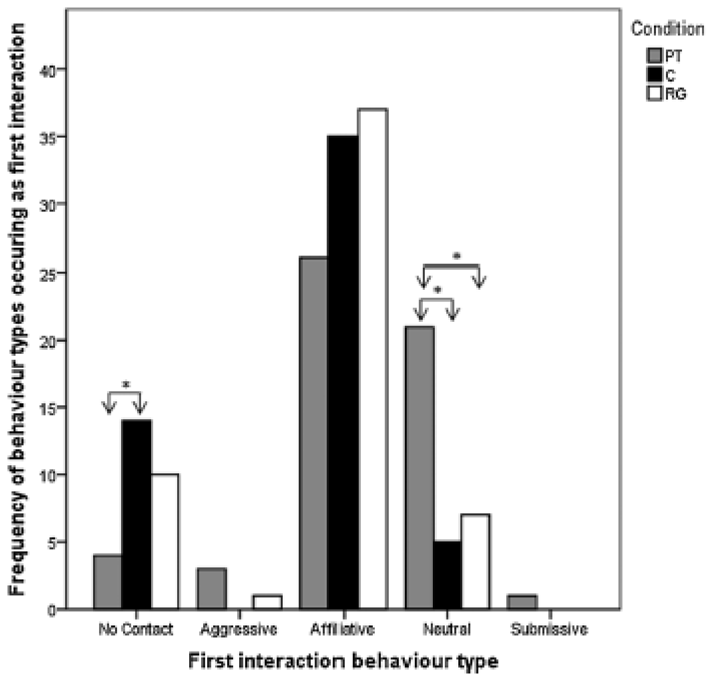

Fig. 4. Frequency of behavior types across five categories occuring as first interaction. Arrows indicate significance. PT, Post-Testing; C, Control; RG, Return to Group.

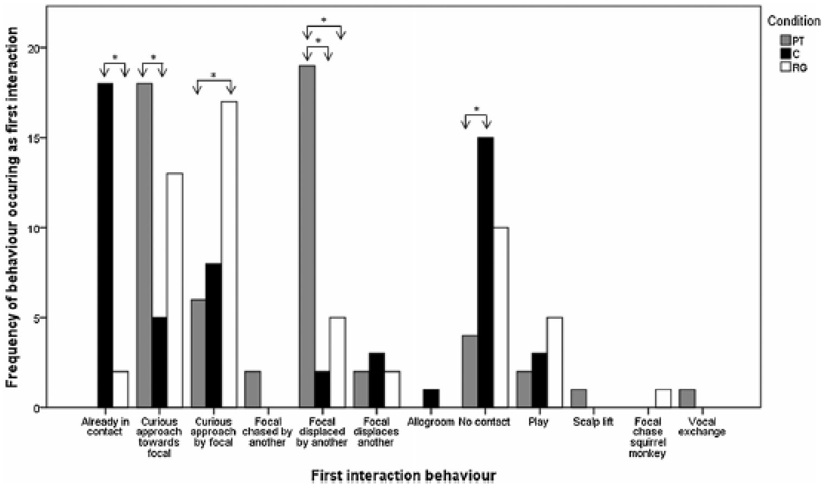

Fig. 5. Frequency of specific behaviors occuring as first interaction across the three conditions. Arrows indicate significance. PT, Post-Testing; C, Control; RG, Return to Group.

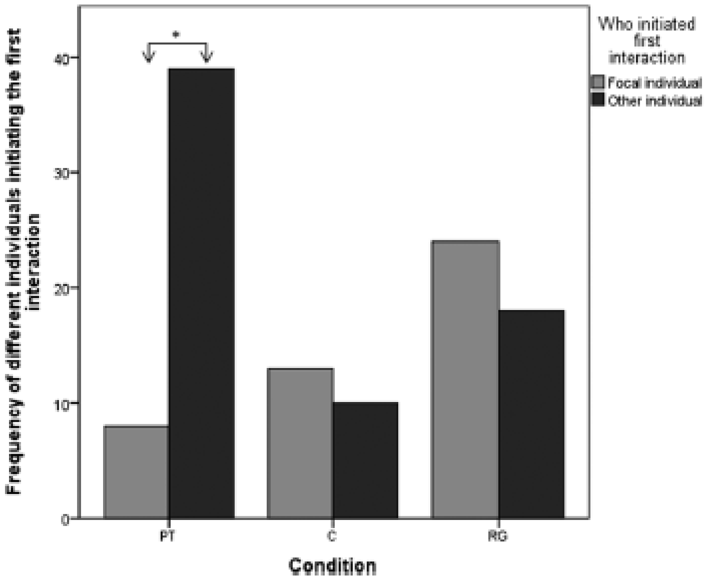

Fig. 6. Frequency that focal subject and other individuals initiated the first interaction across the three conditions. Arrows indicate significance. PT, Post-Testing; C, Control; RG, Return to Group.

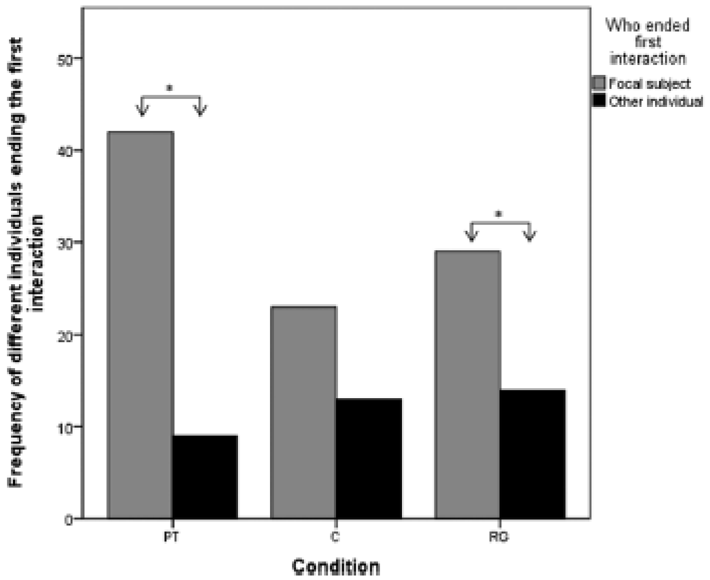

Fig. 7. Frequency that focal subject and other individuals ended the first across the three conditions. Arrows indicate significance. PT, Post-Testing; C, Control; RG, Return to Group.

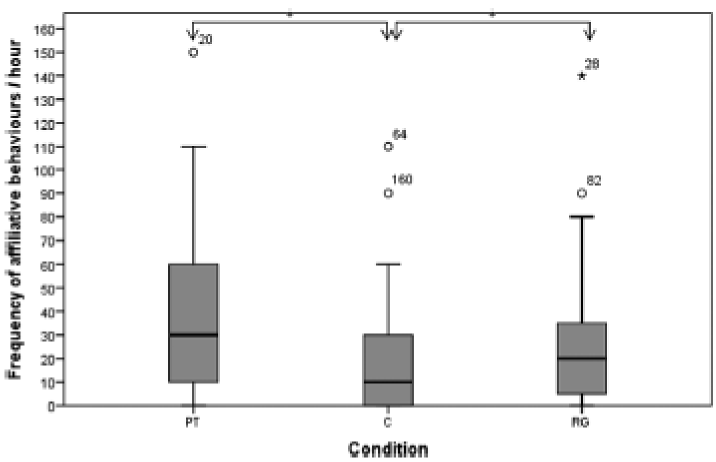

Fig. 8. Frequency of affiliative behaviors experienced by focal subjects/hour across the three conditions. The band in the middle shows median, the bottom and top of the box show lower and upper quartiles, respectively, and whiskers indicate 1.5 interquartile range. Small circles are outliers outside this range. Arrows indicate significant difference. PT, Post-Testing; C, Control; RG, Return to Group.