Case Report

A novel working memory test using capuchin monkeys (Cebus apella) emotional faces

Um novo teste de memória laborativa usando faces emocionais de macacos capuchinhos

Carina Tostes Abreu[1], Maria Clotilde H. Tavares[2], Arianna Marchetti[3], Armida d'Onofrio[4], Antonella Gasbarri[5], Carlos Tomaz[6]*

Received and accepted during the first semester of 2005

ABSTRACT

Several studies with humans have shown that pictures with emotional content are more easily recognizable than emotionally indifferent pictures. To our knowledge, no attempts have been made to investigate the role of emotional facial expressions to facilitate working memory in non-human primates. In this study we developed a pool of 384 pictures of capuchin monkey (Cebus apella) faces classified according to emotional valence (positive/pleasant, negative/unpleasant and neutral/indifferent). The objective was to examine whether working memory can benefit from the emotional content of visual stimuli in the delayed non-matching-to-sample task. Seven adult capuchin monkeys were tested with a computer system and touch screen. Geometric figures (control) and the co-specific faces pictures were used as stimuli. The subjects obtained a similar performance to positive, negative and neutral pictures. However, the monkeys performed above the upper confidence limits around chance to all kinds of stimulus, showing that they are able to learn the tests using emotional faces. Furthermore, the capuchin monkeys had much improved performance when using geometric figures compared to the co-specific pictures. This preliminary study yielded findings that are of relevance for the better understanding of the influences of emotional expressiveness on memory and indicate the possible usefulness of applying the paradigm adopted in this study to investigate emotional working memory in non-human primates.

Keywords: monkeys, Cebus apella; emotional working memory; emotional faces; non-matching-to-sample test.

RESUMO

Estudos com sujeitos humanos têm demonstrado que fotografias com conteúdo emocional são mais facilmente reconhecidas que fotografias neutras. Entretanto, de acordo com o nosso conhecimento, não existem pesquisas investigando o papel das expressões faciais emocionais na facilitação da memória operacional em primatas não humanos. No presente estudo foi desenvolvido um conjunto de 384 fotografias de faces de macacos-prego (Cebus apella) classificadas de acordo com a valência emocional em positivas/agradáveis, negativas/desagradáveis ou neutras/indiferentes. O objeti-

1-PhD student, University of Brasilia – Laboratory of Neuroscience & Behavior and Primate Center. 2- Associate Professor, University of Brasilia – Laboratory of Neuroscience & Behavior and Primate Center. 3 - PhD student, Department of Sciences and Biomedical Technologies, Faculty of Educational Sciences, University of L'Aquila, Italy. 4- PhD student, Department of Sciences and Biomedical Technologies, Faculty of Educational Sciences, University of L'Aquila, Italy. 5 - Full-time professor, Department of Sciences and Biomedical Technologies, Faculty of Educational Sciences, University of L'Aquila, Italy. 6-Full-time professor, University of Brasília – Laboratory of Neuroscience & Behavior and Primate Center, Department of Physiological Sciences, Institute of Biology, Brasilia, DF, Brazil. ctomaz@unb.br *corresponding author

vo desta pesquisa foi de investigar se a memória operacional pode ser facilitada pelo conteúdo emocional do estímulo visual na tarefa de "escolha-diferente-do-modelo-com-atraso" (delayed non-matching-to-sample). Sete macacos-prego adultos foram testados utilizando um programa computacional especifico e com um monitor do tipo tela sensível ao toque. Figuras geométricas (controles) e fotos de faces co-específicas foram utilizadas como estímulos. Os resultados não indicaram diferenças estatisticamente significativas no desempenho dos sujeitos entre as fotos com valências positiva, negativa e neutra. Entretanto, os macacos apresentaram desempenho acima do limite superior da aleatoriedade para todos os tipos de estímulos, demonstrando assim a sua capacidade de responder ao teste utilizando fotografias de faces co-específicas como estímulo visual. Além disso, o desempenho dos animais no teste foi superior com figuras geométricas quando comparadas com fotografias faciais de macacos-prego. Este primeiro estudo demonstra a importância de se entender melhor a influencia da expressividade emocional na memória e indica a possível aplicação do paradigma adotado neste trabalho na investigação da memória operacional emocional em primatas não humanos.

Palavras-chave: macaco-prego, Cebus apella; memória operacional emocional; faces emocionais; non-matching-to-sample test.

INTRODUCTION

From an evolutionary perspective, the benefit of emotion on memory may be considered adaptative as it increases the chances that relevant information will survival and be available in the future (Dolan, 2002). Thus, emotionally meaningful events as a general rule are more easily remembered than events devoid of emotional content. The amygdala seems to regulate this affective component of memory through the modulation of hippocampal activity and via interaction with prefrontal cortices and additional cortical regions that have been shown to play a significant role in emotional memory (Dolan, 2002; Gray et al, 2002; Perlstein et al, 2002; Johansson et al, 2004).

Facial expressions of emotion have an important role in communicating the needs and intentions of people. Accordingly, humans must be specially prepared by evolution and learning to detect and identify the meaning of emotional faces, in order to optimize (or avoid) social interaction (Calvo & Esteves, 2005).

Non-human primates are important and relevant models because they share numerous cognitive and physiological characteristics with humans. The behavioral evidence include similarities in innate action patterns such as body movements and communication signals, as well as highly flexible behavioral tactics and clever problem-solving strategies (Preuschoft, 2000). The capuchin monkey (Cebus apella) has been the focus of several researches due to its behavioral similarities with apes (Antonucci, 1990). Moreover, capuchins show a rich repertoire of facial expressions and body postures that convey an array of messages to co-specifics about their internal state (Fragaszy et al, 2004). Capuchin monkeys are known to display tool-using capacities (Visalberghi, 1993) and to readily solve the working memory tasks: delayed non-matching-to-sample test (DNMS) and concurrent discrimination learning task (Tavares & Tomaz, 2002; Resende et al, 2003).

There are few studies involving pictures of faces and cognition in non-human primates. In a study with females of the rhesus monkeys, Lacreuse & Herndon (2003) demonstrated that higher levels of ethinyl estradiol (EE_2) were only associated with a lower performance in the delayed recognition span test (DRST) when pictures of rhesus monkeys' faces were used as stimulus. These authors speculate that estrogens may produce this effect by enhancing emotional reactivity to socially relevant stimuli.

To our knowledge, no attempts have been made to investigate the role of emotional facial expressions to facilitate working memory in non-human primates. In this study we developed a pool of 384 pictures of capuchin monkey faces classified according to emotional valence (positive, negative and neutral) to examine whether working memory can benefit from the emotional content of visual stimuli in DNMS task. We predicted that working memory would be differentially influenced by stimulus conditions with enhanced performance when emotional valence stimuli (pleasant and unpleasant) was used in comparison to neutral stimuli.

2. METHODS

2.1. Subjects

We studied five female and two male capuchin monkeys (Cebus apella), adults, with estimated ages between 4 and 10 years. The monkeys were housed in groups of two or three in cages measuring 4,0 x 2,5 x 3,0m at the Primate Center of the University of Brasilia. The cages had an internal division with a sliding door to separate the subject during the experimental session. They were tested in their own home cages to avoid disruption of behavior by capture and transport to a different environment.

The monkeys were fed daily, around 7:00 am with fruits, vegetables, eggs, ration and honey. Water was available ad libitum. They were not deprived of food or water during the experimental sessions. The monkeys were treated in accordance with the standards of the Brazilian Institute for Renewable Resources and Environment (IBAMA - Instituto Nacional dos Recursos Renováveis e do Meio Ambiente). The experimental procedures were in accordance with the principles and regulations of the Ethics Committee of the University of Brasilia for the care and use of animals for scientific purposes.

2.2. Apparatus

The equipment consisted of a notebook (Compac Presario 1247) connected to a touch-screen monitor (LG Studio Works 440, Microtouch) and an automated pellet dispenser. This equipment was placed in a wooden trolley, 60 x 60 x 100cm, in order to allow the experimenter to move the apparatus through the cages and position it in front of them. The tests were produced by a custom-designed computer program (SYSMEN) developed by R.S. Chiba. This program controlled stimulus presentation, detected responses and stored data.

2.3. Stimulus Materials

The stimuli were geometric figures and pictures of Cebus apella faces measuring 7 x 7 cm. The geometric figures were chosen from a pool of 400 clipart color figures. About 2000 pictures of capuchin monkeys were taken and edited to keep only the face. The emotional content of each picture was judged by five researchers that had been studying the behavior of capuchin monkeys for at least one year. The pictures were classified as positive (pleasant – Figure 1), negative (unpleasant – Fig. 2) or neutral (indifferent – Fig. 3) according to emotional expressiveness. We used 384 pictures with at least 80% agreement in the emotional valence among the researchers.

Figure 1. Juvenile male playing and making a silent bared teeth display (“grin” or “smile”). Example of **positive** valence picture.

Figure 2. Adult male giving an open mouth threat face. Example of **negative** valence picture.

Figure 3. Adult male. Example of **neutral** valence picture.

2.4. Procedures

We prepared five testing sessions containing 50 trials. The first ten trials were aborted. The other 40 trials were distributed in 10 rounds of each kind of stimulus (geometric figures as well as positive, negative and neutral pictures). To compose each pair of pictures (trial) pictures with the same emotional valence, sex and head position (face or profile) were chosen. Any stimuli were repeated during these 5 sessions.

The capuchin monkeys performed 10 sessions of delayed non-matching-to-sample task (DNMS), in 2 sets over 5 consecutive days, repeating the same sessions of the second set over 5 days (sets A – 1st to 5th sessions and B – 6th to 10th sessions).

2.4.1. Cognitive training

Prior to formal testing, the monkeys learned by successive approximations to touch the screen. Each touch was rewarded by a 190mg banana flavor pellet (Noyes Company). Subsequently, they had to touch a 7 x 7cm geometric figure presented centrally on the screen. Pre-training was accomplished once the monkey touched the stimulus 30 consecutive times.

The monkeys were trained on the DNMS between 30 to 50 times a day, five days a week until they reached a learning criterion of at least 32 correct responses in 40 consecutive trials (80% correct). This training was only with geometric figures.

After reaching the learning criterion, the monkeys were submitted to 4 sessions of pilot-tests. The first session presented 45 trials only with pictures of capuchin monkey faces (15 trials for each emotional valence). Because of the low performance, we included trials with geometric pictures used in the training. The other 3 pilot sessions followed the same scheme of the test sessions.

2.4.2. Delayed non-matching-to-sample

Trials began with the presentation of a sample stimulus in the center of the screen. Touching the sample cleared the screen for 8 seconds after which two comparison stimuli were displayed in any of 16 randomical possible positions. One of the stimuli was identical to the sample (foil) while the other was an unfamiliar image. Subjects were rewarded a pellet and accompanying high-pitched tone for touching the non-matching image of the sample. Touching the foil was followed by a low-pitched tone. The interval between trials varied from 1 to 5 seconds. The computer recorded the performance (correct or incorrect response) as well as the response times.

2.5. Statistical analyses

Statistical analyses were carried out using the Kruskal-Wallis test for comparisons among four stimulus types in performances and response times. When differences were detected we used post hoc Tukey test.

In order to analyze the performance of the subjects in the DNMS task, a binomial test was used to establish 95% confidence limits around chance performance based on the number of test trials. Thus, the upper limit was calculated as 60% for 100 trials ($p < 0.05$) – number of trials per individual per stimulus type.

For the comparisons between sets A and B we used the Mann-Whitney U test for each stimulus type.

RESULTS

The means for correct responses in sets A and B for each stimulus type are presented in Figure 4. The statistical analyses showed better performance for geometric figure compared to all other stimulus types (Kruskal-Wallis; $p < 0.001$). We did not find any differences in performance among emotional pictures (positive, negative and neutral. Kruskal-Wallis; p = 0.406). There was not significant increase in performance from set A to set B in any stimulus type (Mann-Whitney test; p = 0.250 for geometric figure; p = 1.000 for positive; p = 0.639 for negative; p = 0.632 for neutral pictures).

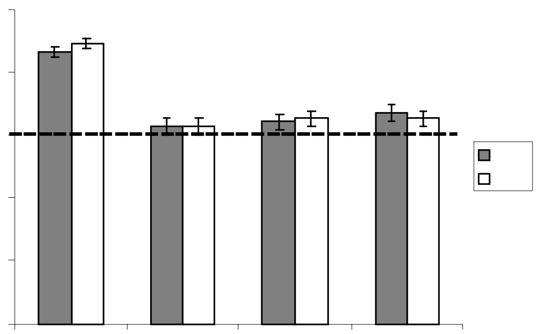

Figure 4. Mean percentage of correct responses on the DNMS test for set A (experimental sessions 1 to 5) and set B (sessions 6 to 10). The bars correspond to SEM. The dash line is the upper limit of 95% confidence interval for chance performance. * Krukal-Wallis test, p < 0.001 for geometric figure compared to all picture types. N = 350 tests.

The monkeys performed above the upper confidence limits around chance performance (60%) in all sample-choice test stimuli (Fig. 4).

The response times for each stimulus type are presented in Table 1. There were no differences for response times among stimulus types (Kruskal-Wallis; p = 0.687).

TABLE 1
RESPONSE TIMES (IN MILLISECONDS) DURING THE WORKING MEMORY TEST.

Stimulus type	Response time (ms) Mean ± SEM
Geometric figure	1297 ± 37
Positive picture	1249 ± 29
Negative picture	1293 ± 32
Neutral picture	1337 ± 58

DISCUSSION

To our knowledge this study is the first to investigate working memory in a non-human primate using monkey facial expressions of emotion. Our results did not show any differences between emotional valence pictures and the neutral ones in a delayed non-matching-to-sample (DNMS) task. However, the monkeys performed above the upper confidence limits around chance to all kinds of stimulus indicating that they are able to learn the task using emotional faces.

The coordinated social interaction of individual primates depends on the ability of individuals to predict what others are likely to do at any given moment; whether, for example, they are likely to fight or flee. There are many sources of information that aid prediction. These sources include the recognition of ritualized displays, the comprehension of learned communicative signals, and the knowledge of various recurrent behavioral sequences that take place in particular contexts (Tomasello and Call, 1997 p.194). Capuchin monkeys have well-developed mobility of the facial musculature, which allows considerable expressive variability, and they have excellent visual acuity for discerning the signals of others (Weigel, 1978). However, most of capuchins' visual signals are accompanied of vocalizations and associated context. In general, the movement and body expression are important to understand emotional valence.

Interpreting the facial expressions of monkeys is not easy. It is not feasible to apply simple concepts such as "aggression" and "fear" to the expressions of animals whose feelings can only be surmised. Studying asymmetries in the timing of facial expressions by rhesus monkeys, Hauser and Akre (2001) listed at least two problems with their classification scheme, composed of a continuum from negative/withdrawal to positive/approach. First, some negative emotions are associated with withdrawal while others are associated with approach. Second, some expressions are associated with relatively more ambiguous emotional states, for example, although lip smacks appear to be associated with a negative emotion when they are produced by subordinates approaching dominants, they can be also produced by individuals involved in grooming, being associated with positive emotion. In this way, classifying the pictures was one of the difficulties. In the present study we decided to use the researchers' experience with the monkeys to judge the valence of the pictures. Capuchin monkeys produce a variety of facial and vocal expressions observed during our studies. Thus, we believe that these researchers are able to see the intention of the monkey in some pictures. However, it was a human assessment which could not agree with the perception of the capuchin monkey when seeing a picture of a co-specific face. One way to solve this problem could be to confirm the valence judged by researchers showing the pictures to the monkeys and observing their behavior and physiological reply. Some physiological signs, like changes in the nasal temperature (Nakayama et al, 2005), movements of the pupil, skin conductance and behavioral expressions could indicate the valence of the picture for the monkeys.

Another difficulty is the assessment of the faces. We chose to use only the faces to reduce the variables. The faces are particularly salient cues in conveying emotional information and it has been proposed that the amygdale shows a considerable specialization in recognizing emotion in facial expressions (Adolphs & Tranel, 2003). The existence of face-selective neurons in the temporal lobe of non-human primates and the responses of the prefrontal cortex has been repeatedly confirmed (Ó Scalaidhe, 1999). The problem here is that in several situations observance of the body (either movement or vocalization) is necessary to contextualize the emotional valence of the face. To minimize this problem, the assessment of the pictures was made using only the face. Moreover, we considered only the pictures with 80% agreement among the researchers. As mentioned earlier in future studies, it's interesting to analyze the physiological and behavioral reactions of the monkeys when assessing the pictures.

Furthermore, the capuchin monkeys presented much improved performance when using geometric figures compared to the co-specific pictures. Although the training tasks used only geometric figures, we made 4 pilot study sessions using pictures to minimize the learning effect. As we can see in the results, there was not significant increase in performance from set A to set B in any stimulus type. Although we didn't find any learning effect, the test using geometric figures was easier for the monkeys. This better performance could be explained by the subjects' previous training experience with geometric figures. One way to test this hypothesis is training naïve capuchin monkeys with pictures of faces and, then, comparing tests containing pictures of faces and geometric figures. Another approach is to divert their attention with co-specific faces which are very attractive for the subjects. An important evidence for this is the fact that performance using geometric figures was better when compared to any valence of emotional pictures.

The response times among the stimulus categories were similar, showing that the monkeys were motivated to answer the test independently of the stimulus type. Moreover, this is strong evidence of the test viability since there were no problems for the animals to carry out the task.

Taken together the results show that capuchin monkeys were capable to perform this new working memory task. This preliminary study yielded findings that are of relevance for the better understanding of the influences of emotional expressiveness on memory and indicate the possible usefulness of applying the paradigm in this study to investigate emotional working memory in non-human primates.

ACKNOWLEDGMENTS

This work was partially supported by a FINATEC grant to C. Tomaz and M. C. Tavares. We thank Úrsula Gomes, Paola Rabelo and Elisa Suganuma for assessment of the pictures. We are grateful to Camila Meireles for reviewing the English text. We also thank Dr. Raimundo Oliveira and Geinaldo Silva for animal care.

REFERENCES

1. Dolan RJ. Emotion, cognition, and behavior. Science 2002; 298:1191-4.

2. Gray JR, Braver TS, Raichle ME. Integration of emotion and cognition in the lateral prefrontal cortex. PNAS 2002; 99 (6):4115-20.

3. Perlstein WM, Elbert T, Stenger VA. Dissociation in human prefrontal cortex of affective influences on working memory-related activity. PNAS 2002; 99 (3):1736-41.

4. Johansson M, Mecklinger A, Treese, A-C. Recognition memory for emotional and neutral faces: an event-related potential study. J Cogn Neurosci 2004; 16 (10):1840-53.

5. Calvo MG, Esteves F. Detection of emotional faces: low perceptual threshold and wide attentional span. Vis Cogn 2005; 12 (1): 13-27.

6.Preuschoft S. Primate faces and facial expressions. Soc Res 2000; 67 (1):245-71.

7.Antinucci F. The comparative study of cognitive ontogeny in four primates species. In: Parker ST, Gibson KR editors. “Language” and Inteligence in Monkeys and Apes. Cambridge: Cambridge University Press; 1990. p. 157-171.

8.Fragaszy DM, Visalberghi E, Fedigan LM. The Complete Capuchin: The Biology of the genus Cebus. Cambridge: Cambridge University Press; 2004.

9.Visalberghi E. Capuchin monkeys: a window into tool use in apes and humans. In: Gibson P, Ingold G editors. Tools, language, and cognition in human evolution. Cambridge: Cambridge University Press; 1993. p. 138-50.

10.Tavares MCH, Tomaz C. Working memory in capuchin monkeys (Cebus apella). Behav Brain Res 2002; 131:131-7.

11.Resende MC, Tavares MCH, Tomaz C. Ontogenetic dissociation between habit learning and recognition memory in capuchin monkeys (Cebus apella). Neurobiol Learn Mem 2003; 79:19-24.

12.Lacreuse A, Herndon JG. Estradiol selectively affects processing of‘conspecifics’ faces in female rhesus monkeys. Psychoneuroendocrinology 2003; 28 (7):885-905.

13.Tomasello M, Call J. Primate Cognition. New York: Oxford University Press; 1997.

14.Weigel RM. The facial expressions of the brown capuchin monkey (Cebus apella). Behaviour 1978; 68:250-76.

15.Hauser MD, Akre K. Asymmetries in the timing of facial and vocal expressions by rhesus monkeys: implications for hemispheric specialization. Anim Behav 2001; 61:391-400.

16.Nakayama K, Goto S, Kuraoka k, Nakamura K. Decrease in nasal temperature of rhesus monkeys (Macaca mulatta) in negative emotional state. Physiol Behav 2005; 84 (5): 783-90.

17.Adolphs R, Tranel D. Amygdala damage impairs emotion recognition from scenes only when they contain facial expressions. Neuropsychologia 2003; 41:1281-9.

18.Ó Scalaidhe SP, Wilson FAW, Goldman-Rakic OS. Face-selective neurons during passive viewing and working memory performance of rhesus monkeys: evidence for intrinsic specialization of neuronal coding. Cereb Cortex 1999; 9:459-75.