Published in final edited form as:

Anim Cogn. 2016 May ; 19(3): 673–677. doi:10.1007/s10071-016-0959-4.

Touchscreen Assays of Learning, Response Inhibition, and Motivation in the Marmoset (*Callithrix jacchus*)

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Abstract

Recent developments in precision gene editing have led to the emergence of the marmoset as an experimental subject of considerable interest and translational value. A better understanding of behavioral phenotypes of the common marmoset will inform the extent to which forthcoming transgenic mutants are cognitively intact. Therefore, additional information regarding their learning, inhibitory control, and motivational abilities is needed. The present studies used touchscreen-based repeated acquisition and discrimination reversal tasks to examine basic dimensions of learning and response inhibition. Marmosets were trained daily to respond to one of two simultaneously presented novel stimuli. Subjects learned to discriminate the two stimuli (acquisition) and, subsequently, with the contingencies switched (reversal). In addition, progressive ratio performance was used to measure the effort expended to obtain a highly palatable reinforcer varying in magnitude and, thereby, provide an index of relative motivational value. Results indicate that rates of both acquisition and reversal of novel discriminations increased across successive sessions, but that rate of reversal learning remained slower than acquisition learning, i.e., more trials were needed for mastery. A positive correlation was observed between progressive ratio break point and reinforcement magnitude. These results closely replicate previous findings with squirrel monkeys, thus providing evidence of similarity in learning processes across nonhuman primate species. Moreover, these data provide key information about the normative phenotype of *wild-type* marmosets using three relevant behavioral endpoints.

Keywords

Repeated Acquisition; Discrimination Reversal; Progressive Ratio; Touchscreen; Marmoset

INTRODUCTION

The common marmoset (*Callithrix jacchus*) is a New World primate that has emerged as an attractive nonhuman animal subject for biomedical research due to its availability, small size, intelligence, lifespan, and reproductive efficiency. Indeed, marmosets have been extensively used for over two decades, playing an important role in the study of age-related diseases, infectious diseases, Parkinson's disease, drug development, reproductive biology, and basic

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The authors have no conflicts of interest to report.

neuroscience (reviewed in Abbott et al. 2003; Mansfield 2003; Okano et al. 2012; Tardif et al. 2011). Recent developments in precision gene editing have further promoted the marmoset as an experimental subject of considerable translational value (Belmonte et al. 2015; Kishi et al. 2014; Sasaki et al. 2009).

Although the development of techniques to manipulate the mouse genome significantly transformed modern biology, numerous biological differences between mice and humans have underscored the limitations of some transgenic rodent models (Hyman 2014; Seok et al. 2013) and have highlighted the potential value of genomic modifications in nonhuman primates. Marmosets have several unique advantages for transgenic modification relative to other nonhuman primates. They reach sexual maturity at 15–18 months and have one of the shortest gestational periods of any primate (~145 days), which allows mature females to have viable offspring—almost always nonidentical twins—twice a year, allowing for the establishment of germline transmission with each generation in a rapid manner for a primate species (Sasaki et al. 2009). Furthermore, marmosets were the first, and to date only, New World primate to have their genome fully sequenced (Marmoset Genome Sequencing and Analysis Consortium 2014).

As genetic techniques continue to be refined, a more detailed understanding of marmoset behavior will be needed to characterize *wild-type in vivo* phenotypes for comparison to those of subsequently developed mutant generations (Schultz-Darken et al. in press). There have been several previous studies investigating complex behavior in the marmoset (e.g., Spinelli et al. 2004; Takemoto et al. 2011; 2015; Yamazaki et al. 2014) and, in particular, reversal learning has been used to evaluate neurochemical signaling in the orbitofrontal cortex thought to mediate response inhibition, (e.g., Clarke et al. 2004; 2011; Rygula et al. 2010). The present studies, however, were designed expressly to characterize the *development* of discrimination learning and response inhibition by using, respectively, the repeated acquisition and discrimination reversal tasks in which subjects learned and reversed novel stimuli daily, rather than serial reversals of the same task (cf. Clark et al. 2011; Takemoto et al. 2015). In addition, the marmoset's sensitivity to motivational variables was evaluated using progressive ratio performance under conditions that varied reinforcer magnitude, a measure that is increasingly included in studies using cognitive tests with animals (e.g. Nettle et al. 2015). These studies were designed to closely mimic recent investigations with squirrel monkeys (Kangas and Bergman 2014) in order to evaluate cross-species similarity with another nonhuman primate and provide information about normative behavior of *wild-type* marmosets under these three commonly used animal models of cognitive behavior.

METHOD

Subjects

Four experimentally-naïve adult male common marmosets (*Callithrix jacchus*) were individually housed in a temperature- and humidity-controlled vivarium with a 12-h light/dark cycle (7am–7pm). Subjects had unlimited access to water in the home cage and were maintained at approximate free-feeding weights by post-session feedings of nutritionally balanced portions of LabDiet® New World Primate Diet and ZuPreem® Soft Marmoset

Diet. In addition, fresh fruit, meal worms, and environmental enrichment were provided daily. Experimental sessions were conducted 5 days a week (Monday-Friday). The experimental protocol for the present studies was approved by the Institutional Animal Care and Use Committee at McLean Hospital. Subjects were maintained in a facility licensed by the U.S. Department of Agriculture and in accordance with guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources (National Research Council 2011).

Apparatus

Experimental sessions were conducted in a modified version of a touch-sensitive experimental chamber designed for small New World monkeys. Schematics and photographs can be found in Kangas and Bergman (2012). The chamber developed for marmosets was modified in 2 ways. First, the custom-built Plexiglas chamber dimensions were reduced (25x30x35 cm), as was the sound- and light-attenuating enclosure (40x60x45 cm). Second, the milk well was mounted on the wall opposite of the touchscreen, rather than underneath it. All other chamber specifications were unchanged. Experimental events and data collection were programmed in E-Prime Professional 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA).

Procedure

Repeated Acquisition—The repeated acquisition task was used to assay the development of discrimination learning (Harlow 1949; Warren 1965). Each session began with concurrent presentation of two 7x7 cm digital photographs, with each stimulus positioned in a different randomly-selected quadrant of the screen. A touch response on one stimulus initiated a 0.15 mL milk delivery (S^+) paired with an 880 ms yellow screen flash, followed by a 10 s intertrial interval (ITI) blackout, whereas a touch response on the other stimulus immediately initiated the 10 s ITI (S^-). The same two stimuli were presented during each of 200 trials comprising each session. Subjects were presented with a new S^+/S^- pair each session, and photographs for each session were randomly selected from our laboratory bank of >10,000 images¹. Thus, the subject was required to learn a new S^+/S^- discrimination each session based on distinguishing features of two visual stimuli that had not been previously viewed. If the subject failed to master the discrimination within the 200-trial session (see below for mastery criterion), the same stimuli were presented during the next day's 200-trial session. The primary dependent variable was the number of trials to acquire the discrimination; the criterion for mastery was responses on the S^+ stimulus in 9 of 10 consecutive trials. Sessions were conducted until 30 discriminations were mastered.

Discrimination Reversal—In the discrimination reversal task, a variant of discrimination learning for examining cognitive flexibility (Easton 2005; Mackintosh et al. 1968) and, more recently, thought to capture critical features of response inhibition and impulsivity (Bari and Robbins 2013), the programmed consequences of responding to the stimuli were reversed after the subject learned the initial discrimination, thereby requiring inhibition of the previously reinforced response following this change in contingency. After 30

¹Copies of the images used are available from the authors

discriminations were mastered, experimental conditions were altered to incorporate a discrimination reversal task. Under the new conditions, the first 100 trials were conducted exactly as described above. However, on Trial 101 the relationship between S^+ and S^- was reversed without signal. That is, during Trials 101–200, the stimulus that was initially S^+ was made S^- , and vice versa. If the subject failed to master the reversal within 100 trials, the same stimuli and contingencies were presented during a 100-trial session the following day. As before, the primary dependent measure was trials to mastery of the new relationship (i.e., number of trials until 90% accuracy across 10 consecutive trials was achieved). This discrimination reversal condition was conducted until 30 reversals were mastered.

Progressive Ratio—Progressive Ratio procedures are used to measure the effort that will be expended to obtain a reinforcer and, thereby, are thought to reflect their motivational value (Hodos 1961). Following the characterization of discrimination reversal performance, subjects first learned to touch a 7x7 cm green box (purple background) in the middle of the screen to produce milk delivery. Next, subjects responded under a progressive ratio schedule of milk reinforcement in which each reinforcer delivery led to an increase in the number of touchscreen responses required for the next reinforcer. The progressive ratio requirement was programmed with a log2 step size (i.e., 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024). Each milk reinforcer was paired with an 880 ms yellow screen flash and followed by a 10 s ITI blackout. Each session was terminated either after 5 min without a response or 45 min, whichever came first. The largest ratio completed defined the session's break point. Steady state performance in individual subjects was defined as 5 consecutive sessions yielding a break point within one of two adjacent step sizes. All subjects initially received 0.15 mL of milk for every ratio completed (the same volume used in the repeated acquisition and discrimination reversal studies). After steady state performance was observed, the reinforcer magnitude was increased for 2 subjects (1.0 mL) and decreased for 2 subjects (0.025 mL). After steady state performance was again observed, subjects were exposed to the other reinforcer magnitude. This manipulation was designed to determine if the expected positive relationship between reinforcer magnitude and motivation to respond (defined by break points) would be observed in marmosets under these touchscreen-based conditions.

RESULTS AND DISCUSSION

The upper panel of Figure 1 presents group average number of trials required to acquire each of the first 30 novel discriminations under the repeated acquisition task. Overall, the rate of acquisition increased across successive discriminations learned until subjects acquired the discrimination quickly each session. This was evident in the number of trials to reach mastery, which decreased steadily until reaching a plateau of approximately 20–25 trials to mastery. Group average acquisition data generally represent findings of individual subjects. There was some variability in acquisition rate across subjects, notably during the first 20 sessions; however, as the error bars indicate, variance diminished across sessions.

The lower panel of Figure 1 presents group average data from the discrimination reversal condition. The filled symbols indicate the number of trials to acquire the initial discrimination. Novel discriminations continued to be mastered each session at a rapid and stable rate following introduction of the discrimination reversal condition. The open symbols

indicate the number of trials to master the discrimination reversal introduced at Trial 101. A similar decrease across sessions in the number of trials to master the reversal was observed. However, the rate of reversal learning reached a plateau at approximately 45–55 trials to mastery, much slower than the rate of acquisition of the initial discrimination in the day's session. Here again, mean performance was generally representative of the individual subjects across the condition. Inspection of within-session responding indicates that this difference in trials to mastery can be reliably accounted for by an initial response perseverance on the stimulus that was initially S^+ (but now S^-) at the onset of reversal for approximately 10–15 trials. Perseveration was then typically followed by a longer period of mixed response allocation than was observed in the initial discrimination acquisition.

Figure 2 shows group average break points as a function of milk reinforcer magnitude (mL). A high correlation was observed ($R^2=0.73$). When the milk volume was 0.15 mL, as in the repeated acquisition and discrimination reversal studies, the group modal break point was 128. Orderly decreases and increases in break point were observed when completed ratios resulted in, respectively, smaller (0.025 mL) and larger (1.0 mL) volume milk reinforcers. Although there was some between-subject variability in break points, this relationship held true for all 4 subjects and resembles similar findings with rats (Rickard et al. 2009).

Interestingly, when comparing the development of marmoset learning and reversal performance with squirrel monkey performance under nearly identical conditions (cf. Kangas and Bergman 2014, Fig. 1), remarkable similarity is evident in both quantitative and qualitative features of the functions. A repeated measures two-way analysis of variance confirmed no statistical difference between marmoset and squirrel monkey in the development of learning ($F[7]=2.71$, $P=.15$). Reversal performance approached but did not reach significance ($F[7]=5.91$, $P=.051$) largely due to the marmoset's requiring more trials to master the first 5 reversals. In progressive ratio experiments, average break point values were also not statistically different ($F[7]=1.52$, $P=.26$) between marmosets and squirrel monkeys responding for 0.15 mL milk delivery (cf. Kangas et al. in press). In addition, the effects of varying reinforcer magnitude on break points are generally similar between primates (unpublished findings).

Taken together, under these relatively complex conditions, marmoset performance was orderly and did not significantly deviate from that of a larger New World primate, the squirrel monkey. This is somewhat contrary to current thinking regarding brain size and cognitive ability among nonhuman primates (see Deaner et al., 2007); however, marmosets have defied this correlation previously (Strasser and Burkart 2012). The present findings demonstrate that marmosets are certainly capable of engaging effectively in a touchscreen-based battery of behavioral assays. Future studies comparing *wild-type* marmosets with those that are genetically modified to influence other phenotypic elements, will provide important information regarding the specificity of such modifications and their effects on cognitive function and other organized complex behavior.

Acknowledgments

We thank Michael Z. Leonard for assistance conducting these studies and Roger Speelman for comments on a previous version of this manuscript. This research was supported by grants K01-DA035974 (BDK) and R01-

DA035857 (JB) from the National Institute on Drug Abuse and R01-MH05190 (JTC) from the National Institute of Mental Health.

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Figure 1.

Upper Panel: Mean number of trials ($\pm$ S.E.M.) to master (90% correct) the first 30 discriminations during repeated acquisition.

Lower panel: Mean number of trials ($\pm$ S.E.M.) to master (90% correct) the first 30 reversals during discrimination reversal (open symbols) conducted during Trials 101–200 following discrimination mastery (filled symbols) acquired during Trials 1–100.

Figure 2.

Mean break point ($\pm$ S.E.M.) as a function of milk reinforcer magnitude (mL). Abscissa is on a log10 scale to emphasize proportional change in reinforcement magnitude. Ordinate is on a log2 scale to emphasize the progressive ratio step sizes arranged.