



A Lesson in Patience: Cooperative Chair Training of Rhesus Macaques

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A Lesson in Patience:
Cooperative Chair Training of Rhesus Macaques

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Abstract

Awake-behaving non-human primates (NHPs), specifically rhesus macaques (*Macaca mulatta*), are often used in experiments involving psychophysics, electrophysiology, and cognition; these tasks involve physical restraint of the animal to some degree in order to ensure safety for the researcher with minimal discomfort to the animal. Traditional methods of training include fitting animals with rigid aluminum or chain-link collars prior to chairing. Long poles or leashes that latch onto the collars are then used to guide and transfer the animals into a special primate chair in which they can be transported to a different laboratory area in which they will perform specific tasks in the context of an experimental design.

The goal of our research was to establish a positive, trusting working relationship between NHP and researcher by using operant conditioning, desensitization, positive reinforcement, and very limited negative reinforcement in order for animals to voluntarily enter transportable box-style primate chairs and allow themselves to be lightly restrained with neck plates. Since the techniques are minimally coercive and do not involve physically handling the animal, researchers were less apprehensive in training animals. All animals trained using operant conditioning were more cooperative, calm, friendly, and willing to work longer periods on tasks. Additionally, because all animals were calm and cooperative, they were able to perform psychophysics tasks without the use of a head restraint system.

Dedication

To Ally Langsdorf who started me on this journey.

Acknowledgments

I would like to thank the following people and entities: Dr. Mehrdad Jazayeri and Dr. Margaret Livingstone for being my thesis advisors, Dr. Carlos Ponce for his helpful comments, Dr. James Morrison for being my research advisor, Dr. Robert Marini for his veterinary advice, Dr. Gerald Schneider for his insights on animal behavior and helpful comments, Dr. Jing Wang for her data on head-free training, the veterinary and animal care staff of the Division of Comparative Medicine at the Massachusetts Institute of Technology for overseeing the health and welfare of the animals, the McGovern Institute for Brain Research at MIT for providing the resources I needed for my research, and Harvard University for providing this opportunity for my graduate education.

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Chapter I

Introduction

Non-human primates (NHPs), especially rhesus macaques (*Macaca mulatta*), have been used extensively as animal models in biomedical and neuroscience research. Rhesus macaques are highly intelligent, curious, social animals that are able to perform complex problem-solving tasks. Awake-behaving NHPs are often used in experiments involving psychophysics, electrophysiology, and cognition; these tasks involve physical restraint of the animal to some degree in order to ensure safety for the researcher with minimal discomfort to the animal. Traditional methods of training include fitting animals with rigid aluminum or chain-link collars prior to chairing. Long poles or leashes that latch onto the collars are then used to guide and transfer the animals into a special primate chair in which they can be transported to a different laboratory area in which they will perform specific tasks in the context of an experimental design.

Not all researchers who join NHP laboratories have the requisite knowledge and experience of working with NHPs. Researchers may lack the constitution and capability to physically handle an average 8.3 kg mature adult male and average 7.4 kg mature adult female animal using traditional techniques. NHPs have a wide range of temperaments which mimic human personality traits such as friendliness, playfulness, and stubbornness. Some animals may not respond positively to traditional training techniques: stressed animals are less willing to perform tasks and may exhibit aggression, self-injurious behaviors, and stereotypies (Coleman & Maier, 2010). An animal that is fearful may also cause stress and fear in the researcher.

The goal of our research is to establish a positive, trusting working relationship between NHP and researcher by using positive reinforcement (Prescott & Buchanan-Smith, 2003;

Schapiro, Bloomsmith, & Laule, 2003), successive approximation strategies (“shaping”) (Peterson, 2004), desensitization, and some degree of negative reinforcement in order for animals to voluntarily enter transportable primate chairs (Mason, 1958), raise their heads up, and allow themselves to be lightly restrained with neck plates. The proposed training methods require steadfast patience, developing from necessity during our own initial training of an animal fearful of and resistant to the pole and collar method. The techniques may require more time and patience than traditional techniques, but they have an invaluable result: stress-free animals should be more cooperative, calm, friendly, and willing to work longer periods on tasks. Since the techniques are minimally coercive and do not involve physically handling the animal, researchers will be less apprehensive in training animals; even researchers who are naïve to training NHPs will be able master these skills in a relatively short period of time. Fearful or aggressive animals can overcome negative associations with chairing and be trained to become compliant in a positive manner. The addition and dissemination of literature documenting these alternative techniques will benefit the scientific community by cultivating a new tradition of cooperative NHP training.

Background

The nature of many neuroscience experiments involving awake-behaving NHPs requires that an animal be able to enter and sit calmly in an enclosed space for lengthy periods of time. There have been a variety of techniques for transferring animals from their home cages to primate chairs, including having multiple researchers manually removing the animal from the cage (Mason, 1958), using a leash attached to a collar to pull the animal into a chair (Glassman, Negrao, & Doty, 1968), and using a long pole to latch onto a collar to guide the animal into a chair (Howell, Hoffman, Votaw, Landrum, & Jordan, 2001; McMillan, Perlman, Galvan,

Wichmann, & Bloomsmith, 2014). Chair design has also evolved from open chairs with neck and waist restraints (Henry & Bowman, 1971; Mason, 1958) to box-style chairs made of Lexan (Bliss-Moreau, Theil, & Moadab, 2013; Carlson, 1972; Ponce, Genecin, Perez-Melara, & Livingstone, 2016; Sledjeski, 1969) where the animal can sit comfortably and have a greater degree of movement while remaining safely enclosed.

While the methods for training NHPs have been continuously refined over the years to be safer for both animal and researcher, and more humane manipulation, current chairing practices utilizing pole & collar are still stressful for animal and researcher. Stressed animals exhibit fear and aggression, leading to self-injurious behaviors such as self-biting, hair pulling, and scratching, and stereotypies such as pacing, twirling, teeth grinding, and eye-saluting (Bellanca & Crockett, 2002; Gottlieb, Capitanio, & McCowan, 2013; Novak, 2003). Stressed researchers are likely to be more reactive and make mistakes which may lead to exposure to macaque bites and scratches. Since macaques are highly intelligent and capable of quickly learning complex tasks, the next logical step in developing a training paradigm would be to use a blend of positive reinforcement, mild coercion in some cases, and systematic desensitization which eventually lead to voluntary cooperation with reward incentives and minimal stress.

Operant conditioning and positive reinforcement training have proven to be successful in training animals for a variety of tasks such as having a macaque voluntarily extend a limb for venipuncture (Coleman et al., 2008; G. E. Laule, Bloomsmith, & Schapiro, 2003) and shifting a troop of monkeys from their home cages to another location for weighing or to a recreation area (Graham et al., 2012). In operant conditioning, animals are trained to perform increasingly complicated tasks by rewarding small segments of successive movements that lead toward the desired goal behavior. For example, when teaching a macaque to not fear a researcher's touch,

the researcher may hold out one hand toward the animal while holding a piece of fruit with the other hand. When the animal reaches for the fruit, the researcher can gently grasp the animal's arm, and then reward it with more fruits. The animal may immediately pull back in fear at first, but it gradually learns that if it holds out its hand or arm, or gently grasps a finger when the researcher presents their hand, it will be given desirable treats. After shaping this basic behavior, the animal can be taught similar tasks such as pressing a button, pulling a lever, or holding still while allowing the researcher to physically manipulate it.

Although rhesus macaques are innately curious, they are also neophobic (Kinnally, Whiteman, Mason, Mendoza, & Capitanio, 2008). Attempting to introduce a highly novel primate chair and carry out a chairing paradigm too quickly may be a frightening experience--especially for more submissive individuals (Chamove, 1983). A cooperative training method encourages an animal to explore and acclimatize at its own pace, therefore allowing a bond of trust to develop between it and the researcher. A growing body of literature has shown that utilizing positive reinforcement and shaping in macaque training have been reproducible and successful (Coleman et al., 2008; Coleman & Maier, 2010; Graham et al., 2012; G. E. Laule et al., 2003; G. Laule & Whittaker, 2007; McMillan et al., 2014; Perlman et al., 2011; Schapiro et al., 2003; Whittaker & Laule, 2012/9). Several publications by Bliss-Moreau et al. (2016; 2013) described the effectiveness and benefits of cooperative restraint training in amygdala-lesioned and anterior cingulate cortex-lesioned animals. Ponce et al.(2016) further expounded upon the methods of cooperative restraint by incorporating them in conjunction with automated chair-training, thereby minimizing the number of in-person hours required in an animal's initial training.

Cooperative chair training for NHPs in biomedical research is still relatively new and not commonly practiced--possibly due to lack of knowledge that such training is possible, long-established laboratory practices, or concerns regarding extensive training times. Our investigation sets out to debunk these perceptions and show that not only is cooperative chair training possible and a vast improvement over traditional techniques, but it is highly beneficial for both the animal's and researcher's well-being.

Chapter II

Materials and Methods

Animals

This study utilized the resources and facilities accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and provided by the McGovern Institute of Brain Research and the Division of Comparative Medicine (DCM) at the Massachusetts Institute of Technology in compliance with the Committee on Animal Care (CAC). Eleven naive, neurologically intact young (2-6 years old) male Indian-origin rhesus macaques weighing 4-7 kg and two naive, neurologically intact young (4 years old) Chinese-origin female rhesus macaques weighing 5-7 kg were enrolled in the study. Animals arrived from five U.S.-based primate facilities. Two of the 13 animals were transferred from other MIT labs. All animals underwent a three-month quarantine, physical exams, and behavioral assessments to confirm sound health. They were pair-housed in quad cages in single-sex rooms and provided with various forms of enrichment (Figure 1). They were fed specified amounts of primate chow (Purina Lab Diet 5038) twice a day, dry treat mix (i.e. raisins, raw peanuts in shell, plain Cheerios) once a day, and provided with water *ad libitum*. Housing conditions were maintained at 20–22 °C, 30–70% humidity, 10–15 non-recirculated air changes h⁻¹ and a light cycle of 12 h light: 12 h dark (Martin et al., 2009).



Figure 1. Macaque housing with enrichment materials in the form of brightly-colored plastic toys, puzzle feeders, and mirrors.

Primate Chairs & Accessories

Box-style Lexan primate chairs, which were procured from Crist Instrument Company, MIT Central Machine Shop, and Brain & Cognitive Sciences Machine Shop, are entirely enclosed except for a removable headcover with a hole drilled in the front to allow the attachment of a water bottle sipper tube, and a small door to allow the researcher to pass food through to the animal without removing the head cover (Figure 2). The chairs are equipped with

pillory-style front and back neck plates for restraint, a guillotine-style sliding door, an adjustable height perch, and a tray filled with biodegradable absorbent material such as Cell-Sorb or Pel-o’Cobs for collecting waste. Chain-link collars (Figure 3) and SafeWrangle Animal Guide catch poles (Figure 4) for control animals were procured from Crist Instrument. Round, rigid aluminum collars were procured from Unifab Corporation (Figure 3).

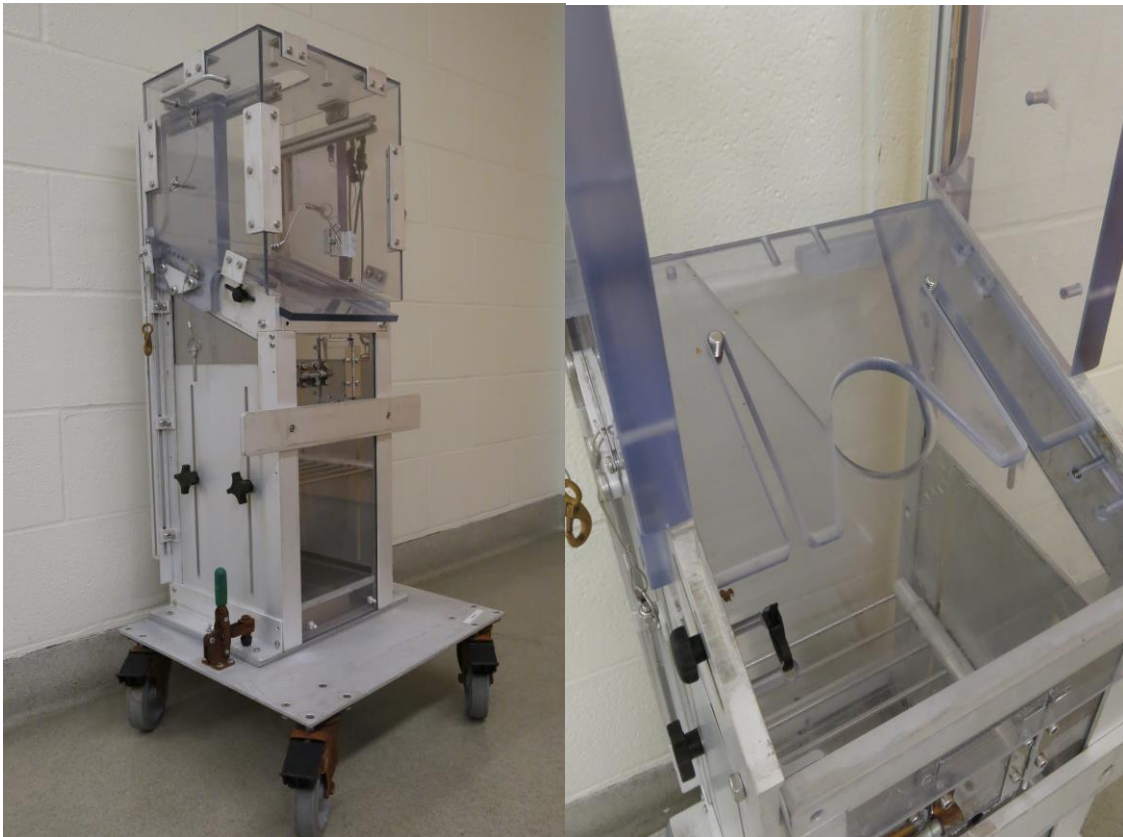


Figure 2. Fully-enclosed Lexan primate chairs.



Figure 3. Primate collars. Left: rigid aluminum collar. Right: chain-link collar encased by flexible PVC.



Figure 4. SafeWrangle Animal Guide poles. Top: 91.5 cm length. Bottom: 60.9 cm length. Both poles may be used together to help bring an animal's head up.

Establishing Trust with Animals

In order to establish a baseline of trust and rapport between researchers and naïve animals, the researcher first spent 30-60 minutes daily in the assigned animal's home cage room, fed the animal fresh fruit pieces (i.e., a choice of apples, grapes, sweet potatoes, oranges, or bananas) and dry treats (i.e., peanuts, raisins, plain Cheerios, marshmallows, dried fruit pieces) by hand at the bottom of the cage, and spoke to the animal in soothing, encouraging tones. Animals that resided with a cagemate were separated from it with vertical cage dividers during the training period, but given visual access. If the animal was initially fearful of the researcher and refused to accept the fruit directly, the researcher left the fruit in the bottom food hopper while maintaining a reasonable distance from the cage so that the animal did not perceive the researcher to be a threat. The researcher noted what fruits the animal favors and used that knowledge for the subsequent steps of chair training. The animal learned that it must come to the bottom of the cage to receive fruits, and that the researcher was a source of positive rewards.

Primate Chair Acclimation & Desensitization

When the animal regularly accepted hand-feeding from the researcher, the researcher began acclimating the animal to the presence of the primate chair by placing the chair in the room near the animal's cage. Depending upon the animal's reaction, the chair was gradually moved closer to the cage until it was situated directly in front of and touching the cage door. The researcher gave fresh fruits to the animal near the chair. When the animal had overcome the initial novelty of the chair, preferred fruits were placed inside of the chair on the perch and on the neck plate so the animal must reach inside to retrieve the treats. If the animal consistently retreated to the upper portion of the cage after obtaining treats, horizontal dividers were placed in

the cage for the duration of the training period in order to acclimate the animal to staying in the bottom portion of the cage.

After the animal learned to positively associate the chair and the researcher as sources of treats, the chair was secured to the cage via bungee cords so the animal could push not the chair away and escape. The door to the home cage was affixed in an open position to allow the animal free access to the chair so it could enter and explore at will. Whenever the animal made motions to enter the chair, the researcher tapped the sides of the chair and used the verbal command, “In the chair.” However, if after a few days, the animal still showed signs of unwillingness to enter the chair, water regulation of the animal began. Water regulation is a standard and approved practice in NHP training by the Institutional Animal Care and Use Committee (IACUC) and other governing institutional animal welfare organizations such as MIT’s Committee on Animal Care (CAC) (Prescott et al., 2010). The animal’s intake of water was gradually decreased from *ad libitum* to 20 ml/kg/day. The researcher ensured that the animal received its daily amount of water by attaching a water bottle with lick tube to the head cover of the chair which encouraged the animal to enter the chair and put its head up to drink. The animal learned that it would only receive water when it enters the chair. Whenever the animal made motions to put its head up to drink, the researcher used the verbal command, “Head up” as well as gave verbal praise and treats.

When the animal consistently entered the chair to retrieve fruits and/or drink water, the researcher shut the door to the primate chair and wheeled the chair a short distance away from the cage. This allowed the animal to acclimate to the confined space while still being reassured that it was receiving water and food rewards, and observed that it remained in an environment with familiar cohorts. If the animal continuously only placed half its body into the chair, the

researcher used the squeeze bars to squeeze its backside fully into the chair. The researcher would place the back neck plate partially into the chair without restraining the animal's neck, allowing the animal to raise or lower its head as it wished; the continued presence of the back neck plate eventually desensitized the animal to it. The researcher gradually decreased the circumference of the opening of the neck plates by slowly sliding the back neck plate closer without restraining the animal, and allowed the animal to lower its head below the neck plates if it wished. When the animal became acclimatized to having the back neck plate within close proximity, the researcher fully closed the neck plate so that the animal was lightly restrained. The researcher then gave the animal abundant amounts of treats, water, and verbal praise (e.g. "Good boy!"). The perch of the chair was adjusted to accommodate for the sitting height of the animal and prevented unnecessary straining of the neck, and the neck plates were adjusted to accommodate for head and neck position to maximize comfort. The chairing procedure should initially not last more than 1-2 hours.

After 2-3 days of following the above steps, the researcher transported the animal to the laboratory space to allow it to grow accustomed to a new environment in which experiments will be carried out. Abundant amounts of fruit, water, and verbal praise were given. The animal sat in the experiment area for 30 minutes. The animal was occasionally apprehensive of the new environment and refuse treats and water; the researcher supervised the animal by sitting in the room with it, encouraged it to eat and drink, and gave verbal praise when it consumed treats and water. The daily time spent in the experiment room was gradually increased by 30-60 minute increments as the animal became acclimatized. If the animal showed signs of continued calmness, cooperation, and positive behavioral signs (e.g. lip smacking, cooing), preliminary training of experimental tasks using 10% diluted fruit juice distributed via a reward tube began.

Control Paradigm: Pole & Collar Training

Two males (Amadeus & Beethoven) and two females (Carmen & Delilah) served as controls. They were sedated with 10 mg/kg ketamine hydrochloride intramuscularly in order to fit them with chain-link collars enclosed by soft PVC tubing. They were trained using a modified version of the pole and collar technique. Control animal training conditions were similar up to the step where it is time for the animal to enter the chair: the researcher squeezed the animal into the chair using the squeeze bars. After one day, if the animal did not put its head up voluntarily, the researcher used a metal catch pole and attached it to the animal's collar in order to bring the head up, then closed the back neck plate so the animal was lightly restrained. When using the pole to bring the animal's head up for the first time, the researcher requested help from a more experienced lab member to hold the chair steady or adjust the animal's perch if necessary. The researcher repeated this chairing technique daily until the animal raised its head up without the need for the pole.

Chapter III

Results

Since the ten experimental animals were individuals of different ages, temperaments, and cognitive skill levels, cooperative training time varied for each animal. All animals were water-regulated prior to chair training.

The dominance or submissiveness of an animal (Table 5) played a role in the ease of its chairing. Usually, the first fight between two individuals establishes a hierarchical relationship between them: the winner of the fight will be dominant and the loser will be subordinate. After dominance is established, the subordinate individual generally avoids the dominant or expresses fear and submission in his/her presence. The dominant attacks the subordinate occasionally, and this maintains or reinforces the dominance relationship between them (Maestriperi & Hoffman, 2012). In our case, animals were carefully selected to be cagemates based upon compatible temperament and age-matched or paired as father-son/mother-daughter (older individual paired with much younger individual). Since our animals are pair-housed with the same cagemate for an indefinite period, social hierarchy is relatively stable. Interestingly, dominant animals were more amenable to chairing and learned at a faster pace (Figure 5), putting their heads up in a mean of 4.6 days compared to 7 days for submissive animals. Fearful, submissive animals took up to 1-2 hours to put their heads up in the early process of chair exploration, contrasting with bolder, dominant animals who took less than five minutes. Chair training time was eventually reduced from one month to two weeks due to technique refinement with dominant animals being fully trained in a mean of 14.4 days compared to submissive animals in 15.2 days (Figure 6). Younger animals with a mean of 2.6 years old were trained in a slightly faster period of time--a mean of 14 days compared to a mean of 16 days--regardless of social hierarchy (Figure 7). An

animal was considered fully chair-trained once they consistently entered the chair voluntarily without the use of horizontal dividers within 5-10 minutes and put their head up within <2 minutes. A summary of cooperative chair training is illustrated in Figure 8.

Animal Name	Age at Time of Training (years)	Weight at Time of Training (kg)	Sex	Social Hierarchy at Time of Training
Amadeus	5	7.4	M	Submissive to cagemate Beethoven
Beethoven	5	9	M	Dominant over cagemate Amadeus
Carmen	4	7	F	Dominant over cagemate Delilah
Delilah	4	7.8	F	Submissive to cagemate Carmen
Elgar	2	6.3	M	Dominant over cagemate Fauré
Fauré	2	5	M	Submissive to cagemate Elgar
Gershwin	2	5.3	M	Submissive to cagemate Haydn
Haydn	2	4.7	M	Dominant over cagemate Gershwin
Ives	6	8.7	M	Dominant over cagemate Janáček
Janáček	5	8.8	M	Submissive to cagemate Ives
Kodály	4	6.3	M	Dominant over cagemate Lalo (no recorded data)
Jiggy	5	7.5	M	Submissive to cagemate from other lab
Gallo	6	8.5	M	Dominant over cagemate from other lab

Table 1. Ages and social statuses of control and experimental animals at time of training.

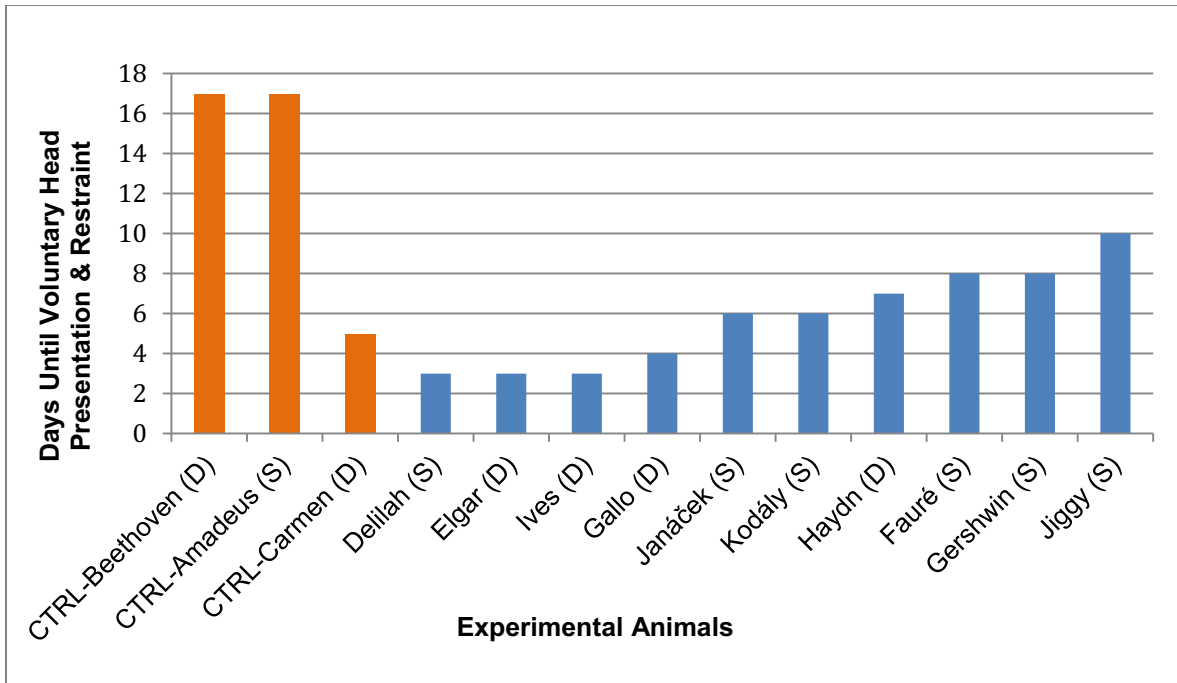


Figure 5. Total days until an experimental animal voluntarily put their head up and allowed themselves to be neck restrained. The mean for dominant animals was 4.6 days and the mean for submissive animals was 7 days. Elgar, Haydn, Ives, Kodály, and Gallo were dominant animals as indicated by the initial D. Delilah, Fauré, Gershwin, Janacek, and Jiggy were submissive animals as indicated by the initial S.

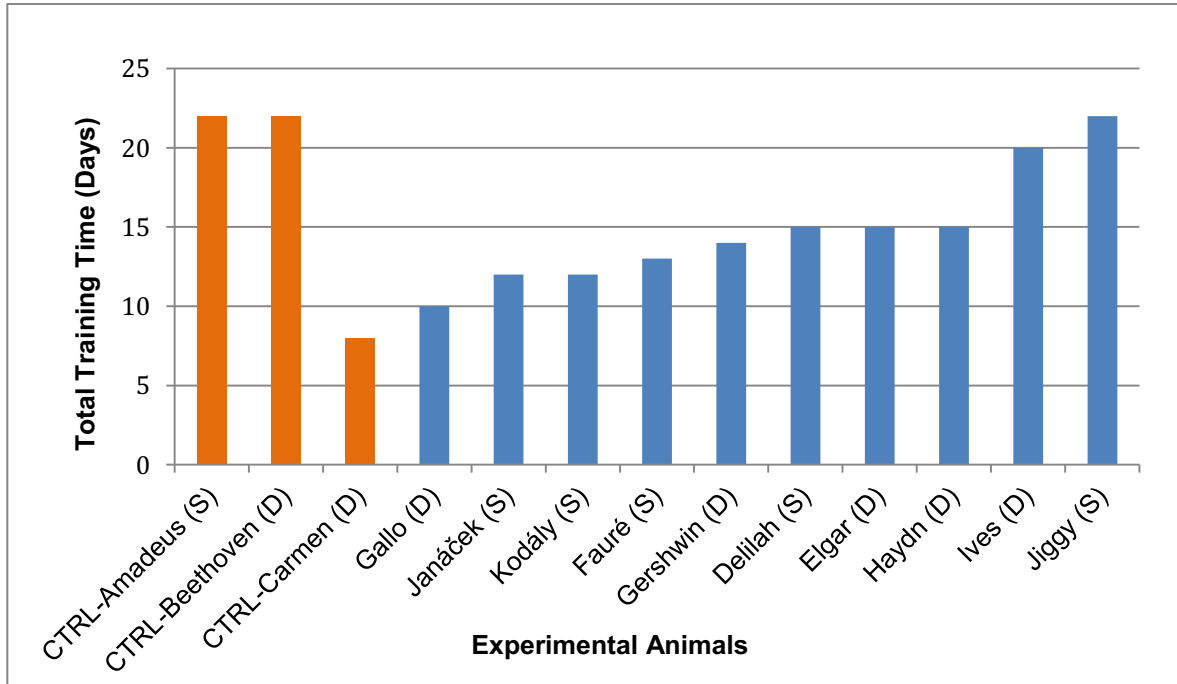


Figure 6. Total chair training time of all experimental animals. The mean of dominant animals was 14.4 days and the mean of submissive animals was 15.2 days. Elgar, Haydn, Ives, Kodály, and Gallo were dominant animals as indicated by the initial D. Delilah, Fauré, Gershwin, Janacek, and Jiggy were submissive animals as indicated by the initial S.

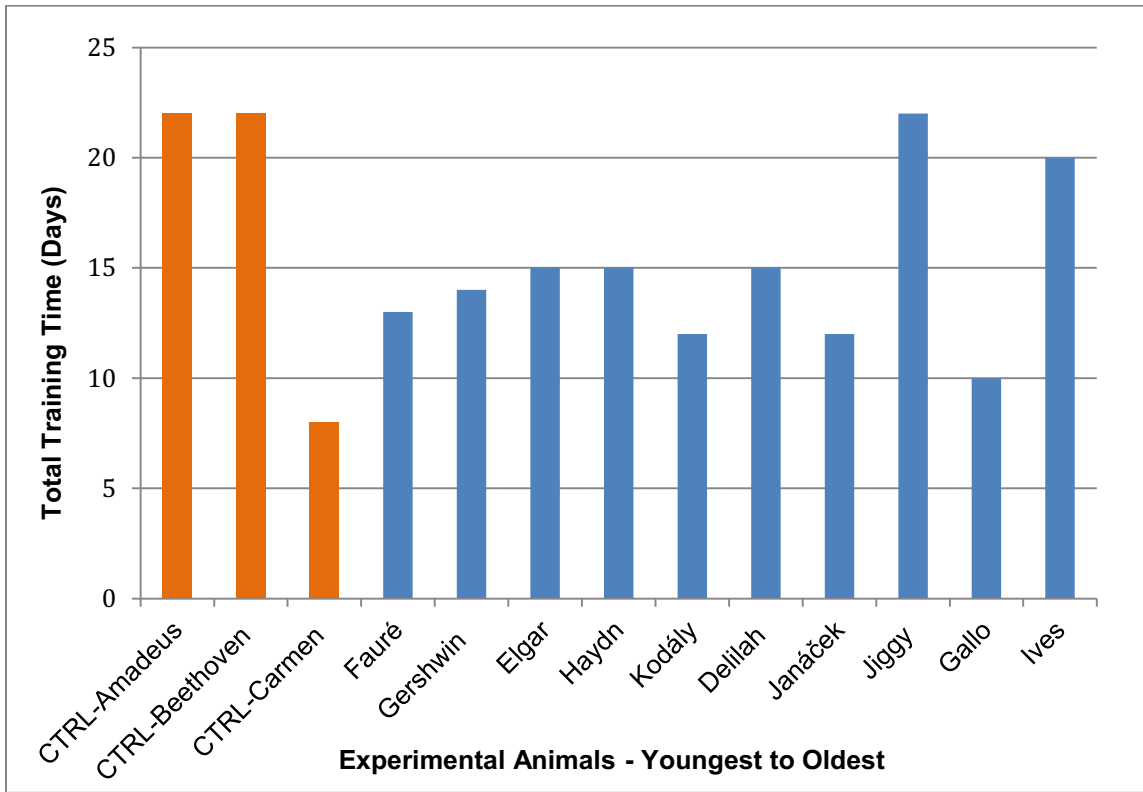


Figure 7. Total chair training time of animals ranked in order (excluding controls) from youngest to oldest. A young animal was considered <4 years old. Youngest animals were 2 years old and oldest animals were 6 years old. Mean age of all animals was 4.0 years. Mean training time of young animals was 14 days, mean training time of older animals was 16 days, excluding controls.



Figure 8. Cooperative chairing procedure. a.) Fresh fruit is placed on top of the front neck plate and on the perch to entice the animal to enter the chair. A water bottle may also be attached to the headcover. b.) The door of the chair is opened and secured to the opened cage door. c.) The animal eventually enters and sits in the chair. d.) There may be some exploratory behavior from the animal before he puts his head up. e.) The animal fully puts his head up, facing the front. f.) The researcher slides in the back neck plate g.) The researcher secures the back neck plate with pins. h.) The animal is fully neck restrained and ready to be transported to the research area. i.) Back view of the secured neck plate.

Data

Control Animals

The control animals Amadeus, Beethoven, Carmen, and Delilah (A-D) were trained first. Training occurred utilizing two researchers--one to advise and assist the less experienced person--per monkey at a time; the animal worked daily with the same researchers in order to ensure consistency for both animal and researchers to develop a bond of trust and familiarity. Researchers' non-human primate experience ranged from 0-10 years. When animals were successfully chaired consistently and consecutively for the span of one week, the researcher was allowed to work by his/herself unsupervised.

Amadeus & Beethoven

Water regulation of Amadeus (social hierarchy: submissive) and Beethoven (social hierarchy: dominant) began one week before chair training. Their water consumption was reduced daily to 1000 ml, 800 ml, 500 ml, 250 ml, and finally to the minimum of 20 ml/kg/day.

After two days of using the squeeze bars to encourage A&B to come into the primate chair, A&B started coming into the chair voluntarily from the third day onward. They were rewarded with their daily allotment of water from a bottle after entering the chair. They were then brought to the research area for 30-60 min where they were given fruits and water while acclimating to the lab's surroundings. Initially, A&B were fearful of their new surroundings, sitting quietly and refusing treats and water until they returned to their home cages. However, after one day of sitting in the lab, animals resumed normal appetite and exhibited appeasement behaviors such as lip smacking and cooing.

Twelve days post-chair training, A&B were sedated with 10 mg/kg ketamine hydrochloride intramuscularly and fitted with chain-link collars enclosed by soft PVC tubing. Animals were given one week to become accustomed to the collar. After a week's time, A&B were chaired and researchers used a long catch pole to grasp the collar in order to bring the animals' heads up. A&B were initially fearful of the pole and tried to avoid collar capture by maneuvering themselves upside down in the chair, hunching down and squeezing into a corner, and grabbing or pushing at the pole. This, in turn, made the situation stressful for the researcher who was careful to avoid hurting the animal while attempting to bring an animal's head up. A&B also occasionally resisted having the neck plate closed around them by putting a hand against the front neck plate and pushing against it.

After two days of using the pole, Beethoven began voluntarily putting his head up after entering the chair. After five days of using the pole, both A&B began voluntarily putting their heads up to be neck restrained after entering the chair. Animals were immediately rewarded with fresh fruit and water and verbal praise after putting their heads up voluntarily or if their heads were brought up with the pole. Chaired animals spent 1-2 hours sitting in the vestibule outside of the home cage room or in the research area in order to acclimate to the environment and learn to be quiet and still during lack of stimulating activity. The daily progress of their training is summarized in Tables 2a & 2b.

Amadeus & Beethoven	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 1	Chaired by using squeeze bars & dividers.	2 hours	No	
Day 2	Chaired by using squeeze bars & dividers, A&B refused treats & water.	2 hours	No	
Day 3	Chaired by using squeeze bars & dividers.	2 hours	No	
Day 4	Chaired without use of squeeze bars.	2 hours	No	
Day 5	Chaired by using squeeze bars & dividers.	2 hours	No	
Day 6	Chaired by using squeeze bars & dividers, brought to lab, A&B shook chairs.	2 hours	No	
Day 7	Brought down to lab; A&B shook chairs, Beethoven put head up voluntarily.	2 hours	No	
Day 8	Brought down to lab	2 hours	No	
Day 9	Brought down to lab	2 hours	No	
Day 10	Brought down to lab; Beethoven put head up voluntarily with researcher out of the room.	2 hours	B after 1 hour	
Day 11	Brought down to lab; Beethoven put head up voluntarily & shook chair	2.5 hours	B after 1 hour	
Day 12	Brought down to lab; both A&B put head up voluntarily	2 hours	A&B after 1 hour	
Day 13	No training to allow for collar adjustment.	N/A	N/A	Collars fitted; training resumed on 6/4.
Day 14	Used pole to bring head up; Beethoven fought pole, Amadeus did not resist.	2 hours	After 1 hour.	

Table 2a. Amadeus & Beethoven's pole & collar training results.

Amadeus & Beethoven	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 15	Used pole to bring head up; Beethoven fought pole, Amadeus escaped chair.	2 hours	After 1 hour.	
Day 16	Beethoven voluntarily put head up; pole used to bring up Amadeus' head.	2 hours	After 1 hour.	
Day 17	Beethoven voluntarily put head up; pole used to bring up Amadeus' head.	2 hours	After 1 hour.	
Day 18	A&B put head up voluntarily.	2 hours	Immediately	
Day 19	A&B put head up voluntarily.	2 hours	After 5 minutes	
Day 20	A&B put head up voluntarily.	2 hours	After 5 minutes	
Day 21	A&B put head up voluntarily.	2 hours	Immediately	
Day 22	No dividers needed to come down; A&B put head up voluntarily.	2 hours	Immediately	

Table 2b. Continuation of Amadeus & Beethoven's pole & collar training results.

Carmen

The pole was used to bring Carmen’s (social hierarchy: dominant) head up for four of her training days post-collar fitting, chair acclimation, and water regulation. She exhibited the same initial resistant behaviors to having her head brought up as Amadeus and Beethoven, and additional more clever behaviors such as bringing her entire body up to the head cover portion of the chair and extending her arm through the opening of the neck plate in order to prevent closure of the back neck plate. On the fifth day of chairing, Carmen began putting her head up voluntarily and consistently to be neck restrained. The daily progress of her training is summarized in Table 2.

Carmen	Behavior in Chair	Session Duration (hours)	Neck Restrained?
Day 1	Chaired by using squeeze bars & dividers, used pole to bring head up, resisted chairing entire time; brought to lab.	1.45	After 1 hour.
Day 2	Chaired by using squeeze bars & dividers, used pole to bring head up. Brought to lab.	1.3	After 30 minutes.
Day 3	Chaired by using squeeze bars & dividers, used pole to bring head up. Brought to lab.	1.3	After 30 minutes.
Day 4	Used pole to bring head up; brought to lab.	1.5	After 10 minutes
Day 5	Used pole to bring head up; brought to lab, vocalized.	1.5	After 15 minutes.
Day 6	Put head up voluntarily, brought to lab.	1.5	After 2 minutes
Day 7	Put head up voluntarily, brought to lab, shook chair, vocalized.	2	After 2 minutes
Day 8	Chaired by using squeeze bars, put head up voluntarily, brought to lab, shook chair, vocalized.	2	After 2 minutes

Table 3. Carmen’s pole & collar training results.

Delilah

Although training Delilah (social hierarchy: submissive) to come to the bottom of the cage and enter the chair was largely uneventful, she was extremely fearful and resistant to pole training to the point where the researcher was concerned she would injure herself in her struggles and thus, discontinued her training for several days. Due to Delilah's unwillingness to submit to traditional pole & collar training methods, she was reassigned to be a subject in the cooperative training paradigm.

It was determined that there were not enough female subjects to draw a significant comparison to male subjects in terms of difficulty of chairing and behavioral differences.

Experimental Animals

Delilah

Once chaired, Delilah was brought to the vestibule outside of the main NHP home cage room where the researcher waited for her to voluntarily put her head up. A water bottle was attached to the head cover of the chair with a bungee cord, and assorted fresh fruits were placed on the front neck plate. Initially, Delilah would only put her head up to drink or take treats while the researcher waited outside of the vestibule. For three training sessions, the researcher stayed in the vestibule standing near Delilah and kept her hand on the back neck plate in order for Delilah to become desensitized to the presence of the researcher and perceived "threat" of the neck plate closing. After three days of repeated systematic desensitization, the researcher would attempt to neck restrain Delilah by slowly sliding in the back neck plate while Delilah was preoccupied with drinking from the water bottle. Delilah was eventually moved to the vestibule outside of the home cage room in order to minimize auditory (e.g. loud vocalizations) and visual

distractions (e.g. threat displays) generated by the other animals. She was initially fearful at the change in environment, staying below the neck plates and refusing fruits and water, but eventually put her head up within a reasonable time period, and allowed herself to be neck restrained.

Delilah's training was postponed for 21 days to allow for recovery from a chronic cephalic implant surgery. When training resumed, Delilah regressed to earlier behaviors of resistance such as extending her arm through the neck plate opening and putting up her head halfway. She was brought to the research area for a brief period where she exhibited curiosity by examining her surroundings, then signs of stress and anxiety by making threat faces when water or treats were offered. She struggled in the chair by pushing at the neck plate and extending her fingers through openings in the chair. However, she acclimated to the research environment within two days: she grew calmer and more cooperative, exhibiting positive, appeasement behaviors such as cooing and lip smacking. After five more training sessions, she learned to receive 10% diluted fruit juice from the liquid reward system, and began preliminary training for psychophysics tasks. The daily progress of her training is summarized in Table 4.

Delilah	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 1	Put head up to drink while researcher waited outside of the cage room.	2	No	
Day 2	Put head up to drink with researcher standing nearby.	1	No	
Day 3	Put head up to drink with researcher standing nearby.	1	After 10 minutes	
Day 4	Put head up to drink while researcher waited outside of the cage room.	1.5	No	
Day 5	Moved to vestibule due to distractions; put head up to drink with researcher standing nearby.	1.5	After 1 hour	
Day 6	Moved to vestibule; put head up to drink with researcher standing nearby.	1	After 10 minutes	
Day 7	Put head up to drink with researcher standing nearby.	1.75	After 1.75 hours	Consumed water <i>ad libitum</i> 4 days prior.
Day 8	Put head up to drink immediately.	1	Immediately	
Day 9	Put head up to drink immediately.	1.25	After 5 minutes	
Day 10	Put head up to drink with researcher standing nearby, brought down to lab, anxious behaviors, refused treats.	2	After 1 hour	Training resumed 21 days post-implant surgery.
Day 11	Put head up to drink with researcher standing nearby, brought down to lab, anxious behaviors, refused treats.	2	After 30 minutes	
Day 12	Put head up immediately, brought down to lab, anxious for 10 min, accepted treats.	2	After 5 minutes	
Day 13	Put head up immediately, brought down to lab, placed close to rig.	2.5	After 5 minutes	
Day 14	Put head up immediately, brought down to lab, placed in rig with door open.	2	Immediately	
Day 15	Put head up immediately, brought down to lab, placed in rig with door closed.	2	Immediately	

Table 4. Delilah's cooperative chair training results.

Jiggy

Jiggy (social hierarchy: submissive) was extremely fearful of the chair during the first three days of acclimatization: he shook when the chair was rolled near the cage,, made fear grimaces, and approached the chair very cautiously by crawling. However, he was brave enough each time to reach into the chair to take the fruits that were placed inside.

Jiggy made the slowest progress out of all the experimental monkeys in chair training: his training period lasted 23 days, excluding chair acclimatization. On day 14 of using the cooperative training method, two researchers used catch poles to bring Jiggy's head up in order to expedite his training. Jiggy was particularly difficult in evading pole capture: he resisted by performing headstands to make it more difficult for the researchers to catch his collar, grabbing and shoving at the pole with his hands and legs, and sticking his arm through the hole of the neck plates. He also expressed stress and anxiety by vigorously shaking the chair and smearing fecal matter upon the walls. In order to facilitate quicker pole capture, one researcher raised and lowered the perch height in order for the second researcher would be able to reach Jiggy's collar more easily. The pole was consistently used to bring Jiggy's head up for two more weeks before Jiggy learned to voluntarily put his head up. The daily progress of Jiggy's training is summarized in Tables 5a and 5b.

Jiggy	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 1	Explored chair.	1.5	No	
Day 2	Explored chair.	1	No	
Day 3	Explored chair.	1	No	
Day 4	Explored chair, put head up with researcher standing nearby.	2	No	
Day 5	Explored chair, put head up with researcher standing nearby.	2	No	
Day 6	Explored chair, put head up with researcher standing nearby.	2	No	
Day 7	Entered and stayed in chair, put head up with researcher standing nearby.	2	No	
Day 8	Chaired, put head up with researcher standing nearby.	1	No	
Day 9	Refused to put head up for new researcher.	2	No	
Day 10	Put head up, neck restrained, anxious behaviors.	2	After 1.5 hours	
Day 11	Put head up with researcher standing nearby.	2	No	
Day 12	Brought down to lab.	1	After 5 minutes	
Day 13	Brought down to lab.	2	After 10 minutes	
Day 14	Moved to vestibule to bring head up, brought down to lab.	2	After 2 hours	Two researchers used pole to bring head up.

Table 5a. Jiggy's cooperative chair training results.

Jiggy	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 15	Moved to vestibule to bring head up, brought down to lab.	1.20	After 20 minutes	Two researchers used pole to bring head up.
Day 16	Moved to vestibule to bring head up, brought down to lab, calm in lab.	1.20	After 20 minutes	Two researchers used pole to bring head up.
Day 17	Moved to vestibule to bring head up, brought down to lab, calm in lab.	1	After 10 minutes	Two researchers used pole to bring head up.
Day 18	Moved to vestibule to bring head up, brought down to lab.	1	After 10 minutes	Two researchers used pole to bring head up.
Day 19	Moved to vestibule to bring head up, brought down to lab.	1	After 5 minutes	Two researchers used pole to bring head up.
Day 20	Moved to vestibule to bring head up, brought down to lab, began preliminary psychophysics.	1	After 5 minutes	Two researchers used pole to bring head up.
Day 21	Moved to vestibule to bring head up, brought down to lab, worked on psychophysics.	1	After 5 minutes	One researcher used pole to bring head up.
Day 22	Moved to vestibule to bring head up, brought down to lab, worked on psychophysics.	1	After 5 minutes	One researcher used pole to bring head up.

Table 5b. Continuation of Jiggy's cooperative chair training results.

Gallo

Gallo (social hierarchy: dominant) acclimatized to the chair within two days and was fully cooperatively chair-trained eight days post-acclimatization--the fastest total training time out of all the experimental animals. Occasionally, he went up into the head cover of the chair, but mostly remained in the proper sitting position. He exhibited no fear regarding chairing or being exposed to novel surroundings--only curiosity as evinced by his immediate willingness to explore and cooperate. Gallo put his up voluntarily regardless of the presence of the researcher nearby. For two days, the researcher experimented with neck restraining Gallo for brief periods (5-10 minutes) before releasing as opposed to long-term restraint. While neck restrained for longer periods, Gallo occasionally presented anxious behaviors which included threat faces, teeth grinding, and spinning in place, but he was otherwise calm for the majority of his chair training. The daily progress of Gallo's training is summarized in Table 6.

Gallo	Behavior in Chair	Session Duration (hours)	Neck Restrained?
Day 1	Entered and sat in chair, put head up with researcher standing nearby.	1	No
Day 2	Entered and sat in chair with researcher standing nearby.	1	No
Day 3	Brought down to lab, put head up;	2	No
Day 4	Brought down to lab, put head up; researcher restrained briefly before releasing, calm.	2	After 2 hours
Day 5	Brought down to lab, put head up; researcher restrained briefly before releasing, calm..	2	After 2 hours.
Day 6	Brought down to lab, put head up with researcher standing nearby, anxious behaviors.	2	After 2 hours
Day 7	Brought down to lab, put head up with researcher standing nearby, anxious behaviors	2	After 10 minutes
Day 8	Brought down to lab, put head up with researcher standing nearby, calm, placed in rig.	2	After 5 minutes
Day 9	Brought down to lab, put head up with researcher standing nearby, calm, placed in rig.	2	After 5 minutes
Day 10	Brought down to lab, put head up with researcher standing nearby, calm, placed in rig.	2	After 5 minutes

Table 6. Gallo's cooperative chair training results.

Haydn

Haydn (social hierarchy: dominant) was an exceptionally curious monkey, enthusiastically exploring the chair on his own during each daily acclimatization session for a period of 15 days. Because he was a small animal during time of training (4.7 kg), he would often come up into the head cover compartment and remain there until the researcher gently prodded him with a long piece of Lexan, signaling him to return to the lower portion of the chair. He would voluntarily put his head up to drink from the water bottle attached to the cover and take fruits with the researcher standing next to him.

After a two-month hiatus, Haydn's training resumed on a regular basis where he was brought down to the lab for the rest of his training. He acclimated quickly to the lab environment, exhibiting positive, appeasement behaviors such lip smacking, cooing, and calling. He was very sensitive to the motion of the back neck plate and therefore would not allow himself to be neck restrained. On day seven, he accidentally neck restrained himself by squeezing through the hole in the neck plates, which were originally in place to prevent him from climbing up into the head cover. After this observation, the researcher adjusted the circumference of the hole of the neck plates so it was large enough for Haydn to comfortably put his head up, yet not allow him immediately duck down; thus, Haydn desensitized himself to the neck plate without interference from the researcher. From the 13th training day onward, Haydn allowed the researcher to neck restrain him within a few minutes without any resistance after entering the chair and putting his head up. He began preliminary training for psychophysics tasks on the 16th day. The daily progress of Haydn's training is summarized in Table 7.

Haydn	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 1	Fearful of being in lab, but eventually put head up to drink & accepted treats with researcher standing nearby.	1.5	No	Training resumed 2 months after acclimatization.
Day 2	Put head up to drink with researcher standing nearby, then ducked down.	1	No	
Day 3	Put head up to drink with researcher standing nearby, then ducked down.	1	No	
Day 4	Put head up to drink with researcher standing nearby, then ducked down.	1	No	
Day 5	Put head up to drink with researcher standing nearby, then ducked down.	2	No	
Day 6	Refused to put head up.	2	No	
Day 7	Accidentally self-neck restrained by squeezing through neck plate hole. Researcher observed outside of room.	2	After 2 hours	
Day 8	Self-neck restrained by squeezing through neck plate hole. Researcher observed outside of room.	2	After 5 minutes	
Day 9	Self-neck restrained by squeezing through neck plate hole. Researcher observed outside of room.	2	After 5 minutes	
Day 10	Self-neck restrained by squeezing through neck plate hole. Researcher observed outside of room.	2	After 5 minutes	
Day 11	Self-neck restrained by squeezing through neck plate hole. Researcher observed outside of room	2	Immediately	
Day 12	After brief defiance, put head up to be neck restrained lab, placed in rig.	2	After 15 minutes	
Day 13	Put head up with lure of water bottle, taken to lab, placed in rig.	2	After 5 minutes	
Day 14	Put head up in lab, placed in rig with door.	2	After 5 minutes	
Day 15	Put head up with lure of water bottle, placed in rig.	2	After 5 minutes	

Table 7. Haydn's cooperative chair training results.

Gershwin

Gershwin (social hierarchy: submissive) was initially fearful of the chair during the first three days of chair acclimatization, preferring to stay near the back corner of his cage. He only took treats from the chair when the researcher had left the room. After becoming accustomed to the researcher's presence, he came into the head cover and contorted himself in order to drink from the bottle, but quickly learned to sit properly in the chair to drink.

After a six month hiatus, Gershwin's training resumed. Gershwin put his head up voluntarily in the lab, but would not keep it up once the neck plate was introduced. After one week of using the cooperative method, the researcher used the catch pole to bring Gershwin's head up in order to expedite Gershwin's training. The pole was only used for two consecutive days before Gershwin consistently and voluntarily put his head up . He started learning psychophysics tasks within 10 days post-initial chair training. The daily progress of Gershwin's training is summarized in Table 8.

Gershwin	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 1a	Accepted treats with researcher standing by, explored and sat in chair.	1	No	
Day 2a	Accepted treats with researcher standing by, explored and sat in chair, contorted into headcover to drink.	1	No	
Day 3a	Accepted treats with researcher standing by, explored and sat in chair, put head up to drink.	1	No	
Day 4a	Accepted treats with researcher standing by, explored and sat in chair, put head head up to drink.	1	No	
Day 1b	Chaired in home cage room.	1	No	Training resumed 6 months after initial start
Day 2b	Brought to lab, put head up to drink with researcher standing nearby.	1	No	
Day 3b	Brought to lab, put head up to drink with researcher standing nearby.	1	No	
Day 4b	Brought to lab, put head up to drink with researcher standing nearby, then stayed below neck plates.	2	No	
Day 5b	Brought to lab, put head up to drink with researcher standing nearby.	1	No	
Day 6b	Brought to lab, put head up to drink with researcher standing nearby.	1	No	
Day 7b	Brought to lab, put head up to drink with researcher standing nearby.	1	No	
Day 8b	Brought to lab, put head up to drink with researcher standing nearby	2	After 5 minutes	
Day 9b	Used pole to bring head up, brought to lab, started psychophysics task.	2	After 10 minutes	Researcher used pole.
Day 10b	Used pole to bring head up, brought to lab, started psychophysics task	2	After 5 minutes	Researcher used pole.

Table 8. Gershwin's cooperative chair training results.

Janáček

Janáček (social hierarchy: submissive) acclimatized to the chair after three days. He was a very cautious and quietly fearful monkey: initially, he would freeze and refuse to take treats after the door closed behind him after he entered the chair, when he was taken to a new environment, and when he was neck restrained. Despite his timidity, he had one of the shortest training times out of all the experimental animals, quickly learning to put his head up and perform basic psychophysics tasks within nine days post-acclimatization. The daily progress of Janáček's training is summarized in Table 9.

Unfortunately, two months post-chair training, an unusual behavior was discovered during Janáček's performance on a psychophysics task: strabismus during saccadic eye movements. An MRI scan later revealed an enlarged lateral ventricle in his primary visual cortex (V1). Due to this abnormality, though it did not affect his physical health or gross behavior otherwise, the attending veterinarian recommended Janáček's discontinuation from the lab's psychophysics training paradigm.

Janáček	Behavior in Chair	Session Duration (hours)	Neck Restrained?
Day 1	Fearful of chair; 30 minutes later, climbed into head cover and froze.	1	No
Day 2	Explored chair, froze when chair door closed, ignored treats..	1	No
Day 3	Explored chair, froze when chair door closed, ignored treats..	1	No
Day 4	Ate treats in chair and put head up only with researcher out of the room.	1	No
Day 5	Ate treats in chair and put head up to drink only with researcher out of the room.	1	No
Day 6	Brought to lab, accepted treats, put head up to drink with researcher nearby, neck restrained.	2	After 30 minutes with lure of water bottle.
Day 7	Brought to lab, accepted treats, put head up to drink with researcher nearby.	2	No
Day 8	Brought to lab, accepted treats, put head up to drink with researcher nearby, placed in rig.	2	No
Day 9	Brought to lab, put head up to drink with researcher nearby, began preliminary psychophysics.	2	After 5 minutes with lure of water bottle.
Day 10	Brought to lab, put head up to drink with researcher nearby, performed psychophysics.	2	After 5 minutes with lure of water bottle.
Day 11	Brought to lab, put head up to drink with researcher nearby, performed psychophysics.	2	After 5 minutes with lure of water bottle.
Day 12	Brought to lab, put head up to drink with researcher nearby, performed psychophysics.	2	Immediately

Table 9. Janáček's cooperative chair training results.

Fauré

Fauré (social hierarchy: submissive) acclimatized to the chair after one day. After three days of using the cooperative training method, the researcher decided to use the pole and collar method in order to expedite his training. Predictably, Fauré was extremely fearful and resistant to pole training: it required the assistance of several other researchers and three hours to bring Fauré's head upon the first day of using the catch poles. Fauré's small size contributed to the difficulty in pole training as he was able to consistently maneuver himself into the bottom corners of the chair to avoid capture. Despite the difficulties in his initial training, Fauré learned to put his head up voluntarily three days after the pole and collar technique was utilized, and use of the cooperative training method resumed per the recommendation of DCM. The daily progress of Fauré's training is summarized in Table 10.

Fauré	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 1	Fearful of chair, did not enter.	1	No	
Day 2	Entered chair, put head up to drink with researcher standing nearby, then ducked down.	2	No	
Day 3	Put head up to drink with researcher standing nearby, then ducked down.	2	No	
Day 4	Put head up to drink with researcher standing nearby, then ducked down.	2	No	
Day 5	Resistant to bringing head up, fought pole.	3	After 3 hours	Four researchers used pole to bring head up.
Day 6	After researchers used pole to bring head up, Faure brought head up voluntarily to drink from bottle. Brought to lab.	2	After 2 hours	Three researchers used pole to bring head up.
Day 7	Two researchers used pole to bring head up after 30 minutes, brought to lab.	2	After 30 minutes.	Two researchers used pole to bring head up.
Day 8	Climbed into head cover, put head up, brought to lab.	2	After 30 minutes	
Day 9	Put head up, brought to lab.	2	After 30 minutes	
Day 10	Put head up after bottle presentation, brought to lab.	2	After presentation of bottle	
Day 11	Brought to lab, started preliminary psychophysics task.	2	Immediately	
Day 12	Brought to lab, training on psychophysics task.	2	Immediately	
Day 13	Brought to lab, training on psychophysics task.	2	Immediately	

Table 10. Fauré's cooperative chair training results.

Elgar

Elgar (social hierarchy: dominant) acclimatized to the chair within one day and voluntarily allowed himself to be neck restrained after three days, making him the animal with the shortest cooperative training time

The first time he was neck restrained, he exhibited anxious behaviors such as vigorously shaking the chair and spinning around. In subsequent chairing sessions, he remained calm and exhibited appeasement behaviors such as cooing and lip smacking. The lure of the water bottle and plentiful fresh fruits aided in enticing Elgar to voluntarily put his head up. The daily progress of Elgar's training is summarized in Table 11.

Elgar	Behavior in Chair	Session Duration (hours)	Neck Restrained?
Day 1	Explored chair, fearful when chair door closed.	1	No
Day 2	Explored chair, brought to lab, accepted treats.	1	No
Day 3	Brought to lab, anxious behaviors after neck restraint, then calm.	2	After 30 minutes
Day 4	Entered chair, did not put head up, fearful.	2	No
Day 5	Brought to lab, put head up.	2	After 1 hour
Day 6	Brought to lab, put head up to drink with researcher standing nearby, calm.	2	After 10 minutes
Day 7	Brought to lab, put head up to drink with researcher standing nearby, calm	2	After 15 minutes
Day 8	Brought to lab, put head up to drink with researcher standing nearby, calm.	2	After 5 minutes
Day 9	Brought to lab, put head up to drink with researcher standing nearby, calm.	2	After 10 minutes
Day 10	Brought to lab, put head up.	2	After 2 minutes
Day 11	Brought to lab, put head up.	2	Immediately
Day 12	Climbed into headcover several times before putting head up.	2	After 5 minutes
Day 13	Brought to lab, put head up.	2	Immediately
Day 14	Brought to lab, put head up.	2	Immediately
Day 15	Brought to lab, put head up, placed in rig.	2	Immediately

Table 11. Elgar's cooperative chair training results.

Ives

Ives (social hierarchy: dominant) acclimatized to the chair after three days. However, he was one of the slowest animals to consistently and voluntarily put his head up. Even though he displayed satisfactory comprehension of what was required, his behavior was inconsistent; he eventually refused to voluntarily put his head up after the seventh training day. Thus, on the ninth training day, he was fitted with a rigid aluminum collar in order to manually enforce the consistency of putting his head up. Though the pole was used to bring Ives' head up, Ives did not fight particularly vigorously nor for a lengthy period compared to Jiggy and Faure. The style of collar used may have been a contributing factor: a rigid collar allowed the researcher to place more torque upon the collar while the rigidity of the collar ensured that Ives did not accidentally hurt himself during struggles to evade pole capture; this subsequently caused less physical discomfort and stress. Ives only fought the pole briefly before allowing the researcher to bring his head up. The daily progress of Ives' training is summarized in Tables 12a and 12b.

Ives	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 1	Chaired, fearful of neck plate, eventually accepted treats with researcher standing nearby.	2	No	
Day 2	Brought to vestibule, fearful, but eventually put head up.	2	No	
Day 3	Put head up to drink with researcher standing nearby, brought to lab, anxious behaviors, then calm.	2	After presentation of water bottle	
Day 4	Put head up to drink with researcher standing nearby, brought to lab, calm.	2	After 45 minutes	
Day 5	Put head up enough to lap at water bottle, brought to lab.	2	After 2 hours	
Day 6	Put head up enough to lap at water bottle, brought to lab.	2	After 1 hour	
Day 7	Brought to head, did not put head up.	2	No	
Day 8	Brought to head, did not put head up.	2	No	
Day 9	Calm, but refused treats while in lab.	2	After 2 minutes	Researcher used pole to bring head up.
Day 10	Brief resistance, brought to lab and ate treats.	2	After 1 minute	Researcher used pole to bring head up.

Table 12a. Ives' cooperative chair training results.

Ives	Behavior in Chair	Session Duration (hours)	Neck Restrained?	Notes
Day 11	Brief resistance, brought to lab and ate treats.	2	After 2 minutes with pole.	Researcher used pole to bring head up.
Day 12	Voluntarily put head up when researcher gestured with pole, brought to lab.	2	Immediately	
Day 13	Fought pole, brought to lab, placed in rig.	2	After 10 minutes with pole.	Researcher used pole to bring head up.
Day 14	Briefly resisted pole capture by putting arm through neck plate hole, brought to lab, placed in rig.	2	After 5 minutes with pole.	Researcher used pole to bring head up.
Day 15	Briefly resisted pole capture by putting arm through neck plate hole, brought to lab, placed in rig.	2	After 5 minutes with pole.	Researcher used pole to bring head up.
Day 16	Briefly resisted pole capture by putting arm through neck plate hole, brought to lab, placed in rig.	2	After 5 minutes with pole.	Researcher used pole to bring head up.
Day 17	Voluntarily put head up when researcher gestured with pole, brought to lab.	2	Immediately	
Day 18	Brief resistance, brought to lab, placed in rig.	2	After 2 minutes with pole.	Researcher used pole to bring head up.
Day 19	Voluntarily put head up when researcher gestured with pole, brought to lab.	2	Immediately	
Day 20	Put head up voluntarily, brought to lab, placed in rig.	2	Immediately	

Table 12b. Continuation of Ives' cooperative chair training results.

Kodály

Kodály (social hierarchy: submissive) acclimatized to the chair after two days. He voluntarily and consistently allowed himself to be neck restrained after eight days of cooperative training and started psychophysics on the ninth day. Although he occasionally demonstrated anxious behaviors such as threat faces, vigorously shaking the chair, and briefly spinning around in the chair after neck restraint, he was largely cooperative and unresisting during his training. The daily progress of Kodály's training is summarized in Table 13.

Kodály	Behavior in Chair	Session Duration (hours)	Neck Restrained?
Day 1	Entered chair and explored, accepted treats.	1	No
Day 2	Entered chair, brought to vestibule, anxious behaviors, put head up with researcher standing nearby.	2	No
Day 3	Brought to lab, put head up with researcher standing nearby.	1	No
Day 4	Brought to lab, put head up with researcher out of the room.	2	No
Day 5	Brought to lab, put head up with researcher out of the room.	2	No
Day 6	Brought to lab, put head up with researcher standing nearby, started preliminary psychophysics.	2	After 1 hour
Day 7	Brought to lab, put head up with researcher standing nearby, worked on psychophysics.	2	After 10 minutes
Day 8	Brought to lab, put head up with researcher standing nearby.	2	No
Day 9	Brought to lab, put head up with researcher standing nearby, anxious behaviors, began preliminary psychophysics.	2	After 10 minutes
Day 10	Brought to lab, put head up with researcher standing nearby, anxious behaviors, performed psychophysics.	2	After 10 minutes
Day 11	Brought to lab, put head up with researcher standing nearby, performed psychophysics.	2	After 5 minutes
Day 12	Brought to lab, put head up with researcher standing nearby, performed psychophysics.	2	After 5 minutes

Table 13. Kodaly's cooperative chair training results.

Chapter IV

Discussion

Behavior in the Chair

Most animals trained under the experimental paradigm were cooperative and able to learn to enter the chair voluntarily with minimal coercion. Animals that were fearful or stubborn during chairing required more time and patience from the researcher. All animals were positively reinforced with plentiful amounts of fresh fruits and dried treats after either voluntarily putting their heads up or having their heads brought up with a pole. Disruptive behaviors, such as spinning, rocking the chair, banging on the chair, flinging Cell-Sorb from pan were ignored by the researcher.

It seemed that some animals were uncertain about putting their heads up not because of an aversion of the neck plate, but possibly due to the factor of whether or not there was enough space to put their head up when the back neck plate was present. If animals were allowed time to grow more familiar with all aspects of the chair, including the undesired behavior of climbing up to the headcover, perhaps they would not find neck restraint as intimidating.

Initially, some animals used a variety of clever tactics to avoid putting their heads up and being neck restrained: grabbing at the water bottle sipper tube and licking water off their hands, putting their heads up just enough to use tip of tongue to reach the sipper tube, immediately ducking under the neck plate after obtain a few sips of water, extending an arm up through the neck plate opening to prevent neck restraint, and putting the middle of the face against the front of the neck plate to obstruct closure of the back neck plate. Overall, the majority of animals were curious about the chair, and allowed to explore at their own pace.

Animals initially fitted with collars, including control animals, had the collars removed after one month of consistently demonstrating putting their heads up for voluntary neck restraint. The cooperative chair-trained monkeys are the only animals in the NHP facility who do not need collars post-training. All the animals remain well-behaved, and voluntarily enter the chair to be neck restrained and head-fixed 1-4 years post-training.

Significance of Results

Cooperative chair training is not only less stressful than traditional methods, it has an immediate benefit and advantage for monkeys learning psychophysics tasks: head-free training. Typically, an animal is surgically implanted with chronic titanium implants compatible with a primate chair head restraint system. The head is immobilized within the restraint system so the animal can only face a forward direction to observe a stimulus in front of them, forcing them to focus only upon the task instead of being distracted by frequent head movements.

The first step for a head-free animal to learn a basic psychophysics task is training them to gaze at a certain location on a computer screen--usually a center fixation point. This is achieved by utilizing a reward system that dispenses a measured amount of fruit juice, either manually by the researcher or automatically by a program, as a reward for the animal correctly focusing on the fixation point. The animal quickly learns that if it gazes at the fixation point for a designated period of time, it will receive a liquid reward, thus encouraging it in continuing the task. Gradual shaping of this behavior leads to progressively more complicated psychophysics tasks.

An animal that has never first undergone operant conditioning to face forward and focus on a computer screen may be stressed and frightened when it finds that its head has been immobilized in a restraint. The animal may struggle in the restraint and injure itself by tearing off an implant. Head-fixing a fearful animal is also stressful for the researcher since they may be preoccupied with the possibility of exposures to macaque bites during the process. In cooperative chair training, an animal has already learned to sit calmly in the chair without the aid of head fixation devices. Both the control and experimental NHPs in this project have been successfully trained to maintain fixation upon a central spot within a 4° eye fixation window throughout multi-second-long trials which require the animal to make saccades either reactively or proactively to visually or memory-guided locations. The accuracy and precision of head-free eye tracking is comparable to head-fixed eye tracking. No additional training is required during the transition from a head-free to head-fixed system (Wang J, Egger SW, Remington ED, Jazayeri M, 2014).

Conclusion

Cooperative chair training encourages the monkey's development of trust with the researcher. The researcher is still at risk of exposure to macaque bites and scratches, but the relative risk is reduced. It is easier to work with a friendly, willing animal. A vital component of cooperative training is the patience of the researcher. Impatience leads to a longer chair training time which delays experimental training time and may further negatively reinforce fearful animals. Allowing monkeys adequate acclimatization and desensitization time to primate chairs greatly reduce undesirable behaviors.

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