3.1. Materials and Methods

3.1.1. Ethics Statement

The use of animals and all procedures were approved by the Animal Care Committee of Mcgill University and affiliated hospitals and research institutes (protocol #2020-8155).

3.1.2. Animals and Management

Sixteen adult Rhesus or Cynomolgus Macaques, aged between 6 and 15 years, housed at the Comparative Medicine and Animal Resources Center (CMARC) of McGill were selected to be enrolled in the study. All monkeys were pair-housed and housed in either two cages (2.03m X 1.14m X 0.91m) side-by-side or one cage and one exercise enclosure (2.49m" X 1.79m X 2.11m). Monkeys were split between two rooms, and cages were distributed around the room so that all monkeys were in visual/auditory contact, but separate pairs had no physical contact. All cages were fitted with structural and occupational enrichment (i.e wooden perches, hammocks, puzzle feeders, mirrors, kongs, etc…) that were subject to change at each full cage clean. Full cage cleans were done every week, in which monkeys were removed from cages, cages were disinfected, and all enrichment/floor substrate was replaced, while smaller cleans were completed every few days to replace substrate and ensure the general cleanliness of the environment.

Artificial lighting was turned on at 7:00AM and off at 7:00PM (note, rooms also had windows to allow some natural light in). Monkeys were provided with a daily ration of monkey chow in the mornings. Additional fruit and vegetables were provided twice daily, once at 8:00AM during positive reinforcement training with the veterinarian technicians, and once at 1:00PM by 1 of 5 CMARC caregivers, each with 1-12 years experience working with the macaques and training in basic macaque behaviour. Produce provided daily would change, but consisted of 1-3 fruits or vegetables (i.e. carrots, sweet potato, apple, etc…) cut into small pieces that could be handed out through the caging. All staff typically ended their workdays by 3:00PM, at which point the monkeys would remain largely without human contact until the next day.

Of the 16 Macaques, many were already enrolled in existing biomedical studies under the various researchers at the Mcintyre Medical Building. Existing studies involved mainly electrophysiology. One to two times weekly, monkeys would be removed from their home cages and brought to a separate lab for a few hours to take recordings. In addition, three monkeys were enrolled in an antibody production project. This involved them being missing from the lab on four separate days throughout the experiment. Monkeys on different trials missed 1-5 days of treatment application, though never on two consecutive days. We did not consider this to be an issue for our project, as the goal was to study the effects of the treatment on laboratory-housed macaques. In order for the treatment to be truly effective, it must apply to monkeys under regular laboratory conditions, which includes being part of existing projects. In addition, monkeys on existing trials were found in both treatment groups and still received treatment the other days of the weeks they missed. As we were only recording weekly averages, we considered them to still receive adequate treatment and therefore no obvious bias was introduced.

3.1.3. Experimental Design

The study was conducted between August 12 and October 30 2020 at the Comparative Medicine and Animal Resources Center (CMARC) of McGill, located at the downtown Campus (Quebec, Canada). Sixteen adult Rhesus or Cynomolgus Macaques were enrolled in a nine-week experiment (plus 2.5 week habituation) consisting of three equal phases: baseline (PRE), treatment application (EXP), and post-experimental (POST) (see Figure 3.1.1.). Of the sixteen macaques, 8 adult females were selected as focal monkeys for the study, while the monkeys they lived with were treated as companions to the focal monkeys. The 8 adult females selected as focal monkeys were blocked by species and randomly assigned to one of the following treatments: Human behaviour (HB) or Monkey-like behaviour (MB).

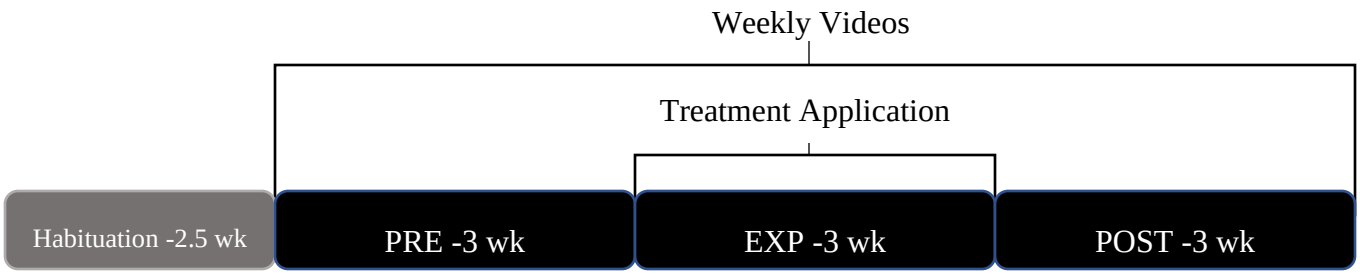

Figure 3.1.1. Timeline of project procedures for the 10.5 week experiment consisting of a 2.5-wk habituation followed by three equal phases of 3-wks. PRE acted as baseline, where weekly videos were taken but no treatment was applied. EXP was the experimental period, in which treatment was applied, live observations were taken, and weekly videos continued. The POST phase acted as the post-experimental condition, in which no treatment was applied but weekly videos continued to see if any effects continued after treatment had stopped.

The trial first began with a thirteen-day habituation (2.5 wks), in which monkeys were familiarized with all experimental procedures and materials. Treatment was only applied during the EXP phase, while PRE and POST acted as baseline and post-experimental condition, respectively. For the purpose of this project, we adapted the usual 1:00 PM feeding time of Monday through Friday to fit our experimental design, while maintaining the general daily routine the monkeys were accustomed to. Weekends already had different procedures from weekdays, as less staff are present. As a result, we kept weekend schedules as normal, and only focussed on weekdays. Caregivers wore their regular PPE (see materials section of SOP # 521: Macaque Husbandry), with a few notable exceptions. Instead of their usual N95 masks and visors, caregivers wore full-face shields that filtered air and allowed their full faces to be visible. Fake-fur wrist bands were also worn to allow for grooming with the monkeys without actual contact. Modified feeding procedures for our treatment were: The caregiver and researcher would enter the first room and set up a chloroplast divider in front of the leftmost cage. This divider was used so that monkeys would not be able to see interactions between the caregiver and other monkeys (allowing us to apply different treatments during the EXP phase to monkeys in the same room). During the PRE and POST phases, the caregiver and researcher would enter the divider and proceed to feed the monkey as they usually would on any normal day, for approximately 2 minutes. During the EXP phase, the caregivers followed an almost identical

routine, with the addition of treatment application. The caregiver and researcher would then move the divider to the next monkey and repeat the process until all monkeys were fed in both rooms.

The treatment application consisted of: once in front of a cage, the researcher would start a timer and the caregiver would follow three feeding times (6 minutes total): they would spend two minutes attempting to interact with the monkey with no food, then two minutes interacting while handing out produce, and finally another two minutes attempting to interact without food being provided. This was done both to see if there was an effect of the food itself versus the treatment itself and to see if there was an optimal time to provide social enrichment. The type of interaction provided depended on the treatment each monkey was assigned to. The goal of this design was to see both the effects of each treatment as well as the effect of extra interaction time provided to the monkeys during the EXP phase.

For both treatments, the goal was to encourage unstructured, monkey-human interactions. For monkeys receiving HB, caregivers would use only human behaviours while interacting with the monkeys. Talking calmly and kindly to the monkeys was encouraged. For monkeys receiving MB, caregivers would imitate monkey behaviours in their interactions. Talking and direct eye-contact was strongly discouraged unless absolutely necessary. All caregivers received training prior to the beginning of the trial to familiarize themselves with each treatment and all experiment procedures. Caregivers were taught three behavioural contexts to utilize: affinitive social/greet, grooming, and submissive/uninteractive (see Table 3.1.1). Caregivers would begin with an affinitive social/greeting context, then they would pick which context to use based on the monkey's context. If the monkey acted aggressive, caregivers would utilize a submissive/uninteractive behavioural context. If the monkey elicited grooming, either by offering a body part of approaching and touching the caregiver, the caregiver would use the grooming behavioural context. Finally, if the monkey acted friendly/neutral, the caregiver would first use the affinitive social context for approximately 30 seconds, then attempt to encourage more interaction by switching to a grooming context.

Table 3.1.1. Caregiver Guidelines, providing behaviours to be performed in each context (chosen based on monkey's reaction).

	HB	MB
Affinitive Social/Greet	Say hello, smile, talk calmly and softly to monkey, greet (say hello), laugh	lipsmacking, playface, neutral face, grunt
Grooming	Inspect/comb through monkey's fur, scratch, eye contact, speak softly	Offer wrist, comb through/inspect monkey's fur, lipsmacking
Submissive/Uninteractive	Maintain posture, but cower slightly, momentarily look away, continuously talk to monkey to calm down (speak slowly/soothingly)	Crouch/get low, avoid eye contact, avoid making sound, backup slightly (a step or two)

3.1.4. Ethogram Creation

In order to create the ethogram, we began by first creating a comprehensive list of all behaviours that may be identified or seen. This was done by compiling ethograms found in Altman 1962, Bayne et al 1991, Partan 2002, Baker et al 2009, Kemp 2014, Cannon et al. 2016, Stolar 2018, and the NC3Rs website on Macaque care and behaviour. Duplicate behaviours were either deleted or definitions were combined to provide the most information possible. For the live observation ethogram, the observer trained during the habituation using the comprehensive ethogram to see what behaviours could be identified during the 6-min interactions. For the weekly video ethogram, two observers trained on pre-trial videos using the comprehensive ethogram to see what behaviours would be visible/possible to code during weekly videos. When a behavioural context was seen to begin, individual behaviours seen would be listed beside the start and end time for that context. Contexts were originally sorted as abnormal, grooming, or social. They were later recoded into abnormal, social (positive-affiliative and grooming and negative-agonistic and submissive), and other (self-grooming) as described in the data handling section. This resulted in the final ethogram used throughout the experiment duration (see Table 3.1.2.).

Table 3.1.2. Ethogram of Macaque Behaviours used for Weekly Videos and Live Observations (Focus and Scans)

Context	Behaviour	Description	Source	Data Collection Method[1]
Abnormal	Abnormal Behaviour	Qualitatively species-inappropriate behaviours, including stereotypic, appetitive, non-injurious self-directed and self-injurious behaviours	Baker et al. 2010	Scan
Abnormal Locomotion	Abnormal Locomotion	Circle, pace, rock, swing, somersault, spin, flip	Bayne et al 1993	Video
	Pacing	Repetitively walking or running back and forth in a circle	Cannon et al. 2016	Video
	Rocking	Any rhythmic motions of the body from a stationary position; animal remains sitting or standing while the upper torso sways back and forth	Baker et al 2009	Video
Cage-Directed	Lick non-food Item	Licking or biting surfaces of environment, walls, or cage	Cannon et al. 2016	Video
Self-Directed	Pluck Hair	Pluck hair from self, using hands or teeth	Bayne et al 1991	Video
	Self-bite	Any vigorous biting of one's own body	Baker et al 2009	Video
	Self-clasp	Clutching or grasping own body	Cannon et al. 2016	Video
Stereotypic	Abnormal Posture	Hold seemingly uncomfortable or unnatural posture	Baker et al 2009	Video
Affiliative	Coo	Tonal (narrow-band) sound (quiet calls, with a variation of ooooh sound)	Partan 2002, NC3Rs n.d.	Focus
	Follow	Walk close behind another monkey, same direction	from pre-trial observations	Video
	Grunt	Quiet, harsh sound usually not repeated (noisy, time modulated structure)	Partan 2002, NC3Rs n.d.	Focus
	Lipsmack	Rapid lip movements, with or without tongue, directed towards a conspecific or person	Kemp 2014	Video, Focus
	Look at	Look in direction of specific individual (in this case, caregiver)	Partan 2002	Focus
	Play	Complex form of behaviour interactions involving many of the other behaviour	Altman 1962, NC3Rs n.d.	Video

		patterns in highly modified form. (social or solitary)		
	Playface	The ears and brow are pulled back, the mouth is open and the top lid is pulled over the teeth	NC3Rs n.d.	Video
	Present	Present part of body (neck, side, or often backside) to caregiver, looking away and becoming still. Often in grooming context, where animal will approach or reposition itself in front of another, presenting an area of the body to be groomed. Also a sign of submission or sexual behaviour	Partan 2002	Video, Focus
	Touch	Monkey has physical contact with another individual or caregiver. Not aggressive	Based on Stolar 2018 (extended to include human contact)	Video, Focus
Aggressive	Bark	Loud, voiced, harsh (broadband) sound (Often the animal will have its head down and ears back, suggesting a degree of fear within the threatening situation)	Partan 2002, NC3Rs n.d.	Focus
	Bite	Focal individual puts mouth and teeth on body part or clothing of caregiver	Stolar 2018	Video, Focus
	Branch Shake	Bounce up and down on branch or other object, holding on with feet so that the substrate moves as well	Partan 2002	Video, Focus
	Chase	Run after other monkey (dominant animals will chase lower ranked animals away from resources where there is high competition, and use aggression to gain access.)	Partan 2002, NC3Rs n.d.	Video
	Displace	Focus animal moves to where another is sitting who then moves away (or opposite)	Kemp 2014	Video
	Display	Monkey does at least 2 threat behaviours at the same time	Stolar 2018	Focus
	Fight	Fast-paced agonistic interaction involving chasing, tumbling, biting, barking, screaming all at once	Partan 2002	Video
	Grab	Use hand to grab hold of other monkey	Partan 2002	Video
	Head Bob (and thorax	The head was jerked very quickly up and down. Arms are sometimes flexed and extended (like a push-up)	Altman 1962	Video
	Head Jerk	Head moved quickly and abruptly up and down	Partan 2002	Video, Focus
	Hit	Meaning obvious/use hand/arm to hit another monkey	Altman 1962, Partan 2002	Video
	Lunge	Sudden and quick movement of upper or entire body towards recipient	Partan 2002	Video, Focus

	Open Mouth Threat (OMT)	Almost always accompanied by direct stare, often by head bobbing. The mandible is opened, but the lips are not bared. The tips of the canine of an adult male are visible but not conspicuous during this gesture. (The open mouth stare is used to threaten other individuals. It is seen during tension between group members, and is often performed towards unwelcome humans from)	Altman 1962, NC3Rs n.d.	Video, Focus
	Push	Meaning obvious, use hand/arm to push another monkey	Adopted from Altman 1962, Partan 2002 DFN of hit/grab	Video
	Slap/Grab	Self-evident. Monkey reaches out to either hit or grab onto the caregiver. Aggressive action	Based on Partan 2002 (Rough Grab)	Focus
	Stare	Direct, prolonged, unwavering look at specific individual. Aggressive	Partan 2002	Focus
	Stiff Arm Threat	Quadrupedal stance. Forelimbs are rigid with hands on ground	Stolar 2018	Focus
	Tense Mouth	Mouth is closed, lip corners drawn back to form straight line. May be seen in a response to aggressive conspecifics.	Partan 2002, NC3Rs	Focus
Grooming	Accept grooming	Monkey being groomed by caregiver. Presenting body part or holding still to allow for grooming.	Based on Partan 2002 (extended to include human)	Focus
	Allogroom	Hands systematically combing through other monkey's hair or combing through/inspecting skin/hair of caregiver (One animal picks through the hair of another with hands or teeth, removing skin, dirt or ticks)	Based on Partan 2002, NC3Rs n.d.	Video, Focus, Scan
	Scratch	Repeated vigorous strokes of the hair	Based on N3CRs n.d. (grooming)	Scan
	Self-Groom	Picking, stroking, and/or licking of one's own body hair	Baker et al 2010	Video, Scan
Submissive	Avoid	Subordinates will avoid or move away from dominant or aggressive animals, to avoid physical contact which may result in injury.	NC3Rs n.d.	Video
	Fear Grin	Focal individual shows all teeth with lips retracted, teeth are clenched (The animal performing the grimace, will look towards the interactant or may turn and look away)	Partan 2002, Stolar 2018, NC3Rs n.d.	Video, Focus
	Flee	Focus animal is moving quickly away from another, usually in response to a threat or other aggressive behaviour	Kemp 2014	Video, Focus

	Freeze	Body suddenly becomes still (Freezing is a fear response to predators, aversive stimuli or unexpected noise from)	Partan 2002, NC3Rs n.d.	Video, Focus
	Lean Away	Lean whole body away from another monkey	Partan 2002	Video, Focus
	Leave	Leave another with whom it had been associated (not rapidly)	Partan 2002	Video
	Yawn	Mouth opens widely in stereotyped gaping movement (Exaggerated yawning with full display of teeth/ Yawning without full display of teeth may simply be an indication of tiredness)	Partan 2002, NC3Rs n.d.	Video, Focus
Neutral	Beg	Monkey extends hand to request food or other object from caregiver (observed in feeding context)	Developed based on pre-trial observations (seen in feeding context)	Focus
	Eat/drink/forage	Feeding behaviours, including eating, foraging, and drinking. Common usage, was not coded for if monkey appeared to be chewing at beginning of video (could be MO, etc..)	Baker et al. 2010	Scan
	NeutralM	Monkey not engaged in any clear activity or paying attention to caregiver	Based on Baker 2010 (Passive or appearing to sleep) and Cannon et al 2016 (Standing immobile, sitting, or lying down)	Scan
Other	Interacting with Caregiver	Monkey is engaged/interacting with the caregiver	Based on Baker 2010 (extended to human)	Scan
	Interacting with other monkey negatively	Aggressive or submissive behaviours directed at another monkey	based on Cannon et al. 2016 (Displaying threat, staring with open mouth, lunging, grimacing, or shaking environmental objects directed toward another individual) and NC3Rs n.d. (submissive behaviours)	Scan

	Interacting with other monkey positively	Affiliative behaviours directed at another monkey	Based on Baker et al. 2010 (Non-contact pro-social behavior directed outside of the cage, to another monkey or human)	Scan
	Locomote	Walk, climb, jump	Cannon et al. 2016	Scan
	Manipulate object	Using hands, feet or mouth to explore inanimate objects or caging	Adopted from Kemp 2014 (Touching, laying with, moving, licking, biting, etc… an object, such as enrichment or enclosure fixings)	Scan
	Not Visible	Counted when the monkey was covered enough such that its behaviour was not visible (ex. The head could be seen popping up behind a barrier but the body language/activity was not visible to the observer	no source, reality of caging/observation method	Scan
	Sexual	Elicitation, mounting, mating, etc…	Kemp 2014	Scan
Body Orienation	Away	Monkey is facing away from caregiver		Scan
	Towards	Monkey is facing towards caregiver		Scan
Cage Position	Back	Monkey is positioned in the back third of their cage		Scan
	Enclosure	Monkey is positioned in secondary enclosure (if available), away from potential interaction with caregiver		Scan
	Front	Monkey is positioned in the front third of the cage		Scan
	Middle	Monkey is positioned in the middle third of their cage		Scan

[1]Where Scan represents Live Observations -Scans, Focus represents Live Observations -Focus, and Video represents Weekly Videos

3.1.5. Observation Procedures

3.1.5.1. Objective 1- Live observations to assess the monkeys' reaction to each treatment during interactions, in the EXP phase only

Scans and focus live observations were taken on focal monkeys during the six minutes of treatment application for each monkey daily. Data was collected by one observer. Scans were

completed every 15 seconds to track monkey body orientation, cage position, and general activity (see Scans -Table 3.1.2.). Throughout the six minutes, continuous observation was also completed to record all human-directed behaviours the monkeys did during treatment application (see Focus -Table 3.1.2.).

3.1.5.2. Objectives 2 and 3 - Weekly videos to assess the mid- and long-term effects of each treatment on the social and abnormal activity of the monkeys across all 3 phases

One-hour-long videos using GoPro (HERO Black 7) on tripods were recorded every Friday afternoon from 4:00 PM to 5:00 PM across all three phases. The goal was to observe any long-term effects of either or both treatments on monkey social and abnormal behaviour when no humans were present. This time was selected as all caregivers ended their days at 3, giving the monkeys time to settle and ensuring our videos had no influence of human presence. The researcher would enter the first room at 3:55 each Friday and set up a camera in front of the leftmost cage such that the majority of the cage was visible (due to room set-up, some cages had corners where the monkeys would not be visible). They would begin filming and hold up a placard with the date, room number, and names of the monkeys in that cage. They would then proceed to the next cage and repeat the process until a camera was in front of each cage in both rooms. Once all cameras were recording, a timer would begin (at 4:00) to ensure exactly one hour of video was recorded. After the hour had elapsed (at 5:00), the observer would then re-enter the rooms to remove all cameras in the same order in which they were set up. The first and last 3-minutes of each video were ignored to exclude any effect of the observer's presence.

Video recordings were analyzed by one trained observer. The observer was trained and assessed on reliability from video recordings of pre-trial data. Overall inter- and intra-reliability of at least 80% was obtained using kappa coefficients once before scoring of trial data commenced and was retested twice throughout scoring (approximately every 2-3 weeks). The observer recorded the start and end time whenever the focal monkey used one of the six behavioural contexts of interest: Abnormal Behaviour, Affiliative Behaviour, Grooming Behaviour, Agonistic Behaviour, Submissive Behaviour, and Self-Grooming Behaviour (see Video -Table 3.2.1.). Continuous observation of each behavioural context was completed for the full hour. A behavioural context was considered to begin when a behaviour unique to that

context began. A context was considered to end or change when either no behaviours from that context were being performed or when a behaviour from a different context was spotted, at which point that context would begin.

3.1.6. Statistical Analyses

3.1.6.1. Data Handling

3.1.6.1.1. Objective 1 - Live observations to assess the monkeys' reaction to each treatment during interactions, in the EXP phase only

Weekly averages were calculated for each variable to control for the effect of caregiver. This was done because while 5 different caregivers participated in the study, they were distributed across each week such that while individual days were influenced by caregivers, whole weeks were identical in the distribution of caregivers. In addition, general activity scans and human-directed behaviours were grouped by type before statistical analyses. For behaviour scans, behaviours were largely grouped into active (eating, locomotion, self-directed, and social/interactive) and inactive behaviours (not moving and not visible). Within active behaviours, self-directed was further split into self-grooming and abnormal, and social/interactive into human interaction and monkey interaction. Finally, monkey interaction was further split into positive monkey interaction and negative monkey interaction (see figure 3.1.2.). For focus observations of human-directed behaviours, behaviours were first grouped into three sections: neutral (begging and look-at), positive (grooming and affiliative behaviour) and negative (aggressive and submissive). Within positive behaviours, affiliative behaviours were further split into vocalizations, touching, and lipsmacking (see 3.1.3.). Measurements are presented as proportion of time (duration in sec divided by total observation time, i.e. 6 min or 360 sec) for scans and frequency per 2 min (either before, during, or after feeding) for focus observations of human-directed behaviours.

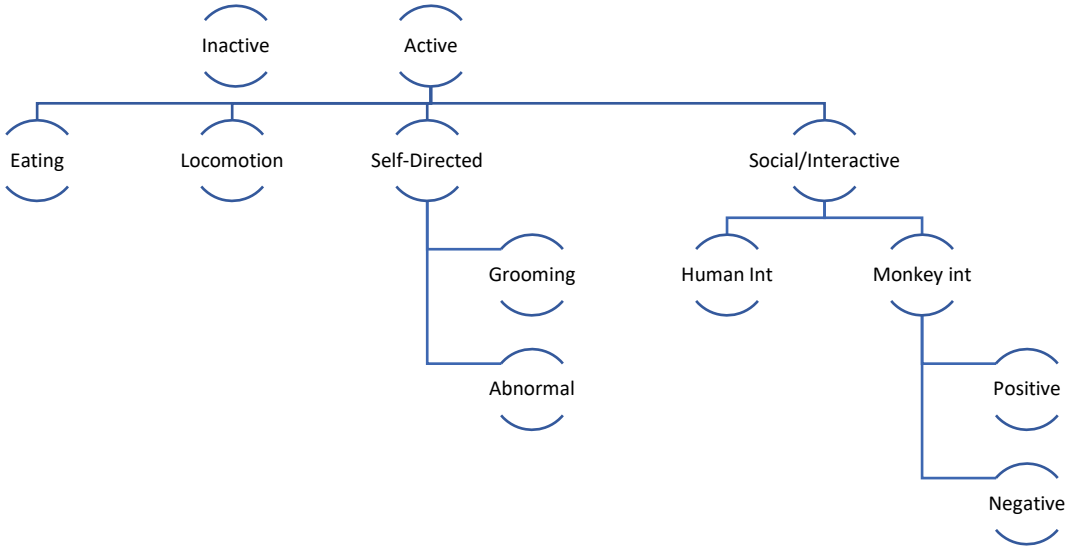

Figure 3.1.2. Scan data from live observation behaviour groupings. Live observations -Scans behaviours found in ethogram were grouped according to the above diagram.

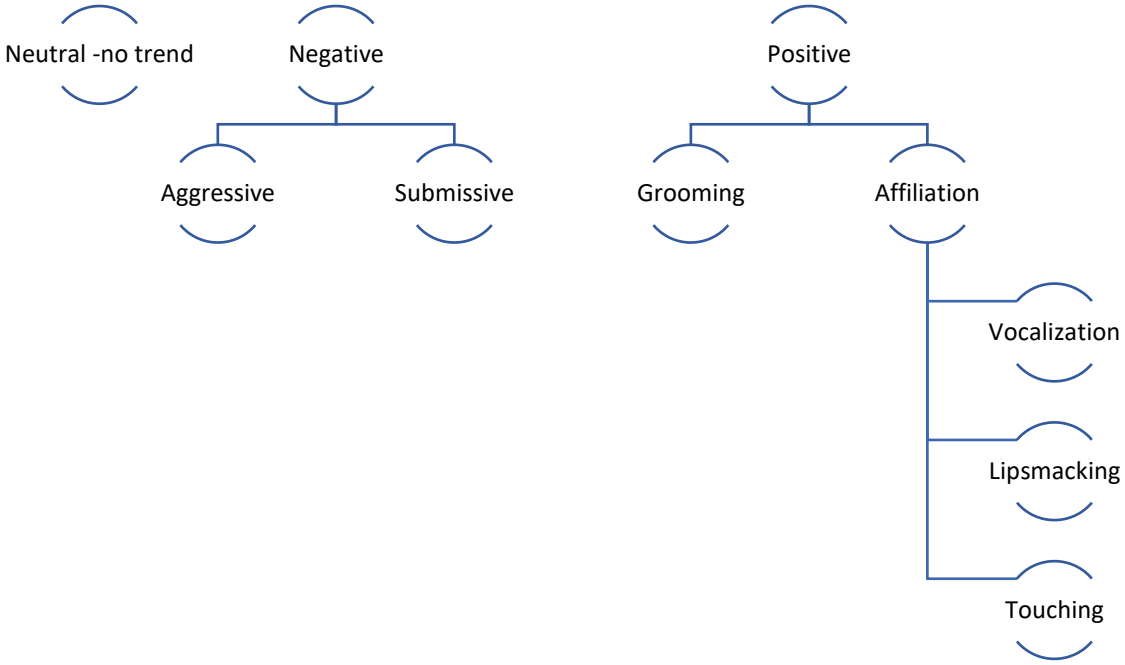

Figure 3.1.3. Focus data from live observation behaviour groupings. Live observations -Focus behaviours found in ethogram were grouped according to the above diagram

3.1.6.1.2. Objectives 2 and 3 - Weekly videos to assess the mid- and long-term effects of each treatment on the social and abnormal activity of the monkeys across all 3 phases

Behavioural contexts were first grouped into their largest categories: abnormal (cage-directed, self-directed, stereotypic, and locomotive), social (positive and negative), and other (self-grooming). Within the social category, positive was further split into affiliative behaviour and grooming, while negative was split into aggressive and submissive (see figure 3.1.4.) Frequency and duration were extracted from data sheets (which recorded start and end times of each context). In addition, durations of behavioural contexts were divided by duration of videos, in all cases one hour, or 3600 seconds in order to produce a proportion of time spent in each context. For social behaviours, individual groups were also divided for the total amount of time the monkey spent in the social behavioural context in order to analyze any shifts in the nature of social behaviours (i.e., positive versus negative, submissive versus aggressive). Hence, data is presented in frequency, duration, and proportion of time (as well as proportion of social time for social behaviours) spent in each of the behavioural context categories.

Behavioural context grouping

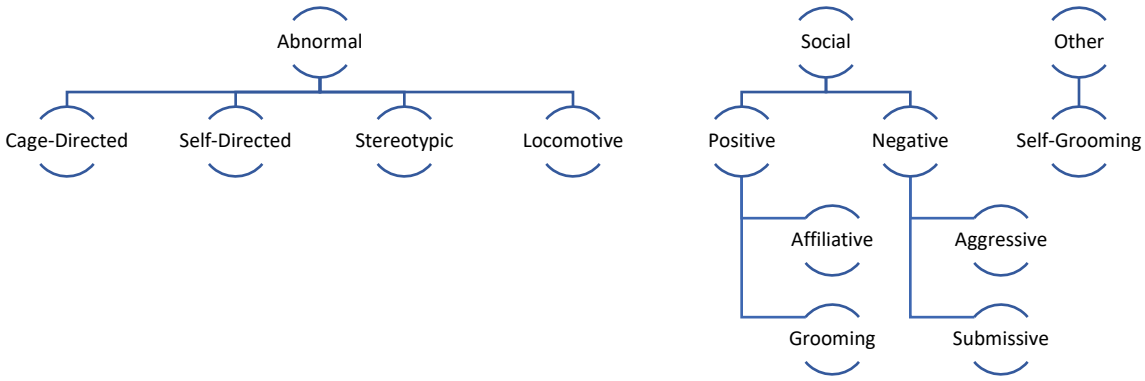

Figure 3.1.4. Weekly video behavioural context data behaviour groupings. Weekly videos behaviours found in ethogram were grouped according to the above diagram.

3.1.6.2. General Model Considerations

Given that only 8 monkeys were enrolled in the study to split between the two treatments, where 5 were Rhesus Macaques and the remaining 3 were cynomolgus, it was not possible to analyze the effect of species. Instead, species was treated as a block effect for analyses. This allowed us to account for the effect of species while still focussing on our main question: assessing the effect of treatment. Only weekly averages were used in the final results to eliminate the need to include caregiver in the final models. Significance was set at 0.10. All models were run using SAS 9.4. Results are presented as means with standard error.

3.1.6.3. Statistical Models

3.1.6.3.1. Objective 1 - Live observations to assess the monkeys' reaction to each treatment during interactions, in the EXP phase only

For live observation analysis, food-time (accounting for the 6 minutes of interactions around which food is provided, specifically before, during, and after food distribution) and week

were considered independent of one another. Since we were explicitly interested in the effect of food-time over and above the effect of week, both are included in the model as main effects. In order to account for their relationship and place in context, an interaction between food-time and week was included. Food-time, as a variable of interest, was included as a fixed effect, while week was considered random. Monkey was considered a random effect as we are not interested in the individual monkeys themselves and they represent significant variation in the model.

The following model was therefore developed to analyze the live observations:

$Y_{ijklmn} = \mu + trt_i + species_j + room_k + monkey_{kil} + week_m + food\text{-}time_n + trt*week_{im} + trt*food\text{-}time_{in} + week*food\text{-}time_{mn} + e_{ijklmn}$

Where: Y_{ijklmn} is the dependent variable, which is the outcome measure of the lth monkey from the j^{th} species and k^{th} room, receiving the jth treatment at the nth time during the mth week. The trt_i is the fixed effect of the ith treatment (j = HB or MB). The $species_j$ is the fixed (block) effect of the jth species (j= Rhesus or Cynomolgus macaque). The $room_k$ is the fixed effect of the kth room (k= 1421B or 1421E). The $monkey_{kil}$ is the random effect of the lth monkey (l= 1 to 8) from the jth species and kth room. The $week_m$ is the random effect of the mth week (m= 1, 2, or 3). The $food\text{-}time_n$ is the fixed effect of the nth food-time (n= before, during, or after feeding). The $trt*week_{im}$ is the random effect of the interaction between the ith treatment and the mth week. The $trt*food\text{-}time_{in}$ is the fixed effect of the interaction between the ith treatment and the nth food-time. The $week*food\text{-}time_{mn}$ is the random effect of the interaction between the mth week and the nth food-time. Finally, e_{ijklmn} is the random error.

In order to account for repeated measures of monkey and week, the best fitting covariance structure (between Auto-Regressive (1) or AR(1) and Compound Symmetry or CS) was selected with a step-by-step elimination process of inclusion and exclusion of different random and repeated factor combinations. The chosen combination for each outcome measure was decided by the best BIC fit statistics. To ensure that the data came from a normal population, for each outcome measure, normality of residuals for each outcome measure was tested using PROC MIXED and PROC UNIVARIATE procedures. A Tukey adjustment was used in order to account for the multiple comparisons associated with each interaction.

3.1.6.3.2. Objective 2 -Weekly videos to assess the long-term effects of each treatment on the social and abnormal activity of the monkeys across all 3 phases

For weekly videos, week was considered nested in phase, as the effect of week is only of interest, and in fact only comprehensible, when nested within each phase. As for the live observation analysis, room was considered as a fixed effect and monkey as a random effect. No interaction was included between treatment and week. Week is not of particular interest to the research question (unlike phase), and upon running the first few variables with the interaction where none was found to be significant, it was removed from the model.

In order to analyze the weekly videos, the following model was developed:

$Y_{ijklmn} = \mu + trt_i + species_j + room_k + monkey_{kil} + phase_m + week(phase)_{mn} + trt*phase_{im} + + e_{ijklmn}$

Where: Y_{ijklmn} is the dependent variable, which is the outcome measure of the lth monkey from the jth species and kth room, receiving the ith treatment during the nth week of the mth phase. The trt_i is the fixed effect of the ith treatment (i = HB or MB). The $species_j$ is the fixed (block) effect of the jth species (j= Rhesus or Cynomolgus macaque). The $room_k$ is the fixed effect of the kth room (k= 1421B or 1421E). The $monkey_{kil}$ is the random effect of the lth monkey (l= 1 to 8) from the jth species and kth room. The $phase_m$ is the fixed effect of the mth phase (m= PRE, EXP, and POST). The $week(phase)_{mn}$ is the fixed effect of the nth week (n = 1, 2, or 3) from the mth phase. The $trt*phase_{im}$ is the fixed effect of the interaction between the jth treatment and mth phase.. Finally, the e_{ijklmn} is the random error.

To account for repeated measures, the best fitting covariance structure (between Auto-Regressive (1) or AR(1), Compound Symmetry or CS, and Unstructured or UN) was selected for each outcome measure by comparing the BIC fit statistics. Normality of residuals for each outcome measure was testing using PROC MIXED and PROC UNIVARIATE procedures. A Tukey adjustment was used in order to account for the multiple comparisons associated with each interaction.

3.1.6.3.3. Objective 3 -Weekly videos to assess the mid-term effects of each treatment on the social and abnormal activity of the monkeys during the EXP phase only

In order to see if there was a difference between monkey behaviour in weekly videos during the phase when treatment was applied (the EXP phase), a subset of the above data was extracted (all data from the three weeks of the EXP phase). The model for this analysis was largely the same as the weekly videos, with one major exception. For this model, there was no longer an effect of phase as all data included in this section came from the same phase. The model below was run for each variable. In cases where the trt*week$_{im}$ interaction was not found to be significant, the interaction was dropped and the model re-run with only main effects for that outcome measure.

This data was analyzed using the following model:

$Y_{ijklmn} = \mu + trt_i + species_j + room_k + monkey_{kil} + week_m + + trt*week_{im} + e_{ijklmn}$

Where: Where: Y_{ijklmn} is the dependent variable, which is the outcome measure of the lth monkey from the jth species and kth room, receiving the ith treatment during the mth week. The trt_i is the fixed effect of the ith treatment (I = HB or MB). The $species_j$ is the fixed (block) effect of the jth species (j= Rhesus or Cynomolgus macaque). The $room_k$ is the fixed effect of the kth room (k= 1421B or 1421E). The $monkey_{kil}$ is the random effect of the lth monkey (l= 1 to 8) from the jth species and kth room. The $week_m$ is the fixed effect of the mth week (n = 1, 2, or 3). The trt*$week_{im}$ is the fixed effect of the interaction between the jth treatment and mth week. Finally, the e_{ijklmn} is the random error.

To account for repeated measures, the best fitting covariance structure (between Auto-Regressive (1) or AR(1), Compound Symmetry or CS, and Unstructured or UN) was selected for each outcome measure by comparing the BIC fit statistics. Normality of residuals for each outcome measure was testing using PROC MIXED and PROC UNIVARIATE procedures. A Tukey adjustment was used in order to account for the multiple comparisons associated with each interaction.