FACULTAD DE CIENCIAS PROGRAMA DE MAGÍSTER EN CIENCIAS

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STUDY OF MEMORY CONSOLIDATED IN A SOCIAL CONTEXT

ANDREW JUSTIN TAYLOR

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Director de Tesis:

Prof. Dr. Alexies Dagnino Subiabre

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ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AM	Amygdala
BLA	Basal Lateral Amygdala
CEA	Central Lateral Amygdala
Dm	Dorsal Medial
HP	Hippocampus
LA	Lateral Amygdala
NAC	Nucleus Accumbens
PFC	Prefrontal Cortex
P	Phase
T	Trial
vM	Ventral Medial

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ABSTRACT

Emotional memory consolidation has been one of the key investigated points in the last decade, understanding the underpinnings from its behavior to its molecular background. As fear memory consolidation has typically been focused on in research in the past decade, giving rise to its behavioral patterns such as freezing in rodent models and understanding of sub-cortical structures involved such the Amygdala and its substructures in the moment of fear memory and hedonic memory consolidation, social memory which is given an emotional salience, has been overshadowed. This study aimed to answer the question of, if it is possible to study memory consolidation in a social context at the behavioral level. As the general objective was to study how memory is consolidated in a social context, the following specific aims of designing a behavioral paradigm to study memory consolidated in a social context, measuring memory consolidation, and measuring social preference, were planted. As the Pavlovian Condition Approach is applied to fear memory conditioning which analyzes behaviors such as avoidance and freezing, and hedonic memory which analyzes behaviors such as place of preference, this new behavioral paradigm, based on parameters of both Pavlovian fear memory and hedonic memory conditioning, analyzed **memory consolidation observed by social preference** which was quantified by **latency**, **cumulative time**, and **frequency**. In order to analyze these variables, with a remodified open field chamber, a four phase a setup was carried out with twelve Sprague Dawley rats. Additionally, a social avoidance preference test was used, with a pairing of the original twelve rats and three rats separated from the other litters at birth, to understand how memory consolidated in a social context, affected sociability with a **new conspecific**, in the aftermath. The findings showed that ***latency*** for only the afternoon of rats which represented 50%, of all rats, had a significant decrease, indicating memory consolidation, in only two trials. Additionally, ***cumulative time*** and ***frequency***, which both, in overall, had no significant difference, indicating no specific place of social preference, hence motivation for a specific social interaction. Finally, findings were inconclusive for the last experiment that looked to understand how sociability is affected by previous memory consolidated in a social context; as there was no strong data to indicate that sociability had improved or worsened based on the previous social interaction. Finally, a neuronal mechanism was proposed to explain the underpinnings of this particular behavior, as well as a new behavioral paradigm that serves the purpose of a new line of investigation to help understand the neurobiology of social memory. Understanding social memory could help in understanding specifically why stress response in social interaction is different for some individuals, such as those on the autistic spectrum.

I. INTRODUCTION

1.1 Memory

Over history and especially today in our modern-day society, memory has been and is one of the key components in social cognition (1) which is defined as how people process, store, and apply information about other people and social situations (2). Findings have shown over decades, primarily four structures involved in memory which are the Prefrontal Cortex (PFC), Hippocampus (HP), Amygdala (AM), and Cerebellum (3). Further research based on these structures, have divided memory into working memory (short term) and explicit and implicit memory (long term) (4). As we analyze social behavior, long term memory in semantics (language) and episodic (experiences) is fundamental in social interaction (4). Moreover, research in the last decade have found strong implications that episodic memory, in the limbic structure which involves PFC, HP, and AM, is strongly consolidated by specific activity of sub structures of the AM such as the Lateral Amygdala (LA), Basal Lateral Amygdala (BLA), and Central Lateral Amygdala (CEA) (5). Therefore, these structures, which are part of the limbic system, have been deemed important for emotional memory consolidation (6) that is a hallmark among many types of research in this day in age. Over the past decade, much research has been focused on the area of emotional memory (7). Considering emotional memory can play a factor in sociability in modern day society, quite a bit of research has been focused on aversive memories such as fear memory (8).

1.2 Fear Memory

Most importantly, fear memory research has been carried out at the behavioral level, correlating fear memory consolidation with freezing behavior in rats (8), and a neuronal mechanism involving structures such as the PFC, LA, BLA , CEA, hypothalamic pituitary adrenal axis (HPA) (8,9,11) and crosstalk among some of the aforementioned structures with the HP (9), Nucleus Accumbens (NAC),Ventral Tegmental Area (VTA) among others (40). As the aforementioned structures are considered to form the limbic system and control

emotional responses (42), this system is modulated, in part, by stress (42) which is commonly defined as an organism´s response before a stressor such as an environmental condition (43); stress is divided into two areas: negative stress (Distress) that is the organism´s inability to cope with a stressor and positive stress (Eustress) an organism's ability to cope with a stressor, assigning it a positive appraisal rather than a negative one originally (44) . Aversive memory such as fear memory is generally correlated with distress (45). Furthermore, continuous distress is commonly correlated with a dysregulation of the HPA axis and an elevated cortisol level in humans (46). In rodents, as cortisol is less appreciable in glucocorticoids, cortisone has been taken as a biomarker measure for stress response in fear memory, being quite comparable to that of humans (47). Additionally, apart from the phenotypical behavior of freezing during fear memory recall, avoidance behaviors (56) they have correlated this freezing behavior during fear memory, with neuronal oscillatory synchrony of 4-6 HZ among the BLA and PFC (15). In this regard, fear memory has been able to be studied not only one, but at various levels.

1.3 Hedonic Memory

On the other hand, pleasurable memories (hedonic memories), originally coined Selye, are correlated with positive stress (Eustress) (48). Evidence in latest research has shown that both aversive and hedonic memories share a similar neuronal mechanism in the moment of consolidation (11), as there are outputs from the BLA to NAC during hedonic and aversive association; furthermore it is known that there is an upregulation of AMPA/NMDA receptors in the NAC during hedonic stimulus and a downregulation to NAC and upregulation to CEA during aversive stimulus (11). In terms of hedonic memory, through natural reward such as food intake, research has also highlighted the mesolimbic circuits´ strong role in regulating food intake alongside the homeostatic circuit (50). The mesolimbic circuit, comprised of subcortical structures such as the NAC, VTA, and HP regulate the motivation/drive, of food intake, as it shares crosstalk with homeostatic circuit, comprised of the hypothalamus which is central for energy balance and several of its nuclei which are involved in energy regulation inside the body (50). It has also been stated that this hedonic circuits´ control is heavily dependent on top-down modulation from the vmPFC in providing inhibitory control-emotional regulation, and executive function (50). Therefore, the involvement of hedonic circuit, involving memory and conditioned learning (50), has

been important in understanding the neurobiological mechanisms underlying conditions such as obesity (50).

Additionally, the other research that has been carried out in hedonic memory consolidation has been done with the use of substances from meta-amphetamines to sucrose (11), with observations at the behavioral level. Furthering the understanding of hedonic memory in addictions, studies have been done to determine the strength of hedonic internet gambling and video game addictions by measuring event related potentials among different frequency bands (16). Lastly, the latest study from 2018, stated that the brain possibly differentiates between an aversive and reward stimulus based on the potentiation in the postsynaptic terminals (14) which might imply unique activity of the PFC during a learning experience. The aforementioned evidence allows a deeper understanding of research that has been carried out in hedonic memory at different levels.

1.4 Emotional Memory´s Relevance for Social Memory

As detailed about fear and hedonic memory, it can be assumed that memory shapes the very fabric of social interaction, as humans are highly dependent on previous interactions to form their perception of someone who they previously met, mainly due to an expectation of reciprocity (17). It is stated that due to our memory of previous interactions, it allows us to successfully generate long term lasting relationship (17). Therefore, emotionally memory plays a fundamental role, as an individual can recall the emotional valence of the social interaction, which is given to an episodic memory, deemed a social memory (56). A social memory, for animals, similar to that of humans, is important in order to express appropriate social behavior such as aggression, avoidance, cooperative behavior, and mating behavior, based on memory recall and recognition (56). It is evidenced that, apart from the encoding of episodic memory in the hippocampus of where(spatial), when, (temporal), what (event information), that there is encoding of social information (who) in CA2 of the hippocampus and retrieval of such social information or social memory engram, from the ventral CA1 region of the hippocampus (56). Additionally, it is stated that from the CA1 of the hippocampus, the NAC receives glutamatergic inputs which elicits social discrimination behaviors of which is observed in avoidance and sexual preference behaviors, respectively, in prairie voles and medaka fish display (56).

Most important, social discrimination, it not without other neuroanatomical structures that works in memory and emotion (57), such as the PFC cortex, comprised of the ventral medial prefrontal cortex (vmPFC) and dorsal medial prefrontal cortex (dmPFC), which has a fundamental role in decision making and attention mechanisms (18) for making a decision before a discrimination task, alongside the anterior cingulate cortex (ACC), both of which have a reciprocal connectivity with the AM (18,19), among all of which have a direct or indirect connectivity with the HP (57)

This could further imply that certain neuropsychiatric disorders that affect the neuronal mechanisms of social memory, such as Major Depression Disorder (MDD), General Anxiety Disorder, Schizophrenia, and even autism, could contribute to generating some impairments in social interaction, apart from contributing to in part, to a persistence in some of these disorders (32). Currently, there are over 300 million people worldwide affected by depression (20), of which only a percentage over the last decade have developed a long-term addiction to the use of antidepressants based on continuous use. Although there has been much advance in years in looking to understanding mechanisms behavior social memory, there is still much unknown. Perhaps a better understanding of these mechanisms could possibly assist in current new alternative therapeutic treatments such as cognitive therapy treatment, neuronal feedback, among others which are growing more common, avoiding the use of medication or improving its effectiveness. Based on the aforementioned, it turns out interesting to understand the underpinnings and mechanisms of memory consolidated in a social context which has yet to be researched in detail.

II. THE PROBLEM

Based on the afore-mentioned evidence, there is very little research in memory consolidated in a social context. As fear memory consolidation has already been correlated with freezing behavior in rodents and avoidance behaviors in rats as in mice , and hedonic memory consolidation by substance use being correlated with behavior oriented towards an increase or decrease in cumulative time, latency and total distance moved, in a specific experimental design, it gives rise to the question of

Is it possible to study memory consolidated in social context at the behavioral level?

2.1 Hypothesis

Memory consolidated in social context is observed by Social Preference

2.2 General Objective

Evaluate if memory is consolidated in a social context.

2.3 Specific Objectives

2.3.1 To design a behavioral paradigm to study memory consolidated in a social context.

2.3.2 Measure memory consolidation.

2.3.3 Measure Social Preference.

III.METHODS AND MATERIALS

3.1 Bioethical Framework

Before an animal rat model was touched in this experiment, the 3RS of Replacement, Reduction, and Refinement were used to evaluate it (27,31). As this experiment looked only at the behavioral level, no replacement techniques were used. Based on the experimental design, only 12 animals were used for total number of 24 trials, optimizing the limited number of rats and assuring a reduction in animal rat model use. Finally, considering this experiment worked with a social memory, with parameters that prevented physical contact of these rats, rats received little or no distress during this experiment, ensuring refinement.

3.1.2 Animal Models

Twelve male Sprague-Dawley rats, approximately 12 weeks old were used. Like previous research carried out (29), the choice of male rats was due to the hormonal changes in female rats which could alter the results given the time frame that was used. They were housed in four separate acrylic chambers in groups of 3, having food bought from the company ACWS (Nº Catalog: RMH 3000 LabDiet ®, Santiago de Chile) and water *ad libitum*, being housed in a room exposed to a light/dark cycle of 12 h (lights on at 8:00 am). The temperature in the housing facility and a place where the experiment was carried out, was always fluctuated between $22 \pm 2^{\circ}C$ (measured with an indoor temperature and humidity gauge, model #00325, AcuRite, CA, U.S.A). To avoid any unnecessary alterations to their behavior; these were the temperature conditions kept for previous experiments in the same or similar line of investigation (7,29).

The following rats were split into two groups R Group (Morning from 9am-1pm) and S Group (Evening from 2pm-6pm).These rats received a classification of R1, R2, R3 (Cage 1-Litter 1) and R4, R5, R6 (Cage 2-Litter 2) with respective markings on their tails according to numbers 1,2,3 and 4,5,6. And for litters 3 and 4, they received the classification of S1, S2, S3 (Cage 3-Litter 3) and S4, S5, S6 (Cage 4-Litter 4) with their respective markings 1,2,3 and 4,5,6. It is important to mention that R1-R6 and S1-S6 were paired in the following order (R1-R5, R2-R4,R3-R6) and (S1-S5, S2-S4,S3-S6). Additionally, 3 rats, separated from group R and S, at PND21, of 30+ weeks, were used for the additional analysis test.

3.2 Experimental Design

3.2 Timeline for the development of the experiment, observed in the following (Fig.1).

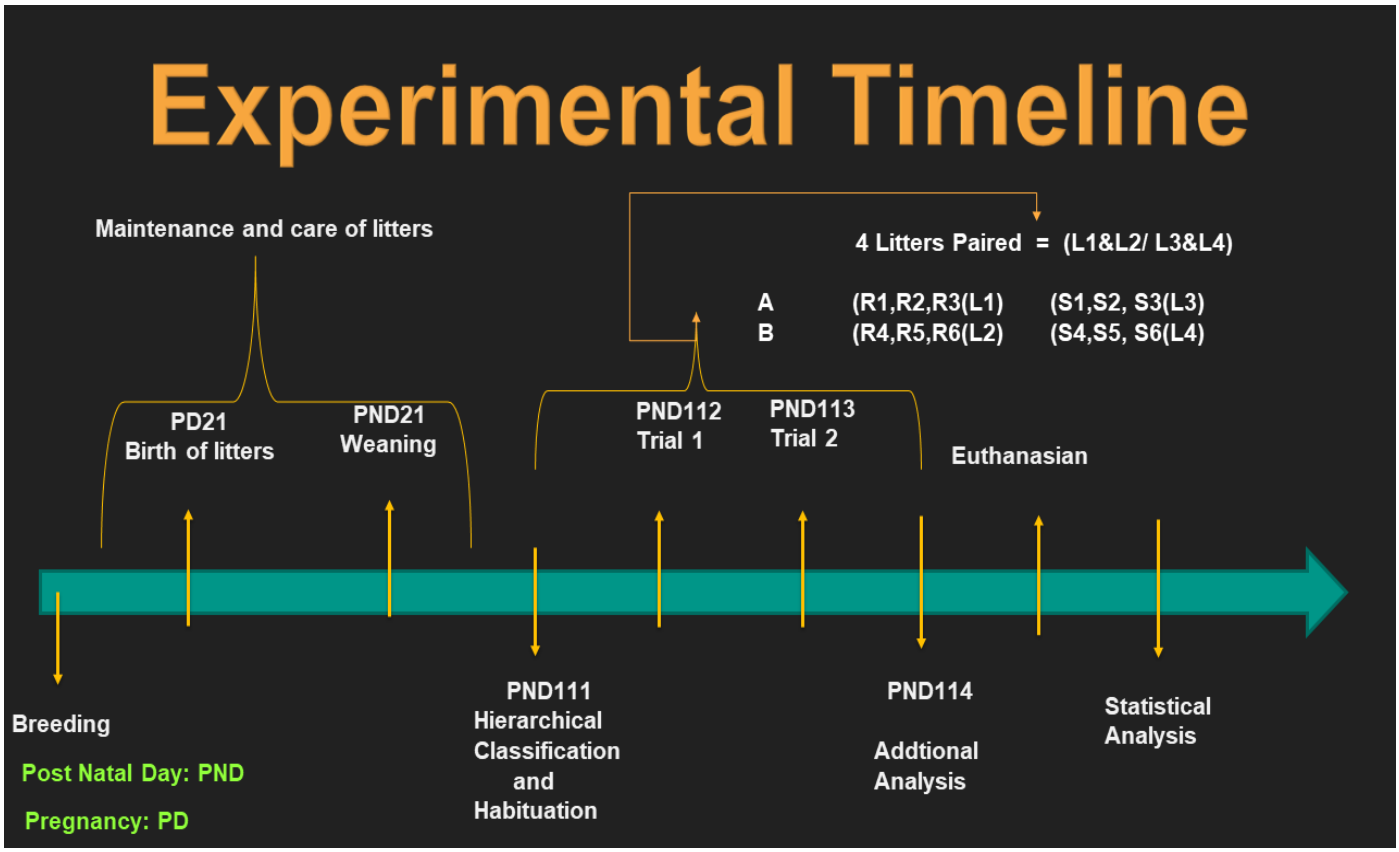

Figure 1. Timeline for experimental design.

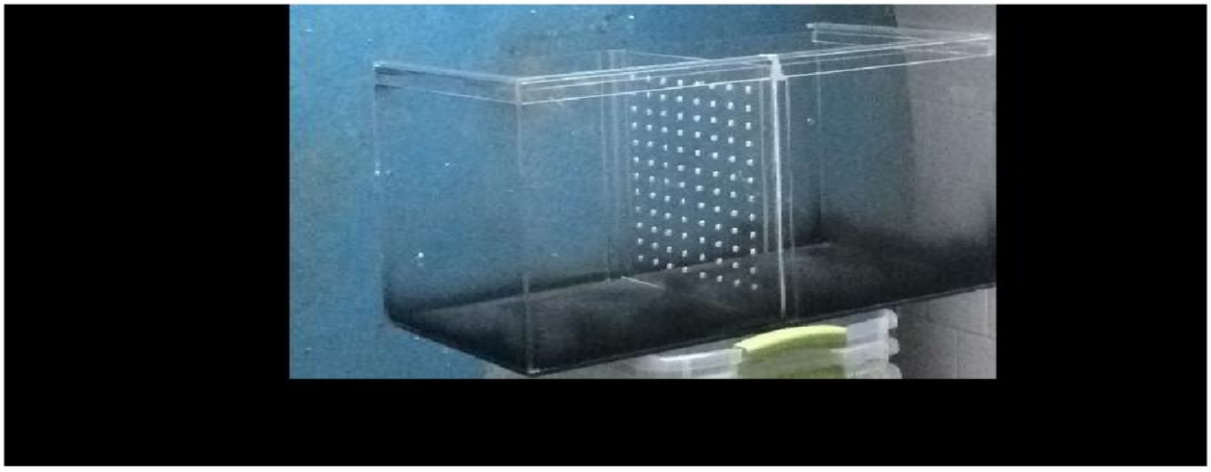

Figure 2. The chamber that was used for this experiment.

3.3 Chamber Setup with equipment

First and foremost, in the fear memory paradigm a chamber is used which is sound proof and light proof to avoid distracting stimulus (7); moreover, the chamber apart from its metallic floor which has a dark base underneath, has small measurements of (33 cm x 25 cm x 28 cm) as only one rat is placed inside. As this paradigm was focused on social interaction, a chamber was created with the measurements of 50x 40x 60 according to the measurements of an open field chamber (31) which allowed social interaction between two rats. The bottom of the chamber was painted black as, previous studies have shown illuminated light areas can be aversive to rats and effect exploratory behavior (52). On one side of the chamber there was an image of a square which represented side A, while on the other side of the chamber, there was an image of a triangle which represented side B, of which both served as visual cues for spatial memory (53).

3.4 Internal Chamber of a Chamber

As to prevent any external stimulus distractions, the chamber was placed inside a bigger chamber where lux (measured with a digital luxmeter, model #LX-1010B, Weafo Instrument Co., Shanghai, China) was maintained between 20 to 45 maximum (24), as to not alter the behavior of the rats during the experiment. Furthermore, the internal walls of the chamber were black painted (52). The internal chamber was kept at 22 ± 2˚C (8) (measured with an indoor temperature and humidity gauge, model #00325, AcuRite, CA, U.S.A). Lastly, but not least, the internal decibels the chamber was measured during every trial, and never exceed 30 decibels (measured with sonometer, model #1100, Quest Technologies, Oconomowoc, WI, USA) which could alter the rats´ behavior (33).

3.5 Classification based on Hierarchy (Dominant and subordinate)

A hierarchical order exists among rats, which normally influences social interaction (25,26). Therefore, to avoid any pitfalls based on hierarchy of two dominant rats interacting socially, dominant rats were paired with subordinate and subordinate were also be paired with subordinate. In order to determine hierarchy, the dominance tube was used with the measurements and instructions from the previous study (25). There were two dominant rats and four subordinate rats for the morning group, as well as two dominant rats and 4 subordinate rats for the afternoon group. The figure below illustrates the aforementioned.

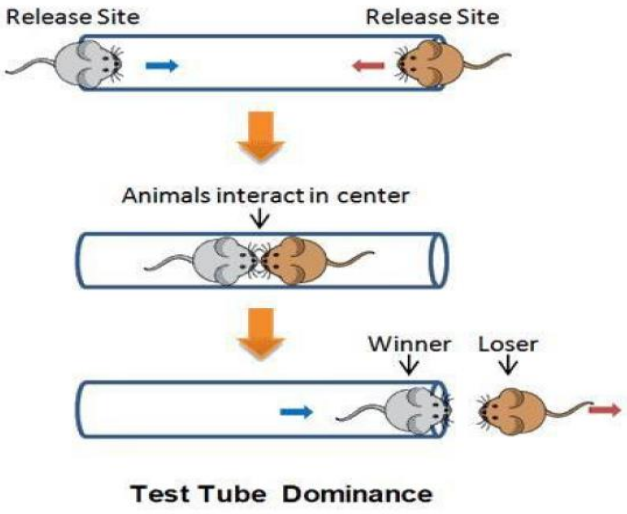

Figure 3. This shows the dominance tube that will be used for hierarchical classification.

3.6 New Paradigm: Memory Consolidated in a Social Context (MCSC)

As there is strong evidence to support a neuronal mechanism which circuitry is used for both aversive and hedonic memory consolidation (8) which is displayed at the behavioral level, with additional support of a social memory engram among these mechanisms (56) and a closely related studies carried out salience incentive to social cues (58), it may be implied that some components, such as the use of particular variables of analysis, may be used in this new paradigm.

3.7 Variables of Analysis

As in fear memory, behaviors of freezing and avoidance to social interaction are observed (22), which implies avoidance to that of social proximity. On the other hand, in hedonic situations, implying social proximity is typically defined as the closeness or distance in which two subjects share (23), is observed by place preference which implies time spent in a given area (58). For this paradigm, taking into consideration a recent experiment carried out with incentive salience given to social cues (58), three variables were decided to be used to fulfill the specific objectives and answer the hypothesis.

Latency was chosen as marker of decision making based on memory recall (38), while cumulative time and frequency were chosen as markers strength of motivation for an area of social interaction apart from memory recall (35,36). This is to say, a decrease in latency among trials would imply quick decision making based on a recall of memory from learning (38), that is to say the memory was consolidated; furthermore, **cumulative time** and **frequency** were the final variables chosen, as an increase or decrease in cumulative time and

frequency into an area of social interaction would imply a motivation or demotivation based on the previous interaction (37,38), that is to say a social place preference.

3.8 Definition of a Trial

Based on aforementioned studies (58), a pavlovian conditioned approach was used, taking into account the variables of latency, cumulative time, and frequency. As mentioned further on, habituation was carried out before the start of the first trial, as to eliminate neophobia which could alter the first trial´s baseline results. Additionally, the hierarchy categorization aforementioned, was also carried out among all 12 rats in MCSC before the start of the first trial. The trial was divided into four phases. **Phase 1** represented the baseline phase which, similar to the research carried out in incentive salience to social cues (58), was used to give a comparison of variable values against the final phase, allowing an understanding of how memory is consolidated in a social context. **Phase 2** represented the initial social interaction with a conspecific, which was considered a phase of association and learning that the place the conspecific was present, also presented a visual symbol different than where it was. **Phase 3** represented an interaction with a novice object, which served the purpose of comparison of place of social preference salience between the novice object and con specific. Finally, **Phase 4** represented the moment in which memory consolidation was observed by a decision in where to go and place preference based on positive or negative valence given to a place of social interaction, which represents motivation. Phase 1 and Phase 4 were compared to understand whether or not a memory was consolidated of the conspecific and if this memory had a strong, weak, or indifferent motivation observed by place preference. Needless to mention, after the removal of each rat, a cleaning with ethanol 0.5% was carried out to remove any odor (54), as not to alter behavior.

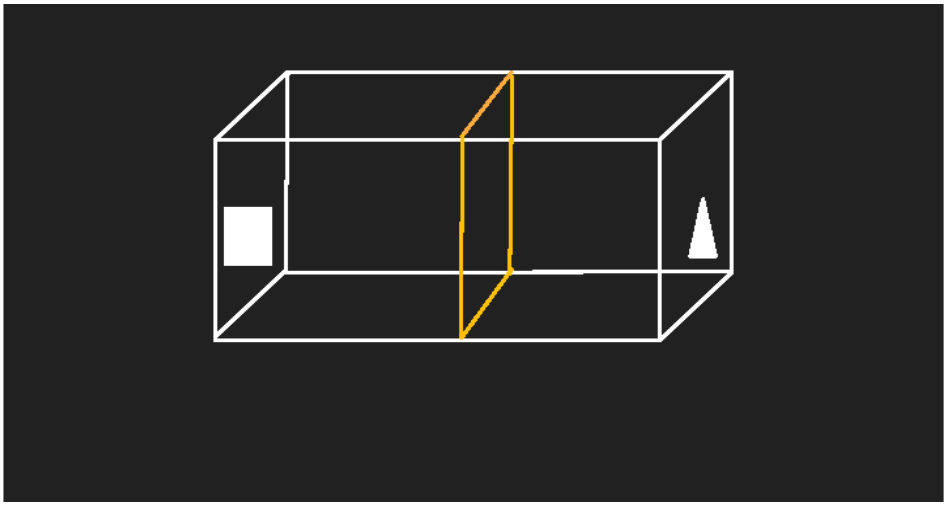

Figure 4. An illustration of the chamber used.

3.8 Handling

Rats received handling before the habituation process and before every phase of the trial, as a way to reduce any negative stress response (49). All rats received approximately 2 minutes of handling in Day O (Habituation), Day 1 (Trial 1), and Day 2 (Trial 2).

3.9 Habituation process for rat models

As it is known, habituation permits the decrease in neophobia which can affect locomotor activity of rats (28, 30). Therefore, each rat will be taken from their cages and placed, by itself, in the respective chamber with the plexis-glass placed for 5 minutes, followed by the removal of the plexiglass for the last 5 minutes.

Total duration of habituation: 10 minutes

3.9.1 PHASES OF TRIAL

The following phases are followed in the same chronological order as 1,2,3, and finally 4.

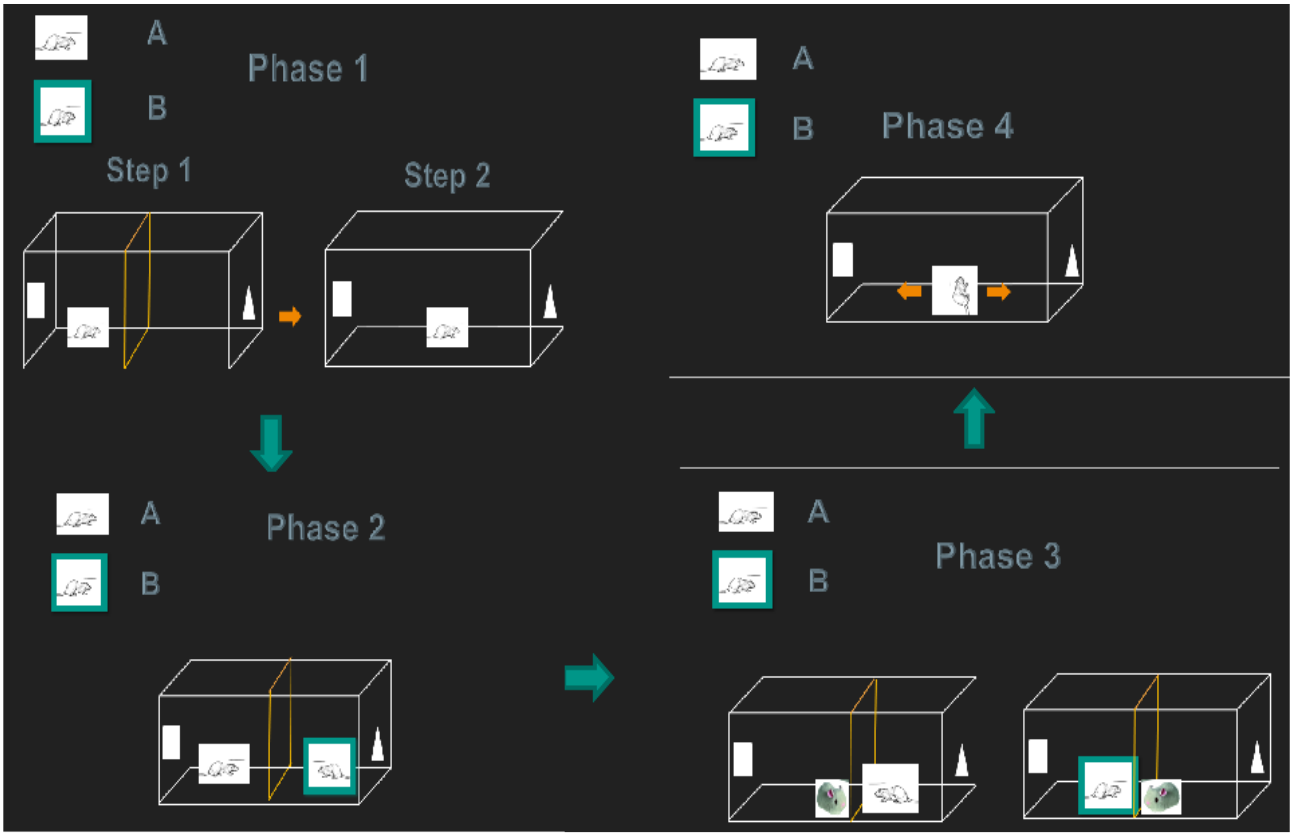

Figure 5**: This is an outline of the four-phase process carried out in this experiment for memory consolidated in a social context.**

Phase 1 (P1/Baseline)

As explained previously in definition of a trial, this was a baseline phase to compare against the final phase 4. The plexiglass was placed first, followed by the respective rat that corresponds to side A(Square)/B(Triangle), placed on its side of the chamber with its respective symbol. The plexiglass was placed for 5 minutes. After the 5-minute period ended, the plexiglass was removed, leaving the chamber without the plexiglass for the remaining 5 minutes. Here, latency to zone of interaction and zone of none interaction, was measured, followed by cumulative time and frequency in both zones. Only the last 5 minutes, with the removal of the plexiglass, was used for analysis. See Figure 5.

Total duration of phase 1: 10 minutes

Phase 2 (P2)

This phase was considered the phase of interaction/learning, introducing both rats corresponding to their categorization of A and B. A plexiglass was inserted into the middle of the chamber. A rat corresponding to side A was placed on its respective side (Square), and a rat corresponding to side B was placed on its respective side (Triangle). Here, latency to zone of interaction and zone of none interaction, was measured, followed by cumulative time and frequency in both zones. This phase lasted approximately 5 minutes. See Figure 5.

Total duration of phase 2: 5 minutes

Phase 3 (P3)

As previously stated, this phase was used as a comparison to understand social place preference between the novice object and conspecific. A novel object (in-animated rat) was placed on side A, close to the plexiglass, with the square in the background, and Rat A placed on Side B (triangle). This phase lasted approximately 5 minutes. Rat A and the Novice object were removed, and Rat B was placed on Rat A´s side (square) of the chamber and the novel object placed on Rat B´s side, with the triangle in the background. Here, latency to zone of interaction and zone of none interaction, was measured, followed by cumulative time and frequency in both zones. This phase lasted approximately 5 minutes. See Figure 5.

Total duration of phase 3: 5 minutes

Phase 4 (P4)

The final phase, as previously mentioned, was used as a comparison against phase 1, to understand if a memory of social memory of the conspecific was consolidated and the strength of motivation from this memory observed in social place preference. At the beginning of this phase, the plexiglass was removed. A rat corresponding to A(Square) or B(Triangle) was placed in the middle of the chamber, facing the wall which did not visual cues. This phase lasted exactly 5 minutes. See Figure 5.

Total duration of phase 4: 5 minutes

3.9.2 Additional Analysis

For this behavioral paradigm, to further understand how social memory of a familiar conspecific affects, social interaction in terms of social place preference, with the new conspecific (58), the social preference-avoidance test by Nestler (29)was used for groups R and S, on the third day after the MCSC behavioral test; here the original variables of latency at first to given area of social interaction, cumulative time in area of social interaction, and frequency in and out of area of social interaction, were measured (29). For this behavioral experiment, habituation was also carried out before starting, 10 minutes. All 12 rats were individually paired with each one of the three older rats.

5-minute session for each rat, 1 hour in total.

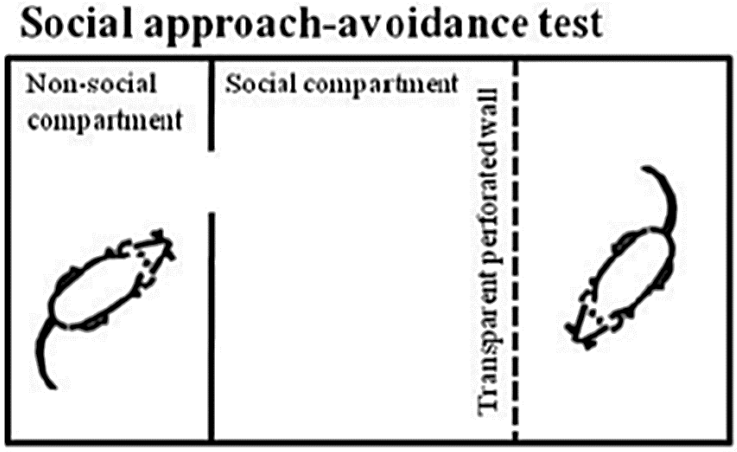

Figure 6. This is the social approach-avoidance test that is used as an additional conduct test.

3.9.3 Software Used

EthoVision XT (Noldus, Wageningen, Netherlands) with a Web Logitech Brio Ultra HD Pro (Logitech, U.S.A), were used to record this social interaction as in fear memory consolidation. *GraphPad Prism 8*(GraphPad Software Inc) *was* used to carry out the statistical analysis for this experiment.

3.9.4 Statistical Analysis

First in foremost, to determine whether to use parametric or non-parametric tests, two criteria were considered in this. The first criteria were the D´agostino and pearrson test and the Shapiro Wilks normality test which showed mixed results (See Appendice C), as for the D´agostino and pearson test, the N=6 for the morning group, and N=6 for afternoon group were too small. The second and final criteria that was used, was a QQ plot; in many cases, for a series of data through the experiment, the tail ends were skewed, amongst great variability among data (see Appendice C). For the aforementioned analysis of the data, it was decided that there was a non-normal distribution, therefore the use of non-parametric tests would be ideal. Considering the data was tested over more than three means, analyzing individual variables such as latency, cumulative time, and frequency over a period of trials, the Freidman Anova test was used for P1 vs P4, P2 vs P3, and the Non INZ and INZ. In terms of P2 vs Social, a Wilcoxon test was used to compare the data among two means. Additionally, a post hoc test of Dunn´s was used to compare difference among specific means where significance was found. Finally, an analysis of correlation among dominant vs subordinate and subordinate vs subordinate, in latency, cumulative time, and frequency was done using the spearman test for correlation. The threshold used was $p<0.05$. For more details, see Appendice D. for all data.

IV. RESULTS

The following results that are shown are based on the variables of analysis, latency, cumulative time, and frequency, among all 4 phases of MCSM and the social approach avoidance test, followed by the correlation results for hierarchy.

4.1 Non interaction vs Interaction Zones

4.1.2 Latency to first (non-interaction vs interaction zones) P1-Baseline

It can be observed in Figure 7. A) that latency differed between Non INZ and INZ from T1 to T2, however there was no significant difference. (p= 0.7715). Figure 7. B) shows the behavior from Baseline of non-interaction (T1) to baseline interaction zone (T2). It can be observed in Figure 7.C) that latency differed between Non INZ and INZ from T1 to T2, and there was a significant difference overall (p= 0.0137) (details in, Append. D p.88). Figure 7. D) shows the behavior from Baseline of non-interaction (T1) to baseline interaction zone (T2). For Zone Details (See Append. A)

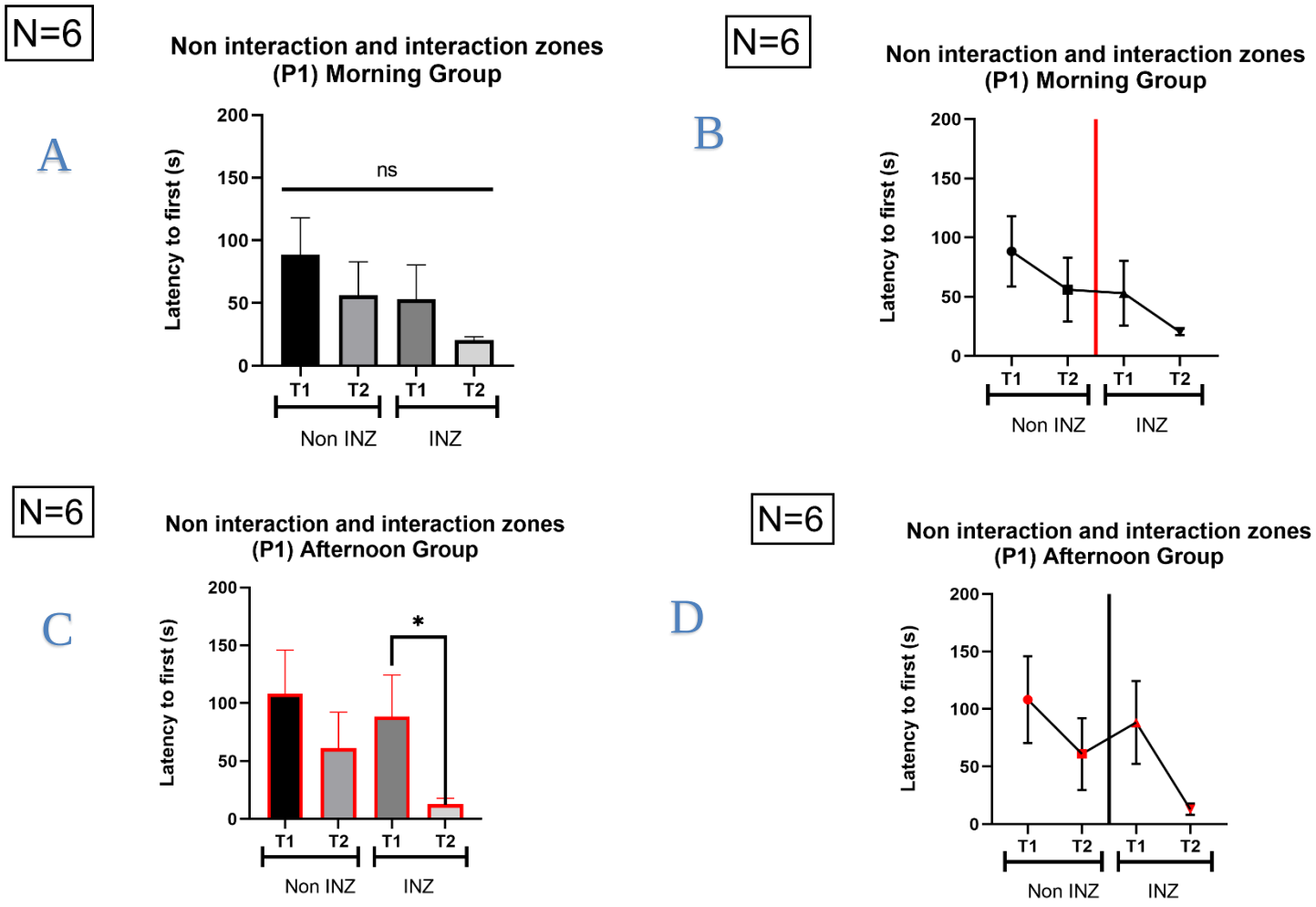

Figure 7: Latency to first (none interaction and interaction zones) A) Latency to first, for morning group (T1 to T2). B) Behavior of latency of morning group over trials (T1 to T2). **C)** Latency to first, for afternoon group (T1 to T2). **D)** Behavior of latency of afternoon group over trials (T1 to T2).

4.1.3 Cumulative time spent in (non-interaction vs interaction zones) *(P1)*

It can be seen, in figure 8. A) that Cumulative time, for the morning group, for latency, differed between Non INZ and INZ from T1 to T2. It can be observed, in Figure 8.B) that cumulative time, for the afternoon group, differed between Non INZ and INZ from T1 to T2. There were no significant differences for both figures A) (p= 0.6172) and B) (p=0.9396).

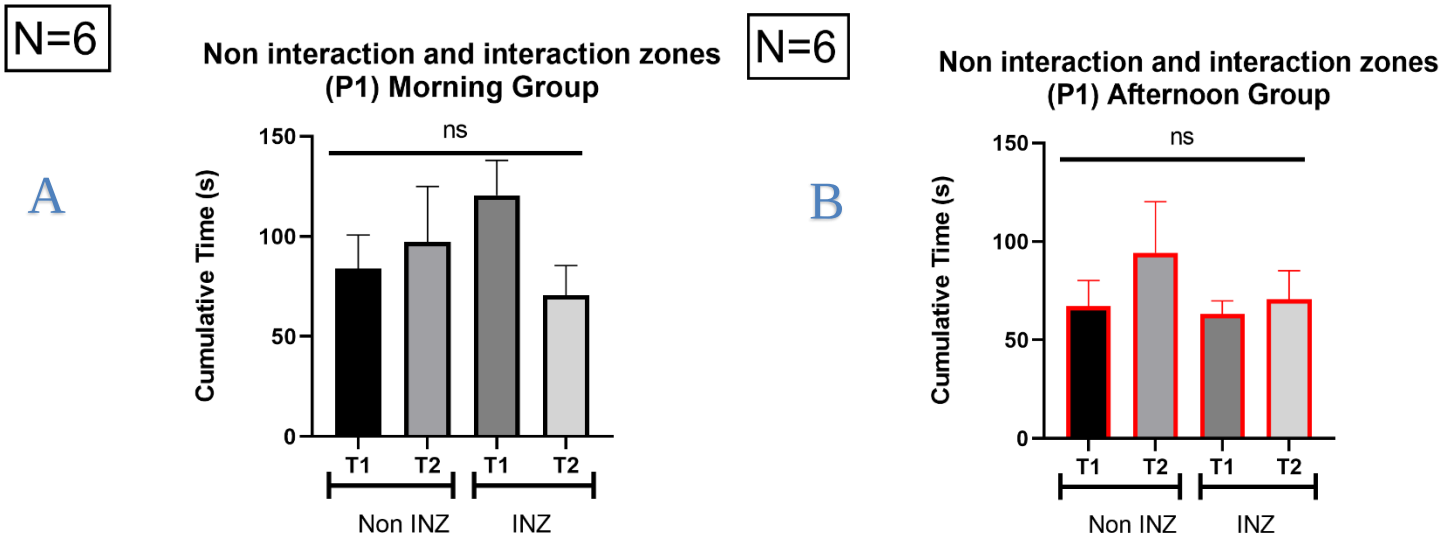

Figure 8: Cumulative time (in non-interaction and interaction zones) A) Cumulative time in non-interaction and interaction zones, for morning group (T1 to T2). **B)** Cumulative time in non-interaction and interaction zones, for afternoon group (T1 to T2)

4.1.4 Frequency into (non-interaction vs interaction zones*) (P1)*

It can be observed in Figure 9. A) that frequency, for the morning group, differed between Non INZ and INZ from T1 to T2. There was a significant difference (p= 0.0034). In figure 9. B) Frequency, for the afternoon group, latency differed between Non INZ and INZ from T1 to T2. There was a significant difference (p= 0.0020). See details in, Append. D (p.89)

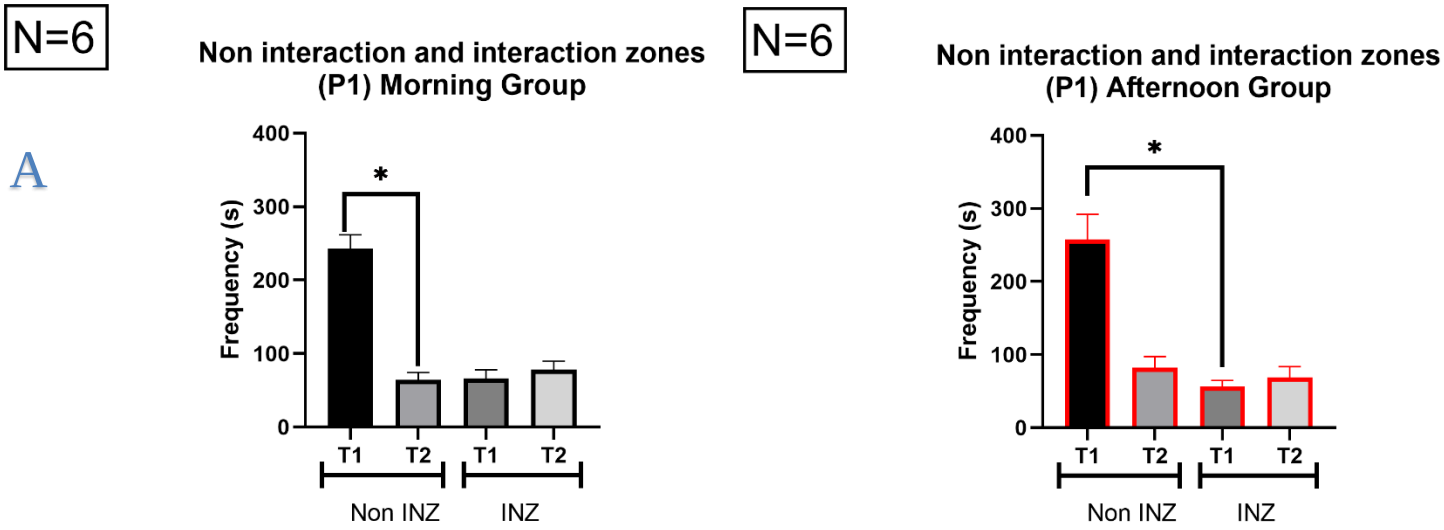

Figure 9: Frequency (into non-interaction and interaction zones) A) Frequency into non-interaction and interaction zones, for morning group (T1 to T2). **B)** Frequency into non-interaction and interaction zones, for afternoon group (T1 to T2)

4.1.4 Latency to first (non-interaction vs interaction zones) *(P4)*

It can be seen in figure 10. A) that latency, for the morning group, differed between Non INZ and INZ from T1 to T2. There was no significant difference (p= 0.7715). It can be observed, in Figure 10 B) that latency, for the afternoon group, differed between Non INZ and INZ from T1 to T2. There was no significant difference (p= 0.4307).

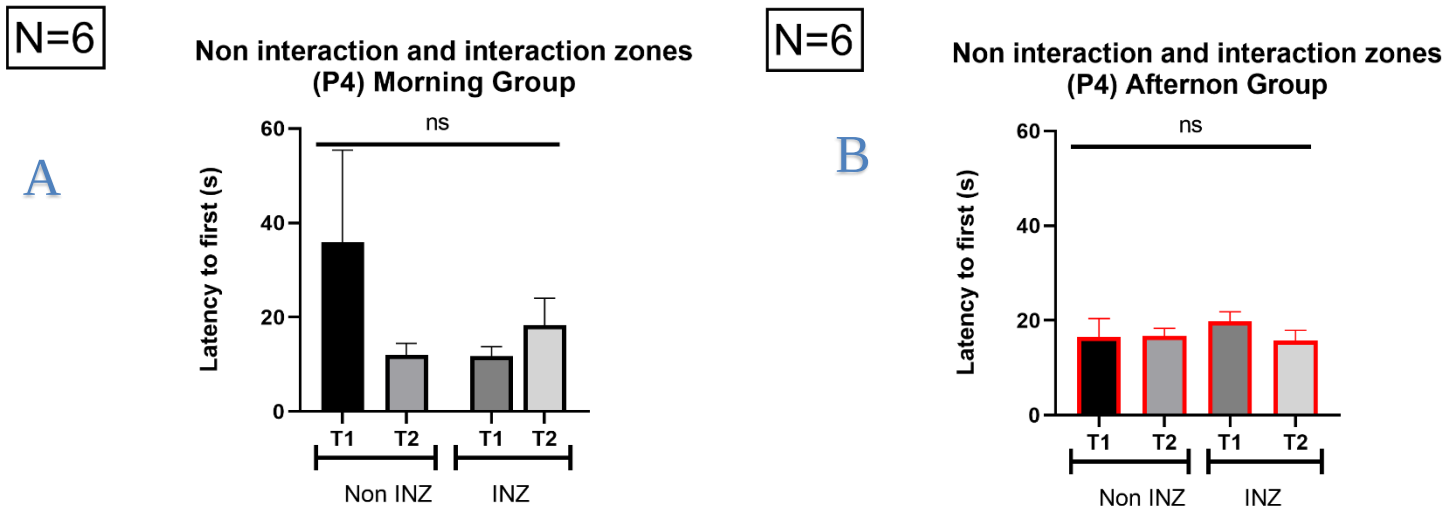

Figure 10: Latency to first (none interaction and interaction zones) A) Latency to first, for morning group (T1 to T2). **B)** Latency to first, for afternoon group (T1 to T2).

4.1.5 Cumulative time spent in (non-interaction vs interaction zones) *(P4)*

It can be observed, in figure 11. A) cumulative time, for the morning group, differed between Non INZ and INZ from T1 to T2. There was no significant difference (p= 0.4307). It can be seen, in figure 11. B) Cumulative time, for the afternoon group, differed between Non INZ and INZ from T1 to T2. There was no significant difference (p= 0.7715)

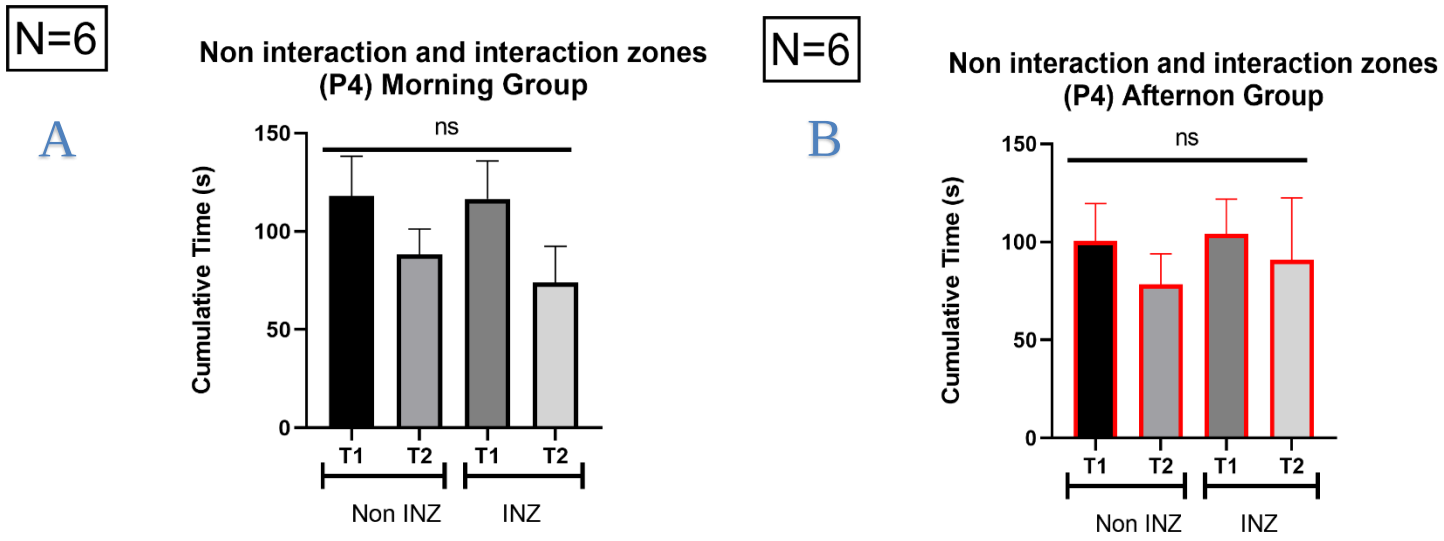

Figure 11: Cumulative time (in non-interaction and interaction zones) A) Cumulative time in non-interaction and interaction zones, for morning group (T1 to T2). **B)** Cumulative time in non-interaction and interaction zones, for afternoon group (T1 to T2)

4.1.6 Frequency into (non-interaction vs interaction zones) *(P4)*

It can be seen in figure 12. A) that frequency, for the morning group, differed between Non INZ and INZ from T1 to T2. There was no significant difference (p= 0.7723). It can be seen, in figure 12. B) that frequency, for the afternoon group, differed between Non INZ and INZ from T1 to T2. There was no significant difference (p= 0.8386).

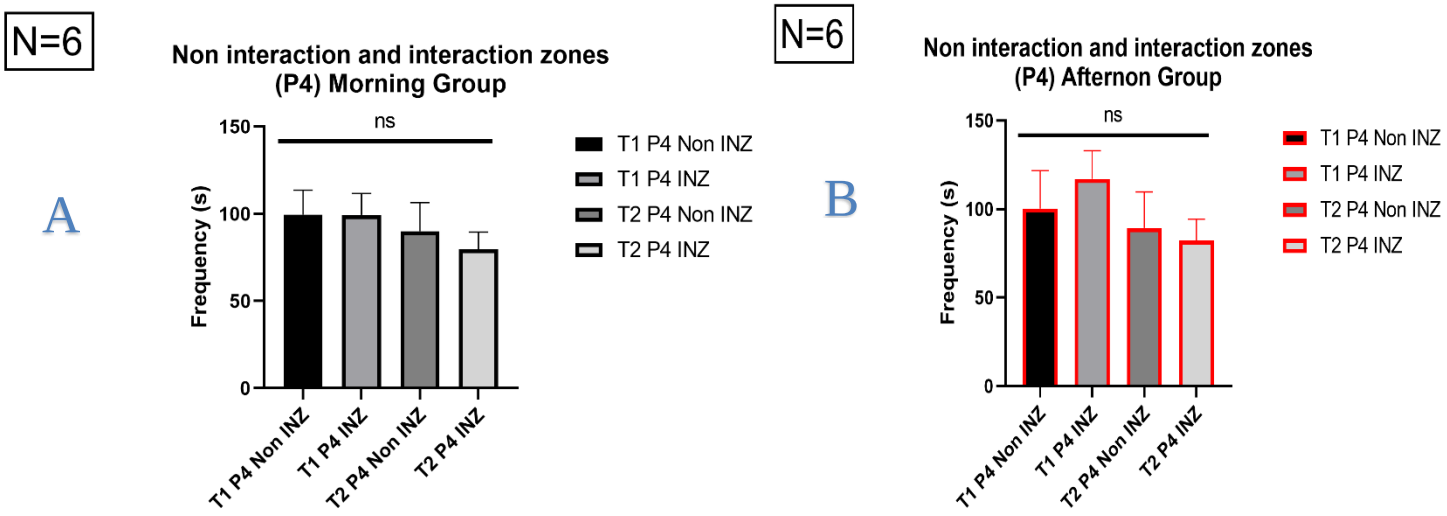

Figure 12: Frequency (into non-interaction and interaction zones) A) Frequency into non-interaction and interaction zones, for morning group (T1 to T2). **B)** Frequency into non-interaction and interaction zones, for afternoon group (T1 to T2)

4.2 Phase 2 (Conspecific interaction) vs Phase 3 (Novice Object Interaction)

4.2.1 Latency to first (P2 vs P3)

As can be seen in figure 13. A) latency, for the morning group, differed between Non INZ and INZ from T1 to T2, however there was no significant difference among means (p= 0.4753). As can be observed, in figure 13. B) latency, for the afternoon group, differed between Non INZ and INZ from T1 to T2, however with no significant difference among means (p=0.1585). See details in Append. D (p. 105).

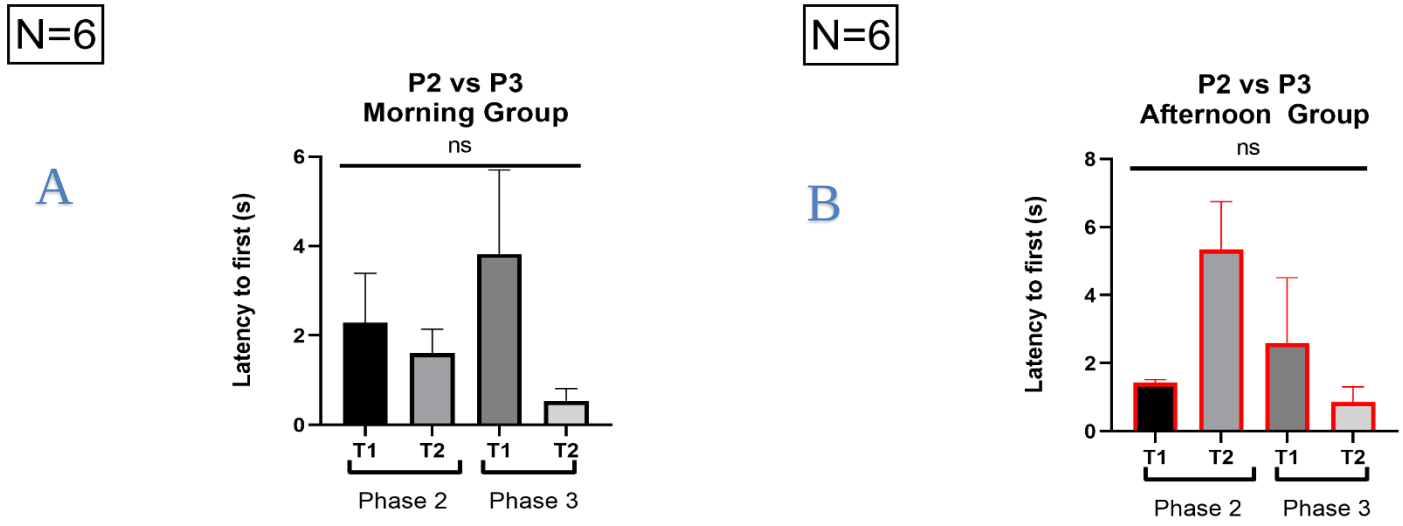

Figure 13: Latency to first (area of social interaction) A) Latency to first, for morning group (T1 to T2). **B)** Latency to first, for afternoon group (T1 to T2).

4.2.2 Cumulative time spent (in the area of social interaction) *(P2 vs P3)*

It can be observed in figure 14. A) that cumulative time, for the morning group, differed between Non INZ and INZ from T1 to T2, however there was a significant difference (p= 0.0057); for more details, see Append. D (p.106). It can be seen, in figure 14. B) that cumulative time, for the afternoon group, differed between Non INZ and INZ from T1 to T2, however, there was no significant difference (p= 0.1555).

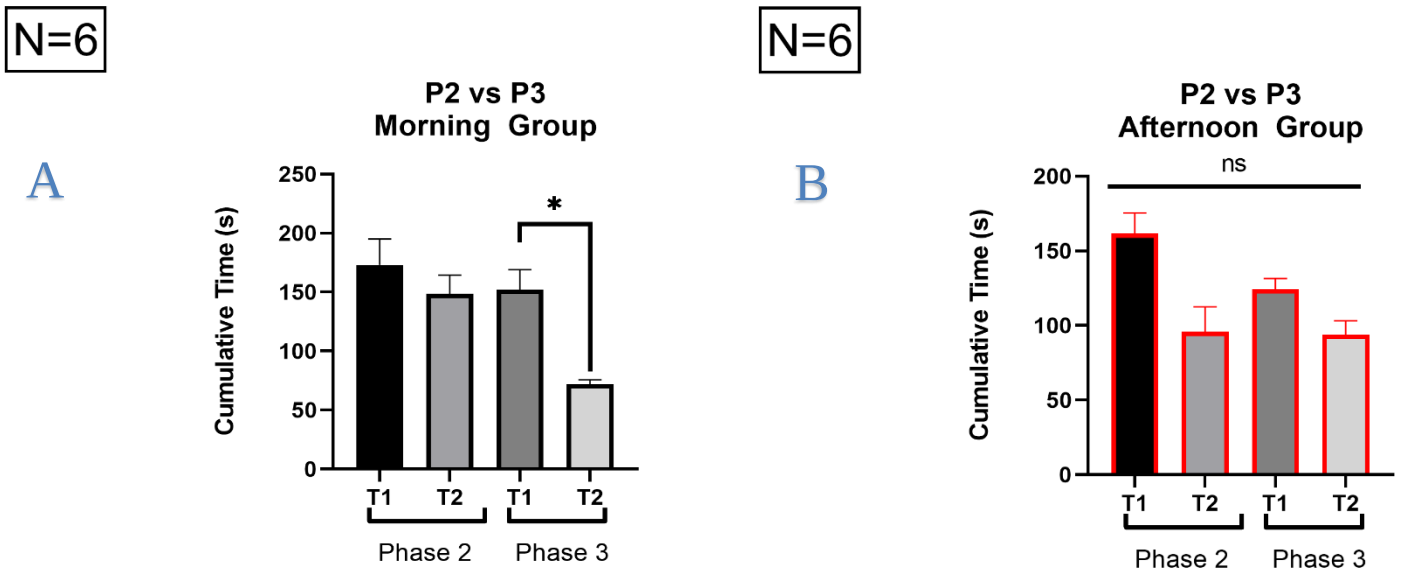

Figure 14: Cumulative time spent (in the area of social interaction) A) Cumulative time spent in area of social interaction, (T1 to T2), for the morning group. **B)** Cumulative time spent in the area of social interaction, from T1 to T2, for the evening group.

4.2.3 Frequency into area of social interaction *(P2 vs P3)*

It can be observed in figure 15. A) that frequency, for the morning group, differed between Non INZ and INZ from T1 to T2; there was no significant difference (p= 0.0951). It can be seen, in figure 15. B) that frequency, for the afternoon group, differed between Non INZ and INZ from T1 to T2; there was no significant difference (p= 0.6329).

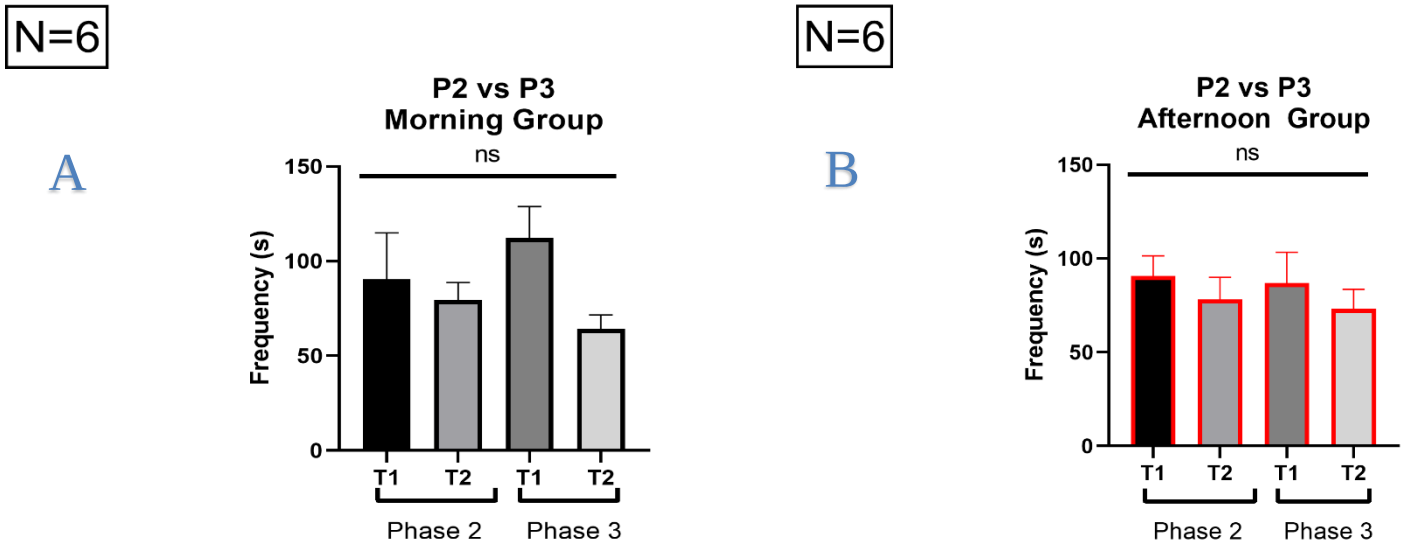

Figure 15: Frequency into the area of social interaction A) Frequency into area of social interaction, from T1 to T2 for the morning group. **B)** Frequency into area of social interaction, from T1 to T2 for the evening group.

4.3 Phase 1 vs Phase 4

4.3.1 Latency to first (P1 vs P4)

It can be seen in figure 16. A) that latency, for the morning group, differed between Non INZ and INZ from T1 to T2, however, there was no significant difference (p= 0.0959). Figure 16. B) shows the behavior of latency of figure 16. A) from T1 to T2. In figure 16. C) latency, for the afternoon group, differed between Non INZ and INZ from T1 to T2, however, there was a significant difference (p= 0.0412). For more details, see Append. D (p.114). Figure 16. D) shows the behavior of latency of figure 16. C) from T1 to T2.

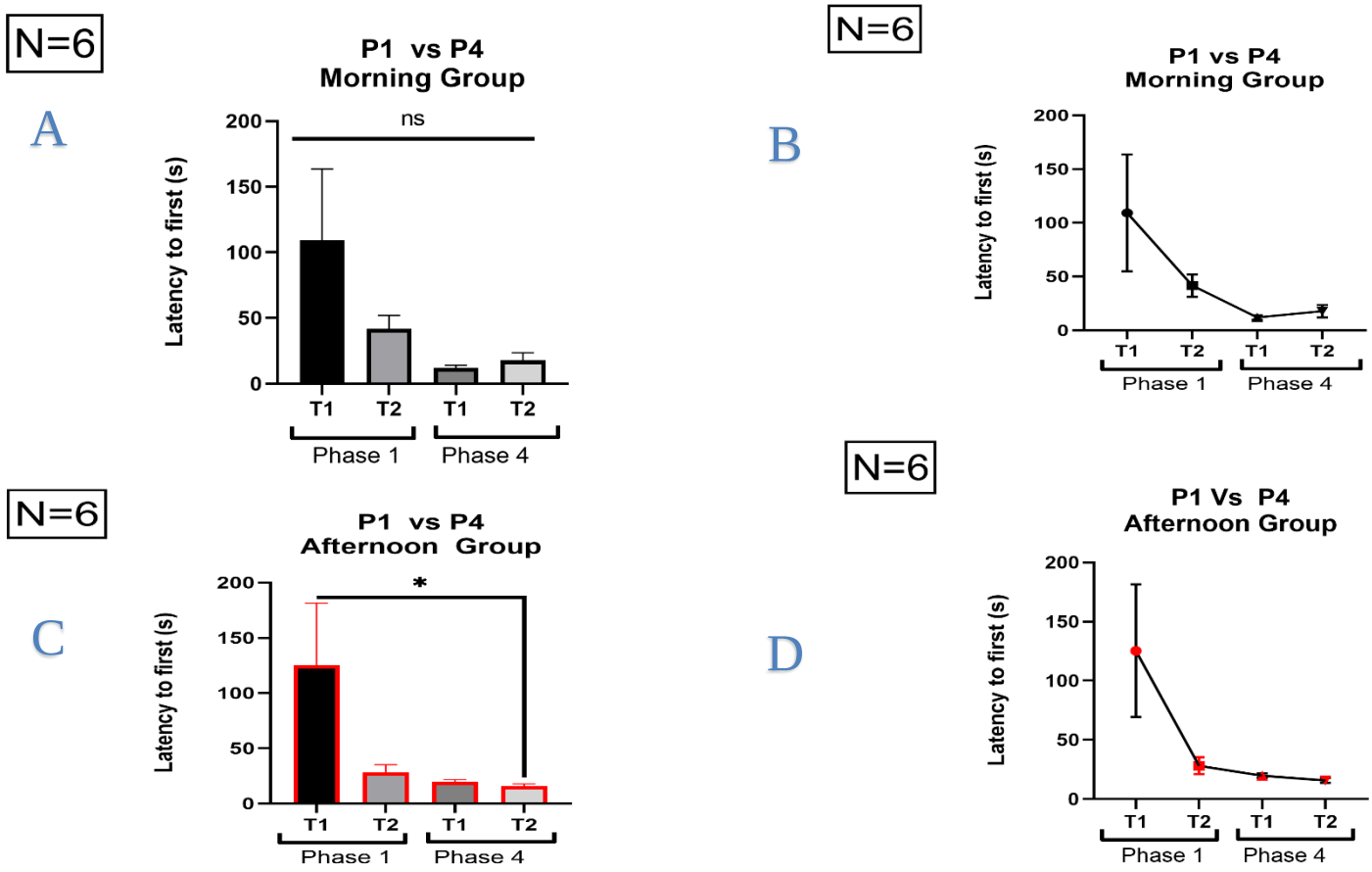

Figure 16: Latency to first (none interaction and interaction zones) A) Latency to first, for morning group (T1 to T2**). B)** Behavior of latency of morning group over trials (T1 to T2). **C)** Latency to first, for afternoon group (T1 to T2). **D)** Behavior of latency of afternoon group over trials (T1 to T2).

4.3.2 Cumulative time spent (in the area of social interaction) *(P1 vs P4)*

It can be seen in figure 17. A) that cumulative time, for the morning group, differed between Non INZ and INZ from T1 to T2, however, there was no significant difference (p= 0.3171). It can be observed, in figure 17. B) that cumulative time, for the afternoon group, differed between Non INZ and INZ from T1 to T2, however, there was no significant difference (p= 0.3751).

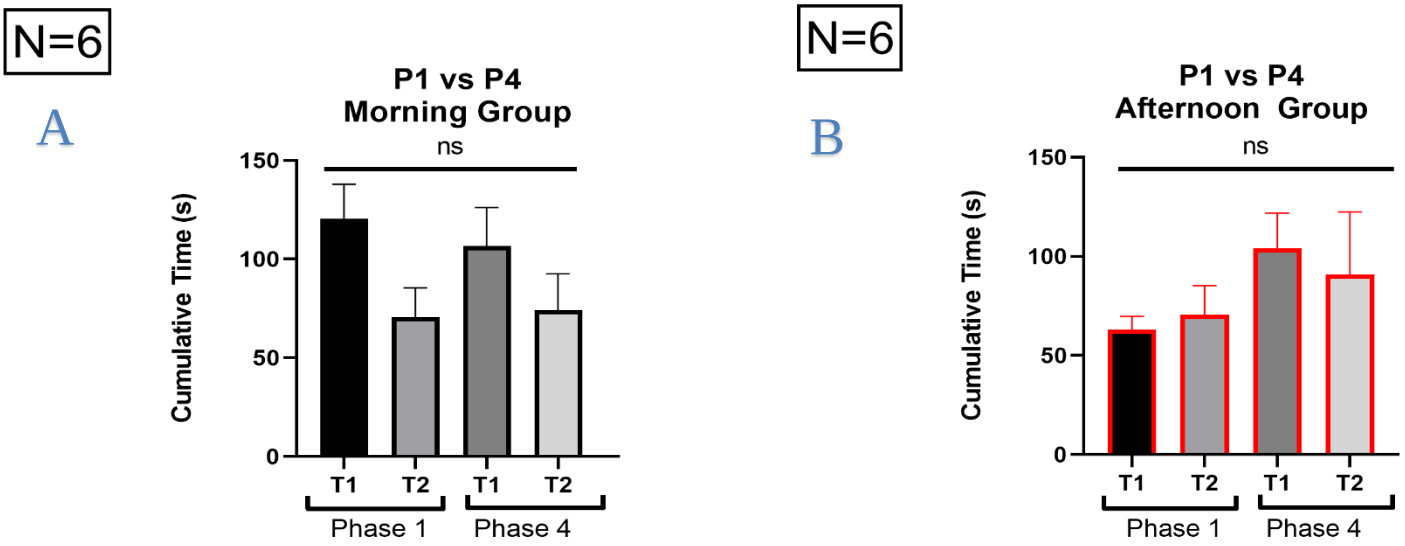

Figure 17: Cumulative time spent (in the area of social interaction) A) Cumulative time spent in area of social interaction, from T1 to T2, for the morning group. **B)** Cumulative time spent in the area of social interaction, fromT1 to T2, for the evening group.

4.3.3 Frequency into area of social interaction *(P1 vs P4)*

It can be observed in figure 18. A) that frequency, for the morning group, differed between Non INZ and INZ from T1 to T2, however there was no significant difference (P= 0.4418). It can be seen, in figure 18. B) that frequency, for the afternoon group, differed between Non INZ and INZ from T1 to T2, however there was no significant difference (p= 0.8386).

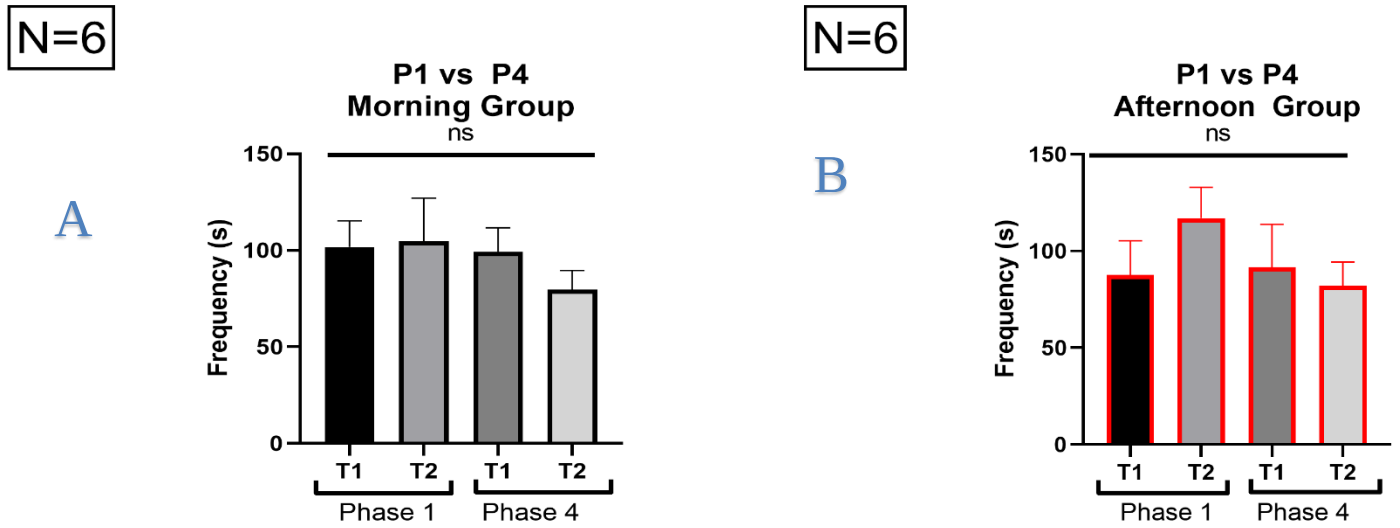

Figure 18: Frequency into the area of social interaction A) Frequency into area of social interaction, from T1 to T2, for the morning group. **B)** Frequency into area of social interaction, from baseline of T1 to T2, for the evening group.

4.4 Correlation of Hierarchy (Dominant and Subordinate, Subordinate and Subordinate)

4.4.1 Correlation of Latency of P4

Figure 19. A) shows the correlation between dominant and subordinate rats, for latency for P4, to area of social interaction, for morning and afternoon rats. There was no significant correlation (p= 0.4279, R= -0.3333). Figure 19. B) shows the correlation between subordinate and subordinate rats, for latency for P4, to area of social interaction, for morning and afternoon rats. There was no significant correlation (p= 0.7500, R= 0.4000).

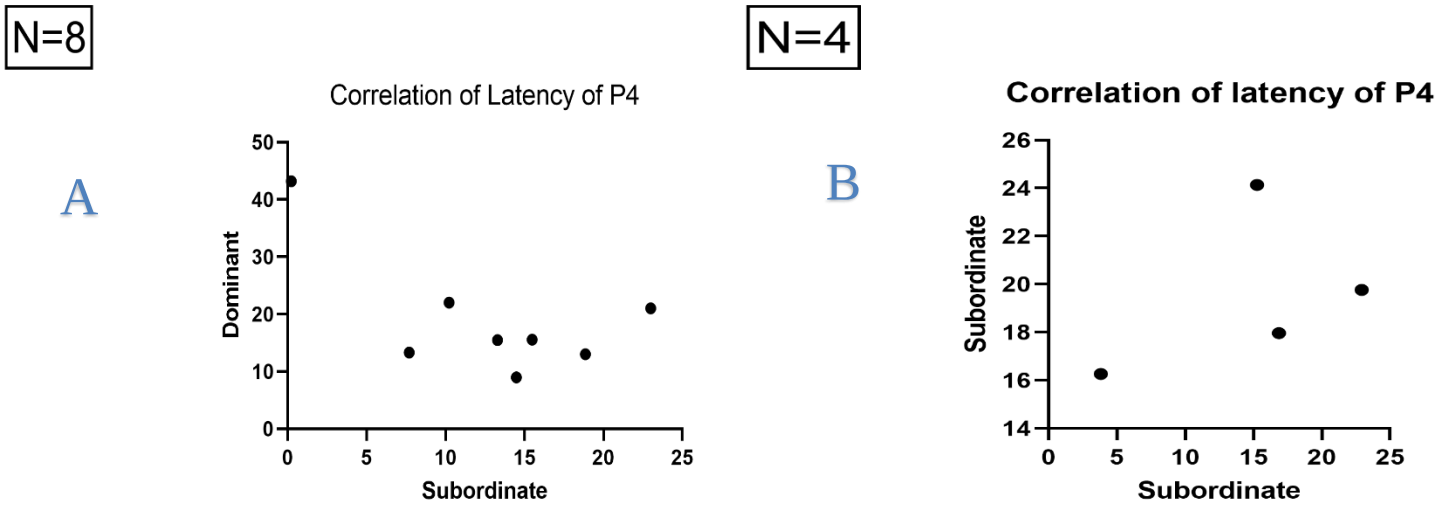

Figure 19: Correlation of Latency of P4 A) shows the correlation of latency of P4, to an area of social interaction, between dominant and subordinate rats, for morning and afternoon groups. **B)** shows the correlation of latency of P4, to an area of social interaction, between subordinate and subordinate rats, for morning and afternoon groups.

4.4.2 Correlation of Cumulative time of P4

Figure 20. A) shows the correlation between dominant and subordinate rats, for cumulative time of P4, in area of social interaction, for morning and afternoon rats. There was no significant correlation (p= 0.5008, R= -0.2857). Figure 20. B) shows the correlation between subordinate and subordinate rats, for cumulative time, in area of social interaction, for morning and afternoon rats. There was no significant correlation (p= 0.3333, R= -0.7778).

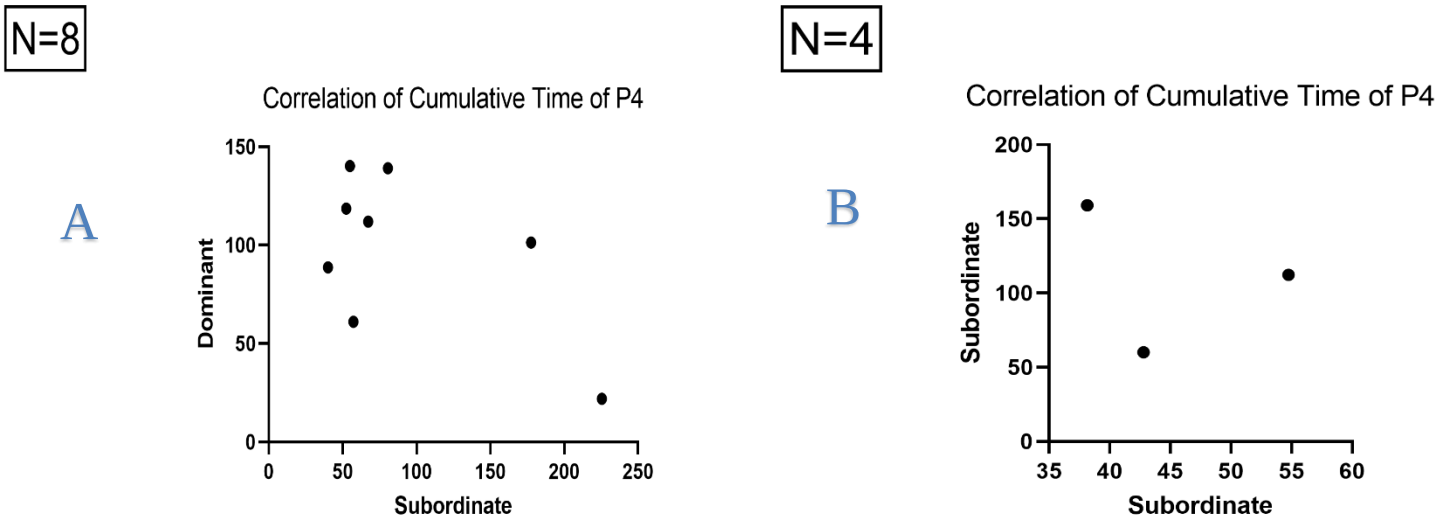

Figure 20: Correlation of Cumulative Time of P4 A) shows the correlation of cumulative time of P4 in an area of social interaction, between dominant and subordinate rats, for morning and afternoon groups. **B)** shows the correlation of cumulative time of P4 in an area of social interaction, between subordinate and subordinate rats, for morning and afternoon groups.

4.4.3 Correlation of Frequency of P4

Figure 21. A) shows the correlation between dominant and subordinate rats, for frequency of P4, into an area of social interaction, for morning and afternoon rats. There was no significant correlation (p= 0.1967, R= 0.5150). Figure 21. B) shows the correlation between subordinate and subordinate rats, for frequency, into area of social interaction, for morning and afternoon rats. There was no significant correlation (p= 0.9167, R= 0.2000).

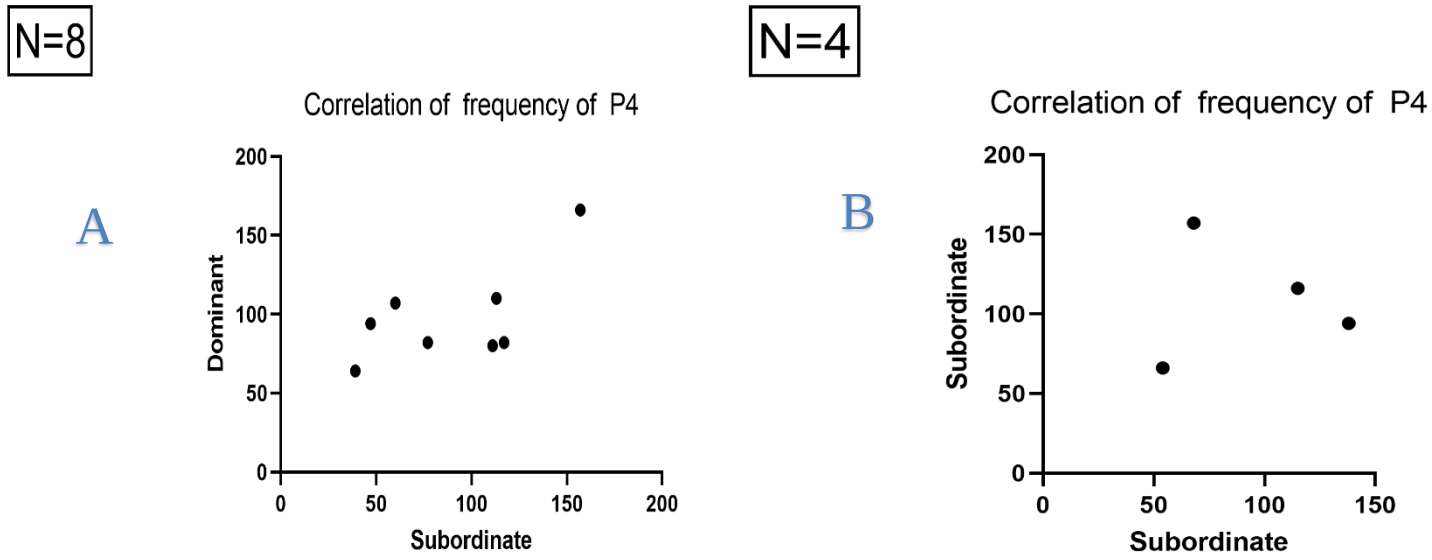

Figure 21: Correlation of Cumulative Time of P4 A) shows the correlation of frequency of P4, into an area of social interaction, between dominant and subordinate rats, for morning and afternoon groups. **B)** shows the correlation of frequency of P4, into an area of social interaction, between subordinate and subordinate rats, for morning and afternoon groups.

4.5 Phase 2 vs Social approach avoidance test

4.5.1 Latency to first *(P2 and Social)*

It can be observed in figure 22. A) that latency, for the morning group, differed between P2T1M and Social T1M; there was no significant difference (P= 0.2500). It can be seen in figure 22. B) that for latency, for the afternoon group, differed between P2T1A and Social T1A; there was no significant difference (p= 0.2500)

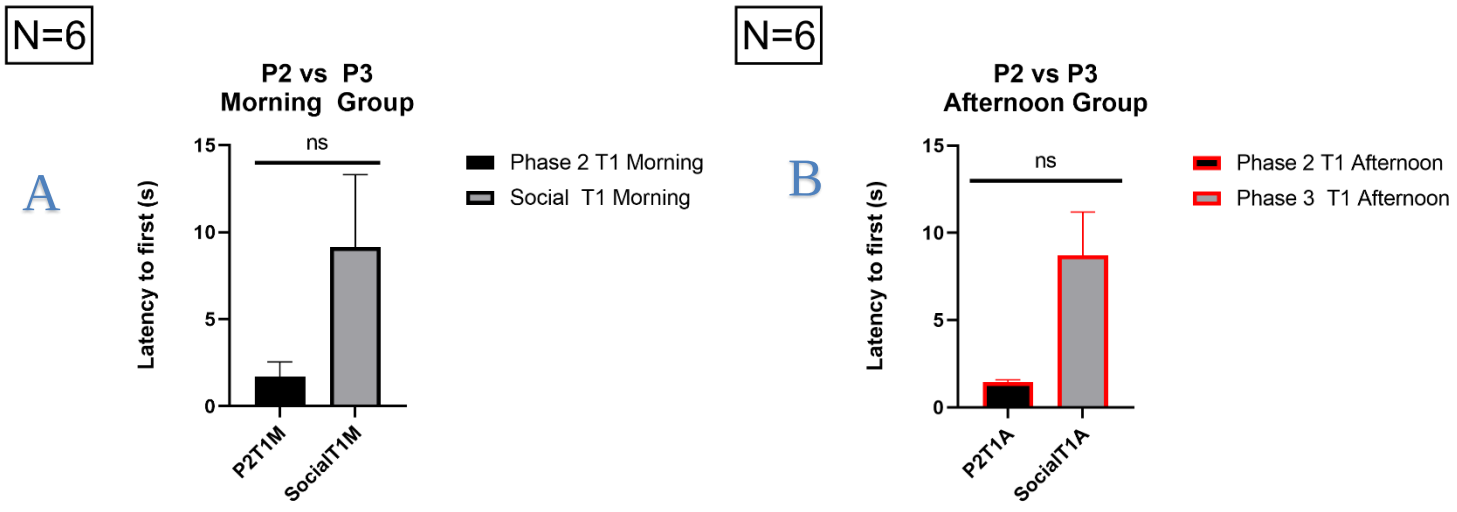

Figure 22: Latency to first (area of social interaction) A) Latency to first (the area of social interaction), in the first trial, for the morning group. **B)** Latency to first (the area of social interaction), in the first trial, for the evening group.

4.5.2 Cumulative time spent (in the area of social interaction) *(P2 and Social)*

It can be observed in figure 23. A) that cumulative time, for the morning group, differed between P2T1M and Social T1M; there is was significant difference (p= 0.9999). It can be seen in figure 23. B) that cumulative time, for the afternoon group, differed between P2T1A and Social T1A; there was no significant difference (p= 0.1563)

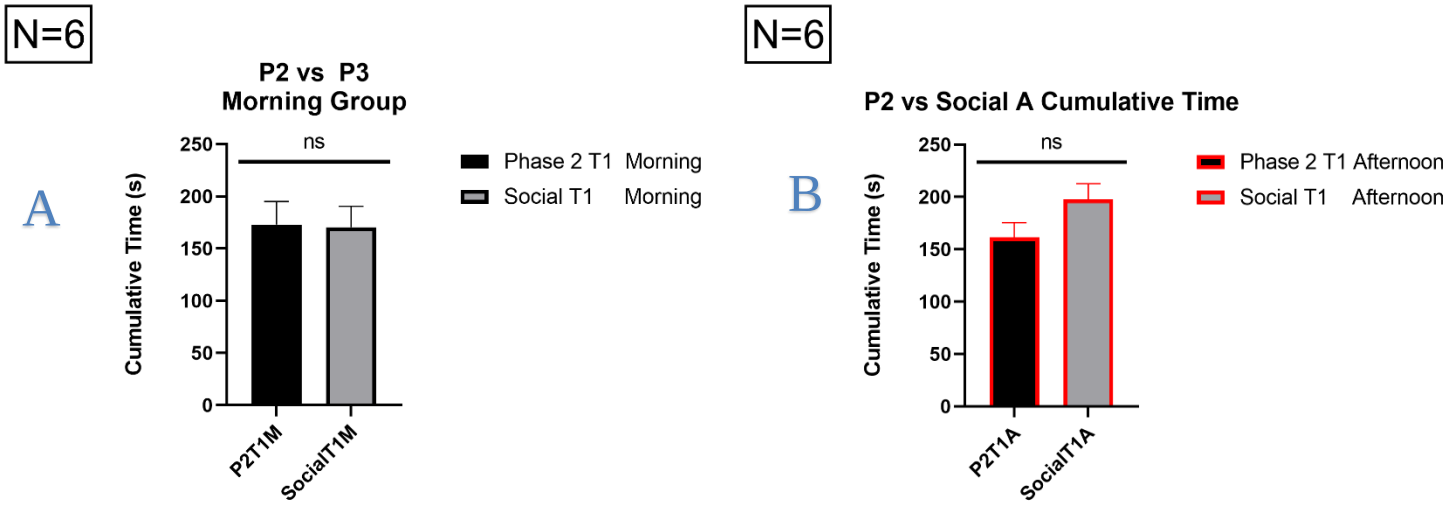

Figure 23: Cumulative time spent (in the area of social interaction) A) Cumulative time spent in area of social interaction, in the first trial, for the morning group. **B)** Cumulative time spent in the area of social interaction, in the first trial, for the evening group.

4.5.3 Frequency into area of social interaction *(P2 and Social)*

It can be seen in figure 24. A) that frequency, for the morning group, differed between P2T1M and Social T1M. There was no significant difference (p= 0.0938). It can be observed in figure 24. B) that cumulative time, for the afternoon group, differed between P2T1A and Social T1A. There was significant difference (p= 0.0313). See Append. D (p. 141)

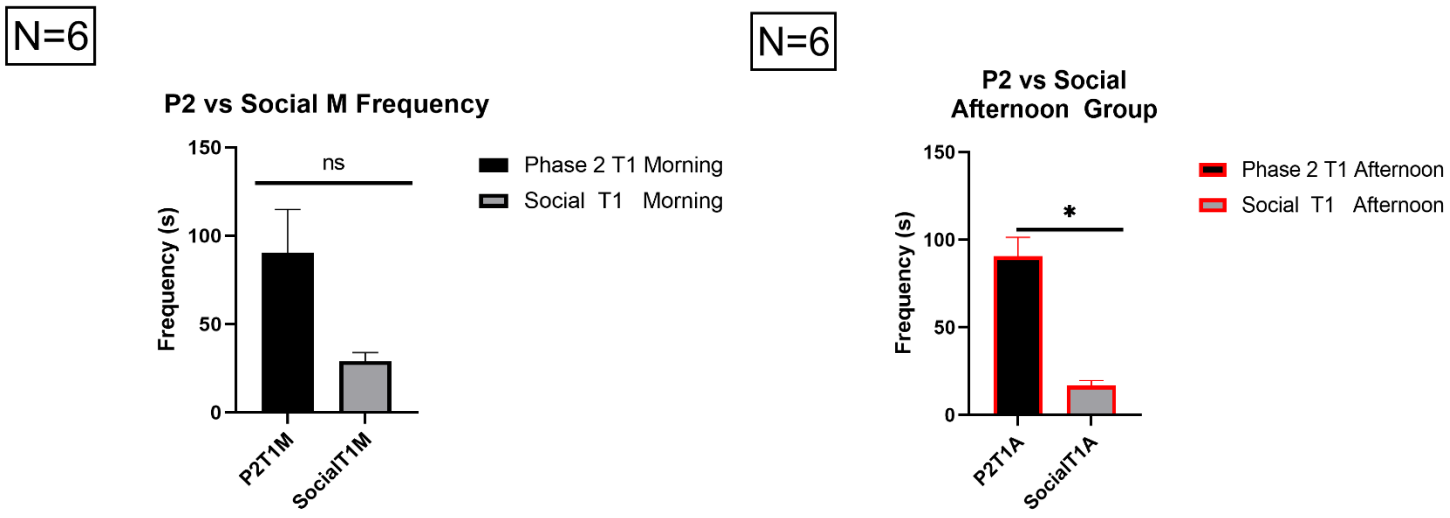

Figure 19: Frequency into the area of social interaction A) Frequency into area of social interaction, in first and only trial, for the morning group. **B)** Frequency into area of social interaction, in first and only trial, for the evening group.

V. DISCUSSION

As previously stated, the focus of this study was to understand, at the behavioral level, how memory is consolidated in a social context. Therefore, based on the aforementioned question, hypothesis, and objectives planted, the following discusses in detail, the findings.

5.1 Non interaction vs Interaction Zones

Learning and memory consolidation can be observed by a change in behavior (35). With that stated, reiterating, it is important to take into consideration that all rats were previously habituated to reduce neophobia (28,30) and looking to eliminate any external variables that could influence behavior, a day before the experiment started, all aforementioned in methods. Initially for non-interaction and interaction zones of P1, from T1 to T2, there is a visual trend ***latency*** that decreases, represented by figures ***(***fig 7.B & fig 7.D). It is important here to look specifically at the non-interaction zone and interaction zones of T2, as this was the baseline for day 2 before a new social interaction, as in T1, they had already had a previous social interaction (P2 and P3), hence it gives an understanding of latency after 24 hours of a previous social interaction. However, in a statistical evaluation, only one group presents statistical relevance (figure 7. C), which could be explained by two scenarios. As latency was described previously as a variable for memory recall of the social interaction (25,26), it is important to take into consideration diurnal rhythm cortisol, or cortisone in rodents, which peaks at the moment of early morning hours until around mid-day (60,61). It is evidenced that memory retrieval is partly impaired or affected in a negative way during periods of higher levels of cortisol (61). As there is also evidence to state that glucocorticoids decrease from 50% to 10% from AM to PM hours, which although, mineralcorticoids, which prevent overstimulation of glucocorticoids, are present throughout the day, there is evidence that suggests memory enhancement is usually better in the PM hours than that of AM (60). This first scenario could explain why the latency wasn´t as significant for the morning group, as that of the afternoon group. The second scenario could have something to do with Type 1 Errors (See Append. B, Type 1 Errors), that was committed in the second day of trials for the morning group. Ultimately, if this error is relevant, then four of the subjects of the morning could have been affected, which shows in their ability to recall the familiar conspecific interaction.

Analyzing **latency** of P4, although there is no significant difference, statistically in either the morning, nor afternoon groups (figure 10. A, figure 10. B), both have inverse behaviors. This result could possibly be explained by the previous two scenarios for the morning group (Figure 10. A), while the evening group doesn´t show statistical significance (Figure 10. B), this possibly could be remedied if more trials could be carried out in a future experiment, as most Pavlovian conditioned approaches use 3 or more trials. By a series of social interactions in the same context, it is possible a social memory engram could be better consolidated (58).

Cumulative time was aforementioned as a variable that indicates social preference of place, hence motivation for having or having had a social interaction. Analyzing P1 and P4(figures 8.A and B, figures 11. A and B), for both morning and afternoon groups, as there is no significant difference, it can be assumed that there was a lack of motivation, to remain in the area of social interaction of the conspecific, not necessarily having a place of social preference. Rats, such as in humans, weigh in on the prosocial benefits (36). Here, there was a similar behavior in terms of ***frequency*** into the zone of social interaction for P4 (see figure 12.A & figure 12.B.), while for P1 there is a significant difference (figure 8.A & figure B). The result of P1 could be explained by the fact that the significant difference has to do with the first day before its social interaction, for why it has more frequency into the non-interaction zone; future trials would be required to see the baseline in day three and possible four.

5.2 Phase 2(Conspecific) vs Phase 3(Novice Object)

Latency, although not significant for morning nor afternoon groups (figure 13.A, figure 13.B) , in this context, considering it was a constant interaction with a conspecific and a new object, it can not necessarily be correlated with memory recall; additionally, both morning and afternoon groups have inverse behaviors in latency (figure 13.A, figure 13.B). The explanation for the morning group, could be the Type I Error committed in the second day, which sparked their interest in interacting with a new conspecific, as rodents have a natural tendency to interact with unfamiliar stimuli (37), hence the decrease in latency. For the afternoon group, it could be that there is little reciprocity in the interactions amongst pairs during the interactions, which is reflected in long latency.

Cumulative time in the area of social interaction, which was at the plexiglass, was considered a variable to analyze the place of social preference, similar to other investigations carried out in substance preference (11). For the morning group, there was a significant difference (figure 14.A); this of course, indicated that the morning group spent most of the time in the area of social interaction with the conspecific in comparison with the novice object; this could be due to the Task I Error, or simply that the interaction was more fruitful for the morning group. Conversely, the afternoon group did not have a significant difference (figure 14.B); Regardless, there seems to be a downward trend for both groups, in cumulative time, possibly referring to a lack there of, motivation, reflected by cumulative time for place of social preference. At the neurocircuit level, the dmPFC, BLA, and NAC (39) and its implications in motivation, could possibly explain the behavior that is being observed during P2 vs P3. As specifically, the NAC which receives modulation from the CA1, is involved in social discriminatory behaviors such as avoidance and sexual preference (56) (see Appendice C); nevertheless, as rats just as in humans, not all interactions are as pleasurable or remain as pleasurable over time, which in part is dependent on prosocial benefit evaluation (36); this could possibly explain why there is significant difference in one group and not the other.

In this context, ***frequency*** into the area of social interaction, which was at the plexiglass, was also considered a variable in motivation (37), hence place of social preference. Regardless, there was no significant difference for both groups (figures 15.A figure 15.B), however there is a downward trend of frequency for both groups from T1 to T2. In part, this result could be explained by an indifference between the novice object and the conspecific or also the experimental design. In this experiment design, a flaw which could have affected the new object learning phase, is that the novice object was placed directly at the plexiglass; in the case of the conspecific, it was permitted to move freely away from the plexiglass; this could have affected the values of latency, cumulative time, and frequency negatively. This possibly can be remedied by resigning new zones and parameters, in Ethovision, that permit calculating and quantifying the latency, cumulative time, and frequency only when both rats (one being the conspecific) are at the plexiglass.

5.3 Phase 1 vs Phase 4

In this context, ***latency*** indicated memory recall. Additionally, a previous study highlighted a decrease in latency for positive rewarded stimulus after learning (38). While there was no significant difference in the morning group, for latency to first (figure 16.A), there is a downward trend of latency from T1 to T2(figure 16.B). Conversely, for the afternoon group, there is a significant difference in latency (figure 16. C), followed by a parallel downtrend in latency (figure 16.D); this is also in accordance with latency to interaction zone, from P1 (figure 7. C & D); the results possibly could be explained by, first, for the morning group by diurnal rhythm cortisone, as levels could have been higher, as previously explained, this could have affected in memory recall (61), or possibly Type I Errors. On the other hand, for the afternoon group, the significance could possibly be due to a better social memory engram consolidation in CA2 of the hippocampus based on vasopressin release from the paraventricular nucleus into CA2 (59,56), of which the paraventricular nucleus of the hypothalamus is modulated by the AM (59), which could be a better perceived connection to the conspecific from the dmPFC which in turn modulates the AM (18,19). Furthermore, as evidence has strongly shown the implication in the VTAs´ outputs to the HP, generating long term potentiation's for long term memory (40), the functional connectivity that exists among the dmPFC, BLA, NAC, VTA, and HP (see Appendix C), this could possibly explain the quick decision making based on long term memory from the HP which in turn could modulate the NAC for this social discriminatory behavior (56) and the dmPFC from which outputs to the BLA,VTA, generating a neuronal loop that ultimately affects the PFC in discerning within milliseconds to seconds where to go based on memory recall. Possibly, a better behavioral test, such as that of a T Maze with two chambers, one for the conspecific, and the other for the novice object, with levers to open the chambers for interaction, could be a more appropriate task to determine memory consolidation of the experience based on an associative task, similar to that of incentive salience to social cues (58).

In this context, ***cumulative time*** indicated motivation, mainly, apart from memory recall. As was observed (figures 17 A & 17 B), there was no significant difference for the morning group or afternoon group. The result of this could be due to indifference between the place of social preference of the conspecific and its own place of social preference with the novice object. Another aspect as previously mentioned, is reciprocal behavior (36) which

was not monitored in this occasion; a possible reciprocal behavior could be nose sniffing, as it has been evidenced that the modulation of both dmPFC and vmPFC affects frequency of nose sniffing which is an indicator of motivation(41) in reward associated behavior. Furthermore, it is known that natural rewards in memory, generally have a faster extinction rate in comparison with synthetic substances (41), which could explain the variability amongst groups in terms of motivation.

In this context, ***frequency*** indicated motivation, hence preference of place. As observed (see figures 18.A & 18.B), there was no significant difference for the morning and afternoon groups. This result is probably due to an inconsistent motivational desire to continue looking for the conspecific, or this behavior could change if more trials were present; this variable, similar to that of cumulative time, could be a good indicator of motivation as previously stated for other non-aversive learning paradigms (37). A possible solution to the aforementioned, is by increasing the number of trials, such as those done, fear memory experiments, (8), 3-4 days. By increasing the number of trials, possibly we will see an even further decrease in latency and an increase in cumulative time and /or frequency. Finally, it could be helpful to study other behaviors that affect this mechanism of social memory, such as behaviors of sniffing (37) or reeling which might indicate aspects of reciprocity (37), appealing to the conspecifics empathy, thus generating a better social interaction which would be a reflection in motivation by an increase in cumulative time and/or frequency.

5.4 Correlation of Hierarchy (Dominant and Subordinate, Subordinate and Subordinate)

First and foremost, as mentioned in methods in classification of hierarchy, both morning and afternoon groups had the equal amount of dominant and subordinate rats. As shown in the results for **latency** of phase 4(figure 19. A, figure 19. B), there is no significant correlation for morning, nor for afternoon groups. In this case, latency represented memory consolidation of the social interaction; this possibly could imply, if there were a correlation, that these memories consolidated in a social context could possibly depend on hierarchy. In the case of dominant vs subordinate, if there were a positive correlation, it could imply that for memory recall, a co-factor such as a perceived threat from the dominant rat by the subordinate, is needed for memory consolidation, making reference to the AM and hypothalamic function in social fear memory consolidation (62). If there were a negative correlation, this would imply impaired memory, memory for one of the groups. Another explanation of this result,

as there was not enough sufficient subordinate vs subordinate for the afternoon group, nor that of dominant vs subordinate for morning or afternoon, both morning and afternoon groups were grouped together. For a better analysis of latency based on memory consolidation, there would need to be a greater N, and a better classification and organization of groups based on morning, afternoon, dominant, and subordinate.

For **cumulative time and frequency,** there was no significant correlation for morning and afternoon groups (figure 20.A, figure 20.B, figure 21. A, figure 21.B). This result, as it mainly indicated motivation through preference of place, a positive correlation would have implied a mutual reciprocity (36) between dominant and subordinate or subordinate and subordinate. On the other hand, a negative correlation would have implied avoidance for one group, while social preference of place for the other.

5.5 Phase 2 vs Social approach avoidance test

During the following behavioral paradigm, a habituation phase was carried out before the behavioral test. In this context, similar to that of P2 vs P3, ***latency*** more than memory recall as it was the first interaction, it was oriented towards that of motivation (37). As observed (figures 22.A and 22.B), with only a sample of 6 (See Appendice B), there was no significant difference in latency. This result can be explained based on the dimensions of the MCSC chamber (p.19) and the social approach-avoidance test (29). As the dimensions for the MCSC chamber were smaller, this could possibly explain this result, as it took less time to get to an area of social interaction. Another explanation could have to do with the fact that the three novice rats were originally from the other litters of R and S, separated at a young age, PND21; this could explain the longer latency getting to the point of social interaction, as these new conspecifics were actually familiars.

In this context, similar to that of P2 vs P3, **cumulative *time*** indicated a measure of motivation, hence place of social preference. As observed (see figures 23.A and 23.B), there is no significant difference for the morning group and afternoon group. This result possibly can be explained for the morning group by Type I Errors, which affected their novice interaction. For the afternoon group, possibly there was indifference to interact with a new conspecific based on motivational aspects. But for both groups, as previously mentioned, it

could have to do with the fact that the three novice rats were originally from their litters, separated at PND21.

In this context, like that of P2 vs P3, ***frequency*** indicated a measure of motivation. As observed (see figures 24.A and 24.B), the results are contradictory as the morning group, statistically were not significant, while the afternoon group was statistically significant. For the morning group, this result could be explained based on the Type I Error which affected novice interaction and the dimensions of the Social Approach Avoidance Test chamber. Furthermore, the afternoon group, the frequency in which its entrance into P2 area of social interaction is probably higher also due to the dimensions of the Social Approach avoidance test Chamber. A remedy to this situation could be to use the same chamber for the novice rats, MCSC, as for the morning and afternoon groups or change the setup as originally proposed in P1 non-interaction and interaction zones.

VI. CONCLUSION

In conclusion, this study was able to answer affirmatively, the initial question of, ***is it possible to study memory consolidated in a social context at the behavioral level.*** Furthermore, the objectives planted were able to be fulfilled. **Latency** was found to be a possibly important variable in understanding memory consolidation, as a modification in the design of the new paradigm and additional trials could effectively show that as latency decreases, it is an indication of memory consolidation of a social context. ***Cumulative time*** and ***frequency*** could be variables to answer the hypothesis of: ***memory consolidated in a social context is observed by social preference***. Nevertheless, without further trials, it is complicated to see if memory consolidated in a social context is hedonic or aversive, or simply, mixed, overtime. Moreover, in the last behavioral experiment which used the social avoidance preference test to understand how sociability is affected by previous consolidated memory in a social context or social memory engram, the results were inconclusive, as there was no strong data to indicate that sociability had improved or worsened based on the previous social interaction. Finally, a neuronal mechanism was proposed to explain the underpinnings of this particular behavior, as well as a new behavioral paradigm that serves the purpose of a new line of investigation to help understand the neurobiology of social memory. Understanding social memory could help in understanding specifically why stress response in social interaction is different for some individuals, such as those on the autistic

spectrum, as research has indicated, autistic individuals tend to have a different stress response during social interaction as in comparison with non-autistic individuals (55).

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VIII. APPENDICES

A. ZONE DETAILS FOR ALL EXPERIMENTS

This section shows images of the actual chambers that were used and the zones, explaining the set up to clarify.

Phase 1)

Figure EXT 1: In **A)** you can see the non-interaction zones and interaction zones which corresponds to RAT A. Both zones of none interaction were considered the zone of non-interaction, while both zones of interaction were considered the zone of interaction. From the aforementioned, the values of the variables, corresponding to latency, cumulative time, and frequency, to and in, both zones, were calculated. In Figure **B),** follows the same explanation of figure A, but with RAT B. It is important to remember that the first phase, before social interaction, it should only be clear to the rat that both sides are different based on the circle and triangle.

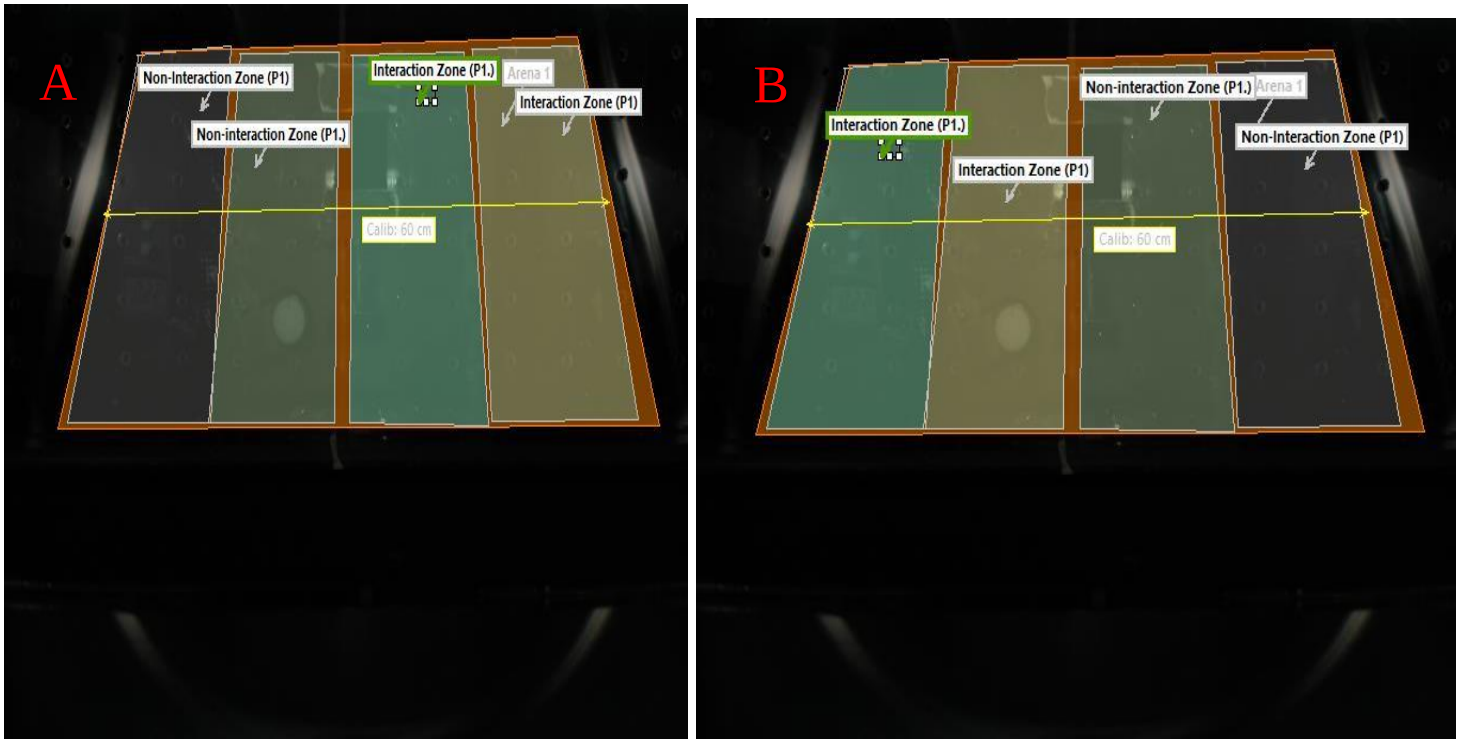


Figure EXT 1: A) This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT A. **B)** This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT B

Phase 2)

In Figure EXT 2: **C)** This side belongs to Rat A; it is divided into two zones, interaction zone which is the sector to the far right, in green, close to the plexiglass where rat A interacted with Rat B (conspecific). The non-interaction zone is in gray, to the far left, close to the Circle Image. **D)** This side belongs to Rat B; it is divided into two zones, interaction zone which is the sector to the far left, in green, close to the plexiglass where rat B interacted with Rat A (conspecific). The non-interaction zone is in gray, to the far right, close to the Triangle Image. In the non-interaction and interaction zones, the values of the variables of latency, cumulative time, and frequency were calculated.

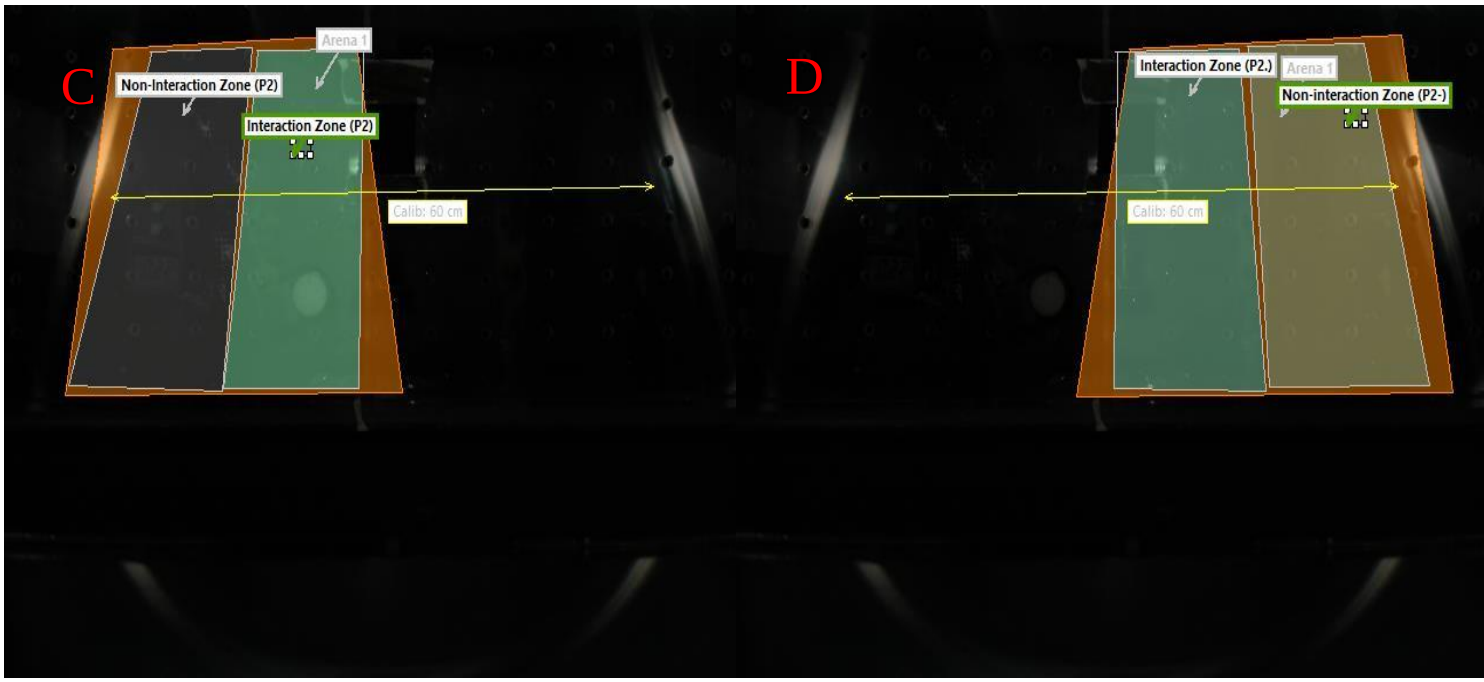


Figure EXT 2: C) This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT A. **D)** This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT B.

Phase 3)

In Figure EXT 3: E) Sides were switched; now this side belonged to Rat A, with the novice object on the other side with the Square. It is divided into two zones, interaction zone which is the sector to the far left, in green, close to the plexiglass where rat A interacted with the novice object. The non-interaction zone is in gray, to the far right, close to the Triangle Image. **F)** Sides were switched; now this side belonged to Rat B, with the novice object on the other side with the Triangle. It is divided into two zones, interaction zone which is the sector to the far right, in green, close to the plexiglass where rat B interacted with the novice object. The non-interaction zone is in gray, to the left right, close to the Square Image.

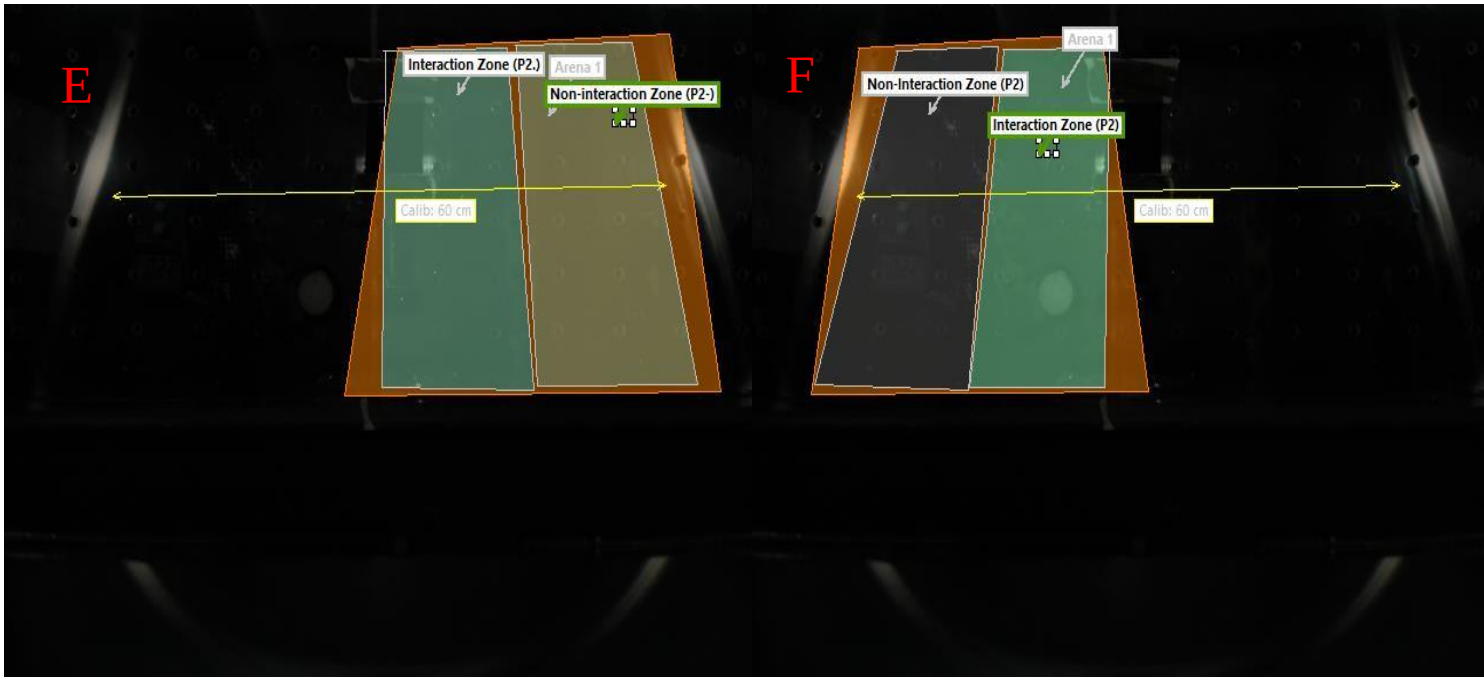


Figure EXT 3: E) This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT A. **F)** This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT B.

Phase 4)

Figure EXT 4: In **G)** you can see the non-interaction zones and interaction zones which corresponds to RAT A. Both zones of none interaction were considered the zone of non-interaction, while both zones of interaction were considered the zone of interaction. From the aforementioned, the values of the variables, corresponding to latency, cumulative time, and frequency, to and in, both zones, were calculated. In Figure **H),** follows the same explanation of figure A, but with RAT B. It is important to remember that this last phase was after social interaction with the conspecific(P2) and with the novice object (P3). The rat was placed facing the wall (vertical) without symbols (Square or Circle).

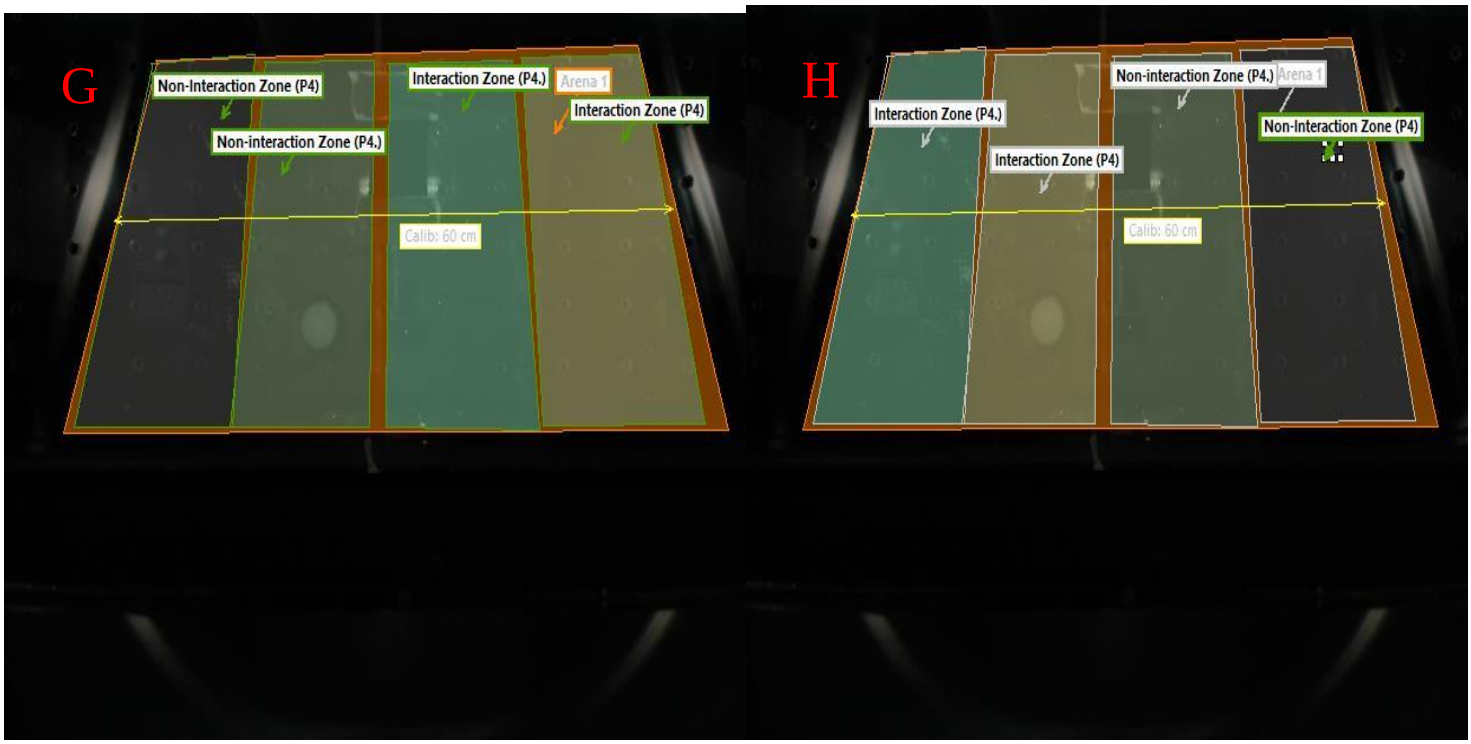


Figure EXT 1: G) This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT A. **H)** This figure shows the non-interaction and interaction zones (non-INZ and INZ) that correspond to RAT B.

Social Avoidance Preference Test)

Figure EXT 5: In figure **I)** The non interaction zone is represented, in green, to the far left, while the middle chamber, in purple, represents the interaction zone, and the chamber to the far left, represents the new conspecific that was placed in an enclosed caging. Latency to both zones were calculated, followed by cumulative time and frequency.

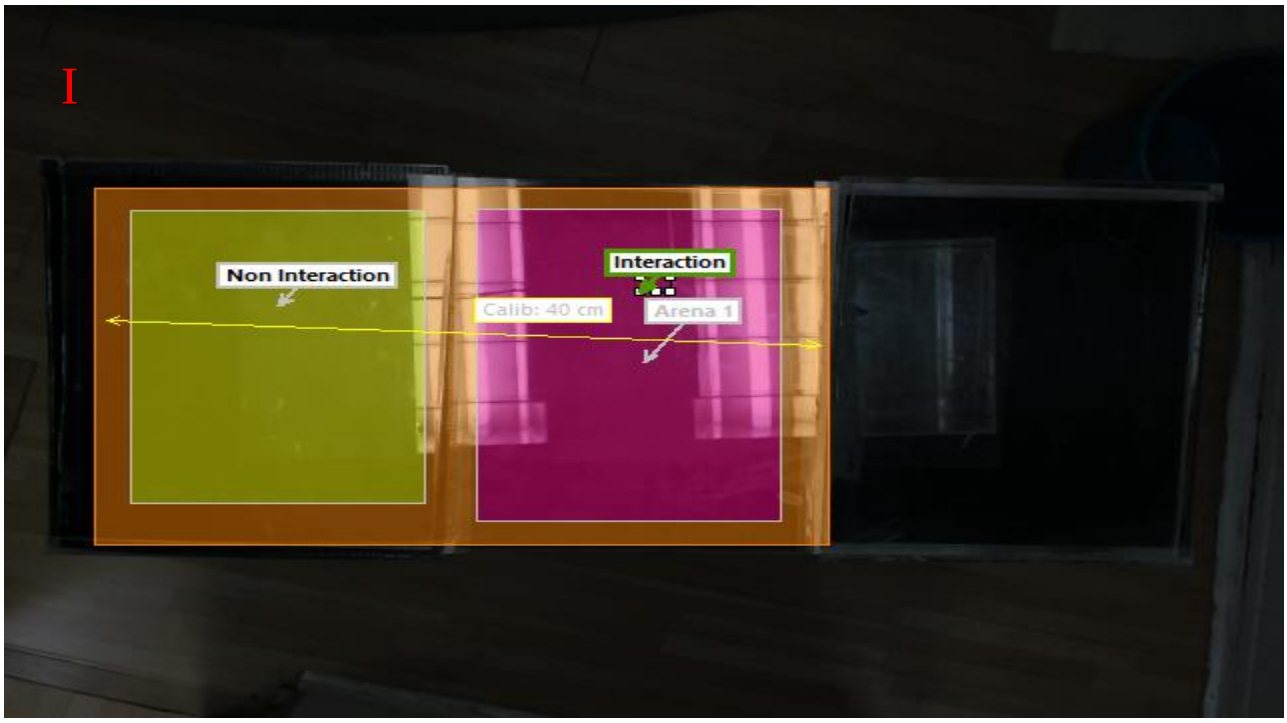


Figure EXT 5: I) This figure shows the non-interaction and interaction zone (non-INZ and INZ) that corresponds to RAT A and B (N=12).

B. EXPERIMENTAL ERRORS

B.1 TYPE 1 ERRORS

A critical error that could have affected the results for the morning group R, was the fact that R2 was not paired with R4 the second day, rather that R2 was paired accidently with R6. In consequence, as R6´s normal pair is R3, this could have also affected the Data of that of R3.

B.2 TYPE 2 ERRORS

4.2Phase 2 (Conspecific interaction) vs Phase 3 (Novice Object Interaction)

4.2.1 Latency to first

R1- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

S1- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

4.2 Phase 2 (Conspecific interaction) vs Phase 3 (Novice Object Interaction)

4.2.1 Latency to first (between *P2 and P3)*

S1- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

S2- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

4.4 *ΔS* Score of Phase 2 vs Social approach avoidance test

4.4.1 Latency to first *(ΔS* score of *P2 vs Social)*

R1- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

R3- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

R5- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

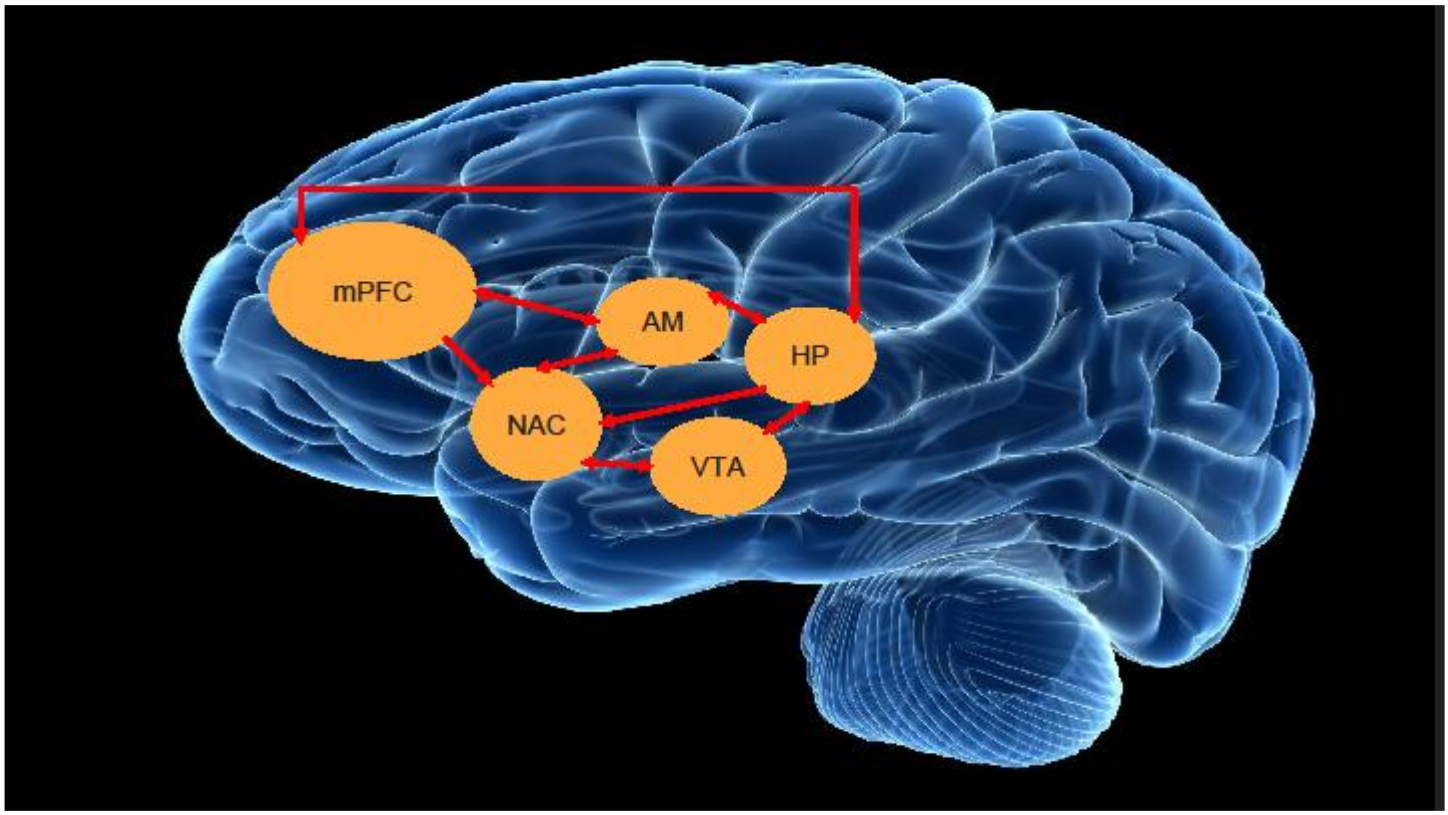
S1- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

S3- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

S5- This rat, during the Ethovision recording presented 0.0000 latency, possibly there was a misplacement of the rat or simply latency for some given reason was not calculated for this rat.

C. NEURONAL MECHANISM SCHEMATIC

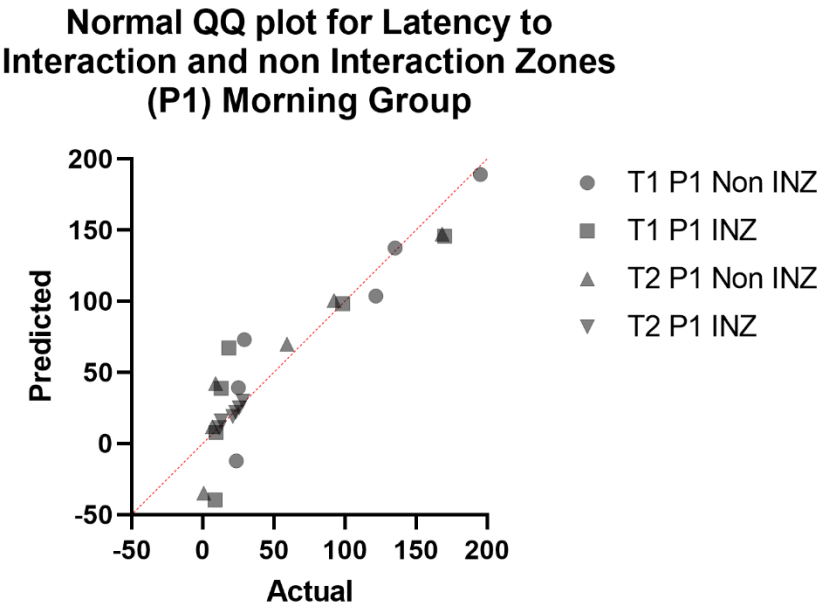
This behavior could be explained by the circuitry between the mPFC, BLA, NAC, VTA and HP; as the dmPFC plays a fundamental role in decision making (35) as it sends outputs to the AM from which the BLA modulates outputs to the NAC for association for positive vs negative valence (7,39). It can be assumed that the first day, within only minutes to an hour for each pair of rats during this social interaction, dmPFC down streams to the BLA glutamatergic projections which in turn sends its efferents to the NAC. As the NAC is made up of medium spiny neurons, GABAergic projections are sent to the VTA which is responsible for dopaminergic projections to the HP, generating Long Term Potentiations (LTPS) (40). As there are outputs from the ventral HP to the medial PFC and AM, these glutamatergic outputs also coincide with motivation based on the hedonic memory previous consolidated through a social interaction. Lastly, but not least, the importance specifically from CA1 of the hippocampus, there is modulation also of the NAC with glutamatergic projections, evidenced through social discriminatory behavior (58)


mPFC
AM
HP
NAC
VTA

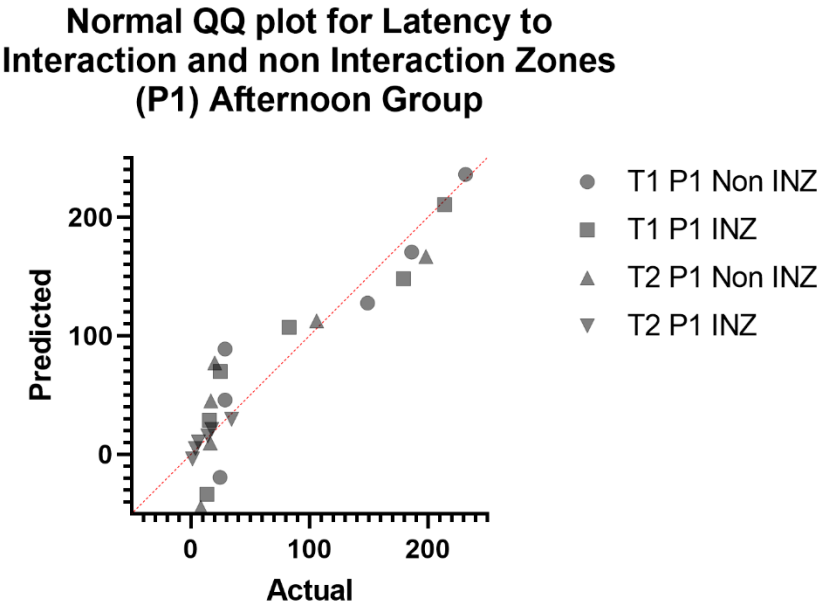
D. DATA

D.1 QQ PLOTS FOR NON-INTERACTION AND INTERACTION ZONES

N=6

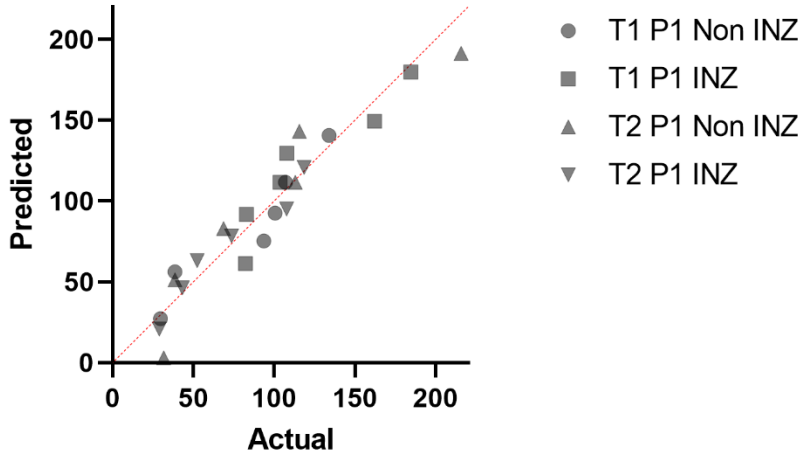


N=6



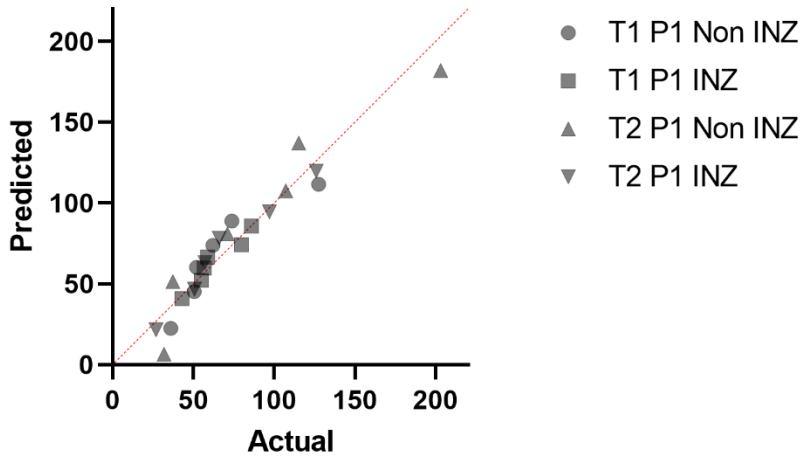
N=6

Normal QQ plot for Cumulative time in Non internaction and interaction zones (P1) Morning Group



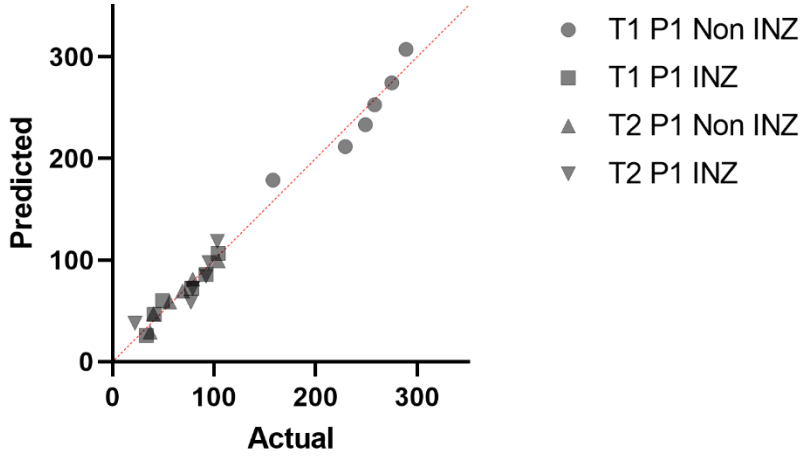
N=6

Normal QQ plot for Cumulative time in Non internaction and interaction zones (P1) Afternoon Group



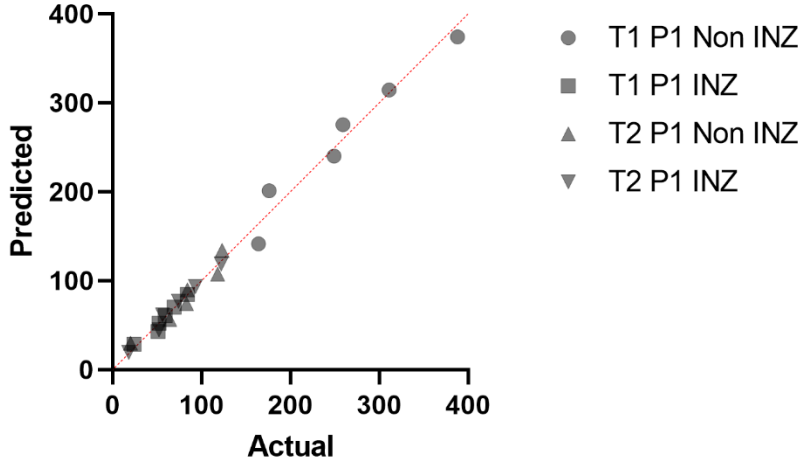
N=6

Normal QQ plot for Frequency into Non internaction and interaction zones (P1) Morning Group

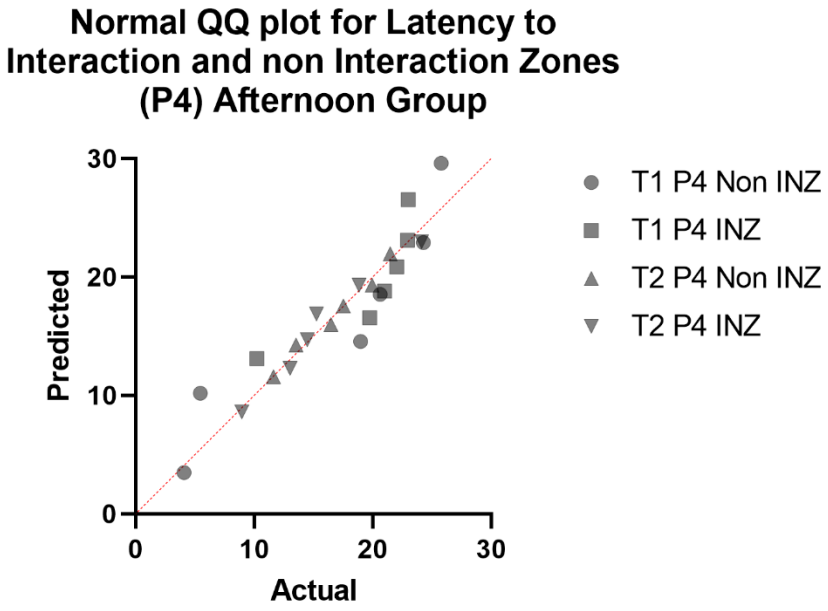


N=6

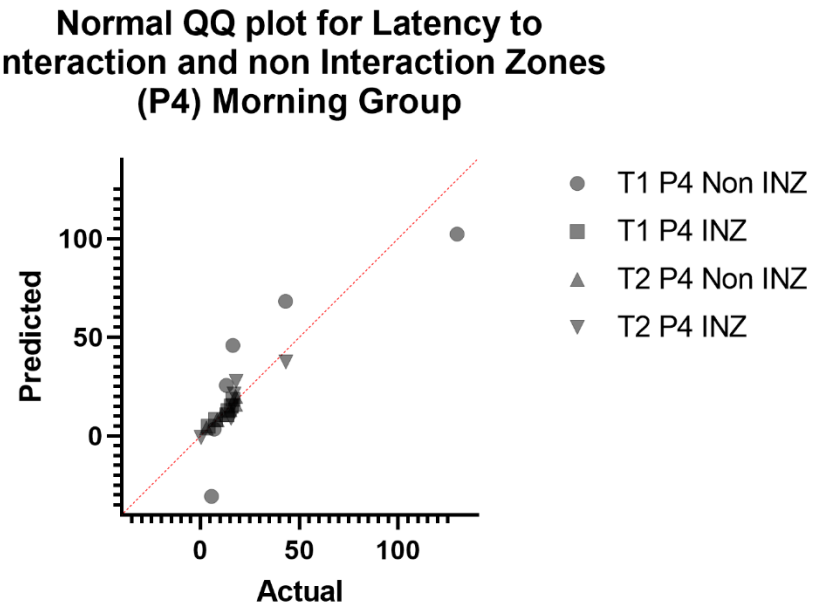
Normal QQ plot for Frequency into Non internaction and interaction zones (P1) Afternoon Group



N=6

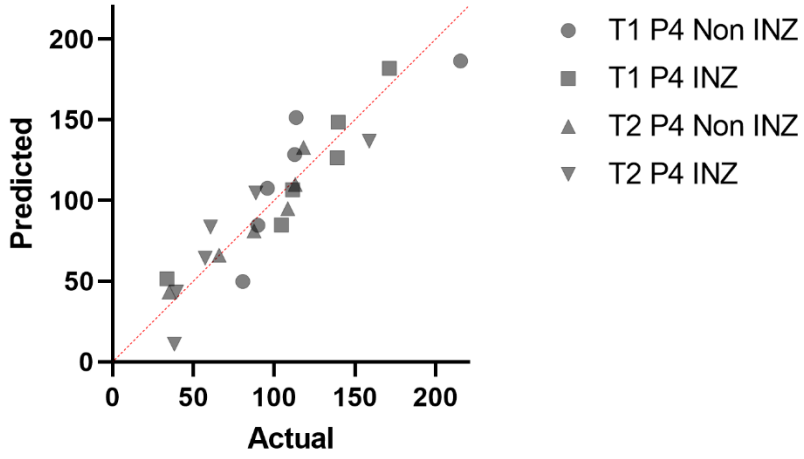

Normal QQ plot for Latency to Interaction and non Interaction Zones (P4) Afternoon Group
Predicted
Actual
0
10
20
30
T1 P4 Non INZ
T1 P4 INZ
T2 P4 Non INZ
T2 P4 INZ

N=6


Normal QQ plot for Latency to Interaction and non Interaction Zones (P4) Morning Group
Predicted
Actual
0
50
100
T1 P4 Non INZ
T1 P4 INZ
T2 P4 Non INZ
T2 P4 INZ

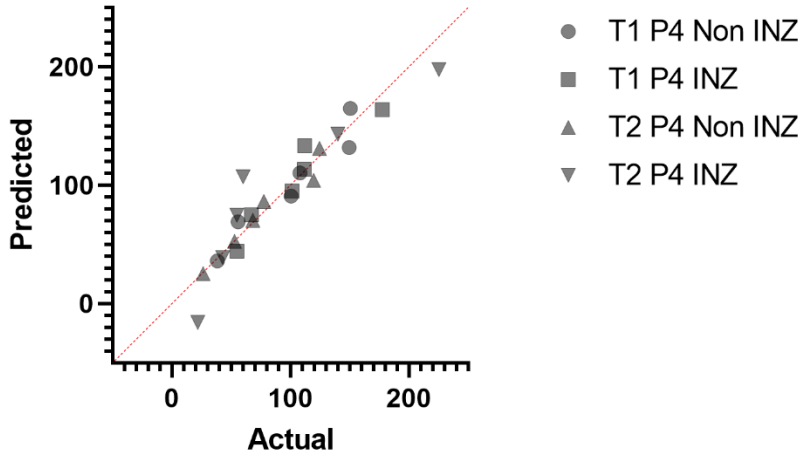
N=6

Normal QQ plot for Cumulative time in Non internaction and interaction zones (P4) Morning Group



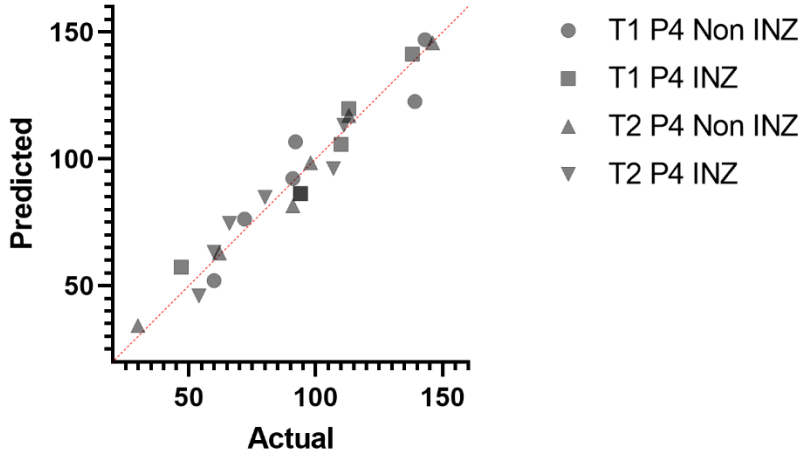
N=6

Normal QQ plot for Cumulative time in Non internaction and interaction zones (P4) Afternoon Group



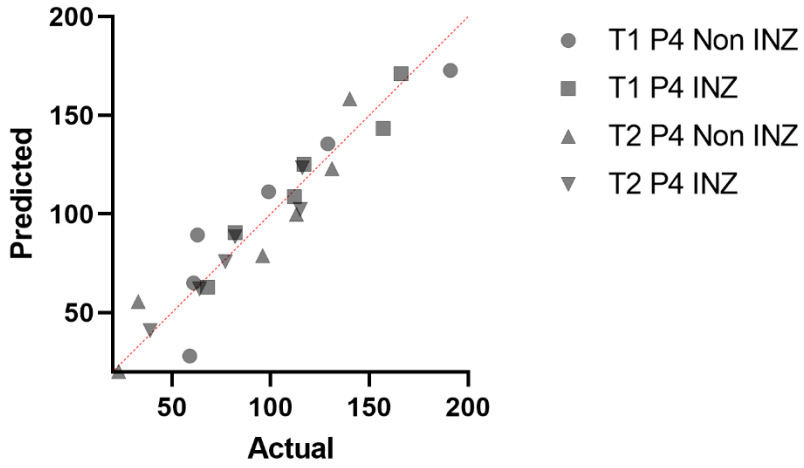
N=6

Normal QQ plot for Frequency into Non internaction and interaction zones (P4) Morning Group



N=6

Normal QQ plot for Frequency into Non internaction and interaction zones (P4) Afternoon Group



D.2 NORMALITY TESTS AND NON-PARAMETRIC DATA FOR NON-INTERACTION AND INTERACTION P4 vs P1

This file can be opened by GraphPad Prism (version 5.00 or later).

This file contains 45 data tables and 45 info tables:

- Friedman test of P1 Zone M Freq:ANOVA results
- Friedman test of P1 Zone M Freq:Multiple comparisons
- Friedman test of P1 Zone A Freq:ANOVA results
- Friedman test of P1 Zone A Freq:Multiple comparisons
- Friedman test of P1 Zone A Lat:ANOVA results
- Friedman test of P1 Zone A Lat:Multiple comparisons
- Friedman test of P1 Zone M Freq:ANOVA results
- Friedman test of P1 Zone M Freq:Multiple comparisons
- Friedman test of P1 Zone A Freq:ANOVA results
- Friedman test of P1 Zone A Freq:Multiple comparisons

Normality and Lognormality Tests of P4 Zone M Lat:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.7071	0.9164	0.8677	0.8379
P value	0.0073	0.4801	0.2170	0.1253
Passed normality test (alpha=0.05)?	No	Yes	Yes	Yes
P value summary	**	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P4 Zone M Lat:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	6,93333	3,39746819798727			
R2	16,39999	45,9235655924163			
R3	5,56666	-30,6507952778995			
R4	129,9	102,273008611233			
R5	42,99996	68,2247451353461			
R6	13,0667	25,6986477409171			
R1	17,73332		20,121186658413		
R2	3,033333		4,07878500825363		
R3	17,73331		16,0119458588779		
R4	10,8333		10,879526269199		
R5	8,433322		8,18802580778875		
R6	14,83333		13,3204453974677		
R1	13,29998			10,7355140693106	
R2	13,96666			12,8089025973561	
R3	3,83332			4,95876434034647	
R4	15,56665			15,0951422827631	
R5	7,69999			8,44927438390356	
R6	16,26665			18,5856523263202	
R1	15,49996				9,00265707112218
R2	0,233332				-0,816063712606372
R3	16,8667				21,266186358989
R4	43,2				37,516044379273
R5	16,33331				15,4337943076776
R6	17,96664				27,6973235955445

Normality and Lognormality Tests of P4 Zone A Lat:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8421	0.9687	0.7155	0.9711
P value	0.1358	0.8838	0.0090	0.8995
Passed normality test (alpha=0.05)?	Yes	Yes	No	Yes
P value summary	ns	ns	**	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P4 Zone A Lat:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	24,29999	22,9212469399523			
S2	25,7999	29,6138336540379			
S3	20,63332	18,537687760109			
S4	18,99993	14,562242239891			
S5	5,46666	10,1786830600477			
S6	4,09999	3,48609634596211			
S1	21,49996		21,9564429821122		
S2	17,53329		17,5657213018184		
S3	19,96669		19,3034185578954		
S4	16,49997		15,9898053648483		
S5	11,6333		11,5990836845545		
S6	13,53337		14,2521081087713		
S1	22,9334			23,1045094253819	
S2	10,23332			13,1091209131489	
S3	21			18,8055203772327	
S4	22,03336			20,8500696227673	
S5	19,76669			16,5510805746181	
S6	23			26,5464690868511	
S1	15,26663				16,8885312946968
S2	18,8666				19,3013974206083
S3	8,96666				8,60360263871971
S4	13,03332				12,2874392460584
S5	24,13332				22,985234027947
S6	14,49998				14,7003053719699

Normality and Lognormality Tests of P4 Zone M CT:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.7356	0.8941	0.9172	0.8126
P value	0.0144	0.3404	0.4856	0.0760
Passed normality test (alpha=0.05)?	No	Yes	Yes	Yes
P value summary	*	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P4 Zone M CT:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	95,7666	107,604623807694			
R2	215,3	186,278655996536			
R3	112,6666	128,384109525639			
R4	89,9665	84,6919452391975			
R5	80,6666	49,7100773367976			
R6	113,5999	151,296788094136			
R1	34,9333		43,1744817885433		
R2	118,1		132,84755154479		
R3	113,0998		109,877908663528		
R4	87,4666		81,1889486237428		
R5	108,4665		94,8330847095905		
R6	65,9999		66,1441246698052		
R1	139,1			126,473339896537	
R2	33,6			51,4143361586911	
R3	111,3665			106,648660103463	
R4	171,3331			181,707663841309	
R5	139,5666			148,333193503491	
R6	104,3998			84,7888064965094	
R1	88,6333				104,512802855947
R2	57,3333				64,2909329968696
R3	38,1666				10,9947195514458
R4	60,6				83,4199870031304
R5	39,59996				43,1981171440526
R6	158,7996				136,716200448554

Normality and Lognormality Tests of P4 Zone A CT:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.9076	0.9385	0.9180	0.8436
P value	0.4211	0.6468	0.4908	0.1397
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P4 Zone A CT:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	38,4667	36,2233365574989			
S2	149,5998	131,955180246442			
S3	108,2332	110,363055185381			
S4	150,6666	164,920896775834			
S5	100,5665	90,7811781479526			
S6	55,8999	69,1890530868914			
S1	68,4333		70,2547082025099		
S2	119,667		104,088034537177		
S3	124,7337		131,176483275464		
S4	52,8666		52,5121187961567		
S5	77,7332		86,3454451308235		
S6	26,36666		25,4236700578696		
S1	54,7666			44,4571109685948	
S2	67,2332			75,0546260675554	
S3	101,3666			95,0956093110232	
S4	111,9332			113,270757355644	
S5	112,2332			133,311740599111	
S6	177,5663			163,909255698072	
S1	42,8				38,7971324721771
S2	54,9333				74,6485974544255
S3	21,89996				-15,9389913889984
S4	140,1997				143,013720861156
S5	60,1666				107,162255878908
S6	225,433				197,749844722332

Normality and Lognormality Tests of P4 Zone M Freq:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8854	0.9876	0.9275	0.8810
P value	0.2950	0.9826	0.5607	0.2738
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P4 Zone M Freq:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	91	92,2730788927619			
R2	92	106,726921107238			
R3	143	146,99734219116			
R4	72	76,335425515034			
R5	139	122,664574484966			
R6	60	52,0026578088401			
R1	91		81,5231042794648		
R2	113		117,171139605074		
R3	146		145,712524155519		
R4	98		98,4768957205352		
R5	62		62,8288603949256		
R6	30		34,2874758444806		
R1	110			105,709927109652	
R2	47			57,4245796450615	
R3	138			141,242087021605	
R4	94			86,281040529436	
R5	113			119,772339003944	
R6	94			86,281040529436	
R1	107				96,0814063498382
R2	111				113,323943998327
R3	54				46,0093893350061
R4	80				84,7877634847033
R5	60				63,2519269834952
R6	66				74,5455698486301

Normality and Lognormality Tests of P4 Zone A Freq:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8383	0.8705	0.9333	0.9296
P value	0.1261	0.2284	0.6054	0.5772
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P4 Zone A Freq:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	59	27,9581088432558			
S2	129	135,630914234156			
S3	191	172,708557823411			
S4	61	65,0357524325104			
S5	99	111,345529245335			
S6	63	89,3211374213316			
S1	131		123,032630484549		
S2	96		78,8197725494185		
S3	113		99,8468941172482		
S4	33		55,6340361821174		
S5	140		158,431388617349		
S6	23		20,2352780493174		
S1	68			62,8968172362996	
S2	117			125,232027634517	
S3	166			171,1031827637	
S4	82			90,6137402813094	
S5	112			108,767972365483	
S6	157			143,386259718691	
S1	115				102,272420595247
S2	77				75,8940413034123
S3	64				62,0609127380865
S4	82				88,439292029921
S5	116				123,392110931866
S6	39				40,9412224014672

Normality and Lognormality Tests of P1 Zone M Lat:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8451	0.8578	0.7450	0.9052
P value	0.1436	0.1818	0.0178	0.4054
Passed normality test (alpha=0.05)?	Yes	Yes	No	Yes
P value summary	ns	ns	*	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 Zone M Lat:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	25,1	39,34654295977			
R2	29,2667	73,1039318829855			
R3	23,59992	-12,1924561368054			
R4	135,3336	137,475863706897			
R5	195,3333	189,014862803472			
R6	121,8337	103,718474783681			
R1	59,3306		69,9768137501636		
R2	92,2977		100,509427912847		
R3	9,166435		42,2868159165031		
R4	6,7665		11,7542017538193		
R5	168,463		147,125011165628		
R6	0,766654		-34,8613814989612		
R1	9,53289			7,84465471625249	
R2	98,4642			98,2306319504142	
R3	18,43289			67,1370244183572	
R4	8,59978			-39,6274235937458	
R5	170,163			145,702710260412	
R6	13,0331			38,9382622483095	
R1	25,79906				25,0511768096027
R2	21,0997				19,0795130466386
R3	11,69978				11,1667266035835
R4	23,1994				21,9195669533614
R5	28,1662				29,8323533964165
R6	13,0331				15,9479031903973

Normality and Lognormality Tests of P1 Zone A Lat:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8297	0.7442	0.8303	0.8964
P value	0.1068	0.0175	0.1082	0.3533
Passed normality test (alpha=0.05)?	Yes	No	Yes	Yes
P value summary	ns	*	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 Zone A Lat:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	28,76659	45,9923485941016			
S2	24,6	-19,445679119224			
S3	28,99996	88,8534244901928			
S4	231,8663	236,023195785891			
S5	186,3997	170,585168072565			
S6	149,1	127,724092176474			
S1	106,163		112,675572801898		
S2	20,2328		77,1935299351547		
S3	198,297		166,847682481746		
S4	8,49974		-44,6392524817463		
S5	16,9331		45,0149000648453		
S6	16,49965		9,53285719810205		
S1	214,06			210,586315045995	
S2	24,9994			69,8384275613232	
S3	179,397			148,003733849287	
S4	15,6662			28,84763281738	
S5	13,5664			-33,7349483793281	
S6	82,8651			107,012939105344	
S1	6,46838				10,4336766028507
S2	14,2997				15,5331333971493
S3	17,53284				21,1560932715024
S4	3,96658				4,81071672849762
S5	1,36663				-3,77412152849984
S6	34,2663				29,7409315284998

Normality and Lognormality Tests of P1 Zone M CT:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8984	0.8932	0.8522	0.9263
P value	0.3643	0.3356	0.1641	0.5519
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 Zone M CT:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	29,5653	27,1683858407884			
R2	133,9968	140,616447492545			
R3	93,631	75,2616159365268			
R4	106,964	111,556870645275			
R5	100,5314	92,5232173968066			
R6	38,666	56,2279626880581			
R1	215,6252		191,297093300524		
R2	112,9649		111,505787284527		
R3	115,5978		143,084453303167		
R4	31,63254		3,07582003280932		
R5	68,6323		82,8671260488068		
R6	38,666		51,2884600301664		
R1	162,1587			149,385953655254	
R2	82,798			91,6739796780795	
R3	103,7641			111,527429556678	
R4	82,298			61,3627806192778	
R5	184,4627			179,697152714056	
R6	107,6983			129,532503776656	
R1	28,83225				20,7744832743517
R2	52,3991				63,1070909899906
R3	73,5319				78,3010923433427
R4	118,597				120,633700058982
R5	43,166				46,3532947746202
R6	107,6983				95,0548885587131

Normality and Lognormality Tests of P1 Zone A CT:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8455	0.9077	0.9080	0.9570
P value	0.1447	0.4215	0.4234	0.7963
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 Zone A CT:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	127,5965	111,601561386147			
S2	51,9654	60,2311450629573			
S3	36,06613	22,4287486138527			
S4	50,5318	45,2702513042918			
S5	73,8322	88,7600586957083			
S6	62,0989	73,7991649370427			
S1	115,1641		137,029392896886		
S2	107,098		107,59872173632		
S3	203,0277		181,962587754053		
S4	71,0985		80,9080749303468		
S5	31,83239		6,54420891261358		
S6	37,2997		51,4774037697807		
S1	56,8316			59,7853625573074	
S2	58,7318			66,6229374426926	
S3	54,9991			52,2458518271858	
S4	43,0653			40,7349249130155	
S5	85,832			85,6733750869845	
S6	79,7651			74,1624481728142	
S1	57,18192				63,1966280253922
S2	50,7324				46,7443729941969
S3	26,89925				21,6259386532559
S4	65,9652				78,1171619746078
S5	97,0973				94,5694170058031
S6	126,0653				119,687851346744

Normality and Lognormality Tests of P1 Zone M Freq:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8863	0.9457	0.9163	0.7996
P value	0.2992	0.7057	0.4789	0.0583
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 Zone M Freq:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	249	233,210216133718			
R2	258	252,789783866282			
R3	158	178,658808588825			
R4	289	307,341191411175			
R5	229	211,62063744752			
R6	275	274,37936255248			
R1	40		47,0531367953355		
R2	37		29,076568856979		
R3	79		81,2801965379978		
R4	104		99,2567644763544		
R5	56		58,8275601647647		
R6	69		69,5057731685686		
R1	49			60,0195397680889	
R2	78			72,3137935652445	
R3	104			106,56730000163	
R4	41			46,4631751247392	
R5	33			25,7660333317036	
R6	92			85,8701582085941	
R1	22				37,6886272479665
R2	103				118,311372752034
R3	79				71,8664544758962
R4	95				97,6599589288073
R5	77				58,3400410711927
R6	92				84,1335455241038

Normality and Lognormality Tests of P1 Zone A Freq:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.9453	0.9284	0.9667	0.9885
P value	0.7022	0.5675	0.8693	0.9853
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 Zone A Freq:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	164	141,545263606262			
S2	176	201,119346220871			
S3	388	374,121403060405			
S4	249	240,139612656327			
S5	311	314,54732044579 6			
S6	259	275,527054010339			
S1	64		56,4939402286243		
S2	83		74,0425785570175		
S3	20		29,701605756002		
S4	123		134,298394243998		
S5	118		107,506059771376		
S6	84		89,9574214429825		
S1	51			42,9244799360857	
S2	84			84,3356557905769	
S3	24			28,6643442094231	
S4	59			60,7353125266907	
S5	52			52,2646874733093	
S6	69			70,0755200639143	
S1	52				44,9059663535128
S2	74				76,7355588681315
S3	18				19,4217950171177
S4	56				61,5977744652018
S5	93				93,4273669798206
S6	122				118,911538316216

Friedman test of P4 Zone M Lat

	Data Set-A
Table Analyzed	P4 Zone M Lat
Friedman test	
P value	0.7715
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	1.400
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P4 Zone A Lat

	Data Set-A
Table Analyzed	P4 Zone A Lat
Friedman test	
P value	0.4307
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	3.000
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P4 Zone M CT

	Data Set-A
Table Analyzed	P4 Zone M CT
Friedman test	
P value	0.4307
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	3.000
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P4 Zone A CT

	Data Set-A
Table Analyzed	P4 Zone A CT
Friedman test	
P value	0.7715
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	1.400
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P4 Zone M Freq

	Data Set-A
Table Analyzed	P4 Zone M Freq
Friedman test	
P value	0.7723
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	1.271
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P4 Zone A Freq

	Data Set-A

Table Analyzed	P4 Zone A Freq
Friedman test	
P value	0.8386
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	0.9661
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone M Lat

	Data Set-A
Table Analyzed	P1 Zone M Lat
Friedman test	
P value	0.2627
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	4.119
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone A Lat:ANOVA results

	Data Set-A
Table Analyzed	P1 Zone A Lat
Friedman test	
P value	0.0137
Exact or approximate P value?	Exact
P value summary	*
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	9.600
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone A Lat:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F

Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	4.000	No	ns	>0.9999	A-B	
T1 vs. T1	0.000	No	ns	>0.9999	A-C	
T1 vs. T2	12.00	Yes	*	0.0437	A-D	
T2 vs. T1	-4.000	No	ns	>0.9999	B-C	
T2 vs. T2	8.000	No	ns	0.4418	B-D	
T1 vs. T2	12.00	Yes	*	0.0437	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	19.00	15.00	4.000	6	6	0.8944
T1 vs. T1	19.00	19.00	0.000	6	6	0.000
T1 vs. T2	19.00	7.000	12.00	6	6	2.683
T2 vs. T1	15.00	19.00	-4.000	6	6	0.8944
T2 vs. T2	15.00	7.000	8.000	6	6	1.789
T1 vs. T2	19.00	7.000	12.00	6	6	2.683

Friedman test of P1 Zone M CT

	Data Set-A
Table Analyzed	P1 Zone M CT
Friedman test	
P value	0.6172
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	1.966
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone A CT

	Data Set-A
Table Analyzed	P1 Zone A CT
Friedman test	
P value	0.9396
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	0.6000
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone M Freq:ANOVA results

	Data Set-A

Table Analyzed	P1 Zone M Freq
Friedman test	
P value	0.0034
Exact or approximate P value?	Exact
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	11.53
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone M Freq:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	13.50	Yes	*	0.0152	A-B	
T1 vs. T1	11.50	No	ns	0.0608	A-C	
T1 vs. T2	11.00	No	ns	0.0834	A-D	
T2 vs. T1	-2.000	No	ns	>0.9999	B-C	
T2 vs. T2	-2.500	No	ns	>0.9999	B-D	
T1 vs. T2	-0.5000	No	ns	>0.9999	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	24.00	10.50	13.50	6	6	3.019
T1 vs. T1	24.00	12.50	11.50	6	6	2.571
T1 vs. T2	24.00	13.00	11.00	6	6	2.460
T2 vs. T1	10.50	12.50	-2.000	6	6	0.4472
T2 vs. T2	10.50	13.00	-2.500	6	6	0.5590
T1 vs. T2	12.50	13.00	-0.5000	6	6	0.1118

Friedman test of P1 Zone A Freq:ANOVA results

	Data Set-A
Table Analyzed	P1 Zone A Freq
Friedman test	
P value	0.0020
Exact or approximate P value?	Exact
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	12.20
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone A Freq:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F

Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	9.000	No	ns	0.2650	A-B	
T1 vs. T1	13.00	Yes	*	0.0219	A-C	
T1 vs. T2	14.00	Yes	*	0.0105	A-D	
T2 vs. T1	4.000	No	ns	>0.9999	B-C	
T2 vs. T2	5.000	No	ns	>0.9999	B-D	
T1 vs. T2	1.000	No	ns	>0.9999	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	24.00	15.00	9.000	6	6	2.012
T1 vs. T1	24.00	11.00	13.00	6	6	2.907
T1 vs. T2	24.00	10.00	14.00	6	6	3.130
T2 vs. T1	15.00	11.00	4.000	6	6	0.8944
T2 vs. T2	15.00	10.00	5.000	6	6	1.118
T1 vs. T2	11.00	10.00	1.000	6	6	0.2236

Friedman test of P1 Zone A Lat:ANOVA results

	Data Set-A
Table Analyzed	P1 Zone A Lat
Friedman test	
P value	0.0137
Exact or approximate P value?	Exact
P value summary	*
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	9.600
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone A Lat:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	4.000	No	ns	>0.9999	A-B	
T1 vs. T1	0.000	No	ns	>0.9999	A-C	
T1 vs. T2	12.00	Yes	*	0.0437	A-D	
T2 vs. T1	-4.000	No	ns	>0.9999	B-C	
T2 vs. T2	8.000	No	ns	0.4418	B-D	
T1 vs. T2	12.00	Yes	*	0.0437	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	19.00	15.00	4.000	6	6	0.8944
T1 vs. T1	19.00	19.00	0.000	6	6	0.000
T1 vs. T2	19.00	7.000	12.00	6	6	2.683
T2 vs. T1	15.00	19.00	-4.000	6	6	0.8944
T2 vs. T2	15.00	7.000	8.000	6	6	1.789
T1 vs. T2	19.00	7.000	12.00	6	6	2.683

Friedman test of P1 Zone M Freq:ANOVA results

	Data Set-A
Table Analyzed	P1 Zone M Freq
Friedman test	
P value	0.0034
Exact or approximate P value?	Exact
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	11.53
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone M Freq:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	13.50	Yes	*	0.0152	A-B	
T1 vs. T1	11.50	No	ns	0.0608	A-C	
T1 vs. T2	11.00	No	ns	0.0834	A-D	
T2 vs. T1	-2.000	No	ns	>0.9999	B-C	
T2 vs. T2	-2.500	No	ns	>0.9999	B-D	
T1 vs. T2	-0.5000	No	ns	>0.9999	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	24.00	10.50	13.50	6	6	3.019
T1 vs. T1	24.00	12.50	11.50	6	6	2.571
T1 vs. T2	24.00	13.00	11.00	6	6	2.460
T2 vs. T1	10.50	12.50	-2.000	6	6	0.4472
T2 vs. T2	10.50	13.00	-2.500	6	6	0.5590
T1 vs. T2	12.50	13.00	-0.5000	6	6	0.1118

Friedman test of P1 Zone A Freq:ANOVA results

	Data Set-A
Table Analyzed	P1 Zone A Freq
Friedman test	
P value	0.0020
Exact or approximate P value?	Exact
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	12.20
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 Zone A Freq:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F

Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	9.000	No	ns	0.2650	A-B	
T1 vs. T1	13.00	Yes	*	0.0219	A-C	
T1 vs. T2	14.00	Yes	*	0.0105	A-D	
T2 vs. T1	4.000	No	ns	>0.9999	B-C	
T2 vs. T2	5.000	No	ns	>0.9999	B-D	
T1 vs. T2	1.000	No	ns	>0.9999	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	24.00	15.00	9.000	6	6	2.012
T1 vs. T1	24.00	11.00	13.00	6	6	2.907
T1 vs. T2	24.00	10.00	14.00	6	6	3.130
T2 vs. T1	15.00	11.00	4.000	6	6	0.8944
T2 vs. T2	15.00	10.00	5.000	6	6	1.118
T1 vs. T2	11.00	10.00	1.000	6	6	0.2236

D. 3 QQ PLOTS FOR P2 vs P3

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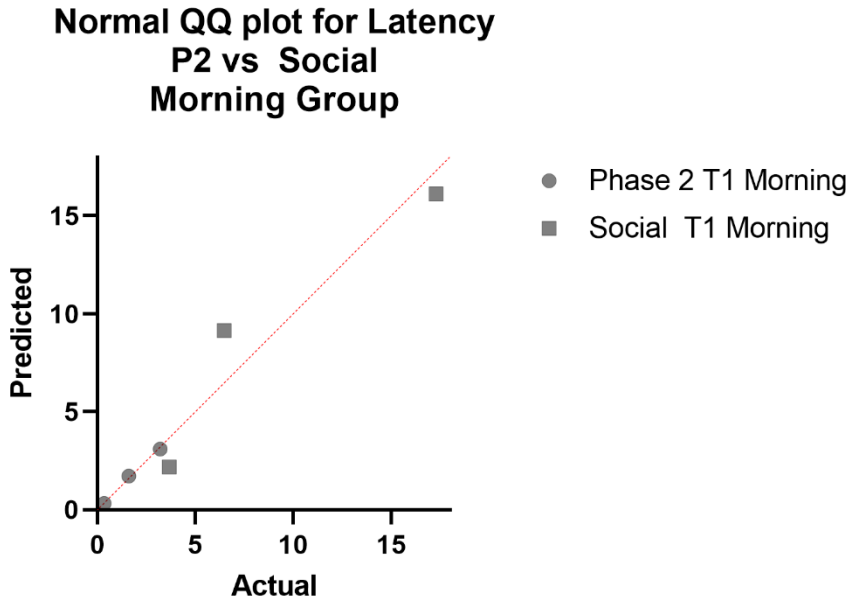

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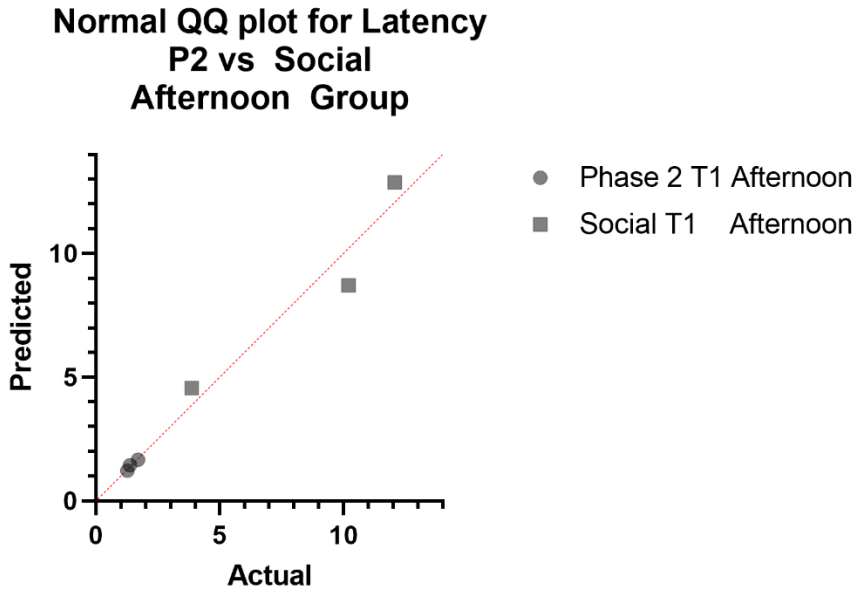
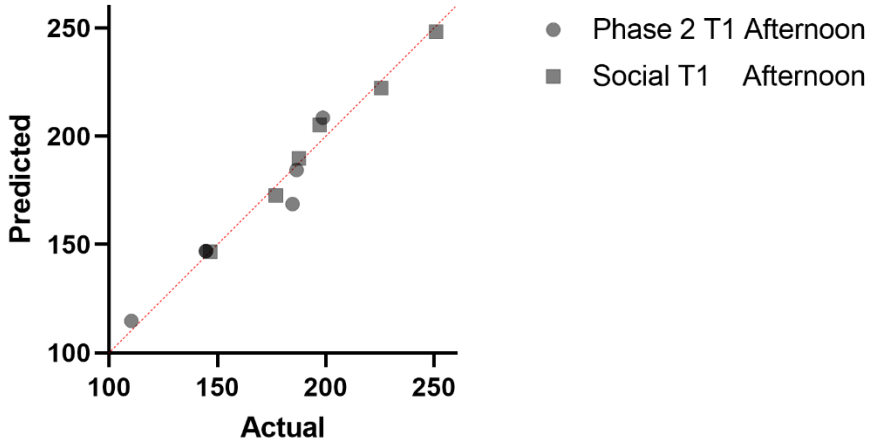


N=6

Normal QQ plot for Cumulative Time
P2 vs Social
Afternoon Group



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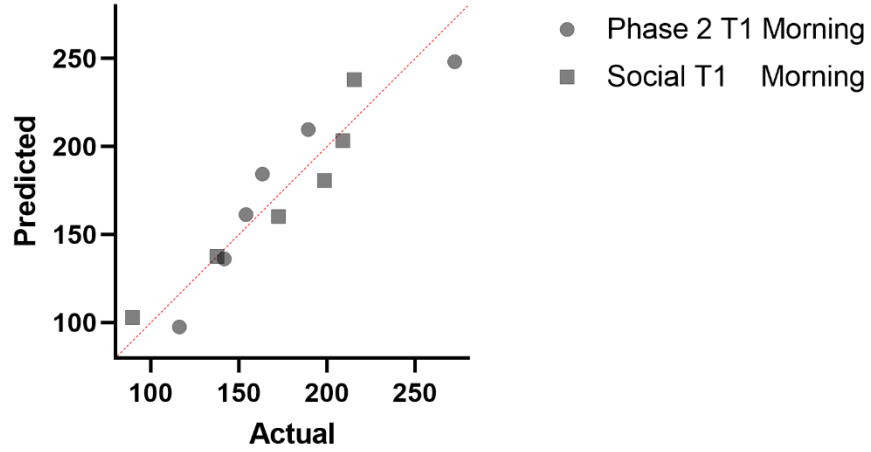
Normal QQ plot for Cumulative Time
P2 vs Social
Morning Group



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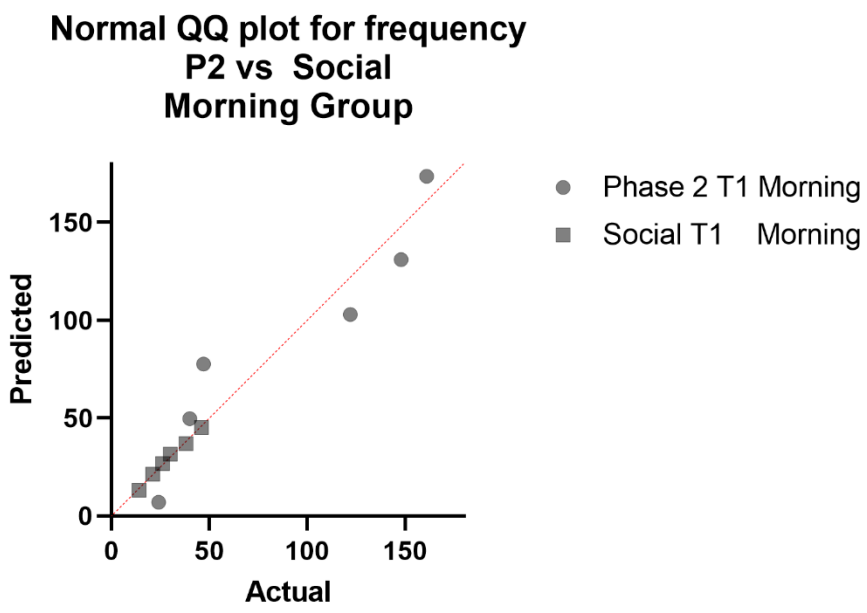
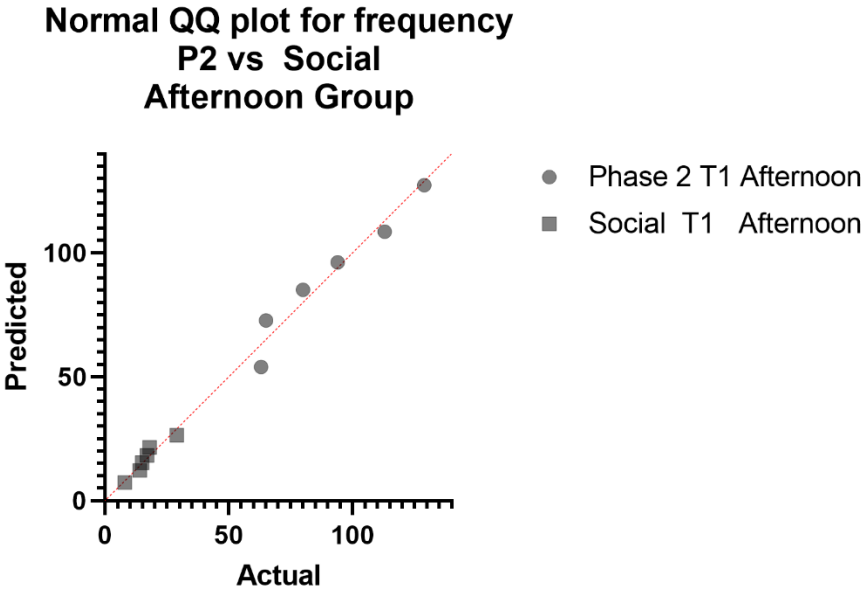

Normal QQ plot for frequency
P2 vs Social
Morning Group
Predicted
Actual
0
50
100
150
Phase 2 T1 Morning
Social T1 Morning

N=6


Normal QQ plot for frequency
P2 vs Social
Afternoon Group
Predicted
Actual
0
50
100
Phase 2 T1 Afternoon
Social T1 Afternoon

D.4 NORMALITY TESTS AND NON-PARAMETRIC DATA FOR P2 vs P3

This file can be opened by GraphPad Prism (version 5.00 or later).

This file contains 22 data tables and 22 info tables:

Normality and Lognormality Tests of P2 vs P3 M Latency:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8570	0.8962	0.8144	0.8731
P value	0.2498	0.4122	0.1306	0.3101
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	4	4	4	4

Normality and Lognormality Tests of P2 vs P3 M Latency:Normal QQ plot

	Actual	T1	T2	T1	T2
R3	1,73322	2,98869564705639			
R4	1,59996	1,57780085294361			
R5	5,46648	4,83004901559033			
R6	0,333333	-0,263552515590326			
R3	0,099999		0,375158536545586		
R4	1,89993		1,94987191810508		
R5	2,63329		2,84144096345441		
R6	1,79998		1,26672758189492		
R3	0,766665			2,63003688540178	
R4	6,53333			5,01995661459822	
R5	0,433332			-0,489038159912926	
R6	7,56666			8,13903165991293	
R3	0,099999				0,361772132258838
R4	0,066666				-0,096909601673721
R5	0,78333225				0,713226492741162
R6	1,2				1,17190822667372

Normality and Lognormality Tests of P2 vs P3 A Latency:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8811	0.9838	0.7563	0.8752
P value	0.3434	0.9236	0.0442	0.3186
Passed normality test (alpha=0.05)?	Yes	Yes	No	Yes
P value summary	ns	ns	*	ns
Number of values	4	4	4	4

Normality and Lognormality Tests of P2 vs P3 A Latency:Normal QQ plot

	Actual	T1	T2	T1	T2

S3	1,36662	1,37386354243347			
S4	1,26666	1,21867075431339			
S5	1,7	1,64796924568661			
S6	1,4	1,49277645756653			
S3	8,96666		8,58731416098752		
S4	2,33316		2,09572083901248		
S5	4,16659		4,44245274518899		
S6	5,89966		6,24058225481101		
S3	0,699999			1,38442788062058	
S4	8,26666			6,98844335080607	
S5	0,066666			-1,78844585080607	
S6	1,36667			3,81556961937942	
S3	1,36667				1,14535292992773
S4	1,86666				1,89453210052091
S5	0,033333				-0,177867600520907
S6	0,166666				0,571311570072272

Normality and Lognormality Tests of P2 vs P3 M Cumulative Time:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8873	0.8980	0.9426	0.9405
P value	0.3041	0.3625	0.6804	0.6634
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P2 vs P3 M Cumulative Time:Normal QQ plot

	Actual	T1	T2	T1	T2
R1	141,795	136,19269860043			
R2	116,267	97,5864475083569			
R3	272,649	248,304552491643			
R4	189,496	209,69830139957			
R5	154,033	161,479302062409			
R6	163,433	184,411697937591			
R1	155,667		156,744577268568		
R2	81,4666		94,1333600700347		
R3	139,833		140,207622731432		
R4	139,262		121,973007642569		
R5	186,43		174,979192357431		
R6	188,198		202,818839929965		
R1	151,72638			142,778415426587	
R2	84,3999			92,9178598914668	
R3	168,133			160,674344573414	
R4	196,866			210,534900108533	
R5	187,666			180,40744469878	
R6	121,567			123,04531530122	
R1	76,4333				78,1509202752829
R2	69,6333				69,9409982289166
R3	67,4999				65,6356197247171
R4	87,5666				84,7241448615529
R5	60,22662				59,0623951384471
R6	69,9999				73,8455417710834

Normality and Lognormality Tests of P2 vs P3 A Cumulative Time:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.9067	0.9342	0.7826	0.8389
P value	0.4153	0.6129	0.0408	0.1276
Passed normality test (alpha=0.05)?	Yes	Yes	No	Yes
P value summary	ns	ns	*	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P2 vs P3 A Cumulative Time:Normal QQ plot

	Actual	T1	T2	T1	T2
S1	186,655	184,521990226064			
S2	110,331	114,792236990025			
S3	184,76	168,794584439943			
S4	144,733	147,065311715142			
S5	144,733	147,065311715142			
S6	198,766	208,533763009975			
S1	98,0666		87,3547015590722		
S2	114,333		104,418931774261		
S3	61,5999		68,5386814108072		
S4	34,2974		39,8113752904082		
S5	139,631		151,962258042925		
S6	127,393		123,234951922526		
S1	134,6			136,398755387206	
S2	116,067			127,93710104464	
S3	113,267			111,801577946127	
S4	156,8			149,317563917236	
S5	113,5			120,2632322887	
S6	110,367			98,8827694160973	
S1	80,9666				89,0993117385986
S2	131,767				125,157406050902
S3	110,667				109,124422143104
S4	75,5333				62,564927282431
S5	87,7332				98,6230215947347
S6	76,4999				78,5979111902291

Normality and Lognormality Tests of P2 vs P3 M Frequency:Tabular results

	T1	T2	T1	T2

Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8620	0.9376	0.9066	0.9868
P value	0.1961	0.6396	0.4141	0.9799
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P2 vs P3 M Frequency:Normal QQ plot

	Actual	T1	T2	T1	T2
R1	40	49,7143382549181			
R2	47	77,6609540898675			
R3	24	7,04692071424313			
R4	161	173,619745952424			
R5	122	103,005712576799			
R6	148	130,952328411749			
R1	75		74,4803843299214		
R2	47		47,4383982661101		
R3	59		63,7780924509213		
R4	105		111,228268400557		
R5	99		94,8885742157454		
R6	91		84,1862823367453		
R1	112			120,714740136326	
R2	52			55,9863424817342	
R3	105			94,6695574342148	
R4	179			168,346990851599	
R5	120			139,56595353484	
R6	105			94,6695574342148	
R1	90				89,4711326543948
R2	52				51,5776638782329
R3	62				60,1244596494587
R4	78				76,4223361217671
R5	64				67,8755403505413
R6	38				38,5288673456052

Normality and Lognormality Tests of P2 vs P3 A Frequency:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.9284	0.8695	0.9815	0.9869
P value	0.5681	0.2244	0.9586	0.9804
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P2 vs P3 A Frequency:Normal QQ plot

	Actual	T1	T2	T1	T2
S1	63	53,9855880592639			
S2	80	85,0854857206423			
S3	65	72,7772114173534			
S4	113	108,55612191598			
S5	129	127,347745274069			
S6	94	96,247847612691			
S1	103		118,320861129887		
S2	94		84,134989220846		
S3	60		58,3354135652021		
S4	28		37,6791388701131		
S5	101		97,6645864347979		
S6	82		71,865010779154		
S1	102			95,3252771441463	
S2	115			114,052706840912	
S3	74			78,3413895225204	
S4	55			59,6139598257542	
S5	142			142,644757737332	
S6	33			31,0219089293346	
S1	73				67,5727420756747
S2	110				108,669453556533
S3	74				78,4272579243253
S4	35				37,3305464434665
S5	89				90,3960831413034
S6	57				55,6039168586966

Friedman test of P2 vs P3 M Latency

	Data Set-A
Table Analyzed	P2 vs P3 M Latency
Friedman test	
P value	0.4753
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	2.692
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	4

Friedman test of P2 vs P3 A Latency

	Data Set-A
Table Analyzed	P2 vs P3 A Latency
Friedman test	
P value	0.1585
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	5.400
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	4

Friedman test of P2 vs P3 M Cumulative Time:ANOVA results

	Data Set-A
Table Analyzed	P2 vs P3 M Cumulative Time
Friedman test	
P value	0.0057
Exact or approximate P value?	Exact
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	11.00
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P2 vs P3 M Cumulative Time:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	1.000	No	ns	>0.9999	A-B	
T1 vs. T1	-1.000	No	ns	>0.9999	A-C	
T1 vs. T2	12.00	Yes	*	0.0437	A-D	
T2 vs. T1	-2.000	No	ns	>0.9999	B-C	
T2 vs. T2	11.00	No	ns	0.0834	B-D	
T1 vs. T2	13.00	Yes	*	0.0219	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	18.00	17.00	1.000	6	6	0.2236
T1 vs. T1	18.00	19.00	-1.000	6	6	0.2236
T1 vs. T2	18.00	6.000	12.00	6	6	2.683
T2 vs. T1	17.00	19.00	-2.000	6	6	0.4472
T2 vs. T2	17.00	6.000	11.00	6	6	2.460
T1 vs. T2	19.00	6.000	13.00	6	6	2.907

Friedman test of P2 vs P3 A Cumulative Time:ANOVA results

	Data Set-A
Table Analyzed	P2 vs P3 A Cumulative Time
Friedman test	
P value	0.1555
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	5.400
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P2 vs P3 A Cumulative Time:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	8.000	No	ns	0.4418	A-B	
T1 vs. T1	3.000	No	ns	>0.9999	A-C	
T1 vs. T2	9.000	No	ns	0.2650	A-D	
T2 vs. T1	-5.000	No	ns	>0.9999	B-C	
T2 vs. T2	1.000	No	ns	>0.9999	B-D	
T1 vs. T2	6.000	No	ns	>0.9999	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	20.00	12.00	8.000	6	6	1.789
T1 vs. T1	20.00	17.00	3.000	6	6	0.6708
T1 vs. T2	20.00	11.00	9.000	6	6	2.012
T2 vs. T1	12.00	17.00	-5.000	6	6	1.118
T2 vs. T2	12.00	11.00	1.000	6	6	0.2236
T1 vs. T2	17.00	11.00	6.000	6	6	1.342

Friedman test of P2 vs P3 M Frequency

	Data Set-A
Table Analyzed	P2 vs P3 M Frequency
Friedman test	
P value	0.0951
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	6.310
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P2 vs P3 A Frequency

	Data Set-A
Table Analyzed	P2 vs P3 A Frequency
Friedman test	
P value	0.6329
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	1.881
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P2 vs P3 M Cumulative Time:ANOVA results

	Data Set-A

Table Analyzed	P2 vs P3 M Cumulative Time
Friedman test	
P value	0.0057
Exact or approximate P value?	Exact
P value summary	**
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	11.00
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P2 vs P3 M Cumulative Time:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	1.000	No	ns	>0.9999	A-B	
T1 vs. T1	-1.000	No	ns	>0.9999	A-C	
T1 vs. T2	12.00	Yes	*	0.0437	A-D	
T2 vs. T1	-2.000	No	ns	>0.9999	B-C	
T2 vs. T2	11.00	No	ns	0.0834	B-D	
T1 vs. T2	13.00	Yes	*	0.0219	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	18.00	17.00	1.000	6	6	0.2236
T1 vs. T1	18.00	19.00	-1.000	6	6	0.2236
T1 vs. T2	18.00	6.000	12.00	6	6	2.683
T2 vs. T1	17.00	19.00	-2.000	6	6	0.4472
T2 vs. T2	17.00	6.000	11.00	6	6	2.460
T1 vs. T2	19.00	6.000	13.00	6	6	2.907

D.5 QQ PLOTS FOR P1 vs P4

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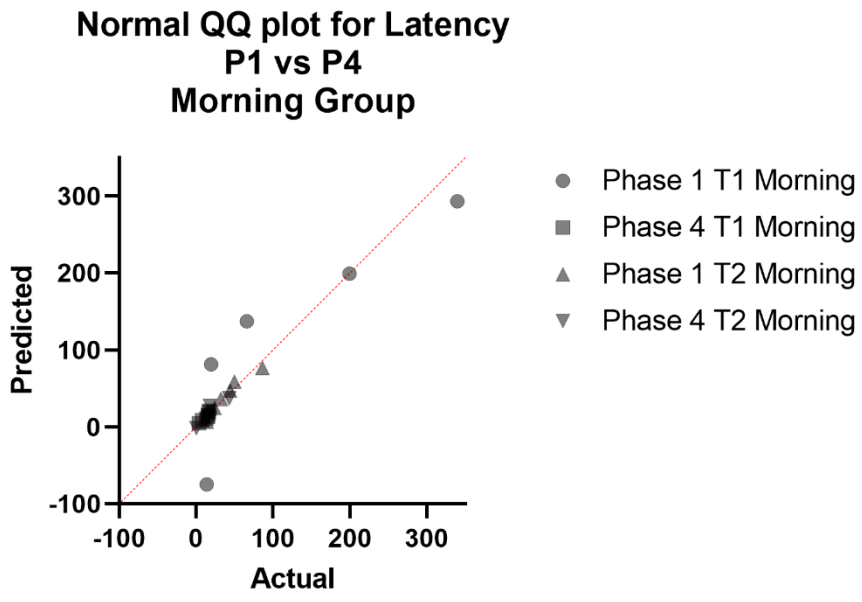

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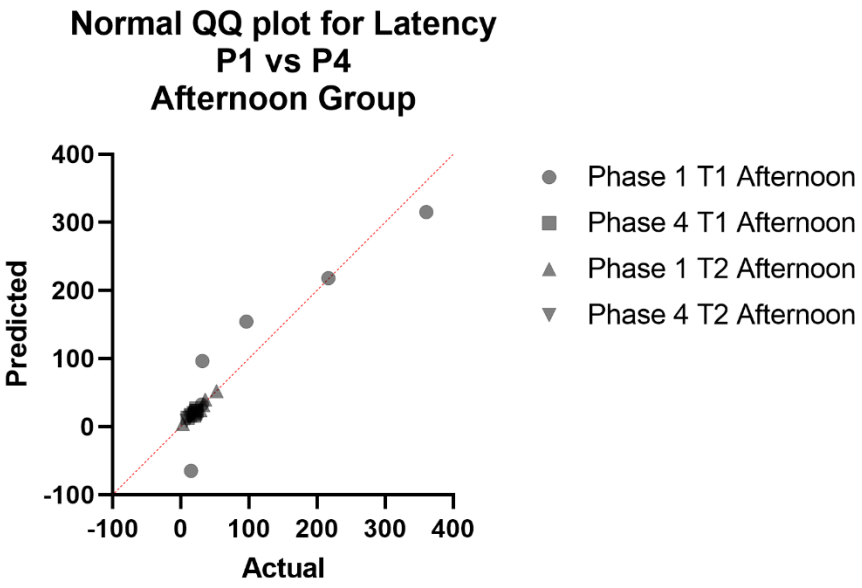

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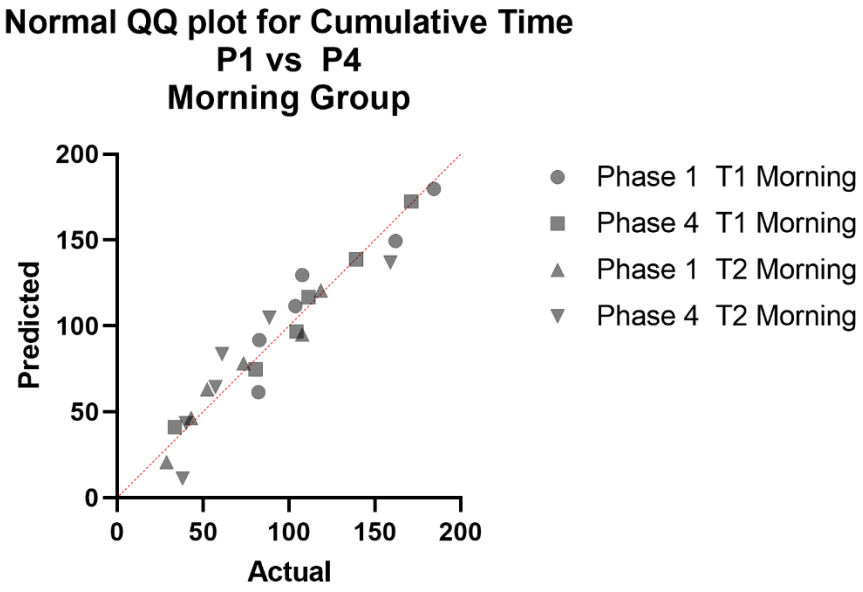
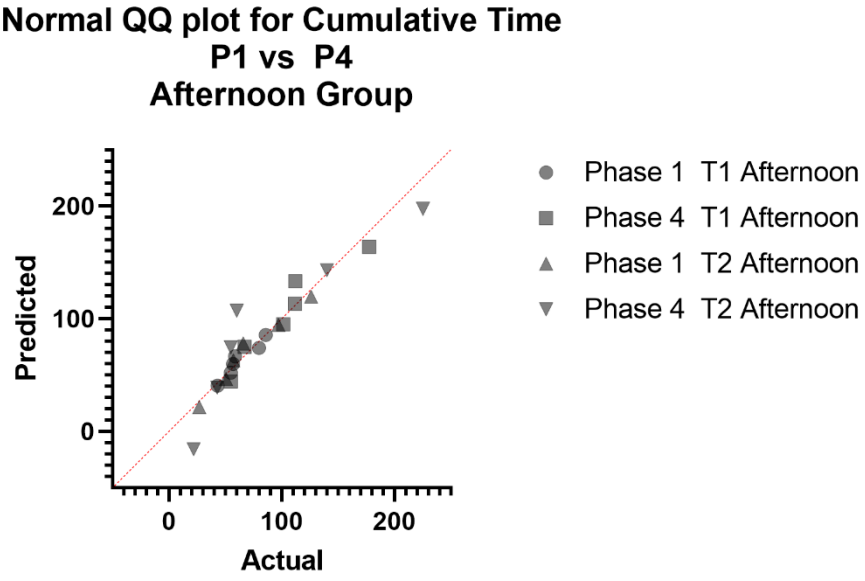
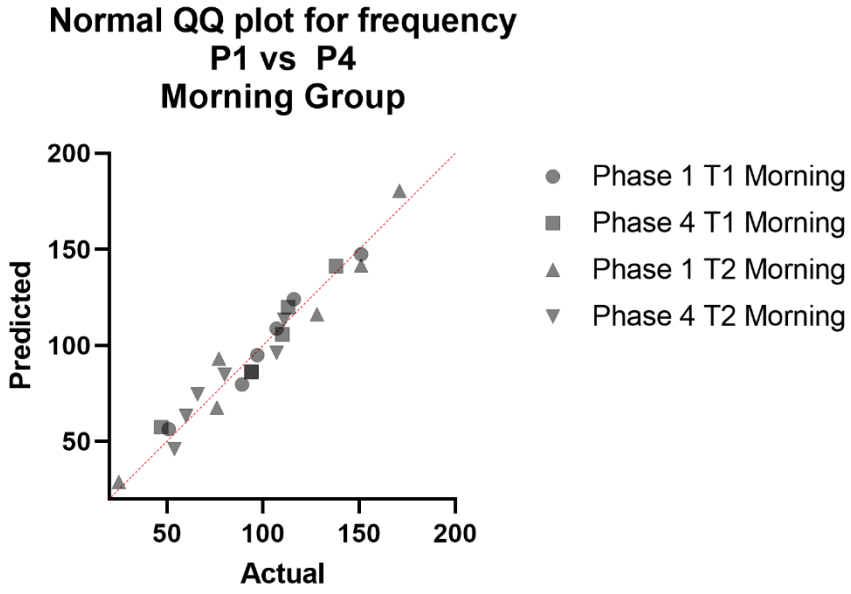

Normal QQ plot for Cumulative Time
P1 vs P4
Morning Group
Predicted
Actual
0
50
100
150
200
Phase 1 T1 Morning
Phase 4 T1 Morning
Phase 1 T2 Morning
Phase 4 T2 Morning

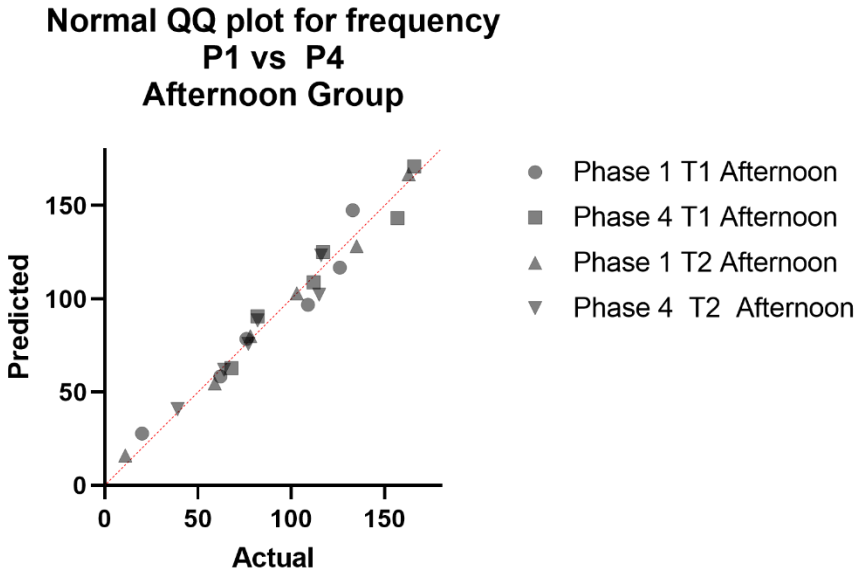
N=6


Normal QQ plot for Cumulative Time
P1 vs P4
Afternoon Group
Predicted
Actual
0
100
200
Phase 1 T1 Afternoon
Phase 4 T1 Afternoon
Phase 1 T2 Afternoon
Phase 4 T2 Afternoon

N=6


Normal QQ plot for frequency
P1 vs P4
Morning Group
Predicted
Actual
200
150
100
50
50
100
150
200
Phase 1 T1 Morning
Phase 4 T1 Morning
Phase 1 T2 Morning
Phase 4 T2 Morning

N=6


Normal QQ plot for frequency
P1 vs P4
Afternoon Group
Predicted
Actual
150
100
50
0
0
50
100
150
Phase 1 T1 Afternoon
Phase 4 T1 Afternoon
Phase 1 T2 Afternoon
Phase 4 T2 Afternoon

D.6 NORMALITY TESTS AND NON-PARAMETRIC DATA FOR P1 vs P4

This file can be opened by GraphPad Prism (version 5.00 or later).

This file contains 23 data tables and 23 info tables:

Friedman test of P1 vs P4 M Latency

	Data Set-A
Table Analyzed	P1 vs P4 M Latency
Friedman test	
P value	0.0959
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	6.254
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 vs P4 After Latency:ANOVA results

	Data Set-A
Table Analyzed	P1 vs P4 After Latency
Friedman test	
P value	0.0412
Exact or approximate P value?	Exact
P value summary	*
Are means signif. different? (P < 0.05)	Yes
Number of groups	4
Friedman statistic	7.800
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 vs P4 After Latency:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	7.000	No	ns	0.7051	A-B	
T1 vs. T1	9.000	No	ns	0.2650	A-C	
T1 vs. T2	12.00	Yes	*	0.0437	A-D	
T2 vs. T1	2.000	No	ns	>0.9999	B-C	
T2 vs. T2	5.000	No	ns	>0.9999	B-D	
T1 vs. T2	3.000	No	ns	>0.9999	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	22.00	15.00	7.000	6	6	1.565
T1 vs. T1	22.00	13.00	9.000	6	6	2.012
T1 vs. T2	22.00	10.00	12.00	6	6	2.683
T2 vs. T1	15.00	13.00	2.000	6	6	0.4472
T2 vs. T2	15.00	10.00	5.000	6	6	1.118
T1 vs. T2	13.00	10.00	3.000	6	6	0.6708

Friedman test of P1 vs P4 Cumulative Time M:ANOVA results

	Data Set-A
Table Analyzed	P1 vs P4 Cumulative Time M
Friedman test	
P value	0.3171
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	3.712
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 vs P4 Cumulative Time M:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F

Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	7.000	No	ns	0.7051	A-B	
T1 vs. T1	3.500	No	ns	>0.9999	A-C	
T1 vs. T2	7.500	No	ns	0.5612	A-D	
T2 vs. T1	-3.500	No	ns	>0.9999	B-C	
T2 vs. T2	0.5000	No	ns	>0.9999	B-D	
T1 vs. T2	4.000	No	ns	>0.9999	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	19.50	12.50	7.000	6	6	1.565
T1 vs. T1	19.50	16.00	3.500	6	6	0.7826
T1 vs. T2	19.50	12.00	7.500	6	6	1.677
T2 vs. T1	12.50	16.00	-3.500	6	6	0.7826
T2 vs. T2	12.50	12.00	0.5000	6	6	0.1118
T1 vs. T2	16.00	12.00	4.000	6	6	0.8944

Friedman test of P1 vs P4 Cumulative Time A:ANOVA results

	Data Set-A
Table Analyzed	P1 vs P4 Cumulative Time A
Friedman test	
P value	0.3751
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	3.400
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 vs P4 Cumulative Time A:Multiple comparisons

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E	Data Set-F
Number of families	1					
Number of comparisons per family	6					
Alpha	0.05					
Dunn's multiple comparisons test	Rank sum diff.	Significant?	Summary	Adjusted P Value		
T1 vs. T2	-1.000	No	ns	>0.9999	A-B	
T1 vs. T1	-7.000	No	ns	0.7051	A-C	
T1 vs. T2	0.000	No	ns	>0.9999	A-D	
T2 vs. T1	-6.000	No	ns	>0.9999	B-C	
T2 vs. T2	1.000	No	ns	>0.9999	B-D	
T1 vs. T2	7.000	No	ns	0.7051	C-D	
Test details	Rank sum 1	Rank sum 2	Rank sum diff.	n1	n2	Z
T1 vs. T2	13.00	14.00	-1.000	6	6	0.2236
T1 vs. T1	13.00	20.00	-7.000	6	6	1.565
T1 vs. T2	13.00	13.00	0.000	6	6	0.000
T2 vs. T1	14.00	20.00	-6.000	6	6	1.342
T2 vs. T2	14.00	13.00	1.000	6	6	0.2236
T1 vs. T2	20.00	13.00	7.000	6	6	1.565

Friedman test of P1 vs P4 Frequency M

	Data Set-A
Table Analyzed	P1 vs P4 Frequency M
Friedman test	
P value	0.4418
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	2.845
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Friedman test of P1 vs P4 Frequency A

	Data Set-A
Table Analyzed	P1 vs P4 Frequency A
Friedman test	
P value	0.8386
Exact or approximate P value?	Exact
P value summary	ns
Are means signif. different? (P < 0.05)	No
Number of groups	4
Friedman statistic	0.9661
Data summary	
Number of treatments (columns)	4
Number of subjects (rows)	6

Normality and Lognormality Tests of P1 vs P4 M Latency:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.7905	0.9320	0.8312	0.8614
P value	0.0482	0.5954	0.1100	0.1940
Passed normality test (alpha=0.05)?	No	Yes	Yes	Yes
P value summary	*	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 vs P4 M Latency:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	17,79918	19,5441932685311			
R2	199,3282	199,072790064802			
R3	65,965	137,313301476186			
R4	19,59948	81,3036818571477			
R5	339,526	293,363916484969			
R6	13,63309	-74,7469331516362			
R1	49,7315		58,8459651164277		
R2	86,0987		76,8517405228682		
R3	24,06619		24,5632948835723		
R4	44,3655		47,0524112268709		
R5	32,3328		36,3568487731291		
R6	13,63309		6,55751947713178		
R1	13,29998			10,9545937919872	
R2	15,49996			13,1009228746795	
R3	3,83332			4,97462150073609	
R4	15,56665			15,4675910897389	
R5	7,69999			8,58792557692777	
R6	16,26665			19,0808951659306	
R1	15,49996				14,8981745505287
R2	0,233332				-1,51921268616834
R3	16,8667				20,790696116138
R4	43,2				37,208083352835
R5	13,29998				8,40073498809814
R6	17,96664				27,2881356785685

Normality and Lognormality Tests of P1 vs P4 After Latency:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8307	0.9740	0.7155	0.9711
P value	0.1089	0.9183	0.0090	0.8995
Passed normality test (alpha=0.05)?	Yes	Yes	No	Yes
P value summary	ns	ns	**	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 vs P4 After Latency:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	216,994	217,964210885648			
S2	31,7992	96,4306699570876			
S3	360,527	315,269307666882			
S4	31,0991	32,6970691143518			
S5	15,23304	-64,6080276668824			
S6	96,3315	154,230610042912			
S1	36,17625		39,8801363623581		
S2	29,1328		24,5065659209734		
S3	33,2324		31,8180574123599		
S4	14,26638		16,4444869709753		
S5	3,33324		4,13573048908044		
S6	52,8328		52,1888928442529		
S1	22,9334			23,1045094253819	
S2	10,23332			13,1091209131489	
S3	21			18,8055203772327	
S4	22,03336			20,8500696227673	
S5	19,76669			16,5510805746181	
S6	23			26,5464690868511	
S1	15,26663				16,8885312946968
S2	18,8666				19,3013974206083
S3	8,96666				8,60360263871971
S4	13,03332				12,2874392460584
S5	24,13332				22,985234027947
S6	14,49998				14,7003053719699

Normality and Lognormality Tests of P1 vs P4 Cumulative Time M:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.8522	0.9263	0.9885	0.8138
P value	0.1641	0.5519	0.9853	0.0779
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 vs P4 Cumulative Time M:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	162,1587	149,385953655254			
R2	82,798	91,6739796780795			
R3	103,7641	111,527429556678			
R4	82,298	61,3627806192778			
R5	184,4627	179,697152714056			
R6	107,6983	129,532503776656			
R1	28,83225		20,7744832743517		
R2	52,3991		63,1070909899906		
R3	73,5319		78,3010923433427		
R4	118,597		120,633700058982		
R5	43,166		46,3532947746202		
R6	107,6983		95,0548885587131		
R1	139,1			138,774573835809	
R2	33,6			41,0685621423457	
R3	111,3665			116,737179146559	
R4	171,3331			172,420104524321	
R5	80,6666			74,7140928308581	
R6	104,3998			96,7514875201074	
R1	88,6333				104,673944155723
R2	57,3333				64,4594196883468
R3	38,1666				11,1729392952489
R4	61				83,5849803116532
R5	40				43,3704558442772
R6	159				136,871460704751

Normality and Lognormality Tests of P1 vs P4 Cumulative Time A:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.9080	0.9570	0.9180	0.8436
P value	0.4234	0.7963	0.4908	0.1397
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 vs P4 Cumulative Time A:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	56,8316	59,7853625573074			
S2	58,7318	66,6229374426926			
S3	54,9991	52,2458518271858			
S4	43,0653	40,7349249130155			
S5	85,832	85,6733750869845			
S6	79,7651	74,1624481728142			
S1	57,18192		63,1966280253922		
S2	50,7324		46,7443729941969		
S3	26,89925		21,6259386532559		
S4	65,9652		78,1171619746078		
S5	97,0973		94,5694170058031		
S6	126,0653		119,687851346744		
S1	54,7666			44,4571109685948	
S2	67,2332			75,0546260675554	
S3	101,3666			95,0956093110232	
S4	111,9332			113,270757355644	
S5	112,2332			133,311740599111	
S6	177,5663			163,909255698072	
S1	42,8				38,7971324721771
S2	54,9333				74,6485974544255
S3	21,89996				-15,9389913889984
S4	140,1997				143,013720861156
S5	60,1666				107,162255878908
S6	225,433				197,749844722332

Normality and Lognormality Tests of P1 vs P4 Frequency M:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.9773	0.9493	0.9275	0.8810
P value	0.9374	0.7344	0.5607	0.2738
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 vs P4 Frequency M:Normal QQ plot

	Actual	T1	T2	T1	T2

R1	107	108,759486248303			
R2	116	124,033850360203			
R3	97	94,9071804183636			
R4	89	79,6328163064642			
R5	51	56,312723631211			
R6	151	147,353943035456			
R1	25		28,8530192007942		
R2	77		93,1313001848386		
R3	76		67,6921581429663		
R4	171		180,480314132539		
R5	128		116,202033148495		
R6	151		141,641175190367		
R1	110			105,709927109652	
R2	47			57,4245796450615	
R3	138			141,242087021605	
R4	94			86,281040529436	
R5	113			119,772339003944	
R6	94			86,281040529436	
R1	107				96,0814063498382
R2	111				113,323943998327
R3	54				46,0093893350061
R4	80				84,7877634847033
R5	60				63,2519269834952
R6	66				74,5455698486301

Normality and Lognormality Tests of P1 vs P4 Frequency A:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
D'Agostino & Pearson test				
K2	N too small	N too small	N too small	N too small
P value				
Passed normality test (alpha=0.05)?				
P value summary				
Shapiro-Wilk test				
W	0.9353	0.9333	0.9900	0.9296
P value	0.6219	0.6054	0.9892	0.5772
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 vs P4 Frequency A:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	62	58,4941727351848			
S2	76	78,5653852129093			
S3	20	27,8505052373942			
S4	109	96,7679481204241			
S5	126	116,839160598149			
S6	133	147,482828095939			
S1	68		62,8968172362996		
S2	117		125,232027634517		
S3	166		171,1031827637		
S4	82		90,6137402813094		
S5	112		108,76797236548 3		
S6	157		143,386259718691		
S1	59			54,7350492081036	
S2	78			80,0300116755019	
S3	11			16,1160360278933	
S4	103			102,969988324498	
S5	163			166,883963972107	
S6	135			128,264950791896	
S1	115				102,272420595247
S2	77				75,8940413034123
S3	64				62,0609127380865
S4	82				88,439292029921
S5	116				123,392110931866
S6	39				40,9412224014672

Normality and Lognormality Tests of P1 vs P4 After Latency:Tabular results

	T1	T2	T1	T2
Test for normal distribution				
Shapiro-Wilk test				
W	0.8307	0.9740	0.7155	0.9711
P value	0.1089	0.9183	0.0090	0.8995
Passed normality test (alpha=0.05)?	Yes	Yes	No	Yes
P value summary	ns	ns	**	ns
Number of values	6	6	6	6

Normality and Lognormality Tests of P1 vs P4 After Latency:Normal QQ plot

	Actual	T1	T2	T1	T2

S1	216,994	217,964210885648			
S2	31,7992	96,4306699570876			
S3	360,527	315,269307666882			
S4	31,0991	32,6970691143518			
S5	15,23304	-64,6080276668824			
S6	96,3315	154,230610042912			
S1	36,17625		39,8801363623581		
S2	29,1328		24,5065659209734		
S3	33,2324		31,8180574123599		
S4	14,26638		16,4444869709753		
S5	3,33324		4,13573048908044		
S6	52,8328		52,1888928442529		
S1	22,9334			23,1045094253819	
S2	10,23332			13,1091209131489	
S3	21			18,8055203772327	
S4	22,03336			20,8500696227673	
S5	19,76669			16,5510805746181	
S6	23			26,5464690868511	
S1	15,26663				16,8885312946968
S2	18,8666				19,3013974206083
S3	8,96666				8,60360263871971
S4	13,03332				12,2874392460584
S5	24,13332				22,985234027947
S6	14,49998				14,7003053719699

D.7 NORMALITY TESTS AND NON-PARAMETRIC DATA FOR CORRELATIONS OF HIERARCHY

This file can be opened by GraphPad Prism (version 5.00 or later).

This file contains 8 data tables and 8 info tables:

Correlation of Dom & Sub Lat

	Subordinate vs. Dominant
Spearman r	
r	-0.3333
95% confidence interval	
P value	
P (two-tailed)	0.4279
P value summary	ns
Exact or approximate P value?	Exact
Significant? (alpha = 0.05)	No
Number of XY Pairs	8

Correlation of Sub & Sub Lat:Tabular results

	Subordinate vs. Subordinate
Spearman r	
r	0.4000
95% confidence interval	
P value	
P (two-tailed)	0.7500
P value summary	ns
Exact or approximate P value?	Exact
Significant? (alpha = 0.05)	No

Number of XY Pairs	4

Correlation of Sub & Sub Lat:XY data

	Subordinate	Subordinate
R3	3,83332	16,26665
S1	22,9334	19,76669
R3T2	16,8667	17,96664
S1T2	15,26663	24,13332

Correlation of Dom & Sub CT

	Subordinate vs. Dominant
Spearman r	
r	-0.2857
95% confidence interval	
P value	
P (two-tailed)	0.5008
P value summary	ns
Exact or approximate P value?	Exact
Significant? (alpha = 0.05)	No
Number of XY Pairs	8

Correlation of Sub & Sub CT

	Subordinate vs. Subordinate
Spearman r	
r	-0.7778
95% confidence interval	
P value	
P (two-tailed)	0.3333
P value summary	ns
Exact or approximate P value?	Exact

Significant? (alpha = 0.05)	No
Number of XY Pairs	4

Correlation of Dom & Sub Freq:Tabular results

	Subordinate vs. Dominant
Spearman r	
r	0.5150
95% confidence interval	
P value	
P (two-tailed)	0.1967
P value summary	ns
Exact or approximate P value?	Exact
Significant? (alpha = 0.05)	No
Number of XY Pairs	8

Correlation of Dom & Sub Freq:XY data

	Subordinate	Dominant
R1	113	110
R4	47	94
S3	157	166
S4	117	82
R1T2	60	107
R4T2	111	80
S3T2	39	64
S4T2	77	82

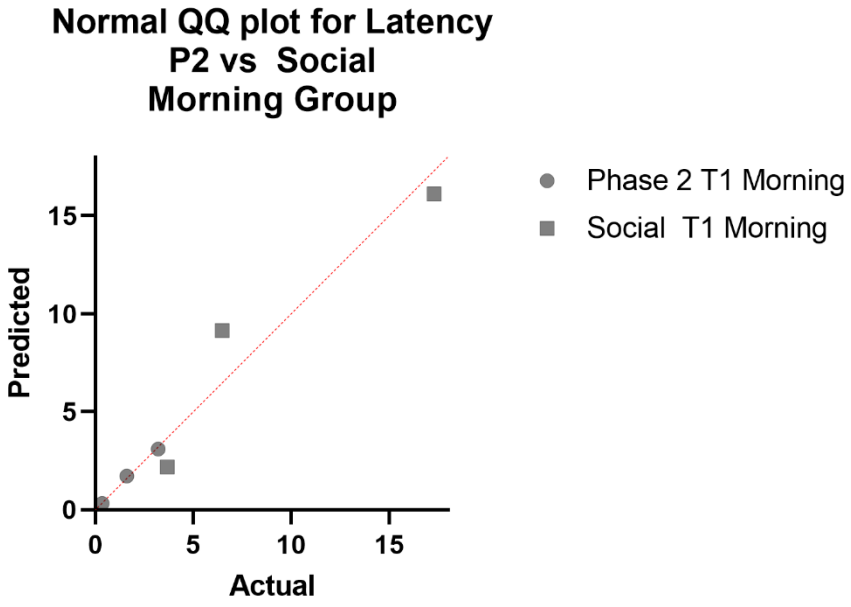
Correlation of Sub & Sub Freq

	Subordinate vs. Subordinate
Spearman r	
r	0.2000
95% confidence interval	

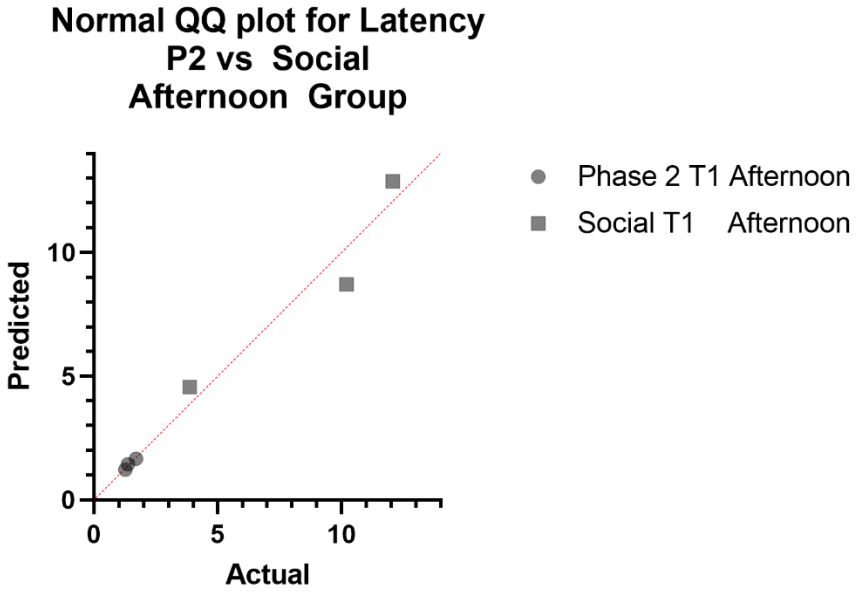
P value	
P (two-tailed)	0.9167
P value summary	ns
Exact or approximate P value?	Exact
Significant? (alpha = 0.05)	No
Number of XY Pairs	4

D.8 QQ PLOTS FOR P2 vs Social

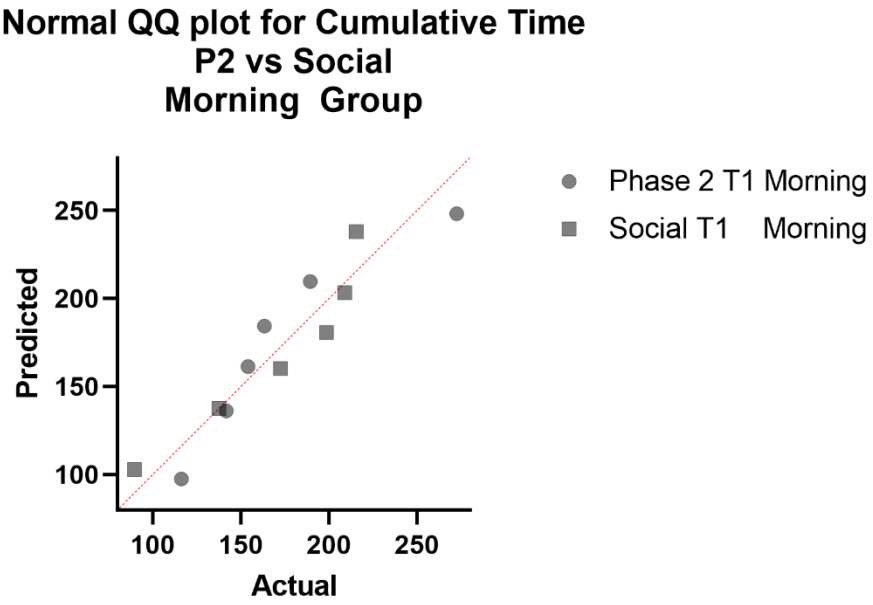
N=3



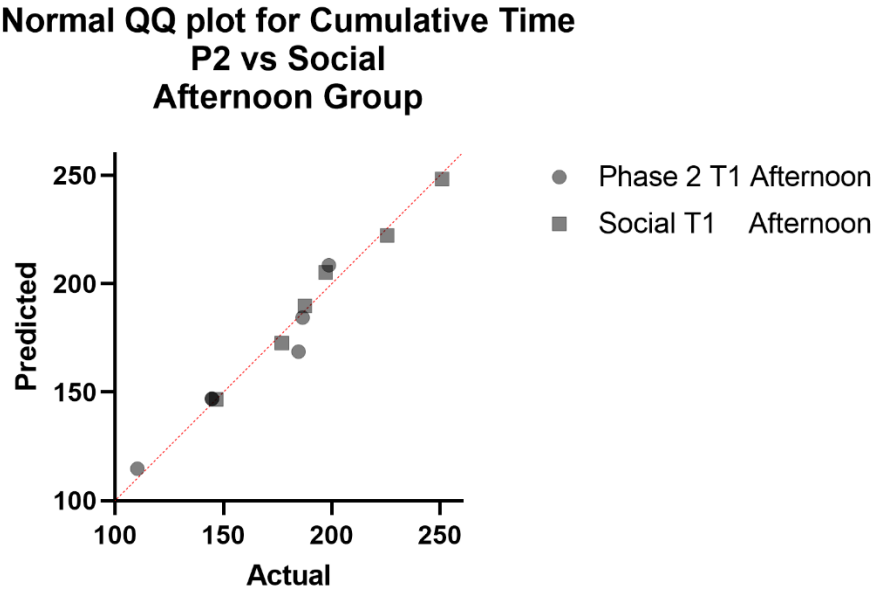
N=3



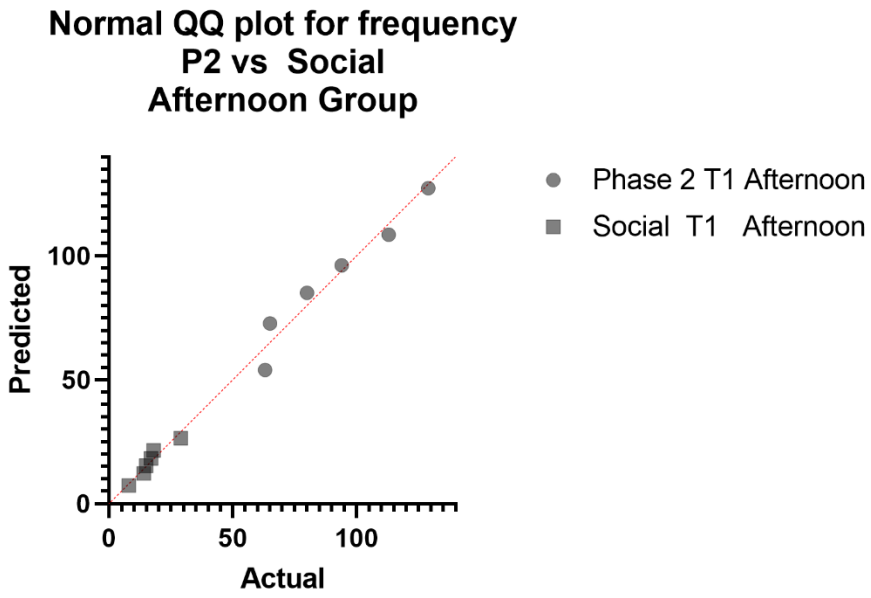
N=6


Normal QQ plot for Cumulative Time
P2 vs Social
Morning Group
Predicted
Actual
100
150
200
250
Phase 2 T1 Morning
Social T1 Morning

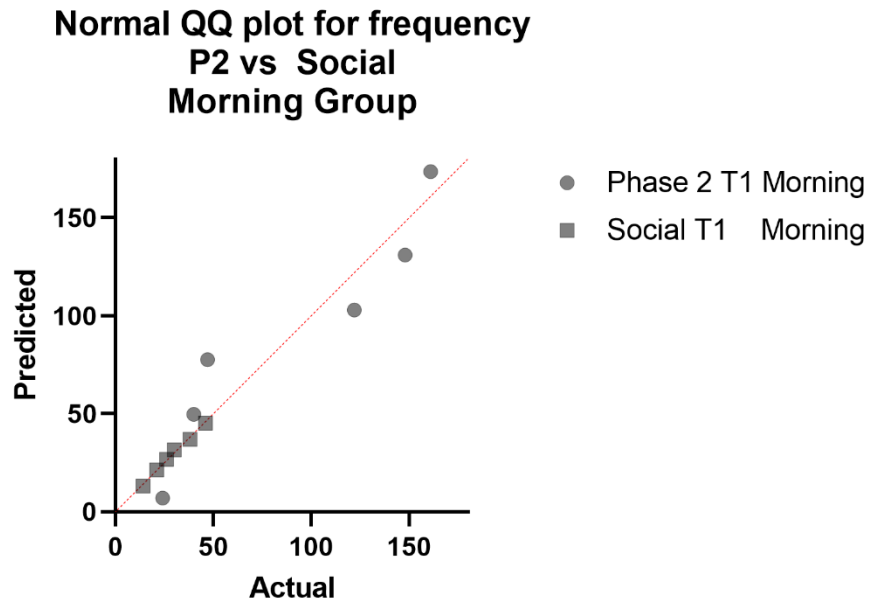
N=6


Normal QQ plot for Cumulative Time
P2 vs Social
Afternoon Group
Predicted
Actual
100
150
200
250
Phase 2 T1 Afternoon
Social T1 Afternoon

N=6


Normal QQ plot for frequency
P2 vs Social
Afternoon Group
Predicted
Actual
0
50
100
Phase 2 T1 Afternoon
Social T1 Afternoon

N=6


Normal QQ plot for frequency
P2 vs Social
Morning Group
Predicted
Actual
0
50
100
150
Phase 2 T1 Morning
Social T1 Morning

D.9 NORMALITY TESTS AND NON-PARAMETRIC DATA FOR P2 vs SOCIAL

This file can be opened by GraphPad Prism (version 5.00 or later).

This file contains 18 data tables and 18 info tables:

Normality and Lognormality Tests of P2 vs Social M Latency:Tabular results

	P2T1M	SocialT1M
Test for normal distribution D'Agostino & Pearson test K2 P value Passed normality test (alpha=0.05)? P value summary	N too small	N too small
Shapiro-Wilk test W P value Passed normality test (alpha=0.05)? P value summary	0.9955 0.8719 Yes ns	0.8963 0.3737 Yes ns
Number of values	3	3

Normality and Lognormality Tests of P2 vs Social M Latency:Normal QQ plot

	Actual	P2T1M	SocialT1M
R2	3,2	3,10085812988231	
R4	1,59996	1,71109766666667	
R6	0,333333	0,321337203451024	
R2	3,66666		2,17867760988964
R4	17,3		16,1102023901104
R6	6,46666		9,14444

Normality and Lognormality Tests of P2 vs Social A Latency:Tabular results

	P2T1A	SocialT1A
Test for normal distribution		
D'Agostino & Pearson test		
K2	N too small	N too small
P value		
Passed normality test (alpha=0.05)?		
P value summary		
Shapiro-Wilk test	0.9118	0.9100
W	0.4242	0.4181
P value	Yes	Yes
Passed normality test (alpha=0.05)?	ns	ns
P value summary	3	3
Number of values		

Normality and Lognormality Tests of P2 vs Social A Latency:Normal QQ plot

	Actual	P2T1A	SocialT1A
S3	1,36662	1,44442666666667	
S4	1,26666	1,22491298532494	
S5	1,7	1,6639403480084	
S3	3,86666		4,55314967842407
S4	10,2		8,71112
S5	12,0667		12,8690903215759

Normality and Lognormality Tests of P2 vs Social M Cumulative Time:Tabular results

	P2T1M	SocialT1M
Test for normal distribution D'Agostino & Pearson test K2 P value Passed normality test (alpha=0.05)? P value summary	N too small	N too small
Shapiro-Wilk test W P value Passed normality test (alpha=0.05)? P value summary	0.8873 0.3041 Yes ns	0.8946 0.3429 Yes ns
Number of values	6	6

Normality and Lognormality Tests of P2 vs Social M Cumulative Time:Normal QQ plot

	Actual	P2T1M	SocialT1M
R1	141,795	136,19269860043	
R2	116,267	97,5864475083569	
R3	272,649	248,304552491643	
R4	189,496	209,69830139957	
R5	154,033	161,479302062409	
R6	163,433	184,411697937591	
R1	215,6		238,123372185171
R2	172,633		160,229867424205
R3	209,1		203,488560441157
R4	89,6332		102,909694481496
R5	198,633		180,803199242461
R6	137,5		137,54450622551

Normality and Lognormality Tests of P2 vs Social A Cumulative Time:Tabular results

	P2T1A	SocialT1A

Test for normal distribution D'Agostino & Pearson test K2 P value Passed normality test (alpha=0.05)? P value summary Shapiro-Wilk test W P value Passed normality test (alpha=0.05)? P value summary Number of values	N too small	N too small 0.9853 0.9748 Yes ns 6

Normality and Lognormality Tests of P2 vs Social A Cumulative Time:Normal QQ plot

	Actual	P2T1A	SocialT1A
S1	186,655	184,521990226064	
S2	110,331	114,792236990025	
S3	184,76	168,794584439943	
S4	144,733	147,065311715142	
S5	144,733	147,065311715142	
S6	198,766	208,533763009975	
S1	146,733		146,709753740331
S2	225,6		222,335991078785
S3	251,066		248,378246259669
S4	187,633		189,809354853204
S5	197,266		205,278645146796
S6	176,966		172,752008921215

Normality and Lognormality Tests of P2 vs Social M Frequency:Tabular results

	P2T1M	SocialT1M
Test for normal distribution D'Agostino & Pearson test K2 P value Passed normality test (alpha=0.05)?	N too small	N too small

Test for normal distribution D'Agostino & Pearson test K2 P value Passed normality test (alpha=0.05)? P value summary	N too small	N too small
Shapiro-Wilk test W P value Passed normality test (alpha=0.05)? P value summary	0.9067 0.4153 Yes ns 6	0.9853 0.9748 Yes ns 6
Number of values		

Normality and Lognormality Tests of P2 vs Social A Cumulative Time:Normal QQ plot

	Actual	P2T1A	SocialT1A
S1	186,655	184,521990226064	
S2	110,331	114,792236990025	
S3	184,76	168,794584439943	
S4	144,733	147,065311715142	
S5	144,733	147,065311715142	
S6	198,766	208,533763009975	
S1	146,733		146,709753740331
S2	225,6		222,335991078785
S3	251,066		248,378246259669
S4	187,633		189,809354853204
S5	197,266		205,278645146796
S6	176,966		172,752008921215

Normality and Lognormality Tests of P2 vs Social M Frequency:Tabular results

	P2T1M	SocialT1M
Test for normal distribution D'Agostino & Pearson test K2 P value Passed normality test (alpha=0.05)?	N too small	N too small

P value summary		
Shapiro-Wilk test	0.8620	0.9875
W	0.1961	0.9823
P value	Yes	Yes
Passed normality test (alpha=0.05)?	ns	ns
P value summary	6	6
Number of values		

Normality and Lognormality Tests of P2 vs Social M Frequency:Normal QQ plot

	Actual	P2T1M	SocialT1M
R1	40	49,7143382549181	
R2	47	77,6609540898675	
R3	24	7,04692071424313	
R4	161	173,619745952424	
R5	122	103,005712576799	
R6	148	130,952328411749	
R1	38		36,9676492243937
R2	26		26,7329036143722
R3	30		31,6004297189612
R4	21		21,3656841089396
R5	14		13,1712967447151
R6	46		45,1620365886182

Normality and Lognormality Tests of P2 vs Social A Frequency:Tabular results

	P2T1A	SocialT1A
Test for normal distribution D'Agostino & Pearson test K2	N too small	N too small
P value		
Passed normality test (alpha=0.05)?		
P value summary		
Shapiro-Wilk test	0.9284	0.9173
W	0.5681	0.4864
P value	Yes	Yes
Passed normality test	ns	ns

Median of differences Median	6.133
How effective was the pairing? rs (Spearman) P value (one tailed) P value summary Was the pairing significantly effective?	-0.5000 0.5000 ns No

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Wilcoxon test of P2 vs Social A Latency

	Data Set-A
Table Analyzed	P2 vs Social A Latency
Column B vs. Column A	SocialT1A vs. P2T1A
Wilcoxon matched-pairs signed rank test P value Exact or approximate P value? P value summary Significantly different (P < 0.05)? One- or two-tailed P value? Sum of positive, negative ranks Sum of signed ranks (W) Number of pairs Number of ties (ignored)	0.2500 Exact ns No Two-tailed 6.000 , 0.000 6.000 3 0
Median of differences Median	8.933
How effective was the pairing? rs (Spearman) P value (one tailed) P value summary Was the pairing significantly effective?	0.5000 0.5000 ns No

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Wilcoxon test of P2 vs Social M Cumulative Time

	Data Set-A

Table Analyzed	P2 vs Social M Cumulative Time
Column B vs. Column A	SocialT1M vs. P2T1M
Wilcoxon matched-pairs signed rank test	>0.9999
P value	Exact
Exact or approximate P value?	ns
P value summary	No
Significantly different (P < 0.05)?	Two-tailed
One- or two-tailed P value?	10.00 , -11.00
Sum of positive, negative ranks	-1.000
Sum of signed ranks (W)	6
Number of pairs	0
Number of ties (ignored)	
Median of differences	9.334
Median	
How effective was the pairing?	-0.2000
rs (Spearman)	0.3569
P value (one tailed)	ns
P value summary	No
Was the pairing significantly effective?	

Wilcoxon test of P2 vs Social A Cumulative Time

	Data Set-A
Table Analyzed	P2 vs Social A Cumulative Time
Column B vs. Column A	SocialT1A vs. P2T1A
Wilcoxon matched-pairs signed rank test	0.1563
P value	Exact
Exact or approximate P value?	ns
P value summary	No
Significantly different (P < 0.05)?	Two-tailed
One- or two-tailed P value?	18.00 , -3.000
Sum of positive, negative ranks	15.00
Sum of signed ranks (W)	6
Number of pairs	0
Number of ties (ignored)	

Median of differences Median	47.72
How effective was the pairing? rs (Spearman) P value (one tailed) P value summary Was the pairing significantly effective?	-0.5798 0.1167 ns No

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Wilcoxon test of P2 vs Social M Frequency

	Data Set-A
Table Analyzed	P2 vs Social M Frequency
Column B vs. Column A	SocialT1M vs. P2T1M
Wilcoxon matched-pairs signed rank test P value Exact or approximate P value? P value summary Significantly different (P < 0.05)? One- or two-tailed P value? Sum of positive, negative ranks Sum of signed ranks (W) Number of pairs Number of ties (ignored)	0.0938 Exact ns No Two-tailed 2.000 , -19.00 -17.00 6 0
Median of differences Median	-61.50
How effective was the pairing? rs (Spearman) P value (one tailed) P value summary Was the pairing significantly effective?	-0.2571 0.3292 ns No

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Wilcoxon test of P2 vs Social A Frequency

	Data Set-A
Table Analyzed	P2 vs Social A Frequency
Column B vs. Column A	SocialT1A vs. P2T1A
Wilcoxon matched-pairs signed rank test	
P value	0.0313
Exact or approximate P value?	Exact
P value summary	*
Significantly different (P < 0.05)?	Yes
One- or two-tailed P value?	Two-tailed
Sum of positive, negative ranks	0.000 , -21.00
Sum of signed ranks (W)	-21.00
Number of pairs	6
Number of ties (ignored)	0
Median of differences	-76.00
Median	
How effective was the pairing?	
rs (Spearman)	-0.2000
P value (one tailed)	0.3569
P value summary	ns
Was the pairing significantly effective?	No