A NEW PARADIGM TO ANALYZE EXTENSIVE POST-REINFORCEMENT OR SOCIAL REINFORCEMENT DELAYS IN FREE-FLYING HONEY BEES (*APIS MELLIFERA*)

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CHAPTER I

ABSTRACT

We have developed a novel paradigm to study and analyze how free-flying honey bees (*Apis mellifera*) react when presented with varying schedules of post-reinforcement delays of either 0s, 300s, or 600s. We measured inter-visit-interval, response length, inter-response-time, and response rate in addition to monitoring temperature. Honey bees exposed to these large intervals react in multiple patterns compared to groups not encountering delays. Three patterns of inter-visit-intervals were observed, and in most cases, the experimental bees had larger inter-visit-intervals when encountering the post-reinforcement delays. Based on response length, we believe adjunctive responses were observed during the delay intervals. We saw no group differences in inter-response time. Honey bees with larger response rates tended to not finish the experiment while the removal of the delay intervals increased subject response rates for those subjects that completed the trials. Our findings and protocol lead us to consider we have in fact studied social pre-reinforcement delays.

CHAPTER II

INTRODUCTION & LITERATURE REVIEW

We have developed a novel paradigm to study what we have defined as a delay of social reinforcement using honey bees *(Apis mellifera*). We broadly define social-reinforcement as a form of reinforcement being delivered to a subject via an interaction with another living entity. Specifically for honey bees, social reinforcement could be provided by other hive mates after performing a variety of behaviors such as recruitment or trophallaxis. We believe honey bees are an excellent candidate for investigations into social reinforcement, as honey bees are a social insect with a complex social structure wherein individuals can be observed working for a common goal. Additionally, honey bees provide a unique opportunity to study novel reinforcement contingencies, for they shuttle their reinforcing sugar water or nectar to their hive and quickly return to a foraging location to fill their crops; only to return back to the hive once their crop is full. Our delay of reinforcement was introduced *after* the bee's crop had been filled. We did not allow her to return to the hive for an extensive interval; thus delaying any social reinforcement incurred during recruitment or when providing her consumed sugar water to the hive or individual bees (Abramson, Wells, Wenner, & Wells, 2011).

We offer our research hypothesis: if large post-reinforcement or social-reinforcement delay intervals are administered, then honey bees will have longer inter-visit-intervals and make more responses per visit/trial compared to bees not exposed to a post-reinforcement delay. We wish to briefly survey the pre- and post-reinforcement literature to solidify our claim that we have developed a novel paradigm.

Pre-Reinforcement Delays

There is no question that contemporary investigations into the effects of delayed reinforcement on behavior have been extensive (Lattal, 2010). An ample amount of literature has been produced on the subject; with far reaching empirical and theoretical developments such as delay-reduction theory, the correlation-based law of effect, and behavioral economics (Lattal, 2010). A variety of species have been used to study the effects of reinforcement delays; including: rats, pigeons, Siamese fighting fish, chicks, rhesus monkeys, chimpanzees, humans, and honey bees (Wood, 1933; Riesen, 1940; Chung, 1965; Lee, & Bitterman, 1990; Beardsley, & Blaster, 1993; Critchfield, & Lattal, 1993; Lattal, & Metzger, 1994; Mazur, 2000; Okouchi, 2009). This literature review only focuses on delays in operant and instrumental conditioning, and does not consider classical conditioning.

Pre-1950

Early exploration into the effects of a delay of reinforcement (ranging from 30s - 300s intervals) saw negligible effects on learning or errors (Hunter, 1913; Watson, 1917; Yarbrough, 1921; Warden, & Hass, 1927). Our current understanding of a delay of reinforcement as having an effect on decreasing learning was not supported until

Hamilton (1929) found a 60s delay required more trials and increased the amount of time rats needed to complete a maze. Hamilton’s results were obtained by a procedural modification which simply used different goal boxes to control for associations of secondary reinforcement and thus effectively delaying all obvious forms of reinforcement (Renner, 1964). Roberts (1930) further expanded our early theoretical understanding of the effects of delays and of reinforcement by proposing that a delayed subject's increase in errors was due to a “recency” variable. “Recency” may be defined as the interval between a behavior and its reinforcement.

Renner (1964) outlines the theoretical development up until the early 1930’s with four concise points: 1) reinforcement delays increase errors and the criteria levels of time and trial scores; 2) associations of immediate rewards in goal boxes had to be avoided; 3) the behaviorist field was in disagreement as to how long of a delay was required to disrupt learning (though it was already apparent that longer delays impacted learning more than smaller intervals); and 4) no theoretical model explaining reinforcement delays had been postulated (Renner, 1964, pp 342).

Hull (1932) was instrumental in producing a theoretical model explaining reinforcement delays with his coherent goal-gradient hypothesis: “[Behaviors become more weak] as they are more remote...from the goal reaction” (Hull, 1932, pp 25-26). This hypothesis did not just address the phenomenon of reinforcement delays, but also encompassed decades of previous research showing subjects prefer immediate rewards (Renner, 1964). Hull’s goal-gradient hypothesis spurred an attempt by many researchers to creatively study and determine what intervals of delay of reinforcement impacted learning by designing stronger experiments which added multiple groups, subjects, and conditions; this trend greatly solidified our modern understanding of reinforcement delays. Wolfe (1934) explored multiple delay intervals (0s, 5s, 30s, 60s, 150s, 300s, 600s, and 1,200s) and determined intervals less than 30s did not disrupt learning while delay intervals longer than a minute significantly decreased learning.

Perin (1943a) was the first to minimize the effects of secondary reinforcement following a response while also developing a quantitative model to predict behavioral reactions to small delays of reinforcement intervals (0s, 2s, 5s, 10s, 30s, and 60s) using rats. Perin (1943b) outlined a provocative point in regards to delay intervals (determined from reciprocal fitted equations for each group's latency to respond): any interval greater than 17s does not allow responses to be learned; refining and roughly confirming Wolfe's (1934) findings. Perin (1943a) expanded this extremely precise interval to14s-21s to account for the variability of his subject's response latencies. The general equation he used to determine this interval is:

response time = constant x 10^(-rate of decrease in response times x training trials) + presumptive asymptote of delay group.

As a delay increases, the constant, rate of decrease in response times, and presumptive asymptote also increase; thus increasing the response time (or response latency). Perin (1943b) next studied small delays of reinforcement using delay intervals of 0s, 2s, 5s, 10s, 20s, and 30s. He found groups with 2s delays were able to perform as perfectly as 0s delay groups and that 30s delays resulted in correct responses not being learned.

Grice (1948) controlled for secondary reinforcement by creating a condition that reinforced subjects in a goal box of the same or different color as the S+. He also studied even smaller intervals of delay (0s, .5s, 1.2s, 2s, 5s, and 10s) than Perin (1943A). Grice (1948) found learning began to be impaired at 10s of delay, and that allowing subjects to be immediately reinforced via secondary reinforcement greatly improved their learning even when exposed to delays of primary reinforcement. The affects of different goal boxes on behavior was further explored years later by Lawrence and Hommel (1961) while using 10s, 20s, 30s, and 60s delay intervals in a psuedo-replication of Grice (1948). Three conditions of goal boxes were utilized: 1) a grey goal box, 2) a black goal box and a white goal box, and 3) a black and white goal box (Lawrence, & Hommel, 1961). Lawrence and Hommel (1961) found rats exposed to a black goal box and a white goal box were able to maintain their accuracy of choice at 10s intervals compared to rats exposed to identical goal boxes. This team of researchers also note that the rats exposed to two different goal boxes were able to maintain their choice accuracy at all delay intervals; though once exposed to a 30s interval the rats began to hesitate before exiting the start box of the runway.

Following the previously described findings, Hull (1949) combined a clear set of 18 postulates and quantitative behavioral laws which have been invaluable for behavioral researchers since its publication. For our purposes, his eighth postulate is the most important: “The greater the delay in reinforcement, the weaker will be the resulting reaction potential” (Hull, 1949, pp 176). A reaction potential here is defined as the likelihood that a correct response will be made following a specific stimulus. Hull (1949) defined this relationship quantitatively as:

$$J = 10^{(-jt)}$$

Here, “j” is the incentive of reinforcement and “t” is time; this was reiterated years later with Chung’s (1965) finding of an inversely exponential relationship between response rates and reinforcement delays (Hull, 1949, pp.176).

1950’s

Nearly every published paradigm described above essentially follows the same basic procedure: a subject elicits or emits a behavior and the reinforcement for this behavior is delayed. There are of course alterations to this basic paradigm, and the 1950’s saw a rise in diversity of reinforcement delay experimental designs; specifically explorations into how various schedules of reinforcement affect behavior when concurrently paired with reinforcement delays.

The effects of variable delays of reinforcement on extinction resistance have been a recurring phenomenon of interest. An occasional delay of reinforcement has been shown to produce a higher resistance to extinction than an immediately and consistently reinforced subject (Crum, Brown, & Bitterman, 1951). Groups exposed to small percentages (25%) of variable reinforcement when paired with 30s delay intervals extinguish faster than higher percentages of variable reinforcement (Wike, & McNamara, 1957). Interestingly, Wike and McNamara (1957) observed no difference in extinction rates between groups scheduled at 50% and 75% partial reinforcement on 30s delay intervals. Variable and partial variable delay schedules of reinforcement have been shown to decrease running speeds in rats as well as increase resistance to extinction (Petterson, 1956). Rats exposed to reinforcement delay intervals of 20s were shown to have retarded learning and be more resistant to extinction than rats exposed to 0s intervals (Marx, McCoy, & Tombaugh, 1965).

Also beginning in the early 1950's was a focus on testing the upper limits of delay intervals on maintaining response rates. Ferster (1953) was able to maintain baseline response rates in pigeons during reinforcement delays up to 60s with very gradual increases in the delay interval. However, without this gradual increase, response rates almost declined to zero on a 60s delay (Ferster, 1953). Dews (1960) was able to maintain response behaviors with delays in excess of 100s in pigeons; and strongly confirmed an inverse relationship between an increase in delay and pecking responses. Rats were found to perform similarly (and seemingly superiorly) to Ferster's (1953) pigeons on 30s delays (Azzi, Fix, Keller, & Rocha e Silva, 1964). These researchers added a signaled delay phase (complete darkness) at delays of 20s and 30s and found this signal served to increase response rates in nearly every case (Azzi, Fix, Keller, & Rocha e Silva, 1964).

1960's & 1970's

The 1960's and 1970's saw a focus on choice experiments utilizing concurrent situational paradigms and the analysis of pairing delays with various schedules of reinforcement. Researchers have shown pigeon's exponential preference for no reinforcement delays (Chung, 1965). This line of research was continued when pigeons were offered a choice between two varying levels of delay (Chung, & Herrnstein, 1967). Subject's response rates were inversely proportional to the reinforcement delay (Chung, & Herrnstein, 1967). These observations were confirmed decades later with choice experiments investigating subject preference for delay, rate, and amount of reinforcement (e.g. Wogar, Bradshaw, & Szabadi, 1992; Mazur, 2000). Pigeons have been observed to show little preference between reinforcement on delay intervals compared to reinforcement provided on fixed-interval schedules (Neuringer, 1969). Any observed preference for fixed intervals were diminished when the delay and fixed intervals were on different durations, and when the delay was unsignaled (Neuringer, 1969).

Responses in rats on variable-interval schedules have been shown to decrease at greater delay intervals (Pierce, Hanford, & Zimmerman, 1972). These researchers also found evidence of a clear difference in fixed-delay and fixed-interval schedules; higher response rates were observed for conditions required to terminate a delay period (Pierce, Hanford, & Zimmerman, 1972). This finding coincides with Neuringer's (1969) utilization of two-key situational paradigms. Morgan (1972) found lever presses by rats were not affected by delays of .75s, 3s, and 12s on fixed ratio schedules requiring up to nine responses; post-reinforcement pauses increased with larger delay intervals. Means of variable delay intervals affect behavior much in the same manner as a variable interval of reinforcement (Hursh, & Fantino, 1973). Mixed/variable delays in reinforcement have been found to be preferred by pigeons compared to constant delays in reinforcement (Cicerone, 1976). Sizemore & Lattal (1978) found delays of reinforcement on variable-interval schedules presented in tandem with fixed-time schedules usually resulted in a decrease in response rates, but there were individual pigeons that did not conform to this trend. Sizemore & Lattal (1978) also report that longer delays on these concurrent schedules resulted in decreases in response rates. A return to baseline performance was observed when the researchers returned subjects to a variable-interval schedule (Sizemore, & Lattal, 1978).

Beginning in the mid 1970's, a distinction between signaled and unsignaled delays of reinforcement became common-place; allowing for a more specified analyses of reinforcement delays. As investigating variable-interval schedules was still a fruitful area of behavioral research, unsignaled delays were first paired with a variety of variable-interval reinforcement schedules (e.g Williams, 1976; Sizemore, & Lattal, 1978). Williams (1967) had pigeons trained on a variable-interval schedule and found an unsignaled delay of reinforcement as little as 3s had a remarkable reduction in responding. While he had no signaled delay group, Williams maintains the unsignaled delays were the primary factor for this change in response rates, yet previous literature had found no noticeable reduction in response rate for any delay less than 10s (Bower, 1961). Richards and Hittesdorf (1978) also found unsignaled delays of reinforcement produced lower response rates in pigeons when compared to various schedules of signaled reinforcement; indeed all subjects responded more slowly during unsignaled conditions. This highly noticeable difference between signaled and unsignaled delays in-part led to the development of the marking hypothesis which supposes learning can be unaffected by 60s delay intervals if the choice response is immediately followed by an intense signal such as light, noise, or handling (Lieberman, McIntosh, & Thomas, 1979). The marking hypothesis is supported by the finding that learning was not observed if handling of the rat subjects was delayed or omitted (Lieberman, McIntosh, & Thomas, 1979). Lieberman, McIntosh, and Thomas (1979) argue that a marker (e.g. a light being turned on) immediately following a response draws the subject's attention to the response and the schedule conditions; thus providing a more “memorable” stimulus. This hypothesis has far reaching implications, for any additional stimulus “marking” the response (e.g. a lever-press clicking) could confound reinforcement delay research.

1980’s, 1990’s, & 2000’s

This interest in determining how unsignaled and signaled delays differ in their effects on behavior was continued into the early 1980’s; notable publications are summarized in Table I.

Table I

Author	Subject	Delay	Findings
Richards, 1981	Pigeons	Unsignaled and signaled delay intervals of 10s, 5s, 2.5s, 1s, & .5s	Higher response rates were observed when subjects were exposed to signaled delays versus unsignaled delays. Some subjects actually responded faster on unsignaled delay intervals of .5s and 1s than immediately reinforced subjects.
Lattal, & Ziegler, 1982	Pigeons	Signaled or an unsignaled delay of .5s	Response rates decreased during the signaled blackout condition while unsignaled delays caused an increase in responses compared to an immediately reinforced condition.
Lattal, 1984	Pigeons	Signaled or an unsignaled delay of 20s	Blackout period during the reinforcement delay interval resulted in higher pecking responses in pigeons when compared to an unsignaled delay.
Thomas, Lieberman, McIntosh, & Ronaldson, 1983	Rats	120s	Subjects have comparable discrimination abilities if a stimulus marker was displayed immediately after and before a response. Stimulus markers signaling the end of a delay interval did not improve learning.

Liberman, Davidson, & Thomas, 1985	Pigeons	3s	Immediate markers are slightly more effective at facilitating learning than delayed markers.
Arbuckle, & Lattal, 1988	Pigeons	Unsignaled delays of less than .5s compared to 5s	5s intervals increased reinforced inter-response times. Pigeons made multiple pecks on .5s unsignaled delays compared to single pecks on 0s delay intervals.

A trend beginning in the late 1980's was to have subjects acquire and learn behaviors while under delays of reinforcement. This trend in determining the upper limits of delay intervals on response acquisition has continued into the 2000's. We have summarized notable publications in Table II.

Table II

Author	Subjects	Delay	Findings
Schaal, & Branch, 1988	Unshaped rats	Signaled .5s-9s & 27s	Maintain baseline response rates at 9s using briefly signaled changes in key color. At 27s intervals, briefly displayed signals did not maintain baseline pecking behavior, but continuous signals during the delay interval were able to maintain the desired behavior.
Lattal, & Gleeson, 1990	Unshaped rats & pigeons	Unsignaled 10s	Successfully observed response in subjects exposed to unsignaled 10s delay intervals.
Dickinson, Watt, Griffiths, & 1992	Experimentally experienced rats	0s-32s & 32s-64s	Acquisition was observed up to 32s of delay, while delays of 64s did not facilitate learning.
vann Haaren, 1992	Rats	Fixed and variable	Fewer responses on fixed 30s resetting delay intervals compared

		resetting delays of 10s or 30s	to subjects on variable resetting delays of 30s.
Wilkenfield, Nickel, Blakely, & Poling, 1992	Rats	Used 4s, 8s, & 16s resetting delays, non-resetting delays, & a “stacked” delay (which provided reinforcement for every response at the end of the delay interval)	Resetting delay procedure saw the highest response acquisition while “stacked” delays produced the smallest about of response acquisition.
Watt, & Griffiths, 1992	Unshaped rats	Up to 64s	Observed lever pressing behavioral acquisition in unshaped rats at 64s delay.
Critchfield, & Lattal, 1993	Experimentally naive rats	30s	Immediate response marking (via an auditory stimulus) assisted, but was not necessary, to train subjects to make the spatial response.
Sutphin, Byrne, & Poling, 1998	Rats	8s, 16s, 32s, and 64s with a no-consequences procedure and a cancellation procedure	Able to acquire the desired behavior for all intervals other than at the prolonged 64s delay.
Byrne, Sutphin, Poling, & 1998	Rats	Up to 30s	Successfully acquire, extinguish, and reacquire lever-pressing behavior with delay intervals up to 30s.
Snycerski, Laraway, Byrne, & Poling, 1999	Rats	60s	Acquisition during early sessions, but responding diminished over later exposures.

Vansickel, White, & Byrne, 2004	Rats	Resetting delays of 10s & 20s	Behavior was acquired and maintained with resetting delays.
Snycerski, Laraway, & Poling, 2005	Rats	Resetting delays 0s, 15s, 30s, & 45 s	3 subjects at 45s were able to reach immediate-reinforcement levels.
Escobar, & Bruner, 2007	Rats	32s	Operant and non-operant pressings were consistent within, but not between subjects; indicating the reinforcement delay played a primary role in response induction.
Anderson, & Elcoro, 2007	Lewis and Fischer 344 rats	Non-resetting 20s	Lewis rats outperformed Fischer rats.

Most notable about Lattal and Gleeson (1990) was the researchers' utilization of a 70% free-feeding weight which they speculated may have been a primary factor for the response acquisition. Wilkenfield, Nickel, Blakely, and Poling (1992) were able to obtain response acquisition with 80% free-feeding weight; calling into question Lattal and Gleeson's (1990) speculation as to their higher level of starvation contributing to their subject's response acquisition. However, pigeons maintained at 90% free-feeding weight have been observed to exhibit less response acquisition (Wogar, Bradshaw, & Szabadi, 1992; Ho, Wogar, Bradshaw, & Szabadi, 1997).

Subject temporal discounting (or the subjective reward value from the subject's perspective) when presented with varying adjusting-delay intervals has been a focus of research during the 1990's and 2000's. We have summarized notable publications in Table III.

Table III

Author	Subjects	Delays	Findings
Raineri, & Rachlin, 1993	Humans	Imaginative 1 month – 50 years	Subjects discount smaller delayed rewards more steeply than larger delayed rewards.
Myerson, & Green, 1995	Humans	Imaginative 0 months-300 months	Subjects discount in a hyperbolic fashion as imagined delay increases.
Green, Myerson, Holt, Slevin, & Estle, 2004	Rats & Pigeons	1s - 32s	Subjects discount food rewards in a declining hyperbolic fashion as delay increases.
Green, Myerson, & Macaux, 2005	Humans	Imaginative Present – 10 years	Hyperbolic relationships between discounting and choice behavior.
Green, Myerson, Shah, Estle, & Holt, 2007	Pigeons	Adjusting 3s or 10s	Subjective value of delayed reinforcement fits a hyperbolic curve.
Valencia Torres, da Costa Arújo, Olarte Sanchez, Body, Bradshaw, & Szabadi, 2011	Rats	Adjusting 20% of baseline	Subject response follows a declining hyperbolic relationship as delay increases.

We found only one study throughout this literature review of pre-reinforcement delays analyzing how a delay of reinforcement affects honey bee behavior (Lee, & Bitterman, 1990). These researchers sought to determine if delays in providing honey bees targets (and thus reinforcement) affected subject performance of correct responses in extinction. By carefully controlling various target elements and variables, the researchers concluded the delay of presentation of the targets had minimal to no effect on response selection in honey bees (Lee, & Bitterman, 1990).

Post-Reinforcement Delays

The majority of the reviewed studies have been briefly described due to their lack of direct influence on our paradigm. However, there are a few investigations concerning post-reinforcement delays that necessitate extensive explanations. We have summarized the post-reinforcement literature in Table IV but have also provided detailed summaries of selected experiments below.

Davis (1954) is arguably the first to publish a finding solely focusing on post-reinforcement delays. His design utilized a T maze with a goal box that was modified to immediately remove a rat subject after 60s of consuming a single reward 1/3g pellet. Essentially, Davis (1954) allowed both experimental and control subjects to eat the pellet without any manipulation, but would immediately remove the control subjects while leaving the experimental subjects in the goal-box for an additional 60s after the experimental subjects had consumed the pellet; thus creating a 60s post-reinforcement delay. Davis hypothesized that the post-reinforcement delay groups would 1) show fewer successful choices, 2) would require more trials to reach criteria, 3) would display less of a tendency to directly repeat its previous choice, and 4) would have longer running times in the maze (David, 1954, pp 276). Davis inappropriately ran a T-test on matched data between control and delay groups and did not come close to "approaching" significance levels as determined by a .05 alpha level. However, much to his benefit, Davis admits that "it must be noted that these different approaches to the data are not independent measures" (Davis, 1954, pp 277). Even so, Davis declared his data disagree with contiguity theory and supposes the reason he did not obtain significant data was because the reward was too small (1/3 gram) to terminate the behavioral sequence and because he used too few subjects (n=21 per group). Contiguity theory posits that learning is improved as the temporal distance between stimuli and reinforcement is reduced.

Fehrer (1956) is the most cited publication on post-reinforcement delays we were able to find. We are of the opinion that Fehrer (1956) is pointed to as the first exploration into post-reinforcement delays due to her "significant" findings. For her first experiment, Fehrer had three groups of rats run a U maze and allowed the water-deprived subjects to drink for either 10s or 40s before they were immediately removed from the goal box. Fehrer's third group (10-D) were allowed to drink for 10s but were left in the goal box for an additional 30s after the water had been removed from the goal box; creating a post-reinforcement delay. Fehrer's extinction trials left all subjects in the goal box for 40s without reinforcement. The learning trials saw no difference between groups; indicating post-reinforcement delays have no negative effect on learning. There were no significant differences in correct responses between groups during extinction trials, but there were significant differences between run-times for the first day of extinction: 10-D subject behavior was extinguished slower than 10s and 40s rats (.87s versus 1.30s and 1.34s, respectively). T-tests on non-independent data revealed significant differences (p<.01) in running time means when 10-D subjects were compared with 10s and 40s indicating that 10-D subjects performed better than conspecifics. Not surprisingly, these differences became less apparent as extinction trials continued. Fehrer also reported how many trials subjects needed to reach a criterion of running the maze in 60s, 90s, 120s, and 180s during extinction trials. She reported her averages between groups for each running-time criteria, but did not report a statistical analysis comparing means between groups. All that was reported was: "group 10-D took reliably longer than others to reach [60s and

90s] criteria, and longer but not reliably to reach the [120s and 180s criteria]" (Fehrer, 1956, pp 170).

Fehrer's (1956) second experiment compared pre- and post- reinforcement groups in a modified open-alley Dashiell maze. This maze was essentially the same as the previously used U maze from experiment one, but half of the U was blocked; thereby creating a modified run-way. Four groups were used: three of which (30s, 10s, and 10-D) were all similar to the previous experiment while the fourth (D-10) was a pre-reinforcement delay group with a 10s interval. Thus, for the group titles, the location of the D either in front of or behind the 10 signifies if group was a pre or post reinforcement delay. No differences in mean running speeds was observed initially, but the final two days of testing saw significant differences (p=.02 for day 4 and p=.01 for day 5) from an analysis of variance between the D-10 (pre-reinforcement) and 10-D (post-reinforcement) groups. The D-10 group ran faster for 66% of the extinction trials, while the 10-D group had shorter run times during the final 33% of trials. Hence, there was slower learning for the D-10 group, while post-reinforcement delays did not affect learning. Fehrer reports that the pre-reinforcement group was more resistant to extinction than post-reinforcement rats.

We were pleased to find an exact replication of Fehrer's (1956) second experiment (Cogan, 1966). Cogan and Fehrer agree that there were no differences between post-reinforcement delay and no-delay groups during training. Yet, Cogan found a decrease in resistance to extinction when the delay groups were compared to the control group; thus failing to replicate (and finding an opposite effect) of Fehrer (1956). This finding also contrasts with Capalidi's (1958) prediction that delay groups would increase their resistance to extinction due to the aftereffects of non-reinforcement; thus calling into question the validity of Cogan's replication.

Bowen (1966) changed Fehrer's paradigm by running rats through a T-maze instead of a U-Maze. Two groups were trained to run to opposite sides of the maze during the first phase of the study. The beginning of the second phase forced all the rats to choose both arms for 50% of their trials (two out of four of the trials per day a subject could freely chose the maze arm, while the final two trials were forced choices). Bowen reinforced one group of rats with a 10s reward if the subject chose the side it was originally trained to prefer during phase one of the study. The other group was also reinforced with 10s of feeding for selecting the side it was originally trained to prefer during phase one of the study, while a 30s post-reinforcement delay was added for selecting the opposite side. Bowen defined a correct response as selecting the originally trained arm, and found rats who were immediately removed from the goal box after having been reinforced for 10s performed significantly better than rats treated with a 30s post-reinforcement delay. Bowen also reports that the post-reinforcement delay only accounted for 18% of the total variance of his study; a rather weak effect which could explain the difference between Cogan and Fehrer's findings (Bowen, 1966).

Mikulka, Vogel, and Spear (1967) added an additional independent variable to Fehrer's (1956) paradigm: the size of the goal box. Rats with either a 0s or 30s post-reinforcement delay were allowed to run into either a small (12 in. x 3.5 in. x 3.5 in.) or large (12 in. x 12 in. x 12 in.) goal box (Mikulka, Vogel, & Spear, 1967, pp 381). They found that the 30s post-reinforcement rats were more resistant to extinction than were rats exposed to the larger goal box at ($p<.01$). However, the statistical test used was an

ANOVA on dependent measures, and thus an inappropriate statistical analysis was used for their data.

Similar to the research question of Mikulka, Vogel, and Spear (1967) is William's (1967) investigation as to the effects of a "confining" goal box at the end of a run-way apparatus on pre and post reinforcement 30s delays. The restrictive goal box was 6 in. x 6 in. x 6 in. while the unrestricting goal box was 6 in. x 18 in. x 12 in. Pre-reinforcement delayed rats were significantly slower in running-speeds than other groups, and no difference between the post-reinforcement delayed rats and immediately reinforced rats was observed ($p<.001$). Contrary to the observation made by Mikulka, Vogel, and Spear (1967) regarding a larger goal box's correlation with higher resistance to extinction, William found no difference between his confining and non-confining goal boxes; though this could be attributed to the larger confining goal box utilized by William (1967).

McCain and Bowen (1967) attempted to determine how a small number of acquisition trials could produce a difference in rat behavior by using three groups: immediately reinforced subjects, subjects exposed to a pre-reinforcement delay, or subjects exposed to a post-reinforcement delay. This paradigm differed from Fehrer's (1956) by using only five training trails followed by 13 extinction trails. Both delay groups were more resistant to extinction compared to the immediately reinforced group (confirming Fehrer's observations), but no significant differences between the delay groups were observed (contrasting with Fehrer's findings) (McCain, & Bowen, 1967). Rosen and Tessel (1968) reiterated these results by showing no difference between post-reinforcement delay and no delay groups' run times on a runway (they did not analyze post-reinforcement affects on extinction as Fehrer [1956] had). Rosen and Tessel utilized an incentive-shift paradigm with four groups; half of which were left in the goal box for 20s. As the size of the reinforcement was moved from 12 food pellets to one food pellet, there was a clear down-shift in run times for all groups following a change in conditions, but there were no differences between groups during this down-shift (Rosen, & Tessel, 1968).

The affects of post-reinforcement delay intervals of 20s were analyzed utilizing an interesting paradigm (Capaldi, Godbout, & Ksir, 1968). This team divided their subjects (rats) into three groups: continuous reinforcement, partial reinforcement, and no reward. After 16 test trials, the procedure was altered to include a 20s post-reinforcement delay for the continuous and partial reinforcement groups. The researchers found a "marginal" level of significance between conditions ($F = 1.82$, $df=8/144$, $p<.10$), though an ANOVA yielded a $p<.05$ (Capaldi, Godbout, & Ksir, 1968, pp 282).

Researchers found an effect on training patterning between no post-reinforcement delay rats and subjects who were post-reinforcement delayed for 15s in a straight-alley runway (Posey, & Cogan, 1970). Post-reinforcement delay rats had no observable patterning effects for start, run, and goal times compared to control rats, and in fact a "tendency toward reverse patterning" was observed for the delay group (Posey, & Cogan, 1970, pp 46). This team also confirmed that post reinforcement delay subjects are less resistant to extinction, confirming Cogan's (1966) previous findings (Posey, & Cogan, 1970). A related-measures ANOVA was used to analyze this data as well, but a more informative analysis was their combination of medians with a one-tailed sign test; we highly commend utilizing medians in this manner.

All previously reported post-reinforcement experiments have utilized rats as subjects. Rabinowitz and Paynter (1969) analyzed differences in learning, relearning, and forgetting in 3rd grade students (n=156) by utilizing 6s, 12s, or 18s of post reinforcement delays. These researchers reported the possibility that children exposed to varying post reinforcement intervals were faster at learning (for both genders), relearning (for only boys), and especially forgetting. On average for this sample, an increase of a post reinforcement interval was associated with faster forgetting compared to pre-reinforcement delays (Rabinowitx, & Paynter, 1969). Rabinowitx, and Paynter (1969) used covariance analyses for their data and found consistently reliable data with seemingly impressive results ($p<.001$); however assumptions as to the linearity of the relationship between the delay interval and the reaction (especially when later studies have found delays of reinforcement match hyperbolic relationships) lend us to question the appropriateness of an ANCOVA.

Table IV

Author	Subject	Apparatus / Method	Post-Reinforcement Delay	Statistical Test	Findings
Davis, 1954	Rats	T maze	0s, 60s	T-test	No difference between control and delayed subjects.
Fehrer I, 1956	Rats	U maze	0s, 30s	T-test	Learning trials saw no difference between groups; indicating post-reinforcement delays have no negative effect on learning. No

					significant differences in correct responses between groups during extinction trials, but there were significant differences between run-times for the first day of extinction.
Fehrer II, 1956	Rats	Modified Dashiell maze	0s, 30s, & a 30s pre-reinforcement group	ANOVA	Post-reinforcement delays did not affect learning. The pre-reinforcement group was more resistant to extinction than post-reinforcement rats.
Cogan, 1966	Rats	Modified Dashiell maze	0s, 30s, & a 30s pre-reinforcement group	ANOVA	No differences between post-reinforcement delay and no-delay groups. Decrease in resistance to extinction when the delay groups were compared to the control groups.
Bowen, 1966	Rats	T-maze	0s or 30s	ANOVA	Subjects immediately removed from the goal box after having been reinforced for 10s performed

					significantly better than rats treated with a 30s post-reinforcement delay.
Mikulka, Vogel, & Spear, 1967	Rats	Run-way apparatus with either a small or large goal box	0s or 30s	ANOVA	30s post-reinforcement rats were more resistant to extinction than were rats exposed to the larger goal box.
Williams, 1967	Rats	Run-way apparatus with either a small or large goal box	30s post- or 30s pre-reinforcement	ANOVA	Pre-reinforcement delayed rats were significantly slower in running-speeds than other groups, and no difference between the post-reinforcement delayed rats and immediately reinforced rats was observed. No difference between his confining and non-confining goal boxes.
McCain, & Bowen, 1967	Rats	Run-way apparatus	20s post- or 20s pre-reinforcement or 0s	ANOVA & T-tests	Delay groups were more resistant to extinction compared to the immediately reinforced group.

					But no significant differences between the delay groups were observed.
Capaldi, Godbout, & Ksir, 1968	Rats	Run-way apparatus	20s	ANOVA	Found a “marginal” level of significance between conditions when subjects were immediately reinforced and then delayed for 20s.
Posey, & Cogan, 1970	Rats	Straight-alley runway	15s	Related-measures ANOVA	Post-reinforcement delay rats had no observable patterning effects for start, run, and goal times compared to control rats. Also confirmed that post-reinforcement delay subjects are less resistant to extinction.
Rabinowitz, & Paynter, 1969	3rd grade human children	difference in learning, relearning, and forgetting	Post- or Pre-reinforcement of 6s, 12s, or 18s	ANCOVA	Varying post reinforcement intervals were faster at learning (for both sexes), relearning (for only boys), and especially forgetting. On average for this sample, an increase of a post reinforcement

					interval was associated with faster forgetting compared to pre-reinforcement delays.

We were disappointed with the statistical methods used by the majority of the research on post-reinforcement delays, and we believe these methods may have contributed to the inconsistency in findings as pointed-out by Cogan (1966). Many ANOVAs, ANCOVAs, or T-tests have been used inappropriately on matched conditions which were clearly not independent of one another; thus invalidating all significance in the findings (Davis 1954; Fehrer, 1956; Bowen, 1966; Cogan, 1966; McCain, & Bowen, 1967; Mikulka, Vogel, & Spear 1967; Capaldi, Godbout, & Ksir, 1968; Rabinowitz, & Paynter, 1969). Some researchers attempted to run matched analyses, but still assumptions as to normalcy and linear relationships were made, and we do not feel comfortable with this practice. The assumption of linearity is especially disconcerting, for hyperbolic and exponential relationships between delay and response have been found in previous literature (e.g. Chung, 1965; Estle, & Holt, 2007). In our opinion, these statistical methods were utilized to show differences in sample conditions (or were requested by peer-reviewers) when in reality an abstract population parameter was being reported. We have attempted to analyze our data in a more statistically sound manner as we have no desire to report about the population of honey bees as a whole, but instead simply analyze our sample of 50 bees.

Detainment:

In an attempt to thoroughly search for similar paradigms, one final direction of behavioral research deserves discussion. Weinberger (1965) detained rats for 0s, 2.5s, 5s, 10s, 15s, and 20s in a Miller-Mowrer shuttle box. The Miller-Mowrer shuttle box contains two areas divided by an aluminum “guillotine gate” which detains the subject in one of the two areas. The floor of both detainment areas could be electrocuted. A light served as a CS predicting a shock, and the gate would be lowered from 0-20s prior to the CS being displayed. Learning acquisition was not related to subject detention time; however, extinction rates increased with longer detainment intervals; similar to post-reinforcement effects.

Having reviewed the available literature, we feel confident that the study of post-reinforcement delays has ceased prematurely and with much disagreement of its mechanisms. We believe that we have developed a novel paradigm to study reinforcement delays utilizing uniquely species-specific behaviors in honey bees (*Apis mellifera*). In addition to this post-reinforcement delay, our study differs from tradition in multiple other manners. Most notably, our subjects were “wild” and could freely choose if they wanted to begin, continue, or stop working with our apparatus. Secondly, counter to the majority of reinforcement procedures, our subjects were not food-deprived. Thirdly, our subjects were allowed to eat as much as they wanted, for our subjects were in a non-resetting interval condition, and there was no limit to the reinforcement they could receive.

CHAPTER III

METHODS

Subjects

Subjects were *Apis mellifera* (n=50) located outside Stillwater, Oklahoma from two different hives (located roughly a half meter from each other). All subjects were experimentally naive prior to shaping. Subjects were randomly assigned to one of five groups receiving varying amounts of post-reinforcement delays. We decided to have 10 subjects per group to remain consistent with previous (soon to be published) studies from our laboratory. All previous studies investigating post-reinforcement delays other than Rabinowitz and Paynter (1969) have utilized fewer subjects than we have opted to use, but modern journal requirements demand higher N's and thus we have compromised. We have no intentions of averaging our data within groups or running standardized statistics, and thus running a power analysis was not an option to determine an appropriate N. We followed a pseudo-ABA design so as to be able to compare each subject with itself as we moved across conditions. We followed this type of ABA design in a previous study from our laboratory assessing how ethanol affects honey bee foraging behavior (Sokolowski, Abramson, & Craig, 2012). This study saw numerous differences between individual subjects; thus comparing each subject to itself will yield more reliable data than simply averaging group data (Sokolowski, Abramson, & Craig, 2012).

A baseline of a 0s delay was administered for the first and last six visits for each bee for every group while the middle 12 visits varied per group depending on the amount of post-reinforcement (expressed in minutes). The groups were as follows: 0-0-0-0, 0-5-5-0, 0-10-10-0, 0-5-10-0, and 0-10-5-0 with each number representing the delay interval encountered by the group for each phase of six visits. Two subjects from different groups were run concurrently in two separate yet attached Skinner boxes so as to control for weather conditions and other unforeseen biases associated with one Skinner box being slightly closer to the hive than the other. Subjects were trapped and terminated as soon as the experiment was concluded so as to control for recruiting and other unforeseen confounds such as pheromone release.

Apparatus

We concurrently utilized two automated computer-controlled Skinner boxes providing 50% sucrose solution which was located approximately 3 meters from a feeding station containing 10% sucrose solution. Data was recorded automatically via a computer program. Subject responses were made when the subject enters a hole in the Skinner box with an infrared sensor which, when triggered, released a prescribed 5μl of sugar water directly in front of the subject's head. Due to the infrared sensor's sensitivity and disruption of functionality brought about by direct exposure to sunlight, we utilized a tarp which was placed in a consistent location every morning before beginning the experiment. A full description of the apparatus can be found in Sokolowski and Abramson (2010). Figure 1 shows the apparatus under the tarp, figure 2 shows the inside of the Skinner box and offers a clear view of the response hole, figure 3 shows two honey bees in the process of shaping prior to being tagged, figure 4 shows the automatic syringe controls, and figure 5 indicates the Skinner boxes' distances from the bee feeder and hives.

Figure 1

Figure 2

Figure 3

Figure 4

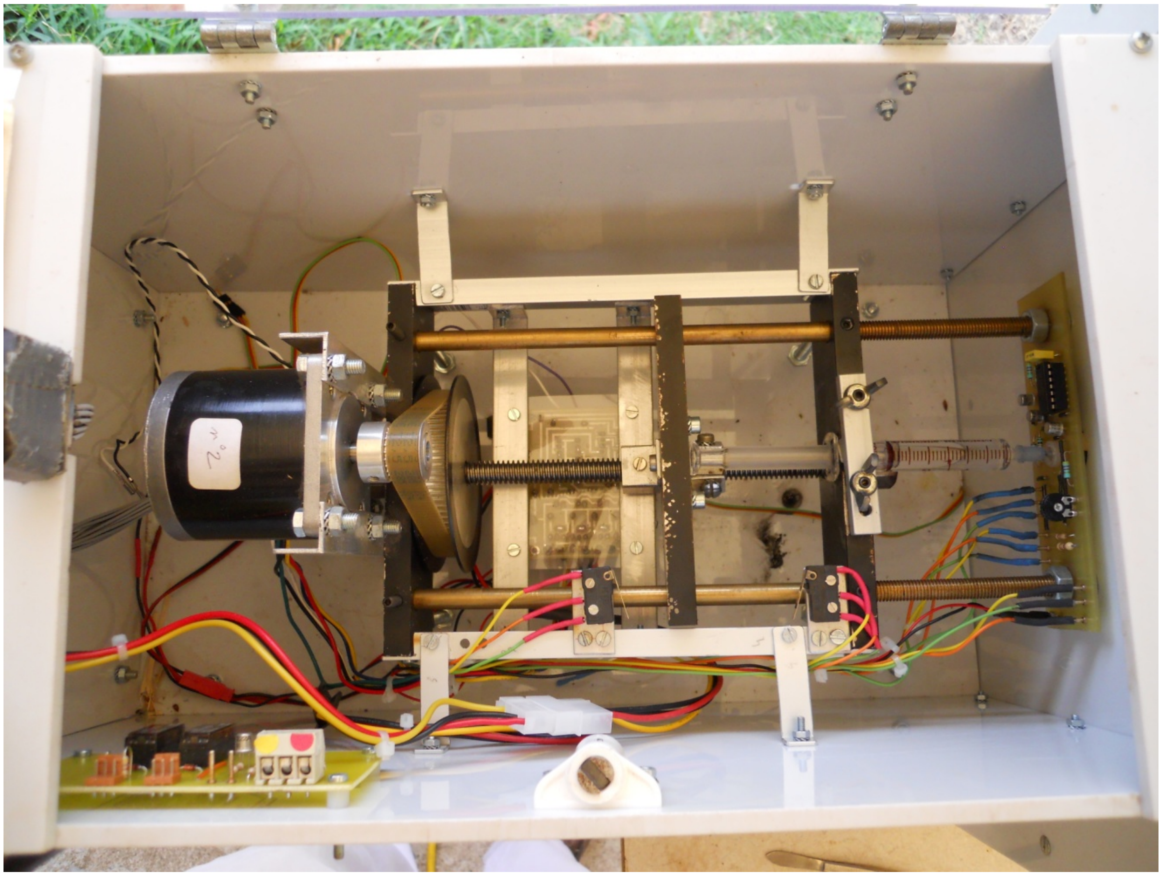


Figure 5

We also used a data-logger which can record a minute-by-minute reading of temperature, humidity, and due-point to control for weather variability. The data-logger was never left in direct sunlight and was placed in the Skinner box to better measure weather variables for the subjects.

Training

Subjects were randomly collected from the nearby feeder station equipped with 10-12% sucrose solution and shaped to use the apparatus described above. The feeding station was never without sucrose solution during the experiment to control for recruiting confounds. Training took no more than 10 visits. We typically focused on shaping two bees by first placing drops of sugar water near the response hole and then in the response hole. Sometimes we were obliged to hand-shape a subject into the response hole while other subjects were able to auto-shape themselves. After these bees had been trained to make the response, the newly trained bees were able to recruit additional potential subjects; exponentially increasing the number of trained bees in a matter of minutes.

Once the bee-in-training consistently returned to the Skinner box, we tagged her so as to be sure we were working with the intended subject. We used a Plunger Marker to securely keep the subject immobilized and attached a colored and numbered bee tag by using a safe, non-toxic adhesive. These materials were purchased from Betterbee® (Greenwich, NY). We attempted to minimize the time the subject was in the Plunger Marker to reduce what we assume to be a punishing aspect of our procedure. Once the subject was tagged, we provided her with three drops of 50% sugar water to combat this assumed tagging punishment before allowing her to return to the hive. We normally tagged numerous bees in one day and returned over the following days to administer our experiment until we needed to spend another day shaping and tagging our future subjects. Frequently, one of the bees would finish her experimental trials before the other concurrently run bee, in which case we shaped and trained more bees whilst finishing the experiment with the remaining bee. We recorded the date that each bee was tagged.

Procedure

We worked with whichever two bees first came to the apparatus consistently each morning after we set up the apparatus. Two bees were run each day for 24 visits/trials apiece. Six baseline trials of a 0s post-reinforcement delay were administered; allowing each bee to serve as her own control. During this time, subjects were allowed to freely exit the Skinner box once their crops filled. Beginning with the 7th and ending with the 12th visit, a bee was confined in the box for either 0, 5, or 10 minutes depending on her group assignment. Conditions and delay intervals changed again at the 13th visit and were held consistent until the 18th visit. Beginning with the 19th visit, we allowed the subjects to once again freely exit the Skinner box; returning to a 0 post-reinforcement delay.

We started the delay interval after the subject had finished feeding and attempted to fly out of the blocked Skinner box. During the delay periods, subjects were free to continue making responses, but these responses did not reset the delay interval. Sometimes a subject would not leave the box after the post-reinforcement delay and in these cases we forced her to exit by gently removing her from the Skinner box with a small fish aquarium net. A session was terminated if the subject failed to return to the apparatus after one hour, or if we saw her return to the 10-12% sucrose solution feeder which we monitored through-out the experiment. In the event of a terminated session, we checked the nearby bee feeder the following day to document the possibility of predation or death as the reason for their lack of return to the apparatus.

CHAPTER IV

RESULTS

We recorded inter-visit-intervals, response length, inter-response-time, the number of reinforcers per visit, visit length, and the response rate per visit as our primary dependent variables. We also recorded the temperature inside the apparatus every minute throughout each trial, as well as the date the subjects were tagged.

Data collection commenced June, 3, 2011 and finished July, 2, 2011. While analyzing our data, we discovered the data out-puts from two of our bees were corrupted. We returned to the field nearly 3 months after the other 48 bees' data had been collected to recollect data from two final subjects on September, 25, 2011 and October, 2, 2011.

Every control 0-0-0-0 bee finished the 24 visits, but only four of the ten 0-10-10-0 bees, five of the ten 0-5-5-0 bees, six of the ten 0-5-10-0 bees, and five of the ten 0-10-5-0 bees finished the experiment by completing 24 visits. All but one of these "drop-out" bees did not return to the Skinner box after a 3600s inter-visit-interval, and this bee was spotted returning to the nearby feeder instead of continuing to work in the Skinner box. Every bee that did not return to the Skinner box was observed the following morning at the bee feeder; ruling out the possibilities of predation or death affecting our data. We observed one bee fall victim to predation, but we re-ran another subject in her place as this attrition was clearly due to a cause other than the delay intervals. We accidently killed two subjects mid-session by closing the door of the apparatus on her; though both of these bees were control 0-0-0-0 bees and were performing on par with the rest of their group at the time of their attrition. We collected data from two more 0-0-0-0 bees to replace the prematurely terminated subjects.

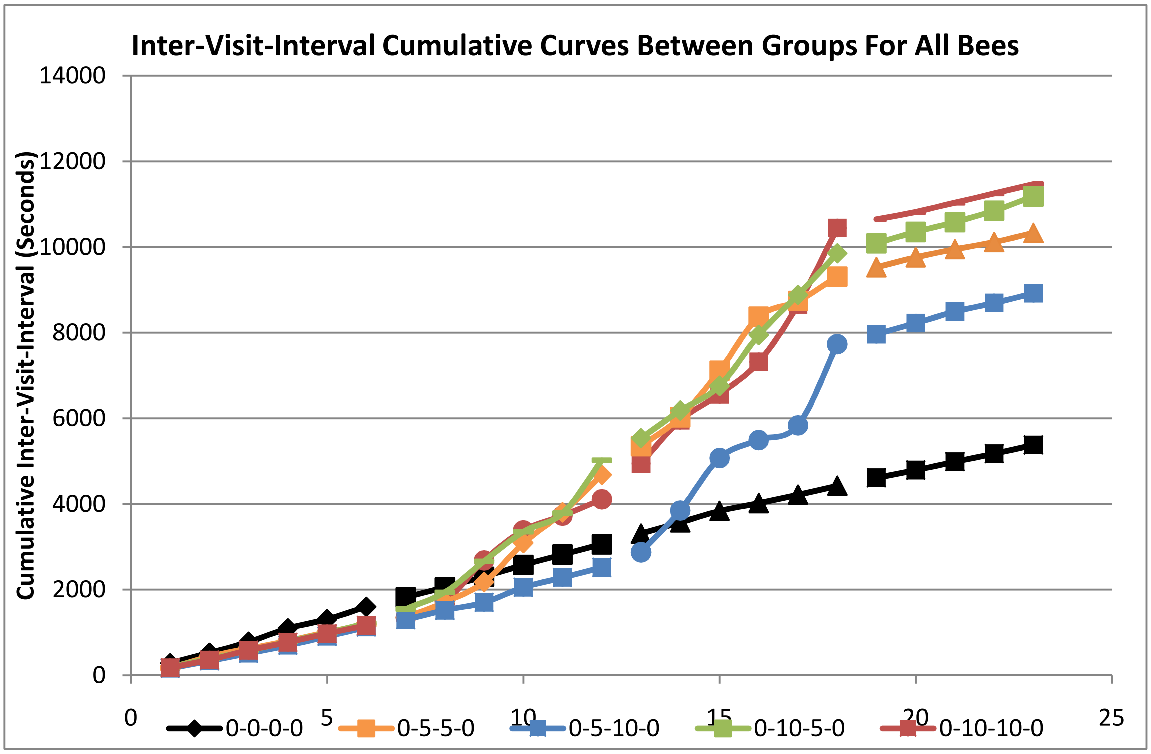
Our paradigm makes data analysis by conventional standards rather tricky. Firstly, our data does not meet the homogeneity assumptions made by traditional mean comparisons (Levene's $F = 13.193$, $p \leq .01$). The control 0-0-0-0 group's inter-visit-interval standard deviations are radically different from each of the experimental groups, for the delay intervals affected most (but not all) of the subjects; hence the greater variability for the experimental groups. Secondly, we utilized a repeated measures experimental design, yet many of our bees "dropped out" and thus a split-plot ANOVA would suffer from catastrophic attrition due to missing data. Moreover, the bees did not really "drop out," they simply did not return to the Skinner box after an hour, and hence their obtained responses should be included in the analysis. Thirdly, the difference between group baseline response rates indicate our sample and group assignment may not have been random, further compromising the validity of any *p*-value obtained from an ANOVA. Due to these complications, we eschewed traditional methods of data analysis and instead utilized a different method that is relatively free of assumptions and incorporates techniques for accommodating the "drop out" non-responses. Specifically, we used Observation Oriented Modeling (Grice, 2011) which is a novel data analysis technique that permitted us to compare our observed results to expected patterns of outcomes and then to evaluate the differences with an accuracy index and a randomization or binomial test. Finally, while not reported for this thesis defense, we are planning an exact replication of this study to hopefully confirm our findings before publication.

Inter-Visit-Interval

For descriptive purposes only, we report simple statistics in Appendix 1 for each group when accounting for every inter-visit-interval. The tabled means and standard deviations clearly reveal differences between the groups. Cumulative curves of the inter-visit-intervals were also plotted and fitted with linear regressions to determine slope differences across each condition and between groups. Graph 1 shows the cumulative curve controlling for attrition for each group while Appendix 2 displays slope values for each condition and group. Every experimental bee's final baseline had a smaller beta-weight than the second delay condition.

Cumulative curve graphs of individual bees were initially analyzed via linear regressions. We observed three types of cumulative curve patterns for individual subjects; linear, exponential, and "S" (see Appendix 3). "Drop out" bees never saw the removal of the delay intervals, and thus never returned to baseline; thereby resembling an exponential "J" curve. Experimental bees which did encounter a return to baseline resembled an "S" curve. Every control 0-0-0-0 bee followed a simple linear pattern. Nine of the ten 0-10-10-0 bees differed from every control subject; seven of the ten 0-5-5-0 bees differed from every control; eight of the ten 0-5-10-0 bees differed from every control; nine of the ten 0-10-5-0 bees differed from every control.

Graph 1



Using Observation Oriented Modeling, an ordinal pattern analysis was conducted for each group to assess if the inter-visit-intervals increased once the delays were initiated and in turn decreased once the delays were removed. For bees in the experimental groups, we predicted the intervals would decrease during the baseline trials as the bees learned to work with the apparatus, and that the inter-visit-intervals would monotonically increase once the delays were initiated, but would instantly decrease and nearly but not fully return to baseline after the delays were removed. This prediction was also echoed by the slope differences obtained from our multiple regression reported in Appendix 2. For each bee, the analysis compares the differences between pairs of intervals to the hypothesized differences, and the percentage of responses that fit the predicted ordinal pattern is determined. Each interval is compared with every other interval for an individual bee (e.g. interval one vs. interval two, interval one vs. interval three…interval one vs. interval twenty-three, etc.); consequently, the number of responses that fit the ordinal pattern can range from 0 to ${}_{k}C_{2}$, where *k* equals the number of visits. For example, a bee completing 23 visits has 253 interval comparisons while a bee completing 18 visits has 153 comparisons to the expected ordinal pattern. The percentage of comparisons matching the expected patterns is computed for each bee, and a binomial probability is also computed.

Table 1 displays individual results of the ordinal analysis and contains proportions of combinations that matched the predicted pattern and the binomial *p*-values. As can be seen, consistent with expectation, seven of the ten control (0-0-0-0) bees did not fit the predicted experimental pattern. However, nine of the ten 0-10-10-0 bees, eight of the 0-5-5-0 bees, eight of the 0-5-10-0 bees, and all of the 0-10-5-0 bees followed the predicted pattern in improbable proximity compared to an arbitrarily selected cut-point of .05 for the binomial *p*-value.

Table 1: Inter-Visit-Interval Individual Results of Ordinal Analysis.

Group	Total	Bee1	Bee2	Bee3	Bee4	Bee5	Bee6	Bee7	Bee8	Bee9	Bee10
0-0-0-0	50%	**41%** **p≤1.00**	**55%** **p≤.08**	69% p≤.00	66% p≤.00	**47%** **p≤.87**	**42%** **p≤.99**	55% p≤.05	**36%** **p≤1.00**	**47%** **p≤.84**	**45%** **p≤.93**
0-10-10-0	74%	**55%** **p≤.08**	83% p≤.00	84% p≤.00	66% p≤.00	75% p≤.00	69% p≤.01	86% p≤.00	71% p≤.00	79% p≤.00	80% p≤.00
0-5-5-5	61%	80% p≤.00	65% p≤.00	65% p≤.00	**34%** **p≤1.00**	69% p≤.01	**33%** **p≤1.00**	84% p≤.00	83% p≤.00	78% p≤.00	74% p≤.00
0-5-10-0	62%	82% p≤.00	62% p≤.02	**39%** **p≤1.00**	62% p≤.02	56% p≤.03	67% p≤.00	64% p≤.00	67% p≤.00	**51%** **p≤.35**	86% p≤.00
0-10-5-0	74%	82% p≤.00	78% p≤.00	77% p≤.00	78% p≤.00	63% p<.00	68% p≤.00	83% p≤.00	80% p≤.00	81% p≤.00	61% p≤ .05

Response Length

An ordinal analysis of response length in Observational Oriented Modeling was conducted to test if response duration was smaller after the subject's crop had filled and the delay interval had been initiated. Every response prior to the delay interval being initiated was compared to every response after the delay interval was initiated, and a randomization test was performed to determine if the differences indicated consistently shorter durations. The 0-0-0-0 control group bees were not considered for this analysis. Individual and group percentages are displayed in Table 2, and most were over 90%. All chance values from the randomization tests were less than .01.

Table 2: Percentage of Adjunctive Responses Which Are Smaller Than Crop-Filling Responses

Group	Total	Bee1	Bee2	Bee3	Bee4	Bee5	Bee6	Bee7	Bee8	Bee9	Bee10
0-10-10-0	91%	79%	94%	83%	93%	93%	96%	97%	*	84%	98%
0-5-5-0	92%	65%	91%	98%	91%	*	99%	100%	74%	*	96%
0-5-10-0	89%	*	*	98%	86%	89%	98%	85%	99%	95%	62%
0-10-5-0	92%	*	97%	97%	97%	89%	98%	100%	95%	96%	91%

*subjects did not make additional responses

We also used an ordinal analysis in Observational Oriented Modeling to determine if the first response of a visit just after a visit containing the above mentioned seemingly adjunctive responses was longer than trials not coming after an additional or adjunctive response. We did not analyze bees that did not make adjunctive responses, nor did we analyze bees that did not return after a single adjunctive response as these subjects had no data to make an ordinal comparison. Table 3 contains group and individual percentages of the first response following a series of adjunctive responses being larger than first responses of a visit not following an adjunctive response (all *c* values < .01).

Table 3: Percentage of Larger First Responses Following an Adjunctive Response

Group	Total	Bee1	Bee2	Bee3	Bee4	Bee5	Bee6	Bee7	Bee8	Bee9	Bee10
0-10-10-0	92%	98%	95%	89%	77%	99%	*	97%	*	64%	100%
0-5-5-0	83%	100%	67%	84%	86%	*	100%	100%	80%	*	81%
0-5-10-0	83%	*	*	91%	8%	98%	82%	41%	86%	98%	64%
0-10-5-0	83%	*	78%	82%	95%	76%	87%	100%	100%	66%	44%

*subjects either did not make an additional response or did not return after an additional response

Inter-Response-Time:

A graphical representation of the collected IRT data did not show any apparent differences between groups; though a slight decrease in IRT group averages per condition could be interpreted (Graph 2). To test if there were indeed no differences between groups, we conducted an ordinal analysis in Observational Oriented Modeling. We predicted a decrease between conditions, but not within conditions, and no difference between groups. An analysis similar to our investigation of the Inter-Visit-Intervals found highly similar results between groups, but not within groups. However, variability in IRTs within groups was observed; Table 4 contains individual binomial *p*-values and percentages of responses following the predicted pattern of the ordinal analysis. Overall, the similar group pattern matched percentages led us to disregard group differences in IRT. After adjusting the predicted patterns within condition, we determined these lower percentages of matches were due to variability within condition and within bees; however, the similarities between groups were still observed.

Graph 2

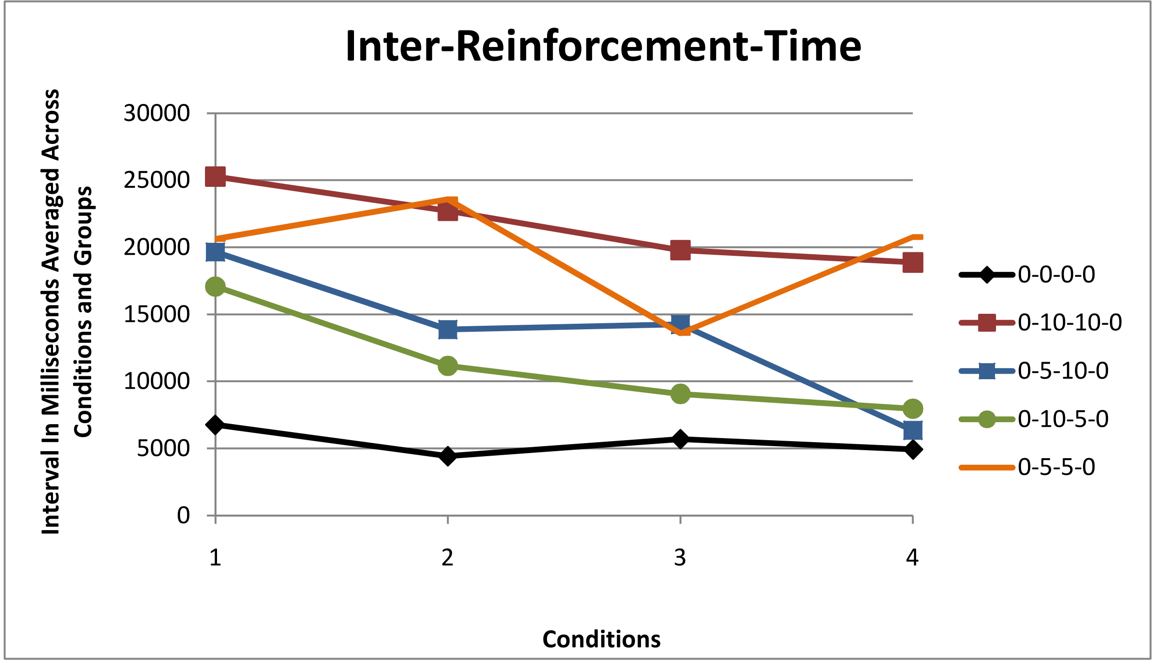


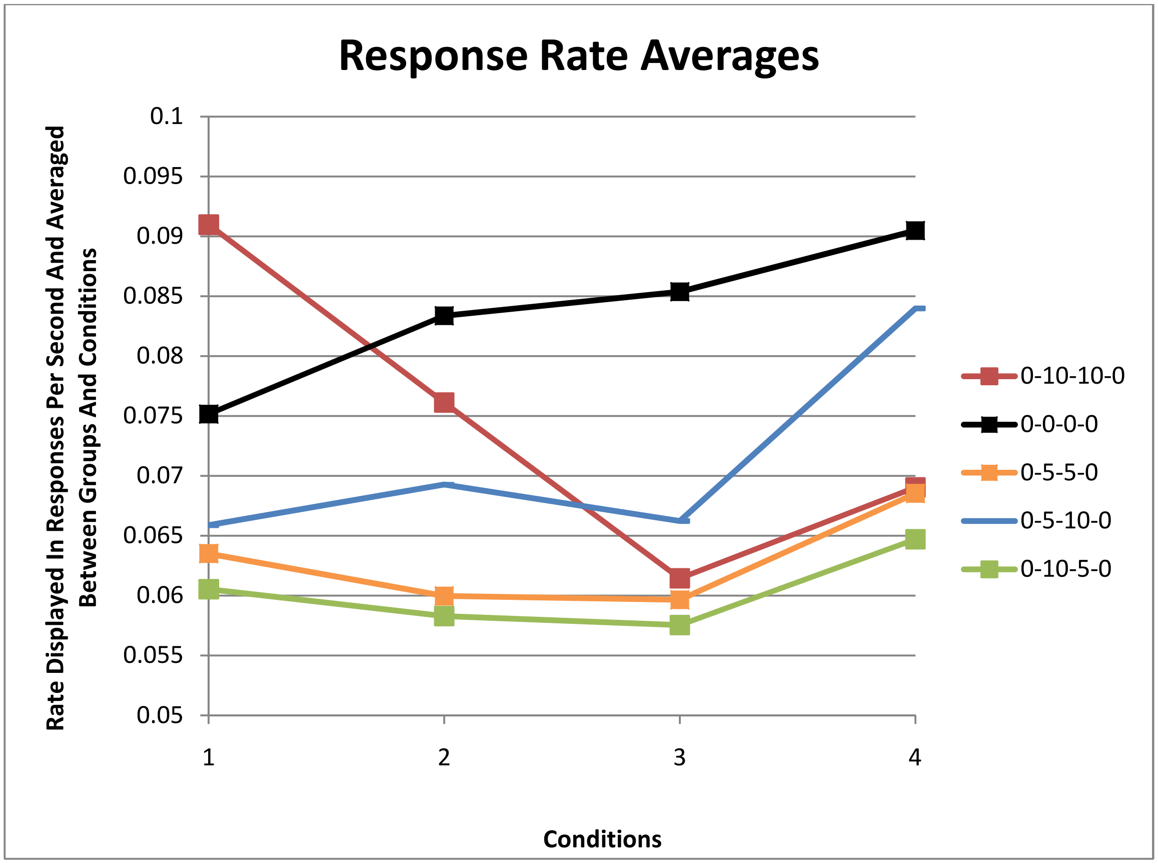
Table 4: IRT Pattern Matching

Group	Total	Bee1	Bee2	Bee3	Bee4	Bee5	Bee6	Bee7	Bee8	Bee9	Bee10
0-0-0-0	47%	**56%** **p≤.02**	**57%** **p≤.02**	42% p≤1.00	**55%** **p≤.05**	27% p≤1.00	44% p≤.98	46% p≤.90	54% p≤.10	54% p≤.10	37% p≤1.00
0-10-10-0	44%	42% p≤1.00	23% p≤1.00	38% p≤1.00	47% p≤.82	**59%** **p≤.00**	28% p≤1.00	50% p≤.56	44% p≤.89	50% p≤.58	27% p≤1.00
0-5-5-0	33%	31% p≤1.00	22% p≤1.00	31% p≤1.00	36% p≤1.00	0% p≤1.00	48% p≤.78	38% p≤1.00	50% p≤.54	16% p≤1.00	29% p≤1.00
0-5-10-0	42%	41% p≤.99	33% p≤1.00	25% p≤1.00	42% p≤.95	41% p≤.99	50% p≤.58	21% p≤1.00	34% p≤1.00	**65%** **p≤.00**	**65%** **p≤.00**
0-10-5-0	44%	22% p≤1.00	50% p≤.48	20% p≤1.00	50% p≤.57	**65%** **p<.00**	59% p≤.00	39% p≤.93	27% p≤1.00	26% p≤1.00	50% p≤ .55

<u>Response Rate</u>:

We predicted an increase within and between conditions, for if learning were present, then response rates could be expected to increase. This prediction was post hoc and was based on the control 0-0-0-0 group's increase in response rates; visible in Graph 3.

Graph 3



An ordinal analysis similar to our investigation of the Inter-Visit-Intervals and IRT found highly similar results between groups, but not within groups. Variability in response rates within groups was observed; Table 5 contains individual binomial *p*-values and percentages of responses following the predicted pattern of the ordinal analysis.

Overall, experimental conditions all differed from the control group, and while differences between experimental groups were observed, these differences were not substantively important.

Table 5: Individual data presenting percentage of observations matching predicted response rate

Group	Total	Bee1	Bee2	Bee3	Bee4	Bee5	Bee6	Bee7	Bee8	Bee9	Bee10
0-0-0-0	66%	**69%** **$p\leq.00$**	**69%** **$p\leq.00$**	**64%** **$p\leq.00$**	**55%** **$p\leq.05$**	**71%** **$p\leq.00$**	**60%** **$p\leq.00$**	**58%** **$p\leq.01$**	**72%** **$p\leq.00$**	**72%** **$p\leq.00$**	**67%** **$p\leq.00$**
0-10-10-0	49%	45% $p\leq.95$	29% $p\leq.100$	34% $p\leq1.00$	43% $p\leq.92$	**71%** **$p\leq.00$**	**67%** **$p\leq.03$**	**58%** **$p\leq..03$**	54% $p\leq.29$	60% $p\leq.03$	44% $p\leq.81$
0-5-5-5	55%	**56%** **$p\leq.02$**	49% $p\leq.62$	**66%** **$p\leq.00$**	52% $p\leq.26$	31% $p\leq1.00$	**72%** **$p\leq.00$**	33% $p\leq1.00$	**60%** **$p\leq.02$**	24% $p\leq1.00$	41% $p\leq.95$
0-5-10-0	57%	**71%** **$p\leq.00$**	**55%** **$p\leq.05$**	**63%** **$p\leq.00$**	43% $p\leq.93$	41% $p\leq1.00$	47% $p\leq.78$	43% $p\leq.94$	52% $p\leq.29$	**67%** **$p\leq.00$**	**68%** **$p\leq.00$**
0-10-5-0	59%	39% $p\leq.99$	**58%** **$p\leq.01$**	**55%** **$p\leq.05$**	45% $p\leq.95$	**83%** **$p<.00$**	**78%** **$p\leq.00$**	58% $p\leq.20$	42% $p\leq.91$	26% $p\leq1.00$	**79%** **$p\leq .00$**

The averages plotted in Graph 3 suggest the 0-10-10-0 group has a far different baseline compared to the other groups when in reality only one bee differed from the other nine. This bee dropped out during the second condition, and thus the group response rate averages were affected by this "outlier." Consequently, we investigated if there were differences in response rates within groups when comparing bees that finished the experiment with bees that did not finish the experiment. Thus, we predicted bees that dropped out had a higher response-rate per visit and tested this prediction with an ordinal analysis in Observational Oriented Modeling similar to how we previously analyzed response length. This analysis compared each response rate from the bees that did not finish the experiment with the response rates from the bees that did finish the 24 trials. As every bee in the control 0-0-0-0 group finished all 24 trials, we did not analyze the control group's response rates. Table 6 displays percentages of drop-out bee's response rates that are larger than response rates from bees that finished the experiment. While a difference between drop-out bees and bees that finished the experiment can be observed when comparing all 24 responses, we do not choose to interpret a predictive quality of response rates as gauged from baseline trials. Phrased differently, we do not believe larger baseline response rates appropriately predict if a subject will not complete the 24 trials.

Table 6: Percentage of response rates of drop-out > completion bees with c-values.

Group	Baseline	All Visits
0-10-10-0	60% $c \leq .00$	60% $c \leq .00$
0-5-5-0	37% $c \leq .10$	45% $c \leq .00$
0-5-10-0	14% $c \leq 1.00$	81% $c \leq .00$
0-10-5-0	60% $c \leq .00$	52% $c \leq .00$

Weather Variability:

As temperature has been shown to affect honey bee behavior (Heinrich, 1979), we recorded temperature at single-minute intervals during the experiment to assess temperature effects on our various DVs. Our data logger did not record temperature data for two out of 50 subjects. Of these 48 subjects, 18 subjects did not complete the 24 trials. Heinrich (1979) reports honey bees are capable of foraging at temperatures as high as 46C without over-heating. The maximum temperature of the 18 subjects that did not finish the experiment ranged from 36.5C – 45.5C while the temperature range for the 30 subjects that did finish the experiment ranged from 26C – 40C. Appendix 4 contains a scatter plot of the temperature associated with each bee's longest inter-visit interval.

An ordinal analysis in Observational Oriented Modeling comparing temperature for bees that finished the trials versus those that did not return to the apparatus within 3,600s was performed. The analysis was run under the prediction that drop-out bees would have higher temperatures compared to the temperature paired with the longest inter-visit-interval for each bee that did finish the trials. Drop-out bees had higher maximum temperatures for 90% of the matches in the ordinal analysis ($c \leq .01$). We then separated by group to determine if drop-out bees had higher maximum temperatures within groups in addition to between groups. As the control 0-0-0-0 group had no "drop-outs," we only analyzed the four experimental groups. Within every experimental group, every "drop-out" bee had a higher maximum temperature than the temperature paired with the longest inter-visit-interval for bees that finished the experiment.

Shaping:

We documented the latency between shaping/tagging a bee and initiating the experiment for each bee. On occasion, we shaped and ran a subject on the same day; however, we also frequently tagged bees days (up to nine) prior to running a subject. A regression of this latency on to whether the subject finished 24 trials found a R^2 value of .30. An ordinal analysis in Observational Oriented Modeling found negligible effects between bees that finished and did not finish the experiment; 30% prediction match, $c \leq .17$. Latency between tagging and data collection was regressed on to the number of additional responses made after the delay intervals were initiated found a R^2 value of .16.

CHAPTER V

DISCUSSION

Inter-Visit-Interval & Temperature Effects:

The main problematic finding for our purposes is the covariance between temperature and inter-visit-interval. Prior to beginning any data collection, we made a schedule for 50 bees and counter-balanced subject order in an attempt to control for weather variability. Data collection would start for each bee usually around 10:00, but control 0-0-0-0 bees would oftentimes finish before 12:00 while experimental groups often finished around 16:00. Thus, temperatures were far higher for the experimental bees and usually during the end of the experiment; thus exacerbating the temperature difference between groups. Regardless, Appendix 4 clearly shows bees did not finish the experiment if the temperature reached above 40.5C; we believe investigating this temperature effect is crucial, but at this time we are unsure of how temperature in combination with our delay intervals may function to dissuade subjects from returning to the Skinner box. Most notably, while temperature certainly affected and accounts for at least some variability in the inter-visit-intervals for groups and individuals, the very clear decrease of the inter-visit-interval cumulative curve’s beta-weights (for individuals and groups) once the delay intervals were removed demonstrates the impact of the delay and functions to show how the delay impact is more salient than that of temperature. Further demonstrating that temperature did not “contaminate” our data would be the control groups’ decrease in inter-visit-intervals across individual bee’s trials, for even though the temperature increased across conditions, a decrease was observed in inter-visit-interval for the 0-0-0-0 control group (as seen in Appendix 2). However, the effect of temperature on the experimental bees is undeniable; thus we propose temperature moderates the inter-visit-intervals. Based on human literature, “frustration” or “aggression” at encountering the delays could easily be exacerbated if faced with dauntingly high temperatures as observed in field studies (Anderson, 1989).

A criticism to be raised against our method of analysis of the inter-visit-intervals would be the relatively low percentage of our observations matching our proposed model. However, our pattern yielded far more correct responses for the first three conditions for each bee, yet the return to baseline typically drastically reduced our percentage match as many of the responses fully returned to or out-performed baseline (differing from our pattern analysis). Also, our criteria are far more strict than any traditional statistical analysis; indeed, any data deemed “significant” in Observational Oriented Modeling will also be significant in any traditional statistical test. Most importantly, in addition to predicting a difference between groups, we also predicted the direction and the order of these differences. No other analytical method we know of is capable of testing such a complicated ordinal prediction while not making numerous assumptions. Regardless, our prediction was incorrect (based on an arbitrary value of $p \leq .05$) for only five out of 40 experimental group bees. Thus, we feel confident stating that the delays increase inter-visit-intervals. Subjects first encountering a 600s delay performed more poorly than subjects first encountering a 300s delay. We did not show a difference in inter-visit-intervals between the 0-5-5-0 and 0-5-10-0 groups, nor did we show a difference in inter-visit-intervals between the 0-10-10-0 and 0-10-5-0 groups.

Response Length:

Our analysis of response length yielded two major findings. Firstly, a difference (an increase) in response length was clearly observed for nearly every response made after the delay had been initiated. Only two out of 34 bees did not match this prediction based on an arbitrary value of more than 66% matching. After comparing the length of the adjunctive responses to the normal crop-filling responses, we do not believe the subjects were receiving reinforcement during their post-delay responses, for the response lengths are usually impossibly small for a subject to make the response and consume the reinforcement. Indeed, we observed sugar water dripping within the apparatus following a string of these adjunctive responses; validating the possibility of this hypothesis. Our second analysis of response length, which found that the first response of a new visit following a string of adjunctive responses was larger than responses not following an adjunctive response, is related to our first response length analysis. We believe the subjects were not consuming some or most of the reinforcement during the responses during the delay interval, and thus when the subjects returned to the Skinner box for the next trial we observed longer response lengths for the first response as the subjects consume the previous visit's left-over reinforcement; hence our utilization of the term "adjunctive response." Thus, a very clear difference between the normal crop-filling responses prior to the initiation of the post-reinforcement delay and the responses during the delay can be observed and inferred from two response length analyses. We believe these additional responses were not made to receive reinforcement, but were adjunctive or “emotional” responses at being unable to leave the Skinner box.

Inter-Response-Time:

The benefit of our focus on individual observations instead of focusing on aggregate data is best exemplified when interpreting IRT data. When considering Graph 2, a slight monotonic decrease in group averages across conditions can clearly be observed for every group other than the 0-5-5-0 experimental condition. However, when considering individual bees, only five of 50 bees (only 10%) followed this aggregate-based prediction as determined by our binomial *p*-value $\leq .05$. Only one 0-0-0-0 control bee followed the predicted pattern, indicating the experimental groups’ 36 bees which departed from our expected pattern did not do so due to the post-reinforcement delays. However, the similarity between the groups’ percent matching dissuades us from claiming IRT is affected by our post-reinforcement delays. Consistent with our findings, pigeons with long delays have previously been observed to have a monotonically inverse relationship between pre-reinforcement delay length and IRT (e.g. Chung, 1965; Chung and Herrnstein, 1967; Sizemore, & Lattal, 1978).

Response Rate:

Our initial analysis of response rate tested if, as observed in the control 0-0-0-0 group, experimental groups’ response rates monotonically increased across visits. Every control 0-0-0-0 bee was found to follow the predicted response rate increase, while only 18/40 experimental bees followed the predicted response rate increase. Experimental group’s matched the predicted pattern approximately equally, though clearly differed from the control 0-0-0-0 group. When considering Table 5, a very clear dichotomy can be observed in the bi-nominal *p*-values for individuals in the experimental groups. However, the 0-10-10-0 group differed furthest from the control 0-0-0-0 predicted pattern, for this experimental group had powerful "outliers" affecting the mean of the group's response rate (as seen in the baseline of Graph 3).

We decided to investigate these differences in baseline and found, for the 0-10-10-0 and 0-10-5-0 groups, that bees with higher response rates tend to drop out more than bees with smaller response rates. Interestingly, these differences in baseline performance for future drop-outs were not observed for the 0-5-5-0 or 0-5-10-0 groups. Because of this inconsistency, we do not believe higher baseline response rates can predict longer inter-visit-intervals when the subjects encounter the delays. However, we decided to compare every visit's response rate for bees that dropped out of the experiment with bees that completed all 24 trials for each group, and found an apparent difference between bees that dropped out and completed the trials for all experimental groups' response rates.

The most obvious finding regarding response rate is the immediate response rate increase once the delays have been removed for group (Graph 3) and individual bees. Clearly, our delays were affecting response rate as the delay removal created a sudden increase in response rate across all groups. Thus, we posit that extensive post-reinforcement delays impair response rate increases traditionally seen in non-delayed subjects, or at the very least serve to increase response rates once the delay has been removed.

Shaping:

The latency between tagging and beginning data collection for individual bees clearly had a negligible impact on the reported effect. Thus, we believe the interval between shaping and beginning data collection have little impact on our positive findings.

Discussion:

There are many other differences between our experiment and those presented in Fehrer (1956) as well as those following her research questions and protocol. Most importantly is Fehrer's focus on extinction trials, for we did not analyze extinction but instead solely studied training/learning. We also used a small amount of acquisition trials (six) before presenting our subjects with reinforcement delays. One of the most crucial differences is the species we worked with. All but one study (Rabinowitz, & Paynter, 1969) we found on post-reinforcement delay literature used rats (Davis, 1934; Fehrer, 1956; Bowen, 1966; Cogan, 1966; Mikulka, Vogel, & Spear, 1967; Williams, 1967; Capaldi, Godbout, & Ksir, 1968); we used honey bees *Apis mellifera*. Thus, any of our observation's inconsistencies with the literature may be attributed to species differences. Another major difference between these paradigms are our delay times. The literature typically reports either 20s or 30s of post-reinforcement delay with the exception of Davis' (1954) analysis of a 60s interval. We used 300s and 600s intervals (a 100x and 200x increase compared to the literature). Our decision to use these large delays follows the tendency of typical extinction trials to last 10 minutes, for we believe any actual behavioral effects caused by post-reinforcement delays would be best observed this way. Finally, our subjects were still able to, and at times did, feed after the "post-reinforcement" delay had been initiated; no other study allows reinforcement to be provided during the delays.

Due to these major differences and as our paradigm has never been reported in the literature, no theoretical hypothesis could be posed a priori. We now offer our research hypothesis post hoc: if large post-reinforcement delay intervals are administered, than honey bees will have longer inter-visit intervals and make "frustration responses" during the delay intervals compared to bees not exposed to a post-reinforcement delay. Honey bees with comparatively higher response rates tend to be exacerbated by the delay intervals. IRT remains mostly unaffected by the delays of reinforcement. The removal of the delay intervals increases subject response rates.

After the conducted literature review, we were unsure how to best label and define the phenomenon we wanted to study. We are hesitant to describe our protocol as an investigation into post-reinforcement delays as our post-reinforcement delays are (or are nearly) as long as a typical extinction trial, but have done so until this point to remain consistent with the available literature. The ability for our subjects to continue feeding once the interval was initiated also leads us to believe we are in fact not using a post-reinforcement paradigm. Our best explanation for these behavioral changes is that subjects were reacting to a delay in *social*-reinforcement. Our subjects were unable to recruit bees after having immediately fed. The bees were also unable to return to the hive to receive social reinforcement via trophallaxis (Abramson, Wells, Wenner, & Wells, 2011). They were also delayed the social-reinforcement incurred when they regurgitate what is left in their social crop for the hive. We also delayed their ability to recruit bees to a foraging location (our Skinner box) after they departed from the hive. Thus, we feel comfortable claiming we have delayed social reinforcement; though we have investigated this phenomenom via a post-reinforcement delay perspective.

Notably, in a paradigm similar to the present study, Wainselboim, Roces, and Farin (2003) investigated how the flow of trophallaxis may be affected by perceived value of a food source. Most importantly for our purposes, this team predicted and found that induced longer visit lengths resulted in slower rates of trophallaxis (Wainselboim, Roces, & Farin, 2003). We were unable to observe if our subjects were engaging in trophallaxis; though if this were the case, a slower rate of trophallaxis may account in part for the longer inter-visit-intervals and also communicate the temporal value of the Skinner box with delays as less of a reinforcer than the Skinner box without the delays.

We were unable to determine if the bees were digesting the sugar solution during their confinement; thus our claim the subjects make “frustration” responses may be incorrect. We also were unable to observe our subjects’ behavior once they returned to and enter their hive. We cannot account for various social behaviors such as recruitment, nor can we speculate as to how these factors could affect our findings. Due to our training procedure, we were unable to observe precisely how many visits were made prior to tagging and the initiation of data-collection for each individual bee, and this could very well be a confound for our findings. However, these faults aside, we feel we uncovered a very interesting phenomenon which deserves more attention.

Future studies should first attempt to replicate our observations. We also suggest varying the delay times more than we have done; perhaps a 150s delay and a 75s delay will yield equally interesting findings. Delaying groups for 30s is also highly recommended to remain consistent with previous literature, and also test the early literature findings of pre- and post-reinforcement delays with a different species. Determining honey bee sensitivity and reactions to these types of delays is crucial before conducting further studies into their sensitivity of various operant paradigms. We also recommend offering utilizing a choice paradigm to investigate if honey bees favor smaller delay intervals. Using an observational hive for future studies is also highly recommended so as to be able to continue to observe the subjects once they leave the Skinner box. Diversifying the honey bee species used to study post or social reinforcement delays is also critical. We also recommend comparing our findings with other hive insects such as ants or termites, and also examine if the reported paradigm could influence the foraging and feeding behaviors of avian and mammalian parents. The applicability of this paradigm on various human behaviors, such as incarceration, seems appropriate; though more comparative research should precede this speculation.

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APPPENDICES

Appendix 1: Inter-Visit-Interval Descriptive Statistics For Each Group

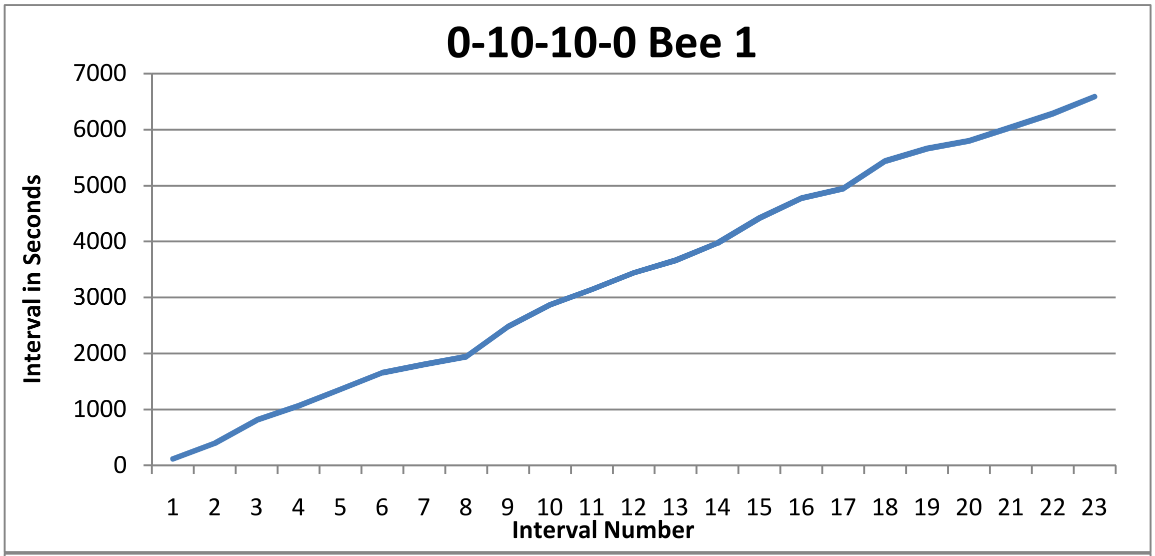
Group	Mean	Median	Mode	Range	SD
0-0-0-0	234s	190s	129s	50s-1377s	183s
0-10-10-0	481s	237s	3600s	60s-3600s	721s
0-5-5-0	441s	197s	3600s	85s-3600s	667s
0-5-10-0	358s	202s	200s	50s-7200s*	561s
0-10-5-0	475s	268.5s	225s	102s-3600s	650s

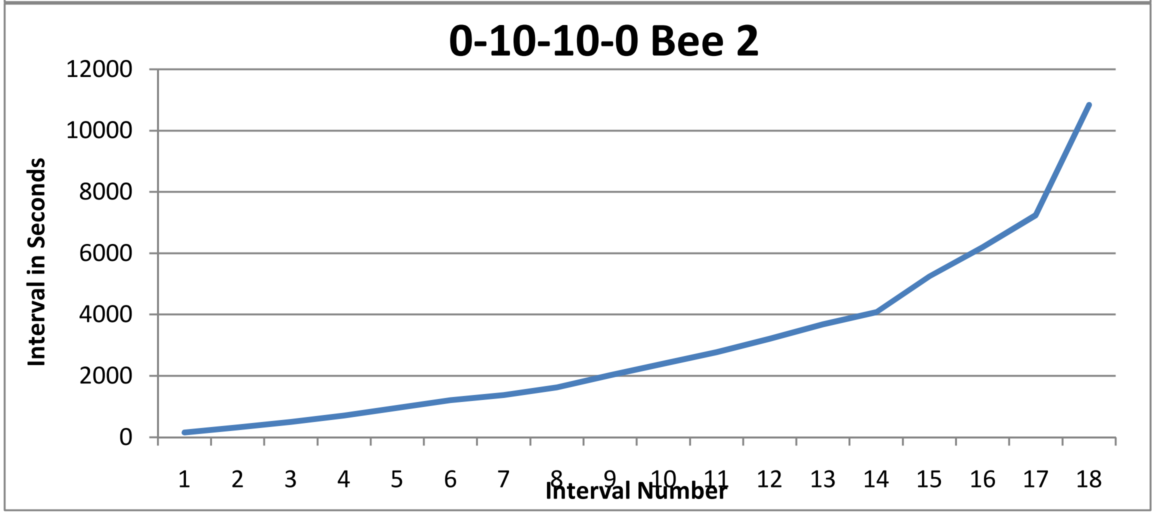
*the first bee we ran was a 0-5-10-0 bee that did not return after two hours of waiting, we decided to change our protocol after collecting data from this first bee so as to only wait 3600s before terminating the session. We have reported this 7200s interval only in the range; all other computations substituted this interval for 3600s.

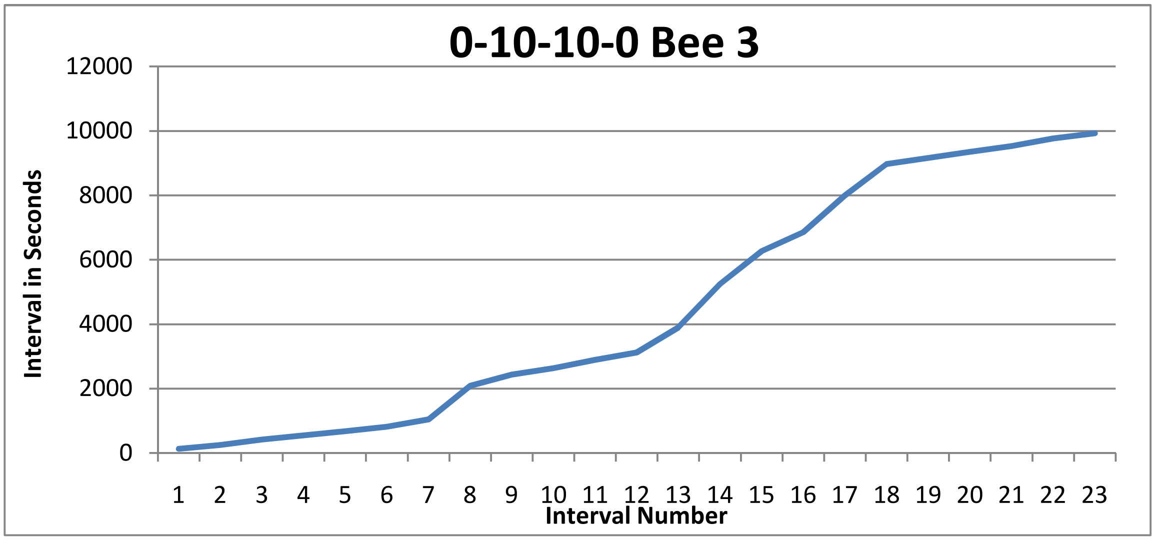
Appendix 2: Slope of Cumulative Curves of Each Condition Per Group

	1	2	3	4
0-0-0-0	264.11	249.8	220.23	192.22
0-10-10-0	198.47	586.34	1039.9	208.6
0-5-5-0	198.28	680.49	835.22	196.19
0-5-10-0	192.47	250.05	876.6	238.8
0-10-5-0	205.31	674.44	882.51	269.04

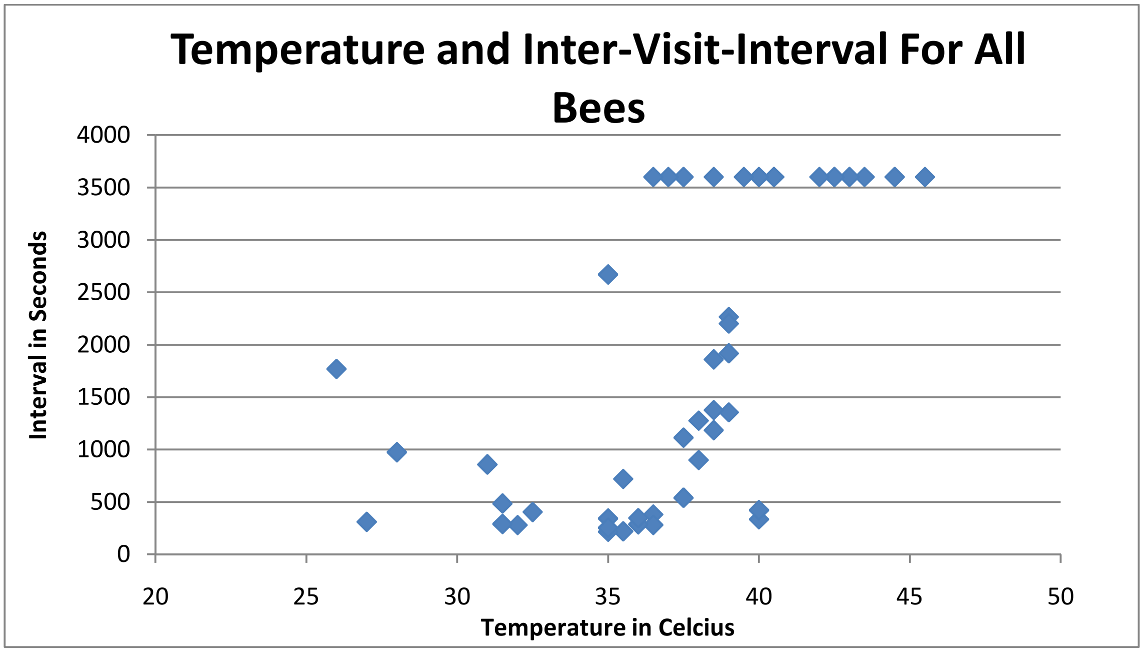
Appendix 3: examples of the three observed inter-visit-interval cumulative curve patterns. Bee 1 exemplifies a linear curve; Bee 2 exemplifies a “J” curve; Bee 3 exemplifies an “S” curve.







Appendix 4



Appendix 5: Acknowledgements

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Title of Study: A NEW PARADIGM TO ANALYZE EXTENSIVE POST-REINFORCEMENT OR SOCIAL REINFORCEMENT DELAYS IN FREE-FLYING HONEY BEES (APIS MELLIFERA)

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Scope and Method of Study: We have developed a novel paradigm to study and analyze how honey bees (*Apis mellifera*) react when presented with varying schedules of post-reinforcement or social-reinforcement delays of either 0s, 300s, or 600s.

Findings and Conclusions: Inter-visit-intervals increase with longer delays over time, “adjunctive” responses are made during the interval, inter response time seems unaffected by the delays as does response rate. However, response rates appear to differ between bees that did and did not finish the trials and increases with the removal of the delay intervals.

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