

# **IS POLYDIPSIA A PREDICTOR OF COGNITIVE IMPULSIVITY?**

**Honors Thesis**

**Presented in Partial Fulfillment of the Requirements  
For the Degree of Bachelor of Science in Psychology**

In the School of Arts and Sciences  
at Salem State University

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Commonwealth Honors Program  
Salem State University  
2020

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## Acknowledgements

I would like to recognize the invaluable guidance that Dr. Carlos Aparicio provided during the entirety of this study. The completion of this thesis would not have been possible without your constant help, support, and endless knowledge. You have taught me so much during the many months we worked together in and outside of the laboratory. I believe that I have improved in many areas and have a deep respect for experimental psychology and the animals we worked with. Thank you for welcoming me onto your well-established research project and entrusting me to work alongside you.

In addition to Dr. Aparicio, I would also like to thank your graduate assistant, Malana Malonson who welcomed me and taught me the ins and out of how to run the procedure correctly as well as how to understand what certain results meant. You provided me with much support during this process that I have appreciated sincerely.

I must also thank the Honors Program for allowing me the opportunity to build new connections and gain the invaluable hands on experience of working inside of a research laboratory.

### Abstract

Two nonhuman animal models of Attention Deficit/Hyperactivity Disorder (ADHD), the Spontaneously Hypertensive rat (SHR) and Lewis (LEW) rats, were used to explore the possibility that schedule-induced polydipsia is a predictor of cognitive impulsivity. A concurrent-chains procedure consisting of 60 choice cycles was used. Each cycle began with one response on the back lever causing two front levers to extend into the experimental chamber. Choice was measured in the initial link with the levers using Random Interval schedules arranging entries to two terminal links. In one terminal link, the left lever produced one food pellet immediately (SSF). In the other terminal link, the right lever produced 4 food pellets (LLF) after a delay of 0.1, 5, 10, 20, 40 or 80 seconds. A bottle of water could be available (B), or could not be (A) available, to the rats in the choice situation according to an ABABA design. The results showed that the rats discounted the value of the LLF as a function of the delay to deliver it. Both strains of rats drank water during the one-minute blackout following 10 choice cycles during the session. But the SHRs drank more water than the LEWs, especially during the delays to the LLF. A negative correlation between polydipsia and discounting rate suggests that: (1) polydipsia is not a predictor of impulsive choice, and (2) polydipsia is not related to motor impulsivity.

## Introduction

Impulsive behavior is characterized by the presence of both motor and cognitive impulsivity such as lack of inhibitory control, rash in decision making, hypersensitivity to delays, and short attention span. Motor impulsivity is driven by behavioral excess as a result of consequences and cognitive impulsivity results in decision making that leads to unwanted consequences (Íbias & Pellón, 2014). Cognitive impulsivity is a fundamental characteristic of Attention Deficit Hyperactivity Disorder (ADHD) in humans, which is marked by hyperactivity, inattention, impulsivity, and learning difficulties (Fox, Hand, & Reilly, 2008). Cognitive impulsivity can be measured in laboratory settings using nonhuman animal models, such as the Spontaneous Hypertensive Rat (SHR) and Lewis (LEW) rats. This is done by determining their likelihood of choosing an immediate smaller reinforcer over a delayed larger reinforcer, indicating impulsivity (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019). When a smaller reinforcer is produced immediately (one food pellet), this is considered to be the smaller-sooner food (SSF), whereas a larger reinforcer (four food pellets) produced after a delay (0.1, 5, 10, 20, 40 or 80 seconds) is the larger-later food (LLF). When the SSF is chosen more frequently than the LLF, this is an indicator of cognitive impulsivity within the animal because they chose the smaller reinforcer when a larger reinforcer was available (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019).

As a result of high systolic blood pressure, SHRs have proven to be suitable nonhuman models of ADHD due to their tendency to be hyperactive, inattentive, and show a sensitivity to a delay in reinforcement that results in impulsive behaviors similar to those seen in humans with ADHD (Fox, Hand, & Reilly, 2008). Similarly, LEWs have shown to

have an increased sensitivity to delay discounting behaviors, which are behaviors that often result in impulsivity (Aparicio, Elcoro, & Alonso-Alvarez, 2015).

Cognitive impulsivity has been studied using both strains of rats responding to concurrent chain procedures to obtain delay discounting functions with the rats choosing between the SSF and LLF. Delay discounting occurs when the value of a larger reinforcer (the LLF) decreases as the delay to the reinforcer increases (Aparicio, Elcoro, & Alonso-Alvarez, 2015). This behavioral process is described by Mazur's hyperbolic decay model.

$$V = \frac{A}{1+kD}.$$

In this equation,  $V$  represents the subjective value of the LLF,  $A$  is the amount of the LLF,  $D$  is the delay to the LLF, and  $k$  a free parameter to estimate how quick the value of the LLF is discounted with the increasing delay to deliver it (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019). When this equation is calculated,  $A$  is the starting point of the curve while  $k$  will indicate how steep the delay discounting rate is. A larger  $k$  value (a steeper slope) will indicate a higher discounting rate, and ultimately impulsivity (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019).

Concurrent chain procedures are used to show if there is a preference between differently valued stimuli that have been associated with food through conditioning (Jimenez-Gomez, 2012). There are many benefits to using concurrent chain procedures including: (1) a clear choice between the SSF and LLF can be analyzed due to the fact that more than one response is used to estimate preference; (2) a variable interval schedule is used which allows for quantitative analysis of responses to be calculated; and (3) different delays to the SSF and LLF can be programmed and changed in random order within session (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019).

The present study used a concurrent chain procedure where SHR and LEW rats were exposed to a choice situation where two reinforcers, 1-food pellet (SSF) and 4-food pellets (LLF), were available in two terminal links, with one terminal link varying the delay to deliver the LLF during the session. An ABABA experimental design was used to introduce a bottle of water in the choice situation to explore the possibility that water will reduce the rats' impulsivity by adding an additional biological important event, causing preference to change from the SSF to the LLF. It is well known that when rats are given free access to water during intermittent food delivery, they begin to develop a pattern of water drinking in relation to food delivery that turns excessive, which has been termed schedule induced polydipsia (SIP) (Íbias & Pellón, 2014). Polydipsia is marked by excessive and nonregulatory drinking that can be induced on various time schedules (Gregory, Hawken, Banasikowski, Dumont, & Beninger, 2015). This behavior has been found to be neither driven by physiological or behavioral mechanisms, leading to SIP being classified as an interim or adjunctive behavior, suggesting that SIP is a model for various psychological conditions such as addiction, obsessive disorder, and impulsivity (Íbias & Pellón, 2014).

Habits are developed through constantly reoccurring behaviors that produce the same outcomes, which over time and conditioning, render that behavior permanent. Past studies have shown that the nucleus accumbens controls the sensitivity of the organism's behavior to outcomes, where the habits develop within the brain (Gregory, Hawken, Banasikowski, Dumont, & Beninger, 2015). Once these behaviors are developed into habits, the medial prefrontal cortex turns some compulsive behaviors, characterized by

automatic and repetitive actions, into psychiatric conditions including ADHD that can be studied through SIP (Gregory, Hawken, Banasikowski, Dumont, & Beninger, 2015).

SIP is developed when hungry animals such as the SHR and LEW rats, respond to intermittent presentation of food provided by a fixed interval (FI) schedule of reinforcement. In the early part of the inter-reinforcement interval, the organism develops an excessive and persistent behavior, like drinking water excessively termed polydipsia (Gregory, Hawken, Banasikowski, Dumont, & Beninger, 2015). Previous studies have argued that not all rats develop SIP. The rats that do develop this excessive drinking pattern as a result of SIP, however, display significant neurological structures in the brain that are different from those developed by rats not showing SIP. For example, the brain of the rats developing SIP has the ability to bind dopamine with receptors in the amygdala, as well as the ability to increase neural activity in the frontal cortical region which is strongly associated with habits and compulsions (Gregory, Hawken, Banasikowski, Dumont, & Beninger, 2015). In addition, these studies have been shown that the rats who developed SIP also had an increased presence of immediate early gene expression (IEG) of the FosB gene in the medial prefrontal cortex and the orbitofrontal cortex, which is consistent with human brain imaging that shows hyperactivity in these specific regions of the brain associated with compulsive behavior (Gregory, Hawken, Banasikowski, Dumont, & Beninger, 2015).

Despite some overlapping similarities, compulsivity and impulsivity are different from one another. While impulsivity is marked by steeper reward discounting, delay aversion, timing impairments, and changes in response criteria; compulsivity is characterized by rigid response strategies in attentional settings, inappropriate persistence

of habits despite the lack of appropriate consequences, and a resistance to extinction (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019). However, compulsivity and impulsivity share some similarities including motor disinhibition and an impaired ability to stop a response (Aparicio, Hennigan, Mulligan, & Alonso-Alvarez, 2019). Thus, while previous studies focused on a possible relation between polydipsia and compulsivity accounting for the neurological status of the organism, the aim of the present study is to determine empirically if polydipsia is a predictor of cognitive impulsivity. This allows an exploration of the possibility that impulsivity is controlled by environmental factors, such as the availability of water in the choice situation, instead of impulsivity being controlled by neurological processes.

## Methods

### *Subjects*

Sixteen experimentally naïve inbred male rats of about 90 days old (Charles River, Wilmington, MA), eight-Spontaneously Hypertensive (SHR) rats and eight experimentally Lewis (LEW) rats, were the subjects. Animals were regularly fed a measured amount of Purina Chow (Mazuri®), based on their weight, with water permanently available with no sought-after weight reduction or gain. When not in the experimental chambers, the rats were housed individually in 42.5 cm x 26.5 cm x 21.5 cm plastic cages that were cleaned regularly. These cages were held in a colony room with a controlled temperature ranging between 68°F and 72°F. In addition, the room was held at a 12:12-h light/dark cycle with the lights automatically turning on at 0700. At the beginning of this experiment, the weights of the SHRs ranged from 241 to 286 g ( $M = 260$  g) and the weights of the LEWs ranged

from 262 to 298 g ( $M = 283$  g). Every day after each session, the rats were weighed, and fed accordingly with 10g ( $\pm 2$ g) of Purina Chow (Mazuri®) based off their weight. When this experiment ended the weights of the SHRs ranged from 299 to 333 g ( $M = 313$  g) and the weights of the LEWs ranged from 385 to 406 g ( $M = 397$  g). Daily sessions at about 12:00 PM lasted sixty minutes, or after fifty sessions, depending on which event came first. This current study was conducted with no notable conflicts of interest, nor did it receive any outside grants or funding. In addition, all aspects of this experiment were approved by the Salem State University's Institutional Animal Care and Use Committee (IACUC 011817-2) based off the NIH (No. 8023) guidelines.

### *Apparatus*

Eight 30 cm x 33 cm x 25 cm modular chambers (Coulbourn Instruments®, E10-11R TC) for rats were used in this experiment. The chambers were individually enclosed in sound-attenuating boxes (E10-23), each measuring at 79 cm x 51 cm x 53 cm and contained an exhaust fan. The front and back walls of the chambers were composed of stainless-steel panels and the side left and right walls of Plexiglas (E10-18NS). The back panel contained a 24-V DC house light (H11-01R), 24 cm above the floor, providing the illumination of the chamber. A white noise generator (E12-08) connected to a 2.6 cm x 4.0 cm speaker, provided a constant white noise within the chamber at 20kHz ( $\pm 3$  dB); it was located on the back panel 20 cm above the floor, 1cm from the left panel and 1 cm below the ceiling. Two retractable levers (E23-17RA), 3.3 cm x 1.5 cm, were mounted on the front panel 6 cm above the floor; the extension/retraction time was approximately 1s. One lever was located 2.3 cm away from the right wall and the other 2.3 cm away from the left wall. A 24-V DC stimulus lights (H11-03R) was mounted 3.5 cm above each lever. A food

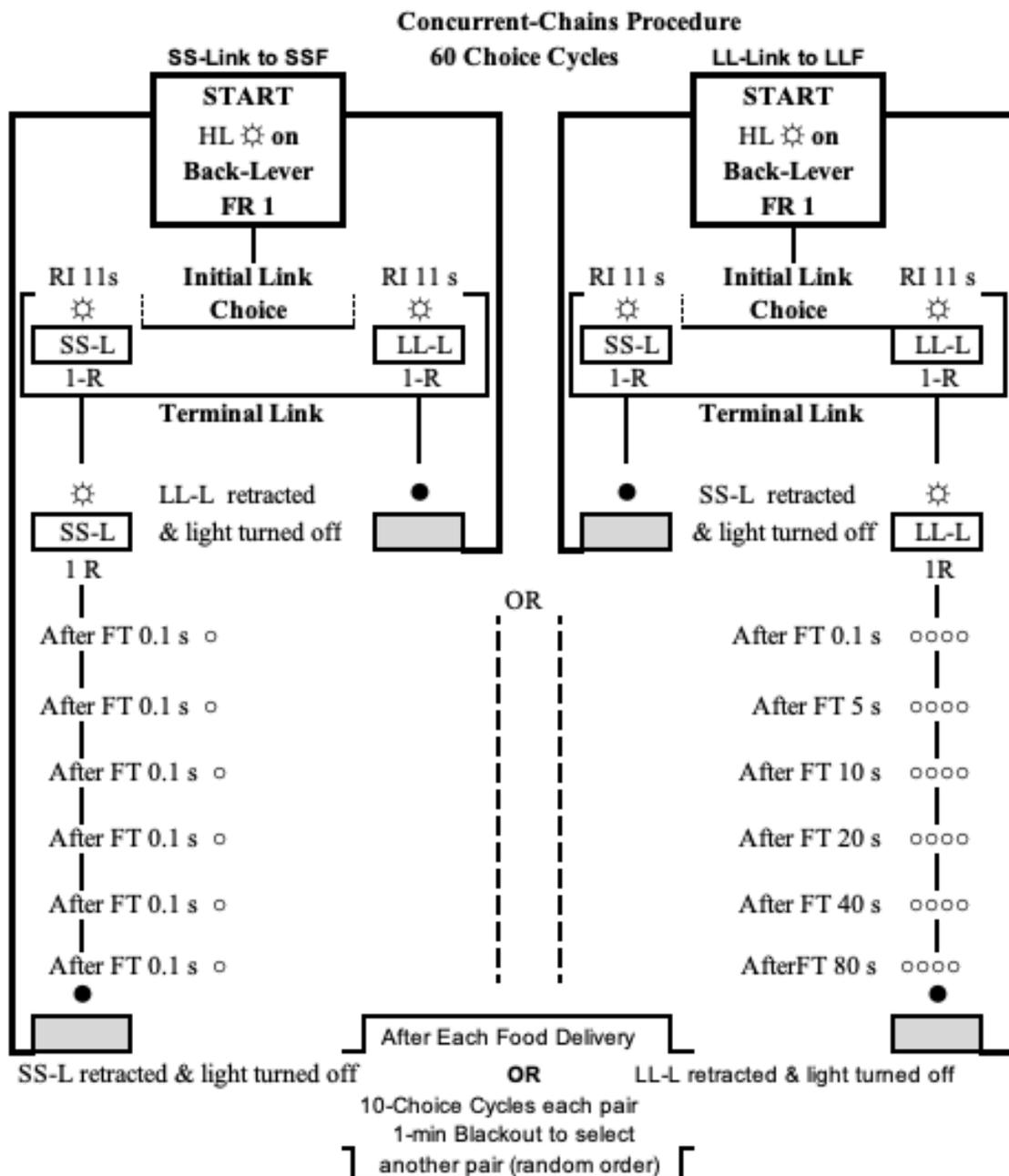
hopper (E14-01R), 3 cm x 4 cm, was centered between the left and right levers and behind the front panel a dry food dispenser (H14-23R) delivered 45 mg of grain-based pellets (BioServ®, F0165) into the hopper. A nonretractable lever (H21-03R), 3.4 cm x 1.5 cm, was placed on the back panel 6 cm above the floor. All levers required a force of approximately 0.2 N to be operated. A second hopper (E24-01) was mounted on the back wall; a photo-operandum buffer (H20-93) was attached to the hopper, 2.6 cm above the floor and 7 cm from the center of the nonretractable lever. It was used to record the behavior of licking the pipeline of a 100 ml bottle of water placed behind the back panel. All experimental events and data collection were programmed with Windows® controlled computers operating on Coulbourn Instruments® software (Graphic State Notation, version 3.03) operating at 0.01 s resolution.

### *Procedure*

A concurrent-chains procedure used two random interval (RI) schedules in the initial link, arranging terminal links entries to one fixed time (FT) response independent schedule delivering the SSR, or to the other FT delivering the LLR. Each session was composed of 60 choice cycles defining six delay components, each consisting of 10 choice cycles. Each cycle began with the house light turning on. A response to the back lever turned the light off, extended both the front levers, and turned the stimulus lights above both levers signaling the choice link. Choice was measured in the choice link with the responses emitted on the left and right levers. When one of the RI schedules timed out, the first response on the lever (left or right) associated with it advanced the cycle to the terminal link, retracting the other lever from the chamber and turning off the light above it. The second press on the available lever started the FT schedule which ended with the reinforcer

(SSR or LLR). The lever advancing the cycle to the terminal link, was not retracted from the chamber preventing it to signal the delay reinforcer (see Figure 1).

Additional presses to the back lever had no other scheduled contingencies but they were recorded. For half of the rats in each strain, the terminal link delivering one pellet (the SSR) was associated with the left lever and the terminal link delivering four pellets (the LLR) with the right lever. For the other half of the rats, the left lever delivered the LLR and the right the SSR. One terminal link used the same FT .01 s to deliver the SSR and the other terminal link used six FT schedules of .01, 5, 10, 20, 40, or 80 s to deliver the LLR. Each FT was operative for ten cycles. When either the SSR or LLR was delivered, each cycle ended, the lever was retracted, the stimulus light turned off, and the house light turned signaling the begin of the next cycle. The session ended after 60 cycles (six delay components) were presented, or 60 min elapsed, whichever event occurred first. Most sessions ended after the 60 cycles, not requiring the full 60 min. The study was conducted according to an ABABA experimental design, where no water available (A) and water (B) conditions alternated in succession. In condition B, a water bottle was continuously available in the experimental chamber during the session.



*Figure 1.* The Concurrent Chains procedure using 60 Choice Cycles. The cycles on the left arm ended with one food pellet (SSF) delivered by a FT 0.1 s schedule. The cycles on the right arm ended with four food pellets (LLF) delivered by FT schedules of 0.1, 5, 10, 20, 40, 80 s presented in random order during the session. A 1-minute blackout followed 10 choice cycle separating the six delay components.

### *Data Analysis*

The data from all sessions were used for the data analysis. The number of responses made by the SHRs and LEWs on the left, or SS- Lever, and right, or LL-lever, were computed separately. With these numbers the proportion of responses were computed as follows: (Response on the LL-lever / (Responses on the LL-lever + Responses on the SS-lever)).

The responses to the nonretractable lever and the number of licks to the pipeline of the bottle were recorded in the session. A 4-parameters logistic (4PL) nonlinear regression mode fitted the data.

$$y = \frac{A_1 - A_2}{1 + (x/x_0)^p} + A_2$$

Where  $A_1$  is the minimum asymptote of responses,  $A_2$  is the maximum asymptote of responses,  $p$  is the slope of the curve,  $x$  the independent variable, and  $x^0$  the inflection point where the responses change in direction. Regression analyses and statistical tests conducted at the alpha level of 0.05 were implemented with Origin®.

### Results

*Figure 2* shows that the proportion of LL choice decreased as a function of the delay of the LLF. Each graph shows that data from sixty days, or four blocks of 15 days each, where the hyperbolic model fitted the mean data from each group. From top to bottom the graphs show the conditions where the bottle of water was available (NW) or was not available (W) in the choice situation. The parameter  $k$  was estimated by the hyperbolic decay model (Mazur, 1987) and represents the degree of impulsivity the rats are exhibited, where small values of  $k$  represent low levels of impulsive behavior and large values of  $k$  high levels of impulsivity. All graphs show the proportion of the LL choice decreased with

the increasing delay to deliver the LLF. In the NW<sub>1</sub> condition, the SHRs showed higher levels of impulsivity ( $k = .035$ ) than the LEWs ( $k = .016$ ). NW<sub>2</sub> proved to be a turning point for both strains' level of impulsivity. The SHRs level of impulsivity began NW<sub>1</sub> at  $k = .035$  and peaked in the NW<sub>2</sub> condition with  $k = .046$ . Following this peak, the SHRs level of impulsivity began to decrease during W<sub>2</sub> and NW<sub>3</sub>, finishing the experiment with a decreased impulsivity level of  $k = .023$ . The LEWs began NW<sub>1</sub> with an impulsivity level of  $k = .016$  that was maintained during W<sub>1</sub>, but slightly decreased to  $k = .014$  during NW<sub>2</sub>. After this decrease, the LEWs began to behave more impulsively during both of the following sessions; W<sub>2</sub> had an impulsivity level of  $k = .018$  and the LEWs ended NW<sub>3</sub> with an increased impulsivity level of  $k = .027$ .

The last redetermination to NW<sub>3</sub> condition shows smaller levels of impulsivity for the SHRs ( $k=.023$ ) than for the LEWs ( $k=.027$ ). The SHRs' proportion of LLF choice decreased significantly when the LLF was delayed 20 s, whereas the LEWs' proportion of LL choice didn't decrease until the LLF was delayed 40 s. Both strains of rats show that the subjective value of the reinforcer decreased with the increasing delay to deliver the LLF. Estimates of the parameter  $A$  (y-intercept) show that the SHRs' choices began at  $A=.737$  and ended at  $A=.651$ ; similarly, the LEWs' choices began with y-intercept at  $A=.702$  and ended with y-intercept at  $A=.657$ ). Together, these results suggest that the SHRs discounted the LLF more steeply than the LEWs.

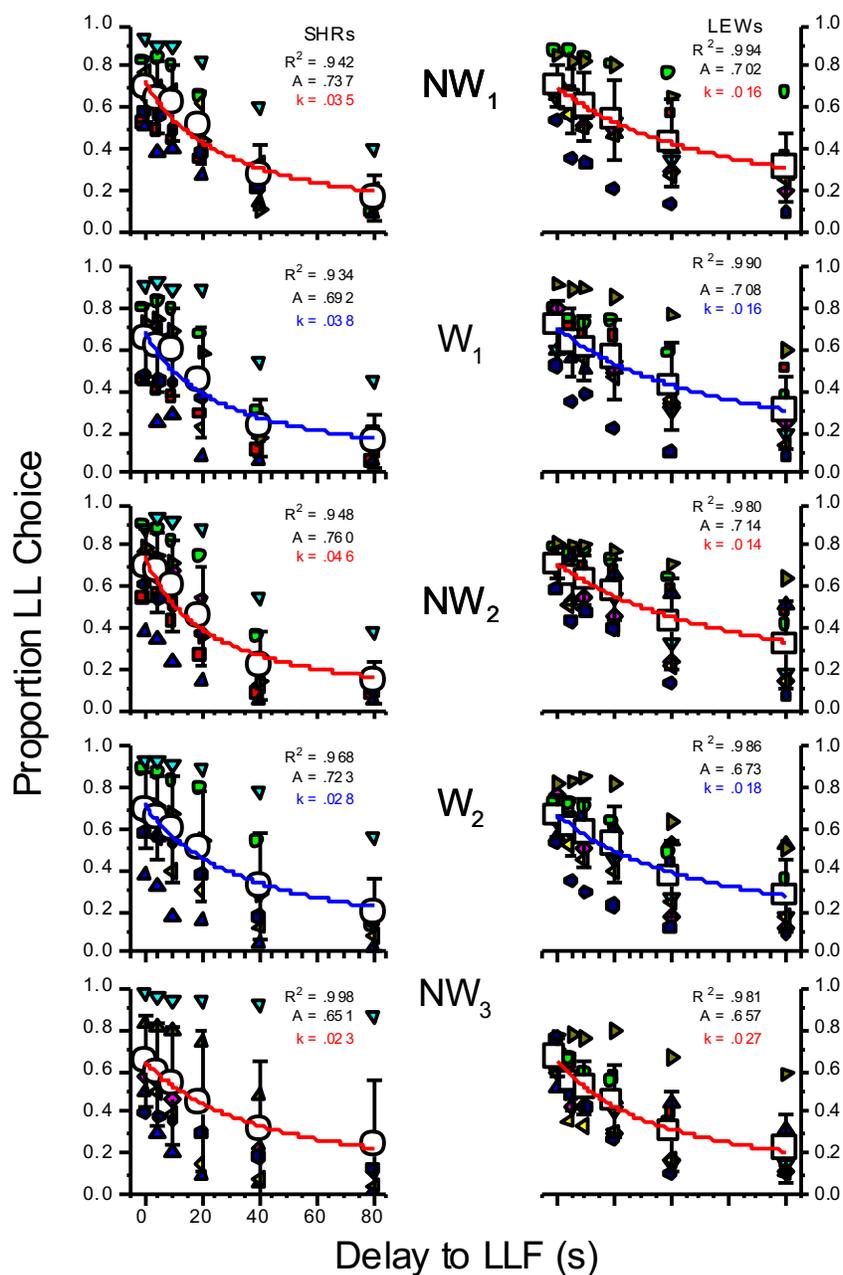
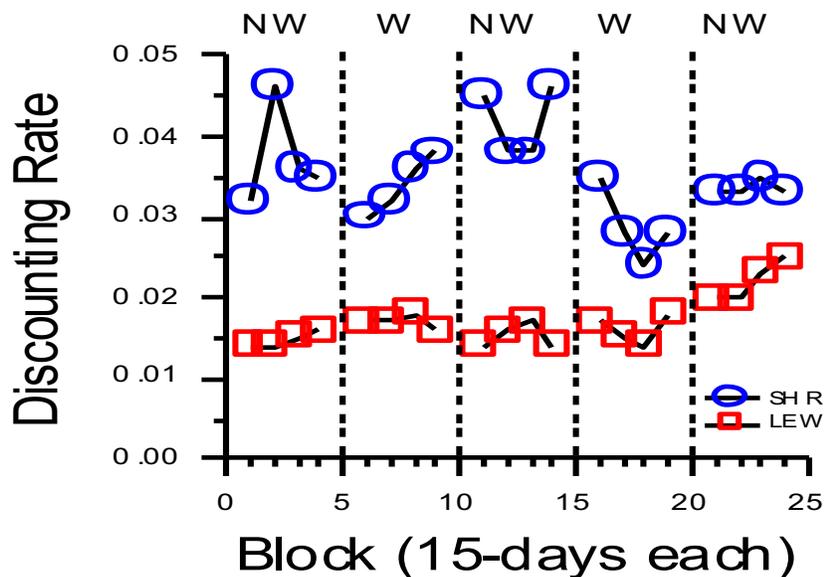


Figure 2. Each graph shows the proportion of LL choice against the block of sessions (15-days each) where the hyperbolic decay model was used to fit the data. The parameter A is an estimate of the y-intercept and the parameter k an estimate of discounting rate (impulsivity).

For each condition, *Figure 3* plots estimates of the parameter  $k$  (discounting rate) as a function of the blocks of sessions (15 days each). The SHRs show higher discounting rates (higher values of  $k$ ) than the LEWs showing small increments in discounting rate across conditions and blocks of sessions. The higher values of  $k$  value observed for the SHRs indicate that they made more impulsive choices than the LEW displaying low levels of impulsivity. The SHR's discounting rates, however, show more variability across block of sessions and conditions than the LEWs' discounting rates. Both strains of rats show higher levels of impulsivity when the bottle of water was not available than that they show when it was available in the choice situation.



*Figure 3.* Estimates of the parameter  $k$  (discounting rate) as a function of blocks of sessions (15-days each). The circles stand for the SHRs and the squares for the LEWs.

The number of licks to the bottle of water that occurred during the one-minute blackout separating delay components, are plotted in Figure 4 as a function of the number of blocks of sessions (15-days each). A 4-parameters logistic (4PL) nonlinear regression model fitted the data. Both strains drank water during the 1-min blackout. In block 1 the SHR licked the bottle 5221.6 times and LEWs 4593.5 times. Both strains show similar number of licks in blocks 2 and 3. However, in block 4 the LEWs liked the bottle more times (9128.1) during the 1-minute blackout than the SHRs (6592.8). Whereas the SHRs' number of licks did not change, the LEW's number of licks increased across clocks of sessions. However, the inflection points ( $X_0$ ) and slopes ( $p$ ) of the fitting curves are similar to one another.

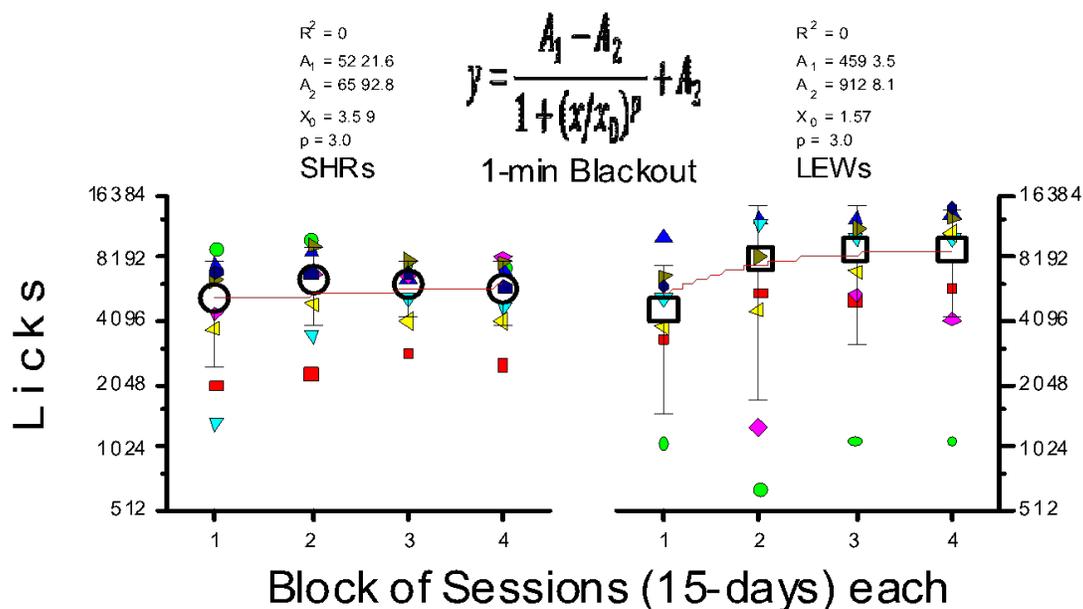
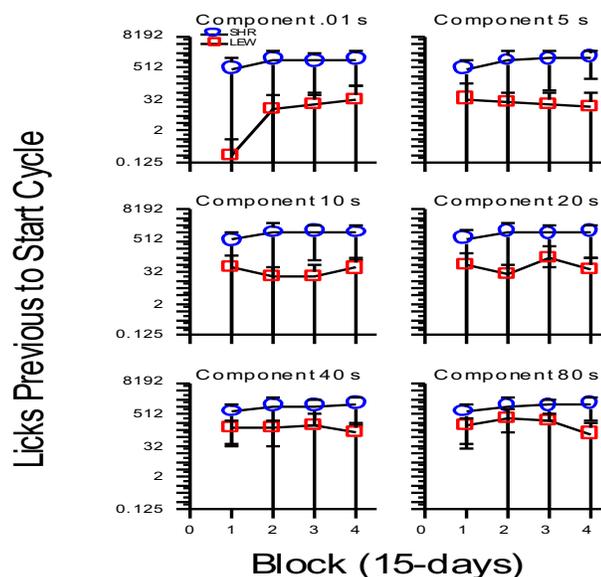
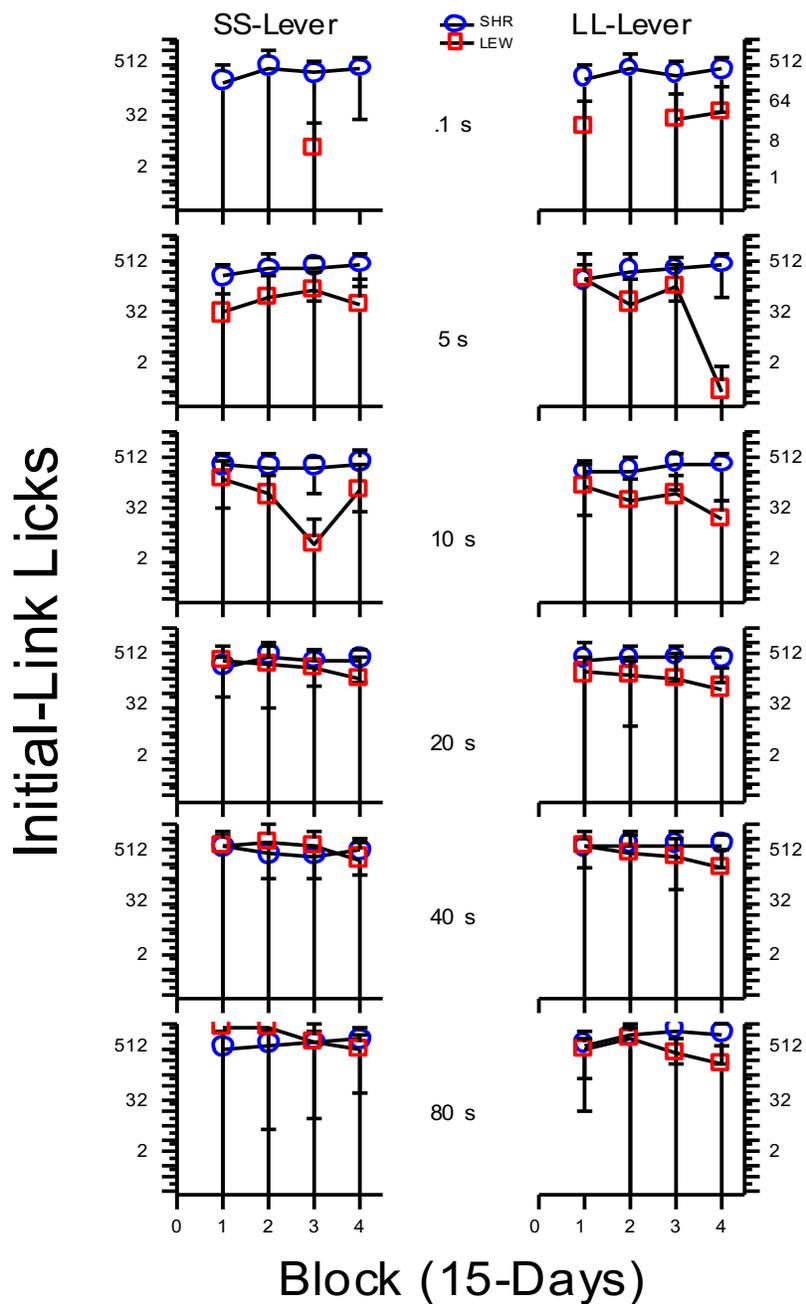


Figure 4. Number of licks as a function of blocks of sessions (15-days each). The circles stand for the SHRs and the squares for the LEWs. The line is the best fitting line using a 4-parameters logistic (4PL) nonlinear regression model. Corresponding parameter appear near the data points.

The number of licks to the bottle of water that occurred before the rats started the choice cycles are plotted in *Figure 5* as a function of the blocks of sessions. The panels are organized by delay component (0.01, 5, 10, 20, 40, and 80 s). All graphs show that the SHRs licked the bottle of water more times than the LEWs, suggesting more motor impulsivity in the former than in the latter strain of rats. Figure 5 shows the number of licks to the bottle of water that occurred in the initial link as a function of blocks of sessions. The left graphs show the data from the SHRs and the right graphs the data from the LEW. From top to bottom the graphs display the licks that occurred in the six delay components before the rats advanced to the terminal links. Once the rats pressed the back lever to start the initial link, the SHRs emitted more licks to the bottle of water in initial link than the LEWs. Note that both strains of rats barely licked the bottle of water in the 0.1-s delay component, advancing to the terminal links quick.

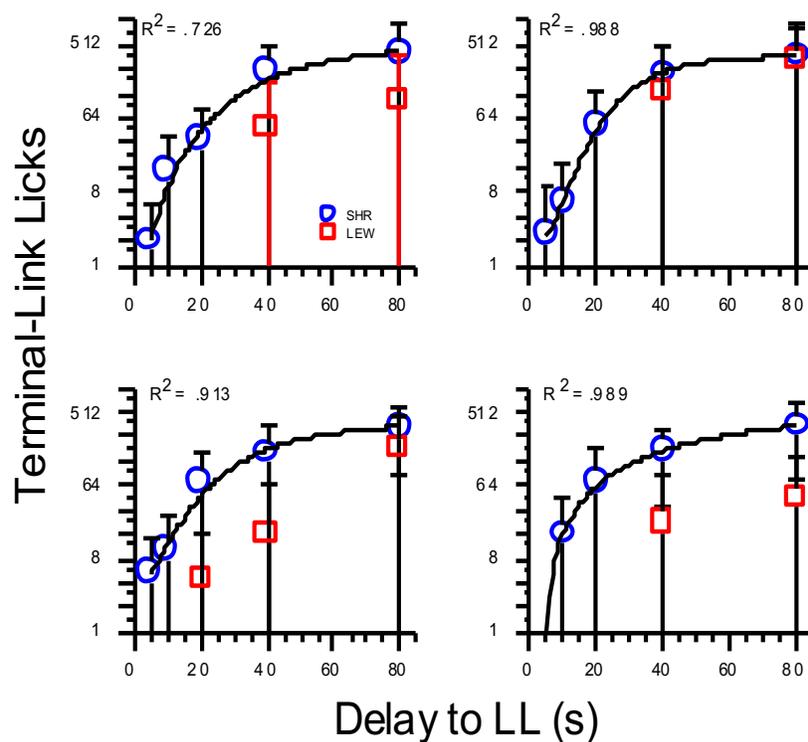


*Figure 5.* Number of licks to the bottle of water previous to start each choice cycle as a function of blocks of sessions. Each graph represents the total number of licks after the 1-minute blackout and before the beginning of the initial link with the selected delay component.



*Figure 6.* Number of licks to the bottle of water during the initial link as a function of blocks of sessions. From top to bottom the panels show the number of licks by delay component (0.1s to 80s) when the SS lever (left graphs) and LL lever were extended into the chamber.

The number of licks to the bottle of water that occurred in the terminal links are plotted in Figure 6 as a function of the delay to deliver the LLF. The panels show the data from the four blocks of session. The circles stand for the SHRs and the squares for the LEWs. Figure 7 shows that when the rats were waiting for the delivery of the LLF, the SHRs licked the bottle of water more times across delays (0.01, 5, 10, 20, 40, and 80 s) to the LLF than the LEWs. These results confirm that the SHRs showed more impulsivity than the LEWs.



*Figure 7.* Number of licks during the terminal link as a function of the delay to deliver the LLF. The panels show the data from four blocks of sessions.

## Discussion

This experiment sought to determine if the development of polydipsia through the availability of water could impact and even predict cognitive impulsivity. Utilizing an ABABA design that alternated water availability, a differentiation between each strain of rat and their resulting behaviors was made possible. Following previous research regarding the presence of water on the behavior of rats, this study was in line with exhibited behavioral changes. In conjunction with past research, SHRs proved to behave more impulsively than their control LEWs and are an appropriate animal model for ADHD.

While previous research determined cognitive impulsivity is derived from various neurological processes, through a concurrent chain procedure, this present study extended those findings to conclude that impulsivity is directly influenced by environmental factors, like the availability of water and can change as a function of learning. The presence of environmental factors allows for the rats to focus their energy on activities, such as licking a water bottle while they wait for their reinforcer, thus decreasing their impulsive behavior. This behavior was seen with both strains as they had increased licking behaviors, especially the SHRs.

Not only were the rat's levels of impulsivity directly influenced by water availability, the ABABA design served as a function of learning for the rats while they waited for the food pellets. All the rats learned to discount the value of the LLF more steeply depending on the length of the delay to the reinforcer. As a result of the increasing delays, the rats began to choose the levers more impulsively to avoid the delay to the reinforcement. Once the rats learned to discount more steeply, their impulsivity changed

as a result to avoid the delay for a larger reinforcement in favor of the smaller soon reinforcement.

These findings will allow for further research to be conducted to expand these results to determine the full extent that water influences behavioral changes in regard to impulsivity as well as how that impulsivity can change with learning. The rat's ability to change their impulsivity through learning based off environmental factors challenges previous research that claimed cognitive and motor impulsivity was driven by neurological processes.

### Conclusion

The present study showed that when the SHRs and LEWs had the bottle of water available in the choice situation, they made less impulsive choices than the impulsive choices that they made when the water was not available. Thus, they were less likely to exhibit impulsive behavior when the water was available. The longer the delay to the four-pellet reinforcer (LLF), the smaller the number of responses on the LL lever. All rats discounted the value of the LLF with the increasing delay to its delivery. The rats learned to choose impulsively with the extended training in the choice situation. It could be concluded that cognitive impulsivity changes with learning. Thus, impulsivity is not only determined by neurological and biological factors, impulsivity changes as a function of training in the impulsive task.

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