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# CHARACTERIZATION OF WHEEL RUNNING IN RATS AS SCHEDULE-INDUCED BEHAVIOR

## CARACTERIZACIÓN DE LA ACTIVIDAD EN RATAS COMO CONDUCTA INDUCIDA POR PROGRAMA

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*A mis queridos padres, Adriana y Lucas,*

*y a mi hermana Pamela,*

*por darme la libertad para elegir lo que quisiera ser, sin dejar nunca de apoyarme.*

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*“El pasado es arcilla que el presente*

*labra a su antojo. Interminablemente.”*

Jorge Luis Borges

Todos los ayeres, un sueño (1985)

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## Abbreviations and acronyms

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **AIP** | Actividad Inducida por Programa |
| **BL** | Baseline |
| **CC** | Conditioning Chamber |
| **CS** | Conditioned Stimulus |
| **DRO** | Differential Reinforcement of Other behavior |
| **EOLPs** | Extra Observing Lever Presses |
| **EOLPs-Ext** | Extra Observing Lever Presses in Extinction condition |
| **EOLPs-VR** | Extra Observing Lever Presses in Variable Ratio schedule condition |
| **FT** | Fixed Time Schedule |
| **GT** | Goal Tracker |
| **HC** | Home Cage |
| **IER** | Intervalo Entre-Reforzadores |
| **IFI** | Inter-Food Interval |
| **LPs** | Lever Presses |
| **LPs-Ext** | Lever Presses in Extinction condition |
| **LPs-VR** | Lever Presses in Variable Ratio schedule condition |
| **OLPs** | Observing lever presses |
| **OLPs-Ext** | Observing lever presses in extinction condition |
| **OLPs-VR** | Observing lever presses in variable ratio schedule condition |

| | |
|---|---|
| **PIP** | Polidipsia Inducida por Programa |
| **REC** | Recovery |
| **SEM** | Standard Error of the Mean |
| **SIP** | Schedule-Induced Polydipsia |
| **SIWR** | Schedule-Induced Wheel Running |
| **ST** | Sign Tracker |
| **TF** | programa de reforzamiento de Tiempo Fijo |
| **VR** | Variable Ratio Schedule |

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CHAPTER III

# CHARACTERIZATION OF WHEEL RUNNING IN RATS AS SCHEDULE-INDUCED BEHAVIOR

## Abstract

Schedule-induced behaviors are distinct from operant behavior that arise excessively under conditions of intermittent reinforcement. They occur without any explicitly programmed contingency of reinforcement and develop a characteristic temporal distribution. Schedule-induced polydipsia (SIP) is generally considered a prototype par excellence.

However, not all behaviors studied in these terms have so clearly established been established as being schedule-induced behaviors. On example is schedule-induced wheel running (SIWR). Currently there are conflicting visions about its suitability as schedule-induced behavior.

Based on this background, the aim of this thesis has been to study the development and maintenance of the SIWR, considering the characteristics of excessiveness, location and temporal distribution within the inter-food interval (IFI). This was achieved by manipulating the experimental conditions, to through two main studies, discussed in Chapters 2 and 3. A third study (Chapter 4) was based on the previous results obtained, and focused on studying another aspect of the schedule-induced behaviors. The reference to its excess as a characteristic feature in the diagnosis of psychopathological disorders related to impulse control.

In Chapter 2, the development of the SIWR in rats under fixed-time (FT) schedules of different length (30, 60, 120, 240, and 480 s) was studied in a first experiment. The order of presentation was counterbalanced among the animals, (except for FT 480 s, which was presented at the end to complete the data set). Rats were also

exposed to a massed-food control condition. The goal was to investigate the influence of intermittentency reinforcement deliveries on maintenance of SIWR.

The results showed that the SIWR was developed under the range of 30-240 s, but not the FT 480 s schedule, with a SIWR was a gradation as a function of inter-food interval (IFI) length. The data also shows that the wheel running adopts a post-reinforcing position within the IFI, presenting a temporary distribution in the form of an inverted U-shaped. Wheel running reduced in the control condition.

Altogether, these data support the idea that wheel running is a schedule-induced behavior. In a second experiment, we proceeded to investigate more about the conditions under which the SIWR develops.

In Experiment 2, the subjects were randomly divided into two groups. All animals were exposed to the same FT schedules as in Experiment 1, except for the FT 480-s. Half of the rats had a wheel in their home cages, while the other half had, a wheel and water bottle during the experimental session.

The presence of the wheel in the home cage caused the reduction of the wheel running rate, as well as the shift of the temporal distribution curve to the right for the FT 30 and 60 s schedules. On the other hand, the availability of a water bottle in the conditioning chambers did not affect the wheel running rate, although the development of the SIP shifted the wheel running curve to a more central position within the IFI, resulting in temporary competition between behaviors.

The results of this first study laid the groundwork for validating SIWR. The results were discussed by comparing them with SIP.

In Chapter 3, the effect of operant contingencies over three behaviors, drinking, running and magazine entering was analyzed, according to Staddon's classification (1977): interim, facultative and terminal behaviors, respectively. At first, the animals

were exposed to an FT 60 s schedule for the establishment and acquisition of stable response rates. Rats were then randomly distributed in two groups. In both cases, the appearance of a protective contingency postponed the appearance of food when the animals performed any of the three responses during the last 1, 3, 5, 10, 20, 40 or 58 s of the IFI, under an FT 60- s schedule. For half of the animals the delay was signaled by the appearance of a tone and the extinction of the lights, while for the other group the delay was not signaled. In a final phase, the delays were eliminated. The average of the last three sessions of the acquisition condition was used as baseline.

The results showed that the three behaviors are affected by protective delays based on their temporary location within the IFIs. Drinking was the behavior that most resisted the disruptive effects of delays, followed by wheel running and, finally, magazine entries. Magazine entries decreased, since the 1s delay, gradually due to the increase in the duration of the delay due to its close relationship with the reinforcer. The wheel running fell from the 1s delay but remained relatively stable with the increase in the duration of the delays. The effects of the delay on drinking were significant given the longer delays (40 and 58 s). The rates of all behaviors increased when delays were suppressed. However, no significant differences were found between signaled and unsignaled delays.

These results indicate that the introduction of contingent delays to the response generates response gradients that reflect the effect of operating contingencies on the responses, showing different sensitivities depending on their temporal location within the IFI.

In Chapter 4, impulsivity levels in sign-trackers (ST) and goal-trackers (GT) rats were studied through exposure to intermittent reinforcement schedules in the development of SIP (Experiment 1) and SIWR (Experiment 2) under different FTs, 5, 30, 60 and 120 s, and, 60 and 120 s, respectively; exposure to schedules was counterbalanced among animals. Subsequently, in Experiment 3, the levels of compulsivity of both groups were measured in an operant observing response task with uncertain reinforcement. Finally, in Experiment 4, cognitive impulsivity levels of both groups were recorded by a delay discounting procedure, the delay values for obtaining the greatest magnification were 5, 10, 20 and 40 s.

The results showed the acquisition of SIP and SIWR in both groups, however, GT rats presented higher levels of drinking and wheel running than the ST group. While ST rats made more magazine entries than GT. Compulsivity and cognitive impulsivity levels did not differ between ST and GT.

These results suggest that the schedule-induced behaviors are not related to the impulsivity traits as previously thought. Furthermore, SIP and SIWR behave as operant behavior, due to these behaviors being behaviors directed towards the goal.

Together, these three studies offer a description of the wheel running as a schedule-induced behavior, presenting the same nature as the SIP, although with different sensitivity to delay to the reinforcer.

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## Resumen

Las conductas inducidas por programa son actividades, diferentes a la conducta operante, que surgen de forma excesiva bajo condiciones de refuezo intermitente, sin que exista ninguna contingencia de reforzamiento explícitamente programada, y que desarrollan una distribución temporal característica, para las que, la polidipsia inducida por programa (PIP) representa el prototipo por excelencia.

Sin embargo, no todas las conductas que se han estudiado en estos términos han conseguido establecer, claramente, su pertenencia a este grupo de conductas. Ejemplo de ello es la actividad inducida por programa (AIP), para la que aún existen visiones contrapuestas sobre su idoneidad como conducta inducida.

Partiendo de dichos antecedentes, el objetivo de esta tesis ha sido estudiar el desarrollo y mantenimiento de la AIP, atendiendo a las características de excesividad, localización y distribución temporal dentro del intervalo entre-reforzadores (IER) mediante la manipulación de las condiciones experimentales, a través de dos estudios principales, recogidos en los Capítulos 2 y 3; un tercer estudio (Capítulo 4), con base en los resultados previos obtenidos, se centró en estudiar otro aspecto de las conductas inducidas, la referencia a su excesividad como rasgo característico en el diagnóstico de trastornos psicopatológicos relacionados con el control de impulsos.

En el Capítulo 2, se estudió, en un primer experimento, el desarrollo de la AIP en ratas bajo programas de reforzamiento de tiempo fijo (TF) de diferente longitud (30-, 60-, 120-, 240-, y 480-s), contrabalanceando el orden de presentación entre los animales, (excepto para TF 480-s, que fue presentado al final para completar el conjunto de datos).

A continuación, se expuso a los sujetos a una condición de control donde los alimentos eran presentados todos juntos al inicio de la sesión, con la intención de estudiar la influencia de la intermitencia en la entrega del reforzador sobre el mantenimiento de la AIP.

Los resultados mostraron que la AIP se desarrollaba bajo un rango de 30 a 240-s, no siendo así para el programa de TF 480-s, con una disminución en la tasa de carrera a medida que la longitud del IER incrementaba. Los datos también muestran que la carrera adopta una posición post-reforzador dentro del IER, presentando una distribución temporal en forma de U-invertida. La introducción de la condición control provocó la reducción de la tasa de carrera.

En su conjunto, estos datos apoyan la idea de que la carrera es una conducta inducida por programa, por lo que en un segundo experimento se procedió a indagar más sobre las condiciones bajo las que se desarrolla la AIP.

En el Experimento 2, los sujetos fueron divididos, de forma aleatoria, en dos grupos. Todos los animales fueron expuestos a los mismos programas de TF que en el Experimento 1, a excepción del TF 480-s, sin embargo, la mitad de los sujetos dispuso de una rueda de actividad en sus jaulas hogar, mientras que, la otra mitad tenía, además de la rueda de actividad, una botella de agua durante la sesión experimental.

La presencia de la rueda de actividad en la jaula hogar provocó una reducción en la tasa de carrera, así como, el desplazamiento de la curva de distribución temporal hacia la derecha para los programas de TF 30 y 60-s. Por otra parte, la disponibilidad de una botella de agua en las cajas de condicionamiento no repercutió sobre la tasa de carrera, aunque el desarrollo de la PIP desplazó la curva de distribución de la carrera hacia una posición más central dentro del IER, expresando una competencia temporal entre las conductas.

Los resultados de este primer estudio sentaron las bases para hablar de AIP. Los resultados fueron discutidos comparándolos con los encontrados para la conducta inducida por excelencia, la PIP.

En el Capítulo 3, se analizó el efecto de contingencias operantes sobre tres conductas, beber, correr y entrar al comedero, conforme a la clasificación de Staddon (1977): conductas de interim, facultativas y terminales, respectivamente. En un primer momento, los animales fueron expuestos a un programa de reforzamiento de TF 60-s para el establecimiento y adquisición de tasas estables de respuesta. Posteriormente, los animales fueron distribuidos aleatoriamente en dos grupos. En ambos casos, la aparición de una contingencia protectora posponía la entrega del reforzador cuando los sujetos realizaban cualquiera de las tres conductas durante los últimos 1, 3, 5, 10, 20, 40 o 58-s del IER, bajo un programa de TF 60-s. Para la mitad de los animales la demora fue señalada mediante la aparición de un tono y el apagado total de las luces, mientras que, para el otro grupo la demora no se señalaba. En una fase final, las demoras fueron eliminadas. Como línea base se tomó la media de las tres últimas sesiones de la condición de adquisición.

Los resultados mostraron que las demoras protectoras afectaban a las tres conductas de forma independiente, en función de su localización temporal dentro del IER. La bebida fue la conducta que más resistió los efectos disruptivos de las demoras, seguida de la carrera y, finalmente, las entradas al comedero. Las entradas al comedero disminuyeron, desde la demora de 1-s, de forma gradualmente ante el incremento en la duración de la demora, debido a su estrecha relación con el reforzador. La carrera cayó desde la demora de 1-s, pero permaneció relativamente estable con el aumento en la duración de las demoras. Los efectos de la demora sobre la bebida resultaron significativos ante las demoras más largas (40 y 58-s). Las tasas de todas las conductas

incrementaron cuando las demoras fueron suprimidas. Sin embargo, no se encontraron diferencias significativas entre las demoras señaladas y no señaladas.

Estos resultados indican que la introducción de demoras contingentes a la respuesta genera gradientes de respuesta que reflejan el efecto de contingencias operantes sobre las respuestas, mostrando diferentes sensibilidades en función de su localización temporal dentro del IER.

En el Capítulo 4, se estudiaron los niveles de impulsividad en ratas sign-trackers (ST) y goal-trackers (GT) mediante su exposición a programas de refuerzo intermitente en el desarrollo de PIP (Experimento 1) y AIP (Experimento 2) utilizando diferentes programas de TF, de 15, 30, 60 y 120 segundos y, de 60 y 120 segundos, respectivamente; la exposición a los programas fue contrabalanceada entre los animales. Posteriormente, en el Experimento 3, los niveles de compulsividad de ambos grupos fueron medidos en una tarea de respuesta de observación operante con refuerzo incierto. Finalmente, en el Experimento 4, los niveles de impulsividad cognitiva de ambos grupos fueron registrados con una tarea de descuento por demora, los valores de la demora para la obtención del refuerzo de mayor magnitud fueron de 5, 10, 20 y 40 segundos.

Los resultados mostraron la adquisición de PIP y AIP en los dos grupos, no obstante, las ratas GT presentaron niveles superiores de bebida y carrera en relación con el grupo ST. Mientras que las ratas ST realizaban más entradas al comedero que las GT. Los niveles de compulsividad e impulsividad cognitiva no difirieron entre ST y GT.

Estos resultados apuntan a que las conductas inducidas por programa no están relacionadas con los rasgos de impulsividad como se pensaba anteriormente; así como que la PIP y la AIP se comportan como conductas operantes, siendo estas conductas dirigidas a la meta.

En su conjunto, estos tres estudios ofrecen una descripción de la carrera como conducta inducida por programa, presentando la misma naturaleza que la PIP, aunque con diferente sensibilidad a la demora del reforzador.

# CAPÍTULO I

# Introducción General

## 1. Conductas adjuntivas y Conductas inducidas por programa

En 1961 Falk observó que la exposición de ratas privadas de alimento a programas de refuerzo intermitente, con acceso libre a una botella de agua en la caja de condicionamiento, provocaba el desarrollo de un consumo de bebida excesivo, sin relación con necesidades fisiológicas o la regulación aparente del comportamiento. Falk (1971) denominó este fenómeno conductual como polidipsia inducida por programa (PIP), y la categorizó dentro de lo que él llamó conductas adjuntivas.

Las conductas adjuntivas son definidas como actividades que ocurren a tasas significativamente más altas, aunque no necesariamente excesivas, respecto a condiciones de control, como resultado de la exposición a programas de refuerzo intermitente, y sin que exista ninguna contingencia de reforzamiento explícitamente programada, presentando generalmente, una ubicación temporal al principio del intervalo entre-reforzadores (IER), periodo post-reforzamiento, con una distribución en forma de U-invertida a lo largo del IER (Falk, 1971; Roper y Posadas-Andrews, 1981; Timberlake, Wahl y King, 1982; Wetherington, 1982). Así mismo, estas conductas adjuntivas pueden ser clasificadas en conductas inducidas o conductas no-inducidas por el programa de reforzamiento.

Como su propio nombre indica, la polidipsia inducida por programa pertenece a las denominadas conductas inducidas por programa, es decir, actividades que surgen de forma excesiva por la intermitencia establecida por el programa de refuerzo (Roper, 1981). Las conductas inducidas se definen como actividades diferentes a la respuesta operante que desarrollan una tasa excesiva de respuesta en programas de refuerzo intermitente, y que muestran una distribución temporal característica (Wetherington y Brownstein, 1982).

Staddon (1977) propuso una clasificación de estas conductas inducidas por el programa basándose en la localización temporal que adoptan dentro de IER y su relación con el reforzador, distinguiendo entre respuestas terminales y actividades de intermedio (también llamadas de *ínterim*). Las respuestas terminales, como por ejemplo las entradas al comedero, ocurren alrededor de la presentación del reforzador y, por lo tanto, en presencia de estímulos relacionados con su presentación y consumición (Robinson y Flagel, 2009); mientras que, las actividades de intermedio preceden a las actividades terminales, con las que generalmente son incompatibles, y se ubican desde inmediatamente después de la entrega del reforzador hasta, aproximadamente, la mitad del IER, como consecuencia de una baja probabilidad de presentación del reforzador (p. ej. la PIP) (Staddon y Simmelhag, 1971).

Por otra parte, Staddon (1977) utilizó el término "facultativo" para referirse a aquellas conductas que no estarían inducidas por el programa, y que, simplemente, completarían el espacio de tiempo entre las actividades de ínterim y terminales cuando la distancia entre los reforzadores era lo suficientemente larga. Estas conductas no aumentarían su frecuencia como consecuencia de la exposición a programas de refuerzo intermitente, sin embargo, presentarían una línea de base no nula en animales privados de alimento (Roper, 1981; Staddon y Ayres, 1975). Así mismo, las conductas facultativas adoptarían una posición intermedia entre las actividades de ínterim y las respuestas terminales dentro de la IER. Como ejemplos de esta clase de conductas Staddon (1977) incluyó el correr en una rueda de actividad y el acicalamiento.

El descubrimiento de la PIP desencadenó la búsqueda e investigación de otras conductas inducidas por programa que presentaran patrones similares. Ejemplos de estas conductas son: lamer una corriente de aire (Mendelson y Chillag, 1970), masticar virutas de madera (Roper y Crossland, 1982), el consumo de sustancias no nutritivas/pica

(Villareal, 1967), la agresión/ataque (Azrin, Hutchinson y Hake, 1966; Looney y Cohen, 1982; Robinson, Flory y Dunahoo, 1990), el escape (Azrin, 1961; Brown y Flory, 1972), la defecación (Rayfield, Segal y Goldiamond, 1982), así como, correr en una rueda de actividad (Levitsky y Collier, 1968).

Aunque Staddon (1977) calificaría a la conducta de correr en una rueda de actividad como conducta no-inducida, atendiendo a su localización temporal en mitad del IER, y atribuyendo su incremento al alargamiento del IER, y no a la intermitencia del programa de reforzamiento (Roper, 1978; Staddon y Ayres, 1975), para otros autores (Levitsky y Collier, 1968), la carrera ha sido considerada una conducta inducida por el programa: Actividad inducida por programa (AIP), donde los animales privados de alimentos desarrollan altas tasas de carrera cuando son expuestos a programas de refuerzo intermitente mientras tienen acceso libre a una rueda de actividad. Este debate sobre actividad inducida versus no-inducida generó en su momento una gran cantidad de investigación, que finalmente fue abandonado sin una resolución clara. Este hecho ha sido la base para el desarrollo de la presente tesis. Las investigaciones previas sobre AIP serán documentadas en un apartado posterior, pero antes, se presentan las características propias de la PIP, por ser considerada la conducta inducida por antonomasia; características de referencia a las que debería responder la AIP en el caso de tratarse de una conducta inducida.

## 2. Características de la polidipsia inducida por programa

### *2.1. Excesividad*

El término polidipsia significa beber con frecuencia y abundantemente. En el caso de la polidipsia inducida por programa este patrón conductual no responde a una necesidad fisiológica destinada a facilitar la ingesta de alimentos, la cual recibe el nombre de bebida prandial, ni actúa como un mecanismo regulatorio, que sería una bebida homeostática. Se trata de un patrón conductual persistente y excesivo que surge bajo unas condiciones fisiológicas (privación de alimentos; p. ej.  entre 80-85% de su peso ad libitum) y experimentales (programas de refuerzo intermitente) determinadas.

Para entender el grado de excesividad de la bebida desarrollado en PIP el estudio original de Falk (1961) muestra como ratas expuestas a un programa de intervalo variable 1-min, en sesiones diarias de 3,17 horas, consumían alrededor de 92 ml de agua, unas tres veces su consumo diario habitual.

### *2.2. Localización temporal*

La polidipsia inducida por programa adopta una localización temporal post-reforzador (Falk, 1971), alcanzando su tasa máxima de respuesta en los primeros momentos de IER, para posteriormente disminuir paulatinamente (Falk, 1966; Flores y Pellón, 1997; Pellón y Blackman, 1992). Sin embargo, esta posición puede retrasarse en el tiempo cuando se manipula la condición experimental restringiendo el acceso a la botella de agua hasta los últimos segundos del intervalo, sin afectar de manera relevante la cantidad de agua ingerida (Gilbert, 1974) ni la distribución de la conducta (López-Crespo, Rodríguez, Pellón y Flores, 2004).

## 3. Actividad inducida por programa

### *3.1. Estudios a favor y en contra*

Como ya se ha mencionado, aún no se ha llegado a un consenso sobre la naturaleza de la carrera; desde que fuera considerada inicialmente como conducta inducida, dado su parecido con la PIP [p.ej. ninguna se desarrolla bajo programas de reforzamiento continuo; ambas dependen de la presentación intermitente del reforzador; y ambas ocurren inmediatamente después de la entrega del refuerzo (Levitsky y Collier, 1968)], una serie de estudios, tanto a favor como en contra de la actividad inducida por programa, fueron desarrollados con base en las características asumidas para las conductas inducidas, con la PIP como prototipo de referencia.

Las investigaciones que sugieren que la carrera podría ser un comportamiento no inducido basan sus conclusiones en las siguientes observaciones: 1) la carrera se ubica en mitad del IER, después de beber y antes de las conductas terminales, claramente anticipatorias de la ocurrencia del reforzador (Staddon y Ayres, 1975); 2) la carrera no está limitada por las condiciones de presentación de los alimentos, al contrario de otras conductas inducidas como la PIP (Penney y Schull, 1977; Staddon, 1977; Staddon y Ayres, 1975; Wetherington, Brownstein y Shull, 1977); 3) un aumento en la tasa de liberación de alimentos provoca un incremento de las actividades terminales y de ínterim, mientras que la carrera disminuye (Staddon y Ayres, 1975); 4) la carrera ocurre principalmente durante los intervalos posteriores a la omisión, independientemente del tiempo transcurrido desde la última entrega del reforzador (Penney y Schull, 1977); 5) la carrera presenta una frecuencia elevada incluso en ausencia total de alimentos, como por ejemplo cuando se impone un procedimiento de extinción (Staddon, 1977).

Por otra parte, los "defensores" de la actividad como inducida por programa han argumentado su idoneidad y, en ocasiones, han hecho referencia a limitaciones experimentales que podrían explicar los resultados obtenidos por los detractores de esta visión.

Ya en 1974 King demostraba que la carrera podía ser una conducta inducida de ínterim cuando, en lugar de comida, los intervalos finalizaban con la presentación de una pequeña cantidad de agua, por lo que la inducción de ciertas conductas podría estar determinada por las características del reforzador. Posteriores estudios indicaron, sin embargo, que también es posible desarrollar actividad inducida por programa utilizando bolitas de comida como reforzador.

Timberlake (1982) rebatía el uso de la condición de extinción como base para explicar la influencia de la presentación intermitente de refuerzos sobre las conductas inducidas, ya que la oportunidad de expresión de la carrera era mayor bajo la condición de extinción que bajo un programa de refuerzo intermitente, al no competir con las conductas relacionadas con la consumición del reforzador. Esta teoría explicaría los resultados encontrados por Staddon (1977) donde la carrera mantenía una alta tasa de respuesta bajo condición de extinción.

White (1985) se centró en investigar las características propias de las conductas inducidas en la AIP, en concreto, se interesó en la influencia de refuerzo intermitente sobre el mantenimiento de la AIP, así como en la distribución temporal de la carrera dentro del IER, encontrando que, bajo condiciones de refuerzo masivo la tasa total de carrera disminuía en tres de los cuatro sujetos, resultando ser menor que la tasa observada bajo el programa de reforzamiento de tiempo fijo (TF) 60-s; volviendo a incrementar tras el restablecimiento del programa TF 60-s. Sus resultados también mostraron una distribución temporal clara en el programa de TF 60-s, donde la ejecución de la carrera era improbable tras la presentación del reforzador, aumentando hasta alcanzar su pico máximo sobre lo que correspondería al segundo 24 aproximadamente, y disminuía gradualmente hacia el final del IER, presentando un patrón bien definido en forma de U-invertida. Estos resultados llevaron a White a concluir que la carrera es una conducta inducida.

### *3.2. Influencia de la longitud de IER*

Las conductas inducidas, y en particular la PIP, muestran una relación bitónica en forma de U-invertida respecto a la frecuencia de presentación de los reforzadores (Falk, 1966; Flory, 1971; Roper, 1980), mientras que las conductas no-inducidas aumentan su tasa en paralelo con los incrementos en la duración del IER, como generalmente se afirma que ocurre con la carrera (Penney y Schull, 1977; Staddon, 1977; Staddon y Ayres, 1975). Sin embargo, en el estudio de Riley, Wetherington, Delamater, Peele y Dacanay (1985) los resultados apuntaron a que la tasa de la carrera podría presentar una función bitónica para el valor del TF. En dicho estudio, el programa más largo empleado fue un TF 180-s, por lo que, los autores creen que con intervalos más largos la tasa de carrera podría descender adoptando la forma de función bitónica.

### *3.3. Competencia temporal entre conductas*

Los estudios que han descrito la ubicación que adoptan las conductas dentro del IER cuando ocurren tanto actividades de ínterim como conductas no-inducidas, generalmente indican que las actividades de ínterim tienden a ocurrir primero y la distribución global de la actividad es multimodal (Reid, Bacha y Moran, 1993; Roper, 1978; Staddon y Ayres, 1975; Staddon & Simmelhag, 1971), con el primer pico representando a la conducta de ínterim y, posteriormente, la conducta no-inducida (Penney y Schull, 1977). Sin embargo, cuando sólo ocurren actividades de ínterim en el intervalo, o cuando únicamente ocurren conductas no-inducidas, la distribución resultante es unimodal, con un desplazamiento del pico máximo de respuesta hacia posiciones más intermedias o tempranas del IER, respectivamente para uno u otro tipo de comportamiento (Roper, 1978; Staddon y Ayres, 1975; Wetherington y Riley). Estos resultados encontrados sobre la carrera, en los que la conducta presenta una ubicación casi post-*pellet* dentro del IER (Riley y cols., 1985; Segal, 1969; White, 1985) se asemejan a los encontrados en otras conductas inducidas, como la PIP.

La prevención de ocurrencia de conductas inducidas desencadenaría la expresión de conductas no-inducidas por el programa (Cook y Singer, 1976); y, de manera similar, las conductas no-inducidas tendrían un efecto sobre las conductas inducidas de ínterim cuando ambas se expresan simultáneamente. En concreto Riley, Peele, Richard y Kulkosky (1981), Roper (1978), Segal (1969), y Wetherington y Riley (1986), han encontrado que la PIP puede disminuir o aumentar simplemente por introducir o retirar, respectivamente, una rueda de actividad.

La mayoría de estos estudios tienden a girar en torno a la disminución o el aumento de la bebida, será en el estudio de Segal (1969) donde se informe sobre el efecto de la bebida sobre la carrera, reportando que el impedimento de beber no influiría sobre la tasa de la carrera, pero produciría un patrón temporal similar al observado en la PIP; lo que le llevaría a apoyar la hipótesis de que la carrera es una conducta inducida por programa.

Staddon (1977) sugirió que estos cambios encontrados en la ubicación temporal de la carrera dentro del IER podrían ser debidos, en parte, a la competencia temporal con la bebida u otras conductas inducidas por el programa. No obstante, esta competencia entre beber y correr puede indicar que ambos comportamientos son de la misma naturaleza (Segal, 1969).

## 4. Las conductas inducidas se comportan como una conducta operante

En una línea de investigación separada, hay un buen conjunto de resultados que respaldarían la idea de que las conductas adjuntivas, como la PIP, se comportan de forma similar a la conducta operante convencional, en términos de ser controlados por sus consecuencias y modulados por variables motivacionales (por ejemplo, Castilla y Pellón, 2013; Lamas y Pellón, 1995; Pellón y Blackman, 1987, 1991; Reberg, 1980; Reid y Staddon, 1990). Estos resultados han llevado a desarrollar una propuesta teórica alternativa para la conducta adjuntiva, según la cual la conducta adjuntiva estaría controlada por eventos ambientales posteriores a su ejecución (Killeen y Pellón, 2013).

Killeen y Pellón (2013) sugirieron que las conductas inducidas por el programa se mantienen mediante la entrega del reforzador al final de los IERs (ver también Ruiz, López-Tolsa y Pellón, 2016), basado en la idea de un gradiente de demora del reforzador extendido en el tiempo (Lattal, 1995), y que la ausencia de contingencias programadas experimentalmente no impide la acción de contingencias que, finalmente, aseguran el mantenimiento de diferentes conductas, pero sin reforzarlas explícitamente (Papini y Bitterman, 1990). La acción potencial de las contingencias respuesta-reforzador parece particularmente sorprendente en el caso de programas que no requieren una respuesta específica para la obtención del reforzador (programas de reforzamiento tiempo fijo o variable), sin embargo, la organización temporal de la conducta es notablemente similar en todos los animales de una misma especie (Anderson y Shettleworth, 1977; Killeen, 1975; Staddon y Simmelhag, 1971), como se ha observado en ratas de laboratorio cuando son expuestas a programas de refuerzo intermitente (Staddon, 1977; Staddon y Ayres, 1975).

Siguiendo a Killeen y Pellón (2013), el papel principal de la contingencia respuesta-reforzador es producir proximidad entre eventos; donde los autores siguen el argumento iniciado por Skinner (1948) de refuerzo adventicio modificando la "contigüidad" para un gradiente exponencial de proximidad. Todas las conductas generadas en los programas de refuerzo intermitente pueden ser explicadas por demoras diferenciales de asociabilidad con el reforzador y diferentes cursos de asociabilidad en el tiempo. Las conductas compiten por su expresión a lo largo del intervalo, presentando así diferentes patrones temporales. Las conductas con gradientes pronunciados tenderán a desplazar a las conductas adjuntivas que presenten gradientes menos acusados. Las conductas adjuntivas predominan durante el inicio y la mitad del intervalo, y recibirán ayuda adicional por su asociación con el reforzador recién consumido; mientras que, las conductas terminales estarán más fuertemente asociadas con el reforzador por su proximidad al final del intervalo.

Estudios recientes que imponen demoras a la respuesta en la entrega de los alimentos, bajo programas de reforzamiento de tiempo fijo, han encontrado que las conductas consideradas por Staddon como inducidas (de ínterim y terminales) son sensibles a los cambios en la proximidad entre las respuestas y la aparición del reforzador (Pellón, Íbias y Killeen, 2018; Pellón y Pérez-Padilla, 2013), apoyando la visión operante de la conducta inducida.

## 5. La polidipsia inducida por programa como modelo animal de impulsividad

La impulsividad puede definirse como la tendencia a responder o tomar decisiones de manera prematura o arriesgada, pudiendo volverse no adaptativa debido a sus posibles consecuencias negativas. El concepto de impulsividad no es unitario, y abarca una amplia gama de comportamientos que van desde la desinhibición motora, la cual podría denominarse como "impulsividad motora", hasta problemas en la toma de decisiones, que recibiría en nombre de "impulsividad cognitiva" (Evenden, 1999).

La excesividad de la bebida en PIP la han convertido en un modelo animal de trastornos psicopatológicos relacionados con el control de los impulsos (impulsividad-compulsividad). Ciertos estudios, han utilizado la PIP como procedimiento para discriminar entre dos poblaciones de ratas en función de su tasa de bebida en altas y bajas bebedoras, considerando la población de altas bebedoras como fenotipo del comportamiento impulsivo/compulsivo; utilizándolas posteriormente en el estudio de diferencias individuales, bien realizado manipulaciones farmacológicas y quirúrgicas, para las que han encontrado diferencias entre estos grupos a nivel cerebral (López-Grancha, López-Crespo, Sánchez-Amate y Flores, 2006; López-Grancha, López-Crespo, Sánchez-Amate, y Flores, 2008; Moreno y cols., 2012; Pellón y cols., 2011), o bien mediante la exposición a la tarea de descuesto por demora, una tarea idónea para la toma de medidas directas de la impulsividad cognitiva (Cardona, López-Crespo, Sánchez-Amate, Flores y Sánchez-Santed, 2011; Cardona y cols., 2006) (para más detalles ver Flores y cols., 2014; Moreno y Flores, 2012). Del mismo modo, los estudios con cepas de ratas que se caracterizan por una alta tasa de conducta han relacionado la PIP con la impulsividad (ratas SHR: Íbias y Pellón, 2011, 2014; ratas romanas de alta evitación: Moreno y cols., 2010).

## 6. Planteamientos y objetivos de la presente tesis

La polémica generada por la carrera como conducta inducida versus no-inducida hace años se terminó “enfriando”, y los estudios en rueda de actividad quedaron relegados al papel de la carrera como reforzador y estudios sobre la anorexia basada en actividad; la idea de esta tesis ha sido “sacarle las telas de araña a la rueda como conducta inducida y echarla a rodar”.

Este debate, surgido hace años, quedo estancado; muchos aceptaron la teoría de Staddon y la calificaron de conducta facultativa, mientras que, otros la consideran una conducta inducida por el programa. Sin embargo, exceptuando unos pocos estudios posteriores, hoy en día se habla de actividad inducida por programa únicamente "de pasada".

En la presente tesis se intenta resolver esta cuestión aprovechando los nuevos conocimientos existentes sobre las conductas inducidas por programa, así como la disponibilidad de nuevas tecnologías que nos permiten recabar un mayor número de datos con mayor exactitud y fiabilidad.

En concreto, el objetivo de esta tesis ha sido documentar las características de la carrera como conducta inducida por programa mediante la manipulación de diferentes variables experimentales (intermitencia en la presentación del reforzador, longitud del IER, preexposición a la carrera en la jaula hogar, interferencia por la concurrencia de otras conductas y exposición a contingencias de demora) y su comparación con la PIP, al ser considerada la conducta adjuntiva por excelencia dentro de las conductas inducidas por programa. Así como, estudiar su competencia como modelo animal de impulsividad/compulsividad.

# CHAPTER II *

# Schedule-induced wheel running

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*This Chapter is based on the publication:

Gutiérrez-Ferre, V. E., & Pellón, R. (2019). Wheel running induced by intermittent food schedules. *Psicológica, 40*(2), 46-61.

## Abstract

Sixteen naïve male Wistar rats were exposed to intermittent food delivery to measure the development of schedule-induced wheel running, using fixed time (FT) 30, 60, 120, 240 and 480 s schedules, counterbalanced across animals according to a Latin square design (except under the FT 480 s, which was always presented last to complete the data set). Rats were also exposed to a massed-food control condition. Wheel running was induced in the range of 30-240 s with a gradation as a function of inter-food interval (IFI) length. The temporal distribution of wheel turns was generally presented in the form of an inverted U-shaped as IFIs progressed, showing maximum responding during the first portion of the interval. The introduction of massed-food resulted in an immediate reduction in wheel running. The rats were subsequently exposed to the different FT schedules (except FT480-s), but this time, half of the animals (n = 8) had a wheel in their home cages, while the other half had concurrent access to a wheel and a water bottle during the experimental session; The development of schedule-induced polydipsia (SIP) under FT 60- and 120-s schedules was also measured for this group. The presence of a wheel in the home cage caused a reduction in wheel running, and a rightward movement of wheel turns curve only for FT 30- and 60-s schedules. The concurrence of water and wheel in the conditioning chambers did not affect the wheel running rate, although the drink induction moved wheel running to a central position within the IFI. The drink induction of the drink and its distribution within the IFI developed in a usual way in all cases, not being affected by the presence of a wheel. These results support the notion that wheel running can be schedule-induced and categorized into the so-called adjunctive behaviors. These data indicate that IFI length affects the development of schedule-induced wheel running and that the rate of wheel running is maintained by intermittent reinforcement, which are common characteristics of schedule-induced behaviors. Likewise, this idea is supported by the occurrence of a similar temporal pattern to that found with other adjunctive behaviors, such as schedule-induced polydipsia, with its maximum manifestation occurring between the beginning and middle of IFIs. The relocation of wheel running within the IFI due to the presence of drink responds to a temporary competition for the expression in the IFI of two adjunctive behaviors.

*Keywords:* Wheel running; Schedule-induced behavior; Inter-food interval length; Temporal distribution; Rats.

## 1. Introduction

In 1961, Falk observed that exposure of food-deprived rats to intermittent food reinforcement schedules, with free access to a bottle containing water in the experimental chambers, caused the development of excessive drinking (schedule-induced polydipsia, SIP) unrelated to physiological needs or apparent behavior regulation. Other similar behavioral patterns were later studied, such as wheel running (Levitsky & Collier, 1968), air licking (Mendelson & Chillag, 1970), wood chewing (Roper & Crossland, 1982), pica (Villareal, 1967), aggression (Looney & Cohen, 1982), escape (Brown & Flory, 1972), defecation (Rayfield, Segal & Goldiamond, 1982) and a long list of other activities (see reviews by Falk, 1977; Pellón, 1990), all in theory being modulated by the degree of intermittency in the reinforcement. These behavioral patterns have been called adjunctive behaviors (or schedule-induced behaviors) (Falk, 1971; Roper & Posadas-Andrews, 1981; Staddon, 1977; Timberlake, Wahl & King, 1982; Wetherington, 1982), and are characterized primarily by meeting the criteria of significantly higher response rates when exposed to intermittent reinforcement (albeit without an explicitly arranged contingency between behavior and reinforcer occurrence) which are usually observed following the reinforcer in the form of an inverted-U throughout the inter-reinforcement interval.

The meeting of the above mentioned criteria to qualify for categorization as an induced behavior has generated discussion with wheel running in particular, and its comparison to SIP as the referenced prototypical adjunctive behavior. Schedule-induced wheel running (SIWR), where animals deprived of food are exposed to intermittent reinforcement while having free access to a running wheel, was investigated initially by White (1985) and Riley, Wetherington, Delamater, Peele and Dacanay (1985). On the one hand, White (1985) was interested in testing 1) the temporal properties of intermittent reinforcement, and 2) the place occupied by wheel running within the inter-food intervals (IFI), finding that, in sessions with massed-food, the final rate of wheel running decreased in three of the four subjects in comparison with the rate observed under a FT 60-s condition. Wheel running rate increased again after the restoration of the FT 60-s schedule. His results also showed a clear temporal distribution, where wheel running was unlikely to occur after food, increasing until reaching its highest peak above what would correspond to the 24-s bin, and gradually decreasing thereafter towards the end of the IFI. These results led White to conclude that SIWR is an induced behavior. On the other hand, Riley et al. (1985) studied the changes in wheel running (amount, rate and temporal distribution) according to variations in the IFI in three rats. Their results showed that an increase in the IFI increased the total amount of wheel running, but did not increase the rate of wheel running and delayed the appearance of the maximum response peak within the IFI. These results tend to support Staddon’s (1977) approach by which wheel running is not considered to be induced but what he instead called "facultative" behavior.

Staddon (1977) distinguished three classes of behaviors based on their temporal location within the IFI, its relationship with the reinforcer and its excessiveness. Induced behaviors (see also Staddon & Simmelhag, 1971) would present a high response rate and would be located either at the beginning of the IFI, that is, activities that occur immediately after the delivery of the reinforcer as a consequence of a low probability of reinforcement in the post-reinforcement period ("interim" behaviors, such as SIP), or at the end of it, occurring consistently before, or just at the moment of, the presentation of the reinforcer, and therefore, in the presence of stimuli related to the release of such a reinforcer ("terminal" behaviors, such as magazine entries). On the other hand, Staddon (1977) used the term "facultative" to refer to those non-induced behaviors that would not increase in frequency as a consequence of exposure to intermittent reinforcement schedules, but that would present a non-null baseline in animals deprived of food (see also Roper, 1981; Staddon and Ayres, 1975). In addition, facultative behaviors would adopt an intermediate position between interim and terminal induced behaviors within the IFI. Among these behaviors, Staddon (1977) included activities such as wheel running or grooming.

Changes in wheel running have been studied according to variations in IFI length. Induced behaviors, particularly SIP, have a bitonic inverted U-shaped relationship with food frequency (Falk, 1966; Flory, 1971; Roper, 1980); while non-induced behaviors increase their rate in parallel with increases in the length of the IFI, as is generally claimed to occur for wheel running (Penney & Schull, 1977; Staddon, 1977; Staddon & Ayres, 1975). Staddon (1977) suggested that changes in wheel running might be due, in part, to temporal competition with drinking or other schedule-induced behaviors. In fact, evidence has been found regarding the interaction between drinking and wheel running (Penney & Schull, 1977; Riley, Peele, Richard & Kulkosky, 1981; Roper, 1978; Staddon, 1977; Staddon & Ayres, 1975). For example, Roper (1978) found that drinking rate declined when competing with wheel running, and recovered when access to the wheel was impeded. This competition between drinking and wheel running might indicate, however, that both behaviors are of the same nature. However, the results reported on this topic tend to revolve around the decrease or increase of drinking by facilitating or preventing, respectively, wheel running (e.g., Wetherington & Riley, 1986). Whereas preventing drinking would not affect wheel running (Segal, 1969).

Studies that have described the location of behaviors within the IFI when both interim and namely non-induced behaviors occur, usually indicate that interim activities tend to occur first and the overall distribution of activity is multimodal (Roper, 1978; Staddon & Ayres, 1975; Staddon & Simmelhag, 1971), with a first peak representing the activity of interim behavior and, later, the non-induced behavior (Penney & Schull, 1977). However, when only interim activities occur in the interval, or when only non-induced behaviors occur, the resulting distribution is unimodal, with a shift of the peak to more intermediate or earlier IFI positions, respectively, for one or the other type of behavior (Roper, 1978; Staddon & Ayres, 1975; Wetherington & Riley, 1986). These are the results obtained with wheel running alone, where it presents an almost post-food location (Riley et al., 1985; Segal, 1969; White, 1985), thus resembling an induced behavior such as SIP. The prevention of the occurrence of induced behaviors would trigger the expression of non-induced behaviors (Cook & Singer, 1976); and, similarly, non-induced behaviors would have an effect on interim behaviors when both are issued concurrently (Riley et al., 1981; Roper, 1978; Segal, 1969; Wetherington & Riley, 1986).

Since wheel running under intermittent reinforcement was initially considered induced given its resemblance to SIP [i.e. both do not occur under continuous reinforcement, both depend on reinforcement being intermittent, and both occur immediately after reinforcer delivery (Levitsky & Collier, 1968)], this was later questioned (Staddon, 1977), and there is still an unresolved debate on whether or not it should be regarded as induced. For example, Segal (1969) supported the hypothesis that it was an induced behavior, because in this study it was found that in the face of concurrent access to water and wheel, the quantity of wheel running increased at the same time as SIP developed, although to a lesser extent, whilst the suppression of access to drink produced a temporal wheel running pattern similar to that of SIP. Investigations that have suggested that wheel running might be a non-induced behavior base their conclusions on observations such as: 1) wheel running locates in the middle part of the IFI, after drinking and prior to anticipatory reinforced behavior (e.g., Staddon & Ayres, 1975); 2) wheel running is not constrained to food contexts, contrary to other induced behaviors such as

SIP (Penney & Schull, 1977; Staddon, 1977; Staddon & Ayres, 1975; Wetherington, Brownstein & Shull, 1977); 3) an increase in the rate of food release causes an increase in terminal and interim activities, while wheel running decreases (Staddon & Ayres, 1975); 4) wheel running occurs mainly during post-omission intervals regardless of the time elapsed since the last meal (Penney & Schull, 1977), being high even in the total absence of food such as when an extinction procedure is imposed (Staddon, 1977). High rates of wheel running in the absence of food can simply be accounted for by considering the fact that animal subjects are substantially deprived of activity in their limited home cages, thus rendering wheel running a form of reinforcement (e.g., Pierce, Belke & Harris, 2018), while pre-organized patterns of behavior and extended temporal contiguity between events can account for other aspects of SIWR (see Killeen & Pellón, 2013).

As a result of the aforementioned background, it seems clear that there is still no unified consensus on wheel running as an induced behavior, and this is an issue that needs to be resolved with further experimentation. Clarifying the nature of wheel running as induced or not is important because if it were induced, it would respond to characteristics related to the reinforcer and thus would be modifiable by environmental variables known to affect other schedule-induced behaviors. Facultative behaviors, in contrast, would not be susceptible to manipulation due to alterations in reinforcement parameters. Consequently, in the present study, we will attempt to clarify the contradictions that have been raised on this issue by reworking previous studies while trying to overcome the criticisms received, and by increasing the sample of subjects, an issue that is extremely necessary for a more thorough analysis and generalization of the results. To address this objective, we will focus on the two criteria that would distinguish an induced from a non-induced behavior: 1) the effect of intermittent reinforcement on wheel running, and 2) the influence of the length of the IFI on the development of wheel running and the location it occupies within the IFI, looking for similarities with SIP. To do this, we will manipulate certain variables such as the intermittency of the reinforcer, the presence of a wheel in the home cage, the length of the IFI, as well as the concurrence of a water bottle and a wheel in the conditioning chambers.

## 2. Method

### *2.1. Subjects*

Sixteen 10-week-old male Wistar rats were purchased from Charles River Laboratories (Lyon, France). They were experimentally naïve and were housed in groups of four in an environmentally controlled room at 21 °C and 65% relative humidity, with a 12-h light-dark cycle (lights on from 08:00 to 20:00 h). After 1 week of habituation to the animal facility, rats were housed individually in 18 cm × 32.5 cm × 20.5 cm transparent Plexiglas cages, with a metal-grid detachable roof that allowed for food to be deposited and a water bottle to be fitted, with food and water available *ad libitum*. After being maintained for 2 weeks in those conditions, rats were gradually reduced to 80-85% of their free-feeding body weight by controlled feeding and then maintained at this level of food deprivation throughout all the experiments, with reference to the standard growth curves provided by the supplier. The animals were weighed daily before experimental sessions, and food was made available by daily feeding of lab chow approximately 30 min after completion of each experimental session. At the start of the experiment, rats were in their 17th week of life and had the following mean body weight: 319.69 g (range: 292–346 g). Water continued to be freely available to all animals in their home cages throughout all the experiments. All procedures were in accordance with the Spanish Royal Decree 53/2013 regarding the protection of experimental animals and with the

European Union Council Directive 2010/63 and were approved by the Bioethics Committee of Universidad Nacional de Educación a Distancia.

## *2.2. Apparatus*

### *2.2.1. Conditioning Chambers*

The experiment was conducted in 8 Letica LI-836 conditioning chambers (Cibertec Inc., Madrid, Spain) measuring 29 × 24.5 × 35.5 cm which were enclosed in soundproofed housing, equipped with their own ventilation and a small observation window at the front. The fan produced an ambient noise of approximately 60 dB in each chamber that functioned as masking noise during experimental sessions. The front panel of each conditioning chamber was made of aluminum, the left wall of transparent Plexiglas and the remaining walls of black Plexiglas, with stainless steel grid floors. The chambers were lit by two 3-W lamps situated at both upper sides of the front panel, and an indirect 25-W light fitted to the interior of the soundproof housing that insulated each chamber. A dispenser was situated behind the front panel to supply 45-mg of standard rat food *pellets* (Bio-Serv, Frenchtown, NJ, USA) in a centered aperture in the front wall of the chamber, situated 3.7 cm from the floor. Magazine entries into the food magazine were detected by the interruption of a photocell beam.

On the exterior of the back panel of the chamber, a wheel of stainless steel was fitted, 32 cm in diameter and 9.5 cm wide with spokes distributed at 1 cm intervals around the rim. The rat had access to the wheel from the interior of the chamber, through a 10 cm in diameter circular aperture in the wall, situated 28 cm from the front panel and 1 cm from the floor. A removable metal trapdoor restricted access to the wheel when necessary. An AZ fag magnetic reed switch recorded each entire revolution of each wheel.

During the schedule-induced polydipsia (SIP) procedure, a bottle filled with 100 ml of fresh tap water was fitted on to the exterior of each chamber' right-hand wall, with a spout to which the rat had access from the interior of the chamber, through a 3.2 × 3.9 cm aperture in the wall, situated 20 cm from the front panel and 7 cm from the floor. The spout was placed 2 cm towards the interior of the aperture to allow for licks rather than continuous drinking. Contact between the animal's tongue and the metal spout completed the electric circuit between the floor metal grid and the bottle spout, and was registered as a lick.

The scheduling and recording of experimental events were controlled by Med PC IV® software.

### *2.2.2. Activity Chambers*

During the procedure where animals had free access to the wheel in their home cages, rats were housed in individual transparent Plexiglas chambers measuring 21 x 45 x 24 cm, equipped with a metal-grid detachable roof where food could be deposited, and a bottle filled with water was permanently accessible. The same wheels used in the conditioning chambers were positioned at the left-hand side of each activity chamber. The structure on which the running wheel was deposited was equipped with a brake mechanism.

The data-programming and collection equipment (MED-PC for Windows, MED Associates Inc., Georgia, VT, USA) was placed in a separate room. Data on wheel turns were recorded at 45min intervals for each subject.

## *2.3. Procedures*

### *2.3.1. Experiment 1: Schedule-Induced Wheel Running (SIWR).*

#### *2.3.1.1. Adaptation to the experimental condition*

When the weights of the animals were stabilized within the criterion-based range, rats were exposed to an adaptation session in the conditioning chambers for 20 min, with 30 food pellets being previously deposited all together in the food magazine, ventilation and illumination provided, but with no experimental contingency in operation. During this session, animals had no access to the wheel.

#### *2.3.1.2. Acquisition SIWR*

The experiment commenced the day after the adaptation session. Rats were exposed to four FT schedules of different lengths (FT 30-, 60-, 120- and 240-s), such that food pellets were dispensed at these regular intervals regardless of the rats' behavior. Sessions were run daily, with a rest interval of two days between successive FT schedules, with each session being composed of a total of 30 food deliveries. All animals underwent all FT schedules, the order of which was established by pairs of rats using a Latin square design. While exposure to the first FT schedule lasted 20 sessions, the remaining schedules were held over 15 sessions due to the stability observed in behavior. On completion of the first four schedules, all animals were exposed to 15 sessions under an FT 480-s schedule for the purpose of completing the data set.

The following measures were recorded for each rat each session: total number of wheel turns and total number of magazine entries, along with the number of responses of both behaviors given at each IFI and every 1-s in each interval.

#### *2.3.1.3. Massed-food control condition*

When exposure to the different FT schedules had been completed, rats were exposed to a single session of 240 min of massed-food presentation; 30 food pellets were presented together in the food magazine at the start of the session, and the total number of wheel turns and total number of magazine entries were recorded every 15 min in order to compare it with the data obtained under the different FT schedules, so as to assess the influence of intermittent food delivery on the development of SIWR.

### *2.3.2. Experiment 2:*

The animals were divided into two groups (n=8). Two rats from each group, previously established by the order of exposure to the different FT schedules, were randomly designated to one group and the remaining pair were assigned to the other group for the remainder of studies.

#### *2.3.2.1. SIWR with wheel running in the home cage*

8 rats were exposed to the same conditions as in Experiment 1. (FT 30-, 60-, 120- and 240-s). However, this time, they had free access to a running wheel in their home cages for 18 hours a day. The FT 480-s schedule was not run because the results found for this schedule during Experiment 1 showed no development of induced activity. The total number of wheel turns and magazine entries were recorded for each experimental session, as well as the total number of daily turns in the home cage.

#### *2.3.2.2. SIWR and SIP*

##### *2.3.2.2.1. Concurrence of SIWT and SIP in the conditioning chambers*

The other group of 8 rats was also exposed to the same conditions as in Experiment 1. (FT 30-, 60-, 120- and 240-s), but on this occasion, the animals had access to a water bottle in the conditioning chambers. In each experimental session, water bottles were filled with 100 mL of fresh tap water.

For each session, in addition to the total number of wheel turns and total number of magazine entries, the total number of licks and the milliliters of water consumed were recorded for each rat, along with the number of wheel turns, magazine entries and licks given in each inter-food interval and in every 1-s during such intervals.

##### *2.3.2.2.2. SIP*

These same rats were later exposed to an extra 30 sessions of FT food delivery, 15 under an FT 60-s schedule and 15 under an FT 120-s schedule, counterbalanced across animals, to measure the development of schedule-induced drinking. Animals had access to water in the experimental chambers without having concurrent access to the running wheel. The following measures were recorded for each rat and each session: total number of licks and milliliters of water consumed, as well as licks given in each inter-food interval and in every 1-s during such intervals.

### ***2.4. Statistical analysis***

SIWR development was analyzed using a one-way repeated measures analysis of variance (ANOVA), with FT schedules (FT 30-, FT 60-, FT 120-, FT 240- and FT 480-s) as the within-subject factor.

Comparisons of each FT schedule with its corresponding massed-control were carried out using paired *t*-tests for related samples.

The effects of wheel running in the home cages (HC), concurrent access to a water bottle and a wheel in the conditioning chambers (CC) and only a water bottle in the conditioning chambers conditions were analyzed using two-way ANOVAs, with one between-subject factor named Condition (No-Wheel (HC) vs. Wheel; No-Water vs. Water (CC); Wheel with Water vs. Only-Water, respectively) and one repeated within-subjects factor named FT schedule (four levels: FT 30-, FT 60-, FT 120- and FT 240-s with wheel running in the home cage, and concurrent access to a water bottle in the conditioning chambers conditions; and two levels: FT 60- and FT 120-s with only a water bottle in the conditioning chambers condition). The lick data in Experiment 2 (concurrent access to water and wheel running) were analyzed using one-way ANOVAs, with FT schedules (FT 30-, FT 60-, FT 120- and FT 240-s) as the within-subject factor.

Comparisons between No-Wheel and Wheel conditions in blocks of 15 minutes were performed by paired *t*-tests for related samples. And, these block data were analyzed using two-way ANOVAs, with one between-subject factor named Condition (No-Wheel (HC) vs. Wheel) and one repeated within-subjects factor named Block (between one and four levels: 1º, 2º, 4º and 8º block with access or no access to wheel running in the home cage condition).

When appropriate, post comparisons were carried out using pairwise comparisons with a Bonferroni correction for *p* values.

Statistical analyses were conducted using data on each respective subject's last three sessions under each FT schedule, with the minimum level of significance set at $p<0.05$. Effect sizes were estimated by $\eta^2$ (ANOVAs) or Cohen d (*t*-tests). All analyses were computed using the SPSS software package (Version 24).

## 3. Results

Figure 1 shows the mean (± Standard Error of the Mean - SEM) number of wheel turns and magazine entries per minute, as well as the mean (± SEM) number of wheel turns and magazine entries per food pellet, given by rats under the different FT schedules and for the massed-food control condition, taking the average of the last three sessions for each FT schedule.

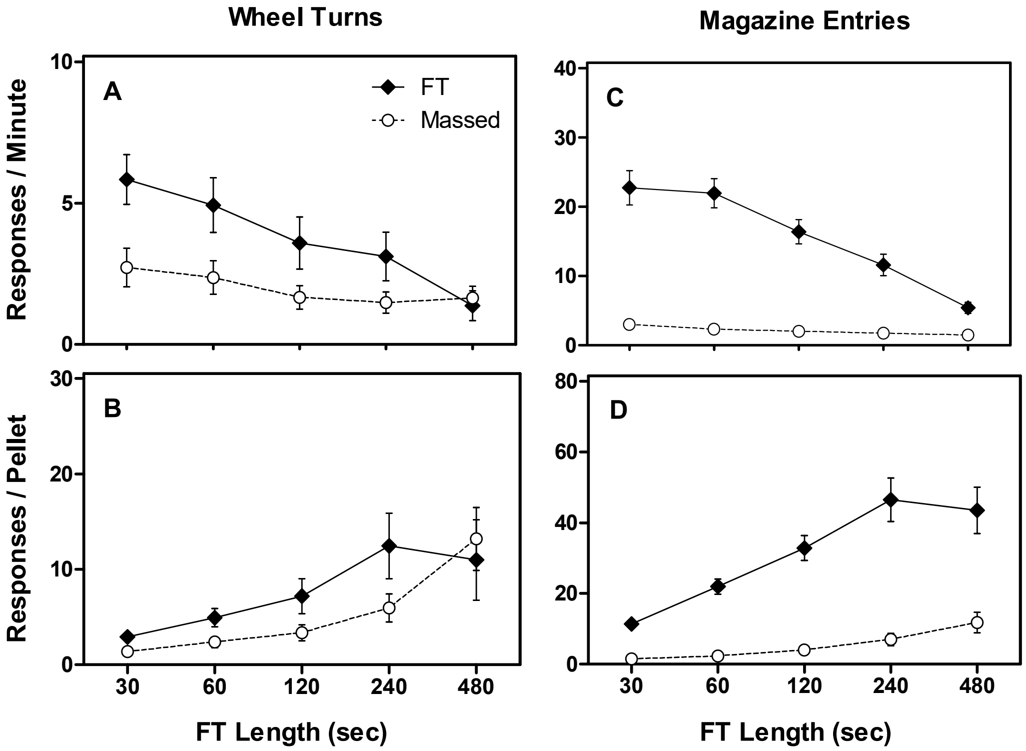

Figure 1. Mean (± SEM) number of wheel turns (A) and magazine entries (C) per minute under each FT schedule and for the massed-food control condition; and mean (± SEM) number of wheel turns (B) and magazine entries (D) per food pellet under each FT schedules and for the massed-food control condition.

Figure 1A compares wheel turns per minute under the five FT schedules. The analysis yielded a main effect of FT [$F(3,41) = 22.79$, $p<0.001$, $\eta^2=0.603$], with a reduction in response rate as IFI length increased. Post hoc comparisons showed that: FT 30-s produced more responses per minute than FT 120-s, FT 240-s and FT 480-s schedules ($p<0.05$, $p<0.01$ and $p<0.001$, respectively), FT 60-s produced more responses per minute than FT 120-s, FT 240-s and FT 480-s schedules ($p<0.01$, $p<0.05$ and $p<0.001$, respectively), and FT 120-s and FT 240-s produced more responses per minute than the FT 480-s schedule ($p<0.01$ in both cases). Paired *t*-tests showed that, when the data of the different FT schedules were compared separately with the data obtained in the massed-food condition, significant differences between means were observed (except for FT 480-s [$t$ (15) = -1.76, $p$=0.099, d=0.44]) as shown by a higher rate of wheel turns with the intermittent delivery of food (FT 30-s [$t$ (15) = 4.10, $p$=0.001, d=1.03]; FT 60-s [$t$ (15) = 4.43, $p<0.001$, d=1.11]; FT 120-s [$t$ (15) = 4.11, $p$=0.001, d=1.03] and FT 240-s [$t$ (15) = 5.03, $p<0.001$, d=1.26]).

Figure 1B depicts wheel turns per food pellet under the five FT schedules. A main effect of FT was found [$F(4,60) = 5.29$, $p$=0.001, $\eta^2$=0.260], with an increase in the number of responses as the length of the interval increased, and therefore the session. Post hoc comparisons showed no statistically significant differences, with only a tendency for FT 30-s to result in fewer responses per pellet than FT 60-s and FT 240-s schedules ($p$=0.06 and $p$=0.07, respectively). Identical results to those found in wheel turns per minute, but for wheel turns per pellet, were obtained in the separate comparisons through paired *t*-tests of the means of the different FT schedules between the intermittent-food condition and the massed condition ($p$=0.001 in FT 30-s and FT 120-s, and $p<0.001$ in FT 60-s and FT 240-s; see detailed results above).

Figure 1C shows the mean number of magazine entries per minute under the five FT schedules. Effects were observed for FT [$F(4,60) = 32.70$, $p<0.001$, $\eta^2$=0.689], with a progressive reduction in response rate accompanying the increases in IFI length. Post hoc comparisons showed differences between: FT 30-s and FT 120-s ($p<0.05$), FT 240-s ($p<0.01$) and FT 480-s ($p<0.001$) schedules; FT 60-s and FT 120-s ($p<0.01$), FT 240-s ($p<0.01$) and FT 480-s ($p<0.001$) schedules; and FT 120-s and FT 240-s produced less responses per minute than the FT 480-s schedule ($p<0.001$ and $p=0.001$, respectively). The paired *t*-tests showed that, when the data of the different FT schedules were compared separately with the data obtained in the massed-food condition, significant differences in their means were observed, with a higher rate of magazine entries per minute under intermittent delivery of food (FT 30-s [$t$ (15) = 7.97, $p<0.001$, d=1.99]; FT 60-s [$t$ (15) = 9.14, $p<0.001$, d=2.28]; FT 120-s [$t$ (15) = 8.40, $p<0.001$, d=2.10]; FT 240-s [$t$ (15) = 6.68, $p<0.001$, d=1.67] and FT 480-s [$t$ (15) = 4.50, $p<0.001$, d=1.12]).

Figure 1D depicts magazine entries per food pellet under the five FT schedules. A main effect of FT was found [$F(2,31) = 17{,}47$, $p<0.001$, $\eta^2=0.538$], with an increase in the number of responses as the length of the IFI increased. The post hoc analyses revealed statistically significant differences between FT 30-s and other FT schedules ($p<0.001$ for FT 60-s, FT 120-s and FT 240-s; $p=0.001$ for FT 480-s), as well as between FT 60-s and other FT schedules ($p<0.01$ for FT 120-s and FT 240-s; $p=0.05$ for FT 480-s). Identical results to those found in magazine entries per minute, but for magazine entries per pellet, were obtained in the separate comparison through paired *t*-tests of the means of the different FT schedules between the intermittent-food condition and the massed-food condition ($p<0.001$ in all cases; see detailed results above).

Figure 2 depicts the mean (± SEM) of total wheel turns and magazine entries made every second (bin) during the IFI for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length), averaged over the last three sessions of exposure to each schedule.

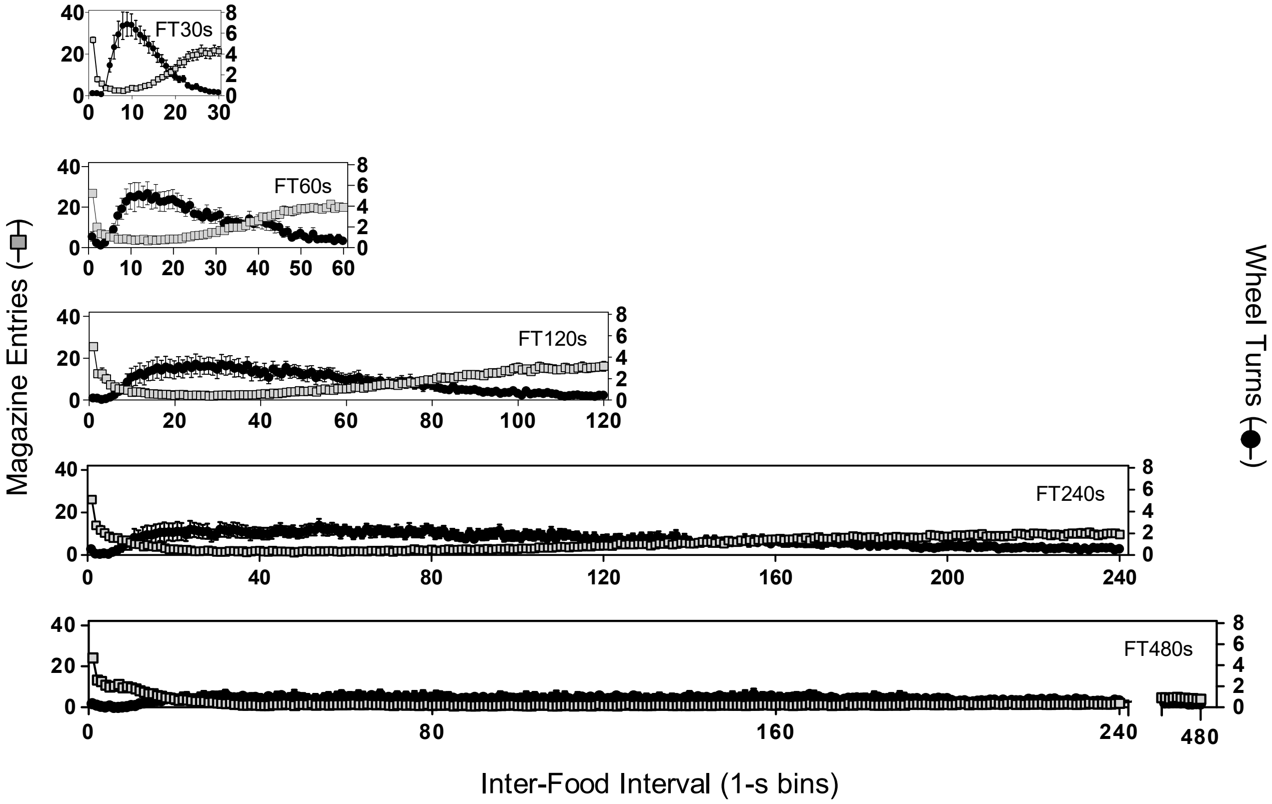

Figure 2. Mean (± SEM) wheel turns and magazine entries given every second (bin) during the inter-food intervals for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length).

Wheel turns were generally distributed in the form of an inverted U-shape as IFIs progressed, showing maximum responding during the first portion of the interval and peaking at bins 9 in FT 30-s, 14 in FT 60-s, 25 in FT 120-s, 54 in FT 240-s, and 32 or 168 in FT 480-s. Rats barely ran towards the end of the IFI. It can be observed that wheel running was more concentrated in the first 3/4 parts of the IFI, except for FT 480-s, where the distribution was flat and low throughout the entire IFI. There was also a tendency towards a shift to the right of the wheel running curve and a "flattening" thereof as a result of the increase in IFI length.

The highest number of magazine entries was found at the beginning of the IFI (0-s bin) in all cases, coinciding with the recollection of food pellets from the magazine as they were released, and then, a steady, accelerated descent was observed as the interval progressed until a more or less sustained rate of responding occurred at approximately half of the IFIs, when magazine entries increased steadily again until the end of the intervals when food pellets were delivered.

Figure 3 shows the mean (± SEM) number of wheel turns and magazine entries per minute, as well as the mean (± SEM) number of wheel turns and magazine entries per food pellet, given by rats under the different FT schedules when rats had no access to the wheel in their home cages (HC) (similar data than those reported in Figure 1 but now only for 8 rats) and when they had access to the wheel in HC, averaged over the last three sessions of exposure to each schedule.

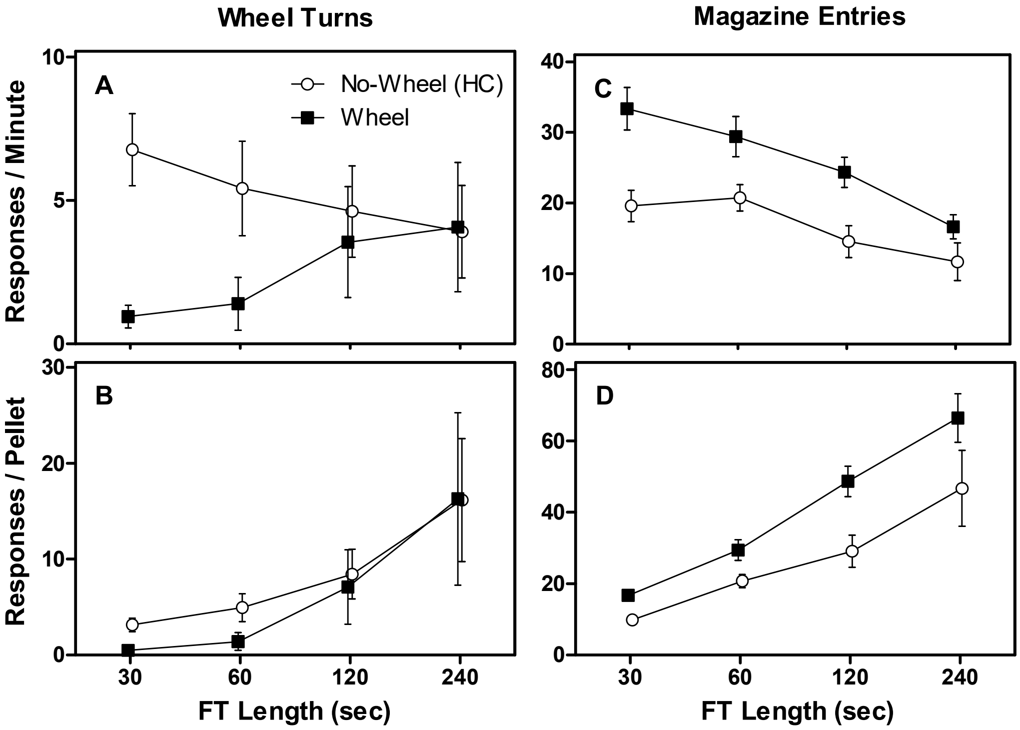

Figure 3. Mean (± SEM) number of wheel turns per minute (A), wheel turns per food pellet (B), magazine entries per minute (C) and magazine entries per food pellet (D), with and without access to the wheel in their home cages (HC) under each FT schedule.

Figure 3A compares wheel turns per minute under the four FT schedules when animals had had the opportunity to run in their home cages and when they had not. The ANOVA displayed effects: for Condition [$F(1,7) = 11.56$, $p=0.011$, $\eta^2=0.623$], with the Wheel condition registering a lower mean rate of running than the No-Wheel (HC) condition; and for the FT schedule × Condition interaction [$F(1,8) = 4.71$, $p=0.05$, $\eta^2=0.0402$]. Post hoc analyses revealed differences in the FT 30-s and FT 60-s schedules, with higher means for the No-Wheel (HC) versus the Wheel condition ($p<0.01$ and $p<0.05$, respectively). FT length resulted in decreases in responding as frequency of food decreased when animals could only run in the conditioning chambers (as shown in Figure 1A) and increases when they could run as well in their home cages. No effect of FT schedule was found [$F(1,10) = 0.22$, $p=0.73$, $\eta^2=0.031$].

Figure 3B depicts wheel turns per food pellet under the four FT schedules. The analysis performed yielded no significant effects (factor: FT schedule [$F(1,7) = 4.30$, $p=0.75$, $\eta^2=0.380$], Condition [$F(1,7) = 1.03$, $p=0.34$, $\eta^2=0.129$] and FT schedule × Condition interaction [$F(1,7) = 0.33$, $p=0.59$, $\eta^2=0.044$]). However, some differences seem to be observed for the short schedules, FT 30-s and FT 60-s, with a higher response rate in the No-Wheel (HC) condition with respect to the Wheel condition.

Figure 3C shows the mean number of magazine entries per minute under the four FT schedules. The ANOVA displayed effects: for FT schedule [$F(3,21) = 18.75$, $p<0.001$, $\eta^2=0.728$], with progressive reductions in response rates accompanying increases in IFI length; for Condition [$F(1,7) = 31.47$, $p=0.001$, $\eta^2=0.818$], with the No-Wheel (HC) condition registering a lower mean value of entering rate than the Wheel condition; and only a trend towards significance for the FT schedule × Condition interaction [$F(3,21) = 2.65$, $p=0.075$, $\eta^2=0.274$].

Figure 3D depicts magazine entries per food pellet under the four FT schedules. The ANOVA showed effects: for FT schedule [$F(1,10) = 27.53, p<0.001, \eta^2=0.797$], with increases in responding as the length of the IFI was increased; and for Condition [$F(1,7) = 18.53, p<0.01, \eta^2=0.726$], with the No-Wheel (HC) condition registering a lower mean value than the Wheel condition. No interaction for FT schedule × Condition was found [$F(1,9) = 1.69, p=0.20, \eta^2=0.194$].

Figure 4 depicts the mean (± SEM) of total wheel turns and total magazine entries made every second (bin) during the IFI for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length) when rats had access to the wheel in their home cages, averaged over the last three sessions of exposure to each schedule.

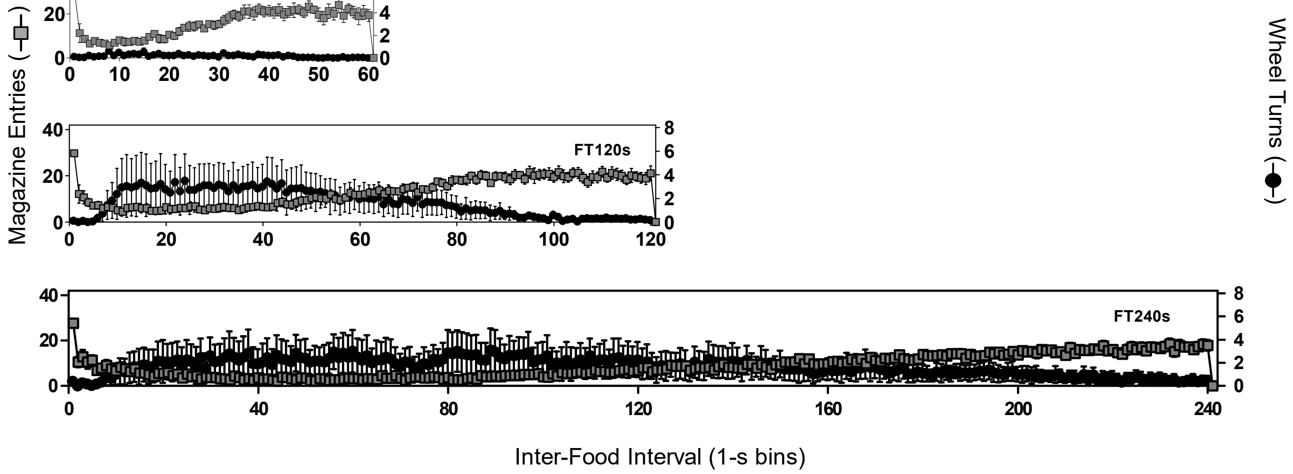

Figure 4. Mean (± SEM) wheel turns and magazine entries given every second (bin) during the inter-food intervals for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length).

When animals had free access to a wheel in their home cages, the number of wheel turns per bin during the experimental session was reduced. In the distribution of the wheel turns, the highest response rate was located on the half (FT 30s-: bin 13) or in the first half of the IFI (FT 60-s: bin 8; FT 120-s: bin 24; FT 240-s: bin 89). The distribution curve was displaced to the right compared to Figure 2, adopting a more central position and a flattened aspect in its upper part for FT 30- and FT 60-s schedules (in the case of FT 60-s, it was not possible to see it in the figure due to the adaptation of the scales of the different graphs). The distribution curves for FT 120-s and FT 240-s maintained parameters very similar to those observed in Figure 2. Rats barely ran towards the end of the IFI. The decrease and absence of response was advanced in comparison to Figure 2. It can be observed that wheel running was more concentrated in the first 2/3 parts of the IFI.

As in the No-Wheel condition in the home cage, the highest number of magazine entries were placed at the beginning of the IFI (0-s bin) in all cases, with the consequent rapid decrease in response, after which the gradual and steady recovery of the response began on the beginning of the second third of the IFI. However, under this new condition (Wheel HC), the magazine entries rate showed no absence of response during the IFI length, with the exception of a short segment in FT 240 s, and in contrast to what was observed in Figure 2.

Figure 5 shows the mean (± SEM) number of wheel turns per minute given by rats under the different FT schedules in blocks of 15 minutes when rats had no access to the wheel in their home cages (HC) (similar data than those reported in Figure 3) and when they did have access, taking the average of the last three sessions of each FT schedule. The aim of this analysis was to find out whether the results reported in Figure 3A, where the short FT schedules (FT 30- and FT 60-s) showed a higher response rate for the No-

Wheel (HC) condition with respect to the Wheel condition, were due to the fact that the activity carried out during the hours of housing in the home cage affected performance during the first minutes of the experimental session.

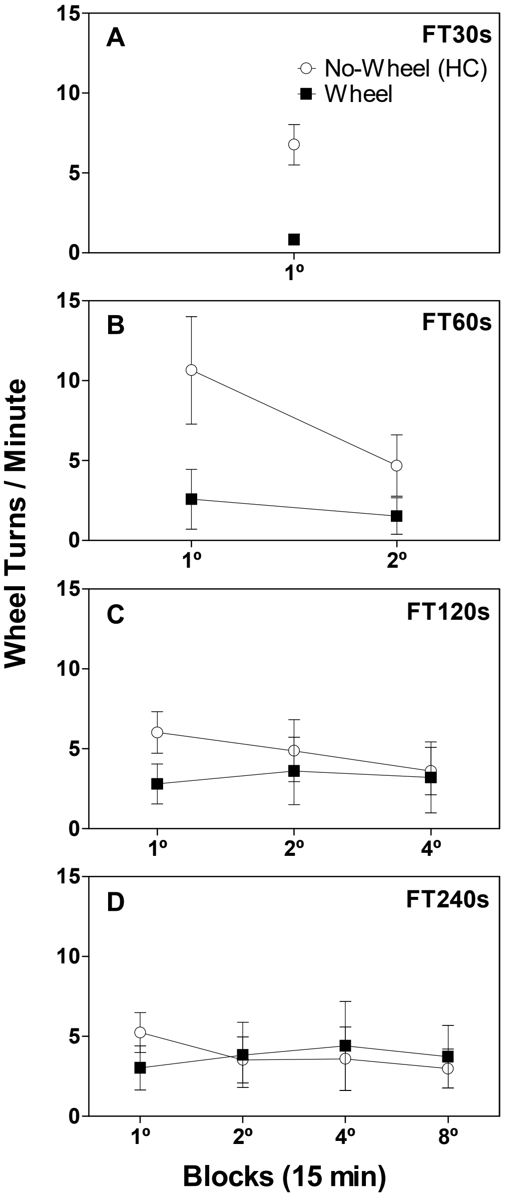

Figure 5. Mean (± SEM) number of wheel turns per minute, in blocks of 15 minutes, under Wheel and No-Wheel condition, for each FT schedule: FT 30-s (A), FT 60-s (B), FT 120-s (C) and FT 240-s (D).

Paired *t*-tests showed significant differences between the No-Wheel and Wheel conditions for the first block of the FT 30-s [$t\ (7) = 5.04, p=0.001$, d=1.78] (Fig. 5A), FT 60-s [$t\ (7) = 3.05, p<0.05$, d=1.08] (Fig. 5B) and FT 120-s [$t\ (7) = 2.76, p<0.05$, d=0.98] (Fig. 5C) schedules, as well as for the second block of FT 60-s [$t\ (7) = 2.35, p=0.05$, d=0.83] (Fig. 5B) schedule, as shown by the higher rate of wheel turns in the No-Wheel condition in comparison to the Wheel condition.

Figure 5B compares wheel turns per minute in blocks of 15 minutes under the FT 60-s schedule when animals had the opportunity to run in their home cages and when they did not. The analysis performed showed effects: for Block [$F(1,7) = 12.82, p<0.01, \eta^2=0.647$], with a reduction in response rate in the second block; for Condition [$F(1,7) = 7.98, p<0.05, \eta^2=0.533$], with the No-Wheel (HC) condition registering a higher mean value of entering rate than the Wheel condition; and for the Block × Condition interaction [$F(1,7) = 13.44, p<0.01, \eta^2=0.658$]. Post hoc analyses revealed differences in the first block ($p<0.05$) and a tendency in the second block ($p=0.051$), with higher rates for the No-Wheel versus the Wheel condition.

Figure 5C depicts wheel turns per minute in blocks of 15 minutes (only blocks 1º, 2º and 4º that match the totals of the other FT schedules) under the FT 120-s schedule. The ANOVA displayed effects: for the Block × Condition interaction [$F(1,9) = 6.48, p<0.05, \eta^2=0.481$]. Post hoc comparisons showed that: the No-Wheel condition produced more responses per minute than the Wheel condition in the first block ($p<0.05$). No effects of Block [$F(1,8) = 1.08, p=0.37, \eta^2=0.133$] or Condition [$F(1,7) = 1.77, p=0.22, \eta^2=0.202$] were found.

Figure 5D shows the mean number wheel turns per minute in blocks of 15 minutes (only blocks 1º, 2º, 4º and 8º match the totals of the other FT schedules) under the FT 240-s schedule. The ANOVA showed effects: for the Blocks × Condition interaction

[*F*(3,21) = 3.10, $p<0.05$, $\eta^2=0.307$]. Post hoc analyzes did not reveal any differences. No effects of Block [*F*(1,9) = 0.23, $p=0.71$, $\eta^2=0.032$] or Condition [*F*(1,7) = 0.01, $p=0.96$, $\eta^2=0$] were found.

Figure 6 shows the mean (± SEM) number of wheel turns and magazine entries per minute, as well as the mean (± SEM) number of wheel turns and magazine entries per food pellet, given by rats under the different FT schedules when rats had no access to water in the conditioning chambers (CC) (similar to the data reported in Figure 1 but just for 8 rats) and when they did have access. The figure also shows the mean (± SEM) number of licks per minute and per food pellet. These data take into account the average of the last three sessions of each FT schedule.

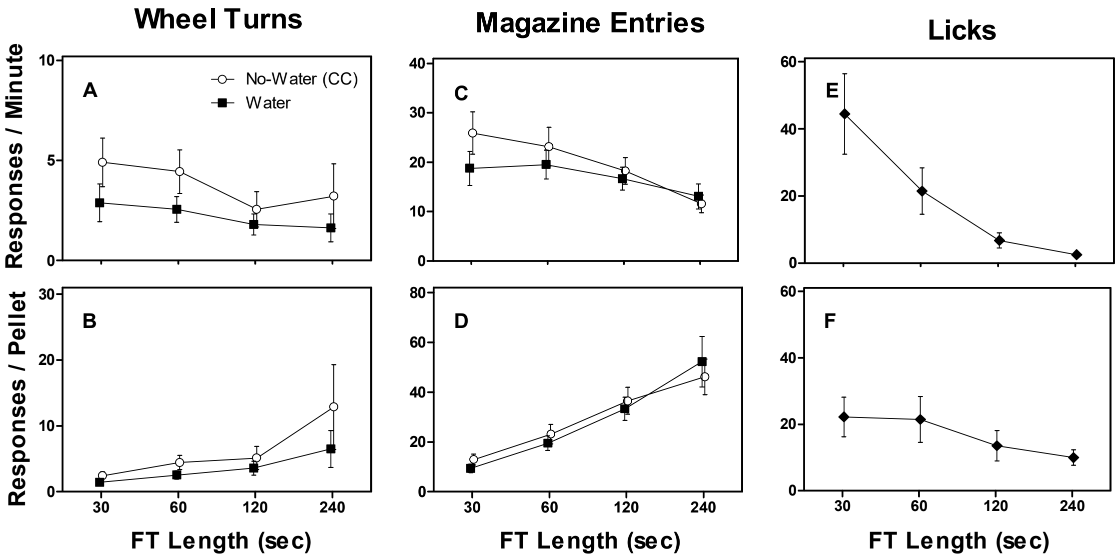

Figure 6. Mean (± SEM) number of wheel turns per minute (A), wheel turns per food pellet (B), magazine entries per minute (C), magazine entries per food pellet (D), licks per minute (E) and licks per food pellet (F), with and without access to a water bottle in the conditioning chamber (CC) under each FT schedule.

Figure 6A depicts wheel turns per minute under the four FT schedules when animals had the opportunity to drink water in the conditioning chambers and when they did not. The ANOVA displayed no effects for: FT schedule [F(1,8) = 2.88, $p$=0.12, $\eta^2$=0.291], Condition [F(1,7) = 1.99, $p$=0.20, $\eta^2$=0.221] or the FT schedule x Condition interaction [F(1,10) = 0.25, $p$=0.71, $\eta^2$=0.035]).

Figure 6B compares wheel turns per food pellet under the four FT schedules. The ANOVA also displayed no effects for: FT schedule [F(1,7) = 4.04, $p$=0.80, $\eta^2$=0.366], Condition [F(1,7) = 1.51, $p$=0.26, $\eta^2$=0.177] or the FT schedule x Condition interaction [F(1,7) = 0.46, $p$=0.53, $\eta^2$=0.062]).

Figure 6C shows the mean number of magazine entries per minute under the four FT schedules. The ANOVA displayed effects for FT schedule [$F$(3,21)= 12.13, $p$<0.001, $\eta^2$=0.634], with reductions in rate as the length of the IFI increased. No statistically significant differences were found for either condition [F(1,7) = 0.52, $p$=0.50, $\eta^2$=0.069], or for the interaction [F(3,21) = 2.01, $p$=0.14, $\eta^2$=0.223].

Figure 6D shows the mean number of magazine entries per food pellet under the four FT schedules. The ANOVA displayed effects for FT schedule [$F$(1,10) = 30.88, $p$<0.001, $\eta^2$=0.815], with the increase of responses before the increase of the length of the IFI. No effects were found for Condition [F(1,7) = 0.03, $p$=0.86, $\eta^2$=0.005] or for the FT schedule x Condition interaction [F(3,21) = 0.88, $p$=0.47, $\eta^2$=0.112].

Figure 6E compares licks per minute under the four FT schedules. The analysis performed showed effects for FT schedule [$F$(1,8) = 12.20, $p$<0.01, $\eta^2$=0.635], with a reduction in the response rate with the increase in the length of the IFI. Post hoc comparisons showed that FT 30-s resulted in higher licks per minute than FT 120-s ($p$<0.05) and FT 240-s ($p$=0.05).

Figure 6F depicts licks per food pellet under the four FT schedules. A main effect of FT was found [$F$(3,21) = 3.86, $p$<0.05, $\eta^2$=0.356], with a reduction in the number of responses with the increase in the length of the IFI. Post hoc comparisons only showed a tendency for FT 30-s to show higher licking than FT 120-s (*p*=0.06).

Figure 7 depicts the mean (± SEM) of total wheel turns, total licks and total magazine entries made every second (bin) during the IFI for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length) when rats had concurrent access to a wheel and a water bottle in the conditioning chambers, averaged over the last three sessions of exposure to each schedule.

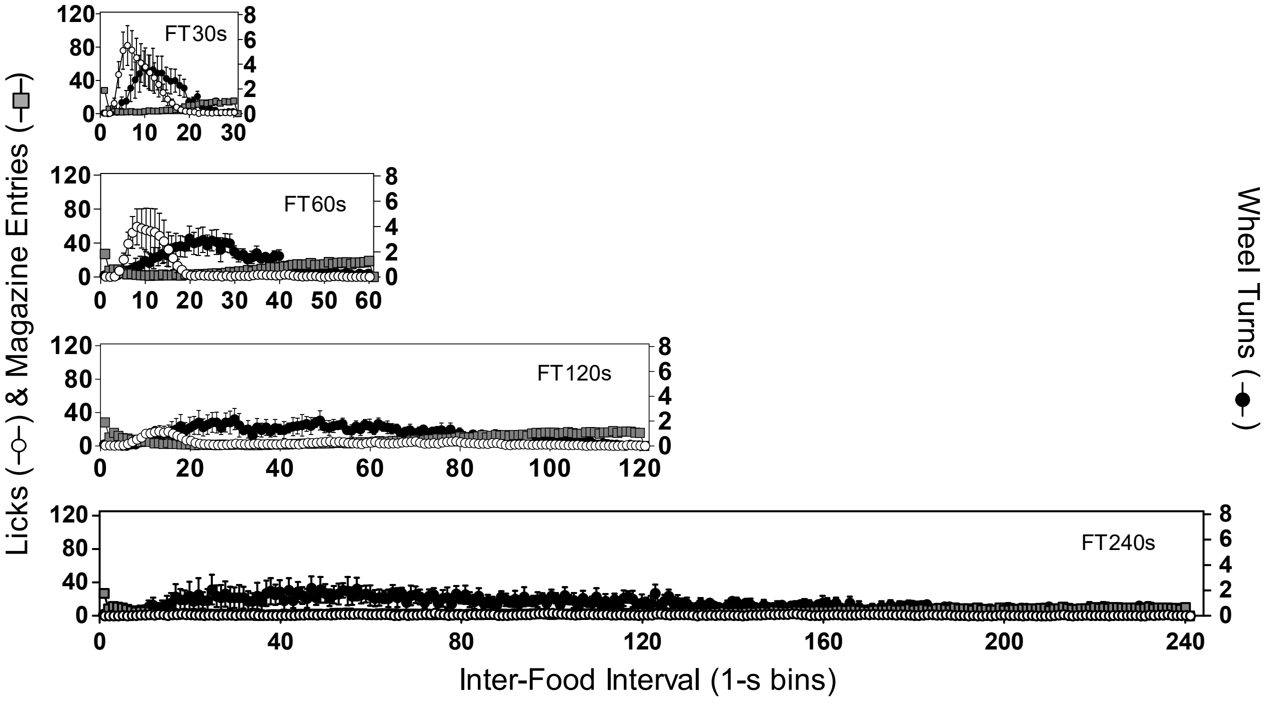

Figure 7. Mean (± SEM) wheel turns, magazine entries and licks given every second (bin) during the inter-food intervals for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length).

In the distribution of the wheel turns, the highest response rate was located in the first third of the interval (FT 30-s: bin 10; FT 60-s: bin 20; FT 120-s: bin 30, and FT 240-s: bin 47). The distribution curve presented a more gradual increase than in Figure 2 (no water bottle in the conditioning chamber), consequently the curve tended to shift to the right occupying a more centered position along the IFI. Rats barely ran towards the end of the IFI. It can be observed that wheel running was more concentrated in the first 3/4 parts of the IFI.

Magazine entries presented their typical distribution, with the highest number of responses at the beginning of the interval (0-s bin), followed by an accelerated decrease and a constant and gradual recovery, in this case from half the IFI.

Licks presented the typical distribution in the form of an inverted U-shape along the IFIs, being located in the first bins. The highest response rate was found in bin 6 for FT 30-s, 8 for FT 60-s, 13 for FT 120-s, and 74 for FT 240-s. In the long programs (FT 120-s and FT 240-s), the animals hardly drank, in fact, drinking did not develop in the FT 240-s schedule, presenting a low and flat rate of licks during the entire IFI.

Figure 8 shows the mean (± SEM) number of magazine entries, licks and mL consumed per minute, as well as the mean (± SEM) number of magazine entries, licks and mL consumed per food pellet, given by rats under FT 60- and FT 120-s schedules when rats had access to a water bottle in the conditioning chambers (Schedule-Induced Polydipsia), and when rats had access to water and wheel running in the conditioning chambers (the data reported in Figure 6). These data consider the average of the last three sessions of each FT schedule.

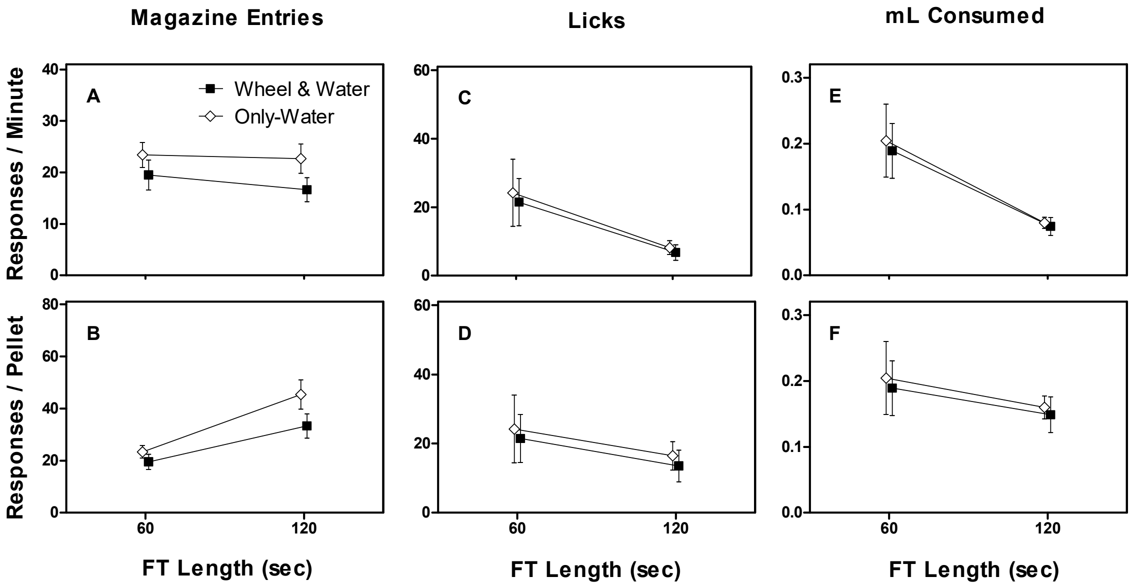

Figure 8. Mean (± SEM) number of magazine entries per minute (A), magazine entries per food pellet (B), licks per minute (C), licks per food pellet (D), mL consumed per minute (E) and mL consumed per food pellet (F), with and without access to a wheel in conditioning chamber (CC) under each FT schedule.

The ANOVA of the three behaviors per minute showed effects for FT schedule [$F(1,7) = 28.94$, $p=0.001$, $\eta^2=0.805$] in magazine entries (Fig. 8A); [$F(1,7) = 9.19$, $p<0.05$, $\eta^2=0.568$] in licks (Fig. 8C); and [$F(1,7) = 11.80$, $p<0.05$, $\eta^2=0.628$] in mL consumed (Fig. 8E), with a higher response rate in FT 60-s than in FT 120-s. No effects were found for Condition or for the Interaction (magazine entries: [F(1,7) = 1.17, $p=0.31$, $\eta^2=0.143$] and [F(1,7) = 1.08, $p=0.33$, $\eta^2=0.134$]; Licks: [F(1,7) = 0.14, $p=0.72$, $\eta^2=0.019$] and [F(1,7) = 0.02, $p=0.89$, $\eta^2=0.003$]; mL consumed: [F(1,7) = 0.18, $p=0.68$, $\eta^2=0.025$] and [F(1,7) = 0.04, $p=0.86$, $\eta^2=0.005$], respectively).

For behaviors per food pellet, analyses showed effects for FT schedule [$F(1,7) = 118.11$, $p<0.001$, $\eta^2=0.944$], only on magazine entries (Fig. 8B), with a lower response rate in FT 60-s than in FT 120-s. No statistical differences were found for all other circumstances: magazine entries [F(1,7) = 1.38, $p=0.28$, $\eta^2=0.164$] and [F(1,7) = 2.67, $p=0.15$, $\eta^2=0.276$], for Condition and Interaction respectively; Licks [F(1,7) = 4.48, *p*=0.07, $\eta^2$=0.390], [F(1,7) = 0.20, *p*=0.67, $\eta^2$=0.028] and [F(1,7) = 0.00, *p*=0.98, $\eta^2$=0.000] (FT schedule, Condition and Interaction, respectively); and mL consumed [F(1,7) = 2.24, *p*=0.18, $\eta^2$=0.243], [F(1,7) = 0.25, *p*=0.63, $\eta^2$=0.035] and [F(1,7) = 0.00, *p*=0.94, $\eta^2$=0.001] (FT schedule, Condition and Interaction, respectively).

Despite these results, in Figures 8A and 8B, a Condition effect is observed, with a higher rate of entry to the feeder under the condition of no wheel access (water only) with respect to the condition of concurrent access to the wheel and bottle, under the FT 120-s program.

Figure 9 depicts the mean (± SEM) of total licks and magazine entries made every second (bin) during the IFI for FT 60-s and FT 120-s schedule (represented in separate panels from top to bottom as a function of increasing FT length), when rats had concurrent free access to a water bottle in the conditioning chambers, averaged over the last three sessions of exposure to each schedule.

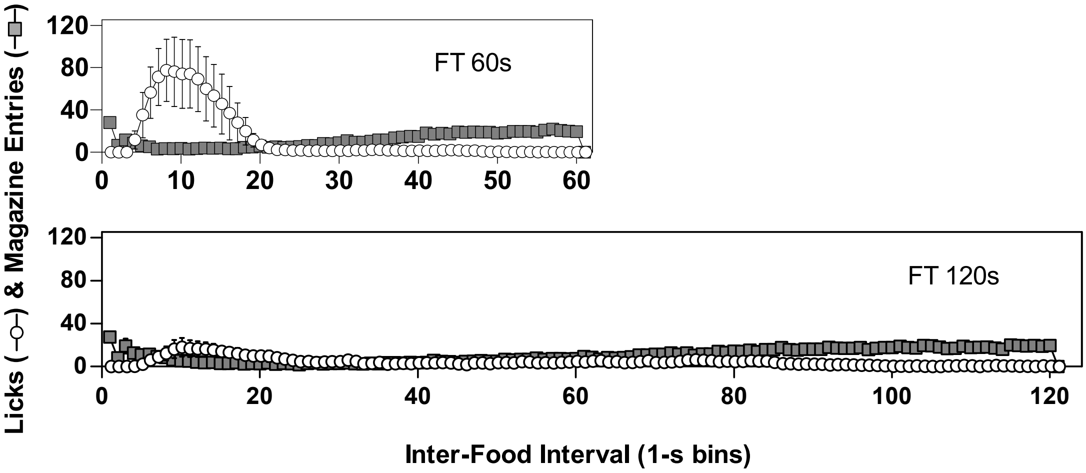

Figure 9. Mean (± SEM) licks and magazine entries given every second (bin) during the inter-food intervals for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length).

Licks present the typical distribution in the form of an inverted U-shape in the early part of the IFI. The highest response rate was found in bins 7 for FT 60-s and 10 for FT 120-s. As can be observed in Figure 7, the animals hardly drank in the FT 120-s schedule; Moreover, the general distribution in the IFI was practically identical to that found for that condition, where the animals had concurrent access to the water bottle and the wheel.

Magazine entries presented their typical distribution, with the highest number of responses at the beginning of the interval (0-s bin), followed by an accelerated decrease and a constant and gradual recovery, in this case from half the IFI.

As on other occasions, the magazine entries described a distribution that started with the maximum response occurring within the IFI (0-s bin), followed by an accelerated descent, and a constant and gradual recovery. In this case, and unlike what was observed in the condition in which the animals also had a wheel in the conditioning chamber, where the recovery of behavior began towards half of the IFI, this recovery took place at the beginning of the second third of the interval, coinciding with the data observed in Figure 4, where the animals had access to a wheel, both in the home cage and in the conditioning chamber.

## 4. Discussion

The purpose of Experiment 1 was to assess whether wheel running can be considered as schedule-induced behavior beyond previous demonstrations, using different FT food schedules and a massed-food control condition. Animals developed SIWR for most of the FT schedules, in a range of 30-240 s (SIWR was not developed under FT 480-s, so it was excluded from subsequent experiments), with a gradation depending on the length of the IFI (Riley et al., 1985) and a decrease in wheel running rate as the IFI increased.

Figure 1 showed a linear downward function as the IFI increased for wheel turns per minute, this being coincident with the results found for other induced behaviors such as SIP, where a fixed duration of the session for different IFIs showed a decreasing monotonic function of the total volume of water ingested with the increase in the length of the IFI (Bond, 1973; Hawkins, Schrot, Githens & Everett, 1972). More precisely, this resembles the transformation in water ingestion rate carried out by Pellón (2012; see a more detailed analysis in Pellón, 1992) on the original data published by Falk (1966, 1967) and Flory (1971), in which the same function was obtained as that found for wheel running rate in the present study. Furthermore, the data on wheel turns per food pellet presented a bitonic relation as a function of an increase in the IFI, although it did not present an inverted U-shape as clearly as is usually observed with other induced behaviors such as SIP. The inverted U-shaped function is characteristic of induced behaviors recorded in conditions where the number of food pellets remains constant for the different IFIs (Falk, 1966; Flory, 1971; Roper, 1980) (for more information see Pellón, 1992, 2012). Altogether, the present data support the idea that wheel running is schedule-induced and cannot be seen as a non-induced behavior, for which the response rate should increase in parallel with increases in IFI length (Penney & Schull, 1977; Staddon, 1977; Staddon & Ayres, 1975).

The results obtained here run counter to those reported by Riley et al. (1985) because their findings pointed towards a clear bitonic rather than linear function, relating the rate of wheel running to IFI length. However, this is more apparent than real. Total wheel turns increased as IFI increased both in Riley et al.'s study and in the present study (here reported as wheel turns per pellet) up to the range of common values tested (30 to

240-s), and then decreased or stabilized at FT 480-s in the present study (a value that was longer than the longest 360-s tested by Riley et al). Differences in the reported results on rate of wheel running are not fully clear but the impression is that Riley et al did not find clear effects of FT length on rate of responding because they first trained all rats on the same FT 60-s food schedule, leading to a lower rate of responding than in any of the other subsequent FT schedules tested, which may then have captured some initial lower level of acquisition than subsequently seen under continuation of repeated and extensive sessions of intermittent reinforcement through different FT values without returning at the end to the initial baseline of FT 60-s. Perhaps more important for discrepancies between Riley et al.'s and our results is the use of a different apparatus, in which the main space was the one with the wheel for Riley et al.'s study (they used a single apparatus consisting of a running wheel with an attached chamber to one of its walls) whilst a side wheel attached to a main chamber was used in our case (see Apparatus section above). The rats' stay in the wheel in Riley et al.'s procedure surely instigated a much higher level of running than in the case of the present study where rats stayed mainly in the open area of the conditioning chambers, crossing to the wheel only to run.

The presentation of a massed-food test allowed us to verify the first criterion (outlined in the Introduction) that must be met in order to consider a behavior as schedule-induced, i.e. an increase in response rate when reinforcers are delivered intermittently. Massed-food presentations caused an immediate reduction in wheel running, and we found significant differences in wheel running rate, this being higher when food pellets were delivered intermittently than when they were all deposited together, thus reinforcing the results previously obtained by White (1985) and extending them to a wider range of FT schedules. This conclusion is further supported by the exception of the data of FT 480-s with a similar rate in massed than intermittent conditions after not having developed high wheel running when food was scheduled intermittently. This reduction in behavior suggests that wheel running itself would be induced by intermittent reinforcement schedules, this being one of the main criteria for the categorization of a behavior as adjunctive (Falk, 1971), and, therefore, contradicting the results obtained by other authors claiming that wheel running was not induced by intermittent reinforcement (Penney & Schull, 1977; Wetherington et al., 1977). For example, Penney and Schull (1977) found that animals drank less and ran more when exposed to a massed-reinforcement schedule than when an intermittent schedule was presented. Their data could be due to the fact that the comparison of the wheel running rate was made under conditions where a bottle of water was concurrently present with the wheel during the different experimental conditions, thus potentially contaminating the results. Drinking could compete with wheel running, presenting itself as a "stronger" behavior, and therefore limiting the expression of wheel running. The apparent increase in wheel turns per food pellet under the massed control of FT 480-s in comparison with other massed-food tests (see open circles in Figure 1B) is simply due to the larger opportunity to run, given the significant increase in session duration as the number of total food pellets was kept constant across the different FT conditions.

For magazine entries, Figure 1 also showed the same functions than those found for wheel turns as the IFI increased, a linear downward function for magazine entries per minute, and a linear upward function for magazine per food pellet; With the exception of the FT 480-s, where magazine entries per food pellet descended, this coincided with the increase in wheel turns mentioned above. On the other hand, the expected results were observed for the massed-food test, with the almost absence of responses as a result of the close relationship between behavior and food delivery.

With regard to the second criterion for schedule induction as post-reinforcer location of the behavior (see Introduction), the temporal distribution of wheel running showed a similar function to that normally seen in SIP, i.e. an inverted U-shaped post-pellet location, presenting a maximum peak during the first part of the IFI (Falk, 1971; Segal, 1969) (except for FT 480-s, which showed a flat distribution throughout the entire IFI, and failed to generate SIWR), followed by a gradual decrease towards the end of the IFI. This well-defined response pattern has been reported by White (1985), and is strong evidence that wheel running constitutes schedule-induced behavior. Similarly, White (1985) concluded that the results, often disparate, that had been obtained by different authors depended on the configuration of the apparatus used. For example, the possibility of access from the wheel to the food receptacle resulted in a pattern of wheel running that barely diminished until the arrival of the next reinforcer (Roper & Crossland, 1982), or the possibility of engaging in alternative behaviors, where for example the presence of a bottle containing water displaced the peak of wheel running to more central positions in the IFI (Penney & Schull, 1977; Staddon & Ayres, 1975). The curve tended to shift to the right and flatten as a consequence of increases in the length of IFI, conditions that have been observed previously in SIP (Íbias & Pellón, 2011). Results reported by Riley et al. (1985) are very similar to those observed here for temporal distributions, and although they preferred to be more conservative and consider running as a non-induced behavior, they also suggested a temporal modulating effect of the food schedule, thus agreeing with the criteria that must be met to regard running as another schedule-induced behavior.

The temporal distribution of magazine entries showed the characteristic pattern of this behavior in the different FT schedules, a maximum peak at the beginning of the IFI, followed by an accelerated descent, and ending with a gradual increase towards the end of the interval, in the face of the proximity of delivery of the next reinforcer (Boakes, Patterson, Kendig & Harris, 2015; Reid, Bacha & Morán, 1993; Staddon, 1977; Staddon & Simmelhag, 1971).

The objective of Experiment 2 (SIWR with wheel running in the home cage condition) was to document the influence of the presence of a wheel in the home cage on the maintenance of the SIWR. The animals had free access to a wheel in their home cages during the time of no-experimental session, trying to match the general conditions under which the SIP is usually studied, that is, where the animals have free access to a water bottle in the home cage.

Figure 3A showed a reduction in wheel running for wheel turns per minute when animals had been pre-exposed to a wheel in their home cages, finding significant differences under short FT (FT 30- and 60-s) schedules. These results would respond to a satiation of wheel running due to the presence of the wheel in the home cage, and the short duration of these schedules. However, analyzes of activity distribution, in 15-minute blocks (see Figure 5) within the experimental session do not support this theory; although significant differences were found between the conditions of No-Wheel (HC) and Wheel, in the first block (first 15 minutes) for FT 60- and 120-s schedules (differences were also found for FT 30-s, but it is not relevant to mention it since, 15 minutes is the total of the experimental session), the same did not happen for the FT 240-s schedule. In this case, we believe that the results found in the first part of the session respond to a state of deprivation of wheel running because the animals had no possibility of exercising in their home cages. Aoyama and McSweeney (2001) found that animals ran more after two days of deprivation of access to the wheel than after one day. Then, as the session progresses, there is a satiation process of the wheel running. Wheel running has the characteristic of functioning as a reinforcer (Belke, 1997), so part of the running rate corresponds to that intrinsic reinforcement (Belke & Pierce, 2016). Attending to this, Bizo, Bogdanov and

Killeen (1998) have shown that reinforcers lose efficacy by satiation, causing a decrease in behavior.

In wheel turns per food pellet, no significant differences were found, however, visually (see Figure 3B), there seems to be an effect of the schedule, with an increase in the number of wheel turns as the IFI increases; as well as a condition effect on the FT-30 and 60-s, where the No-Wheel (HC) condition produced more responses than the Wheel condition.

On the other hand, it is difficult to draw clear conclusions on this issue, since there is no consensus, among other behaviors defined as adjunctive, on the effect of pre-exposure in home cages to the conditions subsequently evaluated; the pre-exposure did not affect drinking (Roper, 1980; Roper & Posadas-Andrews, 1981); delayed or prevented aggression (Looney, Cohen, & Yoburn, 1976; Looney & Dove, 1978); or prevented chewing (Roper & Crossland, 1982).

The reduction of both the wheel running rate and the number of wheel turns per food pellet was accompanied by an increase in the number of magazine entries (rate and -per food pellet), which could indicate some competition between the two behaviors, as has been found for other adjunctive behaviors, such as in the case of SIP (For a reanalysis where emphasis is placed on behavioral competition, see Pellón & Killeen (2015)). Magazine entries were presented in the same way as in Experiment 1, decreasing and increasing depending on the increase in IFI, for magazine entries per minute and per food pellet, respectively. However, the number of magazine entries was higher in the Wheel condition than in the No-Wheel (HC) condition, for both cases.

The temporal distribution of wheel running was affected by the presence of a wheel in the home cage for short FT (FT 30-s and 60-s) schedules, causing a shift of the curve to the right. With this exception, the distribution curves maintained parameters very similar to those observed in the No-Wheel in the home cage condition. On the other hand, the effect of the wheel in the home cage on the temporal distribution of magazine entries resulted in a greater presence of this response during the entire IFI, reaching values close to zero only in a brief segment of the FT 240-s, compared to the No-Wheel in the home cage condition.

In order to study the influence of other induced behaviors on the SIWR and its subsequent comparison, SIP was chosen as a “second behavior” (Experiment 2. *Concurrence of SIWT and SIP in the conditioning chambers*) by adding a water bottle to the experimental condition. SIP developed normally, the animals acquired SIP, and the levels of drinking varied according each FT schedules, figure 6E showed a linear downward function as the IFI increased for licks per minute (Íbias & Pellón, 2011), similarly to wheel running in Experiment 1, and as discussed above (see figure 1 discussion).

Wheel running was barely reduced by the presence of water in the conditioning chamber, although not significantly. These results coincide with the conclusion of Segal (1969), the prevention of drinking would not necessarily increase the amount of wheel running. However, these data tend to deny what Staddon (1977) said, about wheel running rate decreasing, while drinking rate increases when both behaviors are available.

The temporal distributions of the different behaviors within the IFI (Figure 7) adopted a position very similar to that found by other authors (Reid et al., 1993; Roper, 1978; Segal, 1969; Staddon, 1977; Staddon & Ayres, 1975); initially (post reinforcer), we would find drinking (interim behavior), followed by wheel running over the middle of the interval, and finally the magazine entries (terminal behavior). As wheel running does not follow the delivery of the reinforcer, Staddon (1977) does not consider that wheel running is an induced behavior, and calls it a facultative behavior, a behavior that increases before intermittent schedule of reinforcement, but lacks a direct relationship with the reinforcer. However, as we have observed in Experiment 1, and other previous experiments (Levitsky & Collier, 1968), wheel running does occur before intermittent reinforcement when there is no water bottle in the experimental session, adopting a post-reinforcing position, and presenting its maximum peak at an earlier position in the IFI (Riley et al., 1985; Segal, 1969).

This “readjusting” of the behaviors within the IFI that is observed in our work, and responds to a temporary competition between behaviors that has been previously documented by other authors (Roper, 1978; Wetherington & Riley, 1986); when competition occurs, wheel running takes part of the place of drinking, and drinking moves wheel running to a central position within the IFI (Reily et al., 1993; Staddon, 1977; Staddon & Ayres, 1975).

On the other hand, the presence of water in the experimental session affected the temporary distribution of wheel running within the IFI, while the same did not happen for the magazine entries, so it could be concluded that drinking and wheel running have the same nature, that is, they are adjunctive behaviors that compete for their expression within the IFI (Killeen & Pellón, 2013).

The comparison of the data found for licks and mL consumed both in the presence and absence of wheel during the experimental session (Figure 8) does not show significant differences. These data are contrary to those found by other authors (Segal, 1969; Riley et al., 1981; Roper, 1978; Wetherington & Riley, 1986), where the drinking rate was increased when wheel running was prevented. Similarly, the temporary distribution of drinking was not affected by the absence of a wheel (Figure 9), finding an identical distribution to that observed under the condition of concurrence of wheel and water (Figure 8), and contradicting the results found by Wetherington and Riley (1986), who claimed that the restriction of wheel running increased drinking in places previously occupied by wheel running. These results indicate that SIP has a greater “force of expression” (Killeen & Pellón, 2013) with respect to SIWR, since drinking was not affected by wheel running.

## 5. Conclusion

In conclusion, the results found in the present study support the idea that wheel running may be classified within the so-called adjunctive behaviors, as well as acquiring an induced character. According to Staddon (1977), wheel running might be considered an adjunctive behavior but not an induced behavior, as it does not comply with the characteristics of induction, since when exposed to an intermittent reinforcement schedule it would not present excessive rates and it would adopt an intermediate position within the IFI, thus not having a direct relationship with the reinforcer. However, our data indicate that wheel running is a schedule-induced behavior that develops at excessive rates when animals are exposed to an intermittent reinforcement schedule, while under massed control, the rate drops considerably, contrary to Staddon’s suggestion that wheel running should be maintained even in the absence of intermittency. Furthermore, the temporal distribution of wheel running within the IFI presents a pattern similar to that found with other induced behaviors such as SIP, that is, when only the wheel is available during the experimental session, wheel running reaches its maximum between the beginning and the middle of the interval between meals, adopting a post-food position within the IFI.

At all times, we must take into account that wheel running has a different nature than other operant behaviors. Wheel running, by itself, generates its own automatic reinforcement (Skinner, 1957; Vaughn & Michael, 1982). Meanwhile, the induction produced by intermittent reinforcement schedules generates an extrinsic reinforcement, when animals are deprived of food, which motivates wheel running (Baumeister, Hawkins & Cromwell, 1964; Collier, 1970; Jakubczak, 1967; Moskowitz, 1959; Price, 1976; Sclafani & Rendel, 1978; Treichler & Hall, 1962). Therefore, the control exercised on wheel running by the extrinsic reinforcement is altered by the intrinsic reinforcement in SIWR, unlike what occurs in the induction of other behaviors. This intrinsic reinforcement of wheel running implies a higher operant level than in other types of behavior. In this respect, the results found by Belke and collaborators (Belke, Pierce, Fisher & LeCocqc, 2017) show that wheel running does not increase much when it is extrinsically reinforced (with sucrose) in relation to the operant baseline level. This would explain why wheel running is not so explicitly excessive as induced behavior, unlike what is found in SIP, where the drinking rate is very high with respect to its baseline. This also reveals the mistake made by Staddon (1977) when introducing a third category of behavior (facultative) that is not excessive. This fundamental characteristic for the categorization of induced behaviors should be reconsidered in the case of wheel running. Similarly, Staddon's (1977) arguments about the persistence of wheel running, in the absence of intermittent reinforcement, could respond to the motivation generated by the intrinsic reinforcement of behavior. This conclusion was reached by Belke and Pierce (2015) when observing that wheel running maintained a high rate during the extinction phase; After replacing the reinforcer, water with 15% sucrose, for simply water, the wheel running rate barely decreased 26%.

On the other hand, this study has also shown that the possibility of performing two behaviors concurrently, drinking and wheel running, did not alter the response rates of either; Therefore, discarding the hypothesis of Reid and Staddon (1990), whereby the rates of wheel running and drinking decrease and grow, respectively, when both behaviors are available, due to a competitive inhibition of non-induced behavior (wheel running) regarding induced behaviors (drinking); We can conclude that wheel running is not a non-induced behavior.

Moreover, the concurrence of drinking and wheel running, generates a multimodal pattern where drinking is located towards the beginning of the IFI, moving wheel running to a more central position within the interval. In this regard, and accepting wheel running as an induced behavior, the induced behavior does not necessarily have priority over the non-induced behavior, as Staddon (1977) commented, rather we would be talking about two induced behaviors, drinking and wheel running, which compete for their expression when they have the possibility of performing together, which supports the idea that they belong to the same type of behavior with differences in their sensitivity to the delay of the reinforcer, as indicated by the model of Killeen and Pellón (2013), according to which, “different Induced behaviors can be intrinsically more or less memorable, and therefore, have a longer or shorter delay in reinforcing gradients. If they compete with each other for expression, those with the longest memorable gradients have an "earlier" competitive advantage in the IFI”; in the case at hand, drinking would be more memorable than wheel running.

# CHAPTER III

# Delay gradients to food among different schedule-induced behaviors

## Abstract

Male Wistar rats maintained at 80-85% of their free-feeding weights by food restriction were first submitted to a fixed time (FT) 60 s food delivery schedule until they reached stable rates of spout licking, wheel running and magazine entering, and then received a protective contingency by which food was postponed if they licked, ran or magazine-entered during the last 1, 3, 5, 10, 20, 40 or 58 s of inter-food intervals (IFIs) in successive phases of the study. For half of the rats, delays were signaled by tone and blackout, for the other half, delays were unsignaled. In a final phase, delays were removed. Behaviors were affected by protective delays differentially, dependent on their temporal location within IFIs, being licks more resistant to the disruptive effects of delays, followed by running and then magazine entering. All rates increased when delays were removed. No significant differences were observed when compared signaled and unsignaled delays. The introduction of contingent delays upon responding allows the drawing of response-reinforcer gradients that supposedly reflect the effect of operant contingencies over the responses, showing different sensitivities as a function of their temporal location within IFIs.

*Keywords:* Schedule-induced behavior; Response-food delays; Licks; Magazine entries; Wheel turns; Rats.

## 1. Introduction

Adjunctive (or schedule-induced) behaviors are defined as activities that occur at significant high rates as a result of exposure to intermittent reinforcement schedules and without experimentally arranged contingencies between occurrence of behavior and reinforcer delivery, having in general, a post-reinforcement temporal location with an inverted U-shaped distribution along inter-reinforcement intervals (Falk, 1971; Roper & Posadas-Andrews, 1981; Timberlake, Wahl & King, 1982; Wetherington, 1982).

The phenomenon was observed first by Falk (1961) by exposing hungry rats to an intermittent food-reinforcement schedule with access to a bottle filled of water in the experimental chamber. The animals developed excessive drinking (i.e., schedule-induced polydipsia - SIP) not related to physiological needs or apparent behavior regulation. This discovery stimulated the search for other models of behavior that are affected by intermittent reinforcement in the same way, such as wheel running (Levitsky & Collier, 1968), pica (Villareal, 1967), aggression (Looney & Cohen, 1982), and a long list of other activities (see revision by Falk, 1977; Pellón, 1990).

According to Staddon (Staddon & Simmelhag, 1971; Staddon, 1977), schedule-induced behaviors can be divided into terminal and interim activities, depending on their nature and location within the inter-food intervals (IFI). Terminal activities, such as magazine entries, consistently occur before or just at the time of the presentation of the reinforcer, and therefore in the presence of stimuli related to its release (Robinson & Flagel, 2009). Interim activities, where SIP stands, precede terminal activities, with which they are generally incompatible, and are located from immediately after the delivery of the reinforcer until midway into the interval between successive reinforcers, as a consequence of a low probability of reinforcement. For Staddon (1977), the expression of both activities (terminal and interim) would be due to the induction of motivational states by stimulation conditions related to the delivery of the reinforcer.

Moreover, Staddon (1977) used the term facultative behavior to designate certain activities that, according to him, are not schedule-induced and that simply fill in the time between the predominant interim and terminal activities when the spacing of reinforcers is sufficiently long. Within this category of behaviors, wheel running in rats would be included, justified by its temporal position in the middle of the inter-reinforcement intervals and by increasing its response rate with the lengthening of the interval between successive reinforcers (Roper, 1978; Staddon & Ayres, 1975). However, recent studies, about schedule-induced wheel-running (SIWR), in our laboratory (Gutiérrez-Ferre & Pellón, 2019), have found evidence that contradicts the idea of wheel running as a facultative behavior, and supports wheel running as an induced behavior (interim activity). First, we have found that the wheel running rate decreases as the length of the IFI increases, showing a characteristic bitonic function of most schedule-induced behaviors (Falk, 1966; Flory, 1971; Roper, 1980). And secondly, we found that the temporal distribution of wheel running showed a post-pellet location, with a maximum peak during the first part of the IFI, being, the distribution referred by Staddon (1977), a characteristic of conditions where other interim behaviors intervene (Wetherington & Riley, 1986; see Chapter II, Experiment 2, of this Doctoral Thesis).

On a separate line of research, there is a good body of results that would support the idea that adjunctive behaviors such as SIP behave similar to conventional operant behaviors in terms of being controlled by their consequences and modulated by motivational variables (e.g., Reberg, 1980; Pellón & Blackman, 1987, 1991; Reid & Staddon, 1990; Lamas & Pellón, 1995; Castilla & Pellón, 2013). These results have led to the development of an alternative theoretical proposal to adjunctive behavior, according to which adjunctive behavior would be controlled by environmental events after its emission (cf. Killeen & Pellón, 2013).

Killeen and Pellón (2013) suggested that schedule-induced behaviors are actually maintained by the delivery of the reinforcer at the end of inter-reinforcement intervals (see also Ruiz, López-Tolsa and Pellón, 2016), based on the ideas of extended delayed reinforcement (Lattal, 1995) and that the absence of explicit arranged contingencies does not impede the operation of contingencies that ultimately ensure the maintenance of different behaviors, yet not explicitly reinforced (Papini and Bitterman, 1990). The potential operation of response-reinforcer contingencies seems particularly striking in the case of schedules that do not require specific response for the delivery of the reinforcer (fixed- or variable-time schedules), yet the temporal organization of behavior is remarkably similar in all animals of a same species (Anderson & Shettleworth, 1977; Killeen, 1975; Staddon & Simmelhag, 1971), as it occurs in laboratory rats when submitted to intermittent food delivery schedules (Staddon, 1977; Staddon & Ayres, 1975).

Following Killeen and Pellón (2013), the main role of the response-reinforcer contingency is to produce proximity between events; where the authors follow the argument initiated by Skinner (1948) of adventitious reinforcement by modifying "contiguity" for an exponential gradient of proximity. All behaviors generated in intermittent reinforcement schedules can be explained with differential traces of associability with the reinforcer, and different time courses of associability. Behaviors compete for their expression throughout the interval, thus, display different temporal patterns. Behaviors with pronounced gradients will tend to displace the interim behaviors with shallower gradients. Interim behaviors predominate during early and middle of the interval, receiving additional help by their association with the just-consumed reinforcer;

while, the terminal behaviors will be more strongly associated with reinforcement by its proximity to it towards the end of the interval.

Recent studies imposing response-food delays on fixed-time (FT) schedules have found that behaviors considered by Staddon as induced (interim and terminal) are both sensitive to changes in proximity between responses and food occurrence (Pellón & Pérez-Padilla, 2013; see also Pellón, Íbias & Killeen, 2018), in support of the operant vision of adjunctive behavior. The purpose of the present study was to follow up these previous investigations by selecting a wider range of behaviors and delays, in order to best characterize the operation effect of food as a reinforcer on the temporal structure of behaviors within inter-food intervals under schedules that do administer the reinforcer without specific response requirement.

By studying the effect of delays in each one of the three kinds of behavior defined by Staddon (1977) as interim, facultative and terminal responses, we could assess the effects of introducing protective response-food delays in order to see whether it would result in delay-of-reinforcement gradients that allow to question the traditional view of different behaviors belonging to separate categories subject to different principles. The traditional distinction between interim, facultative and terminal behaviors, as proposed in Staddon's (1977) model, is based on the idea that their differential temporal location is due to their different nature, not to the differential applicability of the same behavioral mechanism. We selected drinking, wheel running and magazine entering as examples, respectively, of each one of these categories, and applied similar response-food delays in order to study the way all these behaviors were affected by consecutively increasing the distance that separated behavior and food delivery.

## 2. Method

### *2.1. Subjects*

The same sixteen rats used in the experiments of chapter II, were reused for this experiment. They had previous experience in SIWR, and half of the animals, in addition, had previous experience in SIP, but without contingencies of punishment of any kind. Rats remained housed individually (18 cm × 32.5 cm × 20.5 cm transparent Plexiglas cages) in an environmentally controlled room with an 8:00 am/8:00 pm light/dark cycle, ambient temperature of 21º C, and 60% relative humidity. Weights were maintained to 80–85% of free-feeding weights by a controlled diet. Each rat was weighed before the commencement of each experimental session. Twenty minutes after the completion of experimental sessions, each animal received the appropriate food supplement to maintain its weight within the criterion-based range. Experimental sessions were conducted 5 days a week. Water was freely available in their home cages. All procedures were in accordance with the Spanish Royal Decree 53/2013 regarding the protection of experimental animals and with the European Union Council Directive 2010/63, and were approved by the Bioethics Committee of Universidad Nacional de Educación a Distancia.

### *2.2. Apparatus*

Eight Letica LI-836 conditioning chambers, measuring 29 cm × 24.5 cm × 35.5 cm. The chambers were enclosed in soundproofed housing equipped with a ventilation system and a small observation window in the left wall. The front panel of each chamber was made of aluminum, the left-hand wall and roof of transparent Plexiglas, and the remaining sides of black Plexiglas. A bottle with 100 ml of fresh tap water was attached to the external side of the right wall of each chamber, with its spout being accessible to the rat through a 3.2 × 3.9 cm aperture, located 20 cm from the front wall and 7 cm above the floor. The spout was positioned 2 cm from the wall aperture, in such a way that the rat could lick but not maintain permanent contact with it. Contact between the animal's tongue and the metal spout completed the electric circuit between the 12-bar metal grid that served as the floor and the water bottle spout, and it was registered as a lick. On the exterior of the back panel of the chamber, a wheel of stainless steel was fitted, 32 cm in diameter and 9.5 cm wide with spokes distributed at 1 cm intervals around the rim. The rat had access to the wheel from the interior of the chamber, through a 10 cm in diameter circular aperture in the wall, situated 28 cm from the front panel and 1 cm from the floor. An AZ fag magnetic reed switch recorded each entire revolution of each wheel. 45 mg food pellets were dispensed (Bio-Serv, Frenchtown, NJ, USA) in an aperture in the chamber's front wall situated 3.7 cm from the floor. Magazine entries into the food magazine were detected by the interruption of a photocell beam. The chambers were lit by two internal 3-W lamps situated on the upper part of the front panel to either side of the food tray, and a 25-W ambient lamp fitted to the interior of the soundproof housing that insulated each chamber. The ambient noise produced by the ventilation fan was 60 dB, which served to mask any other external sounds. Licks, wheel turns and magazine entries were recorded using a Med PC IV® software.

### *2.3. Procedure*

#### *2.3.1. Acquisition*

Rats were exposed to a FT 60-s food delivery schedule by which a single food pellet was presented at regular 60-s intervals, independently of behavior. This phase lasted for 30 sessions of 30 min each one.

### *2.3.2. Protective contingency*

In this phase, a protective contingency (DRO —“Differential Reinforcement of Other behavior”— contingencies) postponed food delivery if responses (magazine entries, wheel turns or licks) occurred within the last 1, 3, 5, 10, 20, 40, or 58 s of the IFI. Rats were exposed to 10 sessions for each delay, with the presentation ascending in duration. These delays were resetting, so food delivery would not occur during the punishment of the behavior. Every session terminated after 30 food pellets were delivered.

The rats were randomly separated into two groups, although taking into consideration whether the rats had had previous experience in SIP or not. For half of the subjects (n=8), delays were signaled by turning off all lights of the chamber and by providing a sound (70 dB, 40hz) for the duration of the protective contingency (signaled condition). While, for the other half (n= 8), delays not were signaled (unsignaled condition).

### *2.3.3. Recovery*

As a final experimental phase, delays were discontinued (delay 0 s) and recovery of the initial FT 60-s schedule was implemented for ten sessions.

For each session, total number of licks, total number of wheel turns and total number of magazine entries, as well as the total duration of the session, were recorded for each rat. Licks, wheel turns and magazine entries were also recorded every 1-s bin within inter-food intervals.

### *2.4. Statistical analysis*

The acquisition of behaviors was analyzed using two-way repeated measures analysis of variances (ANOVAs), with one between-subject factor named Group (Signaled vs. Unsignaled) and one repeated within-subjects factor named Session (thirty levels).

The baseline (BL) was established as the average of the last three acquisition sessions.

The effects of drinking, wheel running and magazine entering for the proportion of baseline responses, as well as the reinforcement frequency, were analyzed individually for each delay and recovery phase (delay 0 s), using two-way ANOVAs, with one between-subject factor named Group (two levels: Signaled and Unsignaled) and one repeated within-subjects factor named Session (11 levels: BL and 1-10 sessions).

The proportion of baseline responses for each behavior and group, averaged over the last three sessions of exposure to each delay, was analyzed using a one-way repeated measures analysis of variance (ANOVA), with Delay (first 0 s -acquisition phase, BL-, 1 s, 3 s, 5 s, 10 s, 20 s, 40 s, 58 s and second 0 s -recovery phase, REC-) as the within-subject factor.

When appropriate, post hoc comparisons were carried out using pairwise comparisons with a Bonferroni correction for *p* values. Minimum significance level was set at $p<0.05$. Effect sizes were estimated by $\eta^2$ (ANOVAs). All analyses were computed using the SPSS software package (Version 24).

## 3. Results

Both groups of rats rapidly developed stable response rates (see Figure 1) for the three behaviors recorded (licks, wheel turns and magazine entries) when exposed to the FT 60-s food delivery schedule. This was due to previous experience in FT schedules in the induction of SIP and/or SIWR. Although no differences were observed in the figure between the different sessions, the analysis yielded an effect of Session for wheel turns [$F(5,64) = 3.51$, $p<0.01$, $\eta^2=0.2$] and magazine entries [$F(6,83) = 3.84$, $p<0.01$, $\eta^2=0.215$]; but not for licks [$F(5,72) = 1.86$, $p=0.109$, $\eta^2=0.117$]. There were no remarkable differences between signaled and unsignaled groups (given the random distribution of animals between groups), an observation that was confirmed by the absence of significant ANOVA comparisons among groups for drinking [$F(1,14) = 0.44$, $p=0.519$, $\eta^2=0.030$], wheel running [$F(1,14) = 0.81$, $p=0.385$, $\eta^2=0.054$] or magazine entering [$F(1,14) = 1.08$, $p=0.315$, $\eta^2=0.072$]. No effect of interaction was found for any of the behaviors ([$F(5,72) = 1.08$, $p=0.383$, $\eta^2=0.071$], [$F(5,64) = 1.21$, $p=0.316$, $\eta^2=0.080$] and [$F(6,83) = 1.09$, $p=0.373$, $\eta^2=0.72$], respectively).

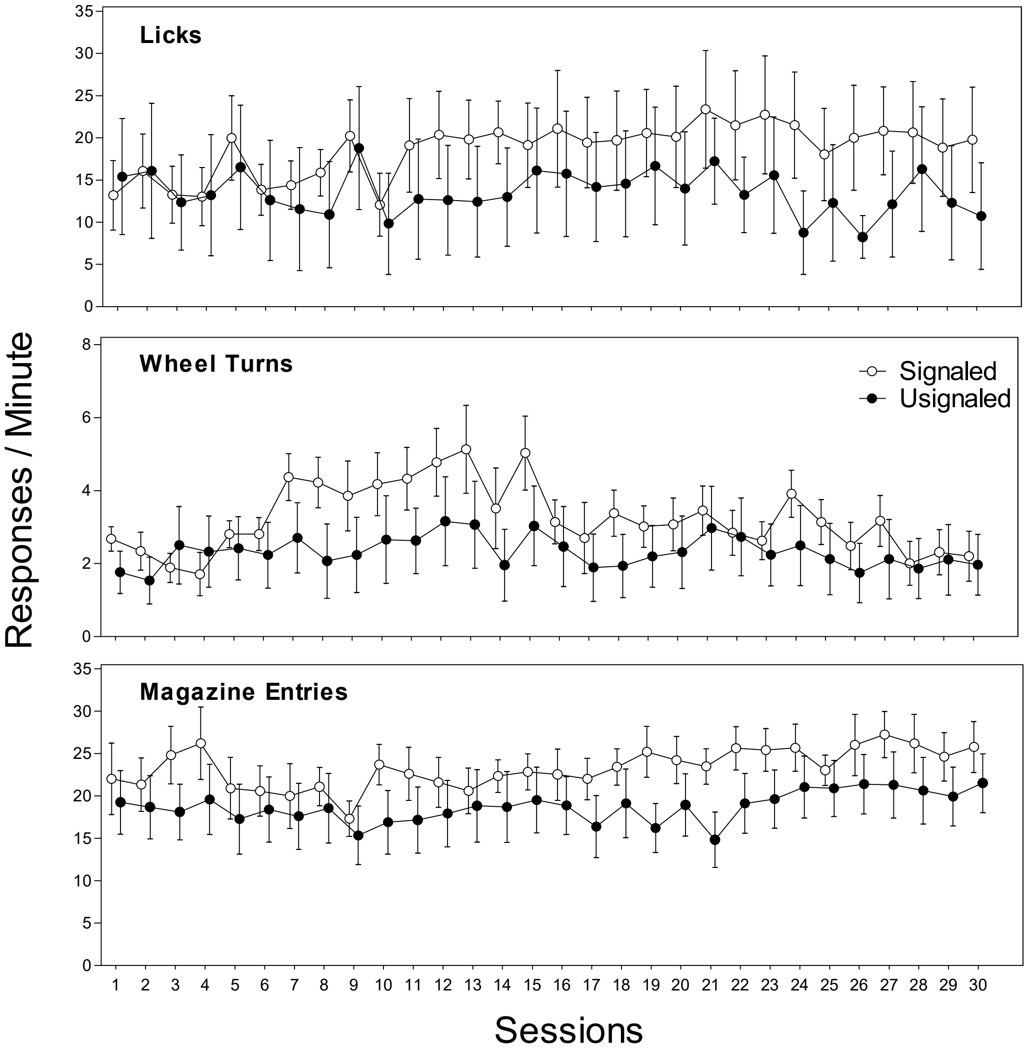

Figure 1. Mean (± Standard Error of the Mean - SEM) number of licks, wheel turns and magazine entries per minute in FT-60s across 30 sessions for each group (Signaled and Unsignaled).

In Figure 2, we can see the mean response rate (± SEM) of each behavior for each delay relative to baseline, along each session of each delay value. The upper panel shows delay effects on spout licking for both signaled and unsignaled groups. Lick-food delays had to increase up to 40 s in the signaled group (open circles), and up to 58 s in the unsignaled group (filled circles), to have systematic decreases in behavior, and in general, it was seen that delays were more effective in reducing licking when they were signaled than unsignaled. The rate of licking was not changed or sometimes was slightly increased relative to the baseline at short delay values (1 to 10 s), more often in the unsignaled condition. Regarding wheel running (middle panel), progressively greater reductions in response rate can be seen with short delays (1 to 3-5 s), and then a maintenance of reduced rates of wheel running until the longest delay tested. Both signaled and unsignaled groups showed similar effects of delays on running rates, except for the 40-s delay condition, where wheel running was slightly lower for the signaled rats. Concerning magazine entries (lower panel), delays affected performance right from the 1-s value and resulted in progressively lower response rates as the delay length was increased up to 40 s, with a very low response rate being maintained with the highest delay value of 58 s. No differences were appreciated in the effects of delays on magazine entries between the signaled and unsignaled groups.

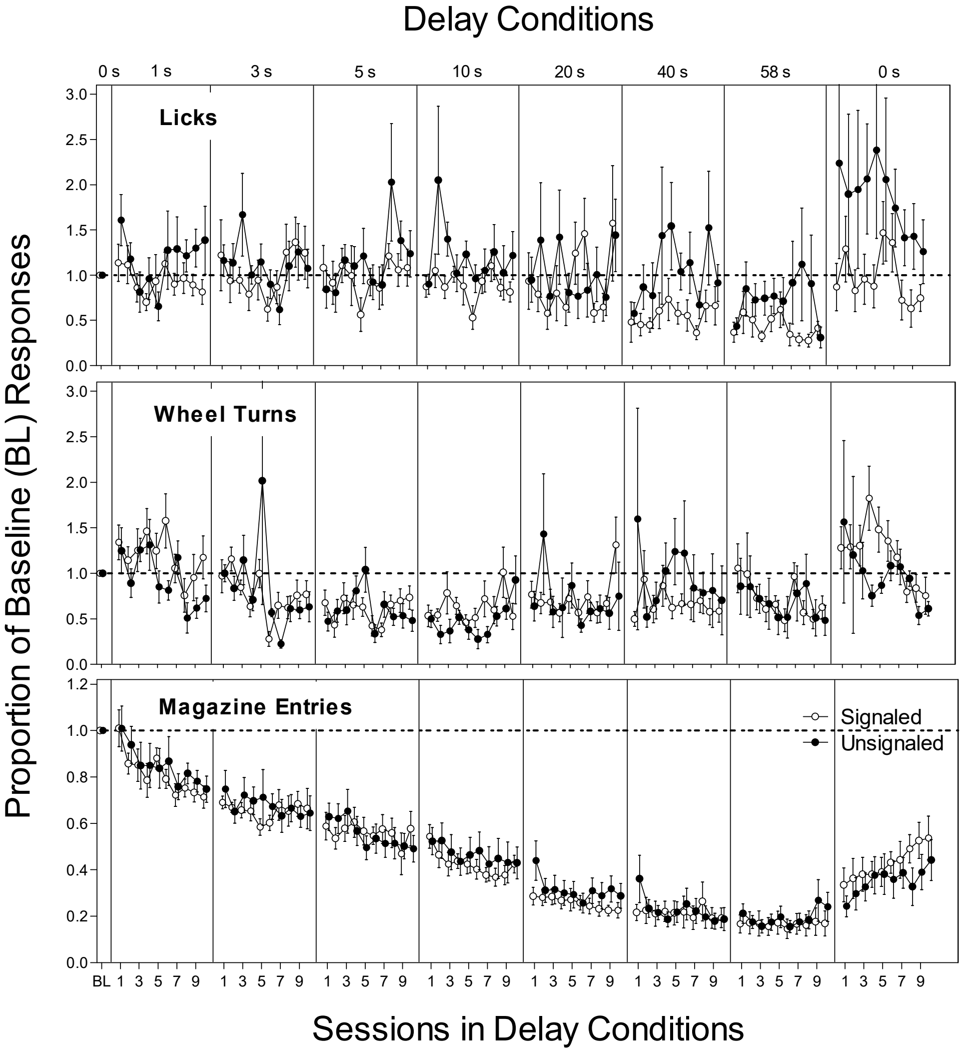

Figure 2. Mean (± SEM) number of licks, wheel turns and magazine entries in proportion to baseline rate of responding for each delay and recovery condition (delay 0 s) along each experimental session, and for each group (Signaled and Unsignaled).

When the delays were removed in the recovery phase of the experiment (as indicated by the right-most cells of the 3 panels of Figure 2), increases in all three behaviors were observed. Lick rate showed a marked increase in both groups during the first sessions, which was reduced over sessions but maintained final levels of licking which were higher than those observed during the latest delay sessions. The unsignaled group even showed recovery licking rates that were higher than during initial baseline.

Wheel running rates also sharply increased during initial sessions of recovery in comparison to the latest sessions with delays, even exceeding initial baseline response rates (particularly in the signaled group), but then resumed. The rate of magazine entries recovered slowly with the pass of the sessions, similarly for both groups, albeit to a greater degree in the signaled group. Magazine entries never recovered the rates observed during the initial baseline.

The analysis found no effects for licks. However, for wheel turns a main effect of Session was found in some of the delays (delay 1 s $p<0.05$; delay 5 s $p<0.01$; and delay 10 s $p=0.001$), with a reduction in response regarding the BL. Except for the delay 1 s, where the post hoc comparisons showed differences between 5th and 10th session ($p<0.05$), post hoc analyses revealed differences between BL and other sessions (for delay 5 s: with 1st and 10th sessions, $p<0.05$; with 2nd, 7th and 8th sessions, $p<0.01$; and with 6th session, $p<0.001$; and for delay 10 s: with 1st, 2nd, 4th, 5th and 6th sessions, $p<0.001$; and with 7th and 8th sessions, $p<0.05$). And, for magazine entries, Session effects were observed for all the delays and recovery condition ($p<0.001$), with a progressive reduction in response accompanying the increases in delay duration, and an insufficient attempt at recovery. Post hoc comparisons showed differences for delay 1 s (between BL and 7th, 8th, 9th and 10th, $p<0.001$), delay 3 s (between BL and all sessions, $p<0.001$, except 5th and 10th, $p<0.01$), delay 10 s (between 1st and 7-9th sessions, $p<0.05$; and between 2nd and 8-9th sessions, $p<0.05$), and for delays 5, 20, 40 and 58 s, as well as for recovery condition (between BL and all sessions, $p<0.001$). All detailed results can be reviewed in Table 1.

| Dependent Variable | Delay | Factor | df | F | Sig. | $\eta^2$ |
|---|---|---|---|---|---|---|
| Licks | 1s | Session | 4,63 | 1,53 | .200 | .098 |
| | | Session*Group | 4,63 | 0,81 | .538 | .055 |
| | | Group | 1,14 | 1,70 | .213 | .109 |
| | 3s | Session | 4,54 | 1,66 | .175 | .106 |
| | | Session*Group | 4,54 | 0,84 | .500 | .057 |
| | | Group | 1,14 | 0,23 | .637 | .016 |
| | 5s | Session | 3,44 | 1,89 | .141 | .119 |
| | | Session*Group | 3,44 | 0,97 | .417 | .065 |
| | | Group | 1,14 | 0,93 | .352 | .062 |
| | 10s | Session | 2,27 | 1,62 | .216 | .104 |
| | | Session*Group | 2,27 | 0,88 | .423 | .059 |
| | | Group | 1,14 | 3,10 | .100 | .181 |
| | 20s | Session | 3,39 | 1,82 | .163 | .115 |
| | | Session*Group | 3,39 | 1,16 | .335 | .077 |
| | | Group | 1,14 | 0,11 | .741 | .008 |
| | 40s | Session | 2,29 | 2,00 | .152 | .125 |
| | | Session*Group | 2,29 | 0,86 | .436 | .058 |
| | | Group | 1,14 | 3,93 | .670 | .219 |
| | 58s | Session | 2,28 | 1,71 | .199 | .109 |
| | | Session*Group | 2,28 | 1,26 | .300 | .082 |
| | | Group | 1,14 | 2,31 | .150 | .142 |
| | 0s | Session | 2,32 | 1,90 | .161 | .120 |
| | | Session*Group | 2,32 | 1,14 | .338 | .075 |
| | | Group | 1,14 | 2,31 | .151 | .142 |
| Wheel Turns | 1s | Session | 4,57 | 3,47 | .013$^{*}$ | .199 |
| | | Session*Group | 4,57 | 1,07 | .368 | .073 |
| | | Group | 1,14 | 2,59 | .130 | .156 |
| | 3s | Session | 1,17 | 2,16 | .157 | .134 |
| | | Session*Group | 1,17 | 0,82 | .402 | .055 |
| | | Group | 1,14 | 0,11 | .746 | .008 |
| | 5s | Session | 4,53 | 5,12 | .002$^{**}$ | .268 |
| | | Session*Group | 4,53 | 2,12 | .950 | .131 |
| | | Group | 1,14 | 0,00 | .987 | .000 |
| | 10s | Session | 3,47 | 5,68 | .001$^{***}$ | .289 |
| | | Session*Group | 3,47 | 2,34 | .079 | .143 |
| | | Group | 1,14 | 1,21 | .290 | .079 |
| | 20s | Session | 2,34 | 2,72 | .071 | .163 |
| | | Session*Group | 2,34 | 1,73 | .186 | .110 |
| | | Group | 1,14 | 0,01 | .914 | .001 |
| | 40s | Session | 2,23 | 0,80 | .437 | .054 |
| | | Session*Group | 2,23 | 1,38 | .268 | .090 |
| | | Group | 1,14 | 0,61 | .449 | .041 |
| | 58s | Session | 2,31 | 2,86 | .068 | .170 |
| | | Session*Group | 2,31 | 0,47 | .650 | .032 |
| | | Group | 1,14 | 0,34 | .857 | .002 |
| | 0s | Session | 2,23 | 1,94 | .171 | .122 |
| | | Session*Group | 2,23 | 1,13 | .332 | .074 |
| | | Group | 1,14 | 0,89 | .362 | .060 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Magazine Entries | 1s | Session | 3,45 | 8,70 | .000$^{***}$ | .383 |
| | | Session*Group | 3,45 | 0,37 | .784 | .026 |
| | | Group | 1,14 | 0,22 | .646 | .016 |
| | 3s | Session | 3,47 | 11,01 | .000$^{***}$ | .440 |
| | | Session*Group | 3,47 | 0,82 | .502 | .055 |
| | | Group | 1,14 | 1,34 | .720 | .009 |
| | 5s | Session | 4,61 | 31,26 | .000$^{***}$ | .691 |
| | | Session*Group | 4,61 | 1,41 | .239 | .091 |
| | | Group | 1,14 | 0,01 | .917 | .001 |
| | 10s | Session | 4,52 | 73,51 | .000$^{***}$ | .840 |
| | | Session*Group | 4,52 | 0,75 | .552 | .051 |
| | | Group | 1,14 | 0,24 | .629 | .017 |
| | 20s | Session | 4,63 | 149,69 | .000$^{***}$ | .914 |
| | | Session*Group | 4,63 | 1,56 | .189 | .101 |
| | | Group | 1,14 | 0,94 | .348 | .063 |
| | 40s | Session | 2,36 | 115,42 | .000$^{***}$ | .892 |
| | | Session*Group | 2,36 | 1,44 | .249 | .093 |
| | | Group | 1,14 | 0,03 | .874 | .002 |
| | 58s | Session | 2,29 | 250,95 | .000$^{***}$ | .947 |
| | | Session*Group | 2,29 | 1,03 | .370 | .069 |
| | | Group | 1,14 | 0,23 | .637 | .016 |
| | 0s | Session | 3,38 | 36,19 | .000$^{***}$ | .721 |
| | | Session*Group | 3,38 | 0,67 | .560 | .046 |
| | | Group | 1,14 | 0,87 | .367 | .058 |

Table 1. ANOVA results for licks, wheel turns and magazine entries in proportion to baseline responses. *$p<0.05$, **$p<0.01$ and ***$p<0.001$.

Figure 3 shows the average rates of food delivery (± SEM) throughout the experiment, depicting each delay value separately, as well as for the recovery condition (delay 0 s). As can be seen, food frequency did not change much with the introduction of delays of increasing length from 1 to 5 s, and even 10 s, but slightly decreased with the introduction of delays of 20 and 40 s, and furthermore with the use of the longest 58-s delay. The ANOVA displayed effects for Session under delay 10 s [$F(4,58) = 6.85$, $p<0.001$, $\eta^2=0.329$], 20 s [$F(4,56) = 17.16$, $p<0.001$, $\eta^2=0.551$], 40 s [$F(2,34) = 12.29$, $p<0.001$, $\eta^2=0.467$] and 58 s [$F(4,57) = 39.18$, $p<0.001$, $\eta^2=0.737$]. Post hoc comparisons showed differences between: BL and 1st-2nd ($p<0.05$) sessions, for delay 10 s; BL and 1st-2nd ($p<0.001$), 3rd-10th ($p<0.01$) sessions, for delay 20 s; BL and 2nd-10th ($p<0.001$) sessions, for delay 40 s; and BL and all sessions ( $p<0.001$), for delay 58 s. The signaled group showed a little lower value of food frequency than the unsignaled group under delays 3 s to 10 s; this was reversed for delays 20 s to 58 s. However, the ANOVA performed revealed significant differences between both groups only for delay 3 s [$F(1,14) = 6.07$, $p<0.05$, $\eta^2=0.303$]. All detailed results can be reviewed in Table 2.

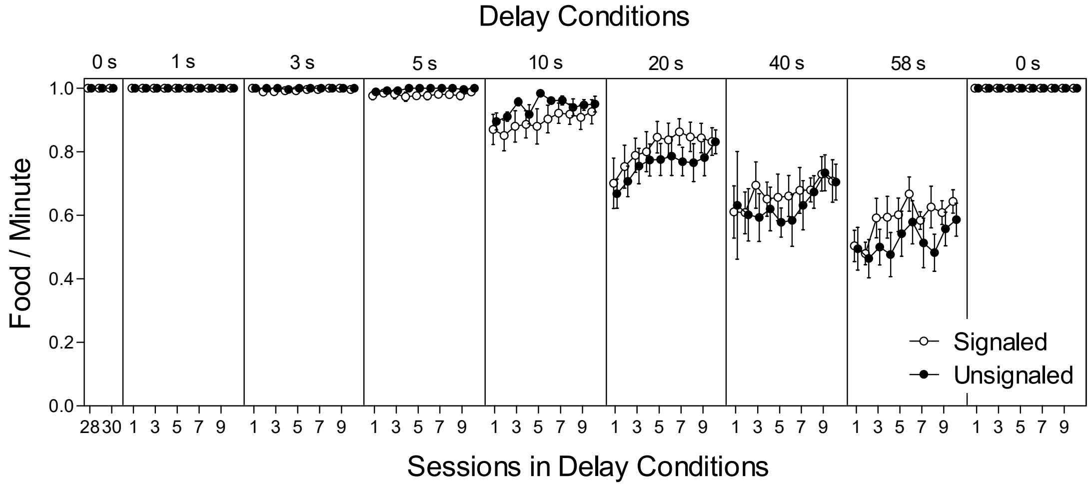

Figure 3. Mean (± SEM) of food delivery per minute for BL, each delay and recovery condition (delay 0 s) along each experimental session, and for each group (Signaled and Unsignaled).

| Delay | Factor | df | F | Sig. | $\eta^2$ |
|---|---|---|---|---|---|
| 1s | Session | 1,14 | 2,00 | .179 | .125 |
| | Session*Group | 1,14 | 0,00 | 1.000 | .000 |
| | Group | 1,14 | 0,00 | 1.000 | .000 |
| 3s | Session | 3,45 | 1,69 | .179 | .179 |
| | Session*Group | 3,45 | 1,30 | .287 | .085 |
| | Group | 1,14 | 6,07 | .027$^{*}$ | .303 |
| 5s | Session | 4,53 | 1,71 | .164 | .109 |
| | Session*Group | 4,53 | 1,33 | .271 | .087 |
| | Group | 1,14 | 2,74 | .120 | .164 |
| 10s | Session | 4,58 | 6,85 | .000$^{***}$ | .329 |
| | Session*Group | 4,58 | 1,41 | .241 | .091 |
| | Group | 1,14 | 1,26 | .281 | .082 |
| 20s | Session | 4,56 | 17,16 | .000$^{***}$ | .551 |
| | Session*Group | 4,56 | 0,65 | .628 | .044 |
| | Group | 1,14 | 0,52 | .482 | .036 |
| 40s | Session | 2,34 | 12,29 | .000$^{***}$ | .467 |
| | Session*Group | 2,34 | 0,40 | .714 | .028 |
| | Group | 1,14 | 0,14 | .716 | .010 |
| 58s | Session | 4,57 | 39,18 | .000$^{***}$ | .737 |
| | Session*Group | 4,57 | 1,00 | .417 | .066 |
| | Group | 1,14 | 0,99 | .337 | .066 |
| 0s | Session | 1,14 | 2,00 | .179 | .125 |
| | Session*Group | 1,14 | 0,00 | 1.000 | .000 |
| | Group | 1,14 | 0,00 | 1.000 | .000 |

Table 2. ANOVA results of reinforcement frequency. *$p<0.05$ and ***$p<0.001$.

Figure 4 shows mean (± SEM) rate of licks, wheel turns and magazine entries for each delay relative to baseline (see x-axis, second delay 0 s), averaged across the last three sessions of each delay value and of recovery condition (see x-axis, first delay 0 s), for each group (signaled and unsignaled). Response totals are expressed as proportion of the baseline value, thus 1.0 reflecting no change as indicated by the dotted horizontal line.

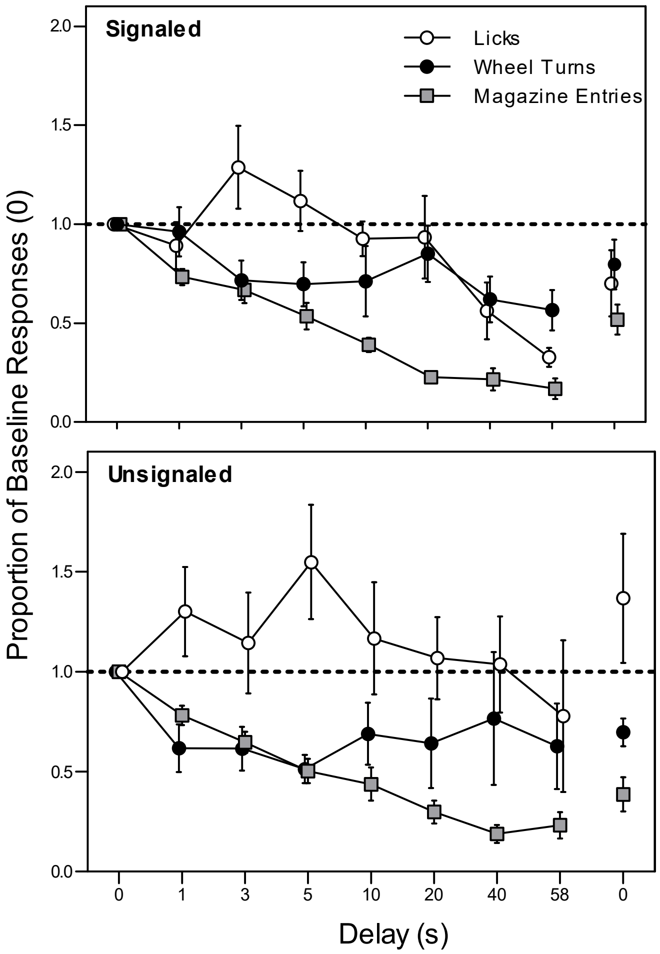

Figure 4. Mean (± SEM) rate of licks, wheel turns and magazine entries in proportion to baseline rate of responding for each delay and recovery condition (delay 0 s), averaged over the last three sessions of exposure to each delay, and for each group (Signaled and Unsignaled).

For the signaled group (upper panel), licks to bottle spout slightly increased with 3-s delays (1.29) and then began to decline as delay length was increased to reach a minimum level at the 58-s delay (0.33). The analysis yielded an effect of Delay [F(3,22) = 4.45, $p<0.05$, $\eta^2=0.388$]. Post hoc comparisons showed that delay 58 s produced lower response rate than BL and delay 10 s ($p<0.001$ and $p<0.05$, respectively). Wheel turns were sensitive to protective delays as short as 3 s (0.72), then the wheel running rate was stabilized for delays values up to 20 s, and finally continued decreasing slowly until reaching 0.56 at the 58-s delay. The analysis showed effect of Delay [F(3,24) = 3.09, $p<0.05$, $\eta^2=0.306$]. Magazine entries began to decline with delays as short as 1 s (0.73) and then decreased monotonically until the 20-s delay (0.23), reaching a minimum level of 0.17 at delays of 58 s. The analysis showed an effect of Delay [F(2,15) = 30.48, $p<0.001$, $\eta^2=0.813$]. Post hoc comparisons showed that: BL produced a higher response rate than all delays (delay 1 and 3 s, $p<0.05$; delay 5 s, $p<0.01$; delay 10 s to 58 s, $p<0.001$) and recovery condition ($p<0.05$); delay 1 s produced a higher response rate than delay 10 to 58 s ($p<0.01$ for delay 10 s, $p<0.001$ for delay 20 s and $p<0.05$ for 40 and 58 s); and delay 3 s more responses than delay 20 s ($p<0.05$).

For the unsignaled group (bottom panel), a similar pattern of results to those described for the signaled group were found. With regard to licks, response rates increased with delays between 1 and 5 s, and then decreased at the 58-s delay (0.78), growing again in the recovery condition. However, the analysis performed yielded no significant effect for Delay [F(3,18) = 1.23, $p=0.323$, $\eta^2=0.150$]. Concerning wheel running, again its rate was reduced at short delays (0.62 at 1 s) and then stabilized for the rest of the delay values, with some minor ups for some of the longest delays, reaching a final reduction of 0.63 for delay 58-s. A significant effect was not found for this behavior either [F(1,10) = 1.10, $p=0.348$, $\eta^2=0.136$]. Magazine entries began to decline with delays as short as 1 s (0.78) and continued declining progressively as delays were lengthened, reaching a response level of 0.23 with the 58-s delay. A main effect of Delay was found [F(3,19) = 43.41, $p<0.001$, $\eta^2=0.861$]. Post hoc comparisons showed that: BL produced a higher response rate than all delays (delay 3 to 10 s, $p<0.01$; delay 20 to 58 s $p<0.001$)and recovery condition ($p<0.01$), except for delay 1 s ($p=0.079$); delay 1 s produced a higher response rate than delay 3 to 58 s (delay 3 to 10 s, $p<0.05$; delay 20 to 58 s $p<0.001$); delay 3 s showed more responses than delay 20 to 58 s ($p<0.001$); delay 5 s showed a higher response rate than delay 20 and 40 s (*p*<0.01); and delay 20 s showed more responses than delay 40 s (*p*<0.05).

Figure 5 shows the temporal distribution of total licks, wheel turns and magazine entries for 1-s bins along the first 60 s of inter-food intervals, represented as the mean (± SEM) of the last three sessions of each delay value, including baseline (top panels) and recovery (bottom panels) phases at 0-s delays, for the signaled (panels to the left) and unsignaled (panels to the right) groups. The averaged differential location within the intervals of the three behaviors can be observed, and also, how the distributions were affected (from top to bottom) as response-food delays changed from 0 s to 1, 3, 5, 10, 20 or 40 s, and back to 0 s.

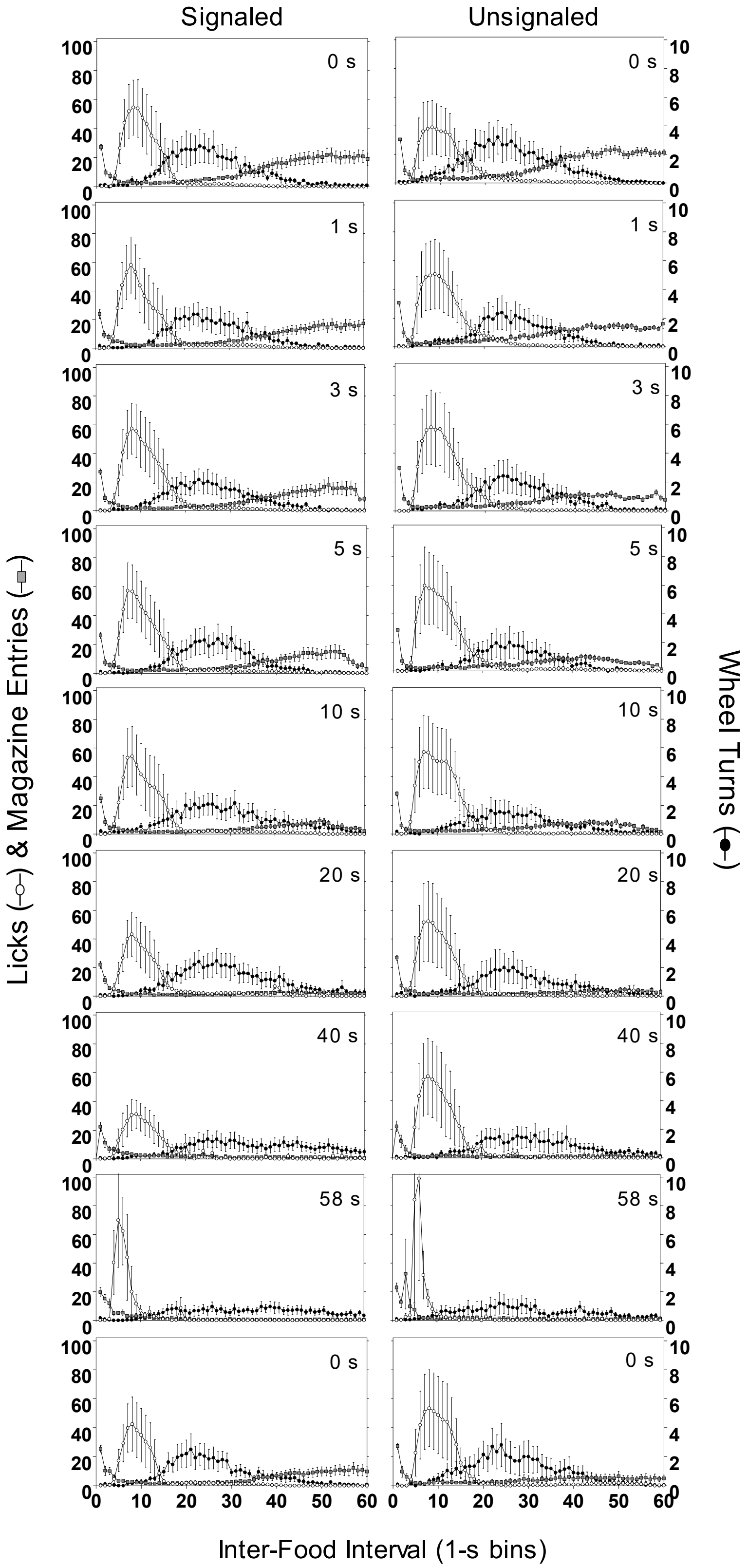

Figure 5. Mean (± SEM) licks, wheel turns and magazine entries given every second (bin) during the first 60 s of inter-food intervals for baseline (top panels), recovery (bottom panels) phase and each delay, for the signaled (panels to the left) and unsignaled (panels to the right) groups.

During baseline (acquisition phase), schedule-induced drinking showed the typical inverted U-shaped function relating licks to the inter-food interval, with maximum licking in the first half of the interval and a peak at 7-s bin. Wheel running showed a similar inverted U-shaped function but displaced to the middle part of the interval, peaking at 22-s bin. Magazine entries showed maximum responding at the beginning of inter-food intervals (0-s bin), reflecting the recollection of food pellets from the magazine as they were released, and then a steady accelerated descent as the interval progressed until a more or less sustained rate of responding, followed by a gradual recovery during the final part of the interval. Signaled and Unsignaled groups coincided at the response peak for all behaviors.

Regarding licks, note that the protective contingency of 20 and 40 s in signaled condition produced a reduction in responses. In addition, the distribution curve narrowed, concentrating most of the responses in the first 10-s bins under delay 58 s, in both groups. The distribution curves of wheel turns gradually decreased accompanying the increases in delay duration, and reaching flattening under delay 58-s, for both groups. Since the graphs collect the data of the first 60 s of the distribution, the increase in magazine entries towards the end of the interval cannot be appreciated. The increase in the duration of the delay extended the length of the interval, and therefore delayed the appearance of the increase in responses when food pellets were delivered.

In the recovery phase, distributions were not completely restored compared to the baseline phase, showing a lower response rate, except in the case of licks (condition not indicated), where there was an increase in the response rate.

## 4. Discussion

Magazine entries, wheel running turns, and licks to the bottle's spout were developed and maintained when rats were submitted to a FT 60-s food delivery schedule. When the three behaviors were established and sustained at steady state, protective response-food delays were introduced. Schedule-induced licking, wheel running and magazine entering were all affected by response-food delays programmed to prevent temporal contact between the behaviors and food delivery, and they did so in an exponential way as a function of delay length.

Magazine entries were affected with delays as short as 1 s and diminished gradually with increases in delay length, this would be consistent with its close relationship with the reinforcer. Wheel turns were affected by response-food delays, causing a decrease in the rate of wheel running from the shortest delay (1 s), but remaining relatively stable with the increase in the duration of delays. This effect has been found in previous studies (Frazier, 1970) and could be due to the intrinsic reinforcement of wheel running, as a consequence of the response-deprivation in the home cage (Pierce, Belke & Harris, 2018). Belke and Pierce (2015) observed that wheel running maintained a high rate during the extinction phase; The wheel running rate barely decreased 26% when water with 15% sucrose (reinforcer) was replaced for simply water. Attending to licks, short lick–food delays (up to 20 s) did not affect responding because licking rarely occurred during the last 20 s of the IFI. Food delays had to reach high values (40 and 58 s) to significantly affect licking. This reduction occurred earlier (40-s delay) in the signaled group (Figure 4). This is consistent with studies showing that signaled delays appear to be more effective than unsignaled ones in reducing schedule-induced drinking (Pellón & Blackman, 1987).

The present results of response-food delays on schedule-induced drinking confirm those obtained by Falk (1964) about the resistance of adjunctive drinking to reduce by contingent delays. Falk (1964) found that only the licks occurring in the last 15 s of average 60-s inter-food intervals postponed food. Our results show negligible effects of delays shorter than 58 s in a 60-s IFI, and only for the signaled group (see comparable results in Pellón and Pérez-Padilla, 2013, using a FT 30-s food schedule). Killeen (1975) found a similar resistance of pigeons' general activity to reduce by protective response–food delays up to 12 s. Altogether, our results can be explained because delay conditions were not in contact with licking until they reached their peak of responding. However, Pellón and Castilla (2000) found that lick-dependent delays as short as 3 or 6 s reduce drinking when the behavior was induced by short IFI (an FT 18-s schedule), but, in this case, any lick initiated a delay, not just the one occurring within the protective delay. All these results emphasize the importance of the lick-food contingency to effective affect schedule-induced drinking (for a more detail consideration of this point, see Pellón and Blackman, 1987).

The increase of the licks in short delays with respect to the baseline (Figure 4) could be explained through behavior competition, the reduction in the rate of wheel turns and magazine entries is accompanied by an increase in the rate of licks in both groups (signaled and unsignaled). Several studies have reported information on this interaction between wheel running and drinking (Riley, Peele, Richard & Kulkosky, 1981; Roper, 1978; Staddon, 1977; Staddon & Ayres, 1975; Wetherington & Riley, 1986). For example, Riley et al. (1981) found that access to wheel running produced a decrease in the rate of drinking, while the suppression of drinking by taste aversions increased wheel running. On the other hand, Segal (1969) noted that restricting wheel running increased drinking, while preventing drinking would not affect wheel running.

The reduction in the response rate should not be attributed to changes in food frequency delivery as it is not overly affected (Figure 3). These data support what was found in other studies on non-explicitly reinforced behaviors (Pellón & Pérez-Padilla, 2013; Lamas & Pellón, 1995). This particularly applies to short delays in which a reduction of response level is observed for magazine entries and wheel running turns, changes that were not accompanied by significant alterations in food frequency; for example, in the case of magazine entries this reduction occurs in delays as short as 1 s, where the food frequency is practically not altered. Also, the differential reduction of licks with 40-s delays between signaled and unsignaled groups was obtained under similar food frequency. Furthermore, a previous comparable study (Pellón, Íbias & Killeen, 2018) on the use of protective response-food contacts on licking or magazine entries employed appropriate controls to overcome the possibility that food rate per se could explain results such as the ones reported here. Even though the potential effect of food delivery rate was not directly controlled in the present experiment, it was done so in a previous work, and based upon those results and the previous literature cited above, we considered not necessary to duplicate such control here.

It has been largely discussed whether magazine entries, here characterized as a terminal activity, are controlled by Pavlovian and/or instrumental conditioning, with interpretations having been put forward that support in exclusivity one of those views (e.g., Harris, Andrew & Kwok, 2013; Gormezano & Kehoe, 1975). According to the operant interpretation, magazine entries are controlled by reinforcement because, in order to obtain the food pellets, animals have to get into the magazine, thus establishing the conditions for reinforcement of magazine entries. This will explain the increase of magazine entries as training progresses and its temporal distribution typical of other terminal activities such as lever pressing (see Figure 5; Pellón & Pérez-Padilla, 2013), and results such as those obtained in this experiment that show reductions of magazine entries by the imposition of delay contingencies (Schaal, Shahan, Kovera & Reilly, 1998).

If the delivery of food becomes marked by the presence of a conditioned stimulus (CS, as in a Pavlovian procedure), the CS might serve in effect as a discriminative stimulus for reinforcement of magazine entry responses. In this sense, Harris et al. (2013) found that magazine entries were suppressed compared to a yoked control when animals were exposed to omission training, where the execution of magazine entries during a stimulus-signal of food delivery suspended food delivery; thus defending that magazine entries are entirely Pavlovian, as these were acquired under a variable-duration CS with omission contingencies, without the intervention of delayed operant reinforcement during the CS.

Furthermore, Pellón and Killeen (2015) noted that the distribution of magazine entries late in the trials looks much like the distribution of lever pressing when that is the contingent response. In addition, if magazine entries are entirely pavlovian, they should show more resistance to instrumental contingencies such as delays of reinforcement (Gottlieb & Begej, 2014). The effect of response-food delays shows that the initial instrumental contingency is not fully overcome by the Pavlovian contingency, thus showing that magazine entries under intermittent food delivery are not solely maintained by Pavlovian conditioning but by instrumental contingencies as well (Pellón & Killeen, 2015). In this experiment, magazine entries were affected by delays as soon as 1s, and the pattern shown during the increase of delay time is similar than other operant behaviors such as lever pressing (see Pérez-Padilla & Pellón, 2013 for comparisons). We think that, perhaps, both mechanisms (Pavlovian and operant learning) are involved; We thus favor a complementary interpretation on the role of operant contingencies in maintaining magazine entries. It could very well be that, in the present experiment, magazine entering was first acquired via instrumental contingency as outlined above because of the necessity to get into the food cup to collect the pellets. The instrumental entering-food contingency can then occur with some training under Pavlovian control (as Harris and associates showed) but this does not imply that the initial instrumental contingency is removed, quite the contrary, as the present results seem to show.

Regarding the temporal distribution of behaviors within the IFI, magazine entries conform to the typical pattern of this behavior in FT schedules (Boakes, Patterson, Kendig & Harris, 2015), namely a peak of responding early in inter-food intervals that reflects the obtaining of the food pellets delivered into the magazine, a rapid retreat and latter acceleration and stabilization as it approaches the moment of the next food delivery. In the case of wheel running, the behavior is located in the middle of inter-food intervals, showing the characteristic pattern observed in previous studies (Reid, Bacha & Morán, 1993; Riley, Wetherington, Delamater, Peele & Dacanay, 1985; Roper, 1978; Staddon, 1977; Wetherington & Riley, 1986; see Chapter II, Experiment 2, of this Doctoral Thesis). Regarding licking, the rats mainly drank on the first part of the interval between successive meals, being this consistent with the characteristic temporal distribution reported in the literature (Boakes et al., 2015; Íbias & Pellón, 2011; Pellón & Pérez-Padilla, 2013; for a review, see Killeen & Pellón, 2013). Details of overall levels of responding and temporal distribution of the three behaviors can be seen in Figures 1 and 5, respectively.

This temporal pattern of behaviors within the IFI replicates the distribution found by Staddon (1977), where interim behavior (drinking) precedes non-induced behavior (facultative activity; wheel running). However, we have found, in previous studies (Gutiérrez-Ferre & Pellón, 2019), that wheel running would not be a facultative behavior, but an interim activity, by adopting an initial position in the IFI when no other adjunctive behavior is present. In fact, Segal in 1969 already proposed the hypothesis that wheel running was an induced behavior, because, in this study, it was found that the suppression of access to drink produced a temporal wheel running pattern similar to that of SIP. These results were already found by Roper (1978), pointing to a temporary competition between behaviors. Following Killeen and Pellón (2013), what would be observed here would be the proximity between events produced by the response-reinforcer contingency, with different delay-of-reinforcement gradients, that is, the behaviors would compete for their expression within the IFI, and they would adopt different positions within it depending on their particular association with the reinforcer. We could conclude, then, that the position occupied by wheel running within the IFI in this study is a consequence of presenting a shallower gradient, while drinking has a more pronounced gradient that displaced wheel running towards the middle of the interval.

In conclusion, differential sensitivities to delays were observed for the three behaviors, in correspondence with their temporal location within IFIs. This, according to Killeen and Pellón (2013), reflects the operation of the reinforcer at a different time distance for each behavior and on their relative strength for association; the behaviors traditionally classified as interim, facultative and terminal (Staddon, 1977) would be subject to the same operating principles but with different parameters that would explain their differential gradients. A principle of temporal proximity supported by the mechanism of delayed positive reinforcement might suffice to explain the apparent different types of behavior sustained under intermittent food reinforcement schedules. The action of delayed reinforcement, as dependent on specific behaviors (the different gradients) is ultimately responsible for the organization of behavior in time. This will account for the present and related findings from our laboratory. The present study extends previous investigations by looking at three different behaviors and by using both signaled and unsignaled delays, with the advantage over Pellón and Pérez-Padilla (2013) of looking at behaviors (wheel running or magazine entering) that do not require specific training (contrary to lever pressing) (see also Pellón, Íbias & Killeen, 2018) but keeping the simultaneous initiation of delays by any of the three behaviors (contrary to Pellón, Íbias & Killeen, 2018).

# CHAPTER IV

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## Schedule-induced behavior in sign- and goal-tracker rats is not related to level of impulsivity

## Abstract

Male Wistar rats, previously divided into goal- (GTs) and sign-trackers (STs), were exposed to intermittent food schedules to measure the development of schedule-induced polydipsia (SIP), and subsequently, to measure the development of schedule-induced wheel running (SIWR), using fixed time (FT) schedules of 15, 30, 60 and 120 seconds and, of 60 and 120 seconds, respectively; counterbalanced across animals according to a Latin square design, in both cases. Later, compulsivity levels of both groups were measured by an operant observing response task with uncertain reinforcement. Finally, impulsivity levels of both groups were measured by a delay discounting procedure with values of 5, 10, 20 and 40 seconds for the larger delayed reinforcer. Adjunctive drinking and wheel running were obtained in all FT schedules with a gradation as a function of inter-food interval (IFI) length, being GTs the ones showing the highest levels of drinking and wheel running compared with STs. However, differences were not found between GTs and STs in the compulsivity levels or in delay discounting. These results suggest that schedule-induced behaviors are not related to impulsivity traits as previously thought; as well as that SIP and SIWR behave as operant behaviors, being these behaviors directed towards the goal.

*Keywords:* Schedule-induced behaviors; Sign trackers; Goal trackers; Delay discounting; Impulsivity; Rats.

## 1. Introduction

Falk (1961) found that food-deprived rats exposed to an intermittent reinforcement schedule developed an adjunctive behavior of excessive drinking, when animals had a water bottle in the conditioning chamber (Falk, 1971). This phenomenon was called schedule-induced polydipsia (SIP). Adjunctive behaviors are thought to occur by the intermittent occurrence of a reward commodity rather than by its direct action, and all of them are displayed in excess and with questionable functionality. The main controversy lies in whether adjunctive behavior corresponds to respondent or operant conditioning, as experimental evidence partially supports both interpretations (Wetherington, 1982).

The discovery of SIP led to the investigation of another series of schedule-induced behaviors that manifest themselves in excess without being directly controlled by the reinforcement schedule, such as aggression (Looney & Cohen, 1982) or wheel running (Levitsky & Collier, 1968). These schedule-induced behaviors were classified by Staddon (1977) in terminal responses and interim activities in relation to the probability of presentation of the reinforcer. Terminal responses occur around the presentation of the enhancer, therefore, in the presence of predictive stimuli; being located at the end of inter-food Interval (IFI). Interim activities occur at times when a reinforcer is unlikely to be delivered, in the post-reinforcement period, located at the beginning of the IFI (see also Staddon & Simmelhag, 1971).

The excessiveness of drinking has converted SIP in an animal model of psychopathological disorders related to impulse control (impulsivity-compulsivity). Certain studies have used SIP as a procedure to distinguish between low- (LD) and high-drinkers (HD), considering HD as a phenotype of impulsive/compulsive behavior, finding differences between these groups at the pharmacological and neuronal level (López-Grancha, López-Crespo, Sánchez-Amate & Flores, 2006; López-Grancha, López-Crespo, Sánchez-Amate, & Flores, 2008; Moreno et al., 2012; Pellón et al., 2011), as well as in the delay discounting task (Cardona, López-Crespo, Sánchez-Amate, Flores & Sánchez-Santed, 2011; Cardona et al., 2006) (for more details, see Flores et al., 2014; Moreno & Flores, 2012). Similarly, studies with rat strains that are characterized by a high behavior rate have related SIP with impulsivity (SHR rats: Íbias & Pellón, 2011, 2014; Roman high-avoidance rats: Moreno et al., 2010).

On the other hand, the Pavlovian Conditioned Approach (PCA), specifically autoshaping, is being proposed as an animal model in the study of impulse control disorders (López, Karlsson, & O'Donnell, 2015; Vargas, Díaz, Portavella & López, 2016), through the evaluation of individual differences in processes of association of signals and reinforcers. The autoshaping procedure allows animals to be classified into two populations, sign- (STs) and goal-trackers (GTs), with different behavioral profiles. STs are associated with sign tracking, they are related to a low impulse control, or a reduced inhibitory capacity once the conditioned stimulus is presented, while, GTs are associated with the pursuit of objectives, approaching the location of the reinforcer (Flagel, Watson, Robinson & Akil, 2007; Robinson & Flagel, 2009).

Impulsivity can be defined as the tendency to respond or take decisions prematurely or riskily and may become non-adaptive due to its potential negative consequences. The concept of impulsivity is not unitary and encompasses a wide range of behaviors that range from motor disinhibition, which could be called “motor impulsivity”, to problems in decision making, which could be referred to as “cognitive impulsivity” (Evenden, 1999). Therefore, in order to accept or reject the hypothesis of ST rats as a possible impulsive phenotype, rats previously selected as sign- and goal-trackers were exposed to tasks that measure impulsiveness of different kinds. Initially, the acquisition levels of schedule-induced polydipsia and schedule-induced wheel running were compared between both groups, as a motor impulsivity measure. Subsequently, an adapted task of model of compulsive checking was used as a compulsivity measure. And finally, the animals were exposed to the delay discounting task, as a cognitive impulsivity measure.

## 2. Experiment 1: Schedule-induced Polydipsia

Recently, the GT and ST rat model has pointed out individual differences between these two groups related to impulsivity. Lovic, Saunders, Yager and Robinson (2011) used different tasks to measure impulsivity and found that STs were more impulsive than GTs; STs performed more premature responses on a 2-choice serial reaction time task and were less efficient on a differential reinforcement of low rates task than GTs.

Schedule-induced polydipsia has been proposed as an animal model of impulsivity and compulsivity, represented by an excessive drinking rate, so the purpose of this experiment was to find differences in the drinking rate between ST and GT groups. STs were expected to display higher overall levels of drinking than GTs, and GTs were expected to make more magazine entries.

### 2.1. Method

#### *2.1.1. Subjects*

Twenty male Wistar rats (16-week-old) previously selected through Pavlovian Conditioning training as sign- (10 rats) or goal-trackers (10 rats) by the University of Seville laboratory (Sevilla, Spain) (for more details on this procedure see López et al., 2015) were received and housed in the UNED (National Distance Education University) laboratory in groups of four. The room had a 12-h light-dark cycle (lights on from 08:00 to 20:00 h) and the temperature (21 °C) and humidity (65%) conditions were controlled. After 1 week of habituation to the new laboratory, rats were housed singly, and two weeks later, rats were gradually reduced to 80-85% of their free-feeding body weight and then maintained at this level of food deprivation throughout the experiment. Every day the animals were weighed before experimental sessions; and supplementary food was supplied to them approximately thirty minutes after the end of experimental sessions. At the start of the first experiment, the rats were in their 21st week of life and had the following mean body weight: ST, 341.4 g (range: 277-398 g); and GT, 315.5 g (range: 270-383 g). Water continued to be freely available in their home cages throughout the study. All procedures were in accordance with the Spanish Royal Decree 53/2013 regarding the protection of experimental animals and with the European Union Council Directive 2010/63 and were approved by the Bioethics Committee of Universidad Nacional de Educación a Distancia.

### *2.1.2. Apparatus*

*Conditioning Chambers*. Eight Letica LI-836 conditioning chambers (Cibertec Inc., Madrid, Spain) of 29 × 24.5 × 35.5 cm were used. Chambers consisted in an aluminum front panel, the left wall of transparent Plexiglas, the remaining walls of black Plexiglas and stainless steel grid floor; the food tray was situated behind the front panel to supply 45-mg of standard rat food *pellets* (Bio-Serv, Frenchtown, NJ, USA) in a centered aperture in the chambers' front wall at 3.7 cm from the floor; and a bottle with 100 ml of fresh tap water was placed on the exterior of chamber right-hand wall, with a spout to which the animal could access from inside the chamber through a 3.2 × 3.9 cm aperture in the wall, situated 20 cm from the front panel and 7 cm from the floor. Each chamber was enclosed in soundproofed housing. A window at the front allowed to observe the interior, and a small fan renewed the air and functioned as masking noise (ambient noise 60 dB). The chambers were lit by a pair of lights 3-W located at both upper sides of the front panel, and an indirect 25-W light fitted to the interior of the soundproof housing.

Magazine entries were recorded by a photo-beam system with photocells situated at both sides of the magazines' entrance. And the licks were recorded when the electric circuit between the floor metal grid and the bottle spout closed, after contact of animal's tongue and the metal spout. Med PC IV® software was used for scheduling and recording of the experimental events. This software was also used in the following experiments.

### *2.1.3. Procedure*

After the stabilization of the animals' weight according to the criterion-based range, the animals received an adaptation session in the conditioning chambers. Ventilation and illumination were present, as well as 30 food pellets being previously deposited all together in the food magazine; however, no experimental contingency was operational; Water bottles were not placed. The session lasted 20 min.

On the following day to the adaptation session, the animals were exposed to the experimental procedure. Four fixed time (FT) schedules of different lengths (FT 15-, 30-, 60- and 120-s) were used, each food pellet was dispensed according to these regular intervals regardless of the rats 'behavior. All animals were exposed to all FT schedules, the order of presentation was established by pairs of rats using a Latin square design. The first FT schedule lasted 20 sessions, while the remaining schedules required a total of 15 sessions due to the stability observed in behavior. Sessions were run daily, with a duration of 30 minutes per session, with a rest interval of two days between successive FT schedules. Water bottles were provided immediately before each session, and removed at the end of the session, to calculate the total of milliliters consumed as the difference between these two moments.

For each rat and session, the total number of licks, the total amount of water (milliliters) removed from the bottle, and the total number of magazine entries, along with the number of licks and magazine entries given at each inter-food interval and every 2-s in each interval were recorded.

### *2.1.4. Statistical analysis*

Behavioral data on schedule-induced polydipsia development were analyzed using a two-way repeated measures analysis of variance (ANOVA), with one between-subject factor named Group (ST vs. GT) and one repeated within-subjects factor named FT schedule (four levels: FT 15-, 30-, 60- and 120-s).

The temporal distribution of each behavior was analyzed individually by a two-way ANOVA, with Group (ST vs. GT) as the between-subject factor, and Bin (levels: 8 under FT 15-s, 15 under FT 30-s, 30 under FT 60-s, and 60 under FT 120-s) as the within-subject factor.

Statistical analyses were performed using the data on the respective subject's last three sessions under each FT schedule.

Pairwise comparisons were used for post hoc comparisons with a Bonferroni correction for $p$ values. The minimum level of statistical significance was $p<0.05$. Effect size was estimated by $\eta^2$. All analyses were computed using the SPSS 25 software package. These same criteria were considered for the remaining experiments.

## 2.2. Results

Figure 1 shows the mean (± Standard Error of the Mean - SEM) number of licks, mL consumed and magazine entries per minute, as well as the mean (± SEM) number of licks, mL consumed and magazine entries per food pellet, given by both groups (ST and GT) under the different FT schedules, taking the average of the last three sessions for each FT schedule.

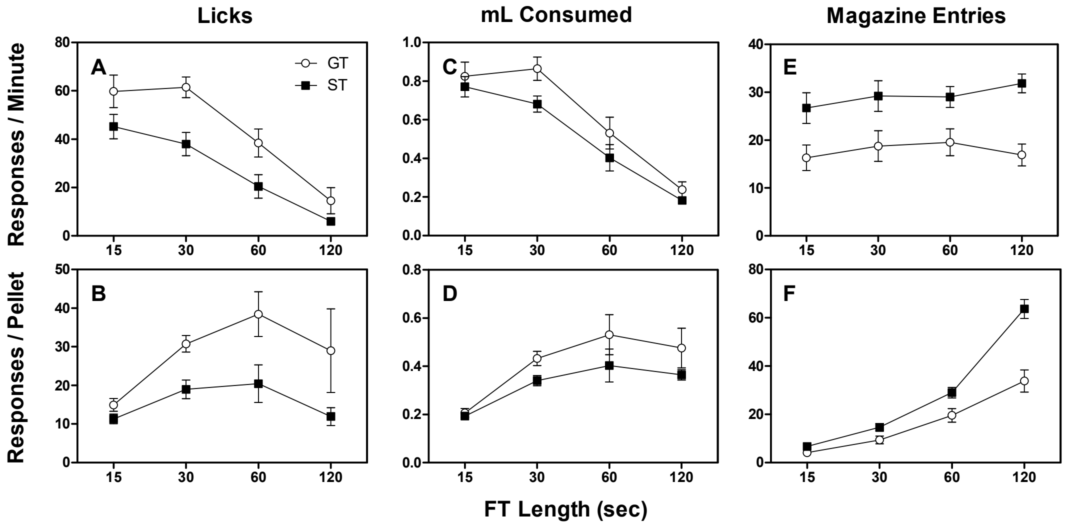

Figure 1. Mean (± SEM) number of licks per minute (A), licks per food pellet (B), mL consumed per minute (C), mL consumed per food pellet (D), magazine entries per minute (E) and magazine entries per food pellet (F) under each FT schedule.

Figure 1A compares licks per minute under the four FT schedules. The analysis performed yielded a main effect for FT schedules [$F(3,54) = 78.27$, $p<0.001$, $\eta^2=0.813$], with a reduction in response rate as IFI length increased, with FT 15-s and FT 30-s presenting greater response per minute rate than the remaining schedules ($p<0.001$), and FT 60-s than FT 120-s ($p<0.001$); and for Group [$F(1,18) = 7.40$, $p<0.05$, $\eta^2=0.291$], with the ST Group registering a lower mean value than the GT Group ($p<0.05$).

Figure 1B depicts licks per food pellet under the four FT schedules. Effects were observed for FT schedules [*F*(2,30) = 6.25, $p<0.01$, $\eta^2=0.258$], with FT 15-s proving lower than FT 30-s and FT 60-s ($p<0.001$ in both cases); and for Group [*F*(1,18) = 6.24, $p<0.05$, $\eta^2=0.258$], with the ST Group registering a lower mean value than the GT Group ($p<0.05$), as in licks per minute.

Figure 1C shows the mean number mL consumed per minute under the four FT schedules. An FT schedules effect was found [*F*(3,54) = 97.29, $p<0.001$, $\eta^2=0.844$], with progressive reductions in response rates accompanying increases in IFI length, with FT 15-s and FT 30-s showing a higher rate of response per minute than the remaining schedules ($p<0.001$), and FT 60-s than FT 120-s ($p=0.001$), coinciding with the results found for licks per minute.

Figure 1D depicts mL consumed per food pellet under the four FT schedules. Effects were observed for FT schedules [*F*(2,33) = 14.77, $p=0.001$, $\eta^2=0.451$], with FT 15-s presenting lower mL consumed per minute rate than the remaining schedules ($p<0.001$).

Figure 1E compares magazine entries per minute under the four FT schedules. The analysis performed showed effects for Group [*F*(1,18) = 12.19, $p<0.01$, $\eta^2=0.404$], with the ST Group registering a higher mean value than the GT Group ($p<0.01$).

Figure 1F shows the mean number of magazine entries per pellet under the four FT schedules. The ANOVA displayed effects: for FT schedules [*F*(1,25) = 180.53, $p<0.001$, $\eta^2=0.909$], with an increase in the number of responses as IFI length increased ($p<0.001$ in all cases); for Group [*F*(1,9) = 12.98, $p<0.01$, $\eta^2=0.892$], with the ST Group registering a higher mean value than the GT Group ($p<0.001$); and for the FT schedules X Group interaction [*F*(1,25) = 18.97, $p<0.001$, $\eta^2=0.513$]. Post hoc analyses revealed differences in all FT schedules, with higher means for the ST group versus the GT group (*p*<0.05 for FT 15-, 30- and 60-s; and *p*<0.001 for FT 120-s).

Figure 2 depicts the mean (± SEM) of total licks and total magazine entries given every two-seconds (bins) during the IFI for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length), averaged over the last three sessions of exposure to each schedule.

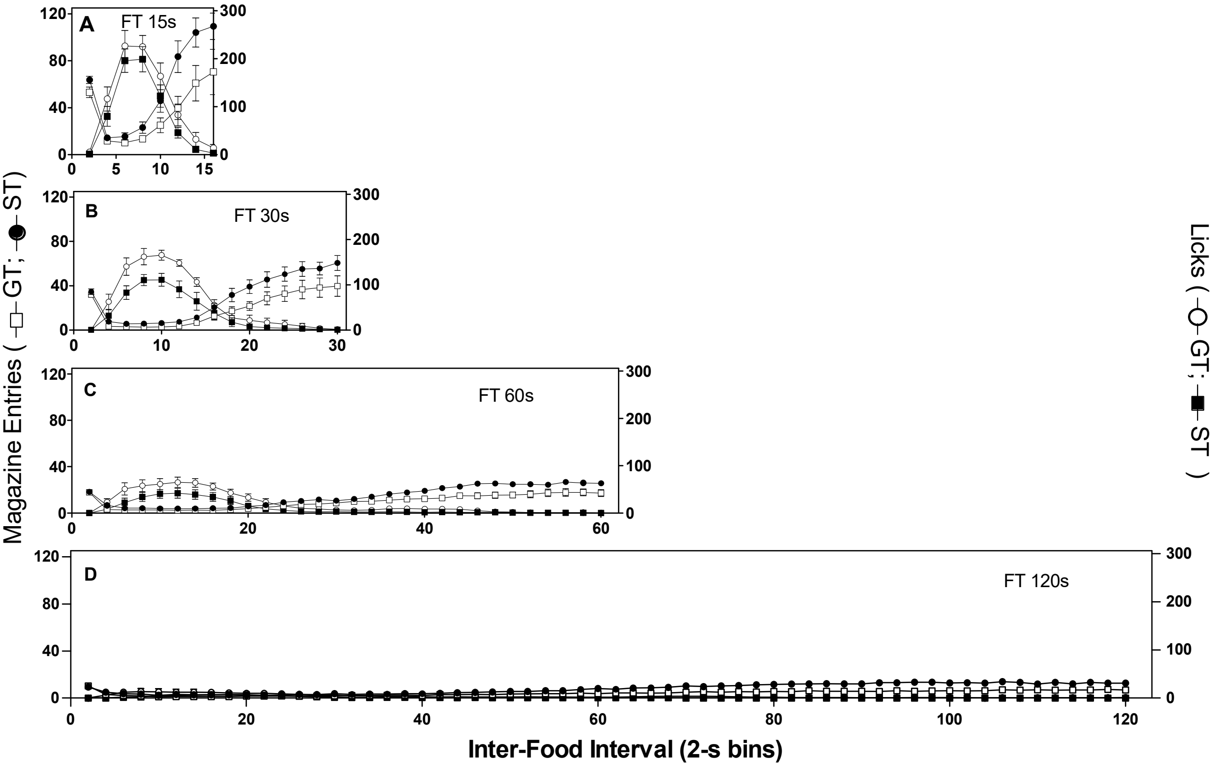

Figure 2. Mean (± SEM) licks and magazine entries given every two-seconds (bins) during the inter-food intervals for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length).

The distribution found for licks is equated to the inverted U-shaped curve along the IFI usually observed in this behavior. The drinking curve shows a tendency to flatten and move to the left, that is, the curve adopts an initial position within the IFI as its length increases. The highest response rate was located at the same time for GT and ST group approximately (bins: 6 and 8 for FT 15-s, bin: 10 for FT 30-s, bin: 12 for FT 60-s, and bins: 8 and 18 for FT 120-s, respectively). In the figure 2D, the program FT 120-s does not apparently develop drinking; this is due to the equity of the axes.

Magazine entries present their typical distribution for this type of studies, highest number of responses at the beginning of the IFI (2-s bin) (FT 60- and FT 120-s for GT group), and towards the end of the IFI (FT 15-s: bin 15 and FT 30-s: bin 30, for both groups; FT 60-s: bin 56, and FT 120-s: bin 106 for ST group), both moments related to the recollection of food pellets from the magazine as they were released; the first peak is followed by a steady accelerated descent as the interval progresses, until a more or less sustained rate of responding up to approximately half of the IFIs, moment when the magazine entries increase steadily again until the end of the interval (second peak of responses).

Figure 2A shows the mean number of licks and magazine entries given every two seconds during the IFI under the FT 15-s schedule. The ANOVA displayed effects: in licks, for Bin [$F(2,38) = 46.62$, $p<0.001$, $\eta^2=0.721$], with the highest number of licks concentrated in 4 to 12 bins segment ($p<0.001$); and in magazine entries, for Group [$F(1,18) = 6.27$, $p<0.05$, $\eta^2=0.258$], with the GT group registering a lower mean value of entering rate than the ST group; and for Bin [$F(2,31) = 35.30$, $p<0.001$, $\eta^2=0.662$], with the smaller number of magazine entries from 4 to 10 bins ($p<0.05$).

Figure 2B depicts the mean number of licks and magazine entries given every two seconds during the IFI under the FT 30-s schedule. The analysis performed showed effects: in licks, for Group [$F(1,18) = 12.60$, $p<0.01$, $\eta^2=0.412$], with the GT group giving more licks than the ST group; and for Bin [$F(2,40) = 52.38$, $p<0.001$, $\eta^2=0.744$], with the highest number of licks given between 4 and 16 bins ($p<0.05$); and in magazine entries, for Group [$F(1,18) = 5.34$, $p<0.05$, $\eta^2=0.227$], with the GT group presenting fewer magazine entries than the ST group; and for Bin [$F(2,31) = 47.09$, $p<0.001$, $\eta^2=0.723$], with the smaller number of magazine entries given between 4 and 16 bins ($p<0.01$).

Figure 2C shows the mean number of licks and magazine entries given over the course of the IFI under the FT 60-s schedule. The analysis yielded effects: in licks, for Group [$F(1,18) = 6.03$, $p<0.05$, $\eta^2=0.251$], with the GT group registering highest number of licks than the ST group; and for Bin [$F(2,35) = 24.82$, $p<0.001$, $\eta^2=0.580$], with the highest licks rate concentrated in segment 6 to 20 bins ($p<0.05$); in magazine entries, for Group [$F(1,18) = 7.17$, $p<0.05$, $\eta^2=0.285$], with the GT group yielding less magazine entries than the ST group; for Bin [$F(3,46) = 58.34$, $p<0.001$, $\eta^2=0.764$], with the lowest number of magazine entries reported in the 4 to 30 bins segment ($p<0.05$); and for Group x Bin interaction [$F(3,46) = 3.00$, $p<0.05$, $\eta^2=0.143$]. Post hoc analyses revealed differences in 36-44 and 52-60 segments ($p<0.05$), and in 46-50 segment ($p<0.01$), with higher means for the ST group regarding the GT group.

Figure 2D depicts the mean number of licks and magazine entries given over the course of the IFI under the FT 120-s schedule. The analysis only found effects in magazine entries, for Group [$F(1,18) = 23.64$, $p<0.001$, $\eta^2=0.568$], with the GT group yielding less magazine entries than the ST group; for Bin [$F(4,78) = 45.51$, $p<0.001$, $\eta^2=0.717$], with the smaller number of magazine entries distributed in the 4-56 bins segment ($p<0.01$); and for Group x Bin interaction [$F(4,78) = 6.94$, $p<0.001$, $\eta^2=0.278$]. Post hoc analyses showed differences from 38 to 62 bins ($p<0.01$ and $p<0.05$ are interspersed between the different bins), and from 64 to 120 bins ($p<0.001$), with higher means for the ST group versus the GT group.

## 3. Experiment 2: Schedule-induced wheel running

Like SIP, schedule-induced wheel running (SIWR) has been considered an adjunctive behavior; presenting the same characteristics observed in SIP (Gutiérrez-Ferre & Pellón, 2019). Therefore, we decided to study whether the results obtained in SIP were replicated in SIWR.

### 3.1. Method

#### *3.1.1. Subjects*

The same animals as for Experiment 1 were used in this procedure. And the same housing conditions were maintained.

#### *3.1.2. Apparatus*

The same conditioning chambers were used as in Experiment 1, with the difference that the water bottles were removed and a wheel of stainless steel (32 cm in diameter and 9.5 cm wide with spokes distributed at 1 cm intervals around the rim) was fitted on the exterior of the back panel of each chamber. A 10 cm in diameter circular aperture in the wall, situated 28 cm from the front panel and 1 cm from the floor, allowed the animal to access from the interior of the chamber. Each entire revolution of each wheel was registered with an AZ fag magnetic reed switch.

#### *3.1.3. Procedure*

The animals were exposed to two FT schedules of different lengths (FT 60- and 120-s). Animals had free access to the running wheel throughout the duration of the session. Just like in the SIP procedure, sessions were run daily, with a rest interval of two days between successive FT schedules; and all animals underwent all FT schedules, the order of which was established according to a fully randomized Latin square design. While exposure to the first FT schedule lasted 20 sessions, the second FT schedule was held over 15 sessions. However, for this procedure the total number of reinforces remained stable (30 pellets), the time of the sessions depending on the FT schedule being variable (30 and 60 min respectively).

The measures recorded were: the total number of wheel turns and the total number of magazine entries, along with the number of responses of both behaviors given at each IFI and every 2-s in each interval, for each rat and each session.

### *3.1.4. Statistical analysis*

SIWR development and the temporary distribution of each behavior within of each IFI were analyzed using a two-way ANOVA, with Group (ST vs. GT) as the between-subject factor, in both cases, and FT schedules (FT 60- and FT 120-s) and Bin (levels: 30 under FT 60-s, and 60 under FT 120-s) as the within-subject factor, respectively.

The data on the respective subject's last three sessions under each FT schedule were used for all statistical analyzes.

## 3.2. Results

Figure 3 shows the mean (± SEM) number of wheel turns and magazine entries per minute, as well as the mean (± SEM) number of wheel turns and magazine entries per food pellet, given by each group under two FT schedules (FT 60-s and FT 120-s) taking the average of the last three sessions of each FT schedule.

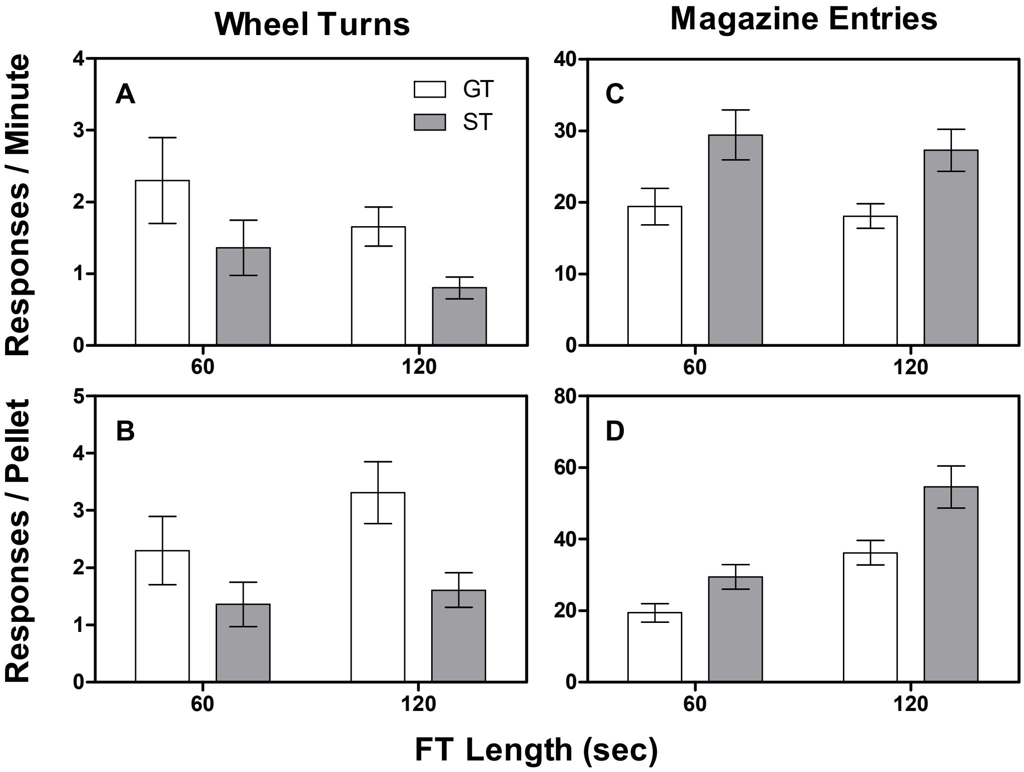

Figure 3. Mean (± SEM) number of wheel turns (A) and magazine entries (C) per minute under each FT schedule; and mean (± SEM) number of wheel turns (B) and magazine entries (D) per food pellet under each FT schedule.

Figure 3A compares wheel turns per minute under the two FT schedules. The analysis performed showed no effects; however, there was a tendency for FT Schedule [$F(1,18) = 4.22$, $p=0.05$, $\eta^2=0.190$], with the reduction of the response rate before the increase of the length of the interval; and for Group [$F(1,18) = 3.71$, $p=0.07$, $\eta^2=0.171$], with the ST group registering a lower mean value of running rate than the GT group.

Figure 3B shows the mean number of wheel turns per food pellet under the two FT schedules. Effects were observed for Group [$F(1,18) = 4.94$, $p<0.05$, $\eta^2=0.215$], with the GT group presenting a higher mean value than the ST group; and a trend was observed for the FT schedule [$F(1,18) = 4.28$, $p=0.05$, $\eta^2=0.192$].

Figure 3C depicts magazine entries per minute under the two FT schedules. The ANOVA displayed effects for Group [$F(1,18) = 7.42$, $p<0.05$, $\eta^2=0.292$], with higher means for the ST group versus the GT group.

Figure 3D compares magazine entries per food pellet under the two FT schedules. The ANOVA showed effects: for FT schedule [$F(1,18) = 67.18$, $p<0.001$, $\eta^2=0.789$], with an increase in the number of responses for FT 120-s regarding FT 60-s schedule; and for Group [$F(1,18) = 7.75$, $p<0.05$, $\eta^2=0.301$], with the GT group registering a lower mean value of entries rate than the ST group.

Figure 4 shows the mean (± SEM) of total wheel turns and total magazine entries given every two-seconds (bins) during the inter-food intervals for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length), averaged over the last three sessions of exposure to each schedule.

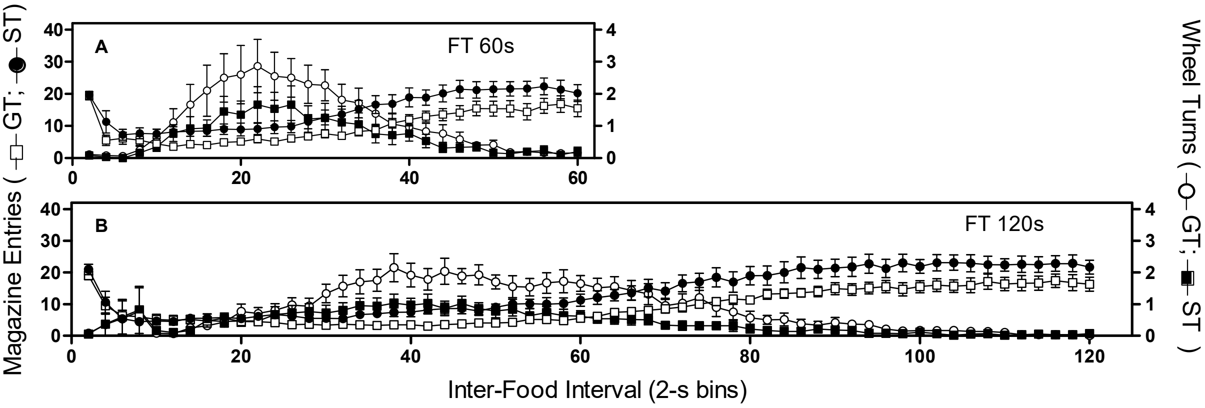

Figure 4. Mean (± SEM) wheel turns and magazine entries given every two-seconds (bins) during the inter-food intervals for each FT schedule (represented in separate panels from top to bottom as a function of increasing FT length).

The distribution of the magazine entries in SIWR was very similar to that found in the SIP, with a higher response rate at the beginning of the IFI (2-s bin) for the GT group in both schedules, and at the end of the IFI (FT 60-s: bin 56, and FT 120-s: bin 104) for the ST group, followed by an accelerated decrease and a constant and gradual recovery towards the middle of the interval.

Wheel turns were distributed in the form of an inverted U-shape along the IFI. Running was mainly concentrated in the 2/4 of the IFI in both groups. Peaking at bins 22 in FT 60-s and 38 in FT 120-s, coinciding the two groups. Rats barely ran over the end, 4/4, of the IFI.

Figure 4A depicts the mean number of wheel turns and magazine entries given every two seconds during the IFI under the FT 60-s schedule. The analysis performed showed effects: in wheel turns, for Bin [$F(2,37) = 13.28$, $p<0.001$, $\eta^2=0.425$], with the lowest number of wheel turns reported at the beginning (2 to 6 bins segment) and the end (42 to 60 bins segment) of the IFI ($p<0.05$); and in magazine entries, for Group [$F(1,18) = 5.27$, $p<0.05$, $\eta^2=0.226$], with the GT group presenting fewer magazine entries than the ST group; and for Bin [$F(4,66) = 29.91$, $p<0.001$, $\eta^2=0.624$], with the lowest number of magazine entries reported in the 4 to 32 bins segment ($p<0.001$).

Figure 4B shows the mean number of wheel turns and magazine entries given over the course of the IFI under the FT 120-s schedule. The analysis yielded effects: in wheel turns, for Group [$F(1,18) = 6.79$, $p<0.05$, $\eta^2=0.274$], with the GT group yielding more wheel turns than the ST group; and for Bin [$F(4,67) = 11.41$, $p<0.001$, $\eta^2=0.388$], with the highest number of wheel turns concentrated in 34 to 60 bins segment ($p<0.05$); in magazine entries, for Group [$F(1,18) = 7.42$, $p<0.05$, $\eta^2=0.292$], with the GT group yielding less magazine entries than the ST group; and for Bin [$F(4,67) = 37.13$, $p<0.001$, $\eta^2=0.674$], with the highest number of magazine entries reported at the beginning (2 and 4 bins) and the end (68 to 120 bins segment) of the IFI ($p<0.001$).

# 4. Experiment 3: Operant observing response task with uncertain reinforcement

The individual differences found in the SIP task have also been related to compulsive behavior (Moreno et al., 2012), understood as the perseverance and repetitive execution of an act. Although our main objective was the impulsiveness characteristic, we applied the following task to inquire whether the differences found in SIP and SIWR could be due to a question of compulsivity and, therefore, whether the GT-ST model is more aimed at this characteristic and not so much to impulsivity.

## 4.1. Method

### *4.1.1. Subjects*

The animals and the housing conditions of previous experiments were maintained.

### *4.1.2. Apparatus*

The same conditioning chambers were used as in previous experiments, but neither the water bottle nor the wheel were present. In this case, two levers were inserted at a distance of 4.8 cm from either side of the feeder, at a height of 4.7 cm from the grid floor. The levers were equipped with a retraction system which, on being deactivated, enabled the animal to respond. Lever pressure required a force of approximately 0.5 N. Further, the presence of a speaker at the top of the front wall of each chamber produced sound signals when necessary.

### *4.1.3. Procedure*

This observing response task is an adaptation of the Eagle's observing response task, as operant model of compulsive checking (Eagle et al., 2014).

#### *4.1.3.1. Training 1: Lever acquisition*

Animals were trained to lever-press for food pellets. The left lever was available and active, as well as the light box, while light situated above the left lever and a tone of approximately 60 dB (25Hz) were presented intermittently throughout the session. The observing lever (right lever) remained retracted. Animals were reinforced with a food pellet on an FR1 (fixed ratio schedule) (until reaching 33 food pellets) followed by a FR3 (until reaching 33 food pellets) and, finally with a VR5-15 (variable ratio schedule) (until reaching 34 pellets) schedule. Each session was ended after 20 min or after the delivery of 100 rewards, whichever happened first. Animals received one session per day. The rats had to get the maximum of rewards (100 food pellets) to access the next training phase. Most of the animals reached the goal in the second session (4 sessions were required by an animal and 6 sessions by another two rats).

#### *4.1.3.2. Training 2: Discrimination training*

Animals were trained to discriminate between reinforced and extinction condition. Each session started with a reinforcement trial followed by an extinction trial. The left lever was always available. The light box, the light situated above the left lever and the tone were intermittently displayed for reinforcement trials and statically for extinction trials. The observing lever remained retracted. Each reinforcement / extinction condition switched on an FT 60-s schedule. Animals were reinforced with a food pellet on a VR10-20 schedule. The delivery of the reward started a new extinction trial automatically. The measures recorded were: lever presses in VR10-20 (LP-VR) and lever presses in extinction condition (LP-Ext). Each session was ended after 20 min or after the delivery of 100 rewards, whichever happened first. Animals received one session per day.

Rats had to obtain a Discrimination Index greater than or equal to 0.65 to access the next phase. Therefore, the number of sessions required to reach the criterion varied in each animal (1-21 sessions, although all the animals were exposed to training a minimum of 6 sessions to ensure the stability of the data).

#### *4.1.3.3. The observing response task*

Rats were exposed to a variable rate schedule (15-s) trials with alternate extinction trials every 60 sec. One lever delivered the reinforcing and the other provided a sound and visual stimulus indicating the type of essay in progress.

The session started with the two levers available and active, as well as the light above the observing lever press (right lever) lit. As in training 2, the session began with a reinforcement trial followed by an extinction trial (FT 60-s schedule, and VR10-20 schedule for reinforced condition). On this occasion, pressing the observing lever press caused the signaling (light box, light situated above the left lever and tone) with the parameters of the current condition for 15 sec. In each trial, further observing lever presses had no consequence but were recorded as extra observing lever presses (EOLPs). The completion of each trial caused the reset of the stimuli. Animals were exposed to 10 sessions in total. The session ended after 20 min or 100 reward pellets, whichever was sooner.

#### *4.1.3.4. Measures recorded*

- Lever presses (LPs): Responses on left lever. There were two categories: presses on active lever gave access to food pellet according a VR10-20 schedule (LPs-VR) and presses on inactive lever (extinction condition) had no consequence (LPs-Ext).

- Observing lever presses (OLPs): Responses on the observing lever (right lever) that turned on discriminative stimuli. Two categories: presses on observing lever activated intermittent signaling for reinforcement condition (OLPs-VR) and presses on observing lever activated fixed signaling for extinction condition (OLPs-Ext).
- Extra observing lever presses (EOLPs): Subsequent responses on observing lever presses when the signaling was already active. Two categories, each corresponding to a condition: reinforcement condition (EOLPs-VR) and extinction condition (EOLPs-Ext).
- Time OLPs: duration in seconds of signaling, calculated as [(OLPs-VR + OLPs-Ext) * 15 sec].
- EOLPs / Sec available: rate of extra observing lever presses by the time available to perform them, calculated as [(EOLPs-VR + EOLPs-Ext) / Time OLPs].
- Rewards. Total reward pellets per session.

### *4.1.4. Statistical analysis*

In the phase of Training 2, the dependent variable was the proportion of number of lever presses given during the reinforcement trials (Discrimination Index), this value was calculated by dividing the total number of responses during the reinforcement trials by the sum of the total number of responses during the extinction and reinforcement trials. These data were tested for statistical significance using a two-way factorial ANOVA, with one between-subject factor named Group (ST vs. GT) and one repeated within-subjects factor named Session (five levels: 1, 2, 3, 4 and 5 last days). Two rats did not reach the criterion (Discrimination Index: ≥ 0.65) so they were eliminated from the future procedure (n=9, in both groups).

For The observing response task, the dependent measure was the proportion of extra observing lever presses during the time available to perform them, this value was calculated by dividing the sum of rate of extra observing lever presses of both conditions (reinforcement and extinction) by duration in seconds of signaling. Data were analyzed using two-way ANOVAs, with one between-subject factor named Group (ST vs. GT) and one repeated within-subjects factor named Session (three levels: 1, 2 and 3 last days).

## 4.2. Results

Figure 5 displays the Discrimination Index, the mean (± SEM) proportion of number of lever presses given by both groups (GT and ST) during the reinforcement trials, in the last five sessions of exposure to schedule.

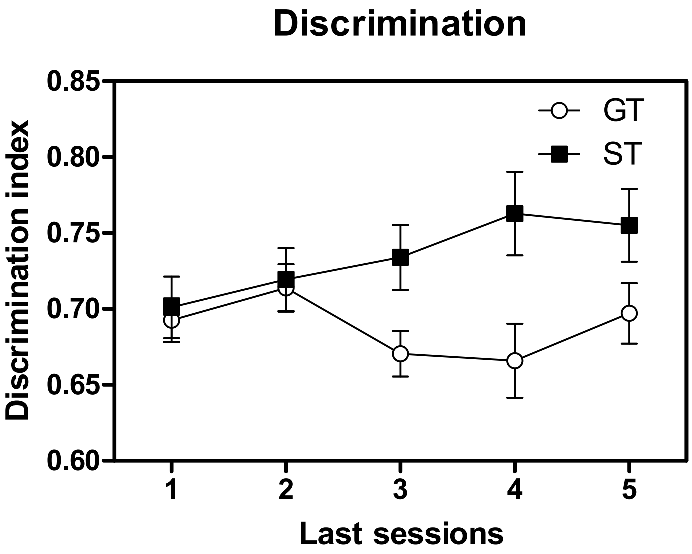

Figure 5. Mean (± SEM) proportion of number of lever presses given by each group (sign- and goal-tracker) during the last five sessions in the Discrimination training phase.

The ANOVA showed effects for the Session x Group interaction [$F(4,64) = 3.56$, $p<0.05$, $\eta^2=0.182$]. Post hoc analyses revealed differences in the 3rd and 4th sessions, with higher Discrimination Index by the ST group versus the GT group ($p<0.05$ in both cases). Although the figure shows differences for Session 5, analyzes indicate only a tendency ($p<0.1$).

Figure 6 shows the mean (± SEM) proportion of extra observing lever presses by the time available to perform them, found for each group (GT and ST), in the last three sessions of exposure to task.

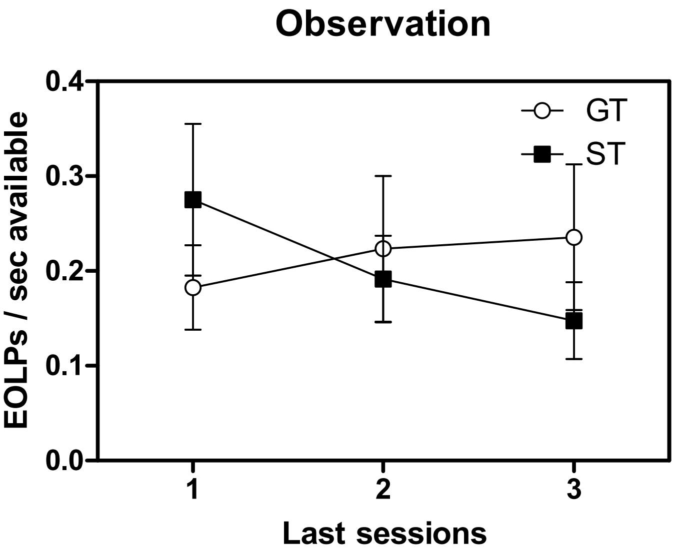

Figure 6. Mean (± SEM) proportion of EOLPs / Sec available for each group (sign- and goal-tracker), in the last three sessions of exposure to task.

No statistically significant differences were found in this task.

## 5. Experiment 4: Delay discounting

Continuing with our inquiries about the possible impulsive nature of STs, and because the SIP and SIWR procedures are usually more related to impulsive motor behavior, we decided to use a self-control procedure, delay-discounting task, as a cognitive measure impulsivity. The task was to choose between a small immediate reward and another delayed reward of greater magnitude, where the frequency with which each option is chosen increases or decreases, respectively, in the face of any increase in the delay that the delivery of the reinforcer of greater magnitude is granted. This task is ideal for taking direct measures of cognitive impulsivity, since each choice is marked by a single response (Fox, Hand & Reilly, 2008).

A previous study (Lovic et al., 2011) found in delay-discounting task that the STs were less impulsive, that is, that the STs preferred the large reward more than the GTs, but only under the longest delay (24 s delay); In our study, we extended the duration of the delay to 40 sec, hoping to find similar results.

### 5.1. Method

#### *5.1.1. Subjects*

The animals and the housing conditions of previous experiments were maintained.

#### *5.1.2. Apparatus*

The same boxes, and under the same characteristics as in Experiment 3 were used.

### ***5.1.3. Procedure***

For this experiment, an adaptation carried out by Ibias and Pellón (2011) of the delay-discounting procedure was used.

#### *5.1.3.1. Pre-training*

The first day the animals received a single session of autoshaping of the levers (remember that the animals had previous experience with levers). During 5 sec, the cue light situated above the levers went on, after that time, it went out and a food pellet was delivered, two seconds later, the light went on again, thus starting a new cycle, until a total of 100 trials. Subsequently, the animals were trained for 4 days (one session per day) in the pressure of levers. In this experiment, only the pressure of the lever was followed by the delivery of food.

#### *5.1.3.2. Training*

During this phase, the lever that was not indicated by the cue light was retracted. The animal had 10 sec to press the lever, after this time, the lack of response was counted as an omission. The presentation of the levers was randomized with a total of 30 trials with each one.

#### *5.1.3.3. Delay discounting task*

The rats were exposed to the delay discounting procedure during 5 sessions (from Monday to Friday) for each delay value, presented in the following order 0, 10, 20, 40 and, again, 0-s. Subsequently, the delay of 5-s and a new delay 0-s for the purpose of completing the set of data. The experimental chambers were counterbalanced, half with the delayed reward being scheduled on the right lever, and the other half on the left lever.

The assignment of ST and GT rats to each of the conditions was also considered. Each session consisted of five consecutive blocks of 12 trials were held, with the first 6 trials in each block being forced-choice and the last 6 being free-choice. When the rat responded on the "immediate" lever, it received one food pellet, but if the rat's response involved a delayed choice, then, four food pellets were delivered after the delay. The animal had 10-s to make a response, otherwise the trial was registered as an omission and the next trial began (see Íbias and Pellón, 2011, for more details).

### *5.1.4. Statistical analysis*

For the Delay discounting task, the proportion of delayed choices of greater magnitude, described as mean of delayed choices of greater magnitude in the last two sessions of the delay / total number of being free-choice trials were registered. Data were analyzed using two-way ANOVAs, with one between-subject factor named Group (ST vs. GT) and one repeated within-subjects factor named Delay (seven levels: 0, 5, 10, 20, 40, 0 and 0-s delays). Statistical analyses were performed using data on each respective subject's last two sessions under each delay. For this experiment, the number of animals in each group was different, two rats had been eliminated in the previous experiment and an experimental death occurred due to the age of the animal (n=9 GT and n=8 ST group).

## 5.2. Results

Figure 7 shows the mean (± SEM) proportion of delayed choices of greater magnitude given by each group (GT and ST) according to the delays established: 0, 5, 10, 20 and 40-s, averaged over the last two sessions of exposure to each schedule.

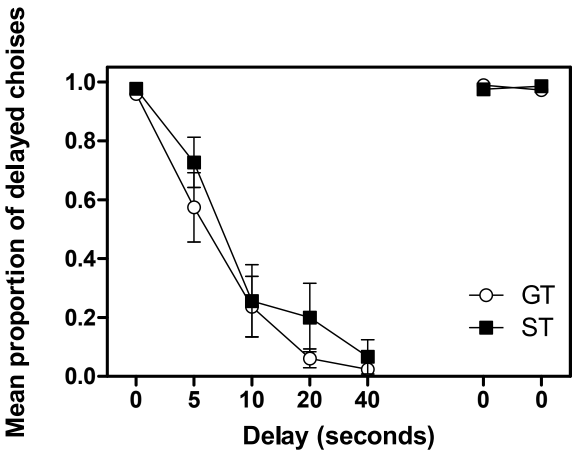

Figure 7. Mean (± SEM) proportion of delayed choices of greater magnitude of each group, in the last two sessions of exposure to task.

The ANOVA revealed statistically significant main effects for Delay [$F(2,35) = 92.55$, $p<0.001$, $\eta^2=0.861$], with a reduction of delayed choices as the delay in receiving the reward of greater magnitude (four food pellets) increased, and an immediate recovery of the election of larger reward when presenting the delay 0-s again, for both groups; No differences were found between goal- and sign-trackers.

## 6. Discussion

The purpose of Experiment 1 was to document SIP in STs and compare its development with GTs, using different FT food schedules. Both groups developed SIP for all the FT schedules, with a gradation depending on the length of the IFI (Falk, 1966; Flory, 1971) and a decrease in drinking rate as the IFI increased (Bond, 1973; Hawkins, Schrot, Githens & Everett, 1972); milliliters consumed rates presented this same trend. Significant differences were found between ST and GT group, with the GTs showing higher levels of drinking compared with the STs (see Figure 1).

Contrary to expectations, the STs had a lower drinking rate than the GTs. These results could be interpreted in two different ways. On the one hand, the initial hypothesis could be rejected, thus, the STs would not represent an impulsivity phenotype. The results observed in this experiment indicate that the GTs have a greater propensity to impulsivity than the STs. These results might be related to the differential dopaminergic activity of the GTs versus the STs. Related to other studies, the GTs present the same behavioral pattern (higher rate of drinking) and dopamine receptor level (greater number of D2 receptors but fewer D1 receptors in the nucleus accumbens) than rats classified as high-drinkers, in comparison to both the STs and low-drinker rats (Flagel et al. 2007; Pellón et al. 2011).

Or, on the other hand, the initial hypothesis could be maintained, but not accepted, arguing that impulsiveness in the ST rats responds to another type of impulsiveness of a more cognitive nature. Another possible explanation for these results suggests that SIP would not be a good animal model of impulsivity. Specifically, Ibias and Pellón (2014) commented that the relationship between impulsivity and adjunctive behavior is not clear; in their study, they found that the most impulsive animals in the control population developed less SIP. Recent studies (Ibias & Pellón, 2014; Moreno & Flores, 2012) suggest that SIP is a model of compulsive behavior.

The temporal distribution of drinking showed an inverted U-shaped post-pellet location, with a maximum peak during the first part of the IFI, as is usually observed in this behavior (Falk, 1971). The distribution curve tended to flatten as a consequence of increases in the length of IFI, characteristic that has been observed previously in other studies (Íbias & Pellón, 2011) and, according to the analyzes, it is totally flat under FT 120-s schedule. Statistical analyzes indicated that the GT rats responded more than the ST rats under FT 30- and 60-s schedules within the IFI.

On the other hand, magazine entries rate did not vary between the different FT schedules, and in the case of magazine entries per food pellets, the increases in the number of responses correspond to a longer duration of the session, that is, a greater amount of time to make responses. The results found for magazine entries also did not match our expectations, we expected to find that the GTs presented a higher rate of magazine entries than the STs, given their characteristic response pattern aimed at obtaining the reward in autoshaping procedure (Flagel et al., 2007; Robinson & Flagel, 2009), however, the ST rats registered a higher mean value than the other group in all FT schedules. What is being reflected here may be the different nature of this behavior. The influence of operant conditioning on schedule-induced behavior versus classical conditioning in autoshaping procedure.

The temporal distribution of magazine entries showed its typical response pattern under FT schedules (Boakes, Patterson, Kendig & Harris, 2015), a maximum peak at the beginning of the IFI, followed by a steady accelerated descent, and the recovery of behavior towards the end of the IFI. Statistical analyzes showed that the ST rats performed more than the GT rats under all FT schedules within the IFI.

In conclusion, the results found in this experiment indicate that adjunctive drinking is a conduct directed towards the goal, that is, the differential pattern in licks and magazine entries of the STs and the GTs support the idea that SIP behaves as an operant behavior (Killen & Pellón, 2013).

The purpose of Experiment 2 was to document SIWR in STs and GTs. Both groups developed SIWR under FT 60- and 120-s schedules, with a higher response rate under the shorter FT schedule (Gutiérrez-Ferre & Pellón, 2019). Significant differences were found between the ST and the GT group, with the GTs showing higher levels of running compared with the STs (see Figure 2).

The results found in SIWR replicate those found in SIP, the GTs presented a higher rate of adjunctive behavior. Therefore, SIWR could also be considered as a good animal model, although it is not yet clear for what, since the conclusions on SIP are perfectly applicable to SIWR (see above). Temporal distribution of wheel turns presented a form of an inverted U-shape along the IFI, observed in previous studies (Gutiérrez-Ferre & Pellón, 2019).

On the other hand, physical activity is usually used in the treatment of disorders that present impulsiveness in its diagnosis (Smith & Lynch, 2012; Vancampfort et al., 2013; Wigal, Emmerson, Gehricke & Galassetti, 2013); Strickland, Feinstein, Lacy and Smith (2016) compared two groups of rats, with and without wheels in their home cages, and found that, effectively, pre-exposure to physical activity decreased impulsivity in the delay discounting task. In this sense, it would be interesting to inquire more about "impulsivity" in SIWR.

Regarding the magazine entries, the results, and therefore the conclusions drawn, also coincide with those found for this behavior in SIP, both per minute, per food pellets and in its temporary distribution within the IFI, with the STs presenting a greater number of magazine entries than the GT group (Figures 3 and 4).

Following the results obtained, the purpose of Experiment 3 was to identify possible differences in compulsivity levels of STs and GTs using an adaptation of the Eagle's observing response task, as an operant model of compulsive checking (Eagle et al., 2014) that could explain the results found in the schedule-induced behaviors, appealing to their characteristic of compulsivity.

Significant differences were found between the groups in the discrimination index. The ST rats registered a higher discrimination index than the GT rats (Figure 5). These results are consistent with the predisposition of the STs to be attracted by the key that predicts the reward (Flagel et al., 2007). However, no differences were found between the GTs and the STs on extra observing lever presses (EOLPs) (Figure 6), so it could be concluded that the differences found between these groups do not imply a matter of compulsivity.

Finally, the purpose of Experiment 4 was to identify possible differences in the impulsivity levels of the ST and the GT group, using a delay discounting procedure as a cognitive measure of impulsivity. In both groups, the preference for the reward of greater magnitude decreased as the delay to receive the greater reward increased (see Figure 7). The analyzes showed no significant differences between STs and GTs. These results could indicate that the PCA does not measure cognitive impulsivity. However, in a previous study (Lovic et al., 2011), the authors observed that the STs preferred the large reward more than the GTs when the delay was long enough (24 s delay), concluding that these results responded to a lower level of impulsivity in the STs. The results of this study would be consistent with the results obtained in Experiments 1 and 2, where the STs showed less impulsivity. However, in our study, we failed to replicate the findings of Lovic et al. (2011). An explanation for these results could come from the results found by Ibias and Pellón (2014), who demonstrated that pre-exposure to SIP decreased subsequent levels of impulsivity in delay discounting tasks.

As a final conclusion, it is suggested that schedule-induced behavior, either in the form of drinking from a water bottle or running on a wheel, is expressed to a greater extent in goal-tracker rats in comparison to sign-tracker ones, and that this is to some extent surprising because it has been long thought that schedule-induced behavior relates to traits of impulsivity, abuse potential and other dysfunctional aspects of behavior. However, recent approaches and evidence suggest that schedule-induced behavior maybe more functional in the sense of serving as adaptive in situations of scarce sources of reinforcement, putting into action patterns of behavior that are naturally linked to the foraging system of animals, being drinking and running especially relevant for rodents, such as laboratory rats. In line with this, schedule-induced behavior does not always clearly relate to impulsive measurements and there is evidence that it relates to consequences following the behavior (Ibias & Pellón, 2014).

# CHAPTER V

# General conclusions

## Conclusions

The aim of this thesis has been to provide empirical data that clarify the debate about whether wheel running can be considered an induced behavior or not; as well as comparing different aspects of schedule-induced wheel running (SIWR) with those observed in schedule-induced polydipsia (SIP), since the latter is the schedule-induced behavior par excellence. If wheel running were induced, it would respond to characteristics related to the reinforcer and thus would be modifiable by environmental variables known to affect other schedule-induced behaviors, however, if wheel running were a facultative behavior, as Staddon (1977) points out, it would not be susceptible to manipulation due to alterations in reinforcement parameters.

In order to achieve this aim, a series of procedures were employed. Through the manipulation of different experimental variables, the characteristics of the schedule-induced behavior were observed for wheel running.

Chapter II consisted of two experiments. In Experiment 1, the criteria that determined whether a behavior can be considered as a schedule-induced behavior or not were analyzed: 1) the development of an excessive response rate when animals were exposed to intermittent reinforcement programs; 2) the influence of the length of the inter-food interval (IFI) on the acquisition of the conduct; 3) the position adopted by the conduct within the IFI; and 4) the effect of intermittent reinforcement on behavior. To meet the first three criteria, the acquisition of SIWR was analyzed under different fixed time (FT) schedules (30-, 60-, 120-, 240-, and 480-s); while for the fourth criterion, the influence of intermittent reinforcement on the SIWR was studied through a massed-food control condition.

The results revealed that the SIWR develops under FT schedules in a range of 30-240 s, but not under the longer schedule (FT 480-s), with a decrease in the wheel running rate as the IFI increases, describing a linear downward function observed in other schedule-induced behaviors, such as SIP. Within the IFI, the temporary distribution of wheel running showed a U-shaped an inverted function, occupying a post-reinforcer position, that is, presenting a maximum peak during the first part of the IFI, location that, for Staddon, occupies the schedule-induced behavior qualified as interim behaviors. The data obtained under the massed-food control condition showed a lower rate of wheel running than under FT schedules, so it is concluded that intermittent reinforcement is necessary for the development and maintenance of the SIWR. The fulfillment of these criteria by wheel running allowed to affirm that this behavior is, in itself, a schedule-induced behavior; more specifically an interim behavior.

Once accepted that wheel running is a schedule-induced behavior, in Experiment 2, the effect of the presence of a wheel in the home cage on the maintenance of the SIWR under different FT schedules (30-, 60-, 120-, 240-s) was studied, in order to equate the conditions under those that the SIP develops; and, on the other hand, the influence of the possibility of performing a new behavior (drinking from a water bottle) during the experimental session on the maintenance of the SIWR under the same FT schedules (30-, 60-, 120- and 240-s) was examined.

The results showed that the presence of a wheel in the home cage significantly reduces the wheel running rate under the FT 30- and 60-s schedules, but not in the longer schedules, revealing a state of satiety at the beginning of the session, so short FT schedules are affected only. The wheel running feature of functioning as a booster influences the development of the SIWR, losing efficacy by satiation, and causing a decrease in behavior. On the other hand, availability of a water bottle during the experimental sessions did not significantly affect the maintenance of SIWR, although SIP developed normally. However, SIP adopted the initial position within the IFI, moving

SIWR towards the middle of it. This responds to a temporary competition between adjunctive behaviors of the same nature, but with differences in their sensitivity to the delay of the reinforcer, that competes for their expression within the IFI (Killeen & Pellón, 2013), thus, drinking presents a more pronounced delay-of-reinforcement gradients than wheel running, which displaces it towards the middle of the interval.

In Chapter III, an attempt was made to deepen the results obtained in Chapter II. To this end, the effect of operating contingencies on three responses was analyzed: drinking, wheel running and magazine entering, according to Staddon's classification (1977) (interim, facultative and terminal behaviors, respectively), through the appearance of a protective contingency, signaled by a tone and light off for half of the animals and unsignaled for the other half, which postponed the appearance of food when the animals performed any of the three responses during the last 1, 3, 5, 10, 20, 40 or 58 s of the IFI, under an FT 60-s schedule, to see if that would result in delay-of-reinforcement gradients that would allow to question the traditional view that the different behaviors belong to separate categories subject to different principles.

The results revealed that the three behaviors are affected by protective delays based on their temporary location within the IFIs, being licks more resistant to the disruptive effects of delays, followed by wheel running and then magazine entering. Magazine entries were reduced by delays as short as 1 s and diminished gradually with increases in delay length, this would be consistent with its close relationship with the reinforcer. The wheel turns rate was decreased from the shortest delay (1 s) but remaining relatively stable with the increase in the duration of delays. This effect could be due to the intrinsic reinforcement of wheel running, that keeps the response as a consequence of the response-deprivation in the home cage. And drinking rarely occurred during the last 20 s of the IFI, so that, food delays had to reach high values (40 and 58 s) to significantly affect drinking. An interesting result was the increase in the drinking rate in short delays respect to the baseline, which could respond to a behavior competition, the reduction in the rate of wheel turns and magazine entries is accompanied by an increase in the rate of licks. However, no significant differences were observed when signaled and unsignaled delays were compared. Regarding the temporal distribution, the different behaviors adopted their usual positions within the IFI when they coincided under the experimental condition. Drinking at the beginning, wheel running in the middle, and magazine entering at the end of the IFI.

From these results, it is concluded that the three behaviors have different sensitivities to delays, in correspondence with their temporal location within IFIs because the reinforcer operates at a different time distance for each behavior and, therefore, different association forces (behavior-reinforcer) are generated for each of the behaviors (Killeen & Pellón, 2013). It can be said that the interim, facultative and terminal behaviors (according to the Staddon classification) are subject to the same operating principles but with different parameters that explain their different delay-of-reinforcement gradients. A principle of temporary proximity supported by the mechanism of delayed positive reinforcement might suffice to explain the apparent different types of behavior sustained under intermittent food reinforcement schedules.

Continuing with the search for similarities between SIWR and SIP, Chapter IV analyzed the possible involvement of SIWR as an animal model of impulsivity. Two groups of rats, goal- (GTs) and sign-trackers (STs), being STs considered by the previous literature as an impulsive phenotype, were exposed to four experimental procedures. In Experiments 1 and 2, individual differences between the GTs and the STs in the development of SIP and SIWR, respectively, were studied under FT schedules. The results revealed that, contrary to expectations, the GTs showed a greater propensity to impulsivity than the STs, presenting higher rates of drinking and wheel running, as well as a lower rate of magazine entries than the STs. These results could point to the fact that the SIWR can be appreciated as an animal model, like the SIP; however, these results suggest that SIWR and SIP are not good animal models of impulsivity. Recent studies (Ibias & Pellón, 2014; Moreno & Flores, 2012) suggest that SIP is a model of compulsive behavior. Another possible explanation for the results found in this study could be that the STs impulsiveness phenotype refers to a different type of impulsivity, cognitive impulsivity. In order to clarify these two possible explanations of the results, in Experiment 3, compulsivity levels of both groups were measured by an operant observing response task with uncertain reinforcement, while, in Experiment 4, impulsivity levels of both groups were measured by a delay discounting procedure. However, differences were not found between the GTs and the STs in the compulsivity levels or in the delay discounting. So, it can be concluded that the schedule-induced behaviors are not related to impulsivity traits as previously thought, as well as that SIP and SIWR behave as operant behaviors (Killen & Pellón, 2013), backed by the differential pattern in licks and magazine entries of the STs and the GTs, being these behaviors directed towards the goal.

As a final conclusion, it can be affirmed that wheel running is a schedule-induced behavior for complying with the criteria of excessiveness, location and distribution within the IFI and susceptibility to the manipulation of the reinforcement parameters, found in other schedule-induced behaviors, such as SIP. SIWR and SIP have the same nature but differ in their sensitivity in the delay to the reinforcer, which leads them to compete for their expression within the IFI.

I hope that this exhaustive analysis of the characteristics of wheel running as a schedule-induced behavior will allow it to take its rightful place within this group of behaviors; as well as the generalization and application of the results in the study of other animal procedures and models, such as activity-based anorexia, where the parameters of wheel running induction are relevant.

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