TESIS DOCTORAL

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SCHEDULE INDUCED POLYDIPSIA IN AN ANIMAL MODEL OF ATTENTION DEFICIT HYPERACTIVE DISORDER

POLIDIPSIA INDUCIDA POR PROGRAMA EN UN MODELO ANIMAL DE TRASTORNO DE HIPERACTIVIDAD CON DEFICIT DE ATENCION

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Acknowledgements

Quiero agradecer a todos aquellos que han contribuido a que este proyecto fuese posible.

En primer lugar a Ricardo Pellón, por su sabiduría y su visión de la ciencia y porque ha sido un modelo a seguir durante estos años, asistí a su clase de Aprendizaje y Condicionamiento en 2004 y desde entonces ha apostado por mí.

En una mención especial al profesor Peter Killeen de Arizona State University (ASU) por sus consejos e inspiración.

Al profesor Armando Machado de la Universidade do Minho, Braga, Portugal y al profesor Federico Sanabria de (ASU), Tempe, Arizona, USA, por haberme acogido en sus laboratorios y por enseñarme, qué preguntas he de hacerle a la ciencia y cómo poder darles respuesta.

A los profesores Concepción San Luís Costas, Mª Carmen Pérez-Llantada y Andrés López de la Llave, a los que debo mi formación en Metodología de las Ciencias del Comportamiento y han sabido dotarme de autonomía y confianza en mí mismo.

A los profesores del departamento de Psicología Básica I de la UNED por su ayuda, en especial a Miguel Miguens por su inquebrantable apoyo y también a Nuria Ortega, Cristina Orgaz y Vicente Pérez por el mismo motivo. A todos mis compañeros del Laboratorio en UNED por su ayuda, es especial a Jorge Ruíz y Antonio Rey por saber transformar lo imposible en fácil. También a todos los investigadores e investigadoras que he ido encontrando y de un modo u otro se han convertido en referencia para mí, en especial a Pilar Flores, Nuria del Olmo y Mª Teresa Gutiérrez.

Y por último a las dos personas más importantes para mí y a quienes dedico esta tesis:

a mis padres.

It is, however, far more necessary to bear in mind that there are many unknown laws of correlation of growth, which, when one part of the organization is modified through variation, and the modifications are accumulated by natural selection for the good of the being, will cause other modification, often of the most unexpected nature.

Charles Darwin, Origin of species,
Chapter IV, Natural Selection.

Title

SCHEDULE INDUCED POLYDIPSIA IN AN ANIMAL MODEL OF ATTENTION DEFICIT HYPERACTIVE DISORDER

Abstract

This thesis investigated the development, maintenance and modulation of Schedule-induced polydipsia (SIP) in the spontaneously hypertensive rat (SHR) through three consecutive studies.

First study: Schedule induced polydipsia in the spontaneously hypertensive rat and its relationship to impulsivity

In an initial study, rats were exposed to different variations of a SIP procedure, in which acquisition parameters were modified by using fixed time (FT) schedules of reinforcement. Food pellets were intermittently delivered regardless of the behaviour of the animals, and FT values were manipulated until locate a range of optimal SIP's development in SHR rats. Differences between SHR and control rats (WKY and Wistar strains) were then evaluated. After SIP procedure a self-control test was conducted in order to obtain the animals' levels of impulsivity.

Eight SHR, 8 WKY and 8 Wistar rats, all males, maintained between 80-85% of their ad-libitum weight by controlled dietary restriction, were exposed to FT schedule's series. FT values were 9, 15, 30, 60, 120 and 180 sec, counterbalancing the order of presentation of schedules among animals. Once SIP procedure had ended, a delay discounting procedure in which rats were faced to successive choices was carried out. During this procedure animals might choose between an immediate reward of 1-pellet food delivery and a 4-pellet food delivery after

a delay of 3, 6, 12 or 24 sec. Mean proportions of "delayed choices" in every delay condition were analysed.

Due to the unrestricted access to water and the intermittent deliveries of food, it was found the development of schedule-induced polydipsia in all FT schedules. We found an inverted U-shaped function relating the number of licks by animals in each of FT schedules. They were obtained optimal development ranges of SIP for each group, major goal of this study. In the impulsivity test SHR rats choosed the small immediate reward more than control rats at longest delays, also making a greater number of omissions in these conditions. In other words, they were found higher impulsivity measures in SHR, measures that may be related to their performance in SIP.

Results of this first study would serve as reference of SIP performance by SHR rats, a topic never before investigated, and conclusions would discuss whether differences in schedule-induced polydipsia should be related to indicators of impulsivity. Therefore, a second study was carried out to investigate this.

Second study: Schedule-induced polydipsia in high and low impulsive Wistar versus Spontaneously Hypertensive and Wistar Kyoto rats

In this second study they were conducted several related experiments, by which, there was evaluated the possible relationship between the different levels of impulsivity and SIP acquisition. To perform this study 8 SHR, 8 WKY and 16 Wistar rats were used. Since it cannot be categorically asserted that the impulsivity found in SHR rats is exactly like the observed in control populations, we investigated to what extent the impulsiveness that may show

the SHR rats, mediated by its motor excess, could be transferable to other impulsive, but not necessarily hyperactive subjects.

Principal aim of this study was grouping subjects by their impulsivity levels, and put these levels into relation with SIP performance.

It was conducted a first delay discounting task, by which animals were classified according to their levels of impulsivity, and then animals were compared, as high or low impulsive, by their results in the SIP procedure. Delays to obtain the higher amount of food in every choice trial were 3, 6, 12 and 24 seconds, and as in Study 1, the bigger but delayed choice consisted on four food pellets versus an immediate one. To complete this evaluation, measures of the relative value of these different amounts of food were taken by using an indifference point procedure.

SIP procedure was conducted after that, using FT schedules of reinforcement with 15, 30, 60 and 120 seconds between food deliveries. As in the first study, the exposure to the schedules between the animals was counterbalanced and subsequent stages were repeated until each animal had completed all schedules.

Finally, and after the SIP procedure, one last delay discounting task was conducted, in order to obtain once again impulsivity measures, this time post-SIP impulsivity measures.

In this study they were found different behavioural profiles that related prior impulsivity, SIP and subsequent impulsivity. Moreover, it was investigated to what extent Wistar rats, as control population, reconstructed reference groups of SHR and WKY rats. At resume, those animals that previously had been classified as high impulsive differed in SIP. On one hand, SHR rats showed both high levels of previous impulsivity and high levels of

SIP, but those Wistar rats classified as high impulsive, at contrast, showed scarce levels of SIP when comparing to both SHR and low-impulsive Wistar rats.

Conclusions would discuss the differential effect of impulsivity on SIP acquisition, and then, the possible influence that SIP procedure may have on final levels of impulsivity.

A third study investigated the role of the dopaminergic system in the development of SIP in the SHR rat, because the exacerbated dopaminergic functioning observed in SHR could be partly responsible for their SIP's results.

Third study: Dopaminergic manipulation of Schedule Induced Polydipsia in Spontaneously Hypertensive Rat, Wistar Kyoto and Wistar rats: ADHD pharmacological treatment effects

SIP acquisition was investigated in 24 male rats of three different strains 8 SHR, 8 WKY and 8 Wistar rats, using a multiple-FT reinforcement schedule with two components of 30 and 90 seconds duration. From the acquisition of behaviour after 40 sessions, with both components randomized in each session, a stable baseline of SIP for each strain was obtained. Then, they were evaluated the effects of the selective and non-selective manipulations over the dopamine receptors (DA) in the maintenance of the induced behaviour.

Principal predictions of this study were related to the possible effects of ADHD pharmacologic treatments over SIP in SHR rats, due their relation with ADHD. Further, pharmacological manipulations on D_1 vs. D_2 receptors or their combined influence provided information about their implication on SIP.

The results showed some insensitivity to both treatments in SHR compared to their controls. Increases in the drugs dose decreased the adjunctive drinking in both FT schedules, but in SHR rats only the higher doses produced an effect.

These results could be discussed in relation to an existing hyperfunction in dopamine in the SHR rat as a putative modulator of the rate of acquisition and maintenance levels of SIP. In turn, results would be related to the hyperactivity and impulsivity that shows this strain, characteristics that have placed this animal as a model of ADHD.

All procedures contained in these three studies were conducted in the laboratory of animal behaviour of the Faculty of Psychology, UNED. All these experiments were carried out using 8 Letica LI-836 conditioning boxes of 29 x 24.5 x 35.5 cm contained in soundproof chambers with proper ventilation and a viewing window in the front. On the outside of the right wall of each box, a water bottle was installed; whose spout the rat would access from the inside of the cage through an opening in the wall of the experimental chamber.

In SIP procedures licks were registered in a MED-PC application under a Windows environment. All procedures dispensed 45mg food-pellets (Bio-Serv, Frenchtown, NJ, USA) into an opening in the front wall of the box, located between the two levers on the panel, which were used exclusively during delay discounting and indifference point procedures.

Altogether, these three studies provide a detailed description of the development of SIP in SHR rats. In this thesis there have been faced two models of excessiveness, in one hand the animal model, the SHR rat, and on the other hand, the behavioural model, the SIP.

Título

POLIDIPSIA INDUCIDA POR PROGRAMA EN UN MODELO ANIMAL DE TRASTORNO DE HIPERACTIVIDAD CON DEFICIT DE ATENCION

Resumen y Conclusiones

En esta tesis se ha investigado el desarrollo, mantenimiento y modulación de polidipsia inducida por programa (PIP) en la rata espontáneamente hipertensa (SHR) a través de tres estudios consecutivos

Primer estudio: Polidípsia Inducida por programa en la rata espontáneamente hipertensa y su relación con la impulsividad.

En un primer estudio se expuso a ratas de laboratorio a distintas variaciones de un procedimiento de PIP utilizando programas de reforzamiento de Tiempo Fijo (TF). Estos programas entregaban de forma intermitente bolitas de comida, independientemente a la conducta de los animales, los valores de los programas de Tiempo fijo (TF) se manipularon hasta obtener un rango de desarrollo óptimo de la PIP en las ratas SHR, y a continuación se evaluaron las diferencias entre estos animales y sus controles. Tras el procedimiento de PIP, se llevó a cabo un test de autocontrol con la intención de obtener medidas sobre los niveles de impulsividad de los animales.

Se utilizaron ocho ratas SHR, 8 WKY y 8 Wistar, todos machos, mantenidas entre el 80-85% de su peso ad-libitum mediante restricción controlada de la dieta. Los animales fueron expuestos a series de programas de Tiempo Fijo (TF). Los valores de TF utilizados fueron 9, 15, 30, 60, 120 y 180 seg., contrabalanceando el orden de presentación de los programas entre los animales y repitiendo sucesivas fases hasta que todos los animales completaron todas las condiciones. A continuación se expuso a los animales a un procedimiento de

descuento por demora, en el cual las ratas se enfrentaban a sucesivas elecciones en las cuales podían escoger entre una recompensa inmediata de una bolita de comida y otra de cuatro bolitas de comida, pero con una demora de 3, 6, 12 ó 24 seg. El promedio de las proporciones de "elecciones demoradas" en cada condición de demora se utilizó para realizar los análisis.

Debido a la libre disposición del acceso al agua, se encontró desarrollo de polidipsia inducida por programa en todos los programas de TF. Como se esperaba, se encontró la característica función bitónica (U-invertida) que relaciona el número de lametones y la bebida consumida con la longitud de los intervalos entre bolitas de comida. Se obtuvieron así los rangos óptimos de desarrollo de PIP en cada grupo de animales. Tras utilizar el test de impulsividad se encontraron medidas más elevadas en las ratas SHR, medidas que podrían relacionarse con su desempeño en PIP. El resultado encontrado fue que en las demoras más largas, las ratas SHR escogieron la recompensa inmediata de menor magnitud más que las ratas Wistar y también realizaron un mayor número de omisiones durante los ensayos forzados del procedimiento.

Los resultados de este primer estudio sirvieron como referencia del desempeño en PIP mostrado por las ratas SHR, un tópico que aún no había sido investigado.

Los resultados se discutieron cuestionando si las diferencias en polidipsia inducida por programa estarían relacionadas con indicadores de impulsividad.

Por tanto, a continuación se llevó a cabo un segundo estudio en el cual se investigó esto.

Segundo estudio: Polidípsia Inducida por programa en ratas Wistar altas y bajas impulsivas frente a ratas espontáneamente hipertensas y Wistar Kyoto.

En este segundo estudio se llevaron a cabo varios experimentos relacionados, mediante los cuales se evaluó la posible relación entre diferentes niveles de impulsividad y la adquisición de PIP. Se utilizaron, 8 ratas SHR, 8 WKY y 16 ratas Wistar. Ya que no se puede afirmar de un modo categórico que la impulsividad encontrada en las ratas SHR sea exactamente la misma que se observa en poblaciones de control; se investigó hasta qué punto la impulsividad que evidencia la rata SHR, mediada por su exceso motor, podría ser transferible a otros sujetos también impulsivos, pero no necesariamente hiperactivos.

El principal objetivo de este estudio fue agrupar a los sujetos de control por sus niveles de impulsividad, y entonces compararlos como altos y bajos impulsivos a partir de sus resultados en el procedimiento de PIP.

Se llevó a cabo una primera prueba de descuento por demora, mediante la cual los animales fueron clasificados de acuerdo a sus niveles de impulsividad. Estos animales fueron entonces comparados como altos y bajos impulsivos, mediante sus resultados en el procedimiento de PIP. Las demoras para obtener la recompensa de mayor magnitud a través de ensayos de elección fueron 3, 6, 12 y 24 segundos, y al igual que en el primer estudio, la recompensa demorada de mayor magnitud consistió en cuatro bolitas de comida frente a una inmediata.

Para completar esta evaluación, se tomaron medidas del valor relativo de estas cantidades de comida mediante un procedimiento de punto de indiferencia.

El procedimiento de PIP se llevó a cabo a continuación. Se utilizaron programas de TF 15, 30, 60 y 120 segundos de duración entre las entregas de comida. Al igual que en el primer estudio se contrabalanceó la exposición a los

programas entre los animales y se repitieron sucesivas fases hasta conseguir que cada animal hubiese completado todos los programas.

Finalmente, tras el procedimiento de PIP, se llevó a cabo un último descuento por demora, con la intención de obtener, una vez más, medidas de impulsividad, esta vez post-PIP.

Se encontraron diferentes perfiles conductuales que relacionaban la impulsividad previa, PIP y la impulsividad posterior. Se comprobó, a su vez, que estos perfiles reconstruían los grupos de referencia, SHR y WKY, distribuyendo al grueso de población control (Wistar). Aquellos animales previamente clasificados como altos impulsivos difirieron finalmente en PIP.

Las ratas SHR mostraron altos niveles de impulsividad previa y mucha bebida adjuntiva, pero las ratas Wistar clasificadas como altas impulsivas mostraron escasos niveles de adquisición de PIP al comparar con las ratas Wistar clasificadas como bajas impulsivas.

Estos resultados permitieron discutir el efecto diferencial de la impulsividad en la adquisición de PIP y también el efecto que esta pueda producir en dichos niveles de impulsividad.

Un tercer estudio investigó el papel del sistema dopaminérgico en el desarrollo de PIP en la rata SHR. Ya que la rata SHR muestra un funcionamiento dopaminérgico exacerbado que podría ser, en parte, responsable de los resultados encontrados en los procedimientos previos de PIP.

Tercer estudio: Modificación dopaminérgica de la Polidípsia inducida por programa, tratamientos habituales para el control del Trastorno de Hiperactividad con déficit de atención (THDA).

Se investigó la adquisición de PIP en 24 ratas macho de tres cepas diferentes, 8 SHR, 8 WKY y 8 ratas Wistar, utilizando un programa de reforzamiento múltiple de TF con dos componentes de 30 y 90 segundos de duración. A partir de la adquisición de la conducta tras 40 sesiones con ambos componentes, aleatorizados en cada sesión, se estableció una línea estable de conducta para cada cepa de ratas. A continuación se evaluó el efecto de la manipulación selectiva y no selectiva de los receptores de Dopamina (DA) en el mantenimiento de la conducta inducida.

Se encontraron dos perfiles diferentes para cada uno de los componentes del programa múltiple que dependían de la cepa de procedencia: un desempeño similar en PIP con el programa de TF 30-s y diferencias en el programa de TF 90-s. Se tomaron estas medidas como línea be base a partir de las cuales aplicar varios tratamientos farmacológicos.

Tras someter a los animales al procedimiento de PIP se aplicaron los principales tratamientos farmacológicos empleados en el control del THDA: Anfetamina y Metilfenidato.

Este estudio se completó realizando una manipulación dopaminérgica selectiva a cargo de agonistas y antagonistas de los distintos grupos de receptores dopaminergicos, los tipos D_1 y D_2.

Las predicciones principales de este estudio se relacionaban con los posibles efectos de los tratamientos farmacológicos para el TDAH sobre la PIP en las ratas SHR, por su relación con el TDAH. Además, las manipulaciones farmacológicas de los receptores tipo D_1 y D_2 ofrecieron información sobre su influencia, tanto individual como combinada en la PIP.

Los resultados mostraron cierta insensibilidad a ambos tratamiento en las ratas SHR frentes a sus controles. En ambos programas de TF el aumento de las dosis de fármaco produjo la disminución de la bebida adjuntiva, pero en las ratas SHR tan solo las dosis más elevadas surtieron algún efecto.

Estos resultados se discutieron en relación a una hiperfunción en dopamina existente en la rata SHR como un posible modulador de la tasa de adquisición y los niveles de mantenimientos de la PIP. A su vez, se relacionaron estos con la hiperactividad e impulsividad que muestra esta cepa de rata, características que la sitúan como un modelo animal de THDA.

Los tres estudios se llevaron a cabo en el laboratorio de conducta animal de la Facultad de Psicología de la UNED. Se utilizarán 8 cajas de condicionamiento modelo Letica LI-836 de 29 x 24,5 x 35,5 cm y contenidas en cámaras de insonorización con ventilación propia y una ventana de observación en la parte frontal. En el exterior de la pared derecha de cada caja, se instaló un biberón de agua, a cuya tetina accedía la rata desde el interior de la caja por una abertura en la pared de la caja experimental.

En los procedimientos de PIP los lametones quedaron registrados en una aplicación MED-PC bajo un entorno Windows. En todos los procedimientos se dispensaron bolitas de comida de 45 mg (Bio-Serv, Frenchtown, NJ, USA) en una abertura de la pared frontal de la caja, situada a 3,7 cm del suelo, entre las dos palancas del panel, que se utilizaron de forma exclusiva durante los procedimientos de descuento por demora y punto de indiferencia.

En su conjunto, los procedimientos incluidos en estos tres estudios ofrecen una descripción detallada del desarrollo de PIP en la rata SHR. En esta tesis se

han enfrentado dos modelos de excesividad, por un lado el modelo animal, la rata SHR, y por otro lado el modelo conductual de excesividad, la PIP.

INDEX

Abbreviations, acronyms and symbols

ADHD – Attention Deficit/Hyperactivity Disorder

ANOVA – Analysis of variance

D1-like – Subfamily of dopamine receptors

D2-like – Subfamily of dopamine receptors

DA – Dopamine

D-AMP – d-Amphetamine

DAT – Dopamine transporter

FT - Fixed Time Schedule

K – Parameter that reflects the steepness of the discount function

MANOVA – Multivariate analysis of variance

mg/Kg – Milligrams per Kilogram

MPH – Methylphenidate

n.s. – non significant

SEM – Standard Error of the Mean

SHR – Spontaneously Hypertensive Rat

SIP – Schedule Induced Polydipsia

WKY – Wistar Kyoto Rat

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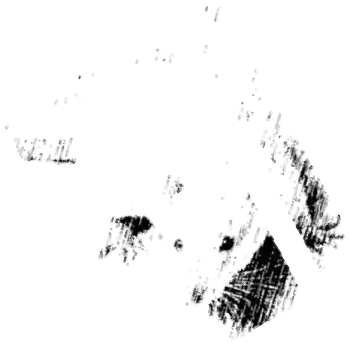
CHAPTER I

GENERAL INTRODUCTION



1. Schedule Induced Polydipsia

Falk (1961) observed that, if rats were allowed free access to a water bottle under intermittent food-delivery conditions, they developed a characteristic drinking pattern during the inter-food intervals. That is, the animals drank a small quantity of water after each food delivery, with this drinking becoming excessive across the experimental session, because of repetition of food administration and prolonged experience of reinforcement. Falk termed such excessive consumption of water, "schedule-induced polydipsia" (SIP).

Intermittent delivery of food is the basis for the development of behavioural expressions along the course of successive inter-food intervals. SIP appears if water is available throughout these intervals, and it belongs to a category of so called “induced behaviours”.

An induced behaviour is any activity, different from the operant response, which shows a characteristic temporal distribution and occurs at high or excessive rate while intermittent behavioural schedule is in course (Wetherington and Brownstein, 1982). Induced behaviours are divided into terminal and interim activities. Terminal activities are located close to delivery of the following reinforcer, are responses related to consummatory behaviour and the reinforcing event used, which will display a range of responses pertaining to a specific behavioural system. Interim activities precede terminal activities, tend to be incompatible with these and arise immediately after delivery of the reinforcer, when probability of its occurrence has decreased dramatically because of its recent delivery (Staddon and Simmelhag, 1971;

Staddon, 1977). Both terminal and interim activities are related to the same behavioural system, albeit, interim activities in a more general manner (Timberlake, 1993).

Besides induced activity, inter-food intervals contain "not induced activities" which consist mainly of exploring, grooming and locomotor activity. These not induced activities grouped to interim behaviour constitute a more global category of "associated behaviours", that is, all activities that occur in addition to terminal activity (Falk, 1971). Every emitted behaviour throughout the intervals will consist ultimately of associated plus terminal activities.

Figure 1 represents the relationship between inter-food interval duration, and the proportion of the interval that is occupied by different behaviours, all of them modulated by the reinforcement schedule. Both interim and terminal activities are facilitated by parameters of the behavioural schedule that modulate the value of the primal reinforcement. These are variables like food-deprivation level (Freed and Hymowitz, 1972) and frequency, magnitude and amount of food (Falk, 1966; Flory, 1971).

General distribution of activities depends on the terminal response. When interval durations are short, as observed in area A, interval time available is entirely distributed between interim and terminal activities, but as descends towards the areas B and C can be observed that, not induced activities takes place, gradually, by the middle of interval, and the longer the interval the higher the probability of occurrence of not induced behaviour, and therefore the less the interim activities (Pellón 1990). It is then, when intervals are long enough to leave some free space between interim and terminal behaviour, when not

induced activities appear, affecting probably the primal schedule and so the induced behaviour, especially when interval's duration becomes excessive.

Terminal activities, e.g. magazine entries in rats, as well as interim activities increases when we descend area A of the figure, and once in area B, time dedicated to terminal activities gets stabilized, and remains relatively constant until a natural decrease produced by excessive interval durations, that will prevent their occurrence, and in turn any interim activity associated. This could be observed descending for area C outside the figure.

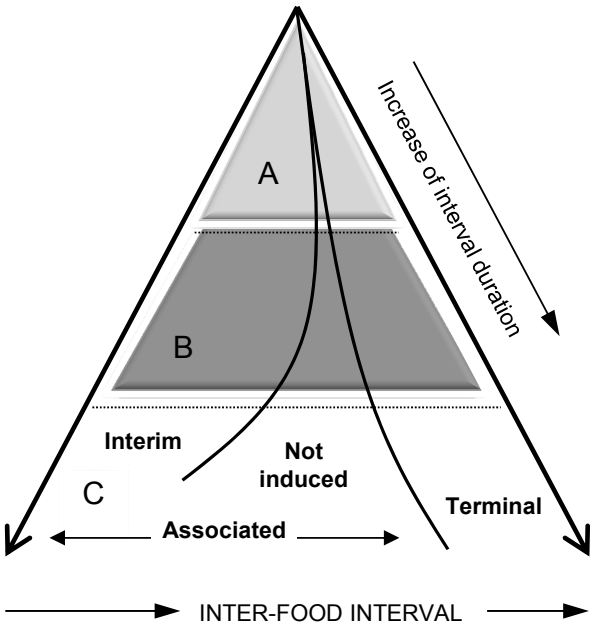


Figure 1. Relationship between inter-food interval duration, and the proportion of the interval that is occupied by different behaviours.

Interim activity may be any response of the same behavioural system activated by the primal reinforcer, among all possible in the experimental scenario, e.g.

drink water with respect to SIP. It is important to note that, interim activities, which are induced by a primal reinforcement, are not reinforced by them. Only an operant response, if any, would be reinforced by this event.

When interim activity becomes quantifiably excessive, one would then be talking of adjunctive behaviour. Excessiveness, in fact, is the principal characteristic that defines adjunctive behaviour, distinguishing it from the rest of possible interim activity.

Although several types of adjunctive behaviour have been observed, such as wheel-running in rats (Levitsky et al., 1968) and induced attack in pigeons (Looney et al., 1982), SIP has become prototype of adjunctive behaviour, having received the largest share of behavioural and neuropharmacological research (see Pellón, 1990, 1992, and Flores and Pellón, 2001 for a revision).

SIP has been observed in a wide range of behavioural schedules, including both interval and time reinforcement schedules (Falk, 1966, 1967). SIP acquisition occurs even regardless of an instrumental control of the behavioural schedule (Falk 1971; Jacquet, 1972; Cohen, 1975), therefore, it has been difficult to define whether adjunctive behaviour, and so SIP, is based in a Pavlovian or an operant learning process (Segal, 1972; Staddon, 1977; Lucas et al., 1988). But, since variables involved in the development and maintenance of SIP can be manipulated, in the same way that they do in operant behaviour, an instrumental structure underlying SIP is undeniable (Killeen and Pellón, 2013).

One determinant variable in SIP, inter-food interval duration, can be manipulated obtaining an inverted U-shaped pattern (Falk, 1961). This pattern represents optimal SIP development against variation of available time to behave between food deliveries. There have been reported optimal ranges of SIP

development from 15- to 60-s between food deliveries, and maximal levels with 30-s (Castilla and Pellón, 2013). Probability of SIP occurrence decreases both above and below this range. E.g. as inter-food interval is reduced by modulation of the reinforcement rate terminal responses tend to occur earlier, leaving less available time to the associate behaviour. This can also be observed in Figure 1, as one move upward from the area C to the area A.

It has also been reported that not all animals adhere with equal strength to SIP, and not whatever schedule where SIP development may be possible generates induced drinking. Actually, variability between animals is a common result in SIP procedures, and this criterion can be used to compare between high versus low drinkers subjects (Moreno et al., 2010 and 2012). This source of variability, principally based on individual differences across animals, can be reduced if needed by using strong schedules, that is, behavioural schedules where environmental conditions disposition is increasing the most the probability of SIP occurrence, e.g. when relatively short inter-food intervals, as described, are used. In these conditions animals emit induced behaviour more vigorously, to the point of stereotyping activities, and also reducing variability (Innis et al., 1983). Otherwise, idiosyncratic characteristics of each animal may play their own role in variability observed in SIP, especially when experimental conditions are not strong enough to hide these individual differences.

This high or low affinity for SIP acquisition, in turn, has let investigators to group animals by their SIP performance both before and after, e.g. pharmacological treatments or behavioural procedures (López-Grancha et al., 2006; Moreno et al., 2010). Therefore, SIP can be used as a tool with which relate

different features present in animals. Thereby, SIP can be used both as a diagnosis task and as an acquisition criterion, as will be explained below.

2. SIP as a model of excessive behaviour

In view of its characteristics, adjunctive behaviour has been study in the framework of a model of excessiveness. SIP has been used with success as criterion for selecting experimental subjects displaying characteristics associated with excess behaviour (Riley et al., 1989; Gilpin et al., 2008), and other disorders where the impulse control fails (López-Grancha et al., 2008) as addictions (Myracle et al., 2005), obsessive compulsive behaviour (Plat et al., 2008; Van Kuyck et al., 2008; Moreno et al 2012 a and b), and schizophrenia (Hawken et al 2011 a and b, 2013).

Several common criteria have placed SIP as an experimental model of such diseases: firstly, such induced drinking consists on an excessive expression of a normal behaviour, and secondly, SIP does not have, apparently, any function or utility for the animal (Falk, 1977). Third criteria is restricted to effects of the pharmacologic, and in a lesser degree behavioural, treatments for control of any disease in which drugs shows similar effects if are applied to both the disease and SIP. On one hand, the excessiveness and absence of a clear function, typical of SIP acquisition, mimics some symptoms of these diseases, and on the other hand, suitable matches in the pharmacologic treatments are used as markers.

Some SIP studies instead of modelling a disease, take SIP performance, but only in relation to any symptom or characteristic involved in it, thus, e.g. have been

reported higher levels of schedule-induced polydipsia in rats with characteristics related to impulsiveness (Cardona et al., 2011), that moreover tends to be a shared symptom of most of diseases related with impulse control.

3. Impulsivity and excessive behaviour

Impulsivity results in a wide spectrum of behaviours which do not always combine in the same way in all individuals. Efforts to arrive at a single, comprehensive definition of impulsivity have basically gone to show that this is no easy task. Although impulsivity can be described as a characteristic of the normal personality, when levels of impulsiveness prove extreme they are associated with psychiatric disorders such as attention-deficit hyperactivity disorder (ADHD), mania, substance abuse and some personality disorders.

By pooling different studies and the results of questionnaires, an attempt has been made to divide impulsive behaviour into various types which, to a lesser or greater extent, successfully bring together the principal factors that intervene in impulsive behaviour and shape the concept of impulsivity as a whole (see, for example, Ainslie, 1975; Barrat et al., 1994).

Behavioural measures of impulsivity can be obtained because humans and other animals acting impulsively pay less attention when performing tasks, committing more mistakes and omissions, and they finally take precipitated decisions when the task requires development of self-control. Impulsivity groups together different aspects as, deficit in inhibitory control, intolerance to delay in reward, precipitated decisions and short attention span (Evenden, 1999), hindering its description since any of these aspects can be found in the

behaviour of both impulsive and non-impulsive subjects. To solve this, have been proposed definitions of impulsiveness to enable a distinction between impulsive behaviour and so called “real impulsivity”, that is, when individuals that behave impulsively are aware of the consequences ensuing from their actions.

This real impulsivity, in turn, can be divided in two types: on the one hand there is "motor impulsivity", in which behavioural excess is mainly to blame for the consequences, and on the other there is "cognitive impulsivity", which entails precipitated decision-making that tends to lead to undesired consequences for the individual (Brunner and Hen, 1997). Yet, despite this division, excessive behaviour, as found in SIP, will in every case have a motor component and a cognitive component.

One way of measuring cognitive impulsivity directly is the self-control task (Mazur, 1987), a task of choosing between a small immediate reward and another delayed reward of greater magnitude. This delay-discounting task is ideal for taking direct measures of cognitive impulsivity, since each choice is marked by a single response. By restricting performance to one decision (a single response) that resolves each test, only the cognitive component of impulsivity is measured (Winstanley et al., 2006). In this type of test, any increase in the delay that precedes delivery of the reinforcer of greater magnitude leads to a decrease in the frequency with which this is chosen and to a corresponding increase in the frequency with which the immediate reward of lower magnitude is chosen. As the delay giving access to the larger reward becomes increasingly longer, choices of this type tend to decrease among impulsive subjects, with an ensuing increase in choices of an immediate reward

of lower magnitude, in comparison to the performance of those, non-impulsive subjects, which show self-controlled choices, and take longer to suffer the effects of the delay on their choices.

4. The Spontaneous Hypertensive Rat

The Spontaneously Hypertensive Rat (SHR) is used as an animal model, both of essential hypertension in non-obese humans (Okamoto and Aoki, 1963), and of resistance to insulin (Swislocki and Tsuzuki, 1993), and possesses traits that tend to come together in impulsive behaviour (Williams et al., 2009; Wooters and Bardo, 2011). In fact, SHR rats accomplish principal characteristics that define ADHD: impulsivity (Evenden and Meyerson, 1999), inattention (Berger et al., 1998) and hyperactivity (Sagvolden et al., 2000), even this animal have been validated as a model of the disease (Adriani et al., 2003; Bizot et al., 2007; De la Peña et al., 2010; Roessner et al., 2010; Langen y Dost, 2011; Meneses et al., 2011), with further evidences of representing better the hyperactive subtype of ADHD (Alsop, 2007 a and b; Sagvolden et al., 2009; and Sutherland et al., 2009), and this accurately predicts the behavioural excess displayed by these animals.

SHRs' hypersensitivity to delay in the reinforcer becomes manifest on their being required to wait before emitting the response sequence to the behavioural criterion, whether by training in low response rates or the introduction of a delay in order to obtain the reinforcer (Evenden and Meyerson, 1999). Impulsivity in SHR rats is manifested in the number of failures committed where it is essential that the behaviour be inhibited in the task. In such self-control situations, SHRs display inattention and impulsivity. Due to their

hyperactivity, these animals' behaviour turns principally on behavioural excess which, in one way or another, eventually manifests itself as impulsive behaviour.

In comparison with its normotensive Wistar Kyoto (WKY) control, the SHR exhibits uses to show impulsivity (Fox et al. 2008; Sanabria and Killeen 2008). Medically speaking, the WKY rat is a normotensive control for the SHR (Festing, 1979) but it has not shown itself to be a good behavioural control in all studies. One of the main characteristics of these animals is their inactivity; another is that they tend to show resistance to antidepressants, which has led WKY rats to being validated as an animal model of post-traumatic stress (Will et al., 2003) and depression (Solberg et al., 2001).

Another important fact about WKY rats is that they display limited genetic concordance with the SHR, which differs depending on the laboratory of origin (Paré et al., 1997). Specifically, comparison between SHR/Crl and WKY/Crl rats (both from Charles River) yields a genome concordance of 76.6%, but when the former are compared to WKY/Hsd rats (from Harlan) this percentage concordance falls to 66.5%. The WKY/Crl rats display neither the hyperactivity nor the impulsivity observed in SHR/Crl rats (Berger and Sagvolden, 1998; Clements and Wainwright, 2006; Sagvolden et al., 2008). WKY/Crl rats also show less sensitivity to delay in the reinforcer than do SHR/Crl rats (Fox et al., 2008; Pardey et al., 2009).

5. Dopaminergic divergences in SHR rat and their relation to SIP

The mutation found in SHRs is known to reduce the effectiveness of dopamine active transporters (DAT) and give rise to dopaminergic hyperfunction in such animals, which is evinced as an increase in learning rates dependent on temporal differences. These animals maintain excessive predictions of reward, which crystallise in their behaviour in the form of hyperactivity. Furthermore, these rats tend to make excessive use of the most recent information in preference to other longer-term information (Williams et al., 2009).

At compared to WKY rats, SHR exhibits an increased D_1-receptor expression in the cortex, striatum, nucleus accumbens and in the olfactory bulb (Díaz and Castellanos, 2006). WKY rats, by their own, display lower DAT levels in the caudate putamen from early ages, well before hypertension appears in the SHRs (Watanabe et al., 1996). In addition to this, implications of the dopaminergic system in ADHD disorder, of which the SHR rat is animal model, can't be left to point.

Several theories are in competition for an explanation of ADHD. There has been suggested that: a response against an environment with low stimulation (Zentall and Zentall, 1983), a maturational process (El-Sayed, et al., 2003), delay aversion (Sonuga-Barke, et al., 1992), or deficits in behavioural inhibition (Nigg, et al., 2005) could be responsible. In addition, a dual pathway theory combines these last two factors, but providing independent contributions on the development of the disorder (Sonuga-Barke, et al., 2002).

Some other theories point to the dopaminergic system as the key of ADHD, at today these theories have the most empiric evidence. A "Dynamic Developmental" theory predicts reduced tonic dopamine levels for individuals with ADHD (Johansen, et al., 2002), and the "Dopamine Transfer Deficit" theory proposes that the transfer of the dopamine release from the reinforcer to the predictive cue is deficient for ADHDs (Tripp, et al., 2008). At resume: "dopamine hypothesis" are the working hypothesis of ADHD, and their usefulness is based on the decrease of symptoms by psychostimulant drugs acting at dopaminergic synapses (methylphenidate (MPH), d-Amphetamine (D-AMP) and Pemoline) (Carey et. al., 1998).

Dopamine receptors are expressed throughout the brain in specific but overlapping areas. These receptors are classified into two subfamilies, D_1- and D_2-like receptors; D_1 receptors show low affinity for dopamine, but D_2 receptors has a high affinity for it (Cooper et al., 2002).

There have been suggested an increased surface expression of DA transporters in SHR at compared to WKY rats (Miller et al., 2012). Faster DA uptake in the ventral striatum and nucleus accumbens, and reduced release of DA in the dorsal striatum supports this idea. In addition, presynaptic dopamine receptors of SHR rats are hypersensitive and their postsynaptic dopamine receptors are hyposensitive (Fujita et al., 2003), and both D_1– and D_2–like receptors density is increased (Le Fur et al., 1981; Kirouak et al., 1993).

Dopaminergic implication in SIP development have been investigated, and the observed results mainly consist in the reduction of this behaviour (Snodgrass and Allen, 1987; Kaempf and Porter, 1987; Pellón et al, 1992; Didriksen and Christensen, 1993; Didriksen et al., 1993; Piazza et al, 1993 and

Flores, 1997). Recently, there has been suggested that D_1 receptors are related to the motor aspects of schedule-induced drinking while D_2 receptors are more involved in the motivational component, without discarding interaction effects between D_1-like, D_2-like receptors (Pellón et al., 2011).

6. The present thesis

Throughout these procedures we investigated, first, the aetiology of SIP in SHR rats by the interaction of two sources of variability, one produced by different inter-food interval durations, and moreover, introducing the variability related to the idiosyncratic characteristics of the animals, in this case, features that place the SHR rat as a model of ADHD. Finally, pharmacological modulation of SIP has supposed an approach to the treatment of the model of excessiveness researched in this project.

CHAPTER II *

Schedule-induced polydipsia in the Spontaneously Hypertensive Rat and its relation to impulsive behaviour

*This Chapter is based on the publication:

Íbias J, Pellón R. (2011) Schedule-induced polydipsia in the Spontaneously Hypertensive Rat and its relation to impulsive behaviour. Behav Brain Res; 223:58-69

Abstract

Eight Spontaneously Hypertensive Rat (SHR), 8 Wistar-Kyoto (WKY) and 8 Wistar rats, all male, maintained at 80%-85% of their free-feeding weight by controlled access to food, were exposed to a series of fixed time (FT) schedules whereby food pellets were regularly delivered regardless of the animals' behaviour. The FT values used were 9, 15, 30, 60, 120 and 180 seconds, with the order of presentation of the schedules among the animals being counterbalanced (except under the FT 120-s and 180-s schedules, which were successively presented as the last two of the series). Due to freely available access to water, the animals developed schedule-induced drinking under all FT schedules, marked by the characteristic bitonic function that relates the number of licks and amount of water drunk to the length of the inter-food interval. Wistar and WKY rats displayed maximum drinking under an FT 15-s schedule, with WKY rats registering lower quantities across all FT values. Among SHR rats, maximum schedule-induced polydipsia was observed under the FT 30-s schedule, with a rightward shift in the bitonic function compared to controls. For long FT values, the temporal distribution of licks within inter-food intervals was shifted slightly towards the right in the SHR rats. In a subsequent study, only the SHR and Wistar rats were used, and the animals were exposed to a delay-discounting procedure. The rats were faced with successive choices, in which they could choose between an immediate reward of one food pellet and another of four food pellets at a delay of 3, 6, 12 or 24 seconds. In the case of the longer delays, SHR rats chose the immediate reward of lower magnitude more often than did their Wistar counterparts, and also committed a greater number

of omissions during the forced-choice trials of the procedure. The results indicate that differences in schedule-induced polydipsia are related to indexes of cognitive rather than motor impulsivity, a finding in line with the theoretical idea that adjunctive behaviour is linked to operant reinforcement processes.

Keywords: Schedule-induced polydipsia; Food frequency; Delay discounting; Impulsivity; Reinforcement; Strain differences; Rats.

1. Introduction

Schedule-induced polydipsia is the leading prototype of a set of so-called adjunctive behaviours, all characterised by occurring in excess and without apparent benefit for the animal (Falk, 1971). This is typically seen when food-deprived rats are exposed to an intermittent food-presentation schedule, in such a way that they drink a small quantity of water immediately after consuming each food pellet, and do so regularly and persistently (Falk, 1961).

Induced behaviours by intermittent reinforcement schedules are divided into terminal and interim activities (Staddon, 1977).Terminal activities are located close to delivery of the following reinforcer, and are responses related to consummatory behaviour and the reinforcing event used. Interim activities precede terminal activities, tend to be incompatible with these and arise immediately after delivery of the reinforcer. If such types of behaviour became quantifiably excessive, one would then be talking of adjunctive behaviour. In line with these characteristics, adjunctive behaviours other than schedule-induced polydipsia have been observed, such as wheel-running in rats (Levitsky and Collier, 1968) and attack in pigeons (Looney and Cohen, 1982).

In view of its characteristics, adjunctive behaviour has been studied in the framework of an animal model of excessiveness. Schedule-induced polydipsia has been successfully used as a criterion for selecting subjects displaying characteristics associated with excess behaviour, such as those observed in impulsive behaviour. In these studies, after the rats were divided into high and low drinkers based on their levels of acquisition of adjunctive drinking, differences were found that were dependent on the surgical and

pharmacological manipulations performed on such animals. For instance, the effects of administration of amphetamine, and dopaminergic activity in general, have been shown to be different in high and low drinking rats (Piazza et al., 1993; López Grancha et al., 2006; Cardona et al., 2006; Pellón et al., 2011). These results show higher levels of schedule-induced polydipsia in rats with characteristics of impulsiveness in cases where these traits result from treatment. In turn, these findings tend to establish schedule-induced drinking as an animal model of impulsivity.

Although impulsivity can be described as a characteristic of the normal personality, when levels of impulsiveness prove extreme they are associated with psychiatric disorders such as attention-deficit hyperactivity disorder (ADHD), mania, substance abuse and some personality disorders. Impulsivity groups together a number of different aspects, e.g., deficit in inhibitory control, intolerance to delay in reward, precipitate decisions and short attention span (Evenden, 1999), and this hinders its description. In order to develop specific measures of those different aspects of impulsivity, procedures tend to minimize the effect of some impulsiveness dimension favouring the expression of another. In line with this, behavioural procedures to measure impulsivity can be divided in two non-exclusive categories: On one hand there are procedures that mainly measure behavioural motor excesses (which could be called "motor impulsivity"); on the other hand there are procedures designed to measure choice behaviour that leads to precipitate decisions (which could be called "cognitive impulsivity") (Evenden, 1999). Impulsive humans and other animals pay less attention when performing tasks, commit more mistakes, commit more

omissions and take precipitate decisions when the task requires development of self-control.

The Spontaneously Hypertensive Rat (SHR) is used as an animal model, both of essential hypertension in non-obese humans, and of resistance to insulin (Swislocki and Tsuzuki, 1993). They are hyperactive animals that show behavioural characteristics of ADHD, basically displaying an exacerbated sensitivity to delay of reinforcement (Sagvolden et al., 2005). This characteristic of impulsivity is manifested in the number of failures committed where it essential that the behaviour be inhibited in the task, as in the case of procedures that punish lever pressing in rats by initiating delays in the reinforcer. For instance, after previous lever-press training with variable-interval food reinforcement schedules, Johansen et al. (2005) introduced response-reinforcer resetting delays of 0.33 to 12 seconds and examined sensitivity to delay in the reinforcer as a measure of impulsivity in different strains of rats. As the duration of the response-reinforcer delay increased, all animals displayed lower response rates, yet this delay-of-reinforcement gradient proved more pronounced in SHR subjects than in their normotensive Wistar Kyoto (WKY) controls, thus yielding an indicator of greater impulsivity. Similarly, these same strains of rat were used to compare acquisition of lever pressing with delayed reinforcement (Hand et al., 2006). In this procedure, when a 15-s delay was introduced in the reinforcer following the criterion-based response (reset by responses on the lever during the delay interval), acquisition of lever pressing in SHR subjects was retarded, as exhibited by a lower response rate and lower asymptotic response level. Once again, lower tolerance to delays in the reward indicated greater impulsivity among SHR rats.

There are other behavioural studies which illustrate the difficulty experienced by SHR rats in showing self-control when taking precipitate decisions (Sanabria and Killeen, 2008). Here, the authors confronted SHR, WKY and Long-Evans (LE) rats with two different tasks. On the one hand, they studied inter-strain differences in a differential-reinforcement- of-low-response-rate task, whereby at least 5 seconds had to elapse between one response and the next in order for the reinforcer to be received. If this did not occur, the delay was reset. On the other hand, they subjected the rats to a task that required maintaining the lever depressed for a time in order to obtain the reinforcer, a time that increased gradually from 0.25 to 2.25 seconds. In both tasks, the SHR animals performed the task worse than did the others, displaying a deficit of inhibitory control, something that is characteristic of patients with ADHD syndrome.

One way of measuring cognitive impulsivity more directly is the self-control procedure, a task of choosing between a small immediate reward and another delayed reward of greater magnitude. This delay-discounting task is ideal for taking direct measures of cognitive impulsivity, since each choice is marked by a single response (Fox et al., 2008). By restricting performance to one decision (a single response) that resolves each test, only the cognitive component of impulsivity is measured (Winstanley, 2006). In this type of test, any increase in the delay that precedes delivery of the reinforcer of greater magnitude leads to a decrease in the frequency with which this is chosen and to a corresponding increase in the frequency with which the immediate reward of lower magnitude is chosen. As the delay giving access to the larger reward becomes increasingly longer, choices of this type tend to decrease among SHR rats, with an ensuing

increase in choices of an immediate reward of lower magnitude, in comparison to the performance of WKY controls (Fox et al., 2008).

In the present study, levels of acquisition of schedule-induced polydipsia were initially compared between SHR rats and their WKY and Wistar controls. Acquisition parameters were varied by using different fixed time (FT) schedules, and inter-strain differences under the various schedules were examined. Bearing in mind the differential characteristics of impulsiveness among these animals, the SHR group was expected to display higher overall levels of adjunctive drinking than their controls. Once this first study had been concluded, measures of cognitive impulsivity were taken in the SHR and Wistar rats: they were offered the chance of choosing between an immediate reward (one food pellet) and another of greater magnitude (four food pellets) delayed 3, 6, 12 or 24 seconds, using a delay-discounting procedure adapted from previous studies (Cardona et al., 2006; Fox et al., 2008). If the differences found between SHR rats and their controls in schedule-induced polydipsia were attributable to differences in impulsiveness, then differences of a similar type should also be observed in a previously validated test for measuring impulsivity, i.e., the delay-discounting task; and, if this was indeed the case, then data would be furnished which would contribute to schedule-induced polydipsia being viewed as a model of animal impulsivity.

2. Schedule induced polydipsia

2.1. Method

2.1.1. Subjects

We used 24 male rats belonging to three different strains - 8 SHR, 8 WKY and 8 Wistar - obtained from Charles River Laboratories (Lyon, France). On arrival at the laboratory they were 10 weeks old. They were housed in groups of four in an environmentally-controlled room with a 12-hour light-dark cycle (light from 08:00 to 20:00 hours), ambient temperature of 17 ºC to 23 ºC, and 60% relative humidity. Once habituated to the animal facility, the rats were housed singly in 18 x 32.5 x 20.5 cm transparent Plexiglas cages, with a metal grid by way of a detachable roof, which allowed for food to be deposited and a water bottle fitted.

At the start of the experiment, the rats were in their 12th week of life and had the following mean weights: SHR, 277 grams (range: 257-287 grams); WKY, 306 grams (range: 290-317 grams); and Wistar, 353 grams (range: 328-387 grams). The animals' weights were gradually reduced by controlled feeding to 80%-85% of their free-feeding body weights and were maintained at such percentage with reference to standard growth curves for each strain. Each rat was weighed daily before the experimental session, and at a minimum of 20 minutes after the session it was given the necessary supplement of food to maintain its weight within the criterion-base range. Water was freely available to all animals in their home cages. All animal care procedures were in accordance with the European Union Council Directive 2010/63 and the Spanish Royal Decree 1201/2005 for minimizing stress and discomfort in animals.

2.1.2. Apparatus

We used 8 Letica LI-836 conditioning chambers measuring 29 x 24.5 x 35.5 cm and enclosed in soundproofed housing, equipped with its own ventilation and a small observation window at the front. The front panel of each conditioning chamber was made of aluminium, the left wall of transparent Plexiglas and the remaining walls of black Plexiglas. On the exterior of the chamber' right-hand wall, a water bottle was fitted, with a spout to which the rat had access from the interior of the chamber, through a 3.2 x 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 16-bar metal grid which served as the floor and the water-bottle spout. Licks were recorded using an MED-PC application under a Windows environment. 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, between the panel's two levers, which were retracted throughout the experiment. The chambers were lit by two 3W lamps situated on the front panel at either side of the food hopper and by an indirect 25W light fitted to the interior of the soundproof housing that insulated each chamber. Exterior noise was masked by a fan that produced an ambient noise of approximately 60 dB in each chamber.

2.1.3. Procedure

The animals' weight was stabilised within the criterion-based range in their 14th week of life. Over two consecutive days, a 30-minute water-ingestion test was conducted, which began after a lump sum of 120 food pellets (5.4 grams) was deposited in each home cage. The measure of water consumption served to establish a baseline level against which subsequent consumption could be compared when the animals were subjected to the schedule-induced polydipsia procedures, which, under the FT 15-s condition delivered 120 food pellets intermittently and not as a lump sum. All animals ate all the food pellets and drank an average of 2.75 ml (SD = 1.03 ml) during the massed-control test, without any observable differences among the respective strains.

On the following day, the animals were adapted to their experimental cages for 15 minutes. Access was allowed to 30 food pellets previously deposited on the food tray but no experimental contingency was in place.

The experiment as such commenced in the animals' 15th week of life. Four FT schedules of different lengths (9, 15, 30 and 60 seconds) were used, such that food pellets were dispensed at these regular intervals regardless of the rats' behaviour. All the animals underwent all the schedules, the order of which was established by pairs of rats of each strain using a Latin square design. While exposure to the first FT schedule lasted 30 sessions, the remaining schedules were held over 15 sessions. Table 1 gives a detailed breakdown of the design used.

On completion of the first four schedules, in view of the results obtained (see below), all the animals were exposed to 15 sessions under an FT 120-s schedule.

The WKY rats whose adjunctive drinking had declined to the baseline level that separates prandial from schedule-induced drinking, were then withdrawn. The Wistar and SHR rats received a further 15 sessions under an FT 180-s schedule.

Subjects	*Order of FT schedules*					
1 & 2	***9***	***15***	***60***	***30***	***120***	***180***
3 & 4	***60***	***30***	***9***	***15***	***120***	***180***
5 & 6	***15***	***60***	***30***	***9***	***120***	***180***
7 & 8	***30***	***9***	***15***	***60***	***120***	***180***
No. of sessions	**30**	**15**	**15**	**15**	**15**	**15**

Table 1. Experimental design followed for each pair of SHR, WKY and Wistar rats in the schedule-induced polydipsia study. The FT 180-s schedule was not run for WKY rats.

In each experimental session, rats were first weighed, water bottles were filled with 100 ml of fresh tap water, and animals were always introduced in the same order. Each session lasted 30 minutes, with millilitres of water consumed and total number of licks being recorded for each rat, along with the number of licks given in each inter-food interval and every three seconds during such interval.

2.1.4. Statistical analysis

To analyse the effects of the animals' strain of origin and FT schedule component on total licks and water consumption, we used a two-way analysis of variance (ANOVA) based on a between-subjects factor determined by strain

(SHR, WKY and Wistar) and a within-subjects factor determined by repeated measures of the different schedules used (FT 9-s, FT 15-s, FT 30-s, FT 60-s and FT 120-s). To analyse the results of the FT 180-s schedule, the measures of the SHR and Wistar subjects were compared directly by means of an *F* test.

We analysed licks per minute during the three 10-minute periods into which the total experimental session was divided using a generalised linear model of repeated measures, with a new factor, namely, repeated measures of the licks in the three 10-minute parts (1st, 2nd and 3rd Part), being added to those used in the previous ANOVA (Strain and FT). The results of the FT 180-s schedule were analysed separately, using the same procedure but only with factors, Strain and Part of Session.

The distribution of animals' licking across each inter-food interval was studied using a generalised linear model with repeated measures, which enabled a two-way ANOVA on licks to be based on the animals' strain of origin and the three-second bins into which inter-food intervals were divided (3 under FT 9-s, 5 under FT 15-s, 10 under FT 30-s, 20 under FT 60-s, 40 under FT 120-s, and 60 under FT 180-s).

Statistical analyses were performed using data on the respective subject's last five sessions under each FT schedule, with the minimum level of statistical significance set at $p<0.05$. Where necessary, post-hoc analyses were performed using the Newman-Keuls test. All analyses were performed using the Statistica 7.0 software package.

2.2. Results

Figure 1 shows the mean (± S.E.M.) number of licks per minute given by each strain of rats under the different FT schedules, taking the last five sessions of each condition. The ANOVA displayed effects: for Strain [$F(2,21)=8.96$, $p<0.01$], with the WKY rats registering a lower mean value than the other two groups ($p<0.01$); for FT schedule [$F(4,84)=24.07$, $p<0.01$], with the mean under the FT 120-s proving lower than those under the remaining schedules ($p<0.01$), and the rate under the FT 60-s proving lower than those under the FT 15-s and FT 30-s schedules ($p<0.01$ in both cases); and lastly for the Strain x FT interaction [$F(8,84)=4.18$, $p<0.01$]. Post-hoc analyses revealed differences in the FT 9-s and FT 15-s schedules, with higher means for the Wistar versus the SHR rats ($p=0.02$ and $p<0.01$ respectively). The WKY group registered a lower rate of licks per minute ($p<0.05$) than did the other two strains under all FT schedules, with the single exception of Wistar rats under the FT 120-s.

Insofar as within-group comparisons were concerned, the WKY rats gave more licks per minute under the FT 15-s than under the FT 120-s schedule ($p=0.02$), without showing differences among the remaining schedules. Among the SHR rats, the lick rate under the FT 30-s was significantly higher than under the FT 9-s ($p<0.05$) and FT 120-s schedules ($p<0.01$) but with no differences vis-à-vis the FT 15-s schedule. The FT 60-s, with intermediate levels of schedule-induced drinking, only differed from the FT 120-s schedule ($p<0.05$). The Wistar rats registered a higher number of licks per minute under the FT 15-s than under the FT 9-s ($p=0.02$), FT 30-s ($p<0.05$), FT 60-s ($p<0.01$) and FT 120-s schedules ($p<0.01$); on the other hand, the lowest number of licks was observed under the

FT 120-s versus the FT 9-s (p<0.01), FT 15-s (p<0.01), FT 30-s (p<0.01) and FT 60-s schedules (p=0.02).

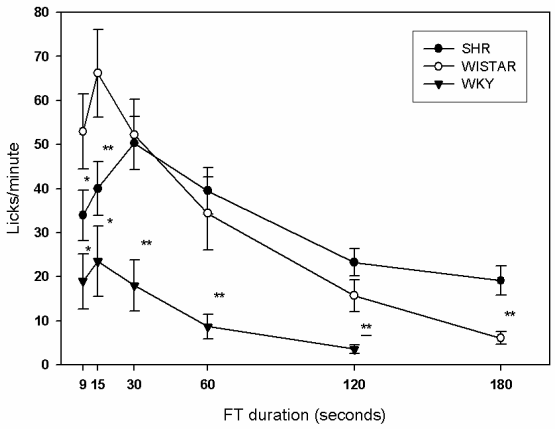


Figure 1. Mean licks (± SEM) per minute given by each group of rats under all FT schedules. Bars denote standard error of the mean.* p<0.05, ** p<0.01, ¨ p>0.05.

Under the FT 180-s schedule, the SHR group registered a higher lick-per-minute rate than the Wistar rats [F(14)=3.58, p<0.01].

Figures 2a and 2b depict: mean (± S.E.M.) millilitres of water consumed by each strain of rats in the last five sessions of each FT schedule; and this same consumption weighted by each animal's weight at the time of the session. In each figure, the horizontal dotted line indicates prandial water intake determined by the earlier home-cage-based consumption test (massed-consumption procedure). The ANOVA of millilitres consumed (Figure 2a) showed effects: for Strain [F(2,21)=14.77, p<0.01], with fewer millilitres consumed by WKY rats (p<0.01); for FT schedule [F(4,84)=27.60, p<0.01], with fewer millilitres being consumed under the FT 120-s than under the remaining

schedules (p<0.01), and more millilitres being consumed under the FT 15-s and FT 30-s than under the FT 9-s (p=0.02) and FT 60-s schedules (p<0.01); and for the Strain x FT interaction [F(8,84)=4.50, p<0.01], with Wistar rats consuming more millilitres than SHR rats under the shorter FT schedules (p<0.05 under the FT 9-s and p<0.01 under the FT 15-s schedules). In the SHR group, the FT 30-s displayed higher measures than did the FT 9-s (p<0.05) and FT 120-s schedules (p<0.01). In the Wistar group, most millilitres were consumed under the FT 15-s vis-à-vis the FT 9-s (p=0.03), FT 30-s (p=0.03), FT 60-s (p<0.01) and FT 120-s schedules (p<0.01). The Wistar rats registered lower drinking levels under the FT 120-s than under any other schedule (p<0.01) except for the FT 60-s, under which this group of rats, in turn, consumed fewer millilitres than under the FT 9-s (p<0.01), FT 15-s (p<0.01) and FT 30-s schedules (p<0.01). The WKY rats consumed more millilitres under the FT 9-s and FT 15-s than under any of the other FT schedules (p=0.02 in both cases), and also consumed more millilitres under the FT 30-s than under the FT 60-s or FT 120-s schedules (p<0.01 in both cases). The analysis that compared millilitres consumed by SHR and Wistar rats under the FT 180-s schedule showed that the SHR group consumed more [F(14)=5.40, p<0.01].

Figure 2b depicts mean millilitres consumed by each strain in terms of weight, expressed in millilitres per gram (ml/gr). This weighted measure of consumption was calculated in view of the fact that the rats' weights were very different depending on their respective strains of origin (see Methods). Effects were observed: for Strain [F(2,21)=19.77, p<0.01], with fewer millilitres per gram being consumed by WKY rats than by the other two groups (p<0.01); for FT schedule [F(4,84)=28,01, p<0.01], with fewer millilitres per gram being consumed

under the FT 120-s than under the remaining schedules (p<0.01); and for the Strain x FT interaction [F(8,84)=3.99, p<0.01], with more millilitres per gram being consumed by SHR than by Wistar rats under the FT 30-s (p=0.03), FT 60-s (p=0.02) and FT 120-s schedules (p=0.03). The WKY group of rats consumed fewer millilitres per gram than did the rest of the rats under all FT schedules (p<0.01), except for the Wistar group under the FT 120-s.

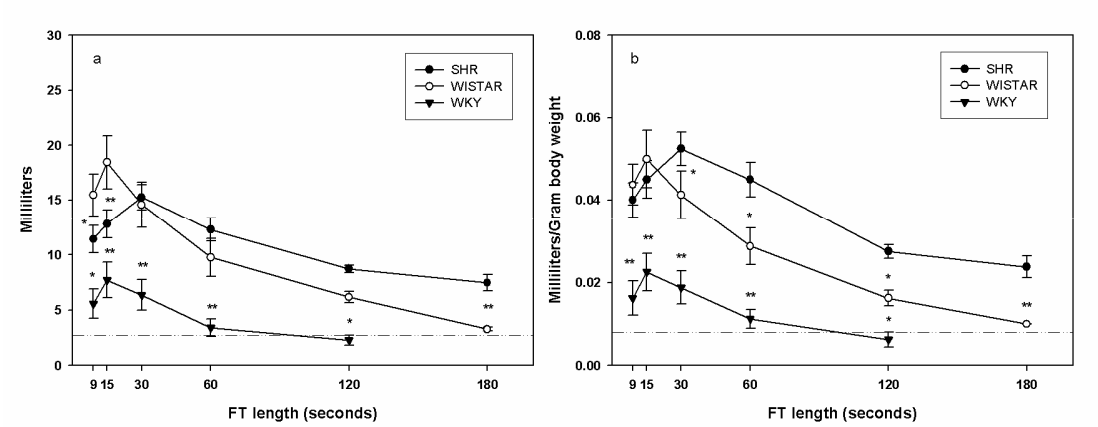


Figure 2. (a) Mean millilitres of water consumed (± SEM) by each group under each FT schedule; and, (b) mean millilitres consumed per gram weight by each group under each FT schedule. Bars denote mean standard error of the mean.* p<0.05, ** p<0.01.

In the SHR group, fewer millilitres per gram were consumed under the FT 120-s schedule than under the remaining schedules, namely, FT 9-s (p=0.01), FT 15-s (p<0.01), FT 30-s (p<0.01) and FT 60-s (p<0.01). In this same group, more millilitres per gram were consumed under the FT 30-s than under the FT 9-s schedule (p=0.01); lastly, the SHR rats displayed no differences between the FT 15-s and FT 30-s schedules, or between the FT 9-s and FT 60-s schedules.

Among the Wistar group of rats, no statistically significant differences were found as between the FT 9-s, FT 15-s and FT 30-s schedules, but differences were however found when the former three were compared to the FT 60-s ($p<0.05$) and FT 120-s schedules ($p<0.01$), and again, when the last-mentioned was compared to the FT 60-s schedule ($p<0.05$).

The WKY rats consumed more millilitres per gram under the FT 9-s and FT 15-s than under the remaining schedules ($p<0.05$ in all cases) and, in addition, consumed more under the FT 30-s than under the FT 60-s and FT 120-s schedules ($p<0.05$ in both cases).

The difference in mean millilitres per gram consumed by SHR and Wistar subjects respectively under the FT 180-s schedule proved statistically significant [$F(14)=6.14$, $p<0.01$].

Figure 3 depicts the mean (±S.E.M.) number of licks per minute given by each strain of rats under the different FT schedules during the three 10-minute parts into which the experimental session was divided. Effects were observed: for Part of Session [$F(2,42)=15.24$, $p<0.01$], with animals giving more licks during the first 10 minutes than during the remaining two 10-minute periods of the session ($p<0.01$ in both cases); for the Strain x Part of Session interaction [$F(4,42)=4.55$, $p<0.01$], with both Wistar (Figure 3b) and WKY rats (Figure 3b) giving more licks during the first 10 minutes of the session ($p<0.01$ in both cases), and SHR rats (see Figure 3a) showing no significant differences in this respect over the entire 30 minutes of the experimental session; for the FT schedule x Part of Session interaction [$F(8,168)=3.66$, $p<0.01$], with animals giving more licks during the first 10 minutes of the session under the FT 9-s, 15-s and 120-s schedules ($p<0.01$ in all cases), giving fewer licks during the last 10

minutes of the session under the FT 30-s schedule (p<0.01), and displaying no differences under the FT 60-s schedule; and finally for the Strain x FT x Part of Session interaction [F(16,168)=1.99, p=0.01], with Wistar rats giving more licks during the first ten than during the remaining 20 minutes of the session under the FT 9-s, 30-s and 120-s schedules (p<0.01 in all cases), WKY rats also giving more licks during the first ten minutes than during the remainder of the session under the FT 9-s, 15-s, 60-s and 120-s schedules (p<0.01 in all cases), and SHR rats displaying no significant differences in terms of licks given across the session under any FT schedule. Under the FT 9-s schedule, Wistar and WKY rats gave fewer licks during the last ten minutes of the session (p<0.01 and p<0.05, respectively) but SHR rats failed to show this difference.

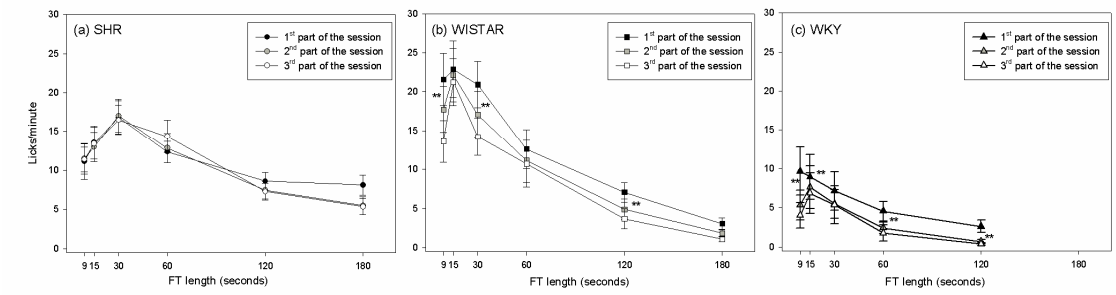


Figure 3. Mean licks per minute (± SEM) in 10-minute periods of the experimental session, for each FT schedule. Data for each strain of rats are shown separately in the following Figures: (a) SHR; (b) Wistar; and (c) WKY. Bars denote standard error of the mean. * p<0.05, ** p<0.01. (N= 24).

Under the FT 30-s schedule, SHR gave more licks during the last ten minutes of the session than did Wistar rats (p=0.03). Under the FT 120-s schedule, SHR

rats registered a higher number of licks in the middle and last parts of the session than did either of the other two groups of rats ($p<0.01$ in both cases). Under the FT 180-s schedule, no differences were observed in terms of number of licks given across the session by either the SHR or Wistar strain.

Figure 4 depicts mean (± S.E.M.) total licks given every three seconds (bins) during the inter-food intervals, for each of the FT schedules. Figure 4a compares the three strains of rat under an FT 9-s schedule. The analysis performed showed effects: for Strain [$F(2,21)=5.53$, $p=0.01$], with differences being seen between SHR rats and the other two groups, and between Wistar and WKY rats ($p<0.01$ in all three cases); for Bin [$F(2,42)=12.07$, $p<0.01$], with more licks being given in the last 6 than in the first 3 seconds of the inter-food interval ($p<0.01$); and for the Strain x Bin interaction [$F(4,42)=4.51$, $p<0.01$]. The post-hoc analyses showed differences among the three strains in the 3-s, 6-s and 9-s periods ($p<0.01$). Although the WKY subjects gave equal numbers of licks throughout the inter-food interval, these were fewer than those given by the remaining animals ($p<0.01$). The SHR and Wistar groups, for their part, gave fewer licks in the first 3 versus the remaining 6 seconds of the interval ($p<0.01$). While both groups displayed the same drinking pattern, the SHR rats registered significantly lower means for all three 3-s bins ($p<0.01$ in all three cases).

Figure 4b depicts the mean number of licks given over the course of the inter-food interval under the FT 15-s schedule. Effects were found: for Strain [$F(2,21)= 6.11$, $p<0.01$], with Wistar giving more licks than SHR and WKY rats ($p<0.05$ and $p<0.01$, respectively); for Bin [$F(4,84)=30.27$, $p<0.01$], with more licks being given in the third three seconds of the interval ($p<0.05$); and for the Strain x Bin interaction [$F(8,84)=5.72$, $p<0.01$], with all rats giving an equal number of

licks on average in the first and last three seconds of the 15-s interval. The SHR and Wistar groups gave more licks at the 6- to 12-second bins of the interval (p<0.01), with Wistar rats registering a greater number of licks than SHR rats at 9 seconds (p<0.01). The WKY group gave fewer licks than did the other rats in these central 3-s bins (p<0.01).

Figure 4c shows the mean number of licks given every three seconds during the inter-food intervals under the FT 30-s schedule. Effects were observed: for Strain [F(2,21)=7.25, p<0.01], with the WKY group giving fewer licks than the other two strains (p<0.01); and for Bin [F(9,189)=61.21, p<0.01], with the greatest number of licks being given in the third and fourth bins (p<0.01 vis-à-vis the rest). A significant Strain x Bin interaction was also in evidence [F(18,189)=4.83, p<0.01]. The highest number of licks was given at the 9- to 12-second bins of the interval, a result observed for all three groups of animals (p<0.01). SHR and Wistar rats displayed no differences in mean licks in any of the bins of this FT 30-s schedule. At the 6- to 15-second bins, the WKY rats gave fewer licks than did the other two strains (p<0.01 in all cases).

Figure 4d shows the mean number of licks given during the inter-food interval under the FT 60-s schedule. Our analysis revealed effects: for Strain [F(2,21)=4.14, p=0.03], with WKY rats giving fewer licks than the other two strains (p<0.05); and for Bin [F(19,399)=50.07, p<0.01], with more licks being given between the third and fourth bins (9 to 12 seconds) than in any other (p<0.01); as well as a significant effect for the Strain x Bin interaction [F(38,399)=3.41, p=0.02]. The post-hoc analyses showed differences under the FT 60-s similar to those observed under the FT 30-s schedule. The highest mean

number of licks in the SHR and Wistar groups was recorded at the 9- to 15-second bins (p<0.01).

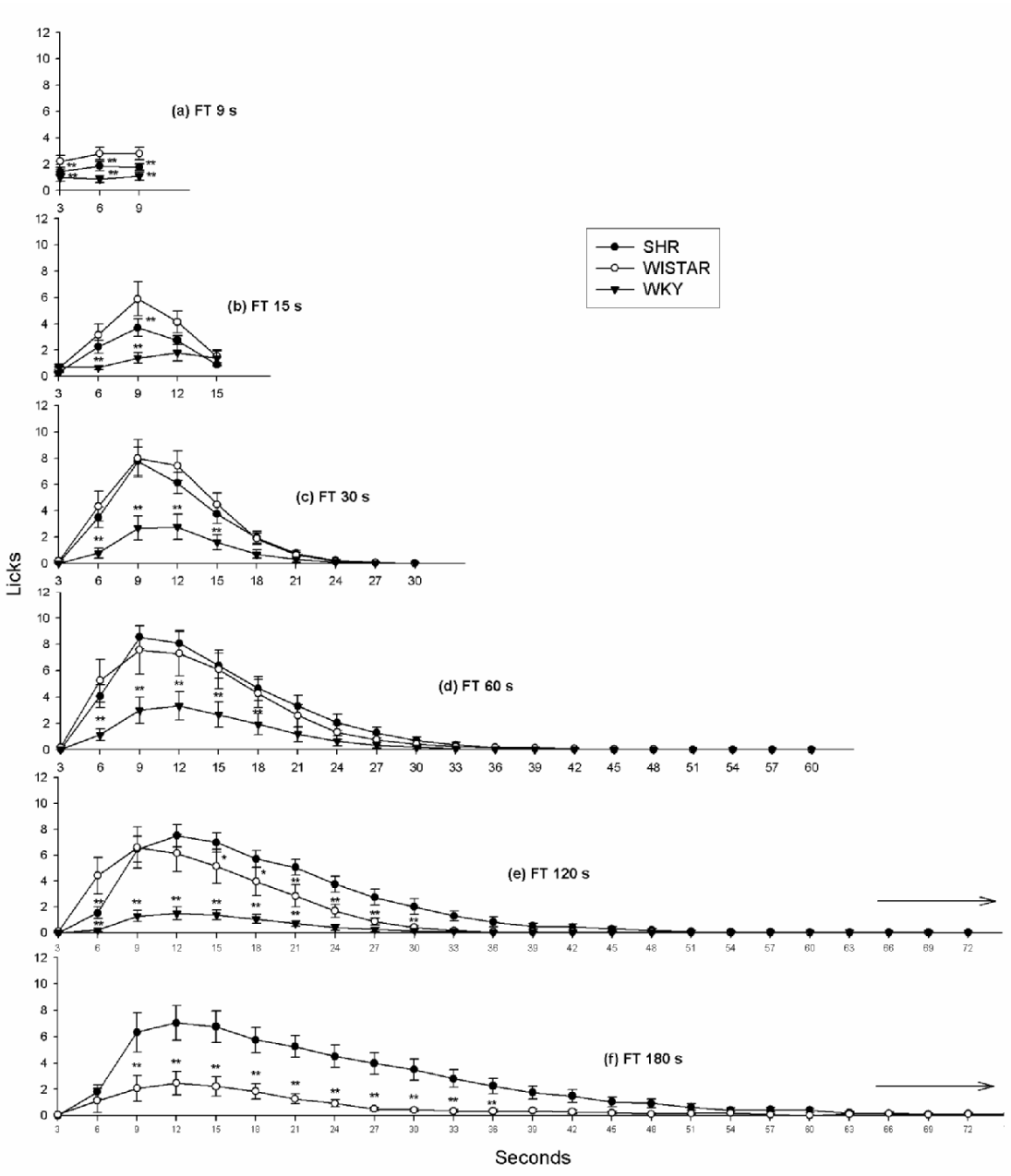


Figure 4. Mean licks (± SEM) given in each three-second bin, for each group of rats and each FT schedule. Bars denote standard error of the mean. * p<0.05, ** p<0.01. (N= 24/ N= 16 (FT180-s)).

While the SHR rats gave more licks in the third than in any other bin ($p<0.01$) and the Wistar rats reached their peak in the third and fourth bins ($p<0.01$ in both cases), as between the two groups there were no differences in any 3-s bin. Under the FT 60-s schedule, WKY rats registered a similar distribution of licks throughout the interval, with levels lower than those of the other two groups at the 6- to 18-second bins ($p<0.01$).

Figure 4e shows the mean number of licks given every three seconds in the inter-food intervals under the FT 120-s schedule. Effects were found: for Strain [$F(2,21)=10.36$, $p<0.01$], with the WKY rats giving fewer licks than the remaining animals ($p<0.01$); for Bin [$F(39,819)=52.58$, $p<0.01$], with maximum licks being located at the 9- to 15-second bins ($p<0.01$); and for the Strain x Bin interaction [$F(78,819)=8.53$, $p<0.01$], with the SHR group achieving the highest number of licks at the 9- to 15-second bins ($p<0.01$ in both cases), and the Wistar group achieving maximum licks in the third bin ($p<0.01$). At the start of the interval, at 6 seconds (2nd bin), the Wistar rats registered a higher number of licks than did the remaining animals ($p<0.01$) but as the inter-food interval progressed, the SHR licked more than did the Wistar rats, with differences in their favour at the 12- to 18- ($p<0.04$) and 21- to 27-second bins ($p<0.01$). The WKY rats recorded a lower number of licks at the 6- to 21-second bins versus the Wistar group ($p<0.01$), and at the 9- to 30-second bins ($p<0.01$) versus the SHR group. In the WKY rats, no differences among 3-s bins were observed across the entire inter-food interval.

Figure 4f depicts the mean number of licks given during the inter-food interval under the FT 180-s schedule, with only the SHR and Wistar groups being compared. Effects were detected: for Strain [$F(1,14)=13.64$, $p<0.01$], with

SHR rats giving more licks than the Wistar rats (p<0.01); for Bin [F(59,826)=26.11, p<0.01], with the highest number of licks being given at the 9- to 18-second bins versus the remaining bins (p<0.05); and for the Strain x Bin interaction [F(59,826)=8.69, p<0.01], with SHR rats giving more licks than Wistar rats at the 9- to 36-second bins (p<0.01; except p=0.01 in the 12th bin). The SHR group gave the maximum number of licks at the 9- to 15-second bins (p<0.03) and again at the 18-second bin (p<0.01), with respect to the remaining bins. The Wistar group of rats reached the maximum number of licks at the 6- to 21-second bins (p<0.03) with respect to the remainder of the interval.

2.3. Discussion

The purpose of Experiment 1 was to document schedule-induced polydipsia in the SHR rat and compares its development with that of Wistar and WKY rats, using FT schedules with different inter-food interval durations. All animals acquired schedule-induced polydipsia, and in all cases the levels of drinking varied according to the FT schedules used (Falk, 1966; Flory, 1971). Differences among the different strains of rat were found, which in some cases depended on the FT schedule. The WKY rats licked and drank less than did the remaining rats under all FT schedules. Among the Wistar and WKY rats, the maximum level of drinking was achieved under the FT 15-s schedule, a peak that shifted towards the right in SHR rats, at around the FT 30-s schedule. Level of acquisition of schedule-induced polydipsia across FT schedules shown the characteristic inverted U-shaped function related to the inter-food interval length (Flores and Pellón, 1995). Distribution of licks in the inter-food intervals

gradually acquired the bitonic form characteristic of adjunctive drinking as the interval grew sufficiently long, with drinking always peaking at the beginning of the inter-food intervals (Flores and Pellón, 1997). In the case of the WKY rats, the number of licks was invariably lower than that of the other rats across all intervals, regardless of the point of time or length. The SHR rats displayed a higher level of drinking than did the other rats in the intermediate and final portions of the inter-food intervals, particularly where these were long (FT 120-s or 180-s). This greater persistence in drinking was likewise observed in SHR rats over the course of the experimental sessions, inasmuch as they generally showed a greater number of licks in the last parts of such sessions than did their Wistar or WKY counterparts.

The initial hypothesis forecast higher levels of schedule-induced drinking for SHR rats under all schedules, a difference that, nevertheless, only started appearing as the duration of the inter-food interval became longer. SHR subjects displayed higher values than both WKY and Wistar rats under the FT 60-s, 120-s and 180-s schedules, mainly on comparing ml/gr consumed (and indeed this was also so under the FT 30-s schedule in terms of this measure).

The results for millilitres consumed and licks given displayed the same trend, i.e., as the duration of the FT interval increased, schedule-induced polydipsia decreased among controls sooner than it did among SHR rats, which were more persistent in their behaviour, despite notable decreases in the frequency of food presentation. Under the short FT 9- and 15-s schedules, SHR rats gave significantly fewer licks and consumed fewer millilitres of water than did Wistar rats.

Since the SHR rats weighed significantly less than the other rats (and the Wistar rats in particular) throughout the experiment, this characteristic might conceivably have influenced the amount of adjunctive drinking, especially under the short FT schedules during which a considerable number of food pellets were delivered in the experimental session. Under the FT 9-s schedule, a total of 200 food pellets were delivered per session, just short of double the amount delivered under the FT 15-s schedule. However, all rats drank less under the FT 9-s than under the FT 15-s schedule, which indicates that this increase in adjunctive drinking cannot be attributed to prandial drinking. The FT 9-s schedule was somewhat peculiar, in that it showed a drinking pattern different to that of all the other schedules. Under the FT 9-s schedule, the rats engaged in bursts of drinking after eating some pellets, accumulating others while doing so (non-systematic observations through chamber peepholes). The FT 9-s schedule showed an equal level of licking across the interval (see Figure 4a). The amount of water ingested could have been influenced by each strain's total intake capacity, determined by its respective body weight. Under the FT 15-s and successive schedules, regular licking episodes were observed after each food pellet was delivered, which then ended before the following food pellet was obtained (see Figures 4 and 6-10). This form of adjunctive drinking fits the initial definition of schedule-induced polydipsia (Falk, 1961), where animals drank a small amount of water after consuming each food pellet and stopped drinking in order to receive the next. As the duration of the FT interval increased, this typical schedule-induced polydipsia pattern was observed during the inter-food intervals, and it was then that differences in schedule-induced polydipsia in favour of the SHR group of rats began to appear. The

finding that SHR, despite being lighter, generally drank more than the other rats runs against the idea that drinking in this procedure is related to body weight. It could be argued that because SHR were lighter food pellets were proportionally larger, thus explaining the larger amount of water consumption solely based on prandial drinking. However, all strains of rats consumed roughly the same amount of water during the massed-food control test at the beginning of the study, thus diminishing the potential contribution of weight differences in prandial drinking.

As shown in the detailed analysis of licking during the inter-food intervals and parts of the session, under the FT schedules in which the SHR rats displayed more drinking, and under the longest schedules in particular (FT 120-s and 180-s), the differences vis-à-vis the other two strains were based on the persistence of the SHR rats' behaviour. Among Wistar and WKY rats, as the FT schedule value increased and the experimental session progressed, licks and, by extension, water consumption declined.

Results on within-session changes similar to those observed for adjunctive drinking among Wistar and WKY rats have been previously documented in operant behaviour with rats and pigeons (Bizo et al., 1998; McSweeney et al. 1995). Contrary to the results obtained with the Wistar and WKY rats, the SHR rats showed no decrease in licks as the experimental sessions progressed, in that they continued licking at a similar amount at the start and end of such sessions. This persistence in behaviour was also seen in the higher number of licks given in the final parts of the inter-food intervals, a finding in line with the greater persistence of SHR rats in levels of terminal behaviour, such as operant lever pressing or food-tray entries (Johansen and Sagvolden, 2005).

One of the principal characteristics of impulsivity is explained as an increase sensitivity to delay in the reinforcer, which translates into more pronounced delay-of-reinforcement gradients. For instance, Johansen et al. (Johansen et al., 2007) ascertained that the delay gradient in the reinforcer is more marked in SHR than in WKY rats. They used a procedure that combined different values of fixed-interval schedules, ranging from 0 to 300 s, so that SHR and WKY rats would make entries into one of 20 holes to obtain water, while entry into any of the other 19 holes might either result in no consequence whatsoever or might be followed by the emission of a noise or fluctuation in the light of the experimental chamber. The results showed greater initial variability and slower extinction of inefficient responses in SHR versus WKY rats.

One could speculate that sensitivity to delay in the reinforcer, a principal characteristic of impulsivity and one that is central to ADHD, would bring about a differential effect under the different FT schedules used in the present study. It would be logical to think that under shorter FT schedules, this sensitivity to delay in delivery of the next food pellet would act as interference in the development of schedule-induced polydipsia and, indeed, under the FT 9-s schedule the SHR rats gave fewer licks than did their Wistar controls. According to Johansen et al. (Johansen et al., 2007), one could predict that, on displaying a more pronounced delay gradient than their controls, SHR rats would necessarily be conspicuous in the distribution of behaviours that occur across the inter-food interval, with this depending, moreover, on the precise duration of the interval. Under the shorter FT schedules (9-s and 15-s), SHR rats would attain lower levels of schedule-induced polydipsia, owing, above all, to the interference produced by the high level of food-anticipatory responses.

Under the longer FT schedules (60-s, 120-s and 180-s), the decline in levels of schedule-induced polydipsia among Wistar or WKY rats could be due to the fact that these animals might be engaging in other behaviours during the inter-food interval by way of terminal (food-related) activities rather than interim drinking. Under these long schedules, with low food frequency, the SHR rats, for their part, would display little terminal activity, thus leaving more time for expression of adjunctive drinking.

It would be ideal if the relationships between characteristics of impulsivity and development of schedule-induced polydipsia could be validated in an independent test of impulsivity, using the same animals as before. A measure of impulsivity that has shown itself sensitive to SHR rats is the delay-discounting task (Fox et al., 2008). In this task, animals are trained to discriminate among rewards of different magnitudes, with measures being taken of the percentage of reinforcers of greater magnitude chosen by the animals as it is increased the delay for their obtain from the occurrence of the choice response. In a task of this type, impulsive behaviour is reflected when animals, faced with an increasing delay in attaining the reward of greater magnitude, opt instead to secure an immediate reward of lower magnitude.

3. Delay discounting

In this experiment, an adaptation of the delay-discounting procedure was conducted in order to ascertain whether the SHR rats used in the above-described experiment would display greater impulsivity than the Wistar rats, in line with previously published results (Fox et al., 2008). If this were indeed to be

the case, then the differences in schedule-induced polydipsia observed in Experiment 1 would be more clearly linked to differences in impulsivity between SHR rats and their controls. For this experiment, the WKY rats were not used because they had aged more prematurely than the others. WKY rats are reported to be hyperresponsive to stress and prone to stress ulcer, being this particularly true for rats provided by Charles River (Paré and Kluczynski, 1997), as in the present study. Four of our WKY rats presented health problems related to gastric ulcers, and serious lesions because of an abnormal teeth growth were found in another WKY rat. Similarly, one SHR rat had an unrecoverable loose of weigh and symptoms of severe rigidity before the beginning of Experiment 2, therefore it was also discarded.

3.1. Method

3.1.1. Subjects

This second experiment was conducted with SHR and Wistar subjects that had taken part in the first experiment. Specifically, we used 7 of the SHR and the 8 of the Wistar rats. At the commencement of the procedure, the animals were in their 57th week of life. The SHR rats had a mean weight of 354 ± 12 grams, while that of the Wistar rats was 431 ± 20 grams. The animals ended the experiment in their 64th week of life.

During this procedure, the animals continued to be housed under the same conditions as in the first experiment, i.e., in the same home cages, in the same room, with the same ambient temperature and humidity, and following the

same light-dark cycle. We also continued using the same 80%-85% food-deprivation criterion with respect to animal's ideal age-based weight.

3.1.2. Apparatus

Although the same conditioning chambers were used as in the schedule-induced polydipsia procedure, in this second experiment the water bottles were withdrawn and the two levers available in each chamber 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, by pressing each lever after it had been inserted into the conditioning chamber. Lever pressure required a force of approximately 0.5 N.

3.1.3. Procedure

Pre-training. Firstly, sessions were held for autoshaping of the right and left lever responses, thereby ensuring that the rats would approach and engage in lever pressing. During these sessions, the cue light situated above the levers was switched on for five seconds, and a food pellet was delivered as soon as it turned off; two seconds later, the light went on again, thus starting a new cycle, until a total of 100 food pellets had been delivered. The cue lights above each lever were presented sequentially, with all the animals beginning on the right lever. Thereafter, training on each of the levers was given using the same sequence with continuous reinforcement, until 50 reinforcers, first on the right

and then on the left, had been obtained on the first day, with this order then being alternated on subsequent days. Under this schedule, each lever press was immediately followed by a food pellet. At the end of four sessions, all the animals successfully managed to complete the schedule on both levers.

Training. During training, the retraction of the levers was activated. Five sessions of 60 trials each were run. When each trial began, the ambient light of the experimental chamber turned on, as did the cue light situated above the lever corresponding to the trial. The light indicated that the retraction of the lever had been deactivated, thereby allowing the animal to press it to obtain a food pellet. Thirty trials on one lever were interspersed with thirty on the other, randomising their presentation. The rat had 10 seconds to respond before the retraction of the lever was reactivated, the lights went off and the experimental chamber underwent a fixed 60-s inter-trial interval (ITI) that also run during the Delay Discounting procedure (see below). If no response was forthcoming during a trial, this counted as an omission.

Delay discounting. Once they had been trained, subjects received 30 sessions of 60 trials in blocks of 5 consecutive days, followed by two rest days. As in Experiment 1, the chambers were programmed in pairs, with the delayed reward being scheduled on the right lever in half the experimental chambers (chambers 1, 2, 5 and 6) and on the left lever in the other half (chambers 3, 4, 7 and 8). During each session, 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. The forced-choice trials began with the ambient light of the experimental chamber and the cue light situated above the lever to be pressed being turned on. As soon as the lever was inserted and the light was switched on, the animal

had 10 seconds to make a response. If it failed to do so, the trial was recorded as an omission and the next trial began. If the rat responded on the "immediate" lever, it received one food pellet, the lights went off and the chamber was returned to the 60-second ITI until the following trial. On the other hand, if the rat's response involved a delayed choice, then, once the response had been made, the lever had been retracted and its corresponding cue light had gone off, the ambient light remained on until the delay elapsed and four food pellets were received: then the ambient light went off and the chamber again returned to the 60-second ITI.

Apart from the ambient light, the lights situated above the levers were also turned on during the choice trials, indicating the animal's access to the choice between the two levers. One the response had been made, both levers were again retracted and the lights situated above them were turned off. If the "immediate" lever was chosen, one food pellet was delivered and the ambient light went off, signalling an end to this trial and passing on to the next trial after the ITI. If the decision was to press the "delayed" lever, however, the ambient light stayed on until the four food pellets had been delivered on conclusion of the delay, followed by the ambient light switching off and the ensuing ITI.

The rats were submitted to the delay-discounting procedure for 6 weeks. During the first two weeks, the delay value was set at 0 seconds, being therefore immediate both the large and the small food magnitudes. Thereafter, the remaining blocks of five sessions were randomised, resulting in an order of presentation of the delayed reward of greater magnitude at values of 6, 12, 3 and 24 seconds.

3.1.4. Statistical analysis

Proportion of delayed choices of greater magnitude. For analysis purposes, we took the mean number of delayed choices made by each animal during the last two sessions of each 0-, 3-, 6-, 12- and 24-second delay. Based on these data, the proportion of choices of the lever which offered the delayed reward of greater magnitude was used as the measure of this dependent variable. To ascertain statistical significance, two-way ANOVA tests were performed, with one factor being strain of origin (SHR or Wistar), and the other comprising repeated measures of the different delays presented (0, 3, 6, 12 and 24 seconds).

Omissions during forced-choice trials. We recorded the omissions committed by the rats at each of the delays used in the last two sessions of the forced-choice trials. These data were pooled for analysis purposes according to: animals' strain of origin; the designated delay; and whether the omission had taken place in a forced-choice trial that gave the option of an immediate or delayed reward. The significance of the differences was ascertained by means of a 3-way ANOVA which, in addition to the factors of strain and repeated measures of omissions at the respective delays, introduced a third factor, denoted "type of omission", that indicated whether the omission had taken place in a forced trial involving immediate or delayed choice. The minimum level of significance was set at 0.05 and the post-hoc analyses were performed using the Newman-Keuls test.

Hyperbolic discounting curve. A curve was fitted for the data obtained, using the least squares method and the following formula (Mazur, 1987):

$$Y = \frac{A}{1 + KD}$$

where *Y* corresponds to the mean proportion of delayed choices, *D* corresponds to the designated delay, *A* is a free parameter that marks the start of the curve (asymptote), and *K* is a parameter that reflects the steepness of the discount function. For all values of the curve, the value of *A* corresponded to the mean proportion of delayed choices under the 0-second condition as the curve start point. A very steep discount curve would supposedly reflect impulsivity. The more steep the discount curve, the higher the value of *K*; accordingly, if SHR were more impulsive than Wistar rats, they would necessarily show higher *K* estimates.

3.2. Results

Proportion of delayed choices of greater magnitude. Figure 5 depicts the mean (± S.E.M.) proportion of delayed choices made by SHR versus Wistar rats, by reference to the delays set at 0, 3, 6, 12 and 24 seconds. Effects were observed: for Strain [$F(1,13)=4.91$, $p<0.05$], with SHR rats choosing the delayed reward of greater magnitude less frequently than their Wistar counterparts; for Delay [$F(4,52)=23.21$, $p<0.01$], with delayed choices in both groups decreasing as the delay in receiving the reward of greater magnitude (four food pellets) grew longer; and for the Strain x Delay interaction [$F(4,52)=2.68$, $p<0.01$], with the SHR rats choosing the delayed lever less frequently at the 12- and 24-second

delays than did the Wistar rats (p<0.01 in both cases), differences that were not observed at the 3- and 6-second delays.

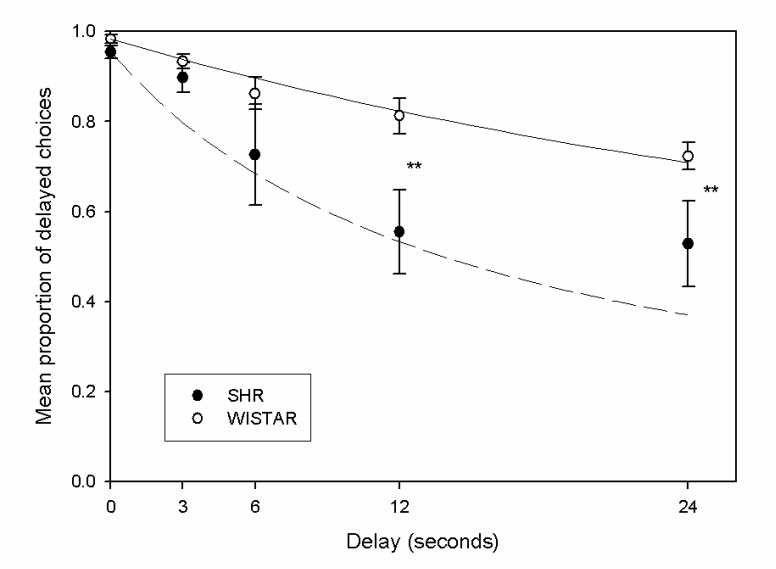


Figure 5. Mean percentage of delayed choices (± SEM) in each group, according to the delays established for rewards of greater magnitude. Bars denote standard error of the mean. Lines correspond to the discount function that best fits the data, the unbroken curve represents the fit of the Wistar group and the dashed curve the fit of the data for the SHR group. For the purpose of a more accurate fit, the delay value of 0 was set at 0.5. ** p< 0.01. (N= 15).

Omissions during forced-choice trials. Figure 6 shows mean (± S.E.M.) omissions committed by each strain in all forced-choice trials across the procedure. We observed an effect: for Strain [F(1,13)= 9.89, p<0.01], with SHR rats committing more omissions than Wistar rats; for Delay [F(4,52)=11.15, p<0.02], with omissions increasing as the delay became longer; and for the Strain x Delay interaction [F(4,52)=3,03, p=0.02], with SHR rats omitting more trials than the Wistar rats at the 6-, 12- and 24-second delays (p<0.01 in all cases). At the 0- and

3-second delays, however, no differences were observed between the rat strains in terms of number of omissions.

With regard to the type of forced trial omitted, the more omissions seen by SHR were on the 6-second delay condition on forced-choice trials of immediate reward and on the 12- and 24-second delay conditions on forced-choice trials of delayed reward. There were no omissions on free-choice trials at any delay condition.

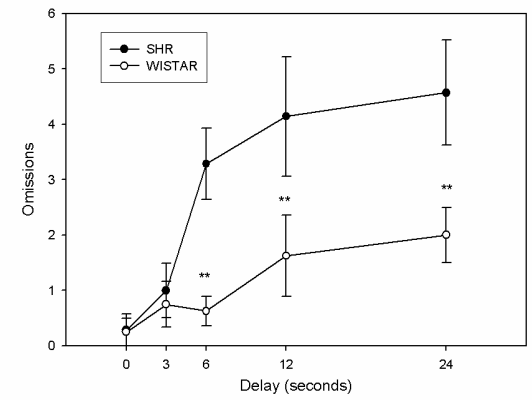


Figure 6. Mean omissions (± SEM) committed during forced-choice trials, according to strain and designated delay. Bars denote standard error of the mean. ** $p < 0.01$. (N= 15).

Discounting curve. The estimates of the parameters of the hyperbolic discount curve after using Equation 1 were as follows. The parameter *K* was almost four times higher for subjects in the SHR group (0.07 ± 0.16) than in the Wistar group (0.02 ± 0.03), while the values of *A* proved similar for both strains (0.95 ± 0.03 for SHR; 0.98 ± 0.03 for Wistar). The model's goodness-of-fit resulted in quantities that proved a satisfactory fit of the data with R^2 values of 0.94 for SHR and 0.99 for Wistar.

3.3. Discussion

The purpose of this second experiment was to take a validated measure of impulsivity among subjects that had previously shown differences in the maintenance of schedule-induced polydipsia during Experiment 1. In the light of the results obtained, SHR rats displayed more impulsivity than Wistar rats in the delay-discounting task, and generally are in agreement with previous findings (Fox et al., 2008). Despite the concordance in the results, the SHR rats in our study displayed a higher level of self-control during the longest delay (24 seconds) than had been reported previously: proportion of delayed choices was about 50% of the opportunities for choice when the delay in the reward of greater magnitude became excessively long in this experiment. In the results of the study by Fox et al. (2008), when this same delay was used, the SHR rats were observed to press the delayed reward solely during the forced-choice trials, reflecting a preference for the lever which delivered the immediate reward in most of the trials. Despite similar differences between SHR rats and their controls being observed in both studies, at the 24-second delay this difference was smaller in ours. This could be due to differences in procedure and the ensuing overlearning of the rats under the 0-delay (no delay) condition in our experiment, which, after 10 training sessions and a further 10 under the 0-delay condition, might have made the rats more resistance to change the larger for the immediate reward as the delay increased. Furthermore, the rats used in the reference study were eight months old and had had previous experience in two lever-press experiments in which the delay in delivery of the reinforcer was likewise increased across the sessions. For their part, the rats used in our study

completed their first year of life while they were performing the delay-discounting procedure and had previously undergone 105 schedule-induced polydipsia sessions in which they had been delivered food pellets regardless of their behaviour. Were one to speculate on a possible differential effect of the age of the rats used in our experiment on the impulsivity that they displayed, one would have to have a baseline, taken at an earlier age, in order to ascertain whether there were indeed differences dependent on the age of the animals at the date of the test. Similarly, one cannot rule out the influence exerted on our results by the schedule-induced polydipsia treatment administered during Experiment 1. Yet, no matter how much influence these differences in procedure might exert, the important thing is that, in our experiment, the SHR rats showed themselves to be more impulsive than the Wistar rats, a result identical to that obtained by Fox et al. when they compared SHR and WKY rats using a similar delay-discounting task.

Differences between the strains were observable in the variability with which they performed the delay-discounting task. This variability, which proved far lower in Wistar than in SHR rats (see Figure 5), relates to the observation that some of the SHR rats displayed a degree of resistance to delay similar to that of their Wistar controls.

This experiment's confirmation of there being a more impulsive condition among SHR rats affords support for the hypothesis that predicted greater impulsivity, in the form of a decrease in choices of a larger reward in response to an increase in delay. More data were furnished in this respect. On the one hand, the study of omissions during the forced-choice trials provided information on another aspect linked to impulsivity, i.e., attention deficit.

Notwithstanding the low number of omissions committed by the rats during the procedure, the SHR rats, being more impulsive, committed a higher number of omissions during the forced-choice trials of the task. The number of omissions, not only rose with increases in the delay leading to the reward of greater magnitude, but also varied in response to variations in this delay. In the case of the 6-second delay, the SHR rats were seen to commit a similar number of omissions with respect to the levers leading to the immediate and delayed rewards, thus contributing the omissions on forced immediate-reward trials to the overall higher number of omissions in SHR versus Wistar rats. This only occurred under the 6-second delay condition, because greater omissions of SHR for the longer delays of 12 and 24 seconds were just on forced-delayed reward trials. This pattern of behaviour might indicate a greater resistance to change the choice alternative in SHR rats, so that they seem to ignore the alternative leading to the small-immediate reward when the delay to the larger alternative choice is not excessive (6 seconds, for example) and to ignore the alternative leading to the large-delayed reward when the value of the delay becomes excessive (from 12 seconds onwards). With very short delay conditions (i.e., 3 seconds) none of the forced options (immediate versus delayed) were ignored.

In terms of the fit of the data yielded by Equation 1, with optimal levels of goodness-of-fit of the data and similar start points in both strains of rats (0 delay), higher levels of impulsivity were also estimated for the SHR rats using the free parameter, *K*, included in the equation. The hyperbole that fitted the data for the SHR rats was more steeped than that estimated for the data on their Wistar controls.

In brief, the differences between SHR rats and their WKY and Wistar controls seen in the schedule-induced polydipsia procedure were confirmed by the results in this second delay-discounting experiment, when the same SHR rats were compared to the Wistar rats.

4. General discussion

In Experiment 1, differences were observed among SHR, Wistar and WKY rats in adjunctive drinking levels induced by FT schedules that varied in the frequency of food presentation. Unlike the other rats, SHR displayed persistence in the above adjunctive behaviour when the values of the intervals under the FT schedules became longer. In Experiment 2, the same SHR rats showed themselves to be more impulsive than the Wistar rats in a delay-discounting task, suggesting that this trait of impulsivity characteristic of SHR rats might be related to the differences previously observed in schedule-induced polydipsia.

On the basis of all the above results, one can speculate a little on the links between schedule-induced polydipsia and impulsivity. On the one hand, one could discuss the role played by the SHR rats' innate impulsiveness in schedule-induced polydipsia procedure; on the other, one could endeavour to give a response as to the precise type of impulsivity that is reflected in adjunctive drinking.

In the former case, and as discussed under Experiment 1, the principal difference observed among strains in the schedule-induced polydipsia procedure was ascribable to the persistence shown by the SHR rats under the long FT schedules (60, 120 and 180 seconds). The reason then cited for the more

steep delay-of-reinforcement gradients in the SHR rats (Johansen et al., 2007) likewise fits the results of the delay-discounting procedure. When the inter-food interval in the polydipsia procedure was increased in line with increases in the value of the FT schedule, the SHR rats' steeper delay gradient explained that they might continue drinking longer due to maintenance of a lower number of competing terminal responses. By virtue of the fact that Wistar and WKY rats display less steep delay gradients, they would be expected to maintain higher terminal activity levels. Despite the absence of measures, such as food-tray entries, which could indicate the type of terminal activity performed by the animals, we nevertheless established that, after the delivery of each food pellet, the SHR rats continued drinking regularly throughout the experimental session, something that did not occur in the case of the other strains.

In the delay-discounting procedure, the SHR rats clearly showed the steeper nature of the delay-of-reinforcement gradient, not only through their decrease in the choice of larger rewards in response to increases in the delay leading to these, but, in particular, through their increase in omissions under these conditions. A trend could be observed among these animals whereby: some forced-choice trials -both immediate and delayed- were omitted in cases where the delay was not very long; and a greater number of trials, almost exclusively involving the lever leading to the delayed larger reward, were omitted in cases where the delay increased. This trend would indicate that the SHR rats ceased paying attention to this lever as the delay grew longer, discriminating less precisely between the blocks of forced- and free-choice trials. These results point in the same direction as those obtained in other studies that have also used the delay-discounting task, albeit with high and low-drinking Wistar rats (Cardona

et al., 2010), with the difference between the two studies lying in the selection of the subjects used. In the reference study, the most impulsive animals were drawn from a single population, instead of being genetically selected as in the case of the SHR rats. On the Wistar rats being divided into high and low drinkers on the basis of the median split yielded by a schedule-induced polydipsia procedure, impulsivity was studied by reference to its incidence in an *a priori* non-impulsive population. The results of that study showed a relationship between high- and low-drinking rats in polydipsia and their performance in the impulsivity task. Whereas high-drinking rats displayed greater levels of impulsivity than did low drinkers (though see (Cardona et al., 2006), in our case greater levels of impulsivity were attained by the most persistent rats in schedule-induced polydipsia. Table 2 depicts correlation indexes of rate of licking and proportion of choice for large-delayed reward, pooled for all subjects that were run in both Experiments 1 and 2 of the present report. As can be seen, increases in FT length led to increasingly negative correlations between amount of licking and level of self-control at any delay value, but correlations between the two measures were highest when both FT length and delay length were the largest. SHR rats licked more than their Wistar controls precisely at FT 120-s and 180-s, and also showed to be significantly more impulsive under 12-s and 24-s delays.

With regard to the type of impulsivity observed in schedule-induced polydipsia, adjunctive drinking could be thought to be something more than excess in motor behaviour, since it is engaged in voluntarily, its maintenance is related to delay-reinforcer gradients (though the presentation of the reinforcer is

not contingent on the behaviour), and it has parallels with substance abuse and impulse control disorders.

		FT schedules				
		9 s	**15 s**	**30 s**	**120 s**	**180 s**
Delays	**0 s**	*.371*	*.422*	*.004*	*-.061*	***-.491***
	3 s	*.171*	*.258*	*-.035*	*-.341*	***-.488***
	6 s	*.136*	*.165*	*-.097*	*-.192*	***-.407***
	12 s	*.229*	*.237*	*-.118*	*-.509*	***-.704****
	24 s	***.271***	***.255***	***-.057***	***-.428***	***-.583****

Table 2. Pearson correlations between licks per minute induced by FT schedules and proportion of delayed-reward choices in the delay-discounting task. Data obtained from all SHR and Wistar rats that undertook both procedures. * $p<0.05$, ** $p<0.01$. (N= 15)

Accepting that there are two basic forms of impulsivity, namely, motor and cognitive impulsivity (Chudasama et al., 2003; Winstanley et al., 2007), SHR rats would show themselves to be more cognitively impulsive (measured by the delay-discounting task: (Fox et al., 2008); and our results) and less motor impulsive (measured by the 5-choice test: Van den Bergh et al., 2006). The greater impulsivity (cognitive) of SHR rats means that they would drink little under schedules with short inter-food intervals and a lot under long interval schedules (compared to the less impulsive control animals). The idea that the highest level of schedule-induced polydipsia is related to higher levels of cognitive but not motor impulsivity might initially seem counterintuitive. As stated above, schedule-induced polydipsia would not appear to be simply a manifestation of behavioural excess, inasmuch as it has been noted that the amount of adjunctive drinking is related to parameters of upcoming

reinforcement (López-Crespo et al., 2004), and theoretical proposals have been advanced which link adjunctive to operant behaviour (Ardoy and Pellón, 2004; Pellón, 2008). Similarities between adjunctive and operant behaviour have repeatedly been shown at a behavioural and pharmacological level (Pellón, 2004, Pellón and Flores, 2011), so that adjunctive behaviour and behaviour governed by access to a subsequent reinforcer (measured by cognitive impulsivity tasks) might well be related, as indicated by the results reported here. Furthermore, the data on the WKY rats indicate that schedule-induced polydipsia would not seem to be a behaviour maintained by the potential reduction in anxiety generated by intermittence in food administration (an alternative explanation to that of positive reinforcement). Accordingly, despite the fact that such rats display an unusually rapid learning of avoidance which has led them to be proposed as an animal model of vulnerability to anxiety (Servatius et al., 2008), they nevertheless displayed adjunctive drinking levels which, in all cases, were lower than those of the other rats. Finally, insofar as impulsivity is concerned, experience of schedule-induced polydipsia might, in itself, also alter animals' impulsive behaviour somewhat, so that, even though SHR rats might exhibit greater cognitive impulsivity than do controls after polydipsia (our results), these differences were not as marked as when the animals had no previous polydipsic experience (Fox et al., 2008).

CHAPTER III *

Schedule-induced polydipsia in high and low impulsive Wistar versus Spontaneously Hypertensive and Wistar Kyoto rats

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*This Chapter is based on the publication:

Íbias J, Pellón R. (2014) Schedule-induced polydipsia in high and low impulsive Wistar versus Spontaneously Hypertensive and Wistar Kyoto rats. Behav Brain Res; *accepted pending revision*

Abstract

Rats belonging to three different strains (15 Wistar, 8 Spontaneously Hypertensive –SHR- and 8 Wistar Kyoto – WKY-) were used to evaluate the possible relationship between different levels of impulsivity and acquisition of schedule-induced polydipsia (SIP). We first measured the rats' levels of impulsivity by means of delay-discounting and indifference-point procedures. Secondly, development of SIP was studied under a series of fixed time 15, 30, 60 and 120 seconds food schedules, which were counterbalanced by means of a Latin-square design. Finally, we re-assessed the rats' levels of impulsivity by replicating the delay-discounting test. The findings showed that, starting from equivalent levels of impulsivity, development of SIP differed among the groups of rats. In comparison with the rest of the animals, the SHRs were observed to attain elevated drinking rates under SIP. On the other hand, the Wistar rats which had initial high impulsivity levels similar to those of the SHRs, displayed the lowest rates of induced drinking. Moreover, low levels of impulsivity in Wistar rats prior to SIP acquisition were reflected into high drinking rates. Relation of SIP and impulsivity is questioned by present results, which gives ground to the understanding of the behavioural mechanisms involved in adjunctive behaviour and its usefulness as an animal model of excessive behaviour.

Keywords: Schedule-induced polydipsia; Delay discounting; Indifference point; Impulsivity; Excessive behaviour; SHR; WKY; Wistar rats.

1. Introduction

Falk (1961) observed that, if rats were allowed free access to a water bottle under intermittent food-delivery conditions, they developed a characteristic drinking pattern in the inter-food intervals, i.e., the animals drank a small quantity of water after each food episode, with this drinking becoming excessive across the experimental session, as a result of repetition in the administration of food and prolonged experience of the reinforcement schedule (Falk, 1961, 1966) (for demonstrations of the phenomenon in our laboratory, see Flores and Pellón, 1995, 2000, Pellón and Padilla, 2013). Falk termed such excessive consumption of water, "schedule-induced polydipsia" (SIP) (since neither physiological- nor behavioural-regulation mechanisms could account for it), with this being proposed as a model of the class of behaviour known as interim (Staddon, 1077) or adjunctive (Falk, 1971).

Although other types of adjunctive behaviour have been observed, such as wheel-running in rats (Levitsky and Collier, 1968) and induced attack in pigeons (Looney and Cohen, 1982), SIP has become the prototype of animal models of adjunctive behaviour, having received the largest share of behavioural (Pellón, 1990, 1992) and neuropharmacological research (Flores and Pellón, 2001).

Polydipsic drinking has been induced in rats as a model of behavioural excess, and attempts have been made to link this behaviour to different disorders in which impulse control fails or the behaviour intensifies for no apparent reason (Piazza et al., 1993; López-Grancha, 2006). SIP has likewise been proposed as a model of addiction (DeCarolis et al., 2003) and obsessive disorder

(Woods-Kettelberger et al., 1993; Plat et al., 2008; Van Kuyck et al., 2008). Several Pharmacological studies (Cardona et al., 2006, 2011) have also evaluated differences in SIP in relation to different levels of impulsivity.

Impulsive behaviour mainly comprises deficit in inhibitory control, precipitate decision-making, hypersensitivity to delay in reward and short attention span (Evenden, 1999). High levels of impulsivity rise to an extreme in several psychiatric disorders such as mania, substance abuse or attention-deficit/hyperactivity disorder (AD/HD).

Bruner and Hen (1997) defined impulsiveness enabling a distinction between impulsive behaviour and real impulsivity, with the principal difference between these categories being that the individual that behaves impulsively is or is not aware of the consequences ensuing from his/her action. This real impulsivity, in turn can be divided in two different types: on the one hand there is "motor impulsivity", in which behavioural excess is mainly to blame for the consequences, and on the other there is "cognitive impulsivity", which entails precipitate decision-making that tends to lead to undesired consequences for the individual (Bruner and Hen, 1997; Evenden, 1999). Yet, despite this division, impulsive responses will in every case have a motor component and a cognitive component.

Impulsivity levels can be assessed prior to any procedure to obtaining a trait impulsivity measure (Perry and Carroll, 2008; Jupp et al., 2013) that, in turn, can be used as a vulnerability marker to diseases where impulse control fails, like drug dependence (McNamara et al., 2010; Broos et al., 2012)

The Spontaneously Hypertensive Rat (SHR) (Okamoto and Aoki, 1963; Swislocki and Tsuzuki, 1993) possesses traits such as hyperactivity, inattention

and elevated sensitivity to delay in the reinforcer, aspects that tend to come together in impulsive behaviour (Watanabe et al., 1996; Wooters and Bardo, 2011). This has led to this animal being validated as a model of AD/HD, a disorder in which impulsivity plays a principal role (Adriani et al., 2003; Russell et al., 2005; Bizot et al., 2007; De la Peña et al., 2010; Langen and Dost, 2011; Meneses et al., 2011). Within the AD/HD spectrum, SHRs fit into the hyperactive subtype in particular (Alsop, 2007 a and b; Sagvolden et al., 2009; Shuderland et al., 2009). In comparison with its normotensive Wistar Kyoto (WKY) control (Festing, 1979; Solberg et al., 2001; Will et al., 2003), the SHR exhibits impulsivity (Fox et al., 2008; Sanabria and Killeen, 2008).

SHRs' hypersensitivity to delay in the reinforcer becomes manifest on their being required to wait before emitting the response sequence to the behavioural criterion, whether by training in low response rates or the introduction of a delay in order to obtain the reinforcer (Evenden and Meyerson, 1999). In such self-control situations, SHRs display inattention and impulsivity. Due to their hyperactivity, these animals' behaviour turns principally on behavioural excess which, in one way or another, eventually manifests itself as impulsive behaviour.

While behavioural excess poses a problem when it comes to inhibiting, extinguishing or reinforcing conditioned behaviour at low rates, this selfsame excess might, on the other hand, promote faster learning of other behaviours. Williams et al. (Williams et al., 2009) put this hypothesis to the test by studying the ability of these animals to modify their behaviour (entering an opening in a panel to obtain water) during a VI 60-s reinforcement schedule, with reference to the duration of the immediately preceding trials. SHRs proved more capable

of increasing or decreasing their behaviour in a matter of minutes than did the WKY rats, but they nevertheless did not seem capable of reducing their behaviour permanently over a period of weeks. Furthermore, these rats tended to make excessive use of the most recent information in preference to other longer-term information (Williams et al., 2009 a and b).

Other evidence of excessiveness in SHRs is to be seen in a slower extinction of behaviours such as lever pressing (Brackney et al., 2012). This has been shown when compared to WKY rats after Fixed Interval (FI) and Variable Interval (VI) reinforcement schedules, with more unreinforced responses (because of the extinction procedure) both in the presence and in the absence of predictive cues of cancellation of the reinforcer (Johansen et al., 2007).

SHR's SIP performance have been investigated (Íbias and Pellón, 2011), after exposing the animals to a counterbalanced series of fixed time (FT) schedules (of 9, 15, 30, 60, 120 and 180 seconds) with available water to generate different levels of SIP, and they were found differences, mainly higher drinking levels among the SHRs under FT schedules with longer inter-food intervals, and higher rates among the Wistar rats under shorter schedules. Once completed this procedure, animals were trained in self-control using a delay-discounting test (Mazur, 1987; Winstanley et al., 2004; Fox et al., 2008), and in this test SHRs made more impulsive decisions and committed more omissions with delays of 12 and 24 seconds to obtain the greater but delayed reward.

This study follows on from some of the questions that arose out of the previous study. In one hand, to what extent the SHRs' impulsivity, mediated by their behavioural excess, was transferable to other impulsive but not necessarily hyperactive, subjects in SIP performance. It would be of interest to take a control

population of Wistar rats and, after screening two groups according to their measures of impulsivity, ascertain to what extent they would share the traits of impulsivity and self-control possessed by SHRs and WKY rats, serving as reference strains of respectively impulsive vs. non impulsive populations.

Moreover, it would be interesting give an account of how sensitivity to delayed reinforcer eventually appear as signs of impulsivity with increasing experience, and thus an increased learning about the delayed reinforcer. Firstly, we measured the levels of impulsivity displayed by the rats, along with value assigned by them to delay under impulsivity test conditions. Secondly, an indifference-point procedure was carried out in which the animals manipulated different delays to obtain different magnitudes of food, and estimations of the value assigned to the delay were obtained, Thirdly, we implemented a SIP procedure using FT schedules with different inter-food interval values and finally we reassessed the rats' impulsivity levels by replicating the delay-discounting test.

2. Methods

2.1. Subjects

We used 32 male rats belonging to three different strains -8 SHR, 8 WKY and 16 Wistar- obtained from the Charles River Laboratories (Lyon, France). On arrival at the laboratory they were 10 weeks old. They were housed for 15 days in groups of four in an environmentally-controlled room with a 12-hour light-dark cycle (light from 08:00 to 20:00 hours), an ambient temperature of 21ºC and

65% relative humidity. Once habituated to the animal facility, the rats were housed singly in 18 x 32.5 x 20.5 cm transparent Plexiglas cages with a metal grid detachable roof that enabled food to be deposited and a water bottle to be fitted, and were kept there without any type of experimental manipulation for 10 days.

At the end of the adaptation period, the animals' mean weight was as follows: 278.12 grams (range 266-288 grams) in the case of SHRs; 339.12 grams (range 302-358 grams) in the case of WKY rats; and 339.21 grams (range 318-373 grams) in the case of Wistar rats. Using a controlled diet, animals were reduced to 82%-85% of their free-feeding weight, which was then maintained throughout the experiment in proportion to the weight established by standard growth curves for each strain. Each rat was manipulated and weighed daily before the experimental session, and a minimum of 20 minutes after the experimental session each animal received the appropriate food supplement to maintain its weight within the criterion-based range.

Water was freely available to all animals in their home cages, and food supplements were daily calculated for every animal depending on their body weight and an estimation of the food pellets amount provided in each procedure. All procedures were carried on 7 days a week, and all animal care procedures were in accordance with the European Union Council Directive 2010/63 and the Spanish Royal Decrete 1201/2005 for minimizing stress and discomfort in animals.

2.2. Apparatus

We used 8 Letica LI-836 conditioning chambers measuring 29 x 24.5 x 35.5 cm and enclosed in soundproofed housing, equipped with its own ventilation and a small observation window. The front panel of each conditioning chamber was made of aluminium, the left wall of transparent Plexiglas and the remaining walls of black Plexiglas. During the schedule-induced polydipsia procedure, a water bottle was fitted on 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 x 3.9 cm aperture in the wall, situated 20 cm from the front panel and 7 cm from the floor (a grid made up of 16 bars). 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 was registered as licks. In procedures in which the levers were used -and only in these- the water bottles were withdrawn, and access to the levers was permitted at all times. Each chamber had two levers available, situated on the front panel and at either side of the food tray, at a distance of 4.8 cm from the latter and at a height of 4.7 cm from the grid floor. Lever presses required a force of approximately 0.5 N to be activated. Licks and lever presses were recorded using an MED-PC application under a Windows environment. 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, between the panel's two levers. The chambers were lit by two 3W lamps situated above and at either side of the front panel, and an indirect 25W light fitted to the interior of the soundproof housing that

insulated each chamber. Exterior sound was masked by a fan that produced an ambient noise of approximately 60 dB in each chamber.

2.3. Procedure

Four experiments were successively conducted. Firstly, after training the rats to press the levers situated in the conditioning chamber, a delay-discounting procedure was implemented to assess the animals' degree of impulsivity. The value assigned by the animals to the delay was then estimated by an indifference-point procedure. The next experimental block consisted of SIP acquisition under different FT schedules, and ended with a new assessment of the rats' impulsivity by re-applying the delay-discounting procedure. On concluding the experiments, we analysed some supplementary variables linked to the initial acquisition of lever-pressing behaviour.

2.3.1. Acquisition of lever-pressing behaviour

Once the animals' weight had been reduced and stabilised at 82%-85% of their free-feeding weight, the rats were placed in the conditioning chambers. A total of 30 food pellets were jointly dispensed into each food tray and were seen to be consumed by all the animals. From the next day onwards, five consecutive autoshaping sessions were held, using the levers in the conditioning chambers. The first day, 25 food pellets were administered, one every 15 seconds, regardless of the animals' behaviour. 3 seconds before each food delivery, the light situated at the side of the corresponding lever was turned on, and was

switched off coinciding with the delivery of the pellets. The fixed-ratio (FR) autoshaping procedure then began. A food pellet was delivered every 30 seconds, with its delivery coinciding with the light over the lever switching off after 3 seconds, as in the preceding session. In addition, any lever press was followed by the switched off of the light and an extra food pellet to be obtained.

This new schedule gave the option of obtaining up to 50 reinforcers (food pellets) under a continuous reinforcement schedule (FR-1). If the animal continued responding on the lever, it could manage to complete the schedule which, after coming to an end on one lever then commenced on the other. If 30 seconds elapsed without a response on the active lever, the animal returned to the autoshaping procedure with a maximum of 25 free food-pellet deliveries every 30 seconds. If, during the autoshaping procedure, the animal passed through to the continuous reinforcement schedule and completed it, it then went on to begin the same procedure on the opposite lever; in all other cases, the following autoshaping session likewise began on the opposite lever. Once all the rats had successfully advanced from autoshaping to the continuous reinforcement schedule on both levers, on the same day, the training sessions began.

Training consisted of four sessions of 50 trials each. The trials took place randomly one either of the levers and were separated from the preceding one by an inter-trial interval (ITI) of 30 seconds. At the beginning of each trial, the lights over one of the two levers (right or left) turned on. On pressing the lever signalled by the light, a food pellet was obtained, whereafter all the lights of the experimental chamber switched off during an ITI of 30 seconds, until the start of the next trial, which might be on the same or on the opposite lever. The criterion

of these sessions consisted of successfully completing 25 trials on each lever. If, following the presentation of a trial, the animal took more than 10 seconds to respond, the trial was computed as an omission and a new 30-s ITI ensued. The session came to an end when the animals had completed 50 trials plus any omissions, or alternatively, if one hour had elapsed from the start of the session. A record was kept of the number of responses and omissions, along with the time taken to complete the respective training sessions. This pre-training procedure lasted for 9 consecutive sessions.

2.3.2. Delay discounting: first assessment

Once the lever-press training had concluded, the delay-discounting procedure began (an adaptation of those used by Winstanley et al., 2004 and Fox et al., 2008). Each delay-discounting session consisted of 40 trials (24 free-choice and 16 forced-choice trials), divided into 4 blocks of 10 trials each.

During the forced-choice trials, the light above one of the levers, A or B, was randomly switched on. The trials on lever A led to a reward of four food pellets (one every 0.5 seconds) for the first lever press after a given delay. The trials on lever B led to a single food pellet being delivered immediately after the response. If the animal took more than 10 seconds to respond after the respective lever light had turned on, the lights of the experimental chamber were switched off and the following trial began, with the preceding one being computed as an omission and a record being taken of the type of trial missed by the animal. Each trial was separated from the next by an ITI of 45 seconds, plus the duration of the delay under each condition. The free-choice trials consisted

of the lights above both levers A and B being switched on, thereby permitting the animal to choose between the two. Aside from the procedure itself, the schedule included no additional lever-related contingency, with the animals being allowed to press the levers freely without any consequences.

The animals' behaviour on both levers was recorded, and the position of levers A and B was balanced as between the chambers, by assigning A as the right lever and B as the left in half the chambers, and reversing the order in the other half.

Firstly, five sessions were conducted with delay 0, with the larger reward immediately following the response on lever A. Once all the animals had achieved a lever A (delayed) preference criterion of 90% or more, an initial estimate of impulsivity was made. Five consecutive sessions were held, in which the value of the delay was doubled in each, with delay values of 1, 3, 6, 12 and 24 seconds being used to obtain the larger reward. We propose that the estimate of impulsivity based on the proportions of choice of lever A (delayed) in these sessions could be considered as a predictor of the impulsivity that the rats would display during the remainder of the delay-discounting procedure.

The preference for lever A (delayed) was re-established by means of three new sessions without any delay (0 seconds), which sufficed to return the animals to an almost exclusive preference criterion for this lever. A second assessment of impulsivity was then made, by programming three blocks of three sessions with delays of 6, 12 and 24 seconds' duration respectively. Under these conditions, the subjects would display impulsivity on the basis of what they learned about each delay, something that could be regarded as a measure

of trait impulsivity. For data-analysis purposes, only the data from the last session of each delay condition was taken into analysis.

In each session, we recorded the percentage choice of levers A and B, and the number of omissions and responses made by each animal on each of the two levers.

This first approach to the delay discounting procedure lasted for 26 consecutive sessions. Task parameters, delays and number of sessions, were chosen trying to minimize possible effects of compromise with delayed reward by an excessive training and the abandonment of the delayed lever when the delay results excessive (Ainslie, 1975; Mar and Robins, 2007), preventing then oversized measurements of respectively self-control and omissions. In turn, the evaluation was planned in two consecutive parts, in order to observe the development of the manifestations of impulsivity due to gradually experience to delayed reinforcement.

2.3.3. Indifference point

The rats were exposed to 40 sessions of 40 trials each. During each session, there were four blocks of 10 trials, with the first four in each block being forced-choice and the following six being free-choice trials.

Each free-choice trial consisted of the lights above the two levers being switched on after an ITI of 45 seconds. Whereas a press on lever B (immediate) led to immediate delivery of a food pellet, pressing the opposite lever A (delayed) led to delivery of four food pellets after the delay that was currently in

force. In the forced-choice trials, only one of the lights turned on, and response was required on the lever situated under it.

The trials in each block were randomly distributed between the two levers, and the distribution of levers A and B among boxes remained the same as in the delay-discounting procedure. On conclusion of each block of trials, the delay was automatically computed for use in the next block, based on the results of the previous six free-choice trials: while more than three choices on lever A (delayed) increased the delay by 30%, more than three choices on lever B (immediate) reduced the delay by 30%. A minimum delay of 2 seconds was established. In cases where the same number of choices had been made on the two levers, the value of the delay in the following block of trials did not vary. Each new session began using the last delay applied in the preceding session.

We measured the delays used by each animal and established an average that reflected the results of the last 100 blocks of trials (last 25 sessions). We also recorded the number of presses on levers A and B, averaging these responses from the initial session for every 20 blocks of trials (5 sessions).

2.3.4. Schedule-induced polydipsia

Four FT schedules of different length were used, namely, 15, 30, 60 and 120 seconds, so that a food pellet was dispensed at these regular intervals irrespective of the rats' behaviour. All the animals performed all the schedules, with the order being established for pairs of rats of each strain by means of a Latin-square design (after the procedure had commenced, one of the Wistar rats had to be withdrawn for medical reasons: its results in the previous procedures

were discarded, and this group was then left with 15 animals). Exposure to the first FT schedule lasted 15 sessions; the remaining schedules were held over 10 sessions. Table 1 gives a detailed outline of the design used for each pair of rats of each of the three strains, which were 10 months old at the start of the SIP procedure.

Subjects	*Order of FT schedules*			
1 and 2	***120***	***15***	***60***	***30***
3 and 4	***60***	***30***	***120***	***15***
5 and 6	***15***	***60***	***30***	***120***
7 and 8	***30***	***120***	***15***	***60***
No. of sessions	15	10	10	10

Table 1. Experimental design followed by each pair of SHR, WKY and Wistar rats in the schedule-induced polydipsia procedure, indicating in each case the order in which the FT 15-, 30-, 60- and 120-s schedules were presented, as well as the number of sessions of exposure to these. Rat 8 in the high-impulsive Wistar group had to be withdrawn from the experiment.

In each experimental session, the rats were first weighed and the water bottles filled with 100 ml of fresh tap water. Each session lasted 30 minutes, with the millilitres of water consumed and total number of licks being recorded for each rat. In the case of water intake, we recorded the millilitres consumed by each animal in each session plus the millilitres consumed in its home cage during the remainder of each of the days on which the experimental sessions were held. A combined measure was thus obtained of the total daily water intake for each rat of each strain during exposure to each FT schedule.

2.3.5. Delay discounting: second assessment

We implemented the same procedure as had been used in the first delay-discounting assessment, first subjecting the animals to four new lever-training sessions, followed by five consecutive sessions under the 0-s delay condition, and concluding with three blocks of three sessions under 6-, 12- and 24-s delay conditions.

2.4. Statistical analysis

Based on the results obtained by the group of Wistar rats (n=15) in the delay discounting procedure, a cluster analysis was used that would demarcate two subgroups of animals. Two-Step cluster method was used, and log-likelihood was the distance measure compared. For classification purposes we took the overall values of the proportion of choices on the lever that led to the delayed reward, according to the 6-, 12- and 24-s delays used. These two groups of Wistar rats were maintained for analysis of the results of the various procedures performed in this study, with comparisons being made between the two subgroups of high- and low-impulsive Wistar rats, and between these and the SHR and WKY reference strains.

Variables measured in each delay discounting task evaluation, mean proportion of choices in lever A, and mean responses in levers A and B, were analysed by MANOVA tests that included a Group factor with four levels (high- and low-impulsive Wistar, SHR and WKY), and another repeated measure with

one level for each of the delays used (0-, 1-, 3-, 6-, 12- and 24-s in case of first estimate, and 0-, 6-, 12- and 24-s in case of the two following evaluations).

In all the delay discounting tests, the data obtained were fitted using the least squares method and the following formula (Mazur, 1987):

$$Y = \frac{A}{1 + KD}$$

where Y is the mean proportion of choices of the delayed reward of greater magnitude, A is a free parameter for designing the beginning of the curve (asymptote), K is a parameter that reflects the slope of the discount function, and D corresponds to the delay used. As the value of the estimate of K increases, the curve becomes more pronounced, so that higher K estimates reflect greater impulsivity. Mean K estimations for each group were compared using several Student t tests.

To compare the delays used by each animal in the indifferent point procedure an ANOVA was performed using one factor (average delay used during the last 100 blocks of trials -25 sessions) and the Group factor. The analysis of lever presses consisted in a MANOVA performed using a two-level factor (presses on lever A or B) and a further factor (blocks of 5 sessions) with 8 levels to compare Group factor.

In SIP procedure they were analysed mean licks/minute and mean millilitres consumed during the last 2 sessions of exposure to each of the 15-, 30-, 60- and 120-s FT schedules. MANOVA tests were carried out including the Group factor and a repeated measure with four levels, one for each of the FT schedules.

Relationship between SIP and previous impulsive levels were investigated using Pearson correlation between the mean licks/minute in each of the FT schedules of 15-, 30-, 60- and 120-s and proportion of delayed choices in each of the delays of 6-, 12- and 24-s used in delay discounting test.

Additional measures, recorded throughout lever-press training, were analysed taking mean of lever-presses including both right and left levers, mean of time for lasting the training session and mean of omissions in each of the four sessions. They were carried out MANOVA tests comparing the Group factor and a repeated measure with four levels, one for each training session. Total mean of each variable was also used to investigate the possible relationship between mean licks/minute in each of the FT schedules of SIP procedure by Pearson correlations.

Post-hoc comparisons, when needed, were made using a Bonferroni correction with minimum significance set at $_{\alpha}$= 0.05. The statistical analyses were performed using the SPSS 17.0 software package.

3. Results

3.1. Delay discounting (pre-SIP impulsivity)

Cluster analysis yielded two groups of Wistar rats, defined as "low-impulsive Wistar" (n=8) and "high-impulsive Wistar" (n=7). Centroids of the combined means (±SD) in 6-, 12- and 24-s delay conditions were respectively 0.66(±0.22), 0.21(±0.17) and 0.06(± 0.04), for each group were respectively 0.45(±0.24), 0.34(±0.12) and 0.10(±0.02) for Wistar low impulsive and 0.25(±0.12),

0.05(±0.05) and 0.02(±0.02) for Wistar high impulsive. These groups differed $p<0.05$ in both 12- and 24-s delay conditions.

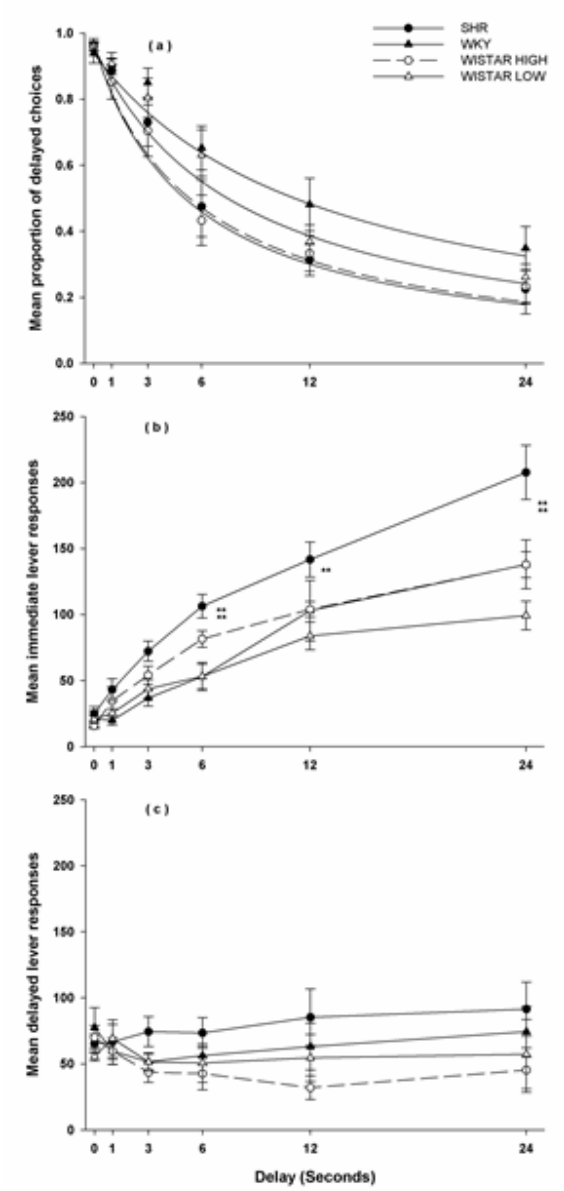


Figure 1. Results of the first estimate of impulsivity in the first delay-discounting test. Upper graphs depict the mean proportion (± SEM) of delayed choices for each strain and group at each delay: (a) compares the groups of high- and low-impulsive Wistar rats; (b) compares the SHR and WKY strains. The values 0, 1, 3, 6, 12 and 24 are the seconds of delay for gaining access to the reward of greater magnitude. Lower graphs compare the four groups' average (± SEM) lever responses: (a) immediate (lever B); (b) delayed (lever A). ** $p<0.01$. (N= 31).

Figure 1, (upper graph) depicts the results of the first estimate of impulsivity during delay-discount training, when the delay was gradually increased across 6 consecutive sessions. This graph compare the mean proportion of choices on the delayed lever (± SEM) for all groups, at all delays. Neither a Group effect nor a Group x Delay interaction was in evidence: there were only trends among the groups of WKY and low-impulsive Wistar rats to display more self-control than did the SHRs and high-impulsive Wistar rats.

Figure 1 (graphs b and c) compare the average lever A (delayed) and lever B (immediate) responses (± SEM) for all groups, at all delays used. Analysis revealed a Group effect [$F(3,27)=8.99$, $p<0.01$], with the SHRs generally registering more responses than any of the other groups. These responses were mostly made on immediate lever as revealed by a Delay x Lever interaction effect [$F(5,140)=51.35$, $p>0.01$], Increased response on the part of SHRs was observed in delays of 3-s, 6-s and 24-s ($p<0.05$ in all cases), these differences were supported by a Group X Delay interaction effect [$F(15,135)=3.25$, $p<0.01$].

Figure 2 depicts the results of the second estimate of impulsivity during delay-discounting training, when blocks of 3 sessions were maintained for any given delay. The upper plots show the average (± SEM) proportion of choices which led to the larger reward at each of the respective delays, Analysis revealed a Group x Delay interaction effect [$F(9,81)=3.37$, $p<0.01$], with the high-impulsive Wistar rats generally showing a preference for the delayed reward of greater magnitude less frequently than did the low-impulsive Wistar and WKY rats with both 12- and 24-s delays ($p<0.01$ in all cases), and the SHRs, in turn, displayed more impulsivity than did either the WKY rats under the 12-s delay

condition (p<0.03) or the WKY and low-impulsive Wistar rats under the 24-s delay condition (p<0.01 in both cases).

Analysis of the average lever B (immediate) and lever A (delayed) responses at all delays used revealed a Delay x Lever interaction effect [F(3,84)= 37.64, p>0.01], i.e., as the delay increased, the distribution of lever-A and -B behaviour changed, so that during the sessions with a 0-s delay the animals pressed lever A (delayed) more frequently (p>0.01), and during the sessions with a 24-s delay this pattern was inverted (p>0.01), but no differences related to any group were observed.

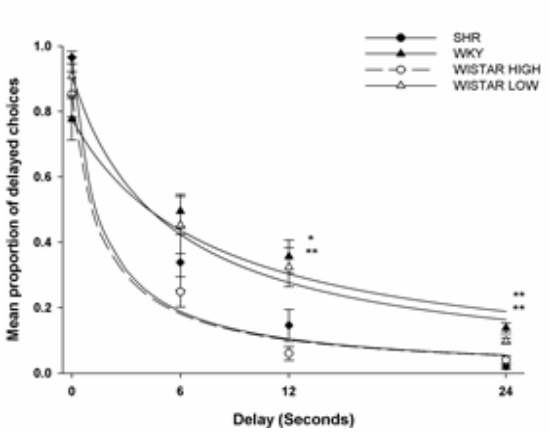


Figure 2. Results of the second estimate of impulsivity in the first delay-discounting assessment. Figure depicts the mean proportion (± SEM) of delayed choices for each strain and group at each delay. The values 0, 6, 12 and 24 are the seconds of delay for gaining access to the reward of greater magnitude. * p<0.05, ** p<0.01. (N= 31)

3.2. Indifference point

Figure 3 shows the results of the indifference-point procedure. Figure 3a corresponds to the mean delay (± SEM) used by each group of rats during the last 25 sessions of the procedure. The trend seen in the results was in line with those obtained in the first delay-discounting test, inasmuch as the WKY rats tended to use longer delays than did the Wistar rats and SHRs. The main Group effect did not prove statistically significant, however.

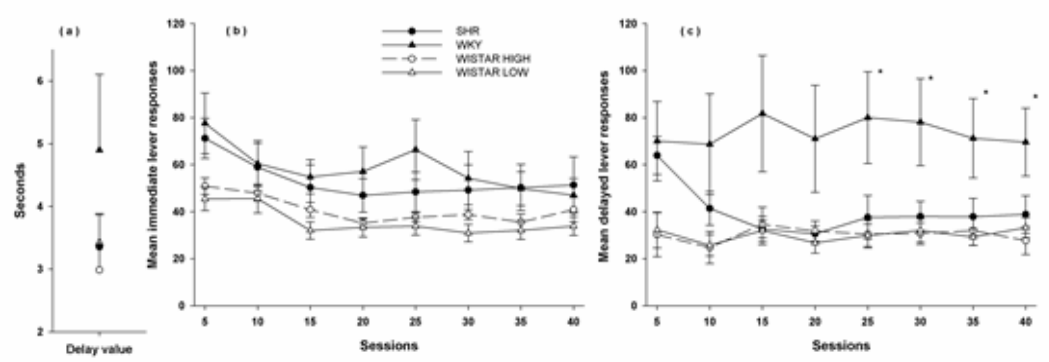


Figure 3. For each group of rats: (a) mean (± SEM) duration of the delay to obtain the delayed reward of greater magnitude across the last 25 sessions of the indifference-point procedure; (b) mean (± SEM) lever B (immediate) responses; (c) mean (± SEM) lever A (delayed) responses. *= $p<0.05$. (N= 31).

Figures 3b and 3c respectively depict the average (± SEM) lever-B (immediate) and lever-A (delayed) presses during the indifference-point procedure in blocks of 5 sessions. No group-related differences were found in lever-B (immediate) behaviour. With respect to lever-A (delayed) presses, a Group effect was in evidence [$F(3,27)=3.95$, $p<0.02$], in that the WKY rats pressed

lever A more than did the high- and low-impulsive Wistar rats (p<0.05 and p<0.04 respectively). There was neither block-of-sessions effect nor any Session x Group interaction for either immediate- or delayed-lever presses.

3.3. Schedule-induced polydipsia

3.3.1. Licks

Figure 4a depicts the average licks per minute (± SEM) for each group of rats in each of the 15-, 30-, 60- and 120-s FT schedules. Both Group [F(3,27)=7.32, p<0.01] and Schedule effects [F(3,81)=41.01, p<0.01] were observed. In general, the high-impulsive Wistar rats gave fewer licks per minute than did the SHRs (p<0.01). There was found a Group x Schedule interaction effect [F(9,81)=2.56, p=0.01]; during the FT 15-s schedule, the low-impulsive Wistar rats registered higher lick rates than did the high-impulsive Wistar rats (p=0.02). During the FT 30-s schedule only the SHRs registered higher lick rates than did the high-impulsive Wistar rats (p<0.01), but the same tendency than in FT 15-s was observed between the wistar rats groups.

During the longer FT 60- and 120-s schedules, the SHRs displayed differences vis-à-vis the rest of the animals, i.e., the high- and low-impulsive Wistar rats (p<0.01 in all cases) and the WKY rats (p=0.02 and p=0.04 respectively for each of the schedules).

3.3.2. Water intake

Figure 4b depicts mean total water intake per group (± SEM) across the different FT schedules, taking the water consumed both in the home cages and in the conditioning chamber. A Group x Schedule interaction effect was found [$F(9,81)=2.02$, $p<0.05$]. Under the FT 15-s schedule, the high-impulsive Wistar rats drank less water than did either the low-impulsive Wistar or SHRs ($p<0.01$ and $p=0.01$ respectively), and the WKY rats drank less water than did the SHRs ($p<0.04$).

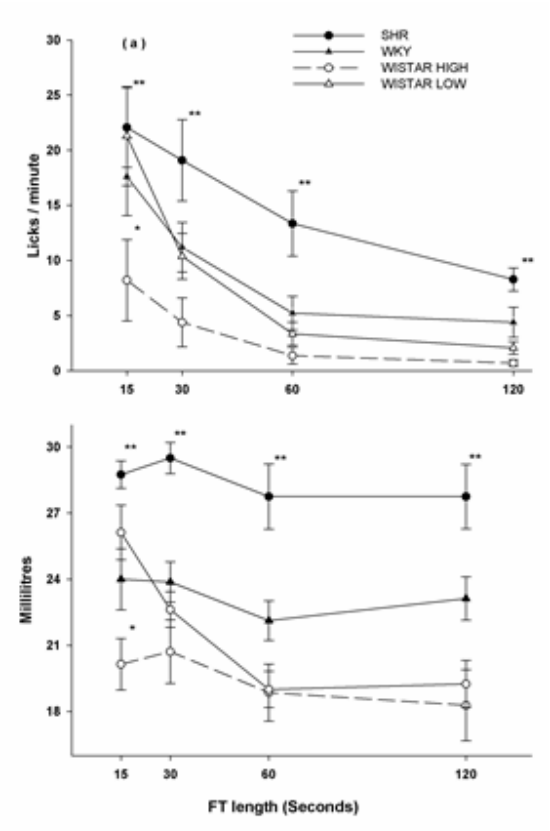


Figure 4. Mean (± SEM) licks per minute for each group of rats under each of the FT 15-, 30-, 60- and 120-s schedules: (a) compares the groups of high- and low-impulsive Wistar rats; (b) compares the SHR and WKY strains. * $p<0.05$. (N= 31)

Under the FT 30- and 60-s schedules, the SHRs drank more water overall than did the rest of the groups (p<0.01 in all cases), and under the FT 120-s this difference was conserved at compare to both groups of Wistar rats (p<0.01 in both cases).

When total millilitres consumed daily by each group of animals were compared separately, a Schedule effect was observed in the low-impulsive Wistar rats [F(3,25)=16.95, p<0.01] but not in the remaining experimental groups, with the former drinking more water under the FT 15-s schedule than under the FT 60- or 120-s schedules (p<0.01 in both cases).

3.4. Delay discounting (post-SIP impulsivity)

The same ANOVA was performed as in the first delay-discount assessment, using identical groups and repeated measures. Figure 5 depicts the results of this second test, conducted after the SIP procedure, There is represented the average (± SEM) proportion of choices which led to the larger reward at each delay used. As found in previous evaluations a Delay effect was revealed [F(3,81)=423.05, p<0.01], with delayed choices of the larger reward decreasing as the delay increased, but without any differences among the groups of animals.

Analysis of the average (± SEM) lever-B (immediate) and lever-A (delayed) presses revealed no statistically significant comparisons between the four groups: the data show similar response patterns to those found in the previous delay-discounting assessment, though more attenuated and without inter-group differences or differences due to increases in the delay.

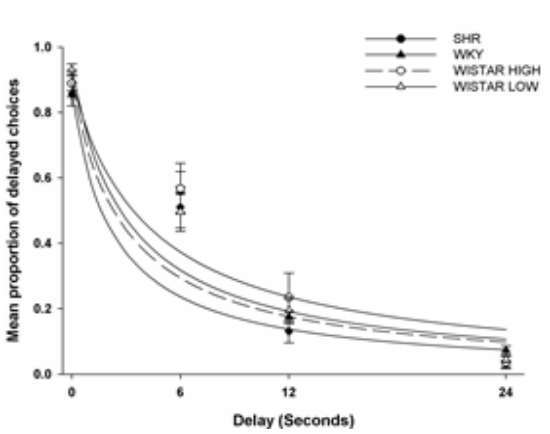


Figure 5. Results of the second delay-discounting assessment. Figure depicts the mean proportion (± SEM) of delayed choices for each strain and group at each delay: The values 0, 6, 12 and 24 are the seconds of delay for gaining access to the reward of greater magnitude. (N= 31)

3.5. Impulsivity coefficients (*K*) in the two delay-discounting tests

Across the procedure and during each of the delay-discounting tests, on fitting the hyperbolic curves, a series of coefficients (*K*) were estimated for each animal and group, which in each case afford a measure of the rate at which the proportion of choices of the larger reward decreased as the delay increased. Table 2 shows the results obtained.

For the first delay-discounting test the following two coefficients were estimated: K_0, to report on the first part of the procedure in which the delays gradually increased from one session to the next; and K_1, to indicate what occurred during the second part of the procedure, in which three sessions were repeated for each delay before proceeding to the next delay, with data on the last session for each delay being used in all cases.

	SHR	**WKY**	**Wistar High**	**Wistar Low**
A_0 ± SD	0.97 ± 0.05	0.94 ± 0.08	0.96 ± 0.04	0.96 ± 0.03
A_1 ± SD	0.97 ± 0.05	0.78 ± 0.17	0.85 ± 0.17	0.91 ± 0.16
A_2 ± SD	0.86 ± 0.10	0.89 ± 0.06	0.89 ± 0.10	0.93 ± 0.05
K_0 ± SD	**0.18 ± 0.09 †***	**0.08 ± 0.06**	**0.17 ± 0.10 †***	**0.12 ± 0.09**
K_1 ± SD	**0.69 ± 0.76**	**0.10 ± 0.07**	**0.59 ± 0.42**	**0.16 ± 0.12**
K_2 ± SD	**0.43 ± 0.45**	**0.23 ± 0.19 †**	**0.33 ± 0.39**	**0.32 ± 0.31**
R_0^2	0.88	0.86	0.84	0.92
R_1^2	0.72	0.87	0.81	0.81
R_2^2	0.76	0.98	0.75	0.79

Table 2. Estimates of *K* coefficients of impulsivity during the first (K_0 y K_1) and second (K_2) delay-discounting assessments, before and after the schedule-induced polydipsia procedure respectively.
Also indicated in all cases are the values of the asymptote (*A*) and of the correlation (R^2) between the estimates and the empirical data. Higher *K* values indicate greater impulsivity. SD = Standard Deviation.
†= column differences $p< 0.05$. *= row differences $p< 0.05$. (N= 31).

The coefficients, K_0 and K_1, differed significantly among the groups during the two parts of the first assessment The SHRs obtained higher *K* estimates than did the WKY [$t(14)=2,19$] and low-impulsive Wistar rats [$t(14)=1,95$] ($p<0.05$ in both cases). The high-impulsive Wistar rats also displayed this difference when compared to the WKY [$t(13)=3,01$] and low-impulsive Wistar rats [$t(13)=2,62$] ($p<0.05$ in both cases). During the second impulsivity assessment after the SIP procedure, no differences were seen between the coefficients (K_2) estimated.

In spite of the wide variability observed in the SHRs and high-impulsive Wistar rats, coefficients that were similar (or tended to be smaller) were estimated during the second delay-discounting assessment (K_2) as compared to the first (K_1). For the WKY and low-impulsive Wistar rats, in contrast, after the experience of SIP the *K* coefficients increased (K_2) in comparison with those

estimated in the first delay-discounting assessment (K_1). The result of these effects was the cancellation, after the adjunctive drinking experience, of the previous inter-group differences in impulsivity.

3.6. Supplementary measures: lever-press training and Pearson Correlations

Over the course of the five initial autoshaping sessions 70% of the SHRs were found to have gained access to the continuous reinforcement schedule during the second session and completed the schedule (50 deliveries of a food pellet) on at least one of the two levers. As from the third day, this criterion had been met by all the SHRs, as opposed to 60% of the Wistar and 10% of the WKY rats. The criterion achieved by the SHRs was matched by the remaining animals during the fifth and last session, in which all the animals gained access to the continuous reinforcement schedule on the two levers and completed the FR-1 schedules on both.

Thereafter, during the first of the four training sessions, as revealed by Group X Session interaction effects, SHRs took significantly less time to complete the session [$F(9,81)=2.25$, $p=0.05$] at compare to WKYs ($p<0.05$), and also registered fewer omissions [$F(9,81)= 2.91$, $p<0.04$] with respect to the available reinforcers ($p<0.05$ with respect to each of the other three groups for both variables).

Analysis of average lever responses showed the same pattern [$F(9,81)=15.94$, $p<0.01$], during the first session, the SHRs registered more lever responses than did the low-impulsive Wistar and WKY rats ($p<0.05$ and $p<0.01$ respectively),

and the WKY rats displayed lower lever-response levels than did the rest of the groups (p<0.01 in all cases). These animals maintained their lever-related behaviour constant during the remainder of the training sessions. During sessions 2 and 3, the SHRs effected more responses to the levers than did the two groups of Wistar rats (p<0.05 in the two cases). During the fourth and last training session, no statistically significant differences were seen among any of the groups.

On further exploring the relationship between SIP acquisition and prior impulsivity levels by means of the Pearson correlation between proportions of choice on the delayed lever and licks/minute for each strain of rats, no constant regularity between the direction and the magnitude of the correlations was detected in the SHRs and WKY rats. In the group of Wistar rats (n=15), in contrast, a pattern of significant positive correlations was found between self-control and drinking rates. In general, for the FT 15-, 30-, 60- and 120-s schedules with respect to the 6- and 12-s delays, correlations were found which systematically associated greater self-control during delay discounting with higher SIP rates (0.55^{*}, 0.72^{**}, 0.71^{**}, 0.64^{*} with respect to the 6-s delay, and 0.63^{*}, 0.64^{**}, 0.86^{**}, 0.42^{-} with respect to the 12-s delay. * p< 0.05 ** p<0.01, - n.s.).

Pearson correlations between mean lever presses across initial lever-press training against mean licks/min in each of the FT schedules of 15-, 30-, 60- and 120-s were evaluated too, and correlations based on SHRs data (0.71^{*}, 0.25, 0.09, 0.43, respectively for each of the FT schedules; * p< 0.05) revealed statistical significance against FT 15-s, schedule under which the higher levels of SIP were observed in SHR, Wistar and WKY rats that failed to show any significant relation.

4. Discussion

In this series of experiments, we examined the relationship between impulsive behaviour and development of SIP in terms of the possible effect of impulsivity on adjunctive drinking measured in delay-discounting tasks, and of the possible modification of performance of the delay-discounting task as a result of experience of SIP, to conclude that impulsivity and SIP do not have the direct relation that we initially thought (Íbias and Pellón, 2011). While a relationship between prior impulsivity and adjunctive drinking exists but it is inversed in animals of a control population, Wistar rats, this is not that clear in the case of SHR and WKY rats.

4.1. Impulsivity changes and SIP experience

On examining impulsivity in the delay-discounting test, it was observed that, initially, when the delay to obtain the larger reward was gradually increased and before the animals displayed differences in the proportion of their choices, i.e., in the first estimate of impulsivity during delay-discounting training (Figure 1), they displayed an early trend towards greater or lesser impulsivity, depending on their membership group.

With respect to behaviour on levers A (delayed) and B (immediate) during these experimental sessions, the SHRs maintained the strategy of increasing their presses on lever B, which led to the immediate reward, as the delay to obtain the larger reward on the other lever grew longer. This would indicate that the animals, on abandoning the delayed lever during the free-choice trials,

were attempting to get more food pellets on the immediate lever by effecting many more responses. This same trend was observed among the high-impulsive vis-à-vis the low-impulsive Wistar rats, with lower behaviour values in both groups.

When it came to assessing the decisions that enabled the animals to learn about the delay, by repeating the same delay condition across several sessions, this strategy was abandoned, and it was then that significant differences were seen in respect of the choice of the delayed larger reward in terms of the 12- and 24-s delays (see Figure 2). At this point, the high-impulsive Wistar rats displayed a choice profile similar to that of the SHRs, and the WKY rats likewise showed no differences from the low-impulsive Wistar rats in the mean proportion of lever-A (delayed) choices.

This result can also be seen on comparing the *K*-coefficient estimates for the rats with respect to the different impulsivity assessments (see Table 2). While differences between K_0 and K_1 estimates were observed in both the SHRs and high-impulsive Wistar rats, they were not in evidence in the groups of WKY and low-impulsive Wistar rats, in which the estimates of the K_1 parameter did not increase on the experience with the delayed reinforcers being prolonged, with respect to the initial K_0 estimates obtained with little training. These data show: firstly, how sensitivity to the delivery of delayed reinforcers developed, both in the SHRs and in the high-impulsive Wistar rats, on the basis of experience of delay, in the form of a steepening of the slope of the delay gradient leading to the reward of greater magnitude; and secondly, how this failed to occur in those animals which showed self-control during the delay-discounting tests, i.e., the WKY and low-impulsive Wistar rats.

Furthermore, the results of the indifference-point procedure showed that, under free-choice conditions similar to those of the delay-discounting test, where the animal itself chose with which delay it would obtain the reward of greater magnitude, differences between the delay gradients attenuated and both SHR rats and Wistar high impulsive rats failed to display delay values that were significantly different from those of the rest of the groups.

During the last delay-discounting assessment subsequent to the SIP procedure, the differences seen in the first assessment disappeared. There was a convergence in the results, such that the previously more impulsive rats (SHR and high-impulsive Wistar) decreased their levels of impulsivity in the second assessment (less so in SHRs) with respect to the first assessment of delay discounting (compare Figures 2 and 5), while these levels *did* rise in those rats which had displayed lower values in the first assessment (WKY and low-impulsive Wistar). SHR and high-impulsive Wistar rats were not at all similar in their development of adjunctive drinking but had changes in impulsivity from the first to the second assessment that were in the same direction. Taking into account just the Wistar rats, however, the adjunctive-drinking experience could have altered the delay-discounting levels, so that the rats that drank the most (initially low-impulsive) increased their levels of impulsivity, and the rats that drank the least (initially high-impulsive) remained in or decreased their levels of impulsivity.

The SIP procedure might therefore have had an effect linked to the increase in impulsivity among animals that initially exhibited greater self-control, not only WKY but also low-impulsive Wistar rats.

4.2 Impulsivity and behavioural excess

SHRs usually exhibit behavioural excess in free operant situations, i.e., more behaviour in absolute terms than WKY rats (Alsop, 2007 a and b). In the present study evidence of faster learning among SHRs was obtained, firstly as an earlier autoshaping, and secondly by rushing of more effectively in the initial training in lever pressing, where prior to the delay discounting test they began to show signs of behavioural excess.

This behavioural excess mainly consisted in higher rates of lever pressing throughout the training sessions, which resulted in a slower decline of unreinforced behaviour when animals were allowed to adjust their behaviour to a minimum response across training. Until the last training session SHRs did not reduce their non-reinforced responses to the same level as the other groups of rats. Wistar rats that were classified as high impulsive, by their own, did not show such an excess.

Under the SIP procedure, the drinking rates of the SHRs were higher under the FT 60- and 120-s food schedules than those of the Wistar and WKY rats. The persistence of induced drinking among SHRs under these schedules could also be viewed as behavioural excess. In SIP acquisition qualitative differences exist between shorter and longer schedules, in terms of an optimal inter-trial interval to SIP development (Falk, 1966). The fact is that animals usually show high levels of SIP under short schedules, but lower levels, or extinction, under long schedules. Results of all the control groups pointed in this direction, these animals showed lower drinking under FT 60- schedule in comparison to FT schedules of shorter inter-food intervals (15 and 30 s), and a very low (if not

absent) drinking in FT 120-s schedule were found in both WKY and Wistar rats (Figure 4).

The results with the SHRs confirm our own earlier observations (Íbias and Pellón, 2011): SHRs again displayed a higher level of polydipsic drinking (especially under schedules with long inter-food intervals) and a high level of impulsivity. However, conclusions about the relationship between impulsivity and adjunctive drinking are shaded by present results with Wistar rats. The subpopulation of high-impulsive Wistar rats that did not differ from the SHRs in their self-control measures drank much less in tests of SIP. These animals showed lower rates of SIP at compare to low-impulsive Wistar rats in FT 15-s and the same tendency in FT 30-s, and in no way showed the persistence of SHR rats in longer schedules. Again, similar prior levels of impulsivity were expressed in different ways in latter development of SIP.

The high levels of impulsivity of SHRs did not differ between pre- and post-SIP treatment, and because this was not the pattern followed by similar impulsive Wistar rats, it can be concluded that the essence of the SIP acquisition-capability observed by SHRs resides in their hyperactivity, not their impulsivity; Pearson correlation between mean lever presses throughout initial training sessions and SIP performance under FT 15-s schedule provides here an evidence.

Moreover, Pearson correlations between delayed choices and SIP performance run in Wistar rats, in addition, would seem to indicate that, in a control population that does not display behavioural excess, impulsivity is expressed in another way in SIP, since the highest drinking rates during the SIP procedure among the Wistar rats were associated with prior low levels of

impulsivity, and vice-versa. This inverse relationship between discount value functions of delayed rewards and level of SIP is solely explain by models of adjunctive behaviour based on drinking being reinforced by next food delivery according to a delay-food gradient (Killeen and Pellón, 2013). At contrast, WKY rats showed a pattern of drinking similar to that of the low-impulsive Wistar rats (Figure 4), and yes these two groups showed similar impulsivity (Figure 2).

4.3. Water consumption

Insofar as total water intake was concerned (see Figure 4b), there was an FT-schedule effect, whereby total daily water intake decreased as the food schedule was lengthened (due mainly to the decline in the level of polydipsic induction, and, despite the fact that the animals displayed increases in water intake in the home cage under these conditions, these increases were nevertheless insufficient to offset the decrease and maintain intake constant. Furthermore, the effect of a decrease in water intake as the food-pellet interval lengthened was greater in some cases than in others, which is evidence in favour of the fact that SIP induction under short time interval schedules ultimately results in raising the general intake of water. This finding can be more clearly seen in the group of low-impulsive Wistar rats which, during the experience under the FT 15-s schedule, drank more water than did the high-impulsive Wistar rats, and this was due to consumption of water during the experimental sessions. The decline in overall drinking as FT length was increased was also observed, though to a lesser extent, in the other groups of rats, most notably in high-impulsive Wistar rats at FT 120 and WKY rats at FT 60.

In all, the SHRs drank more water per day than did any of the other groups of animals (Figure 4b). This could, moreover, be seen during all the FT schedules. Exposure to the SIP schedules generally increased water intake in the home cage independently of the FT value, and although it appeared to compensate for the decreases observed at long FT values during experimental sessions, the increases were not as marked as the decreases. These data seem to run counter interpretations of SIP as normal regulated drinking in defined time cycles (e.g., 24 hours), and possibly reflects again the excessiveness in the behaviour of the SHRs.

4.4. Conclusions and reflections

This study, which sought to conduct in-depth research into some of the aspects of impulsivity that might be linked to development of SIP, showed that in some of the animals, i.e., the SHRs and high-impulsive Wistar rats, signs of impulsivity grew as they had continued experience with contingent delayed reinforcers. Investigating the expression of impulsive behaviour in high-impulsive Wistar rats enabled the exclusive excess activity of SHRs to be isolated as a possible promoting factor of their exacerbated adjunctive drinking. Examination of the relationship between impulsivity and development of SIP in high-impulsive Wistar rats, which have prior levels of impulsivity similar to those of the SHRs though without displaying signs of hyperactivity, showed by way of a result that the impulsivity displayed by the Wistar rats does nothing whatsoever to promote development of SIP but, on the contrary, appears to be an impediment. In the ultimate analysis, the most impulsive subjects in the

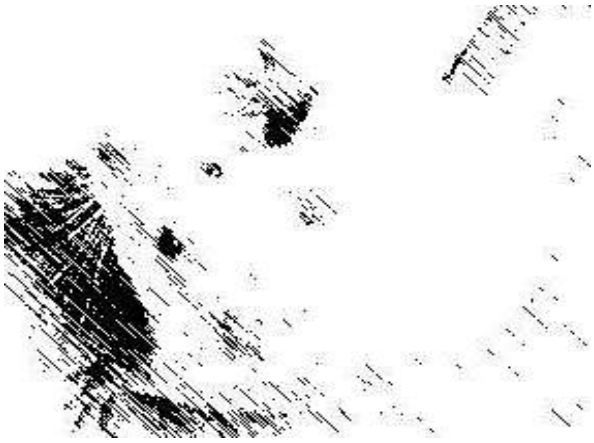
control population proved to be those that developed the least adjunctive behaviour. As mentioned before in this Discussion, this result is of the utmost interest for the explanation of the phenomenon of adjunctive behaviour, since it highlights the relationship between greater impulsivity (characterised by a more pronounced delay gradient to the reinforcer) and adjunctive drinking, pointing in turn to the idea that higher discounting functions of the value of the delayed reinforcers would entail a lower level of development of SIP, an aspect that is only explained by the model of adjunctive behaviour proposed by Killeen and Pellón (2013). Furthermore, the present results are in line with modern conceptions of adjunctive behaviour as a model of compulsive behaviour (Moreno and Flores, 2012), not a reflection of higher impulsivity.

The results of the SHRs pose more questions, and in particular why having a greater delay discount curve than either WKY or high-impulsive Wistar rats, they display higher drinking levels. This cannot be explained on the basis of the above model, unless additional specific factors are assumed to be intervening in the behaviour of the SHRs. One possible explanation of the increased motivation that these animals generally tend to display is based on a disregulation of the dopaminergic system, due to increased levels of circulating dopamine and DAT hyper- or hypofunction in different brain regions (Watanabe et al., 1996; Carey et al., 1998; Langen and Dost, 2011). On the other hand, the dopaminergic system has, in turn, proved to be important in development of SIP (Flores and Pellón, 1995, 1997; Mittleman et al., 1994; Pellón et al., 2011). Hence, to conclude by explaining the reason why SHRs register a very pronounced delay gradient and, by extension, develop high levels of induced drinking, it would be necessary to examine what occurs with the adjunctive drinking of SHRs in

response to manipulation of these excesses and defects in their dopaminergic system. The intervention of the dopamine system in the development of SIP and, in the case of SHRs, the exacerbated expression of this system, might account for the elevated SIP levels present in these animals. The behavioural results, to date, would support this hypothesis, since it has been shown that, in comparison with other strains of rat, the SHR is an animal especially suited to development and maintenance of SIP.

CHAPTER IV *

Dopaminergic manipulation of Schedule Induced Polydipsia in Spontaneously Hypertensive Rat, Wistar Kyoto and Wistar rats: ADHD pharmacological treatment effects



*This Chapter is based on the publication:

Íbias J., Miguens M., Pellón R. (2014) Dopaminergic manipulation of Schedule Induced Polydipsia in Spontaneously Hypertensive Rat, Wistar Kyoto and Wistar rats: ADHD pharmacological treatment effects. *Psychopharmacology, submitted*

Abstract

Schedule induced Polydipsia (SIP) occurs as a reinforcement schedule effect when food is intermittently delivered and animals are partially food-deprived. It consists in excessive water consumption, neither related to prandial drink nor reinforced by food deliveries, and it has been used as a model of excessive behaviour. In this study, Spontaneously Hypertensive rats (SHR), an animal model of behaviour excessiveness, were exposed to the development of SIP and its subsequent modulation by d-Amphetamine, Methylphenidate. The aim of this study was to evaluate the effect of pharmacologic treatments for Attention Deficit Hyperactivity Disorder (ADHD) control and selective D1- and D2-like dopamine receptor agonists and antagonists in SHR's on SIP to investigate the influence of the dopaminergic system in the performance of this behaviour. We used 24 male rats of three different strains [8 SHR, 8 Wistar Kyoto (WKY) and 8 Wistar rats] and a multiple fixed time (FT) schedule with two separate components, of 30 and 90 seconds. Pharmacologic treatment's effects were evaluated after 40 sessions of SIP acquisition. Adjunctive drinking were higher in FT 30-s in all the strains. SHR rats showed higher asymptotic SIP levels in FT 90-s, and a certain insensibility to ADHD treatments when comparing to WKY and Wistar strains. Selective dopaminergic manipulations produced similar variations only in control rats, and despite magnitude differences. Dose-response functions in the different strains of rats are discussed in relation to an existing dopamine hyperfunction in SHR rats. Moreover, the results all together suggest that D1-like receptors play a key role in SIP development.

Keywords: D-amphetamine; Dopamine; Methylphenidate; Schedule-induced polydipsia; SHR; WKY.

1. Introduction

Intermittent delivery of food is the basis for the development of behavioural expressions along the course of successive inter-trial intervals. Schedule induced polydipsia (SIP) appears if water is available throughout these intervals and animals are partially food-deprived (Falk, 1961). Once developed SIP, the animals regularly drink a little amount of water after the delivery of each food, and this phenomenon is observed both if animals control these food deliveries, and if they don't (Falk 1971; Jacquet, 1972; Cohen, 1975; Staddon and Shimmelhag, 1971. This versatility for acquisition, which occurs even regardless of instrumental control of the behavioural schedule, has been a difficulty to define whether adjunctive behaviour is based in a Pavlovian or an instrumental learning process (Segal, 1972; Staddon, 1977; Lucas et al., 1988). However, variables involved in the development and maintenance of SIP can be manipulated in the same way they are in operant behaviour, therefore, an instrumental structure underlying SIP is undeniable (Killeen and Pellón, 2013).

SIP can be observed both in interval and time reinforcement schedules (Falk, 1966, 1967), and they are optimal against non-optimal manipulations, that respectively increase or decrease the probability of SIP acquisition and maintenance, e.g. when variables as inter-trial interval length or the food deprivation are manipulated (Freed and Hymowitz, 1972; Castilla and Pellón, 2013). Otherwise, the idiosyncratic characteristics of each animal may play a role in variability observed in SIP, overall when experimental conditions are not strong enough to hide these individual differences, and high vs. low affinity for SIP acquisition emerges (DeCarolis et al., 2003). Thus, SIP has been induced in rats as a model of behavioural excess (Riley et al., 1989; Gilpin et al., 2008) and

other disorders in which the impulse control fails (Cardona et al., 2011; López-Grancha et al., 2008), as addictions (Myracle et al., 2005), obsessive compulsive behaviours (Plat et al., 2008; Van Kuyck et al., 2008; Moreno et al 2012 a and b), and schizophrenia (Hawken et al 2011 a and b, 2013).

The Spontaneously Hypertensive rats (SHR) accomplish principal characteristics that define ADHD: impulsivity (Evenden and Meyerson, 1999), inattention (Berger et al., 1998) and hyperactivity (Sagvolden et al., 2000). This strain has been validated as a model for studying the disease (Adriani et al., 2003; Bizot et al., 2007; De la Peña et al., 2010; Roessner et al., 2010; Langen y Dost, 2011; Meneses et al., 2011), with further evidences of representing better the hyperactive subtype of ADHD (Alsop, 2007 a and b; Sagvolden et al., 2009; and Sutherland et al., 2009).

Several theories point to the dopaminergic system as the key of ADHD, and posit on one hand, reduced tonic dopamine levels for individuals with ADHD (Johansen, et al., 2002), and on the other hand, that the transfer of the dopamine release from the reinforcer to the predictive cues is deficient for ADHDs (Tripp, et al., 2008). The usefulness of dopamine hypothesis is based on the decrease of symptoms by psychostimulant drugs acting at dopaminergic synapses (methylphenidate (MPH), d-Amphetamine (D-AMP) and Pemoline) (Safer et al., 1988; Seiden et al., 1993).

Psychostimulants, such as MPH and D-AMP are the most common pharmacologic treatments for ADHD (The MTA Cooperative Group 1999). MPH and D-AMP are termed indirect agonists; they do not stimulate catecholaminergic receptors directly, but rather facilitate the action of dopamine and norepinephrine (Solanto, 1998). They act fundamentally through three

mechanisms: inhibition of reuptake, facilitation of release into the synaptic cleft, and inhibition monoamine oxidase (MAO) (Feldman et al., 1997). An important difference between these stimulants is that MPH releases DA from reserpine-sensitive storage pools, while D-AMP facilitates release of DA from newly synthesized store (Clemens and Fuller, 1979).

Dopamine receptors are expressed throughout the brain in specific but overlapping areas, and they differ in their signal transduction mechanism. Based on their pharmacological and functional properties these receptors are classified into two subfamilies, D_1- and D_2-like receptors. The D_1-like and D_2-like receptor mRNAs are present in all DA-containing regions of the rat. Although D_1-like and D_2-like receptors appear to have overlapping distributions, there are some differences in their anatomical locations in the brain (Hartman and Civelli, 1996). Moreover, when D_1 receptors show low affinity for dopamine D_2 receptors have a high affinity for it, suggesting that D_1 require much higher concentration of dopamine than D_2 receptors (Cooper et al., 2002).

Abnormalities in the dopaminergic function, as a reduced release of DA in the dorsal striatum and faster DA uptake in the ventral striatum and nucleus accumbens, as compared to control rats, suggest an increased surface expression of DA transporters in SHR rats (Miller et al., 2012). In addition, presynaptic dopamine receptors are hypersensitive and postsynaptic dopamine receptors are hyposensitive in SHR (Fujita et al., 2003) and D_2–like receptor density is increased when compared to WKY rats (Kirouak et al., 1993), that are the normotensive control strain of SHR rats (Festing, 1979). A higher density of D_1–like receptors is a constant neural substrate of SHR, independent of spontaneous

hypertension, as demonstrated by its persistence in adulthood (Le Fur et al., 1981).

The mesolimbic and nigrostriatal DA systems have been involved in reinforcement learning or habit formation (Wise, 2004; Yin et al., 2008; Belin et al., 2009). Studies that evaluate the dopaminergic system implication in SIP development using pharmacological compounds that modifies dopamine actions generally have shown a reduction of this behaviour (Snodgrass and Allen, 1987; Kaempf and Porter, 1987; Pellón et al, 1992; Didriksen and Christensen, 1993; Didriksen et al., 1993; Piazza et al, 1993 and Flores, 1997). Finally, there has been suggested that D_1 receptors are related to the motor aspects of Schedule drinking while D_2 receptors are more involved in the motivational component, without discarding interaction effects between D_1-like, D_2-like receptors (Pellón et al., 2011).

This study evaluates the interaction of two different sources of variation in SIP. On one hand, the variability introduced by the schedule's manipulation, on the other hand, those divergences belonging to the strain of origin, SHR, WKY and Wistar rats (Íbias and Pellón, 2011, 2014). The experimental variation was produced by manipulating the inter-trial interval length; facing each other the results of two FT schedules with short vs. long inter-food interval lengths, that guarantee respectively high vs. low levels of SIP. Once stabilized, behaviour was manipulated by: 1. pharmacological treatments used for ADHD control, D-AMP and MPH, to evaluate possible effects of these treatments over SIP. 2. modulating dopaminergic variations of SIP expression by the administration of selective agonists or antagonists of both D1 and D2-like receptors. Principal predictions were related to the possible effects of ADHD pharmacological

treatments over SIP in SHR rats, for their relation with ADHD. Furthermore, pharmacological manipulations on D1 vs. D2 receptors, or their combined influence, would provide information about their differential implication on SIP behaviour.

2. Method:

2.1. Subjects

We used 24 male rats belonging to three different strains – 8 SHR, 8 WKY and 8 Wistar rats – obtained from Charles Rivers Laboratories (Barcelona, Spain). On arrival, rats were 10 weeks old. They were housed in groups of four and once habituated to the animal facility were housed singly in 18 cm X 32 cm X 20.5 cm transparent Plexiglas cages, with a metal grid by way of a detachable roof, which allowed for food to be deposited and a water bottle fitted. The room environment was continuously controlled with a 12-h light-dark cycle (light from 08:00 to 20:00 h), ambient temperature of 17-23 ºC, and 60% relative humidity.

At the start of the experiment animals were in their 12th week of life, and they had the following mean (±SD) weights: SHR 291.98±7.85 (range: 277-302 g); WKY 338.88±16.35 (range: 306-061 g); Wistar 372.50±7.33 (range: 359-384 g). Animals' weight was gradually reduced by controlling feeding to 80-85% of their free-feeding body weight, concerning a standard growth curve for each strain. This criterion was maintained throughout all procedure, and before every experimental session, each rat was weighed. A supplement of food was given to

maintain their weights at a minimum of 20 min after this session. . Water was freely available to all animals in their home cages. All animal care procedures were in accordance with the European Union Council Directive 2010/63 and the Spanish Royal Decrete 1201/2005 for minimizing stress and discomfort in animals.

2.2. Apparatus

We have used 8 Letica LI-836 conditioning chambers that were has been described extensively elsewhere (see studies I and II). A water bottle was fitted, and the rat had access to their spout from the interior of the chamber and the contact between the animal's tongue and the metal spout completed the electric circuit between the 12-bar metal grid which served as the floor and the water-bottle spout. The licks were recorded using an MED-PC-IV application under a Windows XP environment. 45-mg food pellets were dispensed (Bio-Serv, Frenchtown, NJ, USA) in an aperture of the chamber's front wall, situated 3.7 cm from the floor, between the panel's two levers, which were retracted throughout the experiment. The chambers were lit by two 3W lamps situated on the front panel on either side of the food hopper, and by an indirect 25W light fitted to the interior of the soundproof housing that insulated each chamber. Exterior noise was masked by a fan that produced an ambient noise of approximately 60 dB in each chamber, and at the top of the front wall of each chamber was a speaker to produce sound signals when necessary.

2.3. Procedure

2.3.1. Behavioural procedure

In order to stabilize baseline rates of behaviour a SIP procedure was carried out. A multiple Fixed Time (FT) schedule was used, in which a food pellet was delivered at regular intervals and regardless of the animal's behaviour. Each experimental session contained two separate components, FT 30-s and FT 90-s between deliveries of food. 20 food pellets were delivered in each component. To facilitate the discrimination of the current FT schedule one of these components was always signaled with a continuous tone of 60 dB that was randomized between animals. The experimental chamber lights were totally turned off for 60 seconds between FT components. And the FT component that initiates each session was also randomized. They were carried out 40 experimental sessions and once behaviour was in a steady state we took to analyze the data from the last five sessions. The rates of licks and magazine entries were computed. Acquisition measures were used as a baseline to compare maintenance levels of SIP in each FT schedule.

2.3.2. Pharmacological procedure

Consisted on a prolongation of behavioural procedure, in which administration of different drug doses were used to create dose-response curves for licks and magazine entries. Procedure was conducted by series of 5 experimental sessions per week (Monday to Friday) followed by two rest days. On Tuesdays and

Fridays they were carried on drug sessions, on Mondays and Wednesdays behavioural sessions without using any drug and on Thursdays were carried on Saline sessions, adequate for dispositions of the current drug in use.

They were conducted acute treatments with the following drugs and doses: D-AMP (0.1, 0.3, 1 and 1.5 mg/Kg), MPH (0.6, 2.5, 5 and 10 mg/Kg), both principal treatments for ADHD with generalized effects on dopaminergic system; SKF 38393 (1, 3 and 5.6 mg/Kg) and Quinpirole (0.003, 0.01, 0.03 and 0.1 mg/Kg) selective agonists for respectively D_1 and D_2 DAT; and SCH 23390 (0.001, 0.003 and 0.01 mg/Kg) and Eticlopride (0.001, 0.003, 0.01 and 0.1 mg/Kg) selective antagonists for respectively D_1 and D_2 DAT. All drug doses were dissolved in 0.9% Saline and they were intraperitoneally administrated in a volume of 1 ml/Kg (except 10 mg/Kg MPH dose at a volume of 2.5 ml/Kg). They were taken average rates per session both for licks and magazine entries to obtain dose-response curves for each drug.

2.4. Statistical analysis

Before evaluating any drug's effect, the results of every saline session and its respective behavioural control session were compared. Different ANOVA analyses, one for each drug treatment, were carried out to discard, in each case, that the possible effects of the injection weren't dwindling animal's results. To investigate drug's effects they were analyzed both rates of licks/min and magazine entries/min, latencies to first lick and durations of SIP episodes, average of these variables in each group and FT schedule were put into analysis. Thereby, the effects of every drug on these variables were analyzed using

MANOVA tests, each one including a strain factor with three levels (SHR, Wistar and WKY) and a repeated measure with one level for each drug dose and their respective saline session, defining this last one as 0 mg/Kg dose. Pos-hoc comparisons were made using a Bonferroni adjust and for general results there was establish an $\alpha = 0.05$. Statistical Analyses were carried out using SPSS 17.

3. Results

3.1. Acquisition of Schedule-induced polydipsia

After 40 experimental sessions, animals reached a steady state of schedule induced licking behaviour in both FT components. We have taken averages of the five last behavioural sessions to analyze possible differences. Results showed no differences between strains in FT 30-s (mean ± SD of licks/min for SHR, WKY and Wistar rats were, respectively 56.81± 18.82, 43.91± 27.29 and 40.16± 25.92); magazine entries' analysis neither showed strain differences in their average rates in FT 30-s (mean ± SD entries/min, respectively 31.54± 15.53, 29.53± 6.38 and 28.16± 10.29).

However, SHR rats showed higher rates of licks/min in FT 90-s [$F(2,21)= 13.349$, $p< 0.01$], an average (± SD) of 24.81± 7.76, when comparing to WKY and Wistar rats ($p< 0.01$ for both comparisons) with respective averages (± SD) of 7.70± 6.12 and 9.11± 8.03, and without differences between them. As found in FT 30-s, no difference was revealed when analyzing magazine entries in FT 90-s, averages (± SD) for SHR, WKY and Wistar rats were, respectively 25.52± 12.40, 31.39± 6.95 and 25.78± 10.80. Magazine entries' averages didn't differ from those observed

in FT 30-s schedule, and despite the increase in licking behaviour, related with SIP procedure, magazine entries, at contrary, didn't change throughout SIP acquisition.

3.2. Pharmacological procedure

Figure 1 and 2 summarize the results of all drugs and doses used in both FT 30-s and FT 90-s components. Neither licks nor magazine-entries' analysis revealed effects for injection or strain x injection interaction with any drug or FT schedule.

Figure 1 represents averages (±SEM) of licks and magazine entries for all groups and drugs used in FT 30-s schedule.

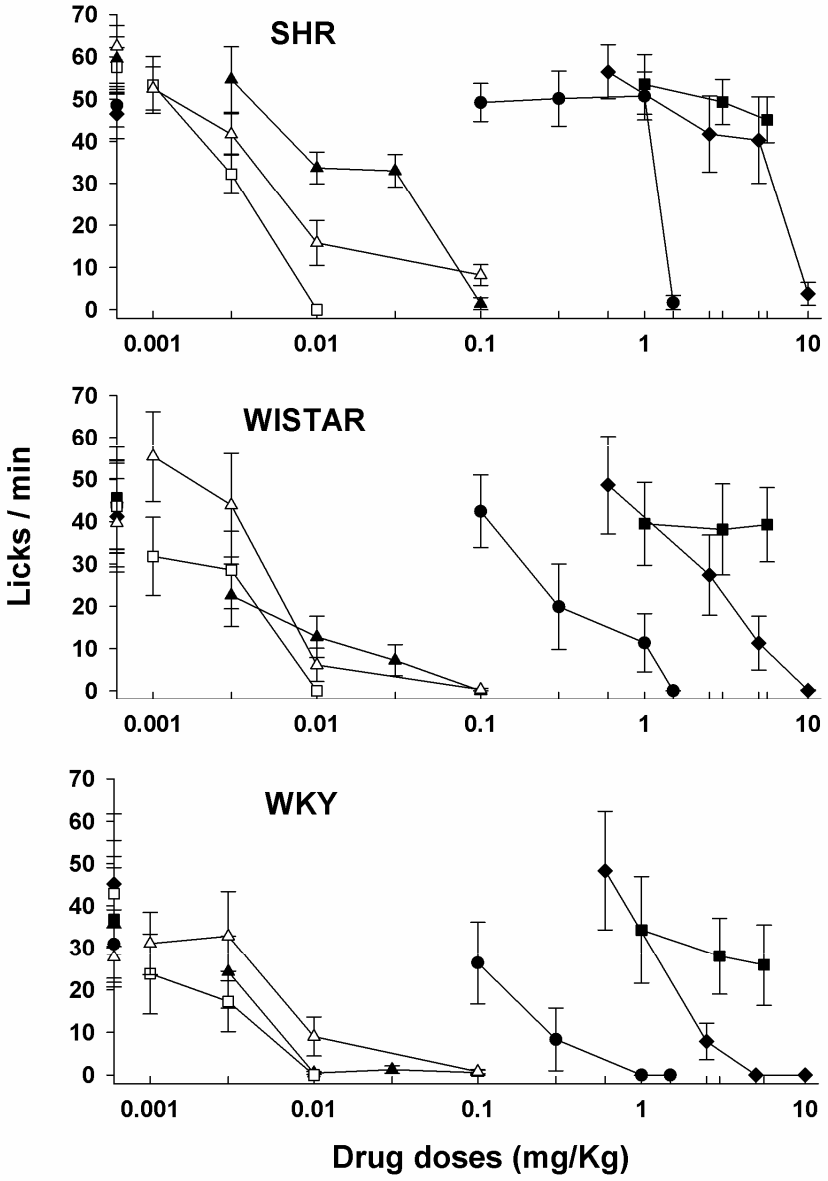
3.2.1. Drug effects on licks and magazine entries on FT 30-s:

ADHD treatments effects (D-AMP and MPH): analysis of licks revealed a strain effect on D-AMP [$F(2,21)= 13.55$, $p< 0.01$]. SHRs showed higher rates of licks at compared to WKY and Wistar rats, especially under doses of 0.3 and 1 mg/Kg, as revealed by strain x dose interaction effect [$F(4,84)= 5.55$, $p< 0.01$]. Highest doses of these drugs decreased licking behaviour in all animals, as revealed by dose effects for WKY on D-AMP [$F(4,28)= 7.26$, $p< 0.01$], and MPH [$F(4,28)= 7.71$, $p< 0.01$], and for Wistar on D-AMP [$F(4,28)= 11.43$, $p< 0.01$], and MPH [$F(4,28)= 10.11$, $p< 0.01$], comparing in all cases both pairs of 0-mg/Kg and lower doses of 0.1- and 0.6-mg/Kg of respectively D-AMP and MPH ($p< 0.05$). SHRs' licks decreased too under the effects of highest doses on D-AMP [$F(4,28)=$

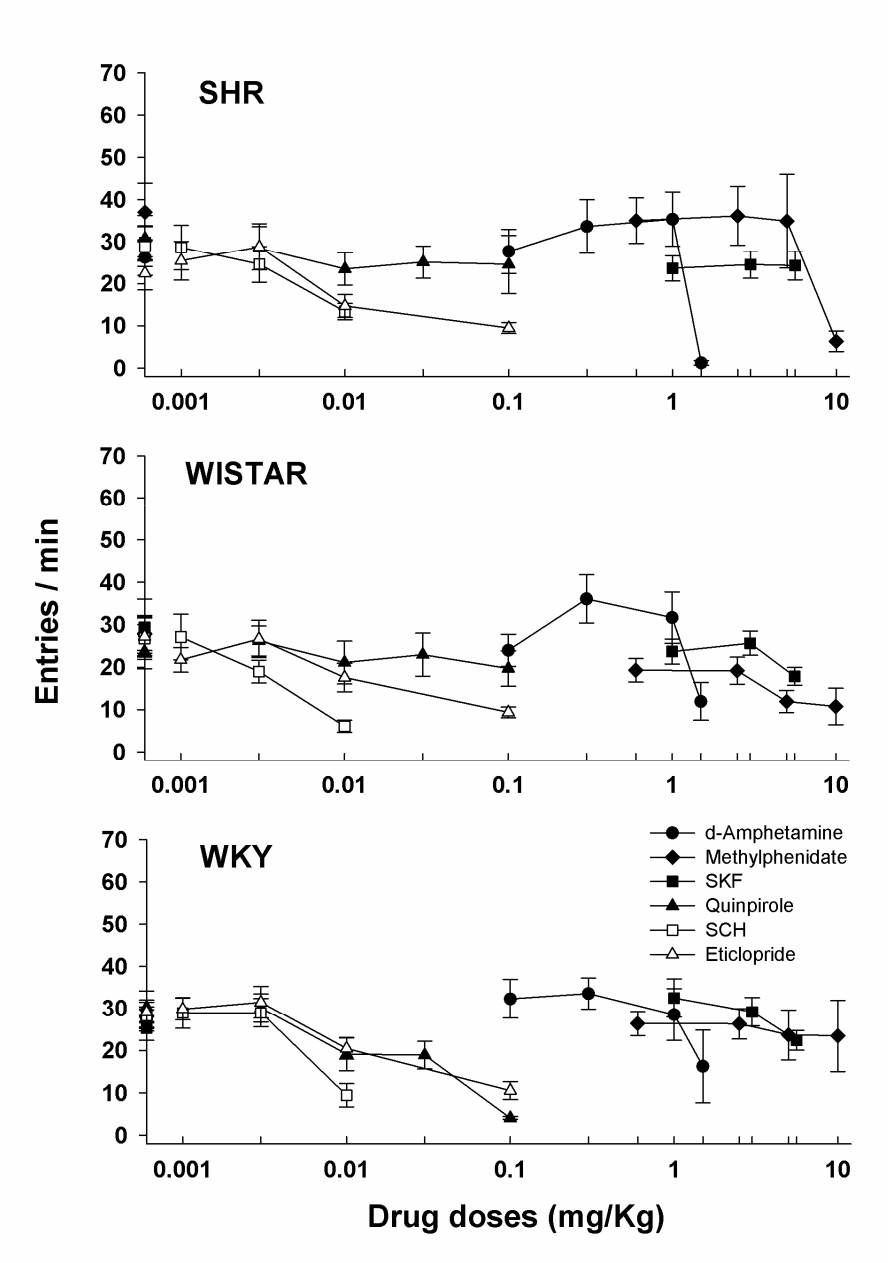
34.86, $p< 0.01$], and MPH [$F(4,28)= 8.14$, $p< 0.01$], but at contrast licks did not decrease at all lower doses, without differences among them ($p< 0.01$). Analysis of magazine entries showed no strain effects, but a strain X dose interaction effect on MPH [$F(4,84)= 2.04$, $p< 0.05$]. SHRs did more entries under 0.6-mg/Kg dose at compare to Wistar ($p< 0.05$). They were found dose effects on D-AMP for both SHR [$F(4,28)= 20.28$, $p< 0.01$], and Wistar [$F(4,28)= 9.83$, $p< 0.01$]. These animals did fewer entries under the highest dose of 1.5-mg/Kg when comparing to 0.3-mg/Kg dose in Wistar's results ($p< 0.05$), and with respect to all lower doses in SHRs ($p< 0.01$), which showed the same effect [$F(4,28)= 4.47$, $p< 0.05$] respect to MPH's highest dose of 10 mg/Kg ($p< 0.01$).

Effects of SKF 38393: analysis of licks revealed no strain, dose or interaction effects, and the analysis of magazine entries showed similar results than for licks, but a dose effect for WKY rats [$F(3,21)= 4.02$, $p< 0.05$], in which a lowest vs. highest dose difference was found ($p< 0.01$).

Effects of Quinpirole: analysis of licks in FT 30-s revealed a strain effect [$F(2,21)=10.30$, $p< 0.01$], SHR made more licks at compare to both Wistar and WKY rats. They were found dose-dependent decreases in licks in SHR [$F(4,28)= 28.30$, $p< 0.01$], Wistar [$F(4,28)= 6.77$, $p< 0.01$] and WKY [$F(4,28)= 6.72$, $p< 0.01$] rats. Analysis of magazine entries revealed no strain effect, but a strain X dose interaction effect [$F(4,84)= 2.50$, $p< 0.05$], WKYs made fewer entries under 0.1-mg/Kg dose at compare to SHR ($p< 0.05$). Only WKY rats revealed a dose effect [$F(4,28)= 16.95$, $p< 0.01$], with dose-dependent decreases in magazine entries.



Effects of SCH 23390: analysis of licks revealed no strain effect, but dose effects for all strains. In SHR [$F(3,21)= 46.00$, $p< 0.01$] a dose-dependent decrease in licks was observed, but both in Wistar [$F(3,21)= 10.39$, $p< 0.01$] and WKY



Panel 1. Show averages (±SEM) of licks (left figures) and magazine entries (right figures) for SHR (top figures), Wistar (middle figures) and WKY (bottom figures) in FT 30-s schedule. (N= 24).

[$F(3,21)= 8.90$, $p< 0.01$] only the highest dose of 0.01-mg/Kg differed when comparing to all lower doses administered ($p< 0.01$). Analysis of magazine entries revealed no strain effect, but dose effects for all strains. A dose-dependent decrease in Wistar rats [$F(3,21)= 9.92$, $p< 0.01$] was found, but both SHR [$F(3,21)= 9.87$, $p< 0.01$] and WKY [$F(3,21)= 17.49$, $p< 0.01$] reduced the number of their entries under the highest dose of 0.01-mg/Kg when comparing to all lower doses administered ($p< 0.01$).

3.2.2. Drug effects on licks and magazine entries on FT 90-s:

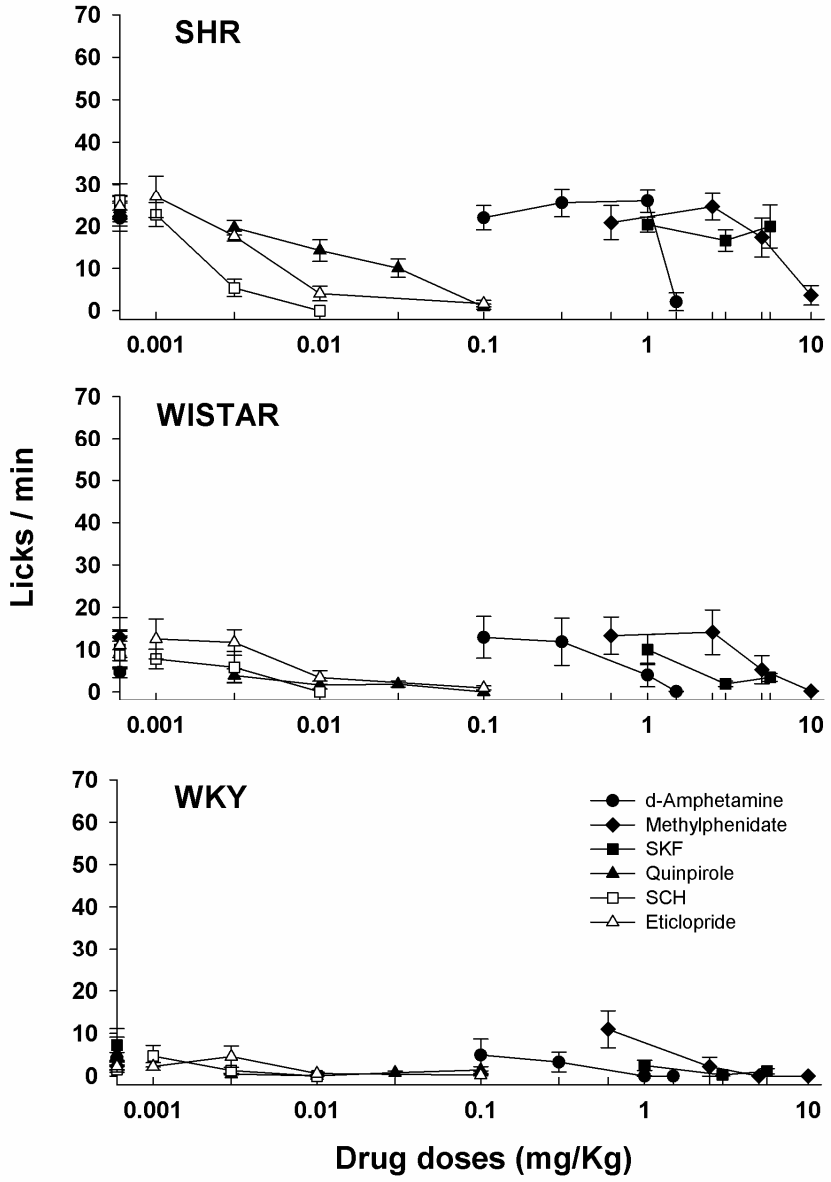
Panel 2 represents averages (±SEM) of licks and magazine entries for all groups and drugs used in FT 90-s schedule.

ADHD treatments effects (D-AMP and MPH): analysis of licks in FT 90-s showed the same pattern than observed for FT 30-s results. They were found strain effects, on D-AMP [$F(2,21)= 29.11$, $p< 0.01$] and on MPH [$F(2,21)=10.55$, $p< 0.01$], and both revealed higher rates of licks in SHR rats. Strain X dose interaction effects on D-AMP [$F(4,84)= 4.69$, $p< 0.01$] and on MPH [$F(4,84)= 2.11$, $p< 0.05$] revealed SHRs showing higher rates of licks: on D-AMP under 0- and 0.1-mg/Kg doses when comparing to Wistar and WKY ($p< 0.01$), and under 0.3-mg/Kg dose, when comparing to WKY rats ($p< 0.01$); and on MPH under 0- and 2.5-mg/Kg doses when comparing to WKY ($p< 0.01$), and under 5-mg/Kg dose at compare to Wistar and WKY (respectively $p< 0.05$ and $p<0.01$). They were found dose effects in SHR on D-AMP [$F(4,28)= 17.99$, $p< 0.01$] and on MPH [$F(4,28)= 6.46$, $p< 0.01$], and in Wistar on D-AMP [$F(4,28)= 5.14$, $p< 0.05$] and on MPH [$F(4,28)= 4.58$, $p< 0.01$], the highest doses of both drugs decreased licks at

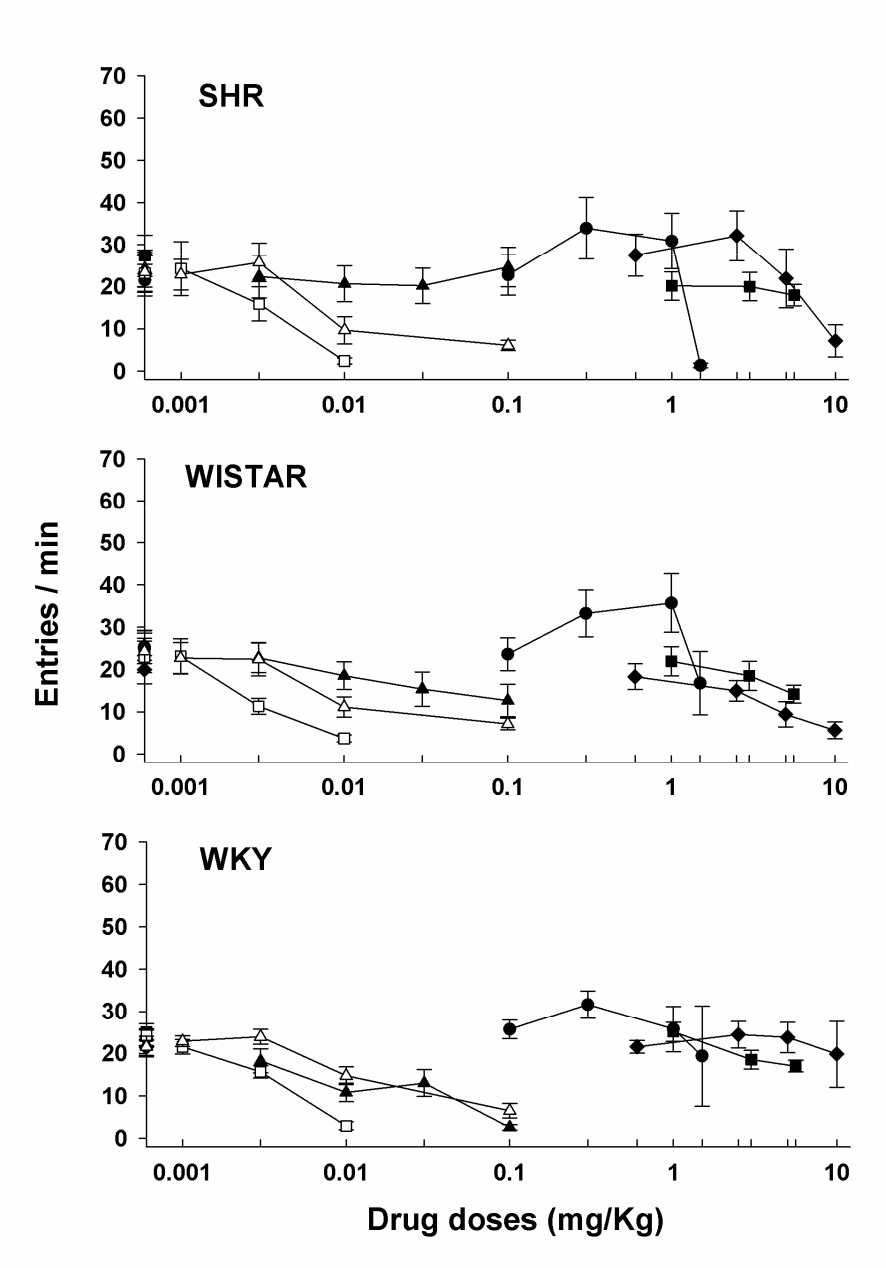
compare to all lower doses ($p< 0.01$ for all comparisons) except under 5.6-mg/Kg dose of MPH in Wistar. The only result found in WKY consisted in a dose effect [$F(4,28)= 5.08$, $p< 0.01$] on MPH, with a dose-dependent decrease in licks.

Analysis of magazine entries revealed no strain effects neither on D-AMP nor on MPH. SHRs made more entries under 2.5-mg/Kg dose of MPH at compare to Wistar ($p< 0.05$) as revealed by strain X dose interaction effect [$F(4,84)= 2,25$, $p< 0.05$]. They were found dose effects both in SHR and Wistar on D-AMP [$F(4,28)=15.55$, $p< 0.01$; $F(4,28)=5.14$, $p< 0.01$, respectively] and MPH, [$F(4,28)=5.73$, $p< 0.01$; $F(4,28)=21.19$, $p< 0.01$, respectively] . Wistar rats showed an increase in entries under 0.3-mg/Kg dose of D-AMP when comparing to lower and higher doses of respectively 0.1- and 1.5-mg/Kg ($p< 0.05$ for both comparisons). SHR rats, by their own, showed decreases in entries under highest doses of both drugs, respect all lower doses ($p< 0.05$) on D-AMP and when comparing to lower doses of 0-, 0.6- and 2.5-mg/Kg on MPH.

Effects of SKF 38393: analysis of licks revealed a strain effect [$F(2,21)= 23.09$, $p< 0.01$] with higher rates of licking in SHR. Only Wistar rats revealed a dose effect [$F(3,21)= 4.02$, $p< 0.05$], where an increment in their licks under 3-mg/Kg dose when comparing to both 0- and 1-mg/Kg doses ($p< 0.01$) was found. Analysis of magazine entries revealed no strain effect, but dose effects. Both Wistar [$F(3,21)= 8.55$, $p< 0.01$] and WKY [$F(3,21)= 17.49$, $p< 0.01$] rats showed dose-dependent decreases in entries. However SHRs [$F(3,21)= 9.54$, $p< 0.05$]showed higher rates of entries under all drug doses when comparing to 0-mg/Kg dose, with no differences among these doses ($p< 0.01$ for all comparisons).



Effects of Quinpirole: Analysis of licks revealed a strain effect [$F(2,21)= 35.97$, $p< 0.01$], SHRs made more licks at compare to both Wistar and WKY rats, but as supported by strain X dose interaction effect [$F(4,84)= 5.42$, $p< 0.01$],



Panel 2. Show averages (±SEM) of licks (left figures) and magazine entries (right figures) for SHR (top figures), Wistar (middle figures) and WKY (bottom figures) in FT 90-s schedule. (N= 24).

these differences were attenuated under 0.1-mg/Kg dose when comparing SHRs to both control strains ($p< 0.01$).

A dose effect was only found in SHR [$F(4,28)= 23.63$, $p< 0.01$], where a dose-dependent decrease in licks was found. Magazine entries analysis revealed no strain effect, but gradual dose-dependent decreases in entries both in Wistar [$F(4,28)= 11.30$, $p< 0.01$] and WKY [$F(4,28)= 19.07$, $p< 0.01$] rats. A strain X dose interaction effect [$F(4,84)= 7,61$, $p< 0.01$] revealed that SHR did more entries under 0.1-mg/Kg dose at compare to both Wistar and WKY ($p< 0.01$ for both comparisons).

Effects of SCH 23390: SHR showed higher rates of licks as compared to Wistar and WKY rats [$F(2,21)= 14.57$, $p< 0.01$],. Dose effects were observed both in SHR [$F(3,21)= 23.91$, $p< 0.01$] and Wistar [$F(3,21)= 3.39$, $p< 0.05$], with gradual dose-dependent decreases in licks. As revealed by a strain X dose interaction effect [$F(3,63)= 9.21$, $p< 0.01$], SHRs did more licks both under 0- and 0.001-mg/Kg doses at compare to Wistar and WKY rats ($p< 0.01$). Magazine entries analysis revealed similar results as found for FT 30-s: no strain effect but dose effects with gradual dose-dependent decreases in entries in SHR [$F(3,21)= 11.48$, $p< 0.01$], Wistar [$F(3,21)= 18.06$, $p< 0.01$] and WKY [$F(3,21)= 50.52$, $p< 0.01$] were found.

Effects of Eticlopride: analysis of licks revealed a strain effect [$F(2,21)= 64.83$, $p< 0.01$], SHRs made more licks at compare to both Wistar and WKY. They were found dose effects both in SHR [$F(4,28)= 22.32$, $p< 0.01$] and Wistar

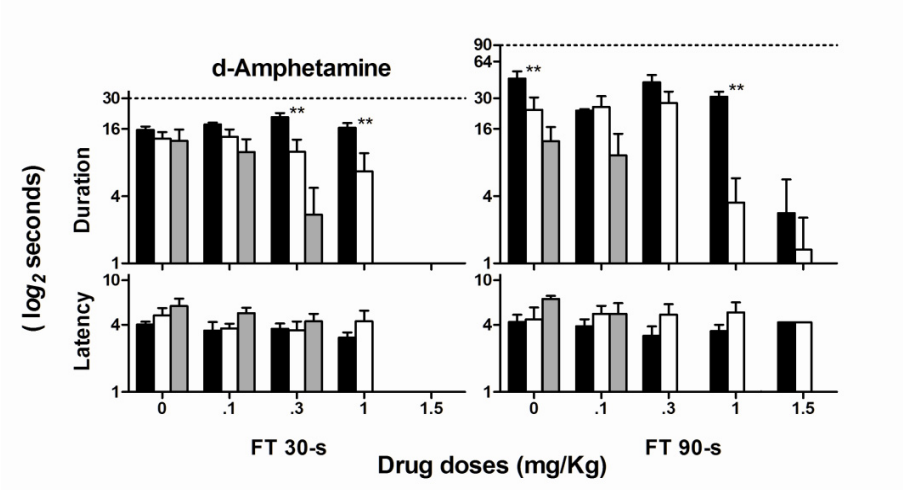
[$F(4,28)= 7.52$, $p< 0.01$] rats. As revealed by a strain X dose interaction effect [$F(4,84)= 7.85$, $p< 0.01$], SHRs under 0.001-mg/Kg dose did more licks at compare to both Wistar and WKY rats, and the same difference was found under 0.003-mg/Kg dose, but only at compare to WKYs ($p< 0.01$ for all comparisons). Magazine entries analysis revealed no strain effect, but dose effects for all strains. A dose-dependent decrease in WKY [$F(4,28)= 27.90$, $p< 0.01$], and decreases under doses of 0.01- and 0.1-mg/Kg at compare to lower doses ($p< 0.01$) both in SHR [$F(4,28)= 19.06$, $p< 0.01$] and Wistar rats [$F(4,28)= 13.69$, $p< 0.01$].

3.2.3. **Latency and Duration of SIP episodes**

Figure 3 represents averages (±SEM) of latency (bottom figures) and duration of the SIP episodes (upper figures) for every group, in each of the FT schedules of 30- (left figures) and 90-s (right figures) on D-AMP treatment. Although the increase in drug doses decreased the lick behaviour both in Wistar and WKY rats, the analysis of latencies neither showed differences among these strains nor respect to SHRs, that is, these drink episodes did not took longer to start in any group regardless of the dose of D-AMP administered. At contrast, they were found differences in the duration of the SIP episodes, as revealed by strain effects in both FT schedules of 30- [$F(2,21)= 13.701$, $p< 0.01$] and 90-s [$F(2,21)= 22.650$, $p< 0.01$].

SHRs performed the longest SIP episodes at compare to Wistar and WKY rats in both schedules, and Wistar rats, by their own, showed the same difference in FT 90-s at compare to WKYs. Differences in favor of SHRs in FT

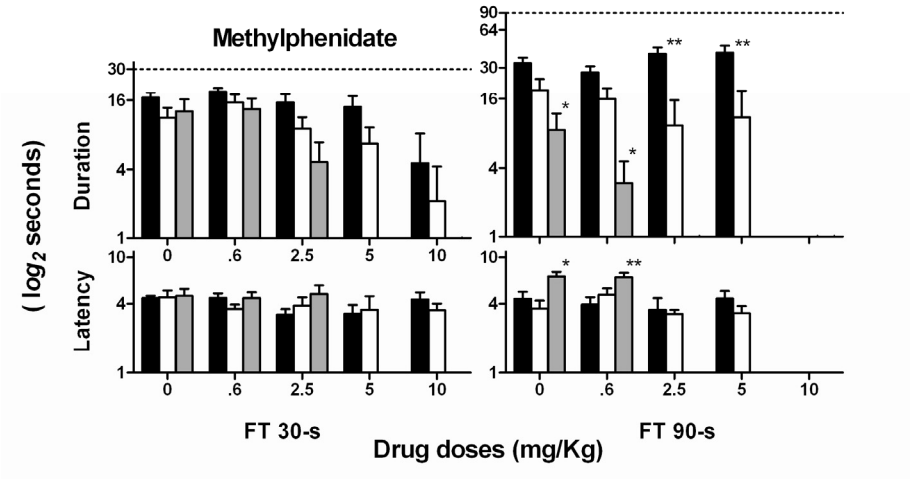
30-s schedule, as revealed by a strain X dose interaction effect [F(8,84)= 4.506, p< 0.01], consisted of SHRs increasing the most the duration of their SIP episodes under the intermediate dose of 0.3-mg/Kg (p< 0.01) at compare to control groups, and also on 1-mg/Kg dose, under which Wistar rats had reduced their SIP episodes' duration and WKY rats did not show adjunctive drinking (p< 0.01).



Panel 3. Mean (± SEM) Latency (bottom graphs) and Duration (upper graphs) of SIP episodes in FT 30-s (left graphs) and FT 90-s (right graphs) on D-AMP. * = *p*< 0.05. ** = *p*< 0.01. (N= 24).

All groups showed gradual dose-dependent decreases in the duration of the SIP episodes. Wistar and WKY rats both in FT 30- [F(4,28)= 10.083, p< 0.01; F(4,28)= 8.152, p< 0.01, respectively] and FT 90-s [F(4,28)= 8.475, p< 0.01; F(4,28)= 5.174, p< 0.01; respectively]. At contrast, SHRs showed no differences in their SIP episodes durations, but under the highest dose of 1.5-mg/Kg, and as their control rats did, they abandoned SIP in FT 30-s [F(4,28)= 28.819, p< 0.01] and

reduced their SIP episodes duration in FT 90-s [$F(4,28)= 10.899$, $p< 0.01$]. In FT 90-s schedule a strain x dose interaction effect on D-AMP [$F(8,84)= 4.937$, $p< 0.01$] revealed that, under low and intermediate doses of 0.1- and 0.3-mg/Kg Wistar rats increased the duration of their SIP episodes, and in fact not only achieving SHRs' durations, but also showing longer durations at compared to WKYs under 0.3-mg/Kg dose ($p< 0.05$), a result that was only observed on D-AMP. WKY rats differed from SHR under all drug doses except under the highest one that prevented SIP in all animals, and from Wistar, as described, only under 0.3-mg/Kg dose. As in FT 30-s similar dose effects in FT 90-s in all groups were observed.



Panel 4. Mean (± SEM) Latency (bottom graphs) and Duration (upper graphs) of SIP episodes in FT 30-s (left graphs) and FT 90-s (right graphs) on MPH. * = $p< 0.05$. ** = $p< 0.01$. (N= 24).

Figure 4 represents averages (±SEM) of latency (bottom part of the figure) and duration (upper part of the figures) of the SIP episodes for every group, in

each of the FT schedules of 30- (left part of the figure) and 90-s (right part of the figure), respect to MPH treatment. Analysis of the duration of SIP episodes showed the same pattern of results found on D-AMP administration. ANOVA showed strain effects in both FT schedules of 30- [$F(2,21)= 8.058$, $p< 0.01$] and 90-s [$F(2,21)= 25.563$, $p< 0.01$] that revealed longer durations of the SIP episodes for SHRs. A strain x dose interaction effect in FT 90-s [$F(8,84)= 6.247$, $p< 0.01$] showed that WKY rats reduced their SIP episodes' duration as MPH dose increased. However, episode duration only decreased under the highest dose of 10-mg/Kg tested in SHR and Wistar, a dose which prevented SIP in all animals. Wistar showed shorter episodes than SHR under doses of 2.5- and 5-mg/Kg ($p< 0.01$), doses in which WKY rats had abandoned SIP. WKY animals showed shorter durations under 0- and 0.6-mg/Kg at compare to respectively SHR and Wistar ($p< 0.01$). Both Wistar [$F(4,28)= 6.134$, $p< 0.01$] and WKY rats [$F(4,28)= 10.364$, $p< 0.01$] showed dose effects in FT 30-s, but differences appeared only when comparing highest vs. lowest doses of MPH ($p< 0.05$). in FT 90-s only WKY rats showed dose-dependent decreases in SIP episodes' duration [$F(4,28)= 4.583$, $p< 0.01$].

Analysis of latencies in FT 90-s revealed a strain X dose interaction effect [$F(3,56)= 10.560$, $p< 0.01$]. WKY rats tended to show longer latencies when comparing to Wistar in the saline session ($p< 0.05$), and SHRs under 0.6- and 2.5-mg/Kg doses (respectively $p< 0.05$ and $p< 0.01$). Under higher doses SHRs and Wistar rats did not differed in their latencies whereas WKY rats abandoned SIP.

With respect to all the rest of drugs used in the study, at resume, latencies did not differed between the strains on any drug. Respect to SIP episodes' durations; they were found strain effects in both FT schedules. Main result of

these analyses consisted of WKY rats showing shorter SIP episodes at compare to SHR after the administration of SKF 38393 and Quinpirole. Both in FT 30-s [$F(2,21)= 4.874$, $p< 0.02$ and $F(2,21)= 7.464$, $p< 0.01$, respectively], and in FT 90-s, [$F(2,21)= 13.532$, $p< 0.01$ and $F(2,21)= 5.165$, $p< 0.01$, respectively]. It was found a strain x dose interaction effect on Quinpirole in FT 30-s schedule [$F(8,84)= 4.571$, $p< 0.01$], SHRs showed longer duration of their SIP episodes when compare to the other groups under the low dose of 0.01-mg/Kg ($p< 0.01$), and only respect WKY under 0.03-mg/Kg dose ($p< 0.01$). After administration of Eticlopride the strain effect only appeared in FT 90-s [$F(2,21)= 3.175$, $p< 0.01$], where a strain X dose interaction effect [$F(8,84)= 3.175$, $p< 0.01$] revealed SHRs showing longer SIP episodes than the other groups under low doses of 0.001- and 0.003-mg/Kg ($p< 0.01$). Differences between Wistar and SHR were only observed on SKF 83390 and Eticlopride, and they were found no differences between Wistar and WKY on any of these conditions.

Dose effects, when appeared, were mainly related to a decrease in the duration of SIP episodes under high drug doses of any drug at compare to lower doses and saline sessions. In SHR and Wistar rats these dose effects were found on Quinpirole, SCH 23390 and Eticlopride both in FT 30- and 90-s. On Quinpirole in FT 30-[$F(4,28)= 43.480$, $p< 0.01$] and FT 90-s [$F(4,28)= 12.406$, $p< 0.01$] for SHR and respectively [$F(4,28)= 9.125$, $p< 0.01$] and [$F(4,28)= 5.224$, $p< 0.01$] for Wistar rats. Reported in the same manner, on SCH 23390, respectively [$F(4,28)= 49.971$, $p< 0.01$] and [$F(4,28)= 22.111$, $p< 0.01$] for SHR and [$F(4,28)= 16.943$, $p< 0.01$] and [$F(4,28)= 10.624$, $p< 0.01$] for Wistar, and on Eticlopride, respectively [$F(4,28)= 11.824$, $p< 0.01$] and [$F(4,28)= 12.309$, $p< 0.01$] for SHR and [$F(4,28)= 11.788$, $p< 0.01$] and [$F(4,28)= 5.931$, $p< 0.01$] for Wistar. WKY rats showed dose effects on

Quinpirole [$F(4,28)= 5.146$, $p< 0.01$] and SCH 23390 [$F(4,28)= 10.082$, $p< 0.01$] in FT 30-s, and on SCH 23390 [$F(4,28)= 13.217$, $p< 0.01$] and Eticlopride [$F(4,28)= 3.128$, $p< 0.03$] in FT 90-s.

4. Discussion

The aim of this study has been to investigate the effects of principal pharmacological treatments to control ADHD symptoms in SHR, Wistar and WKY rats on their performance in a SIP procedure. In addition, differential effects of D_1 and D_2 agonists and antagonists were evaluated too.

Once animals had been acquired SIP after 40 experimental sessions, FT 30-s schedule produced robust levels of SIP without differences between strains. However, SIP in FT 90-s was dramatically reduced both in Wistar and WKY compared to FT 30-s schedule, while it was successfully maintained in SHRs despite a reduction. Altogether, the results on these FT schedules allow us to discuss the results in two directions. On one hand, equal baselines in all groups as observed in FT 30-s enables a way to place SHR rats as a reference of ADHD model, and Wistar and WKY rats as their controls. On the other hand, SIP's resistance under unfavorable conditions, as found in FT 90-s, could be evaluated by comparing the results of FT 30-s versus FT 90-s, and in turn the persistence of SHRs on SIP in this longer schedule.

Independently to the drug treatments FT 30-s, as expected, resulted to be a strong schedule, that is, one in which the experimental conditions provided the most part of the observable variability. Nevertheless, FT 90-s provided a scenario where individual differences could be better observed, and that is what

happened. At this point the question is, why the enhanced performance showed by SHR rats in this schedule was not the observed in control groups? All the more, when excepting the little increase observed in Wistar rats on D-AMP in FT 90-s, no other drug or dose increased SIP in control animals when FT 90-s schedule was running. At contrary, this weak schedule seemed to be ineffective for SIP performance, but not for SHR rats.

4.1. **ADHD treatments**

ADHD treatments produced strain-specific differences on SIP performance. SHR showed insensibility to low and medium-range doses of both D-AMP and MPH, while Wistar and WKY showed dose-dependent reductions in SIP. Therefore, D-AMP-dependent descents on SIP previously reported (Sanger, 1978; Yoburn and Glusman, 1982; Williams and White, 1984; Pellón and Blackman, 1992) were verified in this study. However, differential patterns on these decreases were observed with D-AMP and MPH. In spite of the dose-dependent decreases in SIP observed in Wistar and WKY, SHR did not show any reduction until a dose of such a magnitude that suddenly produced an abrupt decrease in SIP, which in turn placed these animals' adjunctive drinking in those levels observed in the control rats.

The gradual decreases of licking related to D-AMP and MPH in Wistar and WKY rats showed only statistical differences when comparing the highest and the lowest doses of these drugs, whereas differences in SHR were found between the highest doses of both D-AMP and MPH and the remaining doses of each drug, without differences between them or the saline session (see Figure 1).

Given the unspecific effects of both D-AMP and MPH, the excess of surrounding dopamine produced by treatment seem to be less effective in SHRs. It is noteworthy that higher density of D_1-like DA receptors which was associated with lower affinity were found in SHR both in striatal and mesolimbic regions as compared to WKY rats (Carey et al., 1998. In addition, it has been suggested that a dysfunction on the vesicular monoamine transporter in the SHR strain could affect the DA transport into synaptic vesicles (Miller et al., 2012). Taking into account that one of the mechanisms of action of D-AMP and MPH is the facilitation of release into the synaptic cleft (Feldman et al., 1997), a reduced transport of dopamine into the vesicles could be a key factor involved in the low sensitivity to the effects of D-AMP and MPH on SIP in SHR rats. It should be noted that two weeks of D-AMP treatment normalized DA D_1-like receptors decreasing the number of binding sites and increasing the affinity to WKY levels (Carey et al., 1998). Thus, studies on the effects of chronic DA treatment on SIP should be informative of the effects of psychostimulants like D-AMP and MPH on ADHD. Similar results have been reported when analyzing locomotor responses (Myers et al., 1982; Hynes et al., 1985; Fujita et al, 2003).

The highest dose of D-AMP and MPH produced dramatically reductions in SHRs licking behaviour at compared to all the lower doses, and also magazine entries decreased with these high doses, when in turn, was observed the interference of stereotyped behaviours, which at competition with any other behaviour prevented the occurrence of SIP. In these conditions all the animals remained involved in perambulations throughout the whole session, and this was clearly observed in SHRs with D-AMP and MPH, and in a lesser degree in

Wistar rats. This result suggests that both drugs may reduce SIP indirectly, as a consequence of increases in locomotion. In this sense, it has been shown that D-AMP reduces consummatory behaviours as a consequence of dose-dependent increases of unconditioned behaviours (Cole, 1980). Furthermore, SIP ceased on D-AMP treatment when the animals gave up food consumption throughout the session, in turn an expected result, because SIP is maintained as an effect of a primal reinforcement schedule, without which adjunctive behaviour will not happen.

WKY rats showed parallel results to Wistar, but an increased sensibility to drug effects, and a trend towards to show earlier declines in behaviour as a function of the dose was observed in these animals. This strain is considered the normotensive control of SHR and is used as an animal model of depression because of their own characteristics (Solberg et al., 2001; Will et al., 2003). In general, WKY rats show low locomotor activity levels (Gentsch, et al., 1987) and even immobility (Paré, 1992). They usually show high levels of stress (Grauer and Kapon, 1993), anxiety (Carr et al., 2010) and fear (Ledoux et al., 1983) at compare to SHR, and also show resistance to anti-depressive drugs (Lahmame et al., 1997; López-Rubalcava and Lucki, 2000). Thus, the interpretation of the data from this strain may be discussed taking these characteristics into account. However, these animals did not show an increase in their adjunctive behaviour that could be due to the effects of stimulants such as D-AMP, as found in Wistar rats, which by eliminated their differences against SHR under the effects of low and intermediate doses of this drug. At contrast, WKY rats proved high sensibility to D_1 and D_2 dopamine receptor antagonists, compounds with which those described characteristics of these animals appeared to be facilitated.

In case of D-AMP and MPH, observed decreases in SIP, both in Wistar and WKY rats, were generally accompanied by the reduction of the duration of the SIP episodes, but for SHRs this duration remained constant, both on D-AMP and MPH, despite of drug dose increases. WKY rats, proved themselves more sensible to drug effects. Increases in the drugs doses produced, at first, the reduction on the duration of SIP episodes, and later their disappearance. This effect occurred on Wistar rats too, but under higher drug doses than for WKY. Moreover, these animals tended to show longer latencies to initiate SIP episodes after MPH administration compared to the other groups on in FT 90-s. Regardless this, the common result consisted on latencies remaining constant, despite drugs dose increases, as long as SIP episodes had place. In fact, SHR and Wistar rats did not differed in their latencies despite duration reductions observed in Wistar rats. The latter means that even in cases in which the animals reduced their adjunctive behavior, when this happened, it took the same to start.

As it has been observed, SHR increased the duration of their SIP episodes in FT 90-s at compared to FT 30-s, and did it both on D-AMP and MPH treatments. However, neither Wistar nor WKY rats showed such an increase on MPH, but Wistar rats did it under low and intermediate doses of D-AMP. This raises a question about how the SIP occurs, is that, the only way in which these animals may reduce their adjunctive behavior, as observed when FT schedule changed, but without reducing the duration of SIP episodes, as it occurs, is by performing fewer of these episodes.

4.2. Selective D_1 and D_2 agonists and antagonists' effects

Another aim of the study was to analyze on SIP those effects produced by the single administration of selective agonists and antagonists of D_1- and D_2-like dopamine receptors. Our results showed on one hand, that both selective D_1 and D_2 antagonists (SCH 23390 and Eticlopride) reduced SIP in all animals. The decrease was more marked for SHR rats in FT 30-s schedule and further with SCH 23390 than with Eticlopride, wich in turn proved to reduce the duration of SIP episodes both in Wistar and WKY rats under lower doses (0.001- and 0.003-mg/Kg) at compare to SHR. On the other hand, D_1 agonist SKF 38393 didn't seem to affect SIP, at least at the doses tested. In addition, neither latency nor duration of SIP episodes were altered in any group despite increases of SKF 38393 doses. Given the implication of D_1 receptors on SIP (Pellón, et al, 2011) and besides Wistar and WKY rat's results, this could be explained by the high number and low affinity of D_1 receptors found in SHR (Le Fur et al., 1981; Carey et al 1998). These neurochemical characteristics could be indicating that higher doses are needed to produce similar effects to those found in control rats.

D_2 dopamine receptor agonist Quinpirole, dose dependently reduced SIP in Wistar and WKY rats (see Figure 1), but without reducing the duration of the SIP episodes or their latency. As found with D-AMP and MPH treatments, only the highest dose of 0.1-mg/Kg reduced SIP in SHRs, and under low and intermediate doses of Quinpirole (0.01- and 0.03-mg/Kg) these animals showed longer durations of their SIP episodes at compare to control rats, especially with WKY. It has been reported increased drinking after intra-striatal injection of the dopamine D_2/D_3 receptor agonist Quinpirole in the rat (Amato et al 2012). Thus,

the specificity of the effects on different brain regions should be taken into account, but our study does not permit to ascertain these divergences given the systemic nature of the treatment.

D_1 dopamine receptor was primarily in a low affinity agonist state whereas the D_2 receptor was primarily in the high affinity agonist state (Richfield et al 1989). This could explain the different effects of D_1 and D_2 receptors on SIP. Thus, higher doses of D_1 agonist are necessary to show specific behavioral effects. At resume, both D_1 and D_2 antagonists produced similar effects decreasing licks and magazine entries in all animals by increasing drug doses, while D_1 antagonist showed no effect and the D_2 agonist Quinpirole reduced only licks. Taken together D_1 agonists and D_2 antagonists played contrary roles than D_2 agonist and D_1 antagonist in modification of adjunctive behaviour, and this is in concordance with our previous results, showing that D_1 receptors were involved on the motor aspects of SIP while D_2 receptors in the motivational component (Pellón et al., 2011).

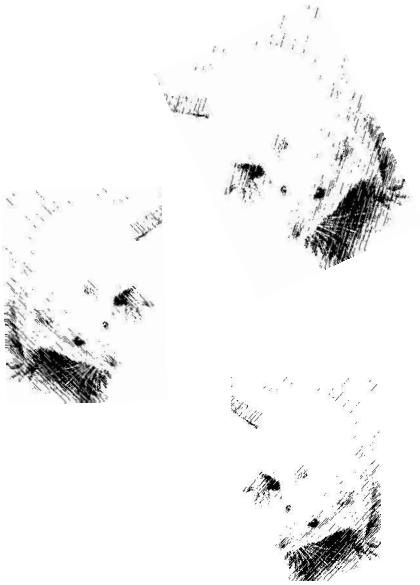
4.3. **Conclusions**

After applied several pharmacological treatments on SIP in SHR rats, those comparisons established between this ADHD animal model and their control rats points to SHR rats as a prototype of an enhanced SIP performance. And it seems that overall; all the described characteristics of these rats converge somehow. Most of the SHR's results have been justified by the persistence of these rats and their excessiveness, but away to downplay these factors, and perhaps promoted by them, a motivational factor may be related to the

indefatigable expression of SIP observed in the SHR rat. Therefore, is possible that the reason to observe such levels of adjunctive behaviour under unfavorable experimental conditions could be related by itself with SIP being instrumentally reinforced. Despite appearing not to have any function, SIP works fine when the dopaminergic system is activated, as usually occurs with reinforced behavior, even more, this facilitation survives above program changes. Thus, the interaction at between a high degree of motivation on instrumental behavior could be giving an explanation of why the SHR rats show the SIP when the rest of the animals have stopped the such behaviour.

CHAPTER V:

GENERAL DISCUSION



1. Global overview

This thesis investigated the excessive phenomenon of Schedule induced Polydipsia in an animal model of excessiveness, the spontaneously hypertensive rat. Throughout every procedure conducted was investigated how SHR rats develop and maintain SIP.

The first of these studies provided a reference from which to observe the limits displayed by SHR rats on the acquisition of SIP in comparison to Wistar and WKY rats. Once obtained these references, the possible influence of impulsivity in the acquisition of SIP was investigated. Through a second study, it was verified that, when impulsivity was measured with a delay discounting test, revealed opposite patterns for SHR and those Wistar rats that despite having been classified as high impulsive, and featuring similar measures of impulsivity to SHR rats, showed at contrary little development of SIP.

A high degree of consistency was found in classifying high- versus low-impulsive Wistar rats according to their levels of adjunctive drink. This allows us to affirm, assuming the reported data that impulsivity found in a subpopulation of control subjects, as impulsive as SHR rats but without displaying characteristics of excessive behaviour, turned out to be optimal as a predictor of low levels of acquired adjunctive behaviour.

Finally, the influence of the main pharmacologic treatments used to ADHD control when applied to maintenance of SIP pointed to SHR rats as being more resistant to those treatments. Together with the behavioural results, this finished confirming the importance of the dopaminergic system in the development and maintenance of SIP. Once discarded impulsivity as

determining factor in the exaggerated pattern of adjunctive drink shown by SHR rats; the dopaminergic divergences that exist between SHR rats and their controls could become responsible for differences found in SIP. These differences mainly consisted on elevated levels of drink for SHR rats, which in turn would be related then to their exacerbated dopaminergic function. Therefore, the importance of the dopaminergic system in reinforced behavior, and in turn in SIP may place SIP also as a reinforced behavior.

2. Development of SIP in SHR

First two studies describe maintenance of SIP in several FT schedules, but it was in study III where acquisition could be investigated, all animals were exposed at the same time to 40 sessions of SIP acquisition, and it provided data related with increases of SIP, from one session to the following, until a steady state of SIP was reached.

These results are represented in figure 1, and the interpretation of these results shows in one hand a faster acquisition in SHR rats, and on the other hand, a delay in the acquisition both in Wistar rats and WKY.

This result leads us to discuss that the development of adjunctive behaviour did not make competition with terminal responses, as can be the magazine entries. SIP procedure only affected licks, and even more, this increase on licks resulted on SIP development, and it was regardless of any redistribution or competition between responses.

Remembering figure 1 belonging to chapter I, which represents the possible distribution of those activities that can occur during the intervals between food deliveries, and combining the results shown in figure 1 of the present chapter, some observations of interest can be made on how SIP works.

The case is consummatory behaviour, the magazine entries, remained constant in spite of the SIP sessions, and it did without variation between the schedules of FT 30-s and FT 90-s. It is easy to deduce, following the figure 1, that in both conditions, we could be in anyone of areas, B or C, where the consummatory behaviour remains constant.

When observing the data corresponding to the adjunctive behaviour, in FT 30-s, one can observe that the training gradually increased the adjunctive behaviour, and did it without disturbing consummatory behaviour. SIP increased session to session occupying therefore, more and more time of the available one during each interval. A representation can be observed in figure 4 belonging to chapter I, in the graph corresponding to FT 30-s. In this same figure, we can observe that when shortening the time until a minimum of 9 seconds, SIP stops displaying a clear topography. It can be induced here that with respect to figure 1 belonging to chapter I, we would be raising up by zone A. Further, we can deduce then, based on our results that 30 seconds are enough time to observe both behaviour expressions without the restriction of any of them. Therefore, we could limit the search into figure 1 to area B.

It is there, where SHR rats establish differences with the rest of the animals, and as it has been reported throughout these studies, when they drank more than the other rats.

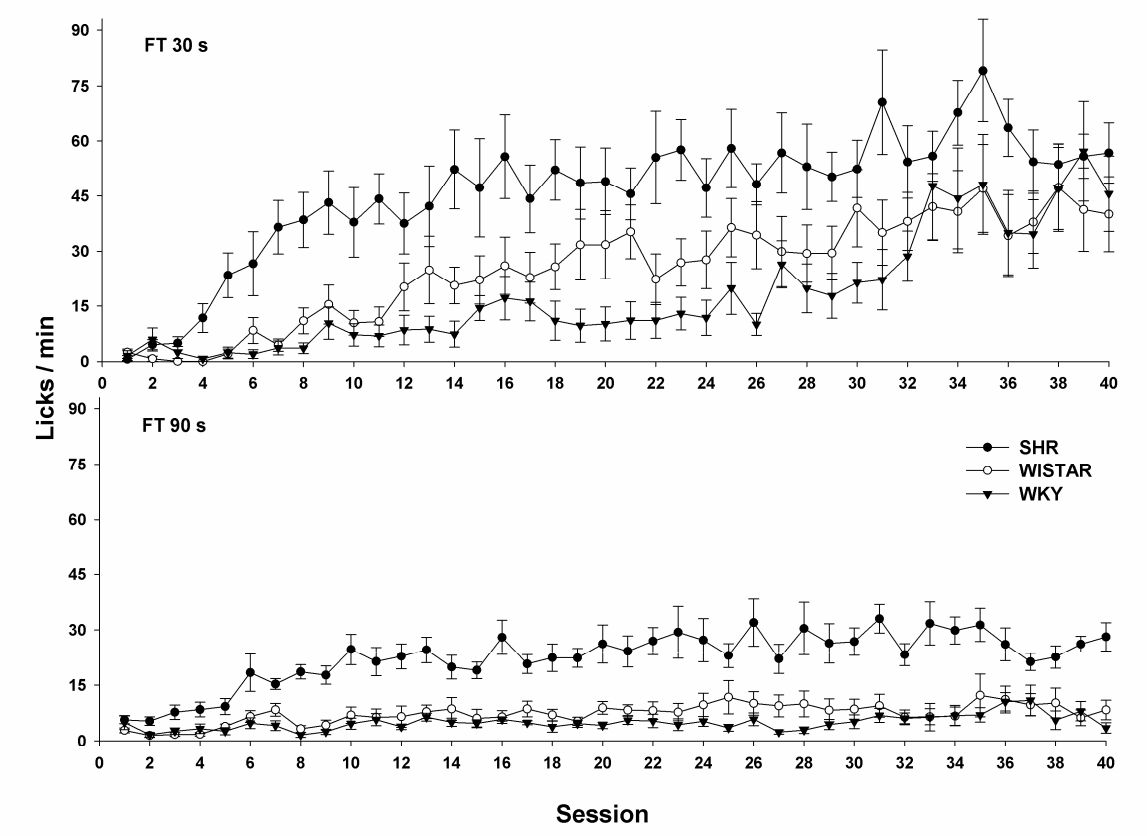
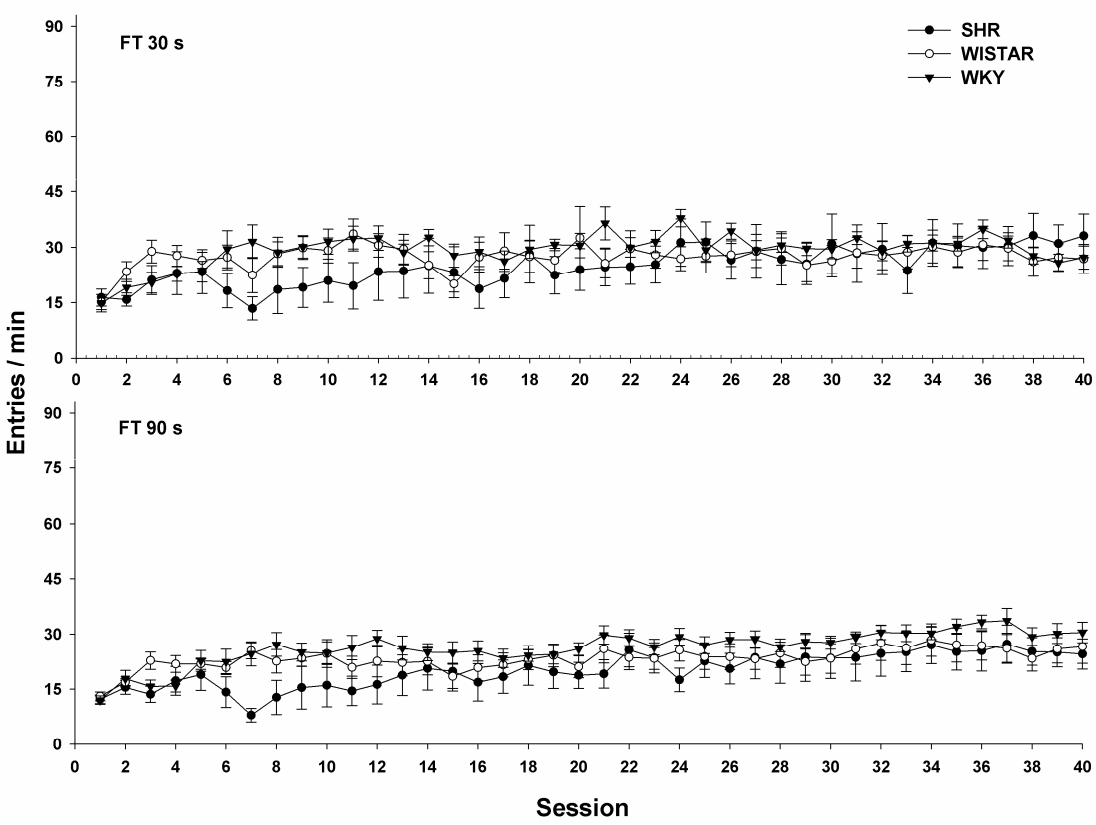


Figure 1. Shows the average (± SEM) of licks/min (left figures) and entries/min (right figures) for each group of animals, for each of the 40 sessions of SIP acquisition, in both FT sub-schedules of 30-s (top figures) and 90-s (bottom figures). MANOVA of FT 30-s schedule revealed a strain effect [$F(2,21)= 6.030$, $p< 0.009$], generally SHRs showed higher SIP rates than WKY respect to total of acquisition sessions ($p< 0.01$). There was found a strain X session interaction effect [$F(78,819)= 2.146$, $p< 0.009$], since the fourth to eleventh session SHRs showed higher levels of SIP at compare to both Wistar and WKY rats ($p< 0.05$ for both comparisons). WKY rats still showed lower SIP rates than SHRs until session 32, but throughout the eight last experimental sessions, they were found no strain differences. Analysing the rates of licking in FT 90-s a strain effect was revealed [$F(2,21)= 21.157$, $p<0.000$], the SHR rats showed higher rates of adjunctive drinking when compared to Wistar and WKY ($p<0.01$ in both cases). As found in FT 30-s a strain X session effect [F (78,819) = 3.265, p <0.000] interaction was found. In FT 90-s, from the second experimental session, SHRs showed higher rates of SIP, and this difference was maintained unchanged during the rest of the procedure at compare to both Wistar and WKY rats (p <0.05 in all cases).



Analysis of magazine entries throughout acquisition revealed no strain, session or interaction effect, in any of the FT schedules.

The increased expression of SIP performed by SHR rats was because they persistently drank water through the whole sessions, and their episodes of drink in those conditions were longer than in control rats, which tended to leave the adjunctive drinking. Still more, the pharmacological manipulation carried out during study III did not make to vary in SHRs the duration of the SIP episodes.

In order to describe possible distribution of responses through SIP procedure, they can be applied models such as the Dynamic Bi-Exponential

Refractory Model (BERM) (Brackney et al., 2011), which enables the instrumental response to be divided into bouts, and these groups of responses to be compared to one another assuming the estimate of some behavioural parameters present in these bouts, such as their duration, number of responses entailed or speed at which such responses occur.

Those models based on bouts of responses describe well the instrumental behaviour and applied to SIP could provide evidence that adjunctive behavior acts like other behaviors reinforced.

Taking data of SIP acquisition on study III, last eight sessions of those showed in figure 1, BERM model were applied. Results concerning both latency and duration of the episodes of SIP did not differ from those observed in figures 3 and 4 belonging to chapter IV when saline sessions were represented. These results mainly consisted on longer durations on episodes of SIP for SHRs in FT 90-s at compared to FT 30-s and an increase in latencies in control's rats in FT 90-s, especially for WKY rats.

Figure 2 show, as an example, the adjustments of data to BERM model for the median subjects of every group, both in FT 30- and 90-s. In general, goodness of fit of the data of these animals was acceptable, mean of the proportion of variance accounted for the model (PVAF) for all subjects of 0.77 respect to FT 30-s and 0.81 respect to FT 90-s.

All animals clearly distributed their licks in bouts when involved in SIP, despite differences in the amount of emitted behavior. All graphs in figure 2 show both intra-bout and inter-bout distribution respect to their inter time responses (IRTs).

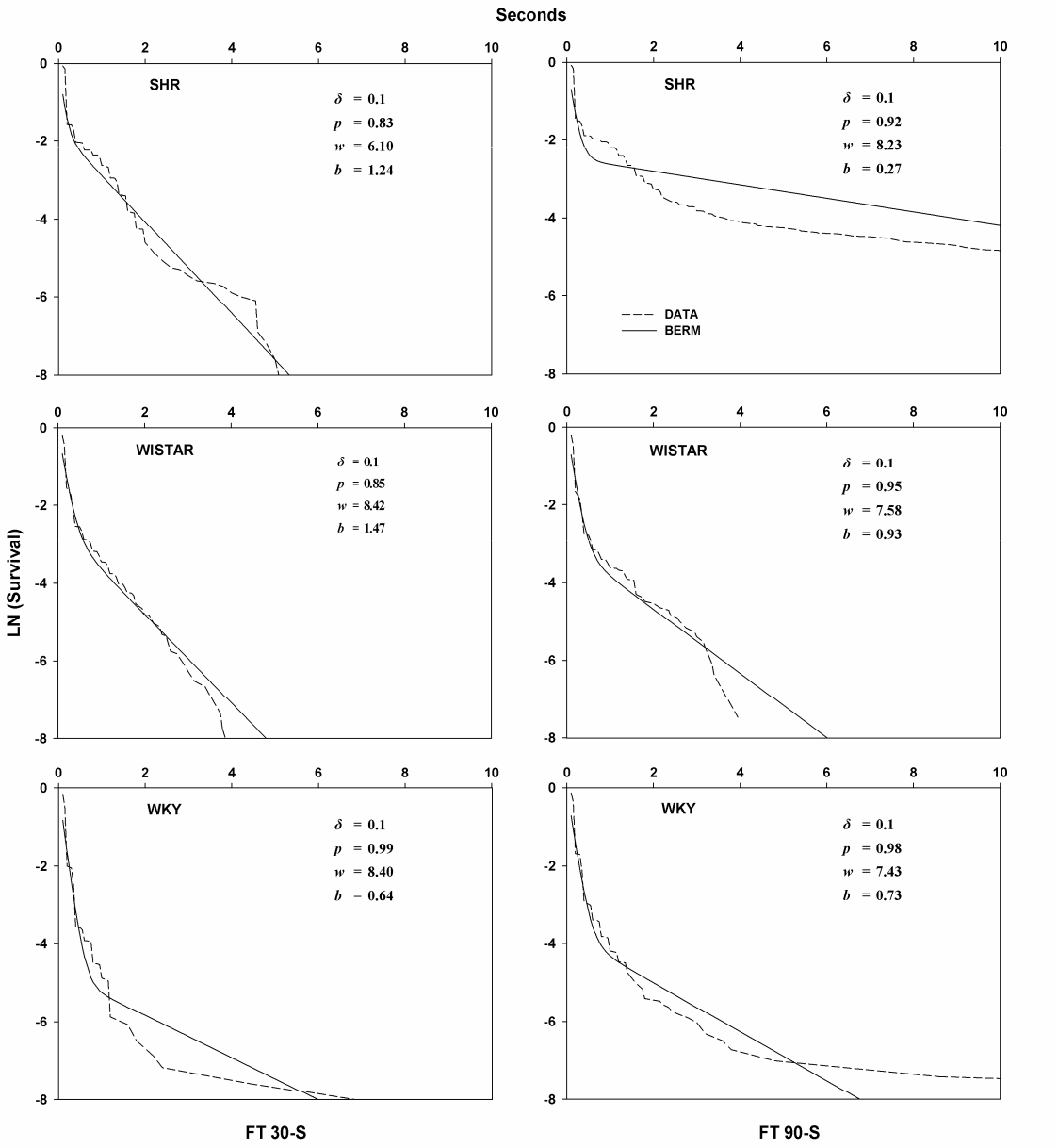


Figure 2. Show predictions of BERM model (continuous line) and data adjusts to the model (dashed line) for median subjects of SHR (top graphs), Wistar (medium graphs) and WKY (bottom graphs) both in FT 30- (left graphs) and 90-s (right graphs). p= probability of continuing in a bout after a response; b= bout-initiation rate, w= within-bout response rate, and δ= minimum refractory period between two responses. Moreover, 1/b + δ= mean time between bouts and 1/w + δ is the mean time between responses within bouts.

As found in reinforced behaviour these distributions differed from one another. Most of the responses occurred in rapid succession, forming the bouts of behaviour, while another intra-bout distribution collected fewer answers, which gave beginning to these bouts of behaviour.

Together, acquisition data and BERM adjustments offers a complementary description of both development and maintenance of SIP.

Finally, BERM estimations altogether with total responses emitted, and duration of the episodes of SIP showed SHR by effecting more bouts but shorter than Wistar or WKY, which is another evidence of the excessiveness usually present in this strain when bout's models are applied to operant behaviour (Hill et al., 2012) and also here in data of our animals on SIP.

3. Impulsivity, SIP and excessive behaviour

SHRs displayed greater levels of impulsivity in delay-discounting tests than do their normotensive controls WKY rats, but the impulsivity displayed by these animals has been linked to behavioural excess. By conducting open field tests, from the fourth week of life SHRs begin to increase their levels of activity and locomotion vis-à-vis WKY rats in measures relating to exploration or horizontal activity, total time in movement and vertical activity, and an increase is likewise seen in stereotype behaviours such as sniffing, grooming and head bobbing. In turn, they display lower values in measures relating to fear or anxiety, such as thigmotaxis. All these differences remain unaltered during the rest of these animals' lives (Hsien Y.L. et al., 2008).

In addition, SHRs usually exhibit more behaviour in absolute terms than WKY rats (Alsop, 2007a, 2007b). Study II have shown that this also occurs when SHRs are compared to WKY and Wistar rats, in terms of total responses made to complete lever-press training.

Another evidence of excessiveness in SHRs is to be seen in a slower extinction of behaviours such as lever pressing. In one study of this type on SHRs, using a VI food-reinforcement schedule with a range of values from 12 to 190 seconds, it was found that after acquisition of lever-pressing behaviour, at the start of the extinction sessions the SHRs engaged in more bouts of behaviour and in turn, took longer to reduce these than did the WKY rats (Brackney et al., 2012), those are the same trends found in our results.

Our study II afforded too evidence relating, in this case, to the slower decline of unreinforced behaviour when the animals were allowed to adjust their behaviour to a minimum response criterion under an FI 30-s reinforcement schedule in which the possibility of obtaining the reinforcer was signaled by a light on the currently active lever. At the start of these training sessions, the SHRs registered significantly more lever-related behaviour, and it was in the last of these sessions when these animals reduced their non-reinforced responses to the same level as the other groups of rats. This deficit in response inhibition and extinction might, in turn, be related to the maintenance of elevated levels of SIP that have been observed in SHRs under reinforcement schedules in which the remaining animals stopped drinking, as are all those FT with durations up to 60 seconds.

In study II too, evidence was obtained of faster learning among SHRs across the period of initial training in lever pressing prior to the delay-

discounting test. Over the course of initial autoshaping the criterion achieved by the SHRs was matched by the remaining animals during the fifth and last session, in which all the animals gained access to the continuous reinforcement schedule on the two levers and completed the FR-1 schedules on both. Thereafter, during the first of the four sessions of the training schedule, SHRs took significantly less time to complete the session and also registered fewer omissions with respect to the available reinforcers.

Under the SIP procedure, the drinking rates of the SHRs were higher under the FT 60- and 120-s food schedules than those of the Wistar and WKY rats. The persistence of induced drinking among SHRs under these schedules could also be viewed as behavioural excess.

In order to complete the analysis of the relationship between impulsivity and SIP, Wistar rats were included in this study as belonging to a control population to be divided according to two levels of impulsivity (high and low), similar to those displayed by SHR and WKY rats respectively. The first conclusion to be drawn on comparing the four groups of rats is that impulsivity is not confined to behavioural excess. While comparison of SHRs and WKY rats might lead one to conclude that this was indeed the case, analysis of the data on the high- and low-impulsive Wistar rats yield other results. In this study, these animals also showed differences in impulsivity, but in contrast, a practically identical degree of behavioural excess.

This absence of a relationship between impulsivity and behavioural excess can again be observed when delay discounting test results are compared to those of SIP. This would seem to indicate that, in a control population that does not display behavioural excess, impulsivity is expressed in another way in

SIP. Moreover, the high drinking showed by high impulsive SHRs might relate to specific alteration in these rats, possibly the facilitation of learning due to their hyperactivity.

4. Enhanced performance of SHR rats in SIP

A common conclusion encompassing all studies is that SHR showed an enhanced SIP acquisition compared to Wistar and WKY rats. Studies I and II have shown optimal levels of SIP in SHR, higher when comparing those schedules where FT durations were of an excessive length for SIP performance in control rats (FT 60-180 s). In turn, those other FT schedules with high environmental control generally did not allow show differences between the groups of rats (FT 9-30 s).

In this regard, when SIP are compared in studies, I and II, one can find similar results, but SHR rats tended to show SIP's levels even higher in study II, fact that could be due to increasing age at time of procedure. Anyhow, since SHR rats, during their adulthood, weigh approximately 100 gr less than their controls, there would be expected that, even increased, their water consumption was less than found in their controls, but this result was never found. This matter was evaluated in study, I, when water consumed by animals was relativized with respect to their weight in grams, that is, when ml/gr were compared, and so differences between strains considerably increased. Furthermore, the results of study II revealed that SHR rats finally drank the most regardless this constant weight difference. Another result of study II, only found in SHR rats, consisted on the increase of measures of consumed water in

home-cages during SIP procedure. SHR rats transferred to their home cage the pattern of excessive drinking, in some way, when they were involved in SIP procedure. Consequently, they drank more water both in the experimental session and in their home cages at compared to Wistar and WKY rats.

5. Concluding remarks

Taken together, the three studies that comprise this thesis have attempted to provide a comprehensive view of the development and performance of SHR in PIP.

The first two studies have focused on defining a developmental etiology of this type of behaviour in animals, and the last of the studies investigated treatment.

Regarding, the first two studies conducted were relevant discarding impulsivity as a determining factor for the development of SIP. It was in the second study where it was found that away from my initial idea, impulsivity not only does not contribute by itself to facilitate the acquisition of SIP but on the contrary, proves to be an impediment to the expression of this behaviour. If there is any reason that could explain the exacerbated development of SIP in SHRs must be sought in its excessiveness and hyperactivity.

Reaching this conclusion helped constrain the reasons, among likely, that make the SHR rats show an exaggerated development of SIP in experimental conditions, as for other animals are not attractive for the development and maintenance of this adjunctive behaviour.

After all, hyperactivity shown by SHR rats is not restricted to a mere locomotor excess, beyond and as has been seen, contributes to these animals in situations in which they have to inhibit their behaviour, that is where they present their deficit. But otherwise, they get to show faster learning in tasks and fewer mistakes during learning, and this is also important because it indicates that the existing hyperactivity in these animals is accompanied by a high level of motivation.

Then, investigate dopaminergic system manipulation of those animals subjected to the procedure of SIP enabled again re-check that the results of SHRs differed again from those found in the control animals, which in turn, they do meet the predictions made by previous literature, ie, the decrease in SIP from gradual increased doses of drugs such as D-AMP or MPH.

Because of the importance of the dopaminergic system in all reinforced behaviour and PIP, this latter result regarding SHR offers an explanation of why the animals remain involved in the induced behaviour, in turn, also provides evidence that this behaviour does not work in a different way than it does when behaviour is operantly reinforced.

In this sense, the next steps will be aimed to study SIP from instrumental models of behaviour based on bouts, as anticipated in the general discussion of this thesis, and this, together with the data reported in these work, will serve to carry out a predictive model of the development of SIP. Such a model could be useful for all researchers who rely on this behaviour to model the different disorders that share with SIP its main feature, the excessiveness.

The differences found in SIP in SHR remained stable over the three studies, and reduced variability presented by these animals compared to their

controls in SIP has been helpful. But, after making all procedures described in this thesis, there are still many issues that have been emerging related, one way or another, with these studies, and also it would be worth investigating.

In the present thesis, it has been used Wistar Kyoto rats as control of SHRs, and as previously reported, these animals are in themselves an animal model of depression, and in turn are the subjects that have shown the most intriguing results.

In fact, the most important thing I learned during these years of experimental work is that those difficulties that lie behind the scientific method are not all about its complicated application, but in getting to describe the findings in a parsimonious way, because it is when it could be said that one knows what it is talking about.

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