



Research report

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

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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 s, 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 s. 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.

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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 [9]. 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 [7].

Induced behaviours by intermittent reinforcement schedules are divided into terminal and interim activities [33]. 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 [18] and attack in pigeons [19].

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 [3,21,28,29]. These results show higher

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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 [6], 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”) [6]. 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 [34]. They are hyperactive animals that show behavioural characteristics of ADHD, basically displaying an exacerbated sensitivity to delay of reinforcement [30]. This characteristic of impulsivity is manifested in the number of failures committed where it is 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. [17] introduced response-reinforcer resetting delays of 0.33–12 s 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 [14]. 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 [31]. 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 s 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 s. 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 [13]. By restricting performance to one decision (a single response) that resolves each test, only the cognitive component of impulsivity is measured [37]. 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 [13].

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 s, using a delay-discounting procedure adapted from previous studies [3,13]. 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-h light–dark cycle (light from 08:00 to 20:00 h), ambient temperature of 17–23 °C, and 60% relative humidity. Once habituated to the animal facility, the rats were housed singly in 18 cm × 32.5 cm × 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 g (range: 257–287 g); WKY, 306 g (range: 290–317 g); and Wistar, 353 g (range: 328–387 g). 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 min 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

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.

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

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 cm × 24.5 cm × 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's 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 cm × 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 3 W lamps situated on the front panel at either side of the food hopper and by an indirect 25 W 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-min water-ingestion test was conducted, which began after a lump sum of 120 food pellets (5.4 g) 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 min. 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 s) 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.

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 min, 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-min 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-min 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

Fig. 1 shows the mean (\pm 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 × 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.

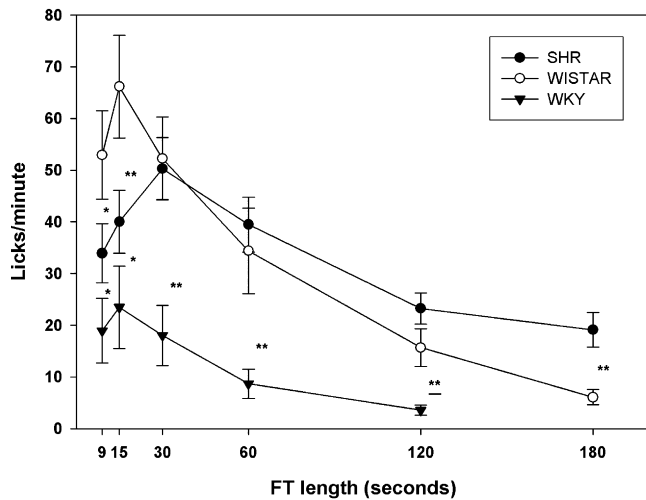


Fig. 1. Mean licks per minute given by each group of rats under all FT schedules. Bars denote standard error of the mean. * $p < 0.05$, ** $p < 0.01$, - $p > 0.05$.

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 ($p = 0.02$).

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

Fig. 2a and b depict: mean (\pm 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 (Fig. 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 \times 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 [$F(14) = 5.40$, $p < 0.01$].

Fig. 2b depicts mean millilitres consumed by each strain in terms of weight, expressed in millilitres per gram (ml/g). 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 Section 2.1). 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 \times 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.

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 [$F(14) = 6.14$, $p < 0.01$].

Fig. 3 depicts the mean (\pm S.E.M.) number of licks per minute given by each strain of rats under the different FT schedules during the three 10-min 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 min than during the remaining two 10-min periods of the session ($p < 0.01$ in both cases); for the Strain \times Part of Session interaction [$F(4,42) = 4.55$, $p < 0.01$], with both Wistar (Fig. 3b) and WKY rats (Fig. 3b) giving more licks during the first 10 min of the session ($p < 0.01$ in both cases), and SHR rats (see Fig. 3a) showing no significant differences in this respect over the entire 30 min of the experimental session; for the FT schedule \times Part of Session interaction [$F(8,168) = 3.66$, $p < 0.01$], with animals giving more licks during the first 10 min 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 min 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 \times FT \times 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 min 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

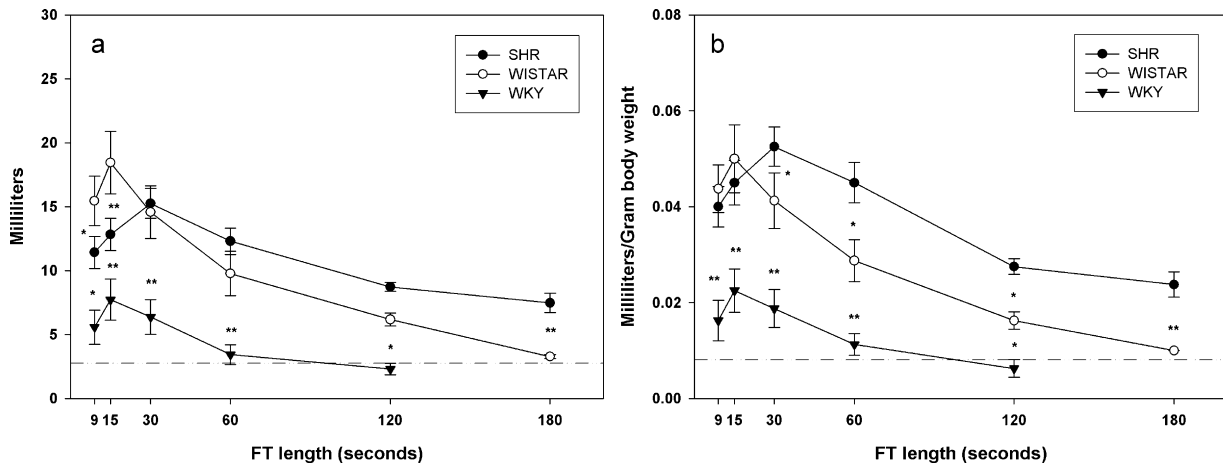


Fig. 2. (a) Mean millilitres of water consumed by each group under each FT schedule; and (b) mean millilitres consumed per gram weight by each group under each FT schedule. Bars denote standard error of the mean. * $p < 0.05$, ** $p < 0.01$.

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. 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.

Fig. 4 depicts mean (\pm S.E.M.) total licks given every three seconds (bins) during the inter-food intervals, for each of the FT schedules. Fig. 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 s of the inter-food interval ($p < 0.01$); and for the Strain \times 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 s 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).

Fig. 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 \times 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 6–12-s bins of the interval ($p < 0.01$), with Wistar rats registering a greater number of licks than SHR rats at 9 s ($p < 0.01$). The WKY group gave fewer licks than did the other rats in these central 3-s bins ($p < 0.01$).

Fig. 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 \times Bin interaction was also in evidence [$F(18,189) = 4.83$, $p < 0.01$]. The highest number of licks was given at the 9–12-s 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 6–15-s bins, the WKY rats gave fewer licks than did the other two strains ($p < 0.01$ in all cases).

Fig. 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 giv-

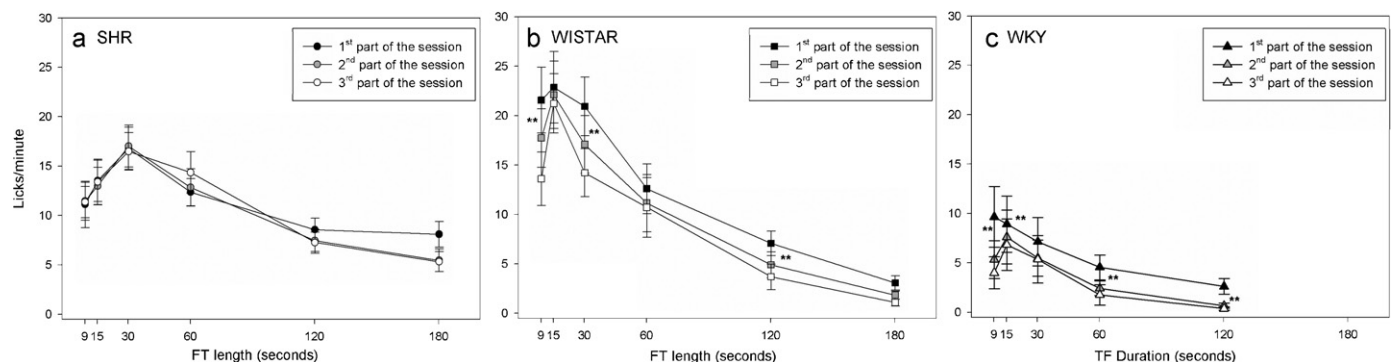


Fig. 3. Mean licks per minute in 10-min periods of the experimental session, for each FT schedule. Data for each strain of rats are shown separately in the following figures: (a) SHR; (b) Wistar; and (c) WKY. Bars denote standard error of the mean. * $p < 0.05$, ** $p < 0.01$.

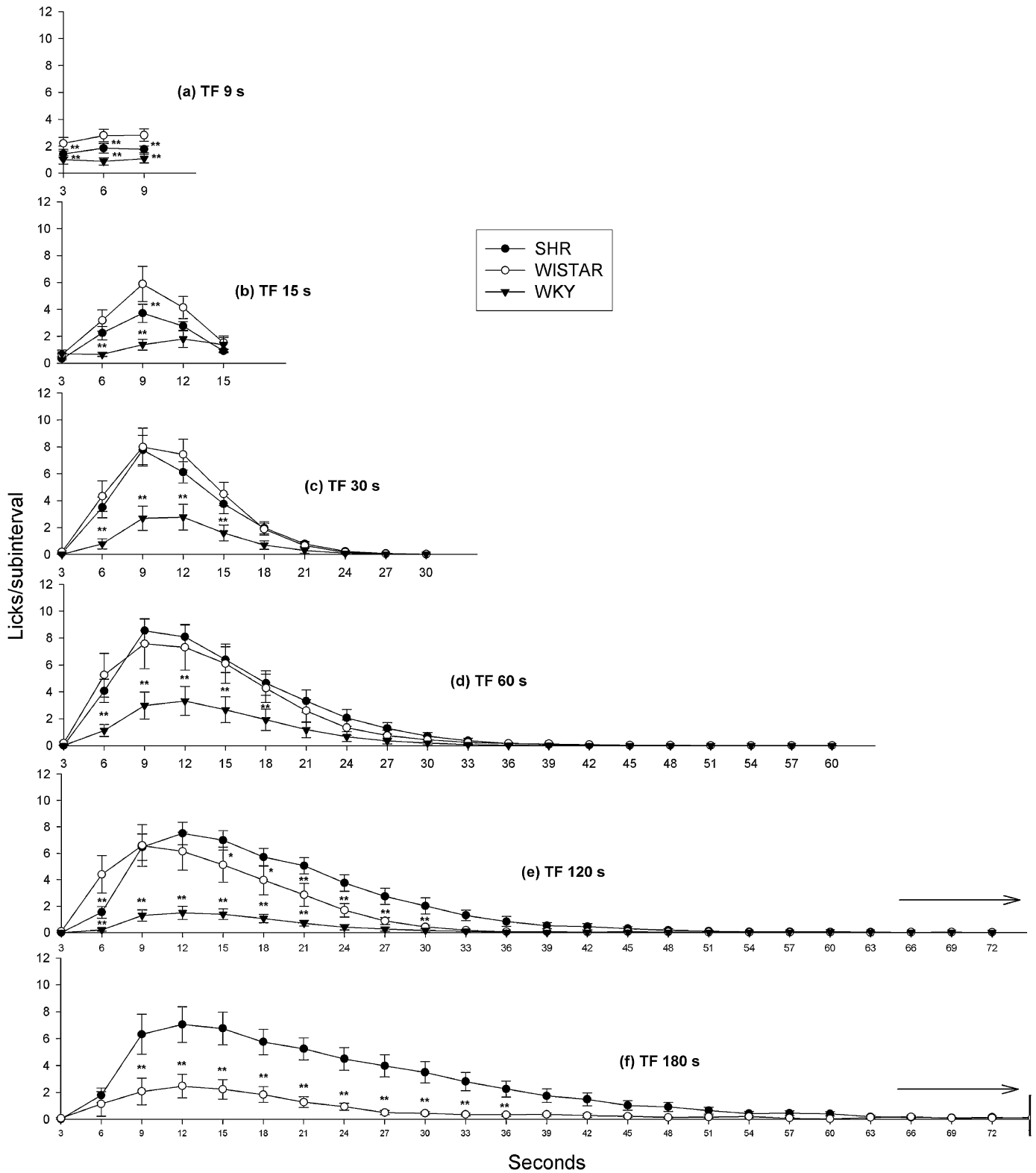


Fig. 4. Mean licks given in each three-second bin, for each group of rats and each FT schedule. Bars denote standard error of the mean. * $p < 0.05$, ** $p < 0.01$.

ing 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 (9–12 s) than in any other ($p < 0.01$); as well as a significant effect for the Strain \times 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 9–15-s bins ($p < 0.01$). 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 6–18-s bins ($p < 0.01$).

Fig. 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 9–15-s bins ($p < 0.01$); and for the Strain \times Bin interaction [$F(78,819) = 8.53$, $p < 0.01$], with the SHR group achieving the highest number of licks at the 9–15-s 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 s (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 12–18- ($p < 0.04$) and 21–27-s bins ($p < 0.01$). The WKY rats recorded a lower number of licks at the 6–21-s bins versus the Wistar group ($p < 0.01$), and at the 9–30-s 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.

Fig. 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 9–18-s bins versus the remaining bins ($p < 0.05$); and for the Strain \times Bin interaction [$F(59,826) = 8.69$, $p < 0.01$], with SHR rats giving more licks than Wistar rats at the 9–36-s bins ($p < 0.01$; except $p = 0.01$ in the 12th bin). The SHR group gave the maximum number of licks at the 9–15-s bins ($p < 0.03$) and again at the 18-s bin ($p < 0.01$), with respect to the remaining bins. The Wistar group of rats reached the maximum number of licks at the 6–21-s 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 [8,12]. 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 [10]. 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 [11]. 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/g 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 Fig. 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 Fig. 4). This form of adjunctive drinking fits the initial definition of schedule-induced polydipsia [7], 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

[2,23]. 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 [16].

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. [15] 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. [15], 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 [13]. 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 [13]. 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 [24], 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 g, while that of the Wistar rats was 431 ± 20 g. 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

3.1.3.1. 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.

3.1.3.2. 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 s 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.

3.1.3.3. 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 s 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-s 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-s 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. Once 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 s, 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 s.

3.1.4. Statistical analysis

3.1.4.1. 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-s 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 s).

3.1.4.2. 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;

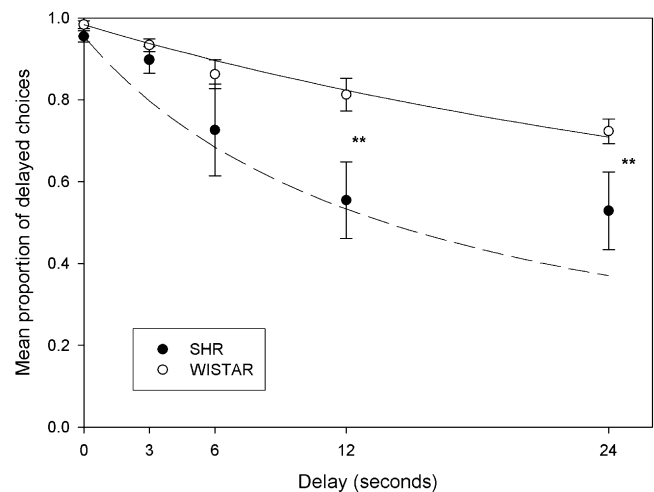


Fig. 5. Mean percentage of delayed choices in each group, according to the delays established for rewards of greater magnitude. Bars denote standard error of the mean. Lines correspond to the discount function that best fits the data, the unbroken curve represents the fit of the Wistar group and the 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. ** $p < 0.01$.

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.

3.1.4.3. Hyperbolic discounting curve. A curve was fitted for the data obtained, using the least squares method and the following formula [22].

$$Y = \frac{A}{1 + KD} \quad (1)$$

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-s 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

3.2.1. Proportion of delayed choices of greater magnitude

Fig. 5 depicts the mean (\pm 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 s. 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 \times 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-s delays than did

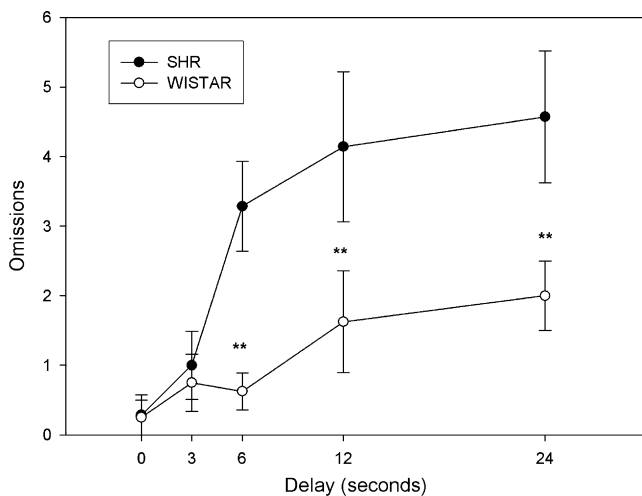


Fig. 6. Mean omissions committed during forced-choice trials, according to strain and designated delay. Bars denote standard error of the mean. ** $p < 0.01$.

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

3.2.2. Omissions during forced-choice trials

Fig. 6 shows mean (\pm 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 \times 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-s delays ($p < 0.01$ in all cases). At the 0- and 3-s 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-s delay condition on forced-choice trials of immediate reward and on the 12- and 24-s delay conditions on forced-choice trials of delayed reward. There were no omissions on free-choice trials at any delay condition.

3.2.3. Discounting curve

The estimates of the parameters of the hyperbolic discount curve after using Eq. (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 [13]. Despite the concordance in the results, the SHR rats in our study displayed a higher level of self-control during the longest delay (24 s) than had been reported previously [13]: 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.

[13], 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-s 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 Fig. 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-s 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-s delay condition, because greater omissions of SHR for the longer delays of 12 and 24 s 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 s, for example) and to ignore the alternative leading to the large-delayed reward when the value of the delay becomes excessive (from 12 s onwards). With very short delay conditions (i.e., 3 s) none of the forced options (immediate versus delayed) were ignored.

In terms of the fit of the data yielded by Eq. (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 s). The reason then cited for the more steep delay-of-reinforcement gradients in the SHR rats [15] 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

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. * $p < 0.05$, ** $p < 0.01$.

Delays	FT schedules				
	9 s	15 s	30 s	120 s	180 s
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*

Wistar rats [4], 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 [3]), 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.

Accepting that there are two basic forms of impulsivity, namely, motor and cognitive impulsivity [5,36], SHR rats would show themselves to be more cognitively impulsive (measured by the delay-discounting task: [13]; and our results) and less motor impulsive (measured by the 5-choice test: [35]). 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 [20], and theoretical proposals have been advanced which link adjunctive to operant behaviour [1,26]. Similarities between adjunctive and operant behaviour have repeatedly been shown at a behavioural and pharmacological level [25,27], 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 [32], 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 [13].

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