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Assessing Impulsivity in Prepubertal Male Rats: A Novel Device and Method to Assess Motor and Cognitive Impulsivity

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The use of animal models in studies of impulsivity has made valuable contributions to our understanding of this behavioral trait as it relates to disorders such as attention deficit hyperactivity disorder. The objective of this work was to develop a paradigm that would make it possible to evaluate both motor and cognitive impulsivity using the same device after a short training period. The operant behavior demanded in this device consists in having rats cross a bridge after receiving a signal to obtain a reward that is available on a goal platform in a Wait-to-Go-signal task, or in crossing a bridge after the animals make a choice between two alternatives in a Delay-discounting task. To test this device and method, a study was conducted using an animal model of dopaminergic dysfunction produced by prenatal alcohol treatment (which has been shown to cause attention deficits and alterations of impulsivity in adult rats). Compared with controls, prepubertal male rats treated prenatally with alcohol showed both higher cognitive and higher motor impulsivity as assessed by the parameters used. Although attention changes proved not to be dependent on prenatal treatment, they were sensitive to the task performed. The device and methods introduced herein thus constitute useful instruments for evaluating impulsivity. Their significant advantages include a short investment in training time, and the ability to assess different types of impulsivity from the vantage point of distinct theoretical perspectives.

Keywords: cognitive impulsivity, motor impulsivity, attention, novel method, inhibitory control

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Impulsivity is a behavioral trait associated with addiction and some behavioral disorders, such as attention deficit hyperactivity disorder (ADHD). Several instruments, tasks, and animal models have been used to study the cognitive and neurofunctional factors that underlie impulsivity. According to Swann, Bjork, Moeller, and Dougherty (2002), the approaches used to assess impulsivity can be classified as (1) choice paradigms, in which impulsivity is assessed based on a choice made between a small, immediate reward and a larger reward delivered after a time delay, and (2) behavior inhibition paradigms, where impulsivity is assessed through rapid responses without an adequate assessment of context, that is, performing premature responses, or the inability to sustain a demanded behavior. As a result, two types of impulsive behavior have been described: cognitive and motor impulsivity.

The choice paradigms are commonly tested through Delay-discounting tasks that involve decision-making behavior. Most studies of this kind use operant chambers equipped with at least two levers. Briefly, pressing one lever produces the immediate delivery (availability) of a reinforcer that is of low value in terms of quantity or quality, whereas pressing the other one results in a high-value reinforcer, but only after a variable postresponse delay (Marusich & Bardo, 2009; Calvert, Green & Meyerson, 2010; Mendez et al., 2010). These methods require several weeks of training for the animals to learn the operant behavior; thus, studies in rodents based on these procedures have been performed only with adult animals. Unlike most paradigms used to assess cognitive impulsivity, the T maze is a method of delay-discounting that involves only a short training time. In this type of test the rat is trained to cross a T maze to reach a food reward and to choose between two arms, one of which leads to a goal compartment that provides a small reward immediately, whereas the other gives access to a goal compartment that holds a larger reward. When the rats choose the arm with a larger reward they are detained between two panels for a waiting period of several seconds before being allowed access to the reward. Studies using this paradigm describe a broad ratio between the size of the smaller and larger rewards (e.g., 1:5), and a reduced number of trials per session (Bizot, Thiébot, Le Bihan, Soubrié & Simon, 1988; Bizot et al., 2007; Bizot, David & Trovero, 2011). We do not know whether this condition reflects a disadvantage for the analysis of behavioral data, but the reduced number of trials per session is probably a result of the animals reaching satiation after only a short time, especially when they select the larger reward.

There are several methods for assessing impulsivity based on anticipatory behavior, the performance of anticipated responses, or the inability to sustain a demanded behavior, all of which define motor impulsivity. Go/No-go–like tasks constitute an approach that has proven useful in achieving the objectives of studies of this kind, which commonly use the operant response of lever-pressing to assess the ability to inhibit responses. In this method, the animal must discriminate between the signals that distinguish the Go period (reinforced responses) from the No-go period (nonrein-

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forced responses). Go and No-go periods are alternated during the session, and the responses in the latter are considered impulsive (Paine, Dringenberg & Olmstead, 2003; Paine & Olmstead, 2004; Anker, Gliddon & Carroll, 2008). One variant of this kind of task is the stop signal reaction time (RT) task, which assesses the ability to inhibit a response when a stop signal stimulus is presented during a Go trial. In this case, the subjects are rewarded when, upon receiving the stop signal, they withhold a response for a preestablished amount of time (Eagle et al., 2009, 2011).

The 5-choice serial RT task (5-CSRTT) is another method used to assess motor impulsivity. Briefly, an animal is trained to detect when a light comes "on" in one of five holes located in a panel. When it introduces its snout into the illuminated hole (correct response) its behavior is reinforced. The holes are illuminated at random following a certain intertrial program. A premature response occurs when the animal introduces its snout into a hole before the signal light comes "on"; this is judged an impulsive response. Nonresponses (omissions) occur when a hole lights up but the animal takes no action, and are classified as signs of attention failure; hence, this method can also be used to assess sustained attention (Dalley, Theobald, Pereira, Li, & Robbins, 2002; Belin, Mar, Dalley, Robbins, & Everitt, 2008; Dalley, Mar, Economidou, & Robbins, 2008).

Another approach based on having the animal introduce its snout into a hole as an operant response is called the Peak-interval (PI) task. Here, the animal is challenged to discriminate the interval time between stimuli, and premature responses are considered an index of impulsivity (Buhusi & Meck, 2002; Meck, 2006; Matell & Portugal, 2007).

Other methods that use the operant response of lever-pressing to assess motor impulsivity include Differential reinforcement of low rates (DRL), in which temporal estimation plays an important role in the animal's inhibitory response (Sanabria & Killeen, 2008; Orduña, Valencia-Torres & Bouzas, 2009), and Fixed consecutive number (FCN), which tests an animal's ability to inhibit a prepotent behavior (Evenden, 1998; Evenden & Ko, 2005; Dellu-Hagedorn, 2006; Rivalan, Grégoire & Dellu-Hagedorn, 2007).

It is beyond the scope of the present study to present an exhaustive review of all the methods used to assess impulsivity; however, the relevant point is that methods which entail operant responses, like pressing a lever or introducing the snout into a hole, require several weeks of training before the test animals adequately acquire the learned behavior. Therefore, most experiments that adopt such approaches in rodents can use only adult animals, or protocols in which the extended training period from infancy to adulthood does not constitute a significant factor in the interaction of the variables to be studied. This consideration is important because it is often necessary to assess animal behavior at earlier stages of their development; for example, before puberty, an age when the activational effect of sexual hormones does not yet exert a significant influence on cerebral functions and behavior. This problem can emerge when a species useful for experiments of this type, such as the rat, have only a short period between weaning and puberty. Moreover, it has been shown that distinct aspects of impulsive behavior (Winstanley, Theobald, Cardinal & Robbins, 2004; Winstanley, Eagle & Robbins, 2006), and motor and cognitive impulsivity (Baarendse & Vanderschuren, 2012; Robinson et al., 2009), may have distinct neurophysiological substrates; so assessing both types of impulsivity is an important challenge in preclinical studies designed to explore behavioral disorders like ADHD on the basis of animal models.

In this study, a device and methods were designed in an effort to develop paradigms that would make it possible to evaluate both types of impulsivity behavior and attention through a more "naturalistic" operant response requirement and after only a short training period, an approach that would be useful in assessing developmental periods that are currently difficult to study with most available paradigms, as described in the literature. This is the case of the transition between infancy and preadolescence in the rat, a period when several developmental disorders like ADHD are most often diagnosed and treated.

With the aim of validating the proposed new device and methods, an experimental study was conducted that consisted in two procedures: one to assess cognitive impulsivity, the other to assess motor impulsivity and attention. For this purpose, a model of dopaminergic system dysfunction produced by prenatal alcohol treatment was used. It is well-documented that dopamine seems to be involved in impulsivity, motor activity, and attention processes. In the model followed, alcohol exposure from 8–20 days of prenatal age produces alterations in the dopaminergic neurons of the ventral tegmental area (Shen, Hannigan & Kapatos, 1999; Choong & Shen, 2004a). Studies have described that after receiving this treatment adult rats show increased impulsivity and attention deficits compared with control rats (Hausknecht, Acheson, Kieres, Shen, Richards & Sabol, 2005). It was on the basis of these data that this model was considered adequate to test and validate the device and methods presented herein.

Method

Device Description

Figure 1 presents a full frontal view of the device developed to train the animals and evaluate impulsivity behavior and attention. This device is called the "Transitional Bridge." It is an original design that was built and programmed as a prototype by professional engineers. As Figure 1 shows, it consists of a main structure with two frontal, lateral pedestals (60 cm high), separated (60 cm)

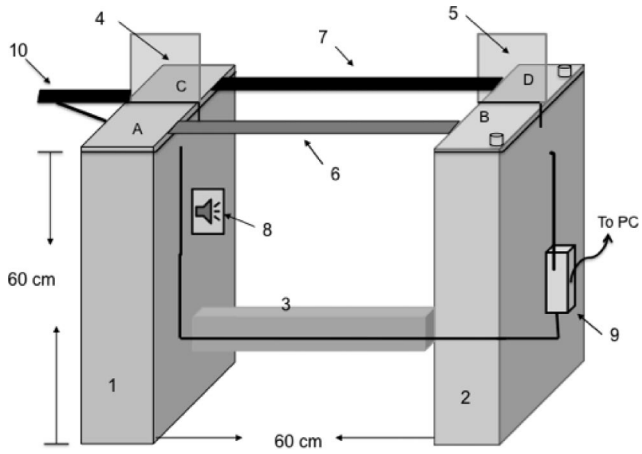

Figure 1. Diagram of the "Transitional Bridge" device for assessing impulsivity and attention behavior.

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but vertically parallel (1, 2). The pedestals are connected by at least one transversal support (3), placed horizontally to provide greater stability. The top of each frontal pedestal (1, 2) has two platforms, each divided into two sections (15 × 15 cm each): A, C for pedestal 1, and B, D for pedestal 2 (letters in Figure 1). Those sections (A, C and B, D) can be operated independently or as a single unit (A-C and B-D, respectively). Movement from one platform to another on each pedestal can be impeded by inserting a removable panel (4, 5) vertically through slots made for this purpose in the midsection of the upper platform and aligned along the longitudinal horizontal plane of the device to form two independent platforms with no possibility of communication between them on either side of the panel: A, C for pedestal 1, and B, D for pedestal 2. The panels (4, 5) are 25 cm tall so that the animals cannot climb over them and cross to the other side of the platform.

Movement from platform A (pedestal 1) to platform B (pedestal 2), and from platform C (pedestal 1) to platform D (pedestal 2) is achieved by means of removable bridges; 6 and 7, respectively. The bridges are assembled horizontally on the upper part of each frontal pedestal, that is, 60 cm above floor level. According to our experience, this height is adequate to prevent the rats from jumping to the floor. The length and width of the bridges can vary according to the specific objectives of each experiment. In the design shown, and for the application of the methods described in this study, bridges 60 cm long × 3 cm wide were used. Previous trials showed that this bridge width did not present any problems in terms of the rats falling off during trials.

The apparatus is equipped with a speaker (8) placed on one side of pedestal 1, which is programmed to emit sounds of different frequencies and tones that serve as signals indicating to the experimental subjects that they are to perform some predetermined behavior. An interface (9) is inserted into the midsection of one of the frontal pedestals (2) as a means of automating stimulation and measuring the behavior of the animal being trained. Detailed information on each subject's behavior is sent digitally to a PC. The data collected include physical detection of the animal's location, transition times from one location to another, latencies of position change after one or more predetermined stimuli, and travel times over the bridges as the subject moves from one platform to another.

Automation of the Apparatus

To automate the recording of animals' behavior under the different experimental designs that can be used with this device, each platform (A, B, C, D) is equipped with a surface-sensor that detects an animal's presence. The electronic signal produced is sent to the interface (9), which measures the time that the animal remains on each platform by registering the moment when it leaves the platform in question. The only means available to the animal to leave a platform is by crossing one of the bridges (6, 7), so as soon as it reaches the platform at the other end of the bridge the pressure that its weight exerts is detected and that signal is also recorded in the interface (9). This procedure makes it possible to automatically register the time that subjects take to cross each bridge, simply by subtracting the end time registered on the previous platform from the arrival time on the following one. The interface (9) sends the information on partial times—in real time—to a PC, where data are stored on the hard drive for later analysis. When the animal is required to respond to an external signal—a sound or light—the interface is designed and programmed to generate stimuli with different characteristics and then record the animal's RTs; for example, the time it takes to leave platform A after emission of a stimulus or combination of stimuli.

Experimental Study to Assess Motor and Cognitive Impulsivity

Experimental Animals

The pharmacological treatment applied was very similar to that described by Choong and Shen (2004a). Briefly, pregnant Wistar rats were treated intragastrically with ethanol (20% vol/vol in distillated water) from gestation Day 8 to 20 at a daily dose of 6.0*g*/kg divided into two doses of 3*g*/kg each (5–6 hours apart), between 10:00 and 16:00 h from Monday to Friday. A single dose of 4.0*g*/kg ethanol was administered at noon on weekends. Control rats, also pregnant, received the same volume of an isocaloric solution (10.5 g/kg sucrose plus distillated water) in two daily doses of 5.25 g/kg sucrose, except on weekends, when the dams received a single dose of 7.0 g/kg sucrose at noon. After gestation Day 20, and throughout the nursing period, dams and offspring were left undisturbed. At weaning (21 postnatal days), the male offspring from at least 3 different litters that received each prenatal treatment were placed in collective cages in groups of 4–5 animals. Rats were maintained on a 12–12 light–dark cycle (lights on at 08:00) during the study.

At 24 days of postnatal age, alimentary restriction was begun to reach and maintain 85% of the normal body weight of rats under standard feeding conditions (i.e., ad libitum). Animal body weight was monitored three times a week and training commenced on Day 28 postnatal age. Different groups were used to test each of the two methods used to assess impulsivity.

Anxiety Assessment

Given that crossing the transitional bridge might generate anxiety in the rats because it is an elevated device (60 cm), the possibility exists that the impulsive behavior targeted in this study could be confounded with different levels of anxiety in different groups. Therefore, anxiety assessment was conducted using two independent groups of preadolescent rats treated prenatally with either alcohol or an isocaloric solution, as described above. The plus-maze and associated procedure described by Pellow and File (1986) were used to assess anxiety. Briefly, the plus-maze consisted of two open arms, 50 × 10 cm, and two enclosed arms, 50 × 10 cm with walls 40 cm high, arranged such that the two arms of each type were opposite each other. The maze was elevated to a height of 50 cm. The procedure consisted in placing each rat at the center of the plus-maze and measuring the time spent in the open or closed arms during a 5-min test.

The care and use of animals in this study, and all procedures involving them, were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Methods for Assessing Impulsivity

It is important to mention that the procedures described below are the result of several previous pilot studies conducted to estab-

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lish the strategies that we consider adequate to assess impulsivity using the present device.

Wait-to-Go-Signal Task (Motor Inhibitory Control)

Motor impulsivity is defined as the failure to inhibit a prepotent response. In the present study, this type of impulsivity was assessed on the basis of anticipated responses to a Go-signal tone. For this application only one bridge was used (6) and panels (4, 5) were placed on the platforms to block transit from area A to C and from area B to D. The bridge width was 3.0 cm, as our experience in several training tests with bridges of different dimensions showed that 3-cm bridges can be crossed without difficulty.

Training procedure. Initially, a rat was placed on platform A and trained by shaping to cross toward platform B, where it would obtain a 45-mg pellet as a reinforcer. The pellet was available in a container placed on platform B (see Figure 1). The acquisition criteria for this behavior required performing at least 15 consecutive crossings from platform A to B with a latency response ≤10 seconds timed from the moment at which the rat was placed on platform A. This criterion was usually satisfied in two days. On the following day, a tone (350 ms, 4 kHz, 40 dB) was generated by the interface one second after the rat was placed on platform A, and only the crossings that occurred after the tone and with a RT ≤2 seconds were reinforced. This training phase consisted of one daily session of 60 trials designed for the rats to establish a relation between tone, crossing, and reinforcement. After two days in this condition, and regardless of the rat's performance, the tone was presented 2 s after placement on platform A. As in the previous phase, only the crossings that occurred after the tone and with a RT ≤2 seconds were reinforced, as they constituted correct responses. The learning acquisition criterion for this task was 32 correct responses of 60 trials in each session. Thirty-two was the mean + 2 standard deviations of the correct responses obtained on the first day of exposure to 2 s of tone delay presentation.

Testing procedure. Once this criterion was achieved, the rats were evaluated for two additional days in this phase under the same conditions. In the two phases that followed, the tone was presented randomly at 2–3 and 3–4 seconds, respectively, and each phase lasted 3 days, regardless of the rat's performance. After each crossing, the rats remained on platform B for 10 seconds, whether they had received a reinforcer or not, before the next trial began.

The following measurements were considered in this task:

Correct responses: Crosses occurring after the tone and within a RT ≤2 s.

Omission responses: Remaining on platform A for more than 2 s after tone emission (inattention measure).

Anticipated responses: Starting to cross before tone emission (impulsivity measure).

Reaction time for correct responses: Time elapsed from tone emission to commencement of crossing within 2 s.

Crossing latency for anticipated responses: Time elapsed from the moment the rat was placed on platform A to commencement of crossing before tone emission.

Delay-Discounting Task (Cognitive Impulsivity)

This task required two parallel bridges (6 and 7, Figure 1). Panel 4 was removed to allow free passage between platforms A and C, but a panel (5) was placed to impede transit between platforms B and D. The bridges were 3.0 cm wide.

Training procedure. At 28 days of postnatal age, animals were placed alternately on platform A or C and trained to cross to platform B or D, respectively, where they obtained a 45-mg pellet that was used as the reinforcer in all trials. Upon the rat's arrival at the target platform (B or D), a 4-kHz, 30-dB tone was emitted at 300 ms and the reinforcer was made available in a small recipient placed in the distal corner of each platform. This procedure was defined as 'immediate reinforcing delivery.' Initially, the alternation trials of bridge crossings were conducted by placing a panel between platforms A and C to force the rat to cross the entire available bridge. After 2 days of training in this alternate crossing regimen, the rat was placed on the exterior edge of a starting platform (component 10 in Figure 1) just between A and B, so it was free to choose any platform and then cross to it using the corresponding bridge from a neutral position. In this condition, rats were tested for their choice-preference using the immediate rewarding delivery (the 45-mg pellet) on both goal platforms. Rats with a crossing preference above 60% for any bridge (40 trials) were discarded from the study (in the present study only one rat met this exclusion criterion).

Testing procedure. On the next day of the choice-preference test, crossings from platform A to B resulted in the immediate delivery of a 45-mg pellet, while crossings from C to D resulted in the delivery of two 45-mg pellets, but only after a delay of 5 seconds. This phase, with the 5-s delay, lasted 3 days. On the next 3 days, the delay in reinforcement delivery (two 45-mg pellets) for crossing from C to D was increased from 5 to 7 s, and then from 7 to 10 s for 3 additional days. Throughout these 6 days, the immediate rewarding delivery (one 45-mg pellet) was maintained for crossings from A to B. Each daily session had 40 trials regardless of the delay in reinforcement delivery.

The crossing choice preference and crossing latency (defined as the time elapsed from the moment the rat was placed on the start platform [10, Figure 1] to the onset of bridge crossing) were measured for each phase of delayed reinforcement (i.e., 5, 7 and 10 s). Although longer delays have been used in other paradigms in adult rats (Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006), we have observed that delays of 7 and 10 sec are sufficient to assess impulse control in prepubertal rats, but not in adult rats (Muñoz and Juárez, unpublished data).

A higher choice percentage of crossings and a shorter crossing latency to the bridge to obtain the immediate reward indicated higher impulsivity in the animals.

Statistical Analysis

Anxiety test. The time spent in the open and closed arms was analyzed by a two-way ANOVA (factor A: alcohol vs. isocaloric prenatal treatment; factor B: type of arm).

Wait-to-Go-signal task. Correct responses, anticipated responses and omissions were analyzed separately by a one-way ANOVA (factor A: alcohol vs. isocaloric prenatal treatment) for the 2- and 2–3-s phases. Similarly, response latency for correct responses and the time elapsed before anticipated responses were analyzed separately, also using a one-way ANOVA (factor A: alcohol vs. isocaloric prenatal treatment) for each phase of sound-time presentation.

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Delay-discounting task. The percentage of choice between the bridge that led to delivery of the immediate reinforcer and the one programmed for consecutive reinforcement delays of 5, 7, and 10 s was analyzed using a two-way ANOVA (prenatal treatment [alcohol, isocaloric] × delay [5, 7, and 10 s]). Starting latency for bridge crossing was analyzed by a three-way ANOVA (prenatal treatment [alcohol, isocaloric] × sessions with different reinforcement delay times [5, 7, 10 s] × type of delay in each session [immediate, delayed]).

Results

Impulsivity Assessment

There were no differences in the amount of time spent in the open and closed arms between the groups with the different prenatal treatments, but the time spent in the open arms was significantly lower than that spent in the closed arms, $F(1, 56) = 41.63$, $p = .0001$ of the plus-maze, regardless of group (see Figure 2).

Wait-to-Go-Signal Task (Motor Inhibitory Control)

As previously mentioned, we were interested in studying impulsivity in the prepubertal period; therefore, the testing procedure was suspended once the rats completed 40 days postnatal age. Because few rats of this age had reached the phase in which the tone was presented 3–4 seconds after trial commencement, only the 2- and 2–3-s phases were included for analysis. When the tone was emitted after a latency of 2 s, the group treated prenatally with alcohol showed a significantly higher number of anticipated responses than the isocaloric group, $F(1, 15) = 25.6$, $p = .0002$ (Figure 3A). In contrast, the number of omissions was lower in the alcohol group than in the isocaloric group, $F(1, 15) = 4.49$, $p = .05$. The alcohol-treated group showed a lower frequency of correct responses than the isocaloric group, but these differences were not significant. Reaction time for correct responses showed no significant differences between treatment groups but, in contrast, the mean time elapsed before an anticipated response was significantly shorter in the alcohol group than in the isocaloric group, $F(1, 15) = 8.76$, $p = .009$ (Figure 3B).

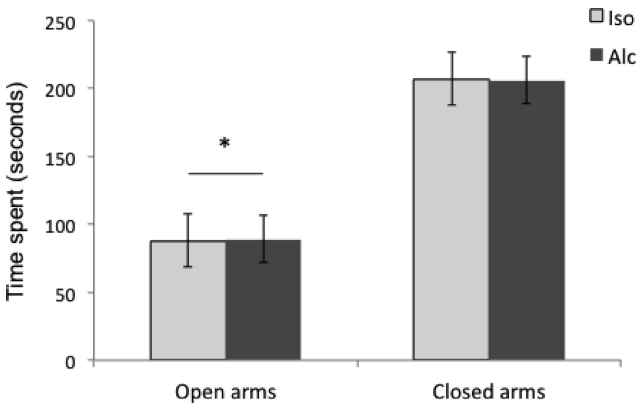

Figure 2. Mean time (± *SE*) spent in open or closed arms of groups of rats prenatally treated with an isocaloric (Iso) or alcohol (Alc) solution. *Significant differences between time spent in open or closed arms regardless of prenatal treatment.

When the tone was emitted randomly after a latency of 2–3 s, correct, omission, and anticipated responses all showed a similar tendency to the results observed in the 2-s phase, and the differences observed were not significant in any of the cases (see Figure 4). On the other hand, RT to correct responses was shorter for the alcohol group than the isocaloric group, $F(1, 15) = 5.28$, $p = .03$, and the time elapsed before making anticipated responses was also significantly shorter in the former than the latter, $F(1, 15) = 80.61$, $p < .0001$ (Figure 5).

Delay-Discounting Task (Cognitive Impulsivity)

The alcohol-treated group showed a percentage of choice significantly lower than the isocaloric group for the delayed reinforcement conditions, regardless of the reinforcement delay, $F(1, 20) = 26.36$, $p = 0001$ (Figure 6). The factor of reinforcement delay was also significant, $F(2, 40) = 93.43$, $p < .0001$, as it indicated that the percentages of choice for reinforcement delays of 7 and 10 s were lower than those observed for the delay of only 5 s, regardless of prenatal treatment.

Starting latency for bridge crossing was shorter in sessions at 10 s than in those at 5 s of reinforcement delay, regardless of group and type of delay, $F(2, 40) = 5.84$, $p = .005$, and also shorter when rats chose the immediate reinforcer than when they opted for the delayed reinforcer, regardless of prenatal treatment and reinforcement delay time, $F(1, 20) = 49.49$, $p < .0001$ (Figure 7). Interaction of the factors of treatment × reinforcement delay time was also significant, $F(2, 40) = 5.28$, $p = .009$, suggesting that starting latency for bridge crossing was shorter in the 10-s sessions than in the 5-s sessions of reinforcement delay only in the alcohol-treated group.

Discussion

The device and methods used in this study to assess impulsivity behavior and attention in rats were designed to train animals to perform a relatively simple behavior in a short time. The tasks employed and the characteristics of behavior performance can vary depending on the experimental design, which confers broad methodological versatility that can be adapted to different experimental requirements.

The considerable reduction in the time required to train the animals and assess behavior that this device and method provide offers significant advantages, because most methods for assessing impulsivity available today require at least three times more training time for the animals to satisfy the criteria required for behavioral assessment. This advantage provides a means of studying developmental periods in which time is a critical factor because of the numerous neurophysiological and cognitive changes that can take place during short periods; for example, preadolescence in rats is a short period between weaning and puberty, and the neuroendocrine changes that occur in this lapse play an important role in maturation, behavior, and cognitive functions that establish marked differences between preadolescents and adults (Tseng & O'Donnell, 2007).

Another objective, and advantage, of this device and method is that they allow impulsivity behaviors to be evaluated from different theoretical perspectives, including both motor and cognitive modalities. This is achieved through simple modifications of certain structural elements in the device according to the requirements of the specific experimental design.

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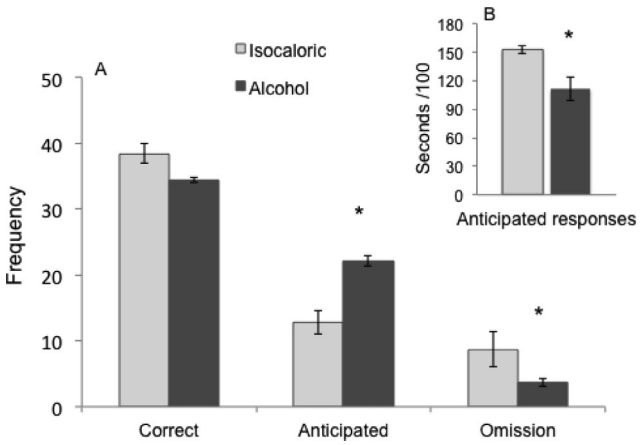

Figure 3. Wait-to-Go-signal task. Mean frequency (± *SE*) of correct, anticipated, and omission responses of groups of rats prenatally treated with an isocaloric or alcohol solution (A), and mean time (± *SE*) elapsed before anticipated responses (s/100) in groups of rats prenatally treated with an isocaloric or alcohol solution (B), when the tone was emitted after a latency of 2 s. *Significant differences between groups (isocaloric vs. alcohol).

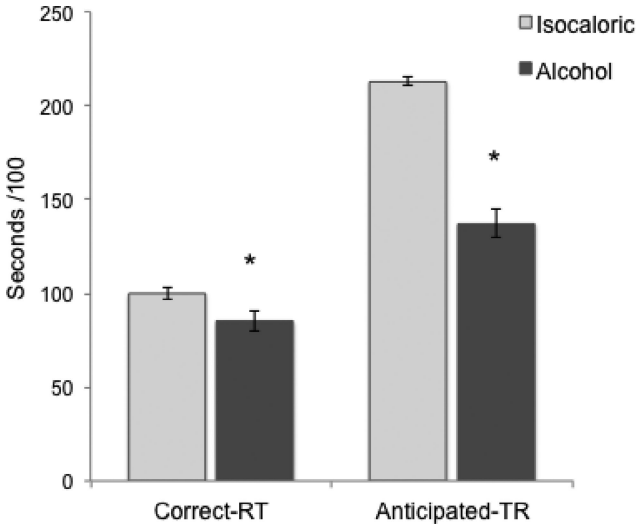

Figure 5. Wait-to-Go-signal task. Mean (± *SE*) RT for correct responses and mean time (± *SE*) elapsed before anticipated responses (TR) of groups of rats prenatally treated with an isocaloric or alcohol solution when the tone was emitted randomly after a latency of 2–3 s. *Significant differences between groups (isocaloric vs. alcohol).

This study reports on two experiments using two different methodologies: one to assess motor impulsivity, the other to evaluate cognitive impulsivity.

The performance of the animals treated prenatally with alcohol, which has been described as provoking alterations of the dopaminergic system (Shen et al., 1999; Choong & Shen, 2004b; Shen, & Choong, 2006; Wang, Haj-Dahmane & Shen, 2006) and behaviors such as impulsivity and attention (Hausknecht et al., 2005), showed the expected results, especially when cognitive impulsivity was assessed, as the animals treated prenatally with alcohol manifested a significantly higher tendency to choose the bridge that resulted in a small, but immediate, reinforcer, compared with those treated prenatally with the isocaloric solution (control group), particularly in the sessions that entailed a longer delay before delivery of the reinforcer.

It is well documented that impulsive subjects prefer a smaller reward delivered immediately over a larger one that arrives after a delay, a finding that holds for both humans and animals. In this study, we found that the longer the reinforcement delay the greater the choice for the bridge with no such delay, regardless of prenatal treatment; therefore, both groups showed the typical devaluation of reward with delay, which would suggest an explicit indicator of the internal validity of the present delay-discounting paradigm.

Motor impulsivity was also affected in the direction predicted, as the alcohol-treated animals showed a higher frequency of, and shorter latencies in, anticipated responses compared with the control group. It is well known that an anticipated response represents a failure of inhibitory control, another behavioral trait associated with impulsivity. Shorter latencies to anticipated behaviors indicate an animal that is less capable of waiting for a permissive stimulus and, therefore, shows poorer inhibitory control. The rats treated prenatally with alcohol also showed shorter latencies in their correct responses in the

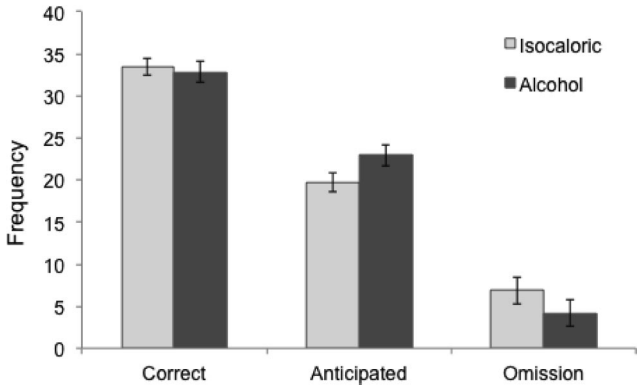

Figure 4. Wait-to-Go-signal task. Mean frequency (± *SE*) of correct, anticipated, and omission responses of groups of rats prenatally treated with an isocaloric or alcohol solution when the tone was emitted randomly after a latency of 2–3 s.

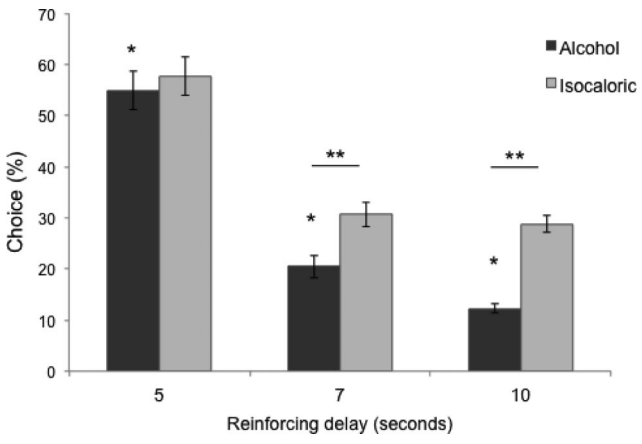

Figure 6. Delay-discounting task. Mean (± *SE*) percentage of choice for bridge crossing with reinforcing delays of 5, 7, and 10 s for groups of rats prenatally treated with an alcohol or isocaloric solution. *Alcohol < isocaloric (treatment main factor). **Delay 7 and 10 < delay 5 (delay main factor).

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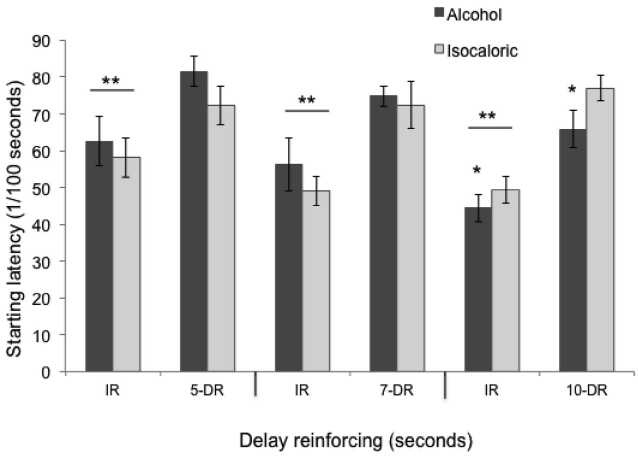

Figure 7. Delay-discounting task. Mean (± *SE*) of starting transfer latency of crossing (s/100) bridges with immediate reinforcing (IR) and delayed reinforcing (DR) of 5, 7, and 10 s, for groups of rats prenatally treated with an isocaloric or alcohol solution. *Alcohol in 10 s-session < alcohol in 5 s-session. **IR < DR regardless of treatment and delay.

2–3-s stimulus (tone) presentation trials, which likely means that they were either more prone to respond, or more attentive to the signal stimulus. This latter hypothesis could be supported by the lower frequency of omissions observed in the prenatally alcohol-treated rats. This result suggests that alcohol treatment may affect impulsivity more than attention behavior, at least in the treatment regimen used in the present study. Similarly, Hausknecht et al. (2005) found a significantly higher frequency of false alarm responses in prenatally alcohol-treated animals than in a control group, and that this tendency was more marked than changes in the attention parameters.

One possible limitation of the present study is that task demands were restricted by the animal's age, and delays longer than 2–3 seconds for the tone presentation in the Wait-to-Go-signal task were not assessed. However, in the present work we were interested in analyzing impulsivity in preadolescent animals; therefore, when the rats were close to reaching puberty we decided to stop the study to avoid the possible influence of the hormonal activational action of puberty, which could have masked the results. In our experience, when adolescent rats continued to execute the task into adulthood, they performed adequately with longer delays.

We cannot discard the possibility that crossing the elevated bridges might generate a certain level of anxiety, which may constitute another limitation. However, the elevated bridge represents the challenge of crossing from one platform to another; even if the elevation is eliminated, the animals cross the bridge spontaneously and crossing does not constitute an operant behavior response to obtain a reward. However, we include experimental evidence showing that there are no differences between rats treated prenatally with alcohol and control rats in the plus-maze task, which supports the interpretation that the observed changes in impulsivity were not attributable to differences in anxiety levels.

Alterations of monoaminergic neurotransmission are related to affectations of impulsive behavior (Pine, Shiner, Seymour & Dolan, 2010; Baarendse & Vanderschuren, 2012), and it is well documented that rats treated prenatally with alcohol under the same pharmacologic scheme of the present study show alterations of the dopaminergic system (Shen et al., 1999; Choong & Shen, 2004b; Shen, & Choong, 2006; Wang et al., 2006). In addition, there is evidence that impulsivity is also affected in these rats; a factor that was assessed by a choice RT task (Hausknecht et al., 2005). This last finding described for adult rats agrees with the results of the present study for preadolescent rats.

The results of this work support the notion that prenatal alcohol treatment, under the scheme described by Shen et al. (1999), could constitute a useful animal model for the study of behavioral alterations related to such disorders as attention deficit hyperactivity disorder. At the same time, they validate the device and method described in the present work; thus, it is suggested that these instruments offer a methodological alternative for assessing impulsivity and attention behavior. Their most notable advantages are (1) shorter training time and (2) the capability to assess both motor and cognitive impulsivity.

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