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TITLE:

Three Laboratory Procedures for Assessing Different Manifestations of Impulsivity in Rats

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KEYWORDS:

animal models, behavioral testing, delay discounting, impulsiveness, self-control, response inhibition, rats

SUMMARY:

We present three protocols that assess different forms of impulsivity in rats and other small mammals. Intertemporal choice procedures evaluate the tendency to discount the value of delayed outcomes. Differential reinforcement of low rates and feature-negative discrimination evaluate response inhibition capacity with and without punishment for inappropriate responses, respectively.

ABSTRACT:

The present article provides a guide for the conduction and analysis of three conditioning-based protocols to evaluate impulsivity in rats. Impulsivity is a meaningful concept because it is associated with psychiatric conditions in humans and with maladaptive behavior in non-human animals. It is believed that impulsivity is composed of separate factors. There are laboratory protocols devised to assess each of these factors using standardized automated equipment. Delay discounting is associated with the incapacity to be motivated by delayed outcomes. This factor is evaluated through intertemporal choice protocols, which consists of presenting the individual with a choice situation involving an immediate reward and a larger but delayed reward. Response inhibition deficit is associated with the incapacity to withhold prepotent responses.

Differential reinforcement of low rates (DLR) and feature-negative discrimination protocols assess the response inhibition deficit factor of impulsivity. The former imposes a condition to a motivated individual in which most wait a minimum period of time for a response to be rewarded. The latter evaluates the capacity of individuals to refrain from food seeking responses when a signal of the absence of food is presented. The purpose of these protocols is to construct an objective quantitative measure of impulsivity, which serves to make cross-species comparisons, allowing the possibility of translational research. The advantages of these particular protocols include their easy set-up and application, which stems from the relatively small amount of equipment needed and the automated nature of these protocols.

INTRODUCTION:

Impulsivity can be conceptualized as a behavioral dimension associated with maladaptive outcomes[1]. Despite the widespread use of this term, there is no universal consensus on its precise definition. In fact, several authors have defined *impulsivity* by giving examples of impulsive behaviors or their consequences, rather than delineating which distinctive aspects govern the phenomenon. For instance, impulsivity is assumed to involve an inability to wait, plan, inhibit prepotent behaviors, or an insensitivity to delayed outcomes[2], and it has been considered a core vulnerability to addictive behavior[3]. Bari and Robbins[4] have characterized impulsivity as the co-occurrence of strong impulses, being triggered by dispositional and situational variables, and dysfunctional inhibitory processes. A different definition was provided by Dalley and Robbins, who stated that impulsivity could be regarded as a predisposition to rapid, often premature, actions without appropriate insight[5]. Yet, another definition of impulsivity, proposed by Sosa and dos Santos[6], is a behavior tendency that deviates an organism from maximizing available rewards due to the acquired control exerted over the organism's responding by stimuli incidentally related to those rewards.

Due to the behavioral processes related to impulsivity, its neurophysiological substrate involves structures in common with those of motivated behavior, decision making and reward valuing. This is supported by studies that show that structures of the cortico-striatal pathway (e.g., nucleus accumbens [NAc], prefrontal cortex [PFC], amygdala, and caudate putamen [CPU]), as well as the ascending monoaminergic neurotransmitter system, participate in the expression of impulsive behavior[7]. However, the neural substrate of impulsivity is more complex than that. Although NAc and PFC are involved in impulsive behavior, these structures are part of a more complex system, and also are composed by substructures that have different functions (for more detailed documentation, see Dalley and Robbins[5]).

Regardless of the controversies about its nature and biological substrate, this behavioral dimension is known to vary across individuals, in which case it can be considered as a trait, and within individuals, in which case it can be considered as a state[8]. Impulsivity has long been recognized as a feature of some psychiatric conditions, such as attention-deficit/hyperactivity disorder (ADHD), substance abuse, and manic episodes[9]. There seems to be a high consensus that impulsivity is composed by multiple dissociable factors, including unwillingness to wait (i.e., delay discounting), incapacity to refrain prepotent responses (i.e., inhibitory deficit), difficulty to focus on relevant information (i.e., inattention), and a tendency to engage in risky situations (i.e.,

sensation seeking)[5,10,11]. Each of these factors can be assessed through special behavioral tasks, which are usually assigned to two broad categories: choice and response inhibition (these may have different labels between each authors' taxonomies). An important feature of such behavioral tasks is that they could be applied across several animal species[2], which allows studying impulsivity in controlled laboratory conditions.

Modeling a behavioral dimension with laboratory non-human animals has a number of advantages including the possibility of measuring specific, operationalized behavioral tendencies, allowing the researchers to largely reduce extraneous variables (e.g., contamination by past life events[4]) and to implement experimental manipulations such as chronic pharmacological administration, performing neurotoxic lesions, or genetic manipulations. Most of these protocols have analogue forms for humans, which make comparisons easy[5]. Importantly, using analogues of these laboratory protocols in humans is effective to aid diagnosis of psychiatric conditions, such as ADHD (especially when more than one protocol is applied[12]).

Like any other psychological measurement, laboratory protocols for assessing impulsivity must comply with particular criteria in order to achieving the goal of providing insight into the phenomenon under study. To be considered as an appropriate model of impulsive behavior a laboratory protocol should be reliable, and possess (at least, in some degree) face, construct, and/or predictive validity[13]. Reliability could refer either that an effect upon the measurement would replicate if a manipulation is conducted two or more times, or that the measurement is consistent over time or across different situations[14,15]. The former feature would be especially useful for experimental studies, while the latter would be so for correlational studies[14]. Face validity refers to the degree in which what is measured resembles the phenomenon that is supposed to be modeled, as to being, for example, affected by the same variables. Predictive validity refers to the ability of a measure to forecast future performance in protocols, which aim to measure the same or a related construct. Finally, construct validity refers to whether the protocol reproduces behavior that is theoretically sound regarding the process or processes assumed to be involved in the phenomenon under study. However, although these are highly desirable features, one should be cautious when stating that a protocol is valid purely based on these criteria[16].

There are several protocols to measure impulsivity in laboratory settings. However, the present article presents only three such methods: intertemporal choice, differential reinforcement of low rates, and feature-negative discrimination. Intertemporal procedures aim to assess the delay discounting (i.e., the difficulty of delayed outcomes to control behavior) component of impulsivity. The basic rationale of this protocol is confronting subjects with two rewards that differ in both magnitude and delay[17]. One alternative provides a small immediate reward (termed *smaller sooner,* SS) and the other provides a larger but delayed reward (termed *larger later,* LL). The proportion of responses to the SS alternative can be used as an index of impulsivity[18]. In differential reinforcement of low rates procedures, the factor of impulsivity to be assessed is response inhibition (i.e., incapacity to withhold prepotent responses) when there is a negative punishment contingency upon inappropriate responding. The rationale of this protocol is introducing subjects to a situation in which the only way of obtaining rewards is to pause their

responding[19]. Finally, feature-negative discrimination procedure evaluates response inhibition when there is no explicit punishment upon inappropriate responding. The rationale of this protocol (also known as Pavlovian conditioned inhibition or the A+/AX- procedure) is to evaluate subjects' ability to withhold unnecessary responses[20].

These procedures stand out in comparison to others as having some convenient features. For example, the procedures presented here are suitable for being conducted in minimally equipped conditioning chambers (also known as 'the Skinner box'). **Figure 1** shows a diagram of a typical conditioning chamber. Conditioning chambers are useful research instruments due to a number of advantages. They allow automated collection of a relatively large volume of data, maximizing the number of subjects assessed for unity of time and space[21]. Moreover, behavioral studies conducted in conditioning chambers require minimal researcher intervention, which reduces the time and effort invested by laboratory staff, unlike other available methods (e.g., non-automated T-mazes, set-shifting boxes)[21]. Minimizing researchers' intervention also help in reducing researchers' bias, decreasing effects of researchers' learning curve, and a reduction of handling-induced stress[22]. Typical conditioning chambers are fairly standardized to be used with medium sized rodents, such as rats (*R. norvegicus*), but can be employed to study other taxa, like similar-sized marsupials (e.g., *D. albiventris, and L. crassicaudata*[23]). There are also commercial conditioning chambers adapted for smaller (e.g., mice [*M. musculus*]) and larger (e.g., non-human primates) species. Setting up and conducting the protocols presented in this article require minimal programming skills and demand a quite low number of attainable input and output devices, unlike more sophisticated alternative methods (e.g., 5-choice serial reaction time task [5-CSRTT][24] and sign-tracking[25]).

[Place **Figure 1** here]

PROTOCOLS:

The three protocols described in this section require the use of rats as subjects. Most laboratory rat strains are suitable; for example, Wistar, Long-Evans, Sprague-Dawley, etc. The Ethics Committee of the Universidad Iberoamericana, following the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996), approved the laboratory protocols to be described.

1. Animal housing and preparation

1.1. Determine the number of rats that will be used. This will depend on several factors, such as the type of design selected, the statistical power desired/required, the costs of conducting the study, and the time available for conducting the study[26].

1.2. Label each rat's tail with an indelible marker for identification purposes.

1.3. House rats either individually or in groups (2-5) with water freely available.

1.4. Restrict rats' food intake in order to motivate them for the protocols. In the case of individually housed rats, a convenient method for food restriction is to reduce weight to 85% of free-feeding weigh (use only for adult rats)[27]. Maintain this goal weight by providing supplementary food after conducting the protocol. For group-housed rats, give access to food for 60 min daily after conducting the protocol[27].

1.5. House the conditioning chambers within the sound and light attenuating shells.

2. Preliminary training

NOTE: Before starting any of these behavioral protocols rats need to become accustomed to the conditioning chambers and food pellets. It is also vital to train the responses with which the animals would operate in the protocol. The three protocols presented here use appetitive motivation to induce behavior indicative of impulsiveness, like most other available alternative tasks (with select exceptions[28]). Conventional food dispensers are well suited to deliver both commercial refined grain and sugar pellets but can even handle "raw" grain under certain circumstances[29].

2.1. Habituation

2.1.1. After starting the food restriction regime, introduce the rats into the conditioning chambers without initiating any protocol for 30 minutes, in order to habituate exploring responses. Put 60 food pellets in the food receptacle at the beginning of the session in order to habituate food neophobia.

2.1.2. Repeat daily until the rats consume all of the food pellets.

2.2. Magazine training

2.2.1. After the habituation stage, introduce the rats into the conditioning chambers for two additional 30 min daily sessions delivering a food pellet every 45 s. This helps the rats identify the source of food pellets.

2.3. Lever-press training

2.3.1. Use this only for intertemporal choice and DRL protocols.

2.3.2. Project one (for DRL) or the two levers (for intertemporal choice) into the chambers and start a continuous reinforcement procedure, that is, deliver a food pellet for every lever press. This procedure is used concurrently with a free food pellet delivery every 45 s (i.e., an alternative FR1-FT45 s schedule of reinforcement[30]), as in the previous stage. Sessions can have durations of 30 min.

2.3.3. Repeat daily after the rats earn 80 rewards for two consecutive days.

2.4. Shaping by successive approximations

2.4.1. Use this method in case the rats do not reach the criterion in four sessions.

2.4.2. Open the isolating shell of the conditioning chamber and observe the rats' behavior. Deliver a food pellet for every response that approximates the target response (i.e., lever pressing). Examples of these approximate responses are approaching, sniffing, or touching the lever.

2.4.3. Once the rats consistently perform the approximate responses, stop delivering rewards upon them and start requiring a response that is closer to the target response. Repeat as necessary.

3. Programming automated protocols

NOTE: The used values (e.g., delays, reward amounts, number of trials, session durations, schedules' values, time-out length, inter-trial interval span, threshold for forced trials, presence/absence of accompanying stimuli, stimuli durations) presented were arbitrarily selected. Readers may want to consult the literature for determining appropriate parameters and conditions for accomplishing their particular goals. Codes for conducting samples of the three protocols presented here in a MED-PC environment are provided in the repository that can be found in the following URL: https://github.com/SaavedraPablo/MED-PC-codes. Such codes can be freely downloaded and modified according to particular needs.

3.1. Intertemporal choice

3.1.1. Select the values for delay and magnitude of reward. For example, choices for the SS alternative deliver one food pellet immediately and choices for the LL alternative deliver five food pellets after a 20 s fixed delay.

3.1.2. Select a finishing criterion. End sessions automatically after completion of some specified criterion. For example: end the session after 40 choice trials or after 50 min.

3.1.3. Combine each alternative with a lever (left or right) within the conditioning chamber counterbalancing the laterality of the alternatives among subjects.

3.1.4. Project both levers into the conditioning chambers and make alternatives SS and LL available upon the accomplishment of a variable-interal schedule[30]. Once the first lever press after a certain interval has elapsed, this activates the associated alternative (delay included). Varying the duration of such an interval in a pseudo-random fashion prevents exclusive preference for a particular alternative.

3.1.5. Retract both levers and activate the consequence associated with the SS or LL alternatives

after accomplishment of a variable-interval schedule of reinforcement.

3.1.6. Perform a time-out condition (signaled by a house-light blackout) after reward delivery. Adjust this duration of this condition to equate the average duration of inter-trial intervals for both alternatives. The next choice trial begins after the completion of the time-out. **Figure 2** shows a diagram of events during two successive trials of an intertemporal choice procedure.

[Place **Figure 2** here]

3.1.7. Implement forced trials. If subjects select one alternative for two consecutive trials, the program will determine that the next trial will be a forced trial of the remaining alternative. That is, in the next trial both levers are available, but only one will operate. This ensures that the subjects experience the outcomes associated with both alternatives.

3.1.8. Finish a daily session whenever a prespecified number of trials have been completed or whenever the maximum time has elapsed.

3.2. DLR

3.2.1. Select the value of the minimum time after which responding will produce a reward. For example, 10 s.

3.2.2. After the beginning of a session or after any lever-press response, start a countdown timer from the selected time value (e.g., 10 s) to zero. If subjects emit a response before the timer reaches the value of zero the timer resets, so that they must wait for a new opportunity to get a reward. If subjects emit a response after the timer reaches the value of zero, deliver a food pellet and reset the timer after 2 s (this allows the animal to consume the food). **Figure 3** shows some possible responding patterns and their corresponding programmed consequences.

NOTE: During the 2 s reward retrieving interval, responses are not counted, which may impact the proportion of burst responses in the rare cases when the rats eat the food quickly enough and happen to respond immediately afterwards or fail to detect the delivery of food. This could be ameliorated by using a cue signaling the 2 s reward retrieving interval[31]. However, previous research has shown that the amount of such responses is negligible even in the absence of signaling cues.

[Place **Figure 3** here]

3.2.3. Finish the session after a time and/or number of rewards criterion.

3.3. Feature-negative discrimination

3.3.1. Select stimuli durations, inter-trial interval durations, and finishing-criterion for sessions. For example, use 8 s durations for conditioned stimuli, variable 92 s inter-trial intervals and

finishing criterion of 24 trials.

3.3.2. Present pseudo-randomly two types of trials, A+ and AX-, at 50% of the times each; A and X represent stimulus types and plus and minus signs represent the presence or absence of food, respectively. A+ trials: turn on one of the focalized lights (stimulus A) for 8 s and then deliver two food pellets (+). AX- trials: turn on one of the focalized lights (either side) for 8 s and concurrently present a tone (stimulus X) but do not deliver food (-). **Figure 4** shows a diagram of the programmed events for each type of trial.

3.3.3. Finish the session after a time and/or number of trials criterion.

[Place **Figure 4** here]

4. Running the protocols

4.1. Conduct the protocol daily, at a standard time, always placing rats in the same operant chamber.

4.2. Set up the protocols in the computer software. Make sure to appropriately label the output file with the subjects' names, condition, and study.

4.3. Clean the inner walls, ceiling, and grill floor of the operant chambers with an ethanol or chlorine solutions, in order to remove odors from previous sessions or previous studies.

4.4. Check that all the crucial inputs and outputs work appropriately by manually activating and monitoring them by means of the computer.

4.5. Check that the food dispenser holds enough food to deliver throughout the session.

4.6. Move the housing cages with the rats inside close to the conditioning chambers.

4.7. Open the housing cage and gently carry each rat to its corresponding conditioning chamber, closing the conditioning chambers and the isolating shells.

4.8. Initiate the program and wait until the program is finished. If data is not saved automatically, save the output files of the session in the computer drive or elsewhere.

4.9. Gently carry the rats back into their corresponding housing cages after the program is finished.

4.10. Give complementary food to the rats according to the selected food restriction regime.

5. Data collection and analysis

NOTE: Codes for extracting and manipulating data from MED-PC output files (saved with the extension .txt) for each procedure are provided in the repository that can be found in the following URL: https://github.com/SaavedraPablo/MED-PC-to-R-codes.

5.1. Intertemporal choice

5.1.1. Record lever presses in the SS alternative and in the LL alternative.

5.1.2. Divide the SS alternative responses by the total responses to obtain the proportion of impulsive responses. Alternatively, divide the SS alternative responses by the LL alternative responses to calculate the ratio of impulsive responses. Take the common logarithm of ratio data points in order to remove skewness from the distribution.

5.2. DRL

5.2.1. Set a counter variable in the program that increases with each unit of time from the beginning of the session.

5.2.2. Record the value of the counter variable in a list of values for each one of the responses as they occur during the session. This will provide a cumulative record of responses; that is, the exact time in which each response occurred during the session.

5.2.3. Get the cumulative record of responses and subtract each value, *i*, from the previous value, *i – 1*, in order to obtain the inter-response times (IRTs), which constitute the variable of interest.

5.2.4. Plot a histogram of IRTs for one rat in one session with 1 s intervals in the X axis, in order to visually inspect the data. For a typical experienced subject, this should see as a bimodal distribution with a portion of the data amassed in the left and another portion of the data clustered near the selected temporal requirement of the DRL protocol. **Figure 5** shows an example of typical performance in the DRL protocol for one rat in a single session.

[Place **Figure 5** here]

5.2.5. Classify types of IRTs. As stated above, the distribution of IRTs for a typical subject is bimodal. One possible interpretation of this shape is that it is composed of the mixture of (at least) two distributions reflecting separate processes[32].

5.2.5.1. Classify IERs indicating attentional lapses.

5.2.5.1.1. Too long IRTs may be indicative of attentional lapses (i.e., periods on which rats were not engaged in the task)[33]. A useful practice for these means is to separate far-right outliers from the rest of the data[32]. To determine a cutoff value to mark the boundary between attentional lapses and the rest of the data, multiply the interquartile range of the rightward distribution by some arbitrary constant (e.g., 3) and add this number to the median of the rightward

distribution[32].

5.2.5.2. Classify responses in either the leftward or the rightward distribution (once the outliers have been removed[32]).

5.2.5.2.1. The leftward distribution or *burst responses* distribution is constituted by too short IRTs, which are interpreted as indicative of hyperactivity[34] or as a lack of attention and/or response feedback[35]. On the other hand, IRTs on the rightward distribution or *timed responses distribution* are considered as indicative of responding in adjustment to the temporal constriction of the protocol[32]. Either use an arbitrary cutoff to classify the boundaries of leftwards and rightwards distributions[31] or use mathematical modeling to do so[32,33,36].

5.2.5.3. Determine the parameters of the timed responses distribution.

5.2.5.3.1. Pay close attention to the rightward distribution in an experienced animal, which usually takes most of the IRTs and is considered as the most important part of the data set.

5.2.5.3.2. Two parameters of interest are the localization of its peak and its spread. The former gives an index of the capacity to inhibit premature responses; shifts to the left of the time criterion may be interpreted as indicative of impulsivity[37]. The latter is indicative of temporal estimation; the narrower the distribution, the greater the timing accuracy[32,40,43]. Estimate these parameters through simple descriptive statistics or by more sophisticated mathematical modeling[40,43,33].

5.2.5.3.3. For a useful guide to fitting DRL to the theoretical distribution proposed by Sanabria and Killeen[33], see the supplementary material provided by these authors.

5.2.5.4. Obtain a global efficiency measure.

5.2.5.4.1. If the finishing criterion of the session is temporal (i.e., session duration will be constant) divide the number of earned rewards by the responses emitted. If the finishing criterion is a specific number of rewards calculate reward rate, which is number of rewards divided by session duration. Note that these global measures say little about how animals are obtaining or losing the rewards in the protocol and must be used only as a rough guide.

5.3. Feature-negative discrimination

5.3.1. Record the frequency or the duration of responses during A+ and AX- trials. The primary measure of conditioned responding may be the mean response frequency[38], the mean response duration[39], or the percentage of trials with at least one response.

5.3.2. After choosing the preferred conditioned responding measure, subtract the value of responding during A+ trials minus responding during AX- trials for each subject in a particular session. This will constitute a negative index of impulsivity[40]; that is, the less the difference

between both values, the greater the impulsivity.

NOTE: The data from this task lend quite well to analyses based on measures from signal detection theory[41,42], which can be used to supplement simple subtraction measures.

REPRESENTATIVE RESULTS:

The three protocols described in this article may be each conducted alone or in conjunction with other procedures; this will depend on the research question, which in turn will determine the study design. Some examples of study designs that are compatible with these protocols are: (1) time series studies, which aim to describe longitudinal changes in performance; (2) quantification of individual variability, which aims to determine the reliability of the measures; (3) cross-sectional correlation studies, which aims to evaluate whether performance in one protocol can be used to predict performance on another protocol conducted afterwards; (4) longitudinal correlation studies, which aim to ascertain whether performance in one protocol can be used to predict performance on another protocol conducted concurrently; (5) non-experimental group comparisons, which aim to assess whether two or more samples from different populations differ with regards to impulsive performance; (6) pretest-posttest comparisons, which aim to determine whether an intervention (e.g., behavioral, pharmacological, chirurgical) is effective in altering (e.g., increase, decrease, stabilize) impulsive performance; (7) experimental simple group comparisons, which aim to evaluate whether an intervention if effective in altering impulsive performance but pretest measuring is not available (e.g., in interventions made in early stages of development intended to impact in adult performance). This list is not intended to be exhaustive and combinations of study designs are possible and encouraged.

As stated above, the intertemporal choice procedure is designed to assess the delay-discounting component of impulsivity. The remaining two protocols are supposed to examine inhibitory capacity, which is assumed to be one of the core components of impulsivity. DRL protocols evaluate response inhibition when inappropriate responding is explicitly punished by reward omission. On the other hand, feature-negative discrimination assesses response inhibition when there is no nominal punishment contingency for inappropriate responses. Next, some representative results of one of each protocol from the present laboratory or elsewhere are described.

Figure 6 shows a comparison of performance in an intertemporal choice procedure from a sample of spontaneously hypertensive rats (SHR) and Wistar rats. The former is a widely accepted rat strain model of ADHD, while the latter is a usual control strain. The SS alternative delivered a single food pellet after a 2 s fixed interval schedule and the LL alternative delivered four food pellets after a 28 s fixed interval schedule (recall that these alternatives were available upon accomplishment of an initial schedule of reinforcement; in this case a variable interval of 30 s). As depicted, the log ratio of lever response rate associated with the SS alternative is higher in SHR compared to Wistar rats. This can be interpreted as SHR presenting a preference for the immediate reward at the expense of a richer but delayed alternative, a sign of high delay-discounting related impulsivity.

[Place **Figure 6** here]

Regarding performance on DRL protocols, **Figure 7** shows longitudinal data of a single rat with a 10 s temporal restraint on responding. As it can be seen, during the first sessions the rat emits a high proportion of burst responses but there is a decrease on further sessions. It also may be seen that in earlier sessions there are few responses near the temporal criterion of the protocol. However, as the animal acquires experience in the task, it eventually learns to respond around 10 s. This represents evidence of the role of learning in performance in this protocol. Note, however, that none of the IRTs lower than 10 s were rewarded; even in the 18th session, there is a great proportion of ineffective responses. Such a performance denotes an important quality of the protocol: at least with these parameters, the task is not easy to master, which is helpful in avoiding problems associated with ceiling effects.

[Place **Figure 7** here]

An example of a pharmacological effect on DRL performance is shown in **Figure 8**. After reaching a steady performance in a DRL procedure with a target time of 10 s, five female rats received a 1 mL/kg subcutaneous injection of saline and were tested in the same procedure 30 min later for eight consecutive days. Then, saline was replaced with an equal volume of 0.05 mg/kg haloperidol and performance was tested for six more sessions. This aimed at testing whether impulsive performance in this procedure was decreased via D2 receptors antagonization. The dose was selected because it is known that haloperidol at 0.075 mg/kg or less does not reduce the motor capacity of animals and shows no side effects that might mask the target behavior[43]. In addition, haloperidol at 0.048 mg/kg virtually did not interfere with receptors other than D2[44]. In **Figure 8**, blue density plots show the distribution of IRTs for rats in the three last sessions of the saline condition and salmon-colored density plots show the distribution of IRTs for the same subjects in the last three sessions of the haloperidol condition. Embedded bar plots depict comparisons between response rates (top) and between reward rates (bottom) within the same time frame of both conditions (color code: blue = saline, salmon = haloperidol).

[Place **Figure 8** here]

As it can be seen in blue density plots, subjects display individual differences regarding the emission of burst responses. While rats 1 and 3 barely produce burst responses, a substantial proportion of rats' 4, 5, and 6 IRTs distribution was constituted by burst responses. The embedded bar plots show that haloperidol reduced overall response rate for three of five subjects, specifically for those subjects with a high proportion of burst responses. This illustrates that haloperidol mainly affects the response rate of those responses with very short IRTs, what can be corroborated with the pink density plots. Also, bar plots show that reward rate decreased for four out of five subjects. In average haloperidol administration slightly decreased both response and reward rates (see right bottom panel), which have been reported in other studies with rats[45] and nonhuman primates[46] using different target times (but see a study by Britton and Koob[47] in which reward rate increased with the same dose). If one only considers global performance measures, this result may seem paradoxical given that this protocol is explicitly

designed to prize low response rates (as its name implies). This result instantiates that a low rate of responding is not sufficient to yield an optimal exploitation of available rewards in this task. Examining the timed responses distribution in the density plots may shed light on the nature of this finding. While the peaks of the timed distributions did not systematically shift to either side with the administration of haloperidol, the spread increased drastically. This may reflect a disruption of temporal estimation, which has been previously reported using other procedures[48].

The expected result was a decrease in impulsivity. Haloperidol is a high-affinity selective dopamine D_2 receptor antagonist that acts mainly in the postsynaptic dopamine receptor. As mentioned above, dopaminergic system plays an important role in impulsive behavior. For instance, D_2 receptor ligand binding in the NAc has been reported to predict increased impulsivity[49]. Also, dopamine NAc depletion decreases the frequency of premature responses in other protocols that measure the response inhibition component of impulsivity[50]. A possible interpretation of the observed results would be that the dose of haloperidol used was not sufficient to decrease substantially inhibition-related impulsivity while disrupting time estimation, causing disorganized responding and reward loss. This highlights the need for a more detailed analysis of IRTs to provide a more thorough interpretation of data, instead of just employing global measures as earlier reports have done.

Concerning feature-negative discrimination, **Figure 9** shows the typical performance of a group of subjects in this protocol through 16 sessions. As is evidenced in the figure, responding in the A+ trials and in the AX- do not differ substantially in early sessions. After a few sessions, however, rats responded differentially in both types of trials, which reveal that the stimulus X is counteracting the response tendency controlled by the A stimulus. Note that subjects withhold magazine approach responses without any punishment in AX- trials. Importantly, subjects show quite robust individual differences in both responding to A+ trials and AX- trials, as shown by the error bars. This is further instantiated in **Figure 10**, which depicts individual examples of extreme cases with regards to the degree of response inhibition displayed in this protocol.

[Place **Figure 9** here]

[Place **Figure 10** here]

FIGURE AND TABLE LEGENDS:

Figure 1. Diagram of a conditioning chamber prototype. The main components of the conditioning chamber include: (1) left lever (2) food receptacle (equipped with lateral infrared diodes to detect head entries) (3) focalized light (4) speaker for tone emission (rear view) (5) house light (rear view) (6) food dispenser.

Figure 2. Diagram of input and output events in two consecutive trials of an intertemporal choice procedure. Diagram of a prototypical intertemporal choice procedure, illustrating an SS alternative choice and an LL alternative choice, in two consecutive trials. Each row depicts the timeline of occurrence of particular output or input events. Spikes in the SS timeline represent

choices of the *smaller-sooner alternative* (upon the accomplishment of the variable-interval schedule). Spikes in the LL timeline represent choices of the *larger later alternative* (*idem*). Asterisks in the Rw timeline represent *reward deliveries.* Elevated plateaus in the OR timeline represent periods of *opportunity to respond* (they are usually signaled, and its duration varies depending on the time that the individual takes to accomplish to the specified criterion); TO stands for the *timeout* that begins after reward delivery and ends with the next trial; during this period both levers are retracted. Note that timeout durations vary depending on the type of trial (SS choice or LL choice) in order to keep inter-trial intervals equated.

Figure 3. Diagram of a hypothetical response pattern and its programmed consequences in a DRL 15 s procedure. Spikes in the R timeline represent the timeline of *responses* spontaneously emitted by the subject. Asterisks in the Rw timeline represent the timeline of *reward deliveries.* Numbers below the Cl row represent a *clock* counting down from 15 s the amount of time remaining before the next opportunity to respond and earning a reward. Note that reward delivery only occurs if a response is given since a minimum time of 15 s has elapsed from the last response.

Figure 4. Diagram of the types of trial used in the feature-negative discrimination procedure. Elevations in the A timeline represent onsets of the *excitatory stimulus*. Elevations in the X timeline represent onsets on the *inhibitory stimulus*. Asterisks in the food timeline represent *food delivery*. (**A**) A+ trials include the presentation of the excitatory stimulus followed by food delivery. (**B**) AX- trials include the presentation of the excitatory stimulus in compound with the inhibitory stimulus without food delivery. Recall that trials must be interspersed randomly and set apart by relatively long inter-trial intervals for better results.

Figure 5. Histogram of IRTs for one rat in a single session on the DRL 10 s protocol. The distribution is bimodal, with one of the peaks at very short IRTs (burst responses) and the other localized near the time criterion of the protocol (timed responses). Note as well that there is an accumulation of a small number of responses to the right and relatively far from the timed distribution (attentional lapses). Data was extracted from the 9th session in the DRL protocol of Rat 6 in a recent unpublished study.

Figure 6. Comparison of preference for Alternative SS in an intertemporal choice procedure for SHR and Wistar Rats. The Y axis displays the log-transformed SS/LL ratios. Boxplots are constituted by data from the average of the last five sessions performance for a group of eight SHR and a group of eight Wistar Rats. Data was adapted from the study conducted by Orduña[37] (**Figure 2** and **Figure 3**) with the author's permission.

Figure 7. Longitudinal progression of performance on a DRL protocol for one rat. Each of the stacked plots displays the estimate of the probability density distribution of IRTs for one subject (Rat 2) along 18 sessions. Data was extracted from a recent unpublished study.

Figure 8. Effect of haloperidol on DRL performance. Each panel shows a comparison between performance on the last 3 sessions in the saline administration stage (blue) and the haloperidol

administration stage (salmon). The primary plots show the IRTs density distributions for individual subjects (Rat 2 died due to causes unrelated to the study) and averaged data (bottom right panel). Embedded plots display comparisons of response rates (**A**) and reward rates (**B**) in both stages with the same color code as the one used for density plots. Data was extracted from a recent unpublished study.

Figure 9. Longitudinal progression of performance on a feature-negative discrimination protocol for a group of rats. Points represent mean conditioned response (magazine approach) durations for six rats in each of 16 sessions. Black points identify responding in A+ trials and grey points identify responding in AX- trials. Error bars represent 95% bootstrapped confidence intervals.

Figure 10. Comparison of response durations in A+ and AX- trials for two extreme individuals on a feature-negative discrimination protocol. Upper panel shows the performance of a high-impulsivity individual (Rat I1) and the bottom panel shows the performance of a low-impulsivity subject (Rat I6). Histograms represent distributions of response durations in the four last sessions; green identifies responding in the A+ trials and purple identifies responding in the AX- trials. Here, impulsivity is indicated by the overlap between distributions.

DISCUSSION:

The present article provided a description of a miscellaneous variety of protocols for screening impulsivity in rats. It is argued that these particular protocols are favored for their ease of programming and data analysis and require fewer operating and stimulus devices than other available alternatives. There are several crucial steps for the effective implementation of these protocols, such as (1) yielding a research question, (2) selecting an appropriate study design, (3) programming the selected protocol, (4) conducting the study, (5) collecting the data, (6) analyzing the data, and (7) interpreting the data. Adequately developing the research question helps narrowing the range of possible ways to approach the topic. A focused research question will likely lead to an appropriate study design, which will inform researchers about the selected topic. One of the cardinal features of these protocols is that they are largely automated. To produce a flawless program to operate the operant chamber and collect data automatically a thoroughgoing code needs to be written. If well conducted (daily run, at the same hour, by the same experimenters, and minding for major extraneous variables), these protocols could yield to fair volumes of data that can be interpreted at a large range of resolutions; for example, in a molecular fashion (response by response), in a trial by trial fashion, within sessions blocks, across sessions, etc.

The protocols presented in this article have been validated elsewhere. For example, using the concurrent-chains version of the intertemporal choice procedure, Orduña[37] found strong evidence that a rat model for ADHD performed poorly compared with animals in a control group (see **Figure 6**). Although this result can be taken as strong evidence in support of the validity of this animal model, there could be, at least, an alternative explanation. Animals could prefer the SS alternative not because of a strong delay discounting but rather due to a poor sensitivity to the magnitude of reward. Subsequent experiments by this author ruled out this possibility

(Experiment 2) and ultimately confirmed that differences in performance between strains are indeed due to differences in delay discounting (Experiment 3). This was elegantly accomplished by using the concurrent chains to evaluate sensitivity to reward magnitude and delay discounting in isolation; that is, assessing preference between varying amounts of rewards maintaining the duration of delay constant and *vice versa*. As it may be recalled, delay discounting is assumed to be directly relevant to impulsivity.

The delay discounting feature of impulsivity has been extensively studied with protocols that manipulate delays or reward amounts in either within or between-sessions fashion[51,52]. Such a practice allows the researcher to mathematically characterize the decay in reward value as a function of the delay. However, using several values of the delay or the magnitude is not necessary for assessing the degree in which a delayed outcome affects the preference for that outcome, as the study of Orduña[37] showed that differences in performance in a delay discounting protocol are due to differences in sensitivity to delay. Moreover, using a single delay value would be desirable if one aims to apply multiple protocols or evaluating subjects within a brief developmental stage. The present article presents the concurrent-chains schedule as a convenient alternative[53], which is considerably straightforward among paradigms to assess delay-discounting associated impulsivity that is easy to program, to conduct, and to interpret.

DRL procedures have also been empirically validated. For example, van den Broek et al.[54] selected impulsive and non-impulsive woman participants based on performance in a figure-matching task. These authors reported that impulsive participants tended to perform poorly in a DRL task compared with non-impulsive participants in several situations. Similarly, Orduña et al.[31] found differences between SHR and Wistar rats in performance on a DRL protocol. However, differences were observed only in early sessions. As sessions passed, the strain differences vanished. This indicates that the protocol (or, again, at least the particular parameters employed) is only able to detect differences in learning rates rather than in steady states of these rat strains. It is important to note that a wide range of target times have been used in the DRL literature. However, it seems that different target times have been related to distinct psychiatric conditions; while shorter target times have been typically used to model impulse control disorders[31-33], larger ones have been used to screen for depression[55-57]. That seems to support the idea that different processes impacting behavior under the constraints of shorter and longer target times[33]. That was the reason for selecting 10-second target times in the Representative Results section. In addition, larger target times need to be introduced progressively over a number of steps, which increases the duration of the protocol.

There are also studies that validate feature-negative discrimination procedures as protocols to assess impulsivity. For example, He et al.[58] found that participants labeled as impulsive perform poorly in a transfer test (i.e., summation) for feature-negative discrimination protocol (but see another study by He et al.[59]). In another study, Bucci et al.[60] assessed feature-negative discrimination performance by SHR and a control strain of rats. Although failing to observe overall differences in performance between strains, these authors found sex differences that mimic those found in humans. Namely, female SHRs showed an impaired performance in the task. This could be compared to clinical data with humans, where females diagnosed with ADHD show

more extreme symptoms than males[61]. A converging line of evidence that validates feature-negative discrimination as a model of impulsivity comes from a study conducted by Meyer and Bucci[40]. These authors reported that performance in a feature-negative discrimination was impaired by lesions in the prefrontal cortex. This brain structure is assumed to play an important role on impulse control[5] and, indeed, lesions in this structure have been documented to impair performance in other protocols to assess impulsivity[62], which provides the feature-negative discrimination procedure with face validity. In spite of the fact that feature-negative discrimination protocol is not as widely used to test impulsivity as other procedures, it was included due to practical reasons, its face and construct validity, and because of the large body of empiric data and theoretical developments that have documented the mechanisms involved in the performance in this procedure[63,64].

The feature-negative discrimination procedure has been a hallmark for inducing a learning phenomenon known as *conditioned inhibition*. In order to unambiguously demonstrate this phenomenon, it is widely believed that two complementary tests have to be jointly passed[65] (although a number of authors have disputed the necessity and sufficiency of those tests[66-69]). In a summation test, the feature stimulus (X in the current notation) would decrease responding elicited by a conditioned stimulus other than that trained along with it (A). In a retardation test, the stimulus X would acquire conditioned responding slower than a control stimulus. However, these are tests for demonstrating that stimulus X is indeed inhibitory according to the theoretical characterization of conditioned inhibition. Tests for conditioned inhibition are not necessary for evaluating the learned capacity of an individual or group to withhold a prepotent approach response in presence of a cue associated with the omission of food.

The procedures described in this article may allow researchers to perform a battery of behavioral tests for impulsivity. As mentioned before, combining multiple impulsivity tests has been shown to synergize the predictive power of the protocols[12], which would be useful on both theoretical and applied grounds. An additional advantage of assessing different manifestations of impulsivity within a single study is providing content validity (a special type of construct validity), under the assumption that impulsivity is a multifaceted phenomenon. However, caution must be exercised when sequentially testing impulsivity with more than one of the tasks presented here, as there have been documented problems associated with such a practice. For instance, carryover effects could occur, which means that performance in a task can be heavily influenced by learning in previous tasks; this type of effect could even arise within different conditions within the same task[70]. Another inconvenient consequence of applying more than two tasks in the same subjects is that, given the life cycle of rodents, tests sometimes would be implemented at different developmental stages[71]. There are some actions to minimize such outcomes, such as counterbalancing the sequence of application of tasks (which anyway would be troublesome for correlational studies, as each particular sequence could not be grouped with the others for analyses) or finding protocols of short duration.

While fairly useful and convenient, the protocols presented in this article have some limitations. For example, several studies have reported weak non-significant correlations between measures from different categories (or even within the same category[72]) of protocols to assess

impulsivity[73-76]. Such finding challenges the concurrent validity of the protocols, prompting some authors to suppose that each category of protocols measure, in fact, independent factors contributing to impulsive behavior[5,10,77,78]. However, others have stressed in the shared features of the protocols and proposed unifying frameworks to account for different forms of impulsive behavior[4,6,20,79,80]. There could be room for doubt in studies showing the absence of correlation and not including intra-class correlations reports or other tests for quantifying the psychometric properties of their measures[14,15]. Although the prevailing belief is that impulsivity is multifaceted, more research with an acknowledgeable statistical power is needed to quantify at what degree. Another known limitation is that processes unrelated to impulsivity may contribute to performance in these protocols[81]. For example, as described above, in the DRL procedure performance is determined not only by response inhibition capability, but also by time estimation. Other authors have suggested that motivational and motoric factors may as well contribute to performance in this protocol[33,82]. Fortunately, ancillary methods have been devised to rule out some of these factors[32,73]. Yet another limitation is that laboratory protocols for nonhuman animals are not exact analogues of those typically used with humans[11]; thus, their validity as translational research methods is moot. However, studies that compared performance of humans in the protocols typically with versions more closely related to those used with nonhumans lead to similar results[83].

Only three protocols were presented. However, a handful of alternative options are available. Examples of these alternatives are the 5-CSRTT (for which there is also a video-article available[84]), the go/no-go task[85], the stop-signal task[86], and the sign-tracking paradigm[87]. The 5-CSRTT has been also validated as a model for ADHD, but it is devised to focus on the inattention feature of this condition (although response inhibition also contributes to performance). This task also requires a customized panel inserted in one of the side walls of the conditioning chamber, requiring at least 5 input and 5 output additional devices (which increases costs). Performance on go/no-go and the stop-signal tasks have shown to be related to several psychiatric conditions involving impulsivity[88-92]. These tasks are fairly similar to feature-negative discrimination protocols, but with the additional aspect that rewards are delivered depending on subjects' performance[20]. Such peculiarity implies slightly more complex coding for automatic operation and data collecting and analysis. Lastly, the sign-tracking paradigm has also been theoretically and empirically related to impulsivity[79]. However, for optimal results, it requires the attachment of a light-emitting devise to the levers[76], which can also increase costs.

The protocols described here could be considered to be promising as performance in these protocols is sensitive to meaningful biological manipulations, such as selective breeding (see **Figure 6**), pharmacological interventions (see **Figure 8**), and brain lesions[40]. However, a review of the literature often reveals mixed results regarding the direction of the effects. Future applications of these methods should systematically study which parameters yield to stronger effects by adopting a parametrical approach. This would enable researchers to select parameters of a given protocol depending on the study design. For example, correlational studies require high reliable inter-individual variation for an appropriate statistical power, while conversely experimental studies benefit from measures with low intra-subject variance but are sensitive to situational manipulations[14]. A research agenda should consider these matters in order to

efficiently contribute to the knowledge about impulsivity.

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The authors have nothing to disclose.

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Figure

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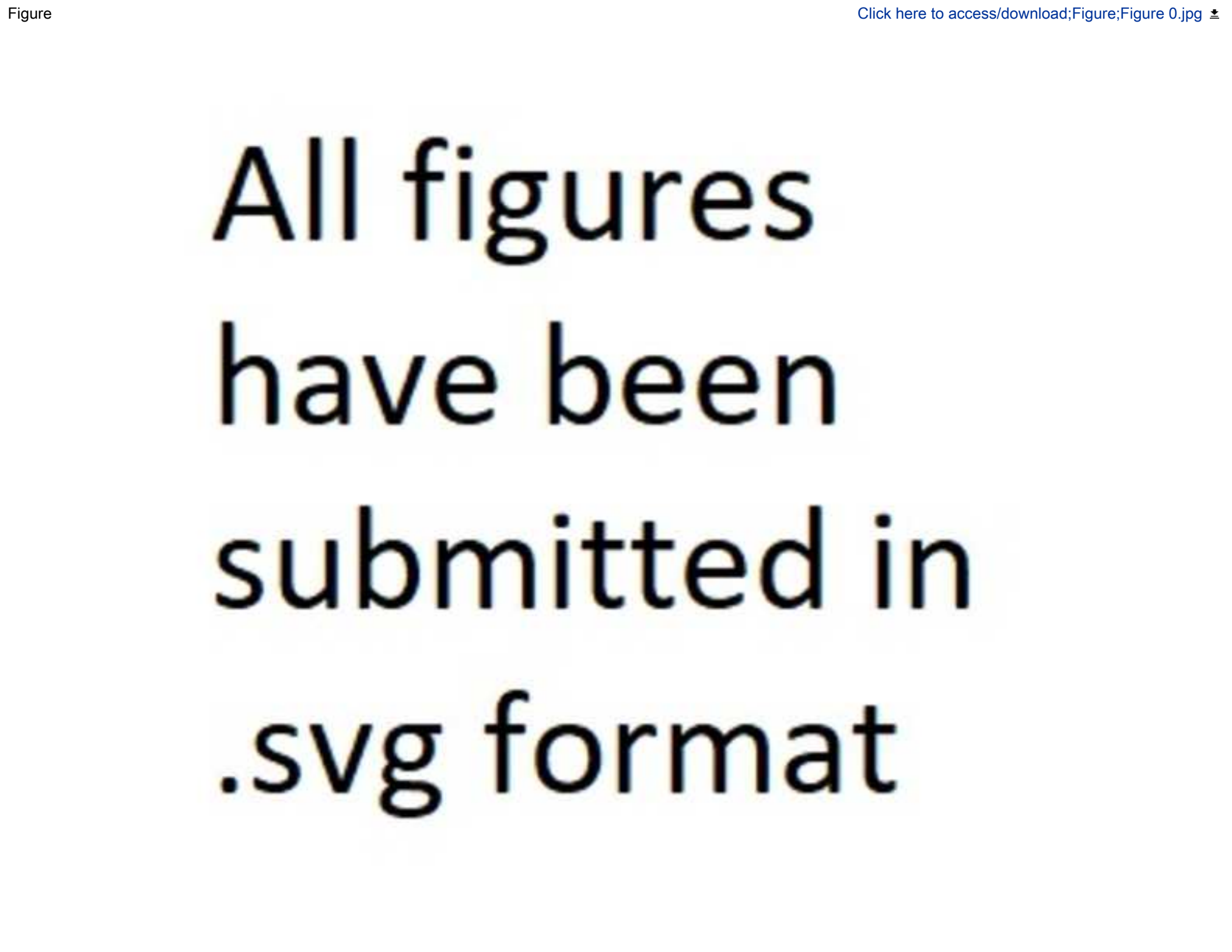

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Name of Material/ Equipment	Company	Catalog Number	Comments/Description
25 Pin Cables	Med Associa	SG-213F	Connect smart control cards to smart control panels
40 Pin Ribbon Cable	Med Associa	DIG-700C	Connects the computer with the interface cabinet
Computer	Dell Comput	T8P8T-7G8MR-4YPQ'	For controlling and monitoring protocols' processes
Conductor Cables	Med Associa	SG-210CP-8	Provide power to the smart control panels via the rack mount power supply
Food dispenser with pedestal	Med Associa	ENV-203M-45 (1293	Silently provides 45 mg food pellets
Head-Entry Detector	Med Associa	ENV-254-CB	Uses an infrared photo-beam to detect head entries into the food receptacl
House Light	Med Associa	ENV-215M	For providing diffuse illumination inside the chamber
Interface Cabinet	Med Associa	SG-6080D	Pod that can hold up to eight smart control cards
Med-PC IV Software	Med Associa	SOF-735	Translate codes into commands for operating outputs and recording/storing
Multiple tone generator	Med Associa	ENV-223 (597)	For controlling the frequency of the tones
Panel fillers	Med Associa	ENV-007-FP	For filling modular walls when devices are not used
Pellet Receptacle	Med Associa	ENV-200R2M	Receives and holds food pellets delivered by the dispenser
Rack Mount Power Supply	Med Associa	DIG-700F	Provides power to the interface cabinet
Retractable Lever	Med Associa	ENV-112CM (10455)	Detects lever-pressing responses; projects into the chamber or retracts as n
Smart Control Cards	Med Associa	DIG-716	Controls up to eight inputs and four outputs of a conditioning chamber
Smart Control Panels	Med Associa	SG-716 (3341)	Connect smart cards to the devices within the conditioning chambers
Speaker	Med Associa	ENV-224AM	For providing tones inside the chamber
Standard Modular Chambers for Rat	Med Associa	ENV-008	Made of aluminum channels designed to hold modular devices
Standard sound-, light-, and temperatu	Med Associa	ENV-022MD	Serve to harbor each conditioning chamber
Stimulus Light	Med Associa	ENV-221M	For providing a round focalized light stimulus
Three Pin Cables	Med Associa	SG-216A-2	Connects smart control panel with each of the input and output devices in t

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Author(s): **Rodrigo Sosa, Pablo Saavedra, Roberto Niño de Rivera, Gustavo Lago, Patsy Moreno, Oscar Galicia-Castillo, César Hernández-Guerrero, Mario Buenrostro-Jáuregui**

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Dear Dr. Steindel:

Thank you for giving us the opportunity to resubmit our manuscript titled "Three Laboratory Procedures for Assessing Impulsivity in Rats", Manuscript No. JoVE59070, for consideration to be published in the Journal of Visualized Experiments after being editorially revised.

General Statements about this Version of the Manuscript

We have addressed the comments in your letter and tracked the changes that were made in the manuscript.

We think that we have reached a point at which we might discuss the content of the video. We have envisaged the video corresponding to this article as one that shows rats' performance on the presented protocols. However, this intention could not be expressed in the protocol as it is, given that the format limits the description of the protocols to imperative sentences describing what the researchers must do. We believe that the video could start with a general description of the aims of the protocols (as is usual), then describing the core features of each protocol (see introduction), then showing briefly how to set up the protocols (see 4th section), then show how rats perform in each of the protocols (for this, we plan to train several rats exclusively for appearing in the video), and finally show how does the data from these procedures typically looks like (see figures 6 to 10) and how to interpret it.

Of course, we are open to any additional suggestions from you.

Point-by-Point Response to Comments

1. Protocol step 1.4: Please include a reference or otherwise elaborate on restricting food intake.
Done.

2. Protocol section 3: If this is to be filmed, please include more specific details on how to modify code (e.g, which files; which lines to modify for each parameter).
We propose not to film neither the coding nor the data extraction steps, as researchers possessing this equipment are usually already familiar with these aspects, so including the details mentioned would not be necessary.