Neuroscience Letters 662 (2018) 351–355

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Influence of intertrial interval on basal and drug-induced impulsive action in the 5-choice serial reaction time task: Effects of D-amphetamine and (±)-2, 5-dimethoxy-4-iodoamphetamine (DOI)

Ciaràn M. Fitzpatrick[a,*], Vojislav S. Maric[b], Simon T. Bate[c], Jesper T. Andreasen[a]

[a] Department of Drug Design and Pharmacology, University of Copenhagen, Universitetsparken 2, DK-2100, Copenhagen, Denmark
[b] Department of Neuroscience, Pomona College, Claremont, USA
[c] Statistical Sciences, GlaxoSmithKline, Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, UK

ARTICLE INFO

Keywords:
Impulsivity
5-CSRTT
Attention
Amphetamine
5-HT2A receptor agonist

ABSTRACT

Impulsivity is a characteristic of a number of neuropsychiatric disorders such as attention-deficit/hyperactivity disorder. The 5-choice serial reaction time task (5-CSRTT) is a rodent paradigm extensively used to assess attention and impulsivity. Notably, 5-CSRTT studies do not typically account for the reduction in premature responding, the measure of impulsive action, occurring upon repeated exposure to test sessions with long or variable intertrial intervals (ITIs).

This present 5-CSRTT study investigated the use of variable ITIs (5, 10 or 15 s) across 15 test days (4 training days followed by 1 drug test day per week for three weeks) as previous experience had shown that 4 training days would be sufficient to induce consistent premature response levels in male C57BL/6J mice. Once a steady state was achieved, the effects of dextroamphetamine (AMPH) and (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) were then assessed using a Latin-square design to determine whether pharmacological-induced impulsive actions depended on ITI length.

Mice habituated to the variable ITI schedule after only 3 days and showed consistently lower premature response levels until the end of the study. AMPH ($p < 0.05$) and DOI ($p < 0.05$) increased the percentage of premature responses at 15 s ITI trials, while only DOI ($p < 0.05$) increased impulsive action at 10 s ITI trials. Additionally, DOI increased omission rates ($p < 0.001$), mean correct latency ($p < 0.01$), reward collection latency ($p < 0.001$), and reduced the total attempted trials ($p < 0.001$).

In summary, we demonstrated that mice habituate to the variable ITI schedule, suggesting that using the variable ITI schedule during training allowed premature response rates to stabilize before commencing pharmacological testing. Moreover, in these habituated mice AMPH and DOI significantly enhanced impulsive action at the long ITI trials only. We propose that experimental design considerations can improve the sensitivity of the 5-CSRTT to detect pharmacologicallyinduced impulsive action.

1. Introduction

Impulsivity is a core symptom in several neuropsychiatric disorders such as attention-deficit/hyperactivity disorder and substance abuse [1,2]. Impulsivity is characterized by deficits in rapid decision making actions without sufficient foresight, intolerance to delay of reward gratification and/or insufficient response inhibition [3,4]. Impulsive action is one form of impulsivity characterized by a tendency to produce an inappropriate, prematurely initiated response [5,6].

In human studies, continuous performance tests are commonly used to quantify attentional function and impulsivity [7,8]. One frequently used rodent approximation is the 5-choice serial reaction time task (5-CSRTT) which allows quantification of visuospatial attention and impulsive action [9]. In the 5-CSRTT, the subject must respond to a brief light stimulus presented in one of five locations. Responses during the intertrial interval (ITI) are defined as premature responses, and are taken as the measure of impulsive action [5,9].

Many neurochemical and pharmacological studies in rodents have employed the 5-CSRTT in order to investigate the neurobiological mechanisms of impulsive action. Evidence from rodent studies has

Abbreviations: 5-HT_{2A}, 5-hydroxytryptamine 2A receptor; 5-CSRTT, 5-choice serial reaction time task; AMPH, dextroamphetamine; DOI, (±) 2,5-dimethoxy-4-iodoamphetamine; ITI, intertrial interval

* Corresponding author.
E-mail address: Ciaran.fitzpatrick@sund.ku.dk (C.M. Fitzpatrick).

http://dx.doi.org/10.1016/j.neulet.2017.10.058
Received 30 July 2017; Received in revised form 25 October 2017; Accepted 28 October 2017
Available online 02 November 2017
0304-3940/ <outputStr5>

suggested that stimulating neurotransmitter activity of the dopaminergic or 5-hydroxytryptamine 2A (5-HT2A) receptor systems promotes impulsive action (see [5] for a comprehensive review). Both the monoamine releasing agent D-amphetamine (AMPH) [10–12] and the psychedelic 5-HT2A receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) [13–15] have been shown to increase impulsive action in both the rat and mouse 5-CSRTT.

When assessing impulsive action, 5-CSRTT investigations typically use a fixed ITI of 5 s. Using the same ITI length for the entire 5-CSRTT training and/or test period causes a gradual drop in premature responding levels, as the animal acquires the task and often develop temporal-mediated response strategies [5,9]. Some studies have incorporated long or variable ITI lengths to induce premature responding [5] and/or to prevent adoption of temporal-mediated strategies [16–18]. These ITI schedules also lead to gradual changes in premature response levels as a result of task experience [19–21]. However, the majority of these studies have utilised these schedules only on experimental manipulation days, implying that the effects of treatment may be confounded by the novelty of the design, or, in the case of repeated within-subject drug testing designs, by gradual habitation to longer or variable ITI lengths.

To reliably assess pharmacologically induced changes in impulsive action, it is therefore necessary to ensure that animals have sufficiently habituated to long or variable ITIs before initiating pharmacological testing. In addition, treatment effects may require that impulsive action be sufficiently challenged, i.e. such effects may depend on the ITI level. Thus, this present study investigated whether repeated exposure to a variable ITI schedule with 5, 10 or 15 s would result in mice habituating to this schedule concerning premature response rates. To validate this as an approach to study pharmacological-induced increases in impulsive action, AMPH [10–12] and DOI [13–15] were administrated.

2. Methods

2.1. Animals

Twelve male C57BL/6J mice, weighing 28–33 g, were used for all described studies. Starting at 8-weeks old, subjects were food-restricted to reach 85–90% of normal free-feeding weight, as adjusted from within-lab growth curve charts [27]. Water was provided *ad libitum*. Mice were placed on a 12:12 h light/dark cycle, with lights switched on at 06:00. Testing was performed between 09:00 and 13:00. Housing and experiments were carried out in the same room with regulated humidity (60–70%) and temperature (22–25 °C). All experiments were carried out in accordance with Principles of Laboratory Animal Care, European Directive 2013/63/EEC and the Danish Animal Experimentation Act.

2.2. Apparatus

5-CSRTT training and testing took place in five-holed, trapezoid-shaped operant chambers, placed within wooden insulated boxes (40 × 34 × 42 cm; Campden Instruments, UK). The back wall of these chambers consisted of a reward tray, where mice could start trials and collect a reward (Mathilde strawberry milk, Arla Foods, Denmark) delivered by a peristaltic pump. The front wall of the chambers contained an infrared touchscreen monitor (24.5 × 18.5 cm) where mice could elicit nose-poke responses. These screens were covered with a black acrylic mask so that there were five equally shaped, sized (4 × 4 cm) and distanced (3.5 cm from the mask's sides, 1.5 cm from the steel floor) apertures. Whisker server (Cambridge, UK) and ABET II (Lafayette Instruments, Indianapolis, USA) software systems controlled the chambers' operations and were responsible for recording, collecting and generating the raw data necessary for further data analysis.

2.3. 5-CSRTT training

Mice were initially trained in the 5-CSRTT [13,29] for 30 min, 5 days a week. Briefly, the stimulus duration trial length was initially set at 32 s, and was successively lowered to 16, 8, 4 and 2 s, following adequately high performance at the previous stimulus duration length. The ITI was set at 5 s for all stimulus duration trials. A *correct* response was registered when a lit aperture was nose-poked during the allotted time. An incorrect or omitted response resulted in a timeout period of 4 s, during which the chamber light would illuminate. As a general criterion to pass, discriminative accuracy *(correct/(correct + incorrect responses) × 100%)* was required to be > 70%, omissions *(omitted responses/total number of trials × 100%)* < 30%, and attempted trials > 50. Mice were required to display these aforementioned performance levels at the 2 s trial for three consecutive days in order to be considered baseline trained.

2.4. Variable ITI and pharmacological challenge

Pilot studies revealed that a within-day variable ITI and stimulus duration of 2 s enhanced premature responses rates while maintaining high motivation and attentional performance. All training and drug testing sessions described in this study were conducted for 45 min, with ITI values of 5, 10 and 15 s and stimulus duration of 2 s. The different ITI trials were presented randomly within each session. Specifically, each session was broken down into sets of 15 trials, where each ITI was presented five times in each set in a random order. This approach ensured that in this study, each ITI was not presented more than 3 times in a row. Testing consisted of 15 days over 3 weeks, and included 4 training and 1 drug testing days per week. Drug testing did not take place until mice habituated to the variable ITI schedule, and pilot studies in our laboratory have indicated that 4 training days are sufficient to achieve this. Four training days took place from Friday to Monday, while the drug was administrated after the training days on Tuesday of each week. This allowed seven days washout between each treatment. The sensitivity of the pharmacological-induced changes on impulsive action was assessed using the monoamine releasing agent dextroamphetamine sulfate ((+)-alpha-methylphenethylamine hemisulfate salt) (AMPH) (Tocris Bioscience, UK) and the 5-HT2A receptor agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) (synthesized at Dept. of Drug Design and Pharmacology, University of Copenhagen). Both drugs were dissolved in isotonic saline and administered intraperitoneally (i.p.) 30 min before testing at an injection volume of 10 ml/kg. Both drugs were administrated at 1 mg/kg based on results from a pilot study and from previously reported results [13,14]. The order of administration of AMPH, DOI and saline treatment to the mice was defined using a Latin-square design, where mice were randomly allocated to treatment sequence.

2.5. Data analysis

Performance in the 5-CSRTT was assessed in terms of measures related to attention (discriminative accuracy, percentage omissions), impulsive action (premature response rates), and task motivation (number of trials attempted; mean correct latency; and reward collection latency). The effect of repeated variable ITI training days on premature response rates was analysed using a repeated measures mixed-model approach, with Day number as the repeated factor. Pairwise Comparisons on the predicted means using Hochberg's Multiple comparison procedure were used to compare percentage premature responses of Day 1 to all other days at the 10 and 15 ITIs. For 5 s ITI there were many zero responses and hence no statistical analysis was performed (Fig. 1). To confirm premature response rate differences between ITIs, separately for the first (Day 1) and last (Day 12), the premature response rates at 10 and 15 s ITI were compared back to the 5 s ITI using an one-way analysis of variance (ANOVA) approach with ITI