Delay Intolerance Task: Different Impact of Timeout Duration on Impulsive and Non-Impulsive Rats

Francesca Zoratto **Giovanni Laviola** **Walter Adriani**
francesca.zoratto@iss.it
+39-06-4990-3171

Sect. Behavioural Neuroscience, Dep. Cell Biology & Neurosciences,
Istituto Superiore di Sanità, viale Regina Elena 299, I-00161 Roma, Italy

ABSTRACT
The intolerance-to-delay (ID) task, classically used to measure behavioural impulsivity in rodent models, requires the setting of specific temporal constraints: daily session length, reward delay intervals, and duration of timeout, i.e. the period following food delivery during which responding is without scheduled consequences. Here we focus on the impact of the timeout (TO) interval, to ascertain whether it affects or not the perception of delays. As expected, individual differences in the preference for large-late *vs* small-soon rewards emerged, with the identification of two distinct rat subpopulations: one with a nearly horizontal curve ("non-impulsive") and another with a very steep slope ("impulsive"). Noteworthy, the reaction to increasing delay length was affected by the TO duration, but only as a function of individual temperament. Interestingly, the use of extended timeout periods further decreased impulsive choice in already delay-tolerant rats. In conclusion, we demonstrate that TO is a key constraint of the ID task and therefore should be handled carefully.

Author Keywords
Cognitive impulsivity, temporal discounting, linear waiting model, animal models.

Ethical Statement
Animal experimental protocols were approved by institutional authorities, on behalf of Ministry of Health, in close agreement with European Community Directives and Italian Law. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to *in vivo* testing.

INTRODUCTION
Impulsivity is a key symptom of attention-deficit hyperactivity disorder (ADHD), a neuropsychiatric syndrome affecting infants and adolescents, and is also common in obsessive-compulsive and addictive disorders. Behavioural impulsivity is usually studied in rodent models with the intolerance-to-delay (ID) task, involving the choice, by nose-poking, between either immediate small amounts of food, or larger amounts of food after a delay [3].

Besides the experimenter-imposed delay, another major constraint within the ID task (to be also set by the experimenter) is the timeout (TO) interval following food delivery (i.e. the period during which nose-poking is recorded but is without scheduled consequences). Therefore, experimental subjects are forced to respond after at least the TO is elapsed. Subjects will spontaneously show a slight interval of further waiting, termed response time (RT). Hence, reinforcers and next responses will always be spaced by a mean inter-trial interval, i.e. the timeout interval plus the mean spontaneous waiting of subjects (mITI = TO + RT).

The introduction of delays is classically expected to generate a subjective state of aversion and to produce the shifting of preference towards the immediate delivery of a smaller-size reinforcer, despite lower payoff in the long term. However, we proposed that the mere absolute value of the delay duration has no universal significance per se, rather its impact on the subject could be dependent on other temporal features within the task [2]. Here, we discuss possible influences exerted by the timeout interval duration on decision making within the ID task. A refinement of ID tasks can be highly relevant to a deeper validation of preclinical models for ADHD and, more in general, of animal models for inhibitory control impairment.

METHODS
Food-restricted (88.13% ± 0.27% of their free-feeding body weight) Sprague-Dawley male rats were tested in operant chambers provided with two nose-poking holes (Coulbourn Instruments, Allentown, PA, USA). Nose-poking in one hole (SS) resulted in the immediate delivery of a small amount of food (one 45 mg pellet, BioServ, Frenchtown, NJ, USA), whereas nose-poking in the other hole (LL) delivered a larger amount of food (five 45 mg pellets) after

a delay, which was increased progressively each day (from 0 s to 7 s, 15 s, 30 s, 45 s, 60 s, 75 s and finally 90 s, 8 daily sessions, preceded by 3 training sessions at delay 0 s). Following food delivery, the magazine light was turned on to signal the length of the timeout (TO), during which nose-poking was recorded but was without scheduled consequences. During the testing phase, a delay was inserted between nose-poking and large-reward delivery. The chamber light was kept on to signal the entire length of this delay. The small reward delivery was unchanged. Rats were assigned to three different timeout intervals (15 s, 30 s or 45 s; n=8 per group) and, consequently, to three different session lengths (20 min, 40 min, 60 min). This was intended to provide animals the opportunity to complete the same number of trials within the session.

Impulsivity can be measured by the steepness of the preference-delay curve. On the basis of the median value of steepness, we differentiated two distinct subpopulations [1]: an "impulsive" one, which shifted quickly towards the SS hole (i.e. with a very steep slope), and a "non-impulsive" one, with little or no shift. Therefore the two subpopulations were analyzed separately. In addition to the classical parameter of choice behaviour (percent LL preference), we calculated the mean spontaneous waiting (termed response time, RT) occurring between the end of each timeout (TO) and the next nose-poke. The pace between reinforcer deliveries and next responses, given by the mean inter-trial interval (mITI = TO + RT), was also calculated. Hence, we have recently proposed that the impact of any given delay may be proportional to this pace and be expressed as delay-equivalent odds, i.e. the extent by which delays are multiples of the mITI [2].

Data were analyzed using repeated-measures parametric analysis of variance (ANOVA). The general model was 8-level delay x 3-level timeout, with timeout (the three different interval durations) as between-subject factor and delay (one per daily session) as within-subject factor. Statistical analysis was performed using Statview II (Abacus Concepts, CA, USA). Data are expressed as mean ± SEM. Significance level was set at $p<0.05$. Since this study was a methodological pilot, the sample size is quite small but a replication study is already planned.

RESULTS

Choice Behaviour

As expected, all animals showed a shift in preference from the large (LL) to the immediate (SS) reinforcer as the delay length increased. However, animals belonging to the group with the shortest TO (TO15 group) experienced a clear-cut intolerance much earlier (already at delays 7.5 s, 15 s and 30 s) than TO30 and TO45 animals. In fact, at these delay values, LL choices were significantly higher in rats belonging to the groups with higher TO (TO30 and TO45 groups) than in the corresponding controls (TO15 group). Therefore, at a first glance, the magnitude of intolerance generated by the introduction of the delays seemed to depend critically on the value which was chosen as TO.

The separate analysis of these two subpopulations revealed an unexpected profile. TO15 animals belonging to the "non impulsive" subpopulation showed an interesting U-shaped curve. At lower delays, rats started shifting, with an apparent recovery of the percent LL preference starting from delay 45 s onward. Thus, at delay 90 s, subjects reached the same values they already showed at delay 0 s. As expected, "non impulsive" rats of TO30 and TO45 never shifted to a clear-cut SS preference, being relatively "tolerant" despite highest delays (see Figure 1). On the contrary, delays had a quite strong impact on animals belonging to the "impulsive" subpopulation and this independently from the duration of the TO (see Figure 2). As a matter of fact, our hypothesis (i.e. the intrinsic value of the delay may be a function of the TO) was true only in the case of "non impulsive" animals.

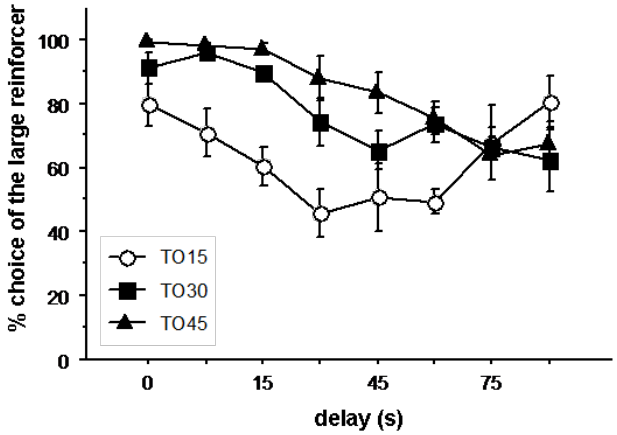

Figure 1. Mean (± SEM) choice (%) of the large reinforcer (LL), shown by "non-impulsive" rats belonging to the three different TO interval groups (n=4 per group).

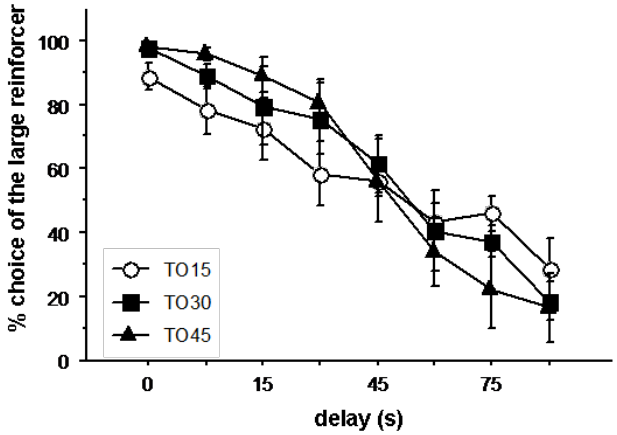

Figure 2. Mean (± SEM) choice (%) of the large reinforcer (LL), shown by "impulsive" rats belonging to the three different TO interval groups (n=4 per group).

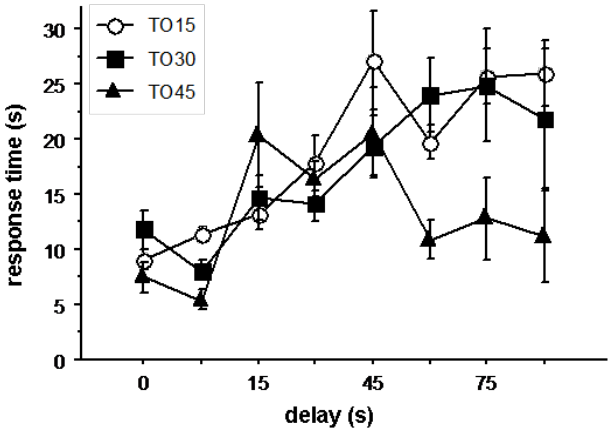

Figure 3. Mean (± SEM) response time (RT), i.e. the spontaneous waiting between the end of a timeout period and the following nose-poke for a reinforcer (either LL or SS), shown by rats belonging to the three different TO interval groups in the ID task (n=8 per group).

Spontaneous Waiting

Data revealed that, in the TO15 group, RT increased sharply (from around 18 s to around 27 s) when the imposed delay changed from 30 s to 45 s. In these animals, a clear recovery of LL preference comes along with a marked increase of RT values. Rats belonging to the TO30 group showed a gradual increase of response time when moving from no delay to a 90 s delay. It appears that the increasing delays directly influenced the length of rats' spontaneous waiting (RT) before next decision. In the TO45 rats, a marked discontinuity (response time increasing from around 5 s to around 20 s) was evident between imposed delays of 7.5 s and 15 s. Moreover, RT decreased (from around 21 s to around 11 s) when imposed delay changed from 45 s to 60 s. Interestingly, compared with the other two groups, TO45 rats expressed markedly lower RT values during all sessions at very high delays (see Figure 3).

DISCUSSION

Present data demonstrate that TO value is a crucial temporal constraint but only within subjects with little or no impulsivity.

Within the "non-impulsive" subpopulation, the main result is that TO30 and TO45 rats show some intolerance at higher delays when compared to TO15 rats. To explain this profile we propose that only delays that are extended enough, compared to the TO value, will generate a considerable drive to support the shift towards SS. Indeed, the specific delay length of 30 s had a quite low impact (i.e. a low odds value) in subjects used to a very long TO (TO45 and TO30 animals). Conversely, we can hypothesise that it was perceived as much more frustrating (i.e. equivalent to higher odds values) in subjects paced by quite a shorter TO (TO15 animals).

The RT profile observed in TO15 rats is consistent with previous work by our group. Indeed, a TO interval around 15-20 s and a session length around 20-25 min have been used so far in our hands. Under these conditions, a clear-cut discontinuity has been repeatedly observed between imposed delays of 30 s and 45 s, i.e. when imposed delay was equivalent to the mITI. Specifically, at that time point, the 15 s of TO plus the 18 s of RT give a mITI value of 33 s. As such, rats apparently begin to react when the delay value exceed the mITI.

The RT profile observed in TO30 animals are in agreement with the "linear waiting model", a formal model of voluntary waiting in experimental animals [4]. Indeed, according to this model, the duration of pauses following food presentation is determined by the preceding inter-food interval. Thus, spontaneous pauses are directly proportional to increasing temporal distances between food deliveries (a behaviour termed "temporal tracking"). Reinforcing events are therefore progressively rarefied.

The use of extended timeout periods further decreased impulsive choice in already delay-tolerant rats and also affected the profile of RT. This possibly reflects the ability of these rats to cope with long paces between reinforcing events. In summary, delay-induced states of aversion may depend on previous adaptation to the rate of reinforcement, at least for non-impulsive individuals.

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