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# Gap in the conditioned stimulus: differential impacts on temporal expectancy in appetitive and aversive conditions in rats.

Lisa Saleille<sup>a</sup>, Lucille Tallot<sup>a</sup>, Bruce L. Brown<sup>b</sup>, Sylvia Wirth<sup>c</sup>, Valérie Doyère<sup>a</sup>

<sup>a</sup> Université Paris-Saclay, CNRS, Institut des Neurosciences Paris-Saclay, 91400 Saclay, France

<sup>b</sup> Department of Psychology, Queens College and the Graduate Center, The City University of New York, Flushing, NY 11367, USA

<sup>c</sup> Institut des Sciences Cognitives, CNRS, 69675 Bron, France

**Abstract: 200 mots**

We analyzed, through a Pavlovian conditioning procedure in rats, the temporal pattern of behavior in appetitive and aversive conditions within subjects, and the difference in inferred temporal working memory functioning with the Gap paradigm. For both conditions, we paired a 60-s conditioned stimulus (CS: tone1 or tone2) with an unconditioned stimulus (US: shock or chocolate pellet) delivered 20s after CS onset. The analysis of behavioral responses and individual-trial data was performed during Probe trials, consisting of CS alone, or trials in which different gaps were inserted, to assess the effect of the temporal manipulation on the behavior. The results showed: (1) An anticipatory peak time in the aversive condition but better accuracy in the appetitive condition, (2) constancy in the Weber fraction suggesting that the difference in peak time was under clock control, (3) a graded effect of gap parameters only in the aversive condition and (4) different gap effects between conditions when a gap was inserted early in the CS. These results highlight behavioral differences between aversive and appetitive conditions and suggest that the temporal working memory mechanism was not engaged in the same manner in each condition.

**Keyword: 3-6**

Interval timing | Pavlovian conditioning | Conditioned suppression | temporal working memory

## Introduction

Keeping track of time is fundamental for being prepared and performing adaptive anticipatory cost-effective behaviors. Following an adaptive scheme, it is lifesaving for animals to compute a time window to escape following hearing a predator's call, or for both humans and animals to process time between lightning and a thunderclap to decide whether there is time to run back home or shelter on site. The shaping of adaptive behavior depends upon forming a long-term memory of predicted time of event arrival, or of the interval between significant events, as well as tracking elapsed time since the last significant event in active working memory to evaluate when the next event is going to occur and adapt action accordingly. This process has been incorporated in scalar expectancy theory (SET, Gibbon, 1977), which hypothesized a pacemaker, which produces pulses, and a switch that, when closed, permits the accumulated pulses to be sent to working memory accumulator as time passes. Upon occurrence of a significant event, the accumulated duration is stored in a reference memory. On the next occurrence, the perceived elapsed time is continuously compared with stored time, and when the ongoing accumulated duration reaches a threshold of similarity with the reference memory, a decision to initiate the behavior is made (so called 'start time'). After time has passed, in the absence of an event, when the two duration values are perceived as different, the decision to stop the behavior is taken (so called 'stop time'). In the search for plausible neurobiological mechanisms, some recent models have hypothesized that interval timing may rely on neural networks specifically related to the task, in addition to core general timing networks (Buhusi and Meck, 2005; Dallérac et al., 2017; Díaz-Mataix et al., 2014; Ivry and Spencer, 2004; Lusk et al., 2016; Matell and Meck, 2004; Tallot et al., 2020). One may thus ask to what extent temporally modulated behavior differs depending on the use of instrumental *vs.* Pavlovian, appetitive *vs.* aversive tasks, as the neural circuitries underlying these tasks may differ (Cain and LeDoux, 2008; Hall, 2002). Differences in some aspects of temporal behavior between tasks could indicate whether they rely on common or different neural clocks.

Interval timing processes have been studied mainly using instrumental appetitive tasks, and to a lesser extent Pavlovian tasks. The latter facilitates the comparison of temporal behaviors in the expectancy

of events of different valences, as the subject can learn that a conditioned stimulus (CS) predicts the arrival of an aversive (e.g., shock) or appetitive (e.g., food) unconditioned stimulus (US), at a fixed time after the CS onset, independently of the subject's behavior. Rat's temporal behavior in both appetitive and aversive Pavlovian conditions conforms to the well-known rules described in an appetitive instrumental setting: (1) When non-reinforced probe trials are introduced in a partial reinforcement schedule (peak interval, PI, task), the averaged responding follows a typical Gaussian shape curve, whether the animal is expecting an electric shock (potentiated startle response, Davis et al, 1989; conditioned suppression of lever pressing for food, Meck and MacDonald, 2007; Boulanger-Bertolus et al., 2015; Tallot et al., 2016; Dallérac et al., 2017), or a food delivery (head entries in a food magazine, Tam et al., 2013; Tam and Bonardi, 2012a). (2) The distribution of conditioned responding follows the scalar property of time (i.e., time precision proportional to the estimated timed interval), as curves from different CS-US time intervals superimpose well when rescaled on a normalized time axis (Kirkpatrick and Church, 2000; LaBarbera and Church, 1974; Tallot et al., 2016). (3) Behavior in individual trials exhibits a three-state pattern, corresponding to a binary response pattern on individual trials (i.e., stable rates of responding transitioning from a low level of responding to a high level and back to a low level with no intermediate rates). The three-state pattern identifies the start and stop times, and has been interpreted as defining a temporal window where the current time is judged as similar to the reference memorized time of US arrival (Gibbon and Church, 1990; Tallot et al., 2016).

The assessment of underlying clock mechanisms, and in particular the potential working memory for time, has often been done in instrumental tasks using a Gap procedure, i.e., a brief interruption in the to-be-timed stimulus (Roberts and Church, 1978; Roberts et al., 1989). Depending on the length of the gap and its position in the to-be-timed stimulus, the time at which the response peaks (peak time) is shifted in time to an extent that is interpreted as reflecting the duration left in working memory when the gap terminates. In reference to SET, three different clock modes have thus been defined (Cabeza de Vaca et al, 1994; Roberts, 1981; Roberts & Church, 1978): (1) the 'Run' mode, in which the switch stays closed during the gap and no shift in temporal behavior is observed; (2) the 'Stop' mode, in which the switch opens during the gap and the accumulated time before the gap is retained in working memory, resulting in a shift in

temporal behavior equal to the gap duration; (3) the ‘Reset’ mode, in which the switch opens during the gap, but the value in working memory is back to zero. Interestingly, Cabeza de Vaca et al. (1994) found graded effects when testing different gap locations and durations in pigeons, with peak time indicating shifts between ‘stop’ and ‘reset’ predictions. They suggested that such graded shifts were consistent with the decay of subjective time stored in working memory that occurred during the gap. However, in conditions reducing ambiguity between the gap and the inter-trial interval, only a stop has been observed, even for long gaps (Kaiser et al, 2002). Results in rats are also mixed. Indeed, Buhusi and Meck (2007) reported a graded effect of gap parameters in an instrumental appetitive procedure, whereas Orduña et al. (2008) did not find the same result in a similar experimental design. The major difference between these experiments was the presence of three gap durations in the experiment of Buhusi and Meck (2007) to test the effect of gap duration, whereas Orduña et al. (2008) tested only the position effect of a single gap duration. Nevertheless, other experiments testing only the effect of duration of a gap found a graded effect (Buhusi et al., 2005; Buhusi and Meck, 2000). In Pavlovian conditioning, the effect of a gap during the CS has been far less studied. To our knowledge, in the appetitive condition, only two studies have tested the effect of a gap during the CS. Tam and Bonardi (2012a) used a single gap in rats and found a shift in peak time between ‘stop’ and ‘reset’. Tam et al. (2013), testing three different gap durations starting at the same position after CS onset, found a graded effect with ‘run’ behavior for shortest gaps and ‘stop’ behavior for the longest gap. Moreover, this experiment showed that the behavior evolves with trial number (the more trials, the bigger the shift observed). The only experiment testing the effect of a gap during the CS on temporal behavior in an aversive condition reported a shift in behavior reflecting a ‘stop’ mechanism (Tallot et al., 2016). Thus, the question remains whether the effect of a gap differs between appetitive and aversive conditions in Pavlovian conditioning, which could give some clues of where neural clock mechanisms may differ, if they do.

The present study aimed at comparing the impact of different gaps in Pavlovian appetitive and aversive conditioning in rats. We designed a procedure for assessing temporal behavior in both conditions in the same animal. While a 60-s tone with a given frequency was followed by delivery of chocolate pellets

in a magazine 20s after the tone onset, another tone frequency was followed by the delivery of a mild foot-shock, in separated sessions. We analyzed timing processes underlying temporal expectancy of the US through the duration of head entry in the chocolate pellet magazine (appetitive condition) and conditioned suppression of lever pressing for food (aversive condition), both for the mean response level function and individual trial behavior. We then characterized the effects of three gap types inserted in different trials of the same session to assess the effect of duration and position of gaps during the CS.

## **1. Material and methods**

### *2.1. Subjects*

The experiment was carried out on twenty-three adult male Sprague-Dawley rats (Envigo, initial weight 290-300g approximatively, France). The experiment was conducted with the accordance of the European Communities Council Directive (2010/63/EU Council Directive Decree) and the Paris-Centre et Sud Ethical Committee (CEEA N°59). Rats were housed by four in Plexiglas cages under a 12/12h light-dark cycle (7:30 am-7:30 pm) with controlled temperature ( $21 \pm 2^{\circ}\text{C}$ ) and hygrometry ( $55 \pm 5\%$ ). Cages were enriched with red tunnels, wood sticks and two types of litter. Food was restricted for a reduction of weight to 85% of the free-feeding weight; water was present *ad libitum*. Sessions were conducted 6 days a week.

### *2.2. Apparatus and stimuli*

Eight operant boxes (30 x 25 x 30 cm, Coulbourn instrument, USA) were used, each equipped with a shock grid floor, a red house light, a speaker, one retractable lever, and two feeding magazines. One magazine next to the lever dispensed grain-based pellets (45 mg, BioServ) and the other magazine, placed on the opposite wall, dispensed sucrose pellets (chocolate flavor, 45mg, BioServ). Two tones were used (3 or 7 kHz, 15 dB above the background) as conditioned stimuli (CS), one tone predicted two sucrose chocolate-flavored pellets and the other predicted a mild foot-shock (0.3-0.5 mA, 0.5 seconds) as unconditioned stimuli

(USs). Operant boxes were placed in sound attenuated enclosures with a ventilation fan (60 dB background). Protocols were controlled by Graphic State software 3.03 (Coulbourn instrument, USA).

### *2.3. Protocol design*

#### *2.3.1. Instrumental training (10 sessions)*

First, one session of magazine training was conducted for each food dispenser: grain-based (aversive condition) and sucrose-based (appetitive condition), during which 15 pellets were delivered at variable intervals (40s, 60, 80s, randomly chosen). Next, one or two sessions on a continuous reinforcement schedule were run, during which each lever press triggered the delivery of one grain-based pellet as a reward. When the criterion of 60 lever presses in 30 minutes, or less, was reached, a variable-interval schedule was run for 10 sessions (VI, 1-60s range). On average, rats realized  $45 \pm 4.82$  lever presses per minute at the end of the training.

#### *2.3.2. Pavlovian conditioning under Fixed-interval training (FI – 23 sessions)*

Rats were trained in two successive Pavlovian conditioning sessions per day, one appetitive and one aversive, with a 10-min break in a cage with water between them. Which CS tone frequency preceded the appetitive or the aversive US was counterbalanced between rats. For each of the two conditions, a total of 12 trials was given, one trial consisting of the presentation of 60s of CS with the US delivery at 20s after CS onset. Trials were presented with a variable inter-trial interval (ITI) of 3 minutes on average (130s, 150s, 170s, 190s, 210s, and 240s); the same ITI distribution remained in effect for all the following phases until the end of the experiment. During the appetitive sessions, the lever was retracted. During the aversive sessions, the lever was extended and the VI schedule was maintained throughout the session. The intensity of shock was modulated individually, depending on the mean suppression ratio (if the suppression ratio was  $<0.7$ , the shock was increased by 0.05mA, with a maximum of 0.5mA). At the end of this training phase, the intensity of shock remained constant for the rest of the experiment.

#### *2.3.3. Peak-interval training (PI – 23 sessions)*



Following the FI protocol, Probe trials were added in a PI training protocol in both appetitive and aversive sessions. Probe trials consisted of the presentation of the CS only (60-second tone). Each session was composed of 12 reinforced trials (CS+US) and 8 probe trials (CS only). The presentation order of trials was randomized, with no more than 3 successive trials of the same type.

#### *2.3.4. Gap testing (23 sessions)*

In addition to reinforced trials (12 per session) and Probe trials (2 per session), Gap trials (6 per session) were inserted. Three different gaps were used, to compare the effect of duration and position of the gap. In “Early” Gap trials, the CS was presented for 5 seconds, paused for 5 seconds, and then resumed for 50 seconds. In “Late” Gap trials, the CS was presented for 10 seconds, paused for 5 seconds, and then resumed for 45 seconds. Finally, in “Long” Gap trials, the CS was presented for 5 seconds, paused for 10 seconds, and then resumed for 45 seconds. As for Probe trials, Gap trials were not reinforced.

#### *2.4. Data analysis*

Only Probe and Gap trials were analyzed for the 23 PI sessions and the 23 Gap sessions in the two conditions. Behaviors (lever-press and head entry) were recorded as timestamped events with 20-ms precision. In the aversive condition, the number of lever presses for each one-second time bin was calculated during the pre-CS period (20s) and the entire duration of the CS (60s). The mean suppression ratio was then calculated for each rat with the following formula:  $y = 1 - [b/(a + b)]$ , where  $b$  represents the mean number of presses for each bin (1s) of the CS, and  $a$  the mean number of presses per bin during the pre-CS period. A suppression ratio of 0.5 represents no suppression, whereas a ratio of 1 represents complete suppression. A minimum suppression ratio of 0.7 (mean during the first 20s of CS) was determined as a criterion to include a given rat in the analysis. For Probe trials, the mean suppression curve of each rat was fitted with a Gaussian function with a ramp using Peakfit 4.12 Software. The following formula was used:  $y = A3 \times \exp(-0.5(\frac{x-A1}{A2})^2) + A4 \times (x - A1) + A0 - 0.5$ .  $A0$  represents the basal level of suppression,  $A1$  the center of the function (peak time),  $A2$  the standard deviation (width),  $A3$  the value of the peak and  $A4$  the slope of the ramp. The same formula was used for Gap trials, in addition to a Gaussian (Amplitude) curve for the

pre-gap period. The formula of this Gaussian was  $y = A0 \times \exp \left[ -\frac{1}{2} \left( \frac{x-A1}{A2} \right)^2 \right]$ . A0 represents the amplitude of the curve, A1 the center and A2 the width.

In the appetitive condition, the duration of head-in was calculated (from timestamps of head entry and head exit from the chocolate pellet magazine) per 1s bin (during 20s pre-CS + 60s CS), for each trial, using a Power Basic program. A minimum ratio of 2.5 (mean during the first 20s of CS/ mean of the 20s pre-CS) was fixed as a criterion to include a given rat in the analysis. As in the aversive condition, the averaged curve of each rat was fitted with a Gaussian function with a ramp, using Peakfit 4.12 software. The following formula was used:  $y = A3 \times \exp \left( -0.5 \left( \frac{x-A1}{A2} \right)^2 \right) + A4 \times (x - A1) + A0$ . Furthermore, a Gaussian (Amplitude) curve was also added for Gap trials.

To determine the behavior during individual Probe trials, we fitted a three-state model on each trial, which delimits start and stop times (Gibbon and Church, 1990). In SET, the start time represents the moment when elapsed time becomes similar enough to the expected arrival of the US, and the stop time when it becomes too dissimilar as time goes on. Empirically, as described in Tallot et al., 2016, start time in the aversive condition represents the start of the suppression (reduction in lever pressing) and the stop time the termination of the suppression (increase in lever-pressing). In our appetitive task, start time was determined as an increase in duration of head entry in the chocolate pellet feeder magazine and the stop time as a following decrease in its duration. Using a Power Basic program, we performed a regression analysis of each trial to determine the proportion of variance ( $\eta^2$ ) accounted for by the three-state pattern on each trial (described in Aum et al., 2004 and used in Tallot et al., 2016). As in Tallot et al., 2016, the data were divided into three categories. First, the “Early onset” trials, where a start time could be measured only when the pre-CS period was included in the analysis. The search for start began 1s after the start of the CS, with a minimal duration of 4 seconds. This category represents trials for which the start time can be found very soon after the CS onset, and thus more controlled by the CS onset than the time at which the US is expected to arrive. The second category was determined as “Late onset” trials, found when the analysis was restricted to the

CS period only. This category represents trials for which the start time was later in the trial, and thus presumably more under temporal control. Finally, in a third category were the remaining trials which could not be fitted with a 3-state pattern, the “None” trials. For each animal, the median and interquartile range for the start, the stop and the spread (variability estimate; spread = stop - start) were calculated.

A total of 14 out of the 23 rats was included in final analysis. Six rats presented a head-in duration ratio <2.5, one rat a suppression ratio <0.7 and two rats had the two ratios below the criterion. Group data (mean  $\pm$  SEM) were subjected to normality test (Shapiro-Wilk). Then, *t*-test and the Cohen’s *d* (effect size factor) were calculated (application of the Wilcoxon signed-rank test if nonnormality were indicated), or Repeated measures ANOVA with the partial eta-squared ( $\eta_p^2$ ) were performed (JASP 0.12.2), with an alpha level of .05. For the Repeated Measures ANOVA, the Greenhouse-Geisser test correction was applied when the condition of sphericity was not met.

## 2. Results

### 3.1. Temporal behavior with probe trials

#### 3.1.1. Mean behavior in both conditions

The animals showed a temporal pattern behavior in both appetitive and aversive conditions (Fig. 1A). In the aversive condition, the mean suppression ratio peaked anticipatorily with regard to the programmed time of US delivery (20s). In contrast, in the appetitive condition, the mean head-in duration increased in the same manner but peaked close to the programmed moment of the US. Determination of the peak time (Fig. 1B) showed a significant difference between the two conditions (aversive:  $15.19\text{s} \pm 0.48$ ; appetitive:  $20.47\text{ sec} \pm 0.58$ ;  $t(13)=7.2, p<0.001, d=1.916$ ), with a significant difference from the FI value (20 sec) in the aversive condition ( $t(13) = -10.302, p<0.001, d=8.707$ ), but no significant difference in the appetitive condition ( $t(13) = 0.839, p=0.417, d=9.704$ ). The width (Fig. 1C) was significantly larger in appetitive ( $12.16\text{ sec} \pm 0.93$ ) than in aversive ( $9.38\text{ sec} \pm 0.52$ ) condition ( $t(13)=-3.15, p=0.008, d=-0.841$ ). No significant difference in the Weber fraction (Coefficient of Variation = width/peak time) was found between conditions (Fig. 1D; aversive:  $0.62 \pm 0.04$ ; appetitive:  $0.60 \pm 0.05$ ;  $t(13)=0.22, p=0.832, d=0.058$ ), suggesting that the behavioral difference between conditions was sustained by a scalar process, with anticipation in the aversive condition under temporal control.

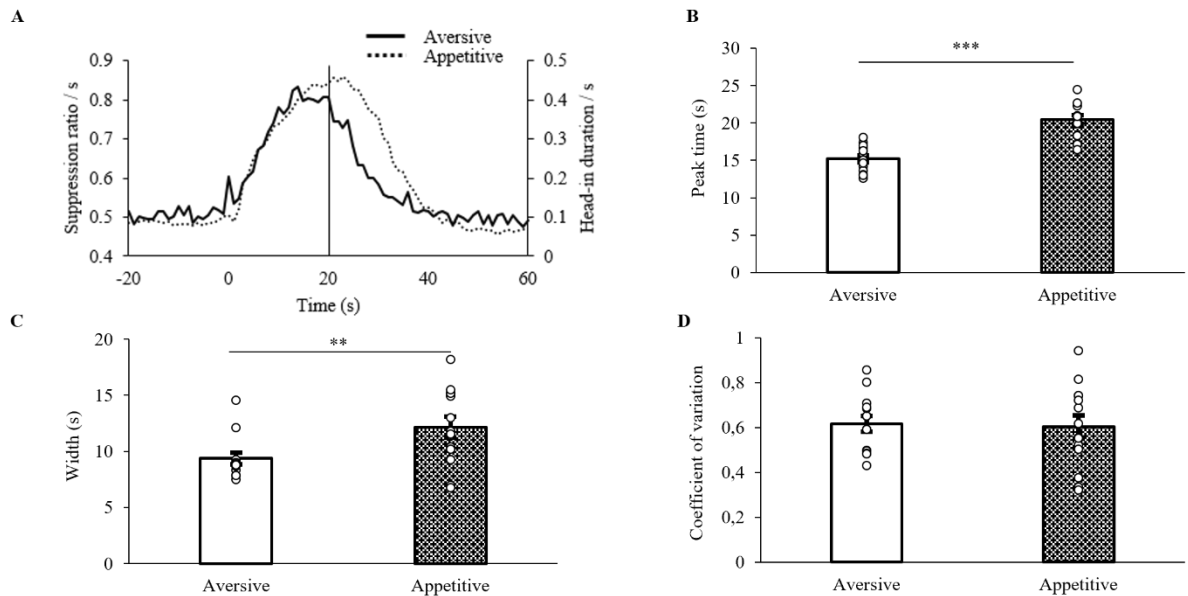


Figure 1: Characterization of the temporal behavior in aversive and appetitive conditions during Probe trials.

(A) Mean temporal curves across time for each condition. The behavior was evaluated by suppression ratio per second in the aversive condition (solid line) and by the head-in duration in chocolate pellet magazine per second in the appetitive condition (dotted line). Black vertical bar indicates the FI value. Mean  $\pm$  SEM and individual data (each dot represents one rat) for the peak time (B), the width (C) and the coefficient of variation (D). \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

### 3.1.2. Trial by trial analysis

To characterize the behavior in individual trials, the analysis of the three-state pattern was undertaken for each condition. As in Tallot et al., 2016, a visual analysis of raster plots in the aversive condition showed a period of decreased lever-pressing framing the moment of the US arrival. Conversely, in the appetitive condition, the time passed in the chocolate pellet feeder magazine increased relatively rapidly after the CS onset and maintained a steady-state level for several seconds until after the estimated US arrival (Fig.2A, top four panels as individual examples). The behavior modulation before and after the expected time of US arrival is clearly visible when behavior for each trial was realigned to the start time or the stop time as  $t_0$  (Fig. 2A, two lower panels). In the aversive condition, the number of lever-presses was superior to 0.5 per second and peaked before  $t_0$ , to drop suddenly and stay low afterward. In contrast, for the realignment

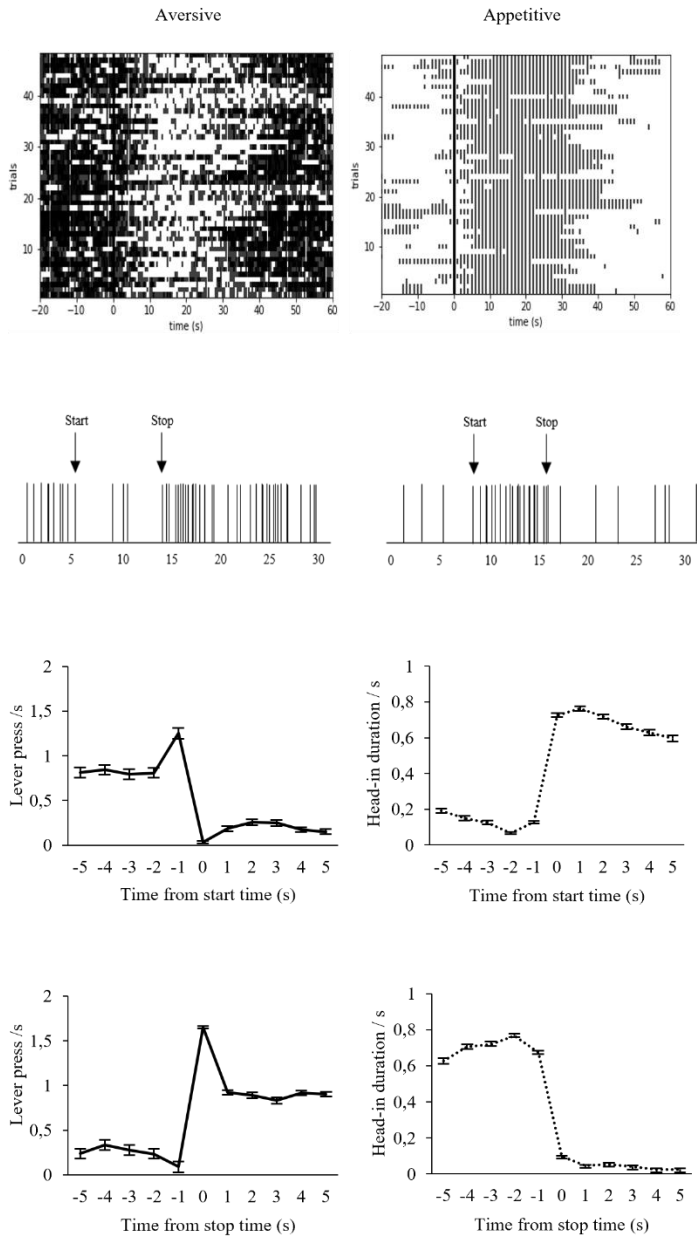
around the stop time, a drastic increase in lever pressing was observed afterward. Concerning the duration of head entry in the chocolate pellet magazine, a mirror image pattern was observed, with an abrupt increase at start time and decrease at stop time.

As in Tallot et al, 2016, we determined three categories of behavior with the 3-state analysis (fig.2B). Globally, the behavior was equally distributed among the three categories in the aversive condition, whereas the majority of trials were of the ‘Late onset’ type in the appetitive condition. A two-way Repeated Measures ANOVA showed no significant effect of US-condition ( $F(1,13)<1$ ), a significant effect of category ( $F(1.36,17.70)=47.7, p<0.001, \eta_p^2=0.79$ ) and a significant condition x category interaction ( $F(2,26)=28.82, p<0.001, \eta_p^2=0.69$ ). A Bonferroni multiple post-hoc comparison showed a significant difference between conditions for ‘Early onset’ ( $p<0.001$ ) and ‘Late onset’ ( $p<0.001$ ) trials, but no difference between conditions for ‘None’ trials ( $p=0.640$ ). However, when the behavior (rate, head-in duration) was averaged within each category, animals showed a typical temporal pattern in all types of trials in the aversive condition (supplementary figure 1, left panel), whereas a typical temporal pattern was evident only for ‘Late onset’ trials in the appetitive condition, which suggests that there was some temporal control in the majority of trials in both conditions.

The analysis of start time, stop time, and spread of ‘Late onset’ trials in both conditions (Fig.2C) showed a significant difference for the start time ( $W=89.0, p=0.003$ ), with an earlier start time for the aversive condition. The stop times did not differ significantly between conditions ( $t(13)=0.36, p=0.73$ ). The spread was significantly different between conditions ( $t(13)=-6.07, p<0.001, d=-1.62$ ), with a smaller value for the appetitive condition. The assessment of correlations between start-stop, start-spread and stop-spread is reported in supplementary table 1. As in Tallot et al., 2016, in the aversive condition, positive correlations were found between start and stop ( $r=0.32, p<0.0001$ ), and between stop and spread ( $r=0.32, p<0.0001$ ), and a negative correlation was found between start and spread ( $r=-0.66, p<0.0001$ ). In the appetitive condition, a positive correlation was found between start and stop ( $r=0.54, p<0.0001$ ), and a negative correlation between start and spread ( $r=-0.73, p<0.0001$ ) as in Brunner et al., 1997. No correlation was

found between stop and spread ( $r=0.04$ ,  $p=0.35$ ). The analysis of the interquartile range indicated that the start time was significantly more variable between trials in the appetitive condition than in the aversive one ( $t(13)=4.38$ ,  $p<0.001$ ,  $d=1.17$ ), but that the variability did not differ significantly between conditions for the stop time ( $t(13)=-0.84$ ,  $p=0.42$ ) and the spread ( $t(13)=-0.9$ ,  $p=0.39$ ) (Fig.2D).

A



B

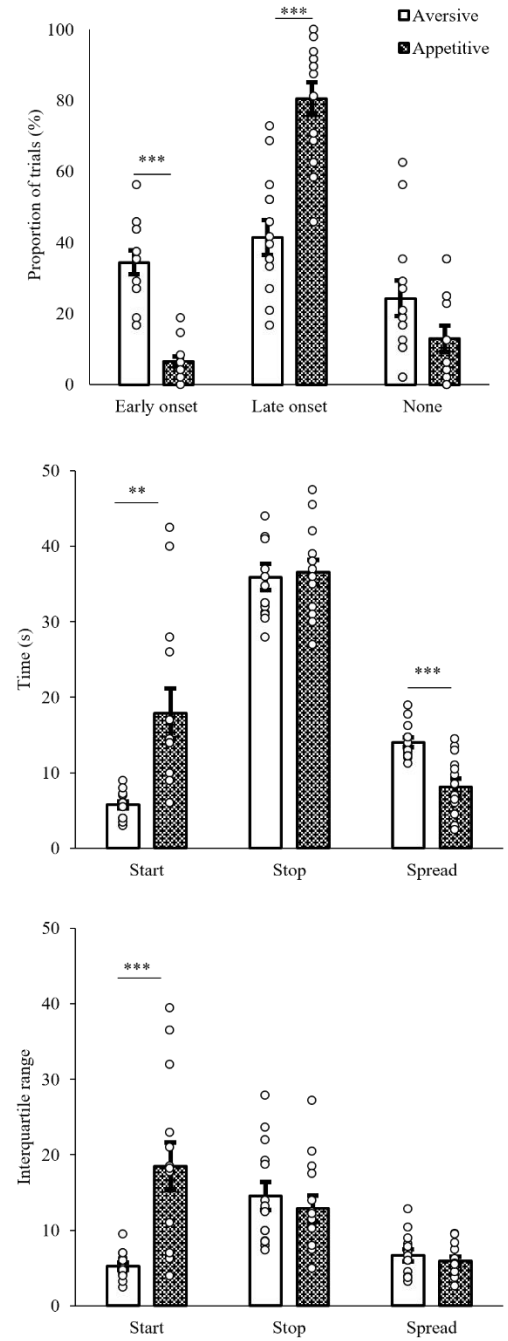




Figure 2: Characterization of the temporal behavior through individual trial analysis in aversive and appetitive conditions.

(A) Top, examples of raster plot representing all Probe trials across time for one rat in both conditions. In the aversive condition, each bar represents one lever press. In the appetitive condition, each bar represents a moment in the chocolate pellet magazine. The large vertical black line represents the CS onset. Below, representation of one trial in both conditions to illustrate the start and stop moments. Bottom, representations of the mean ( $\pm$  SEM) of lever pressing (in aversive) or head-in duration (in appetitive) when realigned to the start and the stop time ( $t=0$ ). (B) Proportions of the 3 categories of trials ('Early onset', 'Late onset' or 'None') for each condition with mean ( $\pm$ SEM) and individual rat data (each dot represents one rat). (C) Mean ( $\pm$  SEM) and individual median data of start, stop, and spread times. (D) Mean ( $\pm$  SEM) and individual median data of interquartile range of each measure. \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

### 3.2. Characterization of behavior during Gap trials

As expected, in both conditions, the introduction of a gap during the CS provoked a shift in temporal behavior to the right, the amount of which depending on the position and the duration of the gap (Fig.3A). Peak time shift magnitude was calculated as the difference between the peak time during Gap trials and the peak time during Probe trials (Fig.3B). A two-way repeated measures ANOVA showed no significant effect of condition ( $F(1,13)=4.31, p=0.058, \eta_p^2=0.249$ ), a significant effect of Gap type ( $F(2,26)=19.74, p<0.001, \eta_p^2=0.60$ ) and a significant condition x Gap type interaction ( $F(1.27, 16.6)=12.08, p=0.002, \eta_p^2=0.49$ ). A Bonferroni multiple post-hoc comparison indicated a significant difference between conditions for the ‘Early’ gap ( $p=0.025$ ), with a bigger shift to the right in the appetitive condition. No significant difference was obtained between the two conditions for the ‘Late’ gap ( $p=0.665$ ) or the ‘Long’ gap ( $p=1.00$ ). A calculation of ratios for the width of curves (width gap/width probe) (supplementary figure 2) and for peak rate (peak rate gap/peak rate probe) (supplementary figure 3) did not reveal any significant interaction between condition x Gap type ( $F(2,26)=2.49, p=0.103, \eta_p^2=0.16$  for width ratio and  $F(1.27, 16.47)=0.18, p=0.730, \eta_p^2=0.014$  for peak rate ratio).

We tested which rule the behavior may have followed during the Gap trials, and whether the gap produced a differential effect depending on the position and length of the gap during the CS. In the aversive condition (Fig. 3C), A repeated measures ANOVA indicated a ‘Gap type’ effect ( $F(2,26)=40.15, p<0.001, \eta_p^2=0.755$ ). The Bonferroni multiple post-hoc comparison indicated significant differences between all pairs of comparisons, showing that the peak time of the ‘Long’ gap was greater than that of the ‘Late’ gap ( $p<0.001$ ), which was greater than that of the ‘Early’ gap ( $p=0.004$ ). These results point to a graded effect of the gap on the temporal behavior depending on the position and duration of the gap. When contrasting the peak time in each condition against a predicted peak time under ‘Run’, ‘Stop’ or ‘Reset’ modes, we found that rats adopted a behavior between ‘Run’ and ‘Stop’ when a gap was inserted ‘Early’ in the CS,

whereas a 'Late' gap induced a 'Stop' mode behavior. Finally, a 'Long' gap induced a behavior between 'Run' and 'Stop' (see table 2 for statistical results).

In the appetitive condition, a repeated measures ANOVA indicated no significant Gap type effect ( $F(2,26)=1.670$ ,  $p=0.208$ ,  $\eta_p^2=0.114$ ), indicating no graded effect. Analyzing the strategy used, 'Early' and 'Late' gaps induced a 'Stop' mode behavior, whereas a 'Long' gap induced a behavior between 'Run' and 'Stop' (see table 3 for statistical results).

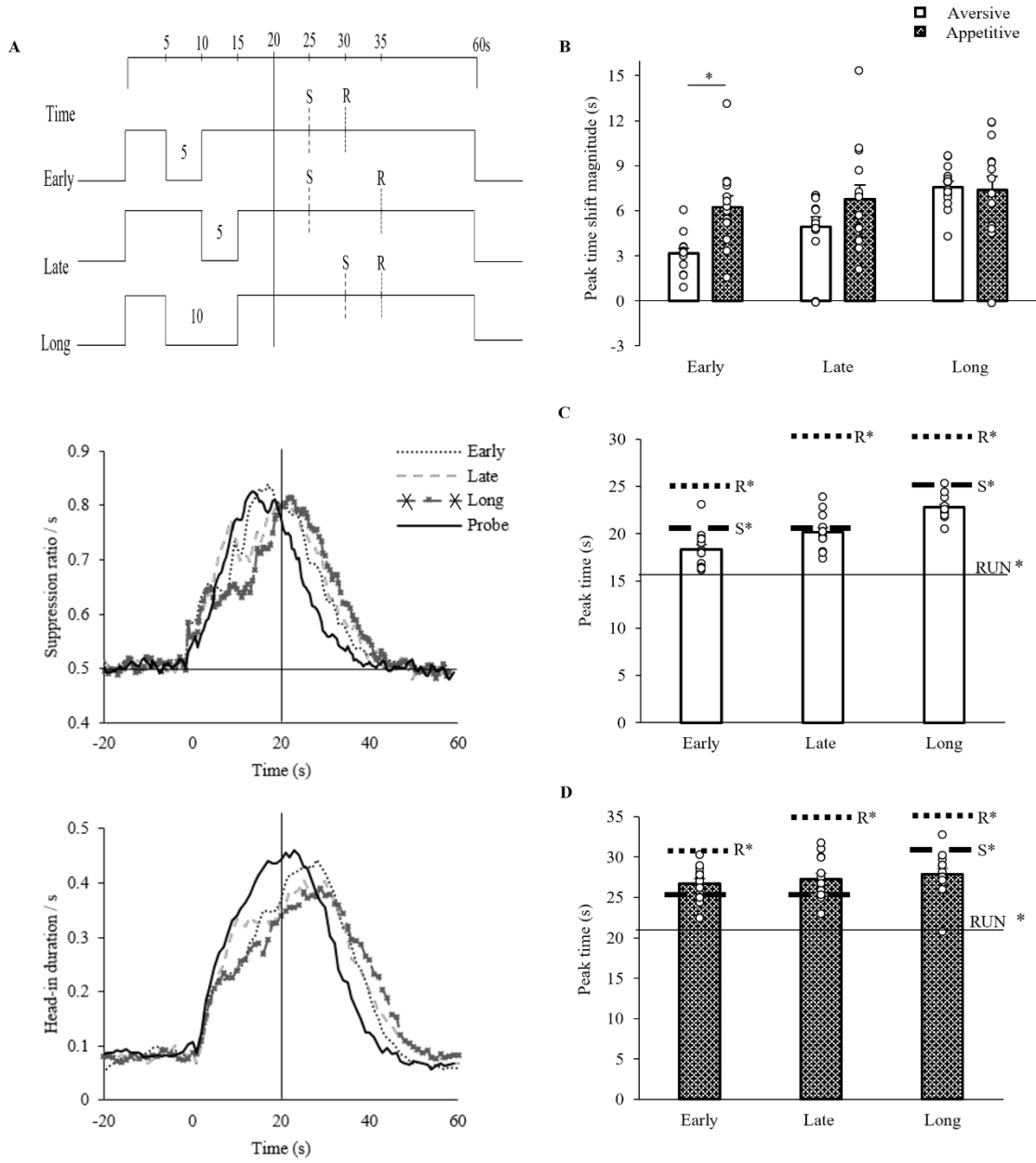


Figure 3: Characterization of the temporal behavior during Gap trials.

(A) Top, diagram of the GAP protocol. The first line represents a time scale of the 60-s CS. For each Gap paradigm ('Early', 'Late' and 'Long'), the gap in the CS is represented with its duration. The vertical solid line indicates the CS-US interval value, 20s (i.e., 'RUN' behavior). In each GAP paradigm, times corresponding to 'Stop' (S) and 'Reset' (R) behaviors are indicated by dashed and dotted lines, respectively. Below, representation of the averaged curves for all rats of suppression ratio per second in the aversive condition and the head-in duration in chocolate pellet magazine per second in the appetitive condition, during the Probe trials and three types ('Early', 'Late', 'Long') of Gap trials. The black vertical line represents the FI value. (B) Mean ( $\pm$  SEM) and individual rat data (each dot represents one rat) peak time shift magnitude in seconds for Gap trials as compared to Probe trials for both conditions. (C) Mean ( $\pm$  SEM) and individual rat data (each dot represents one rat) peak time value for the Gap trials in the aversive condition. (D) Same as (C) for the appetitive condition. For both conditions, Run\* represents a significant difference from the 'Run' behavior, S\* represents a significant difference from the 'Stop' behavior mode, and R\* a significant difference from the 'Reset' behavior. \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

### 3. Discussion

In this experiment, we have shown that temporal behavior patterns differ between aversive and appetitive conditions. A premature peak time was observed in the aversive condition, an anticipatory effect that was under temporal control as the Weber fraction was similar to the one obtained in the appetitive condition. Individual trial analysis, through the three-state pattern of behavior, showed a difference for the start time, both in its median value and inter-trial variability, between the two conditions. However, despite an earlier start time response in the aversive condition, the stop time was similar to the one in the appetitive condition. The insertion of gaps induced, as expected, a shift to the right for the response curve in both conditions. However, the effect of the gap differed depending on the condition, the position, and the length of the gap, with a graded effect observed only in the aversive condition, indicating a potential difference in the working memory functioning.

The anticipatory peak time observed here in the aversive condition confirms previous results using various behavioral measures (Drew et al., 2005; Meck and MacDonald., 2007; Tallot et al., 2016). This result raises the question of the process implicated in the premature expectancy in the aversive condition. A previous report mentioned an anticipatory peak-time in an appetitive condition where two instrumental timing tasks were superimposed (Meck and MacDonald, 2007). These authors hypothesized an earlier return to the baseline task as soon as the criterion was passed, resulting in the observed anticipatory result. In contrast, when a single task is used in appetitive situations, peak time is typically close to the FI value, whether in a Pavlovian (our present results; Kirkpatrick and Church, 2000; Tam et al., 2013; Tam and Bonardi, 2012b) or instrumental (e.g., Meck, 1984) tasks. However, an anticipatory peak time was also observed in a simple Pavlovian aversive task in goldfish (Drew et al., 2005). Furthermore, the quality of the CS, such as its frequency or intensity, is known to influence the conditioned response, including its temporal characteristics, in particular at CS onset (Zielinski and Walasek, 1977; Boulanger-Bertolus et al, 2015). Our analyses comparing both appetitive and aversive conditions in the same animal permitted the demonstration that the difference observed in the temporal behavior was accompanied by an earlier start time in the

aversive condition, with no difference in stop time. Thus, the anticipatory peak time in the aversive condition in our study was unlikely due to a sooner return to baseline in the double-task situation, but more likely related to the aversiveness of the situation. Boulanger-Bertolus et al. (2015) suggested that temporal behavior in an aversive conditioning may reflect the sum of two response patterns, one related to the onset of a CS with acquired aversiveness, and the other related to temporal conditioning, the combination of which results in a premature peak time. Our individual trial analysis here revealed that the proportion of ‘Late onset’ trials in the aversive condition - similar to that previously reported (Tallot et al. 2016) - was lower (41%) than in the appetitive condition for which approximately 80% of trials were in the ‘Late onset’ category. Nevertheless, the shapes of the mean curves followed a temporal pattern for all three categories in the aversive condition, whereas only the trials in the ‘Late onset’ category showed temporal control in the appetitive condition (supplementary figure 1). These observations concur with the dual-process hypothesis applicable to an aversive cue, and consistent with the variable response pattern observed under that condition, while a single temporal process dominates behavior in the appetitive condition.

Another difference between aversive and appetitive conditions was observed during the Gap procedure. While in both conditions all gaps induced a shift to the right compared to behavior during Probe trials, the magnitude of the shift differed depending on the duration and location of the gap in the aversive condition only, with a larger shift after a longer gap and a later gap as previously reported in a parametric appetitive instrumental study (Cabeza de Vaca et al., 1994). The lack of a graded effect in the present appetitive condition is in accordance with Orduña et al's (2008) experiment, but in opposition to Tam et al.'s (2013) observations. Some authors (Buhusi et al., 2005; Zentall and Kaiser, 2005) have proposed that the magnitude of the gap effect may depend on the relative salience of the gap, that is, the more intense the stimulus is, the more salient the gap would be, and the greater the memory decay of the pre-CS duration would be. Such a hypothesis may explain discrepant results in the literature, as well as between conditions in our experiment. Although CS physical characteristics were the same in both conditions, the emotionally acquired salience value through conditioning likely differed, with a weaker value in the appetitive than in the aversive condition, to the extent that little loss of memory of the pre-gap duration was induced in the appetitive case

whatever the gap properties. As the present study utilized a within subject manipulation of US, a contrast effect may have further reduced the salience in the appetitive condition relative to the aversive condition.

Nevertheless, even though the existence of a graded effect in our aversive condition is suggestive of a memory decay mechanism during the gap, the observed behavior never followed a 'Reset' rule, nor even fell between 'Stop' and 'Reset', in contrast to the literature (Buhusi, 2003; Buhusi et al., 2005; Cabeza de Vaca et al., 1994; Meck, 1984). As previously proposed by Buhusi et al (2006), one parameter that may control the observed behavior is the proportion of time the gap takes with respect to the FI (Buhusi et al., 2006). A previous report showed that a gap of 1/5 of the FI duration induced a 'Stop' behavior in a Pavlovian aversive conditioning in rats (Tallot et al., 2016). In the present study, although the longest gap was larger in proportion (1/3 of FI duration), we did not observe a behavior shift beyond 'Stop'. An alternative explanation relates to the sensory property of the to-be-timed stimulus, as suggested by Orduña and Bouzas (2011) who reported a 'Stop' behavior for gaps introduced during an auditory stimulus, but a 'Reset' behavior for gaps introduced during a visual stimulus. According to these authors, hearing being a fundamental modality for rats, it is possible that the memory decay for this modality could be weaker than for other modalities (Orduña and Bouzas, 2011). However, an important observation is the behavior between 'Run' and 'Stop' that we consistently observed in 'Early' and 'Long' conditions. According to the SET model (Gibbon, 1977), the gap effect can be influenced by the switch latency, which controls the number of clock pulses transferred to the accumulator (Church, 1984; Maricq et al., 1981; Meck, 1983). Different factors, such as arousal or emotional value, can influence switch latency (Lejeune, 1998; Zakay, 2000; Zakay and Block, 1995, 1996). To account for a behavior closer to 'Run' than to 'Stop', one may hypothesize that latency may have been longer in opening the switch at the onset of the gap than in closing the switch at the offset of the gap, thus reducing the absolute duration of the gap at the functional level. The emotional value acquired by the CS, likely higher than the one triggered by the gap, would then produce a difference between switch latencies. Thus, a combination of two sources of influence, a difference in switch opening/closing latencies and memory decay during the gap, may have resulted in the observed behavior in the aversive condition.



In sum, the present results reveal several differences in timing between aversive and appetitive conditions. First, the effects of gap insertion point to potential differences in switch opening/closing latencies and in the rate of temporal working memory decay during the gap. Second, peak time was earlier in the aversive condition, which may reflect multiple factors contributing to behavior in that condition. Finally, start times, but not stop times, differed between conditions, suggesting that the former timing mechanisms may rely on separate brain networks, whereas a common network may sustain the latter whatever the context.

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