Individual trial analysis evidences clock and non-clock based conditioned suppression behaviors in rats

Lucille Tallot [a,*], Daphné Capela [a], Bruce L. Brown [b], Valérie Doyère [a,*]

[a] Univ Paris-Saclay, Univ Paris-Sud, CNRS, Institut des Neurosciences Paris-Saclay (Neuro PSI), UMR 9197 Orsay, France
[b] Department of Psychology, Queens College and the Graduate Center, The City University of New York, Flushing, NY 11367, USA

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ABSTRACT

We analyzed the temporal pattern of conditioned suppression of lever-pressing for food in rats conditioned with tone-shock pairings using either a 10 or 15 s conditioned stimulus (CS)-unconditioned stimulus (US) interval with a CS duration that was three times the CS-US interval. The analysis of average suppression and of individual trials was performed during Probe CS-alone trials and when a short gap was inserted during the CS. The pattern of suppression followed the classical temporal rules: (1) scalar property, (2) a shift in peak suppression due to a gap, compatible with a Stop rule, (3) a three-state pattern of lever-pressing in individual trials, with abrupt start and stop of suppression. The peak of the average suppression curve, but not the middle time, was anticipatory to the programmed US time. The pattern of lever-pressing in individual trials unraveled two types of start of suppression behavior: a clock-based biphasic responding, with a burst of lever-pressing before suppression, and a non-clock based monophasic reduction of lever-pressing close to the CS onset. The non-clock based type of behavior may be responsible for the anticipatory peak time, and the biphasic pattern of lever-pressing may reflect the decision stage described in clock models.

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1. Introduction

Interval timing, the capacity to estimate time intervals in the range of seconds to minutes, is critical in everyday life, in particular to prepare for action in a most efficient manner. For example, being capable of estimating the time between the lightning and the thunderclap will help you decide whether you have time to run back home or should protect yourself on site, decisions that may save your life in extreme situations. In Pavlovian aversive conditioning, the laboratory experimental equivalent, the subject not only learns that a conditioned stimulus (CS) predicts the arrival of an unpleasant noxious stimulus (unconditioned stimulus, US), but also when the US is due to arrive. Although the CS-US interval is learned very rapidly, the emergence of a temporally organized behavior related to the expectancy of US arrival may take tens to hundreds of pairings, depending on the behavioral index analyzed (Balsam et al., 2002; Bevins and Ayres, 1995; Davis et al., 1989; Díaz-Mataix et al., 2013; Drew et al., 2005; Shionoya et al., 2013).

The question of the neurobiological bases of timing remains. While the neurobiology of Pavlovian aversive conditioning has been very well described over the years from a cellular to a network level (Herry and Johansen, 2014; LeDoux, 2014), the study of timing characteristics has mostly been limited to instrumental appetitive conditioning. As the neural circuitry underlying instrumental appetitive conditioning differs from the one involved in Pavlovian aversive conditioning (Herry and Johansen, 2014; Hollerman et al., 2000; LeDoux, 2014), one may wonder whether temporally modulated behavior may also differ depending on the type of task used. Alternatively, if timing is subserved by the same neuronal circuit whatever the task, we would expect no such differences. Therefore, differences in some aspects of temporal behavior depending on the type of task used could inform us on the possible existence of a single internal clock.

The study of interval timing in a Pavlovian aversive task has been sparse. LaBarbera and Church (1974), using a conditioned suppression paradigm (Estes and Skinner, 1941) in which foot-shock USs were given at regular intervals while rats were lever-pressing for food, showed that well-trained animals suppressed their lever-pressing following a temporal pattern that resembled the one seen in typical fixed interval (FI) instrumental appetitive tasks (Dews, 1970; Schneider, 1969). In other studies, when a foot-shock US was delivered at a fixed time after the onset of a CS and non-

reinforced probe trials were interleaved as in a peak interval (PI) paradigm, the pattern of suppression followed the typical Gaussian shape (Boulanger-Bertolus et al., 2015; Meck and MacDonald, 2007). Davis et al. (1989) also reconstructed this pattern in a potentiated startle preparation. Finally, Balsam et al. (2002) reported the expected bell-shaped curve of conditioned activity to an electrical shock in a Pavlovian preparation with goldfish. In rat studies, it was also observed that the temporal patterns conformed to the scalar property (e.g. temporal precision is proportional to the timed interval) as suppression curves for different intervals superimposed well when rescaled on a normalized time axis (Boulanger-Bertolus et al., 2015; LaBarbera and Church, 1974; Meck and MacDonald, 2007). These data suggest that the processes underlying temporal control of behavior in Pavlovian aversive conditioning may be the same as those in instrumental appetitive tasks. However, the time of maximal average suppression in rats was earlier than the programmed time of US arrival, suggesting that the peak of expectancy for the US was anticipated (Boulanger-Bertolus et al., 2015; Meck and MacDonald, 2007). This anticipation contrasts with the results classically reported in the instrumental appetitive PI task, for which the peak time falls in the temporal vicinity of the programmed reinforcement time or slightly after (Aum et al., 2007, 2004; Buhusi and Meck, 2006; Roberts et al., 1989). In our study, we aimed at exploring the question of whether (1) the mean suppression curve reflects a single temporally controlled behavior, which could be anticipatory because of the nature of the task, or (2) several behaviors may be at play, so that the envelope of the mean suppression curve peaks at an earlier time.

Since Gibbon and Church (1990) report, it is well established that rats' behavior, trained in an appetitive instrumental PI task, follows a binary response pattern on individual trials: stable rates of responding transition from a low level of responding to a high level and back to a low level with no intermediate rates (e.g. Aum et al., 2004; Balci et al., 2009; Church et al., 1994; MacDonald et al., 2012; Matell et al., 2006; Matthews et al., 2012). The times of state changes are called start and stop times, respectively, and are under the control of putative decision thresholds as incorporated in Scalar Expectancy Theory (SET, Church et al., 1994). The SET model is one of the foremost internal clock models of the past twenty years of timing research. It is based on the presence of a pacemaker that produces pulses that are accumulated across the duration of a salient event in an accumulator. The accumulated durations are saved in memory to be later compared to currently measured intervals. As time elapses, if the contents of both accumulator and memory are sufficiently similar (above a set threshold) a decision to start responding is made, and then, when they become sufficiently dissimilar, a decision to stop responding is made. The recent literature has highlighted the importance of analyzing start and stop behaviors, as they may be independently manipulated, and may thus be more informative than the molar measure of peak time based on mean response rate functions (Balci et al., 2009; MacDonald et al., 2012; Matell et al., 2006; Matthews et al., 2012; Taylor et al., 2007). Whether the same type of start and stop behavior underlies the mean temporal bell-shaped curve of conditioned suppression is not known.

Interval timing processes in instrumental tasks have also often been analyzed using a gap procedure, where the impact of introducing a brief interruption in the to-be-timed stimulus is studied to enable assessment of underlying clock mechanisms. When a gap is added in a PI task, depending on the length of the gap and its position in the to-be-timed stimulus, the response of the animal is shifted in time (Cabeza de Vaca et al., 1994; Meck and Church, 1987; Roberts and Church, 1978; Roberts, 1981; Roberts et al., 1989; Swearingen and Buhusi, 2010). Three timing modes have been inferred: Run, Stop or Reset. In the Run mode, the clock continues to time during the gap, so there is no temporal shift in behavior. In

Stop, the clock does not time during the gap but memory is retained of the time elapsed before the gap, so behavior is shifted by the duration of the gap. And finally, in Reset, the gap returns the clock to zero, and timing starts anew after the gap, i.e. from the second onset of the to-be-timed stimulus (Buhusi et al., 2006; Kaiser et al., 2002; Roberts and Church, 1978; Roberts, 1981). Gap trials have received a good deal of attention in interval timing research but studies have been limited to appetitive instrumental paradigms (Cabeza de Vaca et al., 1994; Meck and Church, 1987; Orduña et al., 2008; Roberts and Church, 1978; Roberts, 1981; Roberts et al., 1989). In Pavlovian aversive tasks, the effect of a gap has been mainly studied through the use of trace fear conditioning, in which a gap is inserted between the CS offset and the US. The decrement in the conditioned response produced by that type of gap is well known, but little attention has been paid to temporal control. The impact of a gap interrupting the CS temporarily on CS-US interval processing has never been assessed, and it is thus not known whether in well-trained animals under Pavlovian aversive conditioning it would interrupt the timing of CS-US interval, and produce a Stop or Reset type of behavior as in instrumental tasks.

In the present study, we used a conditioned suppression paradigm with auditory fear conditioning in rats to assess the timing processes underlying temporal expectancy of the US. In those well-trained animals, we looked at the temporal pattern of the mean response rate function as well as individual trial behavior, and assessed the effects of a gap during the CS, while comparing two CS-US intervals.

2. Materials & methods

2.1. Subjects

Behavioral experiments were carried out on 20 adult male Sprague-Dawley rats (Harlan Laboratories, France) in accordance with the guidelines of the European Community Council Directives of September 22nd 2010 (2010/63/UE) and the French National Committee (2013/118) for the care and use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering. Rats were housed in standard laboratory cages five by five and maintained on a 12/12hr light/dark cycle. Rats were weighted daily (initial weight of approximately 300–350 g) and reduced at 85–90% of their normal weight for the whole duration of the experiment. Training was run six days a week.

2.2. Apparatus and stimuli

Training took place in a set of four identical conditioning chambers (30 × 25 × 30 cm, Coulbourn Instruments, USA), equipped with a shock floor, a speaker, a lever and a food magazine that dispensed 45 mg grain-based pellets (BioServ), and placed in sound attenuating enclosures with a ventilation fan (60 dB background noise). Behavioral protocols were controlled by Graphic State software (Coulbourn Instruments, USA).

2.3. Conditioned suppression training and gap testing

2.3.1. Instrumental training (9 sessions)

Following one day of magazine training (30 pellets were presented at random intervals), a lever press response for food was shaped in one or two sessions on a continuous reinforcement schedule where each lever press produced the delivery of one pellet as a reward. When a criterion of 60 lever presses in 30 min or less was met, a partial reinforcement schedule was added for seven sessions with a variable interval of 30.5 s (VI, 1–60 s range). The rats