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# The effect of recording interval length on behavioral assessment using the forced swimming test



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### KEYWORDS

Forced swimming test;  
Interval length;  
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Rats

**Abstract** The forced swimming test is a method used in the assessment of depressive-like behavior in rodents. Changes in the original forced swimming test procedure developed by Porsolt et al. and their influence in the results is a controversial issue and has been discussed in many studies. Animal's behavior is usually recorded by partial interval recording, dividing the total recording time into equal intervals and manually recording the predominant behavior. Despite the influence of the recording method in the subsequent results, this issue has not been further studied nor normalized. The aim of this study was to assess whether the representativeness of the data is influenced by the recording interval length, by recording behaviors (immobility, swim and climbing) in the same subjects at 3, 5 and 10 s recording intervals. We used a non-pathological sample of male and female adult Wistar rats. Our results show no differences in the use of these three recording intervals in the registration method of the forced swimming test, for the main three behaviors measured.

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### PALABRAS CLAVE

Test de natación forzada;  
Longitud del intervalo;  
Método de registro;  
Ratas

### El efecto de la longitud del intervalo de registro en la evaluación conductual mediante el test de natación forzada

**Resumen** El test de natación forzada es un método utilizado para evaluar el comportamiento depresivo en roedores. Las modificaciones del procedimiento del test de natación forzada desarrollado por Porsolt y su influencia en sus resultados es un tema que suscita controversia y ha sido analizado en numerosos estudios. El comportamiento del animal se analiza generalmente mediante el registro de intervalos parciales, dividiendo el tiempo total de grabación en intervalos iguales y registrando manualmente la conducta predominante durante ese tiempo. A pesar

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de la influencia del método de registro en los resultados posteriores, esta cuestión no se ha analizado ni normalizado. El objetivo de este estudio fue comprobar si la representatividad de los datos se ve afectada por la longitud del intervalo de observación, registrando a 3, 5 y 10 seg la conducta (inmovilidad, natación y escalada) de los mismos sujetos. Se utilizó una muestra no patológica de ratas Wistar macho y hembra adultas. Los resultados mostraron que no existen diferencias significativas entre estos intervalos analizados en la prueba de natación forzada para las 3 principales conductas registradas.

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## Introduction

The forced swimming test (FST) is a method used in the assessment of depressive-like behavior in rodents that was developed by Porsolt, Bertin, and Jalfre (1977). It is based on learned helplessness, as the animal is repeatedly exposed to an aversive stimulus, water, which it cannot escape. When the animal is introduced in an inescapable cylinder filled with water, it learns that there is nothing it can do to escape and, therefore, it reduces the time of regular escape behaviors (swimming, climbing and diving) in subsequent trials and spends more time making no movements. In the animal model of depression, the total time of immobilization is higher than in control condition and it has been seen that antidepressants reduce the differences in total time of immobility between control and experimental groups (Porsolt, LePichon, & Jalfre, 1977).

An animal model of a particular psychological condition must meet certain criteria to be applicable to humans: it should resemble the human condition in its etiology, biochemistry, symptoms and treatment (McKinney & Bunney, 1969). As reviewed in the text of Belzung and Lemoine (2011), most authors have focused on these external validity criteria. In this review, five basic criteria of validity of an animal model, which differ slightly from the classical criteria, are proposed. These criteria are homological validity, pathogenic validity, mechanistic validity, face validity and predictive validity (Belzung & Lemoine, 2011). According to many of these criteria, major depressive disorder has been reproduced in animals in order to study the underlying neurobiological mechanisms. This model has been developed by replicating aspects of the depressive syndrome that are not intrinsically human features, i.e. anhedonia, helplessness and behavioral despair (Krishnan & Nestler, 2011).

The validity of the FST has been questioned many times. Several authors have compared the results of FST with other depression-measuring tests, such as sucrose preference test (Grillo et al., 2011; Hong et al., 2012; Karson, Demirtas, Bayramgürler, Balci, & Utkan, 2013), and have found consistent results. Nevertheless, there is controversy about the validity of FST to measure behavioral 'despair', as habituation has been proposed as an explanation to immobility (Hawkins, Hichs, Phillips, & Moore, 1978) due to a process of familiarization (Borsini, Volterra, & Meli, 1986). There are multiple variations on the original methodology of the FST that lead to differences in the results (Borsini & Meli,

1988). While most of the researchers record behaviors manually (Grillo et al., 2011; Kawai, Ishibashi, Kudo, Kawashima, & Mitsumoto, 2012; Sirianni, Olausson, Chiu, Taylor, & Saltzman, 2010; Ulloa, Díaz-Valderrama, Herrera-Pérez, León-Olea, & Martínez-Mota, 2014), others use automated devices (El-Alfy et al., 2010; Uz, Dimitrijevic, Imbesi, Manev, & Manev, 2008). Therefore, behavioral results used to be based on subjective recording.

Animal's behavior is usually recorded by partial interval recording (PIR), consisting on dividing the total recording time into equal intervals and manually recording the predominant behavior in each interval (Borsini & Meli, 1988). Some authors have also tried to automate the recording process by developing software that measures different mobility parameters, such as Ethovision 3.0 by Noldus (Hédou, Pryce, Di Iorio, Heidbreder, & Feldon, 2001) or CVA software by ProTrack (Gersner, Gordon-Kiwkowitz, & Zangen, 2009), although the preferential method is still the trained observer. In Fiske and Delmolino (2012), advantages and disadvantages of different recording methods are mentioned. Using PIR, some authors conclude that the smaller the interval, the lower the absolute and relative errors (Wirth, Slaven, & Taylor, 2014). Also, it has been observed that the length of the recording interval inversely affects the representativeness of data (Repp, Roberts, Slack, Repp, & Berkler, 1976). Finally, regarding recording methods, it has been shown that PIR method is a more sensitive recording method than momentary time sampling (MTS) (Harrop & Daniels, 1986).

We have found in the literature (summarized in Table 1) that many studies using FST do not include information in their methodological section about the recording method. Most of the few authors who describe the recording method usually record behavior at intervals of 5 s. There are exceptions such as Su, Hato-Yamada, Araki, and Yoshimura (2013), who quantify the duration and frequency of the behavior, and Grillo et al. (2011), who recorded at intervals of 3 s. Despite the influence of the recording method in the subsequent results, this issue has not been further studied nor normalized.

In the present study, we assessed the relevance of using a longer or shorter interval in the recording method of the FST. To do this, we compared the behavioral results of the FST in the same sample of animals at 3, 5 and 10 s recording intervals. We expected to find more accurate results in the shortest interval and a masking effect in the widest one.

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