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Original Article

A Modified Method for Objective Analysis of Forced Swim Test Using Student Physiograph

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Abstract

Background: Forced swim test (FST) is used for preliminary screening of antidepressant drugs. In this test, the antidepressants decrease the duration of immobile phase (despair). The immobility time obtained by observer may not be accurate either because he is not trained or due to bias. This is an attempt to make use of student physiograph to accurately measure the duration.

Aims and Objectives: To evaluate the utility of student physiograph as an objective tool to detect and record immobility time in forced swim test.

Materials and Methods: A total of 15 laboratory bred swiss albino mice of either sex weighing between 20-25 g were used for the study. A set up was made using Student physiograph, force transducer, student organ bath, bucket with holes at the bottom and thermocol block. In this set up, the movements of the mice which were placed inside the bucket with water produced turbulence which was transmitted to the outer jacket and recorded on the physiograph using force transducer and thermocol block. The mice were placed in the bucket containing water and the total duration of immobile phase was recorded. Next day, the same mice were used for the forced swim test but after administration of Amitriptyline 25 mg/kg i.p 30 minutes prior to the study. The duration of immobile phase was calculated separately by observer, by using the graph obtained by this set up and using the video clip by digital camera.

Results: The mean duration of immobility in naïve mice using student physiograph was found to be 193.8±3.09 sec which was close to the mean duration of immobility as obtained from video recording (i.e. 194.87±3.47 sec) but significantly different from mean obtained from observer data (i.e. 179.73±5.81 sec). Similarly, after Amitriptyline administration, the mean duration of immobility was decreased to 107±2.32 sec which was close to the mean obtained from video recording i.e. 108.47±2.81 sec but significantly different from mean obtained from observer data (i.e. 92.8±2.14 sec).

Conclusion: This modified method of forced swim test eliminates errors and bias in obtaining duration of immobile phase and also it is possible to keep the record for re-evaluation and future reference.

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Introduction

The first antidepressant drugs were detected by serendipity in clinical trials. Iproniazid was developed for the treatment of tuberculosis. The observation of mood-elevating effects was followed by the detection of the inhibition of the enzyme monoamine oxidase (1). Gone are those days where drugs used to be discovered accidentally or by simple hypothesis. Now, a detailed in vitro and in vivo investigation (part of preclinical studies) is necessary for drug discovery. Forced swim test (FST) also called as despair swim test was proposed as a model to test for antidepressant activity by porsolt et al in 1977 (2). This test is for initial screening before going for more complex preclinical tests and clinical evaluation of antidepressant drugs. The mice, which were forced to swim in a restricted space from which they cannot escape, are highly active, vigorously swimming in circles and trying to climb the wall. Later it remains immobile. Immobility is defined as when no additional activity is observed other than that required to keep the rat's head above the water. The immobility phase is then measured in during this 5 min test period and has been found to be reproducible in different groups. This immobility phase reflects a state of despair which can be reduced by several antidepressants (3).

The main advantages of this procedure are that it is relatively easy to perform and that its results are easily and quickly analyzed. But, the main drawback or limitation is observer's bias and record keeping. The observer might not be able to differentiate between the mobile and immobile phase or may become biased for obtaining good results. And the researcher at a later stage cannot go back and re-evaluate/measure the immobile phase in that particular animal. So, to overcome these limitations many modifications of this method were proposed. One such modification was 'open space swimming test' by Sun and Alkon et al (4). Rats were free to swim (or not to swim) for 15 min and then returned to their home cage. The same procedure was followed 24 h later for 3 days. The swimming/drifting path was recorded with a video tracking system. The measurement is considered to be more objective than the forced swimming test. Buckett et al. (5) described an automated apparatus for behavioral testing of typical and atypical antidepressants in mice. A multichannel system can test 10 mice simultaneously. Each mouse is placed in the beam of a Doppler radar head and horn assembly. The moving mouse causes reflections of a frequency differing from the transmitted signal. Within the Doppler head these reflected waves are mixed with a proportion of transmitted waves to produce a difference signal proportional to the activity of the mouse within the beam. The output of each Doppler head is fed to an amplifier whose gain has been calibrated to compensate for differences in sensitivity between individual heads. The method is claimed to eliminate human error and bias and to allow the testing of large numbers of compounds. Nomura et al. (6) published a modification of the despair swim test in mice involving a small water wheel set in a water tank. Mice placed on this apparatus turned the wheel vigorously but, when they abandoned attempts to escape from the water, the wheel stopped turning. The number of rotations of the water wheel was counted.

All the above modifications require complex instruments and above that cost is a major constraint. So we have made an attempt to develop a modified set up by which objectivity and sensitivity of forced swim test can be achieved. For this purpose, we have used physiograph which is available in all medical colleges (7). The aim of the following experiment is 1) To see whether student physiograph can be used to detect immobility time in forced swim test (FST). 2) To compare the duration of immobility as assessed by observer, by using physiograph and by using digital camera.

Material and Methods

Animals:

laboratory bred swiss albino mice of both sex weighing around 20-25 g were used for the study. The animals were housed under standard laboratory conditions at 25°C, commercial pellet diet with water ad libitum and normal photo period (12 hr dark/12 hr light). Experimental protocol has been approved by the Institutional Animal Ethics Committee (IAEC).

Instruments:

Student physiograph, Force transducer, Student organ bath, Plastic bucket with holes at the bottom, thermocol, Stop watch (Racer), digital camera (Sony DSC-W830).

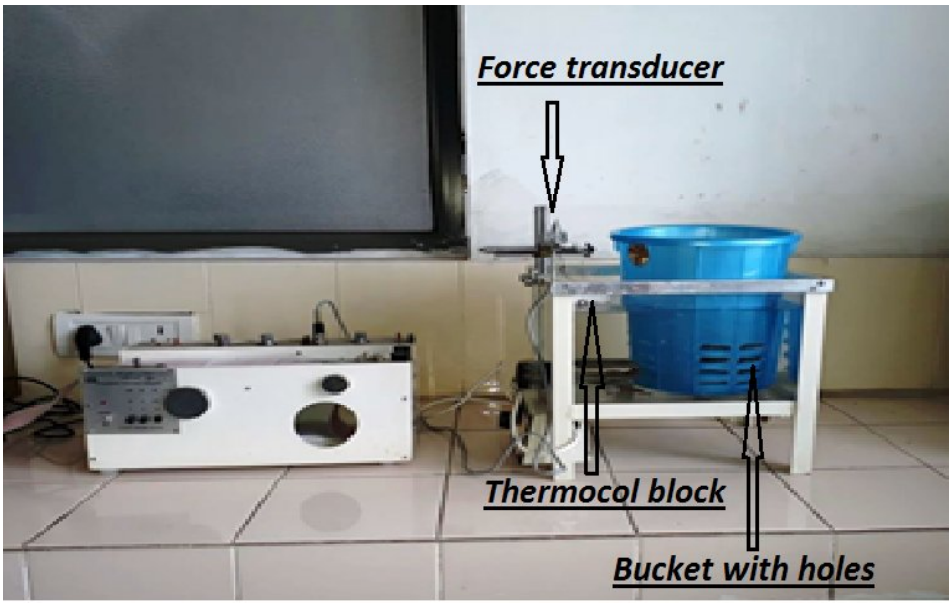
Drugs:

Amitriptyline 25 mg/kg

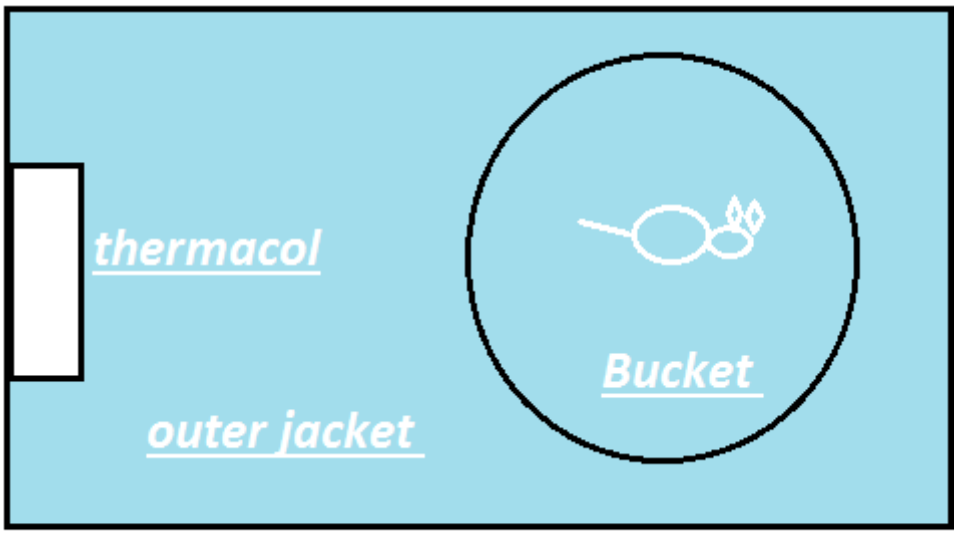
Experimental design and methodology:

In this method, fifteen mice weighing 20-25 g were used. They were brought to the laboratory one day before the experiment and kept in separate cages free access to food and water ad libitum. Naive mice were individually forced to swim inside a vertical bucket (height: 40 cm; diameter: 18 cm, containing 15 cm of water maintained at 25°C). In this period of time mobile phase and immobile phase were recorded separately using stop watch. Next day, a modified set up was made by making holes at the bottom in the bucket and kept inside the student organ bath with water as shown in picture 1A and 1B.

We used student organ bath for keeping bucket.



Picture 1A: A modified model for objective analysis of forced swim test using student physiograph.



Picture 1B: A schematic top view of the modified model for objective analysis of forced swim test using student physiograph.

Outer part of student organ bath is known as water bath or outer jacket (It is made up of steel, glass or Perspex (transparent thermoplastic resin). It holds water and other parts of organ bath. The outer jacket and bucket are connected through the holes at the bottom of bucket. Now, the mice were individually placed inside the bucket. The movement of mice created some turbulence in water. This turbulence was carried to outer jacket through holes at the bottom of bucket and it created waves in water of outer jacket. Mice were observed for 5 minutes. These waves were recorded with the help of force transducer and student physiograph which is connected to thermocol block floating in outer jacket. Simultaneously the movements of the mice were also recorded by using a digital camera. The duration of and immobile phase was calculated separately by observer, using the graph obtained by this set upand using the video clip by digital camera. Next day, the experiment was repeated using same mice but after prior administration of 25 mg/kg of Amitriptyline i.p half an hour before the test.

Statistical analysis:

The statistical analysis among the groups, I (Observation by volunteer), II (Student physiograph) and III (Video recording) was done using unpaired student t test. P value less than 0.05 was considered to be significant.

Results and Discussion

In this method, the duration of immobile phase in the 5 minutes forced swim test was measured using the recording from our modified set up. The sample of the recording is shown in picture 2.

The total length of graph paper was 30 cm (300 mm) the speed was kept at 1 mm/sec so that the whole length of graph paper would pass under stylus in 5 minutes (which was equal to the observation period of FST). So, while measuring the mobile and immobile phase, the length to time conversion factor would be 1 mm = 1 second. Simultaneously, duration of immobile phase in mice before and after Amitriptyline administration as obtained from observer's data and by video recording was also noted. The results are as shown in Table I and II.

We collected data (Immobility phase in seconds)

TABLE I : Immobility time in naïve mice in forced swim test by different methods.

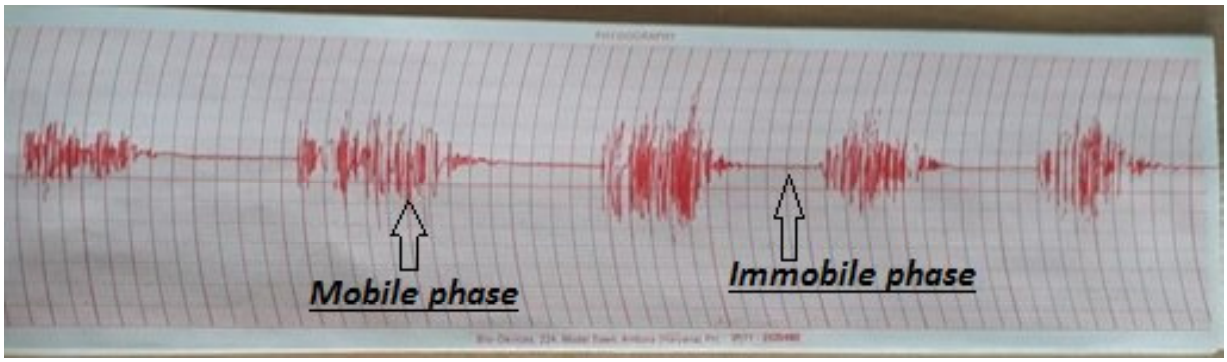
	Observation by volunteer	*Student physiograph*	*Video recording*
Mean	179.73	193.8	194.87
SD	5.81	3.09	3.47

n=15, All values are in seconds.

TABLE II : Immobility time in Amitriptyline treated mice in forced swim test by different methods

	Observation by volunteer	*Student physiograph*	*Video recording*
Mean	92.8	107	108.47
SD	2.14	2.32	2.81

n=15, All values are in seconds.



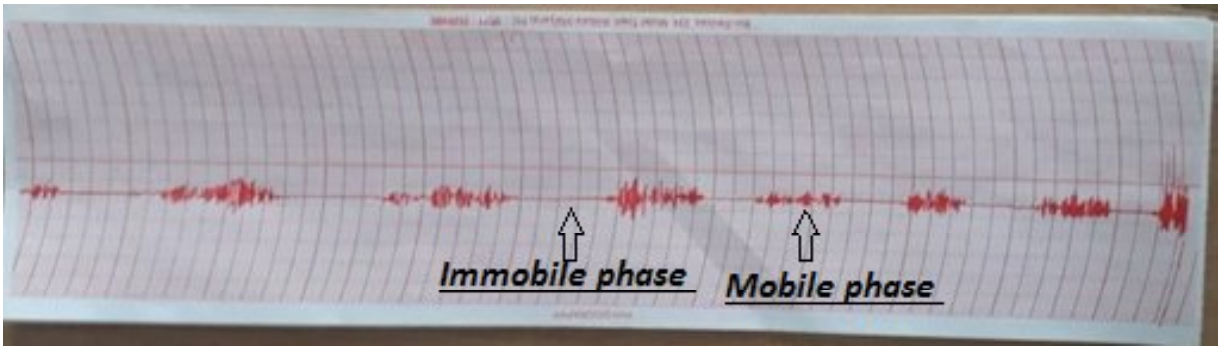
Picture 2 : Sample of recording from a modified method using studentphysiograph (sensitivity at 200 mVolt).

from three different sources namely observer, from graph and from video recording. The mean duration of immobility in naïve mice from graph was 193.8±3.09 sec which was close to the mean duration of immobility as obtained from video recording i.e. 194.87±3.47 sec. The mean duration of immobility as obtained from observer's data was 179.73±5.81 sec which was significantly different from data collected using student physiograph ($P<0.05$) and data obtained from video recording ($P<0.05$) respectively. The mean duration of immobility in the same mice was decreased after administration of 25 mg/kg of Amitriptyline i.p half an hour before the test. The mean duration of immobility measured by using graph was found to be 107±2.32 sec which was close to the mean obtained from video recording i.e. 108.47±2.81 sec. But, the mean duration of immobility as obtained from observer's data was just 92.8±2.14 sec which was significantly different from data collected using student physiograph ($P<0.05$) and data obtained from video recording ($P<0.05$) respectively. Clearly, this shows that the mean duration of immobility was obviously less when measured by live observer. The reason behind this might be error in detecting immobility by observer as he is going to observe the animal only once. With video recording, the immobility time can be measured by different observers by repeated observation and with student physiograph also different observers can go through the graph repeatedly so as to get accurate readings.

The most important aspect of analysis of immobile phase and usually the biggest source of variability between observers in the FST is the correct identification of movements that are counted as valid movements (8). The standard definition for mobility in the FST is any movements other than those necessary to balance the body and keep the head above the water (9). Mice generally float in water readily, however they still manifest small movements to balance their bodies and keep their heads above the water. These behaviors are not an attempt to escape and should not be scored as mobility. Also, after a single bout of mobility, even though essentially immobile, mice can still drift in the water as a result of momentum. These movements also should not be scored as mobility. When we started recording responses; we got fluctuations even when the animal was immobile, creating confusion. This confusion between mobile and immobile phase can be totally reduced by changing the sensitivity of physiograph, where immobile phase (movements other than those necessary to balance the body and keep the head above the water – responsible for errors by observers) is recorded as straight line which helps to calculate correct duration of immobile phase as shown in picture 3.

Further, this forced swim test is a basic and preliminary method for evaluating the efficacy of antidepressant drugs (10, 11). The characteristics of the FST make it an important tool in academic research and drug discovery in industrial settings where reliability and high throughput screening of novel compounds are essential.

Our study indicated that either observer error or bias (Favoring the test towards positive result) or inter-



Picture 3: Recording from a modified method using student physiograph with reduced sensitivity (sensitivity at 2 mVolt).

observer variability was responsible for gross change in the mean duration of immobility measured. This may make a whole process of drug discovery erroneous. This error or bias can be easily eliminated by using this modified method. Also, the major advantage of this modified method is that we can re-evaluate and reconsider the readings by looking into these recorded graphs.

Recently, an advanced commercial automated behavior analysis systems have been introduced that can accelerate the data collection process. (12, 13 and 14) But, then these automated systems require extensive validation by human scoring. Additionally, automated parameters may have to be readjusted when using different strainsor with mice of different sizes or behavioral responses.

Conclusion

This modified method of forced swim test eliminates errors and bias in obtaining duration of immobile phase and also it is possible to keep the record for re-evaluation and future reference.

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