Circadian Mechanisms of Prefrontal Cortex Dependent Memory in Trace Fear Conditioning

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The following is an excerpt from a longer piece. For the full text, please visit https://scholar.colorado.edu/concern/undergraduate_honors_theses/x059c890d, or scan the QR code.

Abstract

Circadian rhythms are physiological and behavioral processes that follow an approximately 24-hour cycle. They arise from internal biological clocks, which are synchronized mainly by environmental light. Fear-based mental disorders, such as post-traumatic stress disorder (PTSD), are associated with disruptions in circadian rhythms. Understanding this relationship is important for developing more effective treatments. In delayed fear conditioning, the most common form of fear conditioning, the presentation of a tone (conditioned stimulus) is paired with a foot shock (unconditioned stimulus), causing experimental subjects to associate tone presentation with shock delivery. In trace fear conditioning, a brief temporal gap occurs between the tone and foot shock, causing different brain circuits to get engaged. Past work in our laboratory and others has shown circadian rhythms in the extinction of delayed conditioned fear. More recent work in our laboratory has shown that the recall of trace conditioned fear similarly exhibits a rhythm, with recall being stronger during the inactive phase of the rest-activity cycle. The purpose of this experiment was to test whether stronger recall for trace fear is dependent on the time of conditioning or the time of recall. Additionally, I analyzed brain tissue to assess whether rhythms in behavior were reflected in circadian and activity-dependent gene expression in the prefrontal cortex. Rats were fear conditioned at either ZT4 (the inactive phase) or ZT16 (the active phase) and then underwent fear recall after 24 h (same time of day as training), 36 h (opposite time of day as training), and 48 h (same time of day as training). Results showed an interaction between the time of training and the interval between training and testing for trace fear conditioning. Specifically, ZT4 rats showed strong fear recall at all intervals, whereas ZT16 rats showed increased recall over a greater recall interval. The mRNA expression of *Per1*, a key circadian gene, showed opposite results, with the ZT4 expression decreasing over recall intervals and ZT16 expression remaining constant. For *c-Fos*, an immediate early gene whose expression is used to approximate recent neuronal activation, mRNA expression tended to be higher at ZT16 regardless of the time of training. Notably, however, ZT4-36 h rats showed higher *c-Fos* expression than ZT16-36 h rats in the infralimbic but not prelimbic prefrontal cortex, suggesting differential engagement of the prelimbic cortex during trace fear recall. These results suggest that learned fear may be consolidated more quickly during the inactive phase, leading to signs of prefrontal engagement at later recall intervals during the active phase. In turn, these findings pave the way for circadian assessments, treatments, and prevention for fear-based mental disorders in clinical settings.

Lay Summary

Circadian rhythms are physiological and behavioral processes that follow an approximately 24-hour cycle. They arise from internal biological clocks, which are synchronized mainly by environmental light. Fear-based mental disorders, such as post-traumatic stress disorder (PTSD), are associated with disruptions in circadian rhythms. Understanding this relationship is important for developing more effective treatments. In trace fear conditioning, a brief temporal gap occurs between the presentation of the tone (conditioned stimulus) and foot shock (unconditioned stimulus). Recent work in our laboratory has shown that the recall of trace conditioned fear exhibits a rhythm, with recall being stronger during the inactive phase of the rest-activity cycle. The purpose of this experiment was to test whether stronger recall for trace fear is dependent on the time of conditioning or the time of recall. Additionally, I analyzed brain tissue to assess whether time-of-day differences in behavior were reflected in circadian and activity-dependent gene expression in the prefrontal cortex. Rats were fear conditioned at either ZT4 (the inactive phase) or ZT16 (the active phase) and then underwent fear recall after 24 h, 36 h, and 48 h. Results showed that for behavior the time of testing for trace fear conditioning, ZT4, is what was the most important, and ZT16 rats increase in freezing behavior over a time interval. *Per1* mRNA expression in the PFC showed opposite results with the ZT4 expression decreasing over recall intervals and ZT16 expression remaining constant. The time of recall interval was the most important for *c-Fos* neuronal activity in the PFC. These results show that learned fear behavior is expressed more during the inactive phase training, ZT4, but the neuronal circuitry shows more memory consolidation during the ZT16 recall intervals. These findings suggest that learned fear may be expressed differently through underlying neuronal circuits than through behavior in clinical settings.