URC97119

LIGHT-INDUCED ALTERATIONS IN STRIATAL NEUROCHEMICAL PROFILES

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Introduction

Much of our present knowledge regarding circadian rhythms and biological activity during space flight has been derived from those missions orbiting the Earth. During space missions, astronauts can become exposed to light/dark cycles that vary considerably from those that entrain the mammalian biological timing system to the 24-hour cycle found on Earth. As a spacecraft orbits the Earth, the duration of the light/dark period experienced becomes a function of the time it takes to circumnavigate the planet which in turn depends upon the altitude of the craft. Orbiting the Earth at an altitude of 200-800 km provides a light/dark cycle lasting between 80 and 140 minutes, whereas a voyage to the moon or even another planet would provide a light condition of constant light (Stampi, 1994). Currently, little is known regarding the effects of altered light/dark cycles on neurochemical levels within the central nervous system (CNS).

Many biochemical, physiological and behavioral phenomena are under circadian control, governed primarily by the hypothalamic suprachiasmatic nucleus. As such, these phenomena are subject to influence by the environmental light/dark cycle. Circadian variations in locomotor and behavioral activities have been correlated to both the environmental light/dark cycle and to dopamine (DA) levels within the CNS. It has been postulated by Martin-Iverson et al. (199 1 & 1992) that DA's role in the control of motor activity is subject to modulation by circadian rhythms (CR), environmental lighting and excitatory amino acids (EAAs). In addition, DA and EAA receptor regulated pathways arc involved in both the photic entrainment of CR and the control of motor activity. The cellular mechanisms by which DA and EAA-receptor ligands execute these functions, is still unclear. In order to help elucidate these mechanisms, we set out to determine the effects of altered environmental light/dark cycles on CNS neurotransmitter levels. In this study, we focused on the striatum, a region of the brain that receives a number of dopaminergic and glutamatergic input and is known to be involved in the modulation of locomotor and behavioral responses.

Methods

Animal Entrainment and Tissue Extraction. Adult male Long-Evans Hooded rats (200-250 g) were maintained on a 12 hour light/dark (12L:12D) cycle (lights on at 0600 hours; lights off at 1800 hours) in laboratory quarters with free access to food and water. After 3 days of synchronization, rats were exposed to constant dark or constant light conditions for 24 and 48 hours, respectively. Control animals remained in the 12L:12D. After 24 and 48 hours, animals were decapitated either one hour prior to the onset of day light (subjective night) or one hour prior to the onset of night (subjective day). These times to sacrifice the animals were chosen to allow for the possible accumulation of the metabolic changes over the light or dark period. The brains were rapidly removed, submerged in ice-cold 0.9% saline and dissected on ice. Wet tissue weights were determined and sections were frozen at -80°C. Frozen sample aliquots from striatum were sonicated on ice in 0.1 M $HClO_4$ (l 0µL/mg wet tissue weight) for three 10-second intervals. The homogenate was centrifuged at 12,000 rpm for 30 minutes at 4°C.

Dopamine and Glutamate Analysis. Monoamine and amino acid levels were determined simultaneously according to the method of Gamache et al., (1993). Analytes were separated on an HR-80 column (ESA, Inc., Bedford, MA) and maintained at 33°C with mobile phases flowing at 1.0 mL/min. Four serial coulometric electrodes with applied potentials of 70, 140, 210 and 280 mV were used for the measurement of dopamine, while four serial electrodes all set at 450 mV were used for analysis of o-phthalaldehyde-β-mercaptoethanol (OPA-βME) derived glutamate. For dopamine analysis, 20 µL of the supernatant was injected into a high-performance liquid chromatography (HPLC) system with 8 electrochemical sensors (CEAS model

5500, ESA, Inc., Bedford, MA). The monoamine mobile phase consisted of 0.05 M monobasic sodium phosphate; 250 mg/L heptanesulfonic acid; 8% methanol v/v and was adjusted to pH 3.0 with HPLC grade phosphoric acid. For glutamate analysis, 30 µL of supernatant was combined with 50 µL of OPA-βME derivatizing solution and 20 µL was injected into the HPLC for analysis. The amino acid mobile phase consisted of 0.1 M dibasic sodium phosphate, 3.1% actonitrile (v/v) and 25% methanol (v/v) adjusted to pH 6.8 with phosphoric acid. OPA stock was prepared weekly. 27 mg of OPA was dissolved in 1.0 mL of methanol. Following the addition of 5 µL of βME, the solution was diluted to 10 mL with a borate-EDTA solution containing 0.1 M sodium tetraborate (pH 9.3) and 10 µM EDTA. The stock solution was stored at 4°C and protected from light. The working solution was prepared daily by diluting the stock solution with 3 parts borate/EDTA solution and placed into the refrigerated autosampler in an amber vial.

Kynurenic Acid (KYNA) Analysis. 20 uL of the supernatant was injected into the HPLC for analysis. KYNA levels were measured according to an isocratic method. The mobile phase consisted of 50 mM Na_2HPO_4 in 5% methanol at pH 6.26 (adjusted with H_3PO_4). A 15 cm x 4.6 mm^3 um C_{18} reverse-phase column (HR- 150, ESA, Inc.) with a flow rate of 1 mL/min was used. The electrochemical sensors were set at 150,200,300,600, 750, 1000, 1040 and 1090 mV.

Results

Striatal DA levels following continuous light exposure are shown in Figure 1. DA levels during the subjective night (samples taken at 5:00 a.m., est) were elevated relative to controls after 24 hours. However, following 48 hours of continuous light DA levels were suppressed. When compared to 12L:12D controls, animals held in 24 or 48 hours of continuous light registered no change in DA levels during the subjective day (samples taken at 5:00 p.m., est). Striatal dopamine levels following continuous dark exposure are shown in Figure 2. In 24 hours of constant dark, an increase in DA was observed in both daytime and nighttime levels. This increase was more pronounced in the night-time levels. In both subjective day and subjective night, DA levels remained elevated at 48 hours of continuous dark.

Striatal glutamate levels following continuous light exposure are shown in Figure 3. During both the subjective day (5:00 p.m.) and subjective night (5:00 a.m.), glutamate levels peaked at 24 hours of exposure and then decreased at 48 hours. A similar profile was observed during constant dark shown in Figure 4. Clearly, there was a faster rise to peak levels during both the subjective day and subjective night at 24 hours as compared to constant light.

Since glutamate levels were found to vary, attempts were made to determine whether similar changes were occuring in other endogenous EAA receptor ligands. Thus, kynurenic acid levels in rat striatum were measured during constant light and the results are shown in Figure 5. Exposure to constant light conditions suppressed KYNA levels during both the subjective day and subjective night. However, KYNA levels did not vary significantly during the subjective night. During the subjective day, KYNA levels decreased significantly within the first 24 hours. These light-induced levels remained low as long as continuous light conditions were maintained. In contrast, an increase in striatal KYNA levels was observed within 24 hours of constant dark conditions (Figure 6). This increase was transient however, in that there is a decrease to initial control values within 48 hours. Subjective night-time levels did not vary in the first 24 hours of constant dark, however, these levels rose rapidly to reflect daytime levels within 48 hours. Clearly, at the end of 48 hours, striatal KYNA levels were low during constant light and are elevated to approximately day-time control levels in constant dark.

Discussion

In the present study, we attempted to characterize the alterations in neurotransmitter levels within the striatum associated with variations in the environmental light/dark cycle. In 1993, Engber et al. suggested key roles for DA and EAA receptor-regulated pathways in the control of motor responses. They suggested that DA receptor-mediated pathways exhibit varying degrees of sensitivity to EAA receptor blockade and that some DA receptor-mediated responses require simultaneous NMDA receptor stimulation. These findings agreed with earlier reports by Martin-Iverson et al. (1991& 1992) which suggested DA and EAA involvement in both locomotor

activity and the photic entrainment of circadian rhythms. However, the mechanisms by which dopaminergic neurons repond to alterations in the light/dark cycle is currently not known.

Results from these experiments contribute to a number of implications with regards to the response of the striatum to alterations in environmental lighting. Clearly, striatal DA, glutamate and KYNA undergo alterations in response to constant lighting conditions. Although a number of reports exist regarding glutamate's role in excitotoxic damage within the basal ganglia, little data is available explaining its impact on other transmitters with the region. Based on experimental evidence from Difazio et al. (1992) demonstrating glutamate's involvement in basal ganglia DA release, light-induced interactions between these two transmitter systems may exist presynaptically. Subsequently, these alterations may trigger biochemical and metabolic changes, such as an up- or down-regulation of transmitter receptor proteins in response to these transmitter levels, which in turn may facilitate the observed biological response. Both DA and glutamate levels were elevated at 24 hours constant light and dark, respectively. Reports from Knapp et al. (1987) demonstrated DA's role in increasing the sensitivity of retinal cells to glutamate excitotoxicity. Thus, elevated DA levels working in concert with elevated glutamate levels may subject the neuronal system to excitoxic damage unless some compensatory mechanism is activated and maintained. Interestingly, KYNA levels, a tryptophan metabolize and endogenous EAA-receptor antagonist, were found to be suppressed at 48 hours of constant light. This suggests a loss of KYNA's neuroprotective antagonistic activity at EAA receptors and a potential increase in EAA excitotoxic susceptibility during light photoperiods. Clearly, an alteration in the natural balance between endogenous DA and EAA receptor ligands may confer varying degrees of susceptibility or protection relative to excitotoxicity in the CNS.

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Acknowledgement

This project was supported by NIH grants NS34194, RR03034, and GM08248 and NASA grant NCCW-0083.

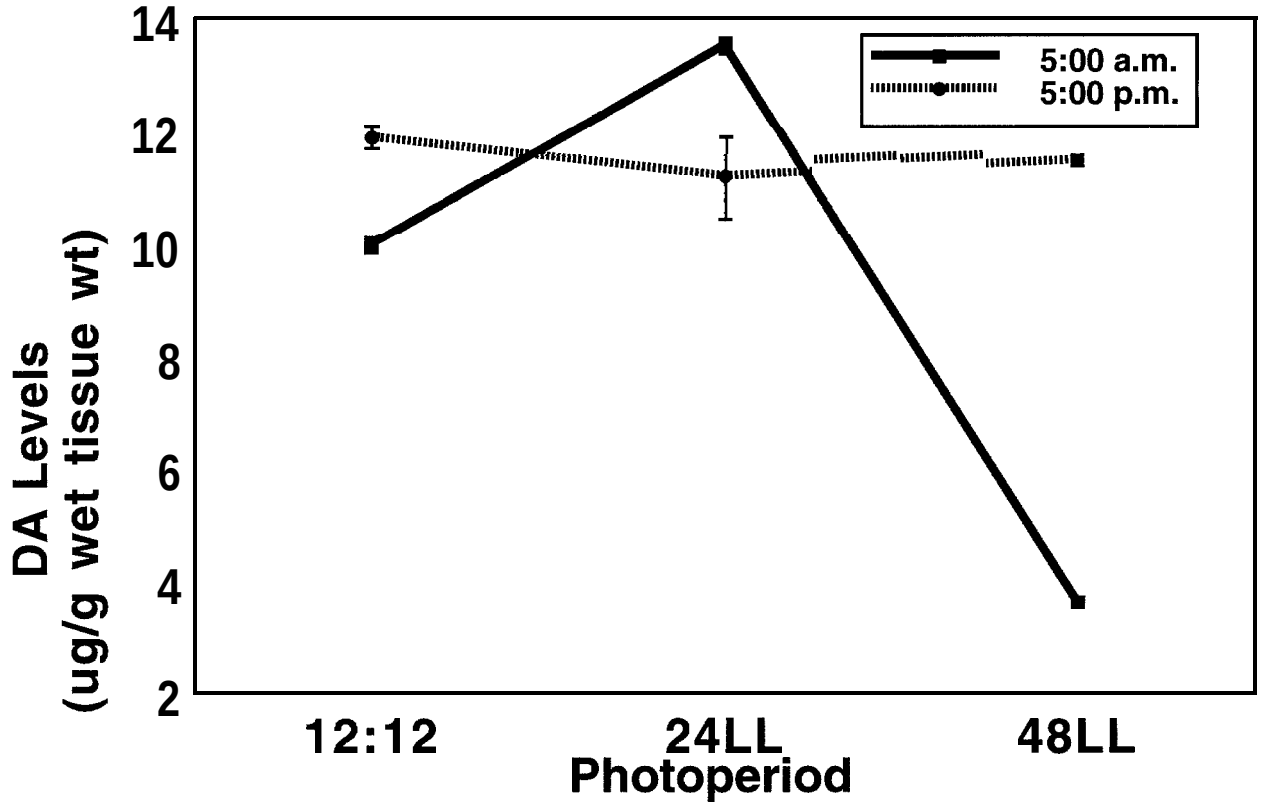

Figure 1 Dopamine **levels in rat** striatum **following exposure to continuous light.**

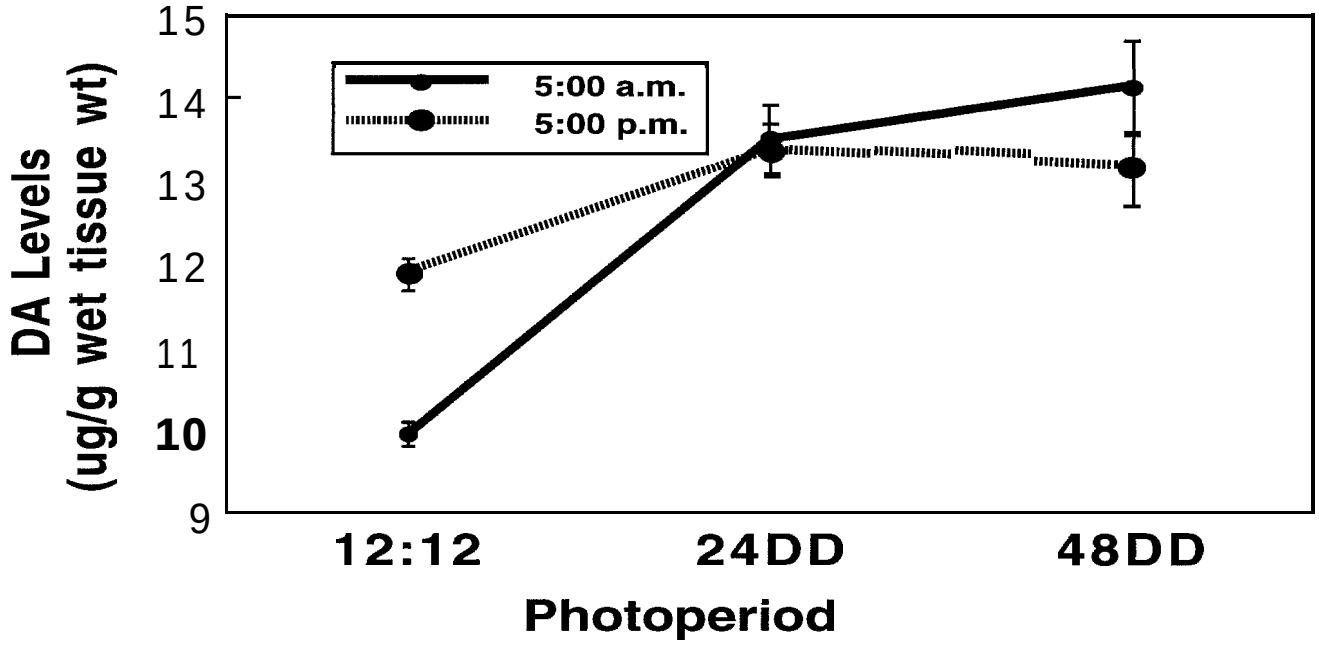

Figure 2. Dopamine levels in rat striatum **following** exposure to continuous dark.

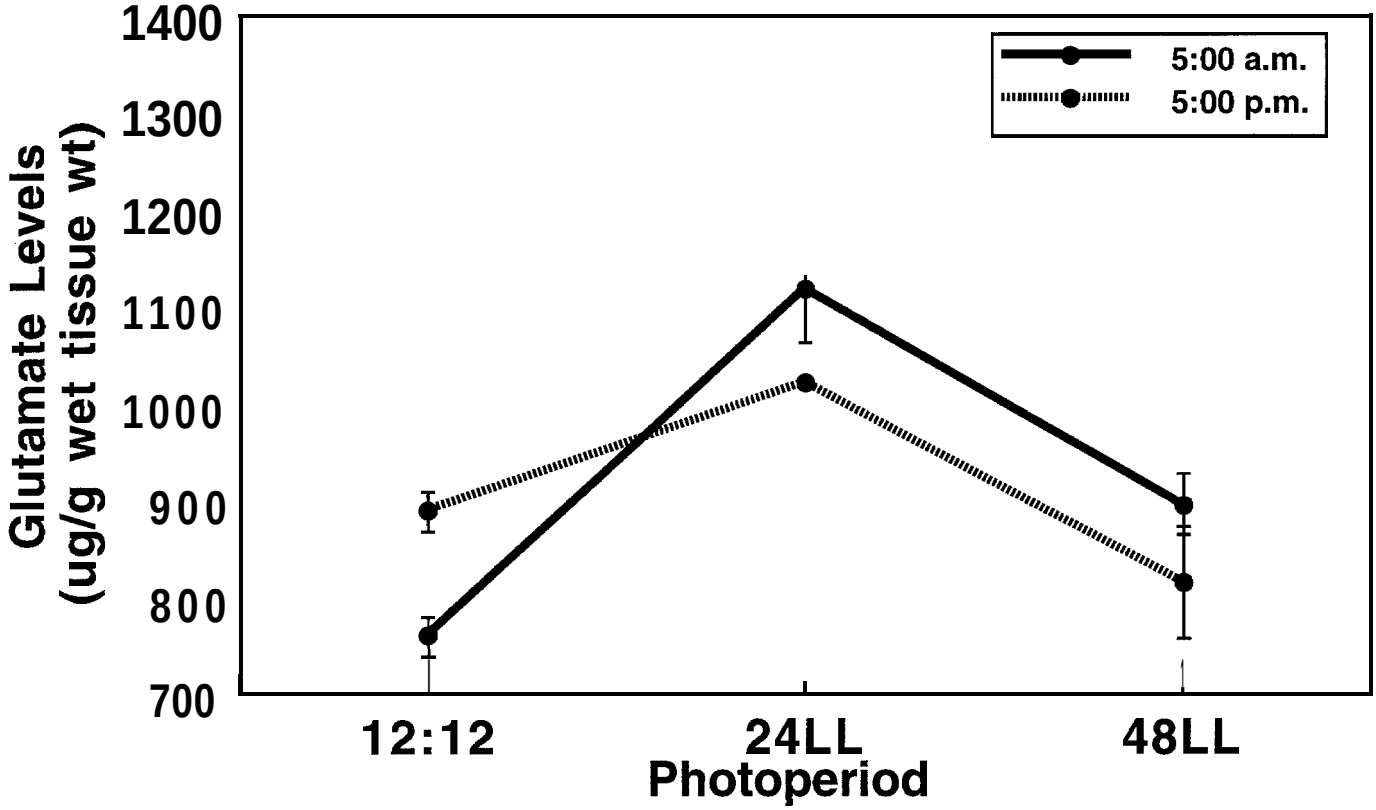

Figure 3. Glutamate levels in rat striatum following exposure to continuous light.

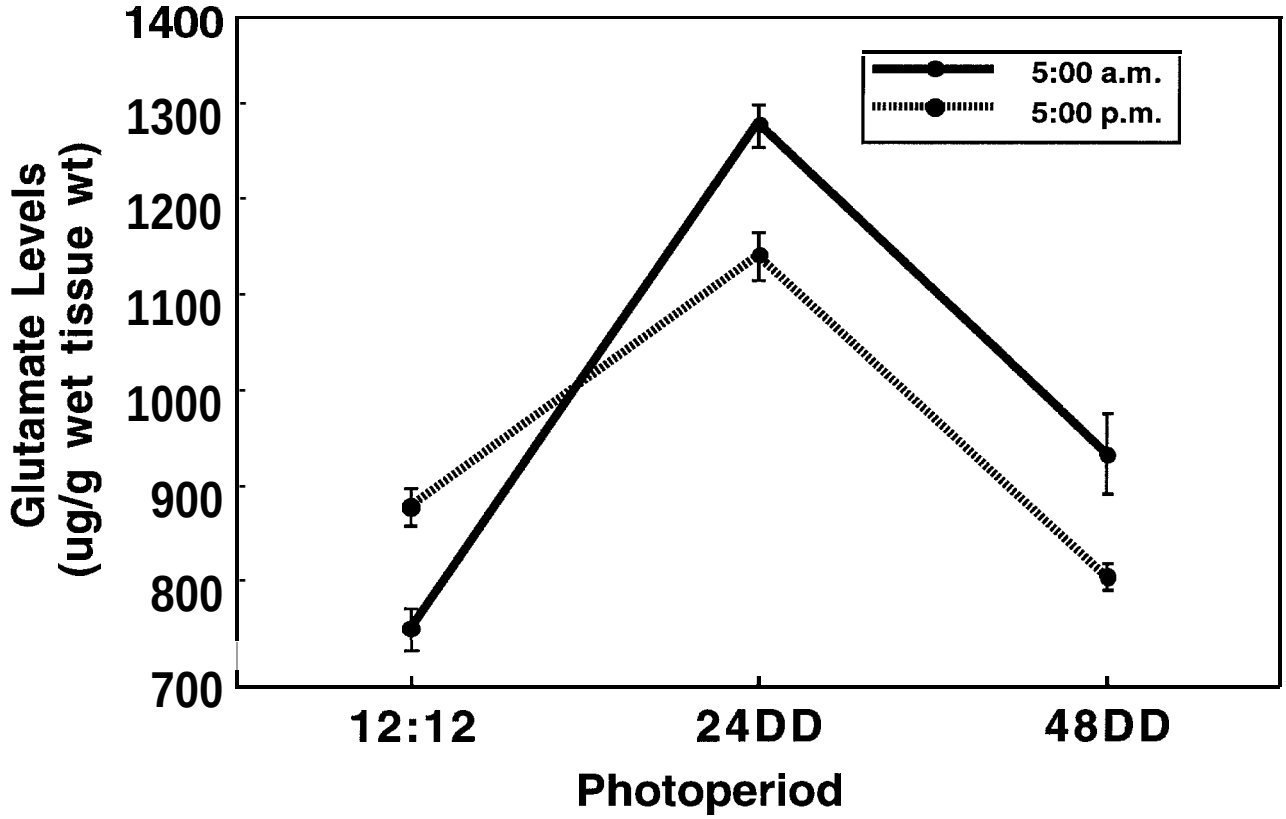

Figure 4. Glutamate Levels in rat striatum following exposure to continuous dark.

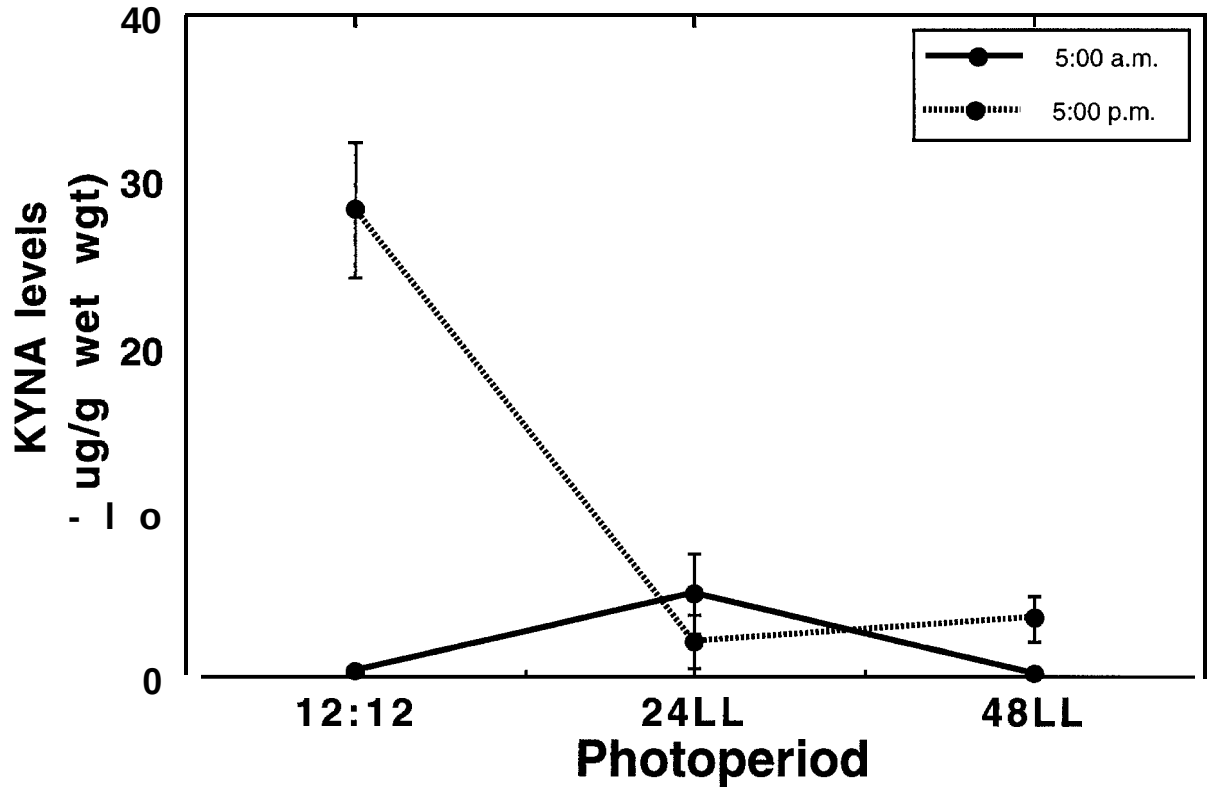

Figure 5. Kynurenic **Acid levels in rat** striatum **following exposure to continuous light.**

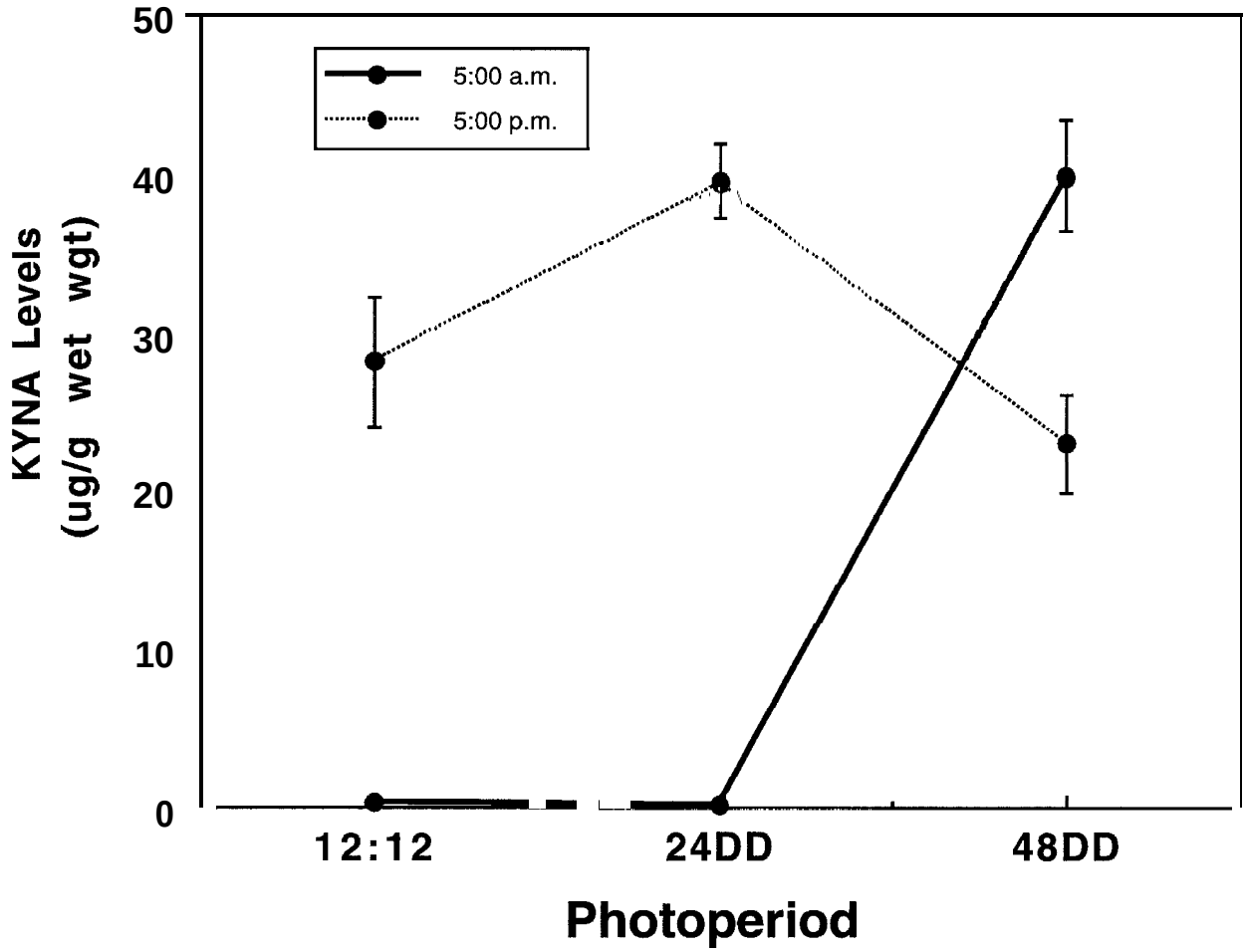

Figure 6. Kynurenic **Acid levels in rat** striatum **following exposure to continuous dark.**