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Circadian Rhythm of Brain GABA Levels in the Cockroach, *Leucophaea maderae*

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Circadian Rhythm of Brain GABA Levels
in the Cockroach, *Leucophaea maderae*

A senior honors thesis
presented to
the University Honors Program
Western Kentucky University
Bowling Green, Kentucky

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&

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May 1995

Approved By

Blaine R Ferrell

Darwin Dall

Walker Rutledge

Sam Tr^c Ferrell, Honors Director 5/4/95

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This thesis is dedicated to David, Matthew, and Chelsea McCay; Darrell and Martha Martin; and Ralph and Kathryn Gibson. Without their love and support, we would not have achieved this goal.

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Modified from (Paga, 1990).

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Modified from (Page, 1990).

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ABSTRACT

Brain levels of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) were determined at three-hour intervals in roaches acclimated to a 12-hour light/12-hour dark cycle (light onset 0600 h) at $25 \pm 2^\circ$ C using high performance liquid chromatography coupled with fluorometric detection. GABA levels were found to be highest at noon and lowest at 1800 h. A difference between the high and low levels at these two times remained during the second day under constant dark conditions, indicating that GABA levels do indeed vary on a circadian basis. Brain GABA levels were highest at a time of day that corresponded with the time of day of greatest clock output measured electrophysiologically. The fact that these two parameters correspond and occur at a time of day when locomotor activity and eye sensitivity to light are low is suggestive that clock output is inhibitory.

INTRODUCTION

Organisms live in environments that exhibit cyclic fluctuations in light and temperature, variations that occur daily and throughout the year. Organisms have developed circadian systems or "biological clocks" that work to synchronize them with these cyclic environmental changes by altering such things as physiology, biochemistry, and/or behavior (Page, 1990). Biological clocks have been found in eukaryotic organisms studied and have been discovered to share three main characteristics. First, these clocks are based on endogenously generated, self-sustaining oscillations that have a period length close to, but not exactly, twenty-four hours. Second, these circadian pacemakers can be adaptively synchronized (i.e. entrained) with environmental patterns such as photoperiod (light/dark) cycles. Thirdly, the periodicity of the clock does not change with changes in temperature.

Because of apparent similarities that exist in the circadian systems of many different species, it has been possible to exploit simple invertebrate systems in order to gain insight into the organizational principles that underlie more complex circadian systems (Scharrer, 1987). The cockroach has proven to be a particularly good model for studying circadian systems, and consequently, more is known about its circadian organization than that of any other organism. The circadian system of the cockroach consists of four functionally defined elements (Figure 1): two mutually-coupled pacemakers located in the optic lobes that generate the primary timing cue for rhythms, photoreceptors located in the compound eyes for transduction of light information used in entrainment, a coupling pathway that transmits information from the photoreceptor to the pacemaker, and another coupling pathway that transmits information from the pacemaker to the activities and mechanisms it controls. In the cockroach, the circadian system controls locomotor

activity (Nishiitsutsuji-Uwo & Pittendrigh, 1968), retinal sensitivity to light (Wills, Page, & Colwell, 1985), eye cell morphology (Ferrell & Reitcheck, 1993), and cytochrome oxidase activity (Lavialle, Chabanet, & Dumortier, 1989). The pattern of locomotor activity is easy to detect and shows a distinct daily rhythm.

In 1990, Colwell and Page showed that the optic lobe is a self-sustained circadian oscillator by removing the optic lobe from *Leucophaea maderae* and monitoring its activity with a suction electrode. The removed optic lobes exhibited spontaneous neural activity in tissue culture for three to five days while totally isolated from any sort of neural input, and that activity exhibited a circadian periodicity. Furthermore, it has been suggested that the output of the optic lobe oscillators is inhibitory in that the output inhibits activity from occurring at an inappropriate phase in the circadian cycle.

It is believed that the regulation of circadian systems involves biochemical substances. Since clock output is believed to be inhibitory, the predominant inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), was chosen for this study. Inhibitory neurotransmitters act by damping or suppressing the firing of target neurons. Inhibitory neural networks "act as the brakes on the entire nervous system" by "preventing a runaway spree of excitatory neural firing" (Gottlieb, 1988). Thus, they help to control the responses of excitatory networks that assess information about the external environment in which an organism lives. GABA, a modified primary amino acid, has been found in high levels in the brains of mammals and arthropods (Gottlieb, 1988). GABA is thought to be the main inhibitory neurotransmitter in many organisms and is the only inhibitory neurotransmitter known in some systems, such as that of crustaceans. In humans, impaired GABA transmission has been implicated in such neurological and

psychological disorders as Huntington's disease, epilepsy, Parkinson's disease, Alzheimer's disease, and alcoholism. Because GABA is so prevalent in the brains of such a wide variety of organisms and since it is considered to be one of the main inhibitory neurotransmitters, it is believed that GABA fluctuations may be involved in the mechanism whereby clocks regulate circadian rhythms of behavior, physiology, and biochemistry. The identification of a biochemical substance that varies on a circadian basis would be an important first step in trying to determine which cells regulate the circadian rhythm.

In studying a compound that is believed to affect or be affected by the circadian system, one must first establish whether or not that compound exhibits a daily rhythm in organisms acclimated to a 12-hour light/12-hour dark cycle (LD 12:12). If the compound does not exhibit a daily pattern, the compound does not affect or is not affected by the circadian system. If a daily rhythm is found to exist, the next step is to establish if the rhythm exhibited by the compound persists on a circadian basis--in the absence of entrainment cues (i.e. light/dark and temperature cycles). If it does, it can be said that the compound is involved in the circadian system of the animal.

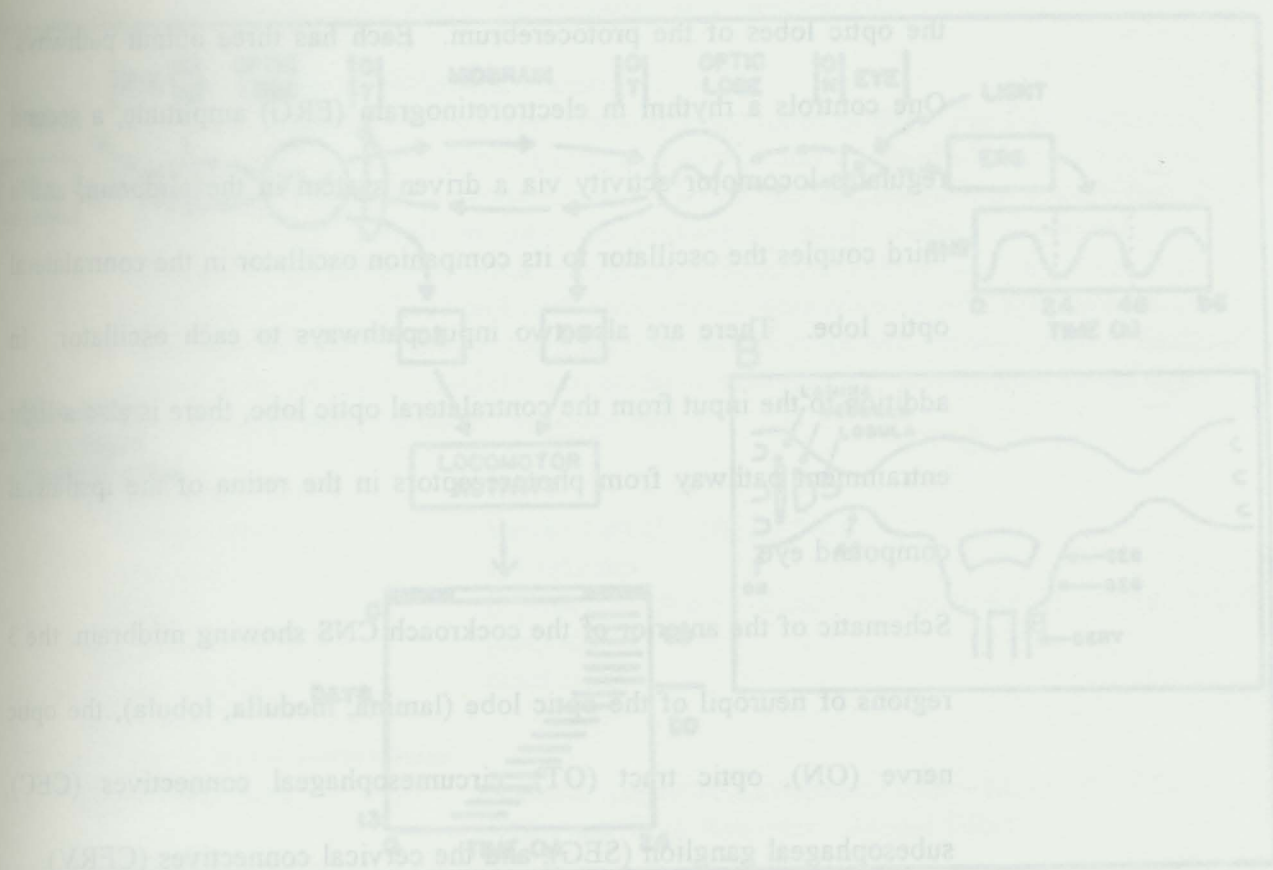
When organisms are entrained to the LD cycle, the onset of light resets their clocks to a 24-hour period each day. However, when the organisms are subjected to total darkness (DD), their perception of a day, controlled by the circadian system, is not exactly 24 hours. In total darkness, the organisms' perception of a particular time and activity will not correspond to ambient time as it does during the LD cycle. In fact, depending on the organism, activity will begin at either a slightly earlier or slightly later time each day that the organism is in total darkness. Thus, the activity levels, which are an indication of the organisms' circadian systems,

must be closely monitored. This is an important consideration for experimentation. When carrying out studies using DD conditions, one must perform tests at the organisms' perceived time (circadian time: CT) and not necessarily at a set time on a 24-hour clock. If a test were carried out at 1800 hours under LD conditions, for example, that same test may have to occur at 1745 hours (i.e., CT 1800) under DD conditions, depending on the shift in onset of activity of the animals.

In this study, we measured GABA levels in the brains of a large African cockroach, *Leucophaea maderae*, in order to determine if daily and circadian rhythms of this inhibitory neurotransmitter exist. We completed this analysis using high performance liquid chromatography (HPLC) coupled with fluorometric detection.

FIGURE 1 Brain Schematic

A Schematic model of the circadian timing system of *Xenopus laevis*. There are two bilaterally paired driving oscillators, each located in one of the optic lobes of the protocerebrum. Each has three subunits: a pacemaker, a driver, and a responder. The pacemaker controls a rhythm in electroretinogram (ERG) amplitude, which is shown in the graph. The driver controls the oscillator's activity via a driver neuron, which in turn couples the oscillator to its companion oscillator in the contralateral optic lobe. There are also two inhibitory pathways to each oscillator, one from the contralateral optic lobe and one from the retinal ganglion cells (RGCs) in the retina of the ipsilateral eye.



Modified from (Page, 1990)

FIGURE 1. Brain Schematic

- A. Schematic model of the circadian timing system of *Leucophaea maderae*. There are two, bilaterally paired driving oscillators, each located in one of the optic lobes of the protocerebrum. Each has three output pathways. One controls a rhythm in electroretinogram (ERG) amplitude, a second regulates locomotor activity via a driven system in the midbrain, and a third couples the oscillator to its companion oscillator in the contralateral optic lobe. There are also two input pathways to each oscillator. In addition to the input from the contralateral optic lobe, there is also a light entrainment pathway from photoreceptors in the retina of the ipsilateral compound eye.
- B. Schematic of the anterior of the cockroach CNS showing midbrain, the 3 regions of neuropil of the optic lobe (lamina, medulla, lobula), the optic nerve (ON), optic tract (OT), circumesophageal connectives (CEC), subesophageal ganglion (SEG), and the cervical connectives (CERV).

Modified from (Page, 1990).

A

LIGHT

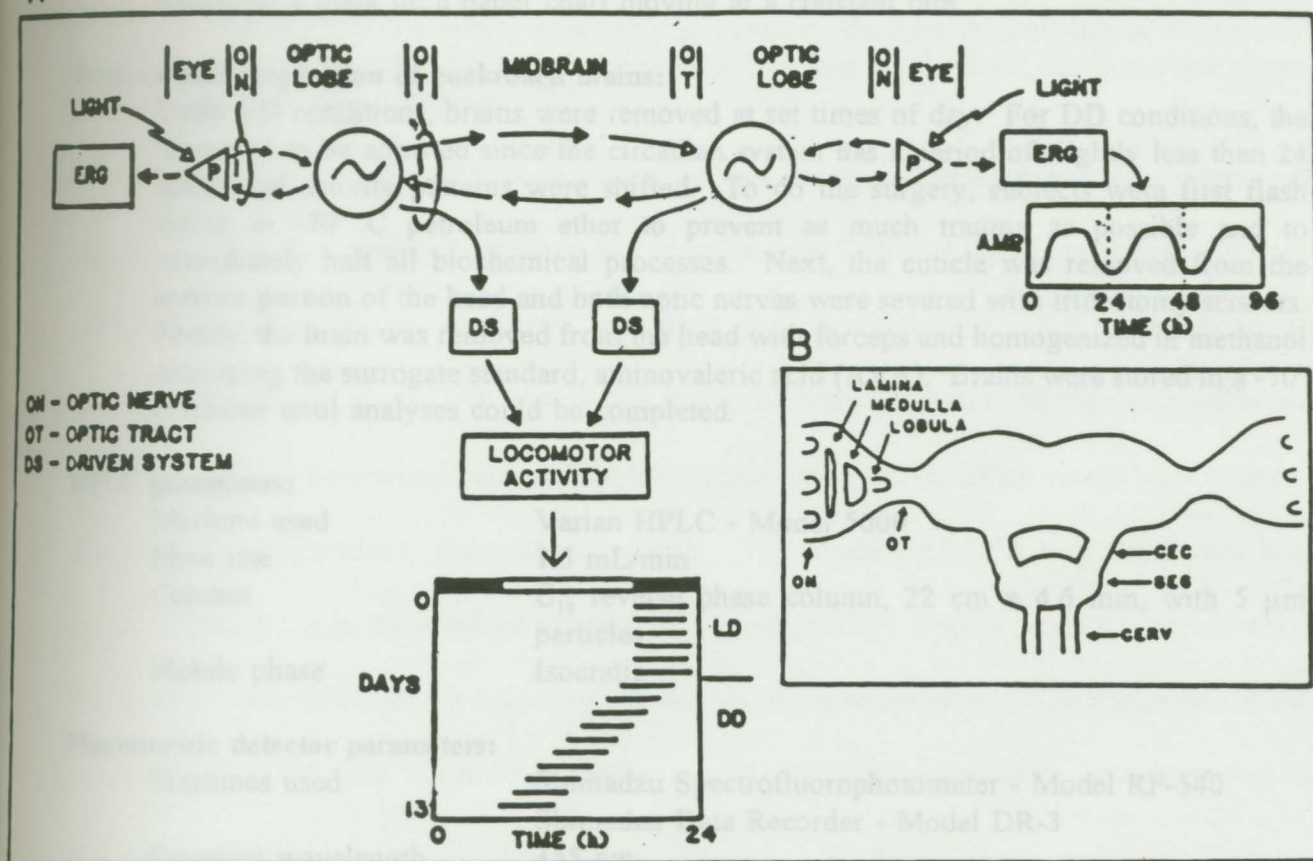
ERG

ON - OPTIC
OT - OPTIC
DS - DRIVE

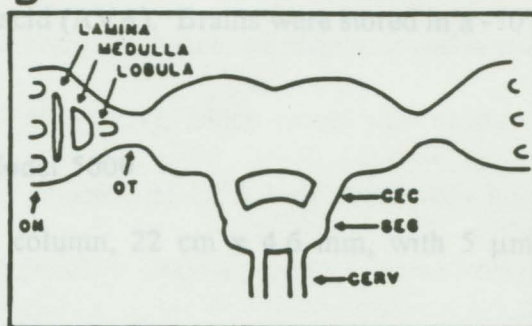
MATERIALS AND METHODS

Male *Leucophaea maderae* were used in these experiments and were housed in temperature-controlled chambers. For the light/dark portion of the experiments, lights came on at 0600 hours and were extinguished at 1800 hours. For the constant darkness portion of the experiments, the animals were subjected to constant darkness.

A



B



MATERIALS AND METHODS

Subjects:

Adult male *Leucophaea maderae* were used in these experiments and were housed in photoperiod and temperature-controlled chambers. For the light/dark portion of the experiments, lights came on at 0600 hours and were extinguished at 1800 hours. For the circadian portion of the experiments, the animals were subjected to constant darkness.

Measurement of activity:

Small plastic running wheels similar to those found in hamster cages were used to record the activity levels of the roaches. Each time the wheel turned, magnets mounted on the wheel closed a reed switch connected to an Esterhine-Angus event recorder, thus producing a mark on a paper chart moving at a constant rate.

Removal and preparation of cockroach brains:

Under LD conditions, brains were removed at set times of day. For DD conditions, the times had to be adjusted since the circadian system has a period of slightly less than 24 hours, and activity patterns were shifted. To do the surgery, subjects were first flash frozen in -70°C petroleum ether to prevent as much trauma as possible and to immediately halt all biochemical processes. Next, the cuticle was removed from the anterior portion of the head and both optic nerves were severed with iridectomy scissors. Finally, the brain was removed from the head with forceps and homogenized in methanol containing the surrogate standard, aminovaleric acid (AVA). Brains were stored in a -70°C freezer until analyses could be completed.

HPLC parameters:

Machine used	Varian HPLC - Model 5000
Flow rate	1.5 mL/min
Column	C_{18} reverse phase column, 22 cm x 4.6 mm, with 5 μm particles
Mobile phase	Isocratic

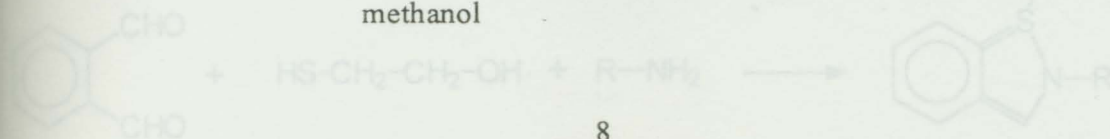
Fluorometric detector parameters:

Machines used	Shimadzu Spectrofluorophotometer - Model RF-540 Shimadzu Data Recorder - Model DR-3
Emission wavelength	355 nm
Excitation wavelength	440 nm
Flow cell volume	12 μL

Solution preparation:

Mobile phase 55% phosphate buffer (pH 2.8 using H_3PO_4), 45% acetonitrile

OPA 5.5 mg ortho-phthaldialdehyde (OPA), 40 μL ethanethiol, 4 mL methanol



Solution preparation (cont.):

Borate 0.1 M boric acid solution (pH 10.5 using NaOH)

Standards All standards were made with methanol and the stated concentrations of GABA and AVA.

0.2 µg/mL GABA: 4.5 µg/mL AVA

0.5 µg/mL GABA: 4.5 µg/mL AVA

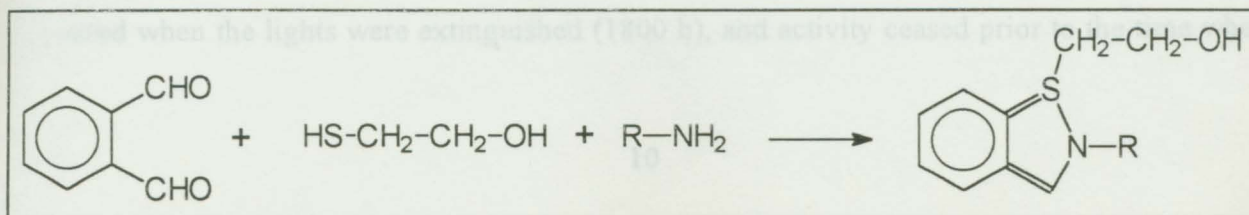
1.0 µg/mL GABA: 4.5 µg/mL AVA

Standards were stored in a -70° C freezer until analyses could be completed.

Derivatization procedure:

10 µL of sample (brain or standard) was mixed with 50 µL of the borate solution. 100 µL of the OPA solution was added and allowed to react for 90 seconds before the reaction was halted with the addition of 100 µL of glacial acetic acid. This mixture was then injected into the HPLC as quickly as possible. Because the half-life of derivatized GABA is only eight minutes, the sooner the injection took place, the larger the peaks were on the chromatogram.

Aminovaleric acid (AVA) was chosen as the surrogate standard and was present in constant levels in both standard solutions and brain samples. AVA is a primary amino acid with similar chemical properties as GABA, but AVA does not naturally occur in the brain of *Leucophaea maderae*. Since GABA and AVA do not naturally fluoresce, all standards and brain samples had to be derivatized with ortho-phthaldialdehyde (OPA), which reacts with primary amino acids to form highly fluorescent products. The reaction of OPA will occur with any primary amine group in the presence of a reducing agent (thiol) to yield a product (Hearn, 1991). This reaction with OPA, shown below, required a pH in the range of 9-10.5 and allowed for the detection of GABA and AVA levels present in the standards and brain samples using a spectrofluorometer.



Each day that samples were analyzed, the first run completed was a methanol blank. A series of three working standards of GABA and AVA were subsequently analyzed, and a standard curve was established using the three standards mentioned previously. The GABA versus AVA ratio of peak heights was plotted against GABA concentrations to formulate the standard curve. All standards were run in triplicate each day to establish accuracy and precision. Retention times for GABA and AVA were approximately 4.5 minutes and 6 minutes respectively.

After a standard curve was established, brain samples were analyzed. All brain samples were run in triplicate. From the chromatograms of the brain extracts, the GABA to AVA peak height ratio was calculated, and GABA levels were determined by means of the standard curve. To determine if the GABA peak in the chromatogram of a brain was truly representative of GABA levels and not of a mixture of substances, a brain sample was "spiked." Equal volumes of a brain sample and of the high standard (1.0 $\mu\text{g/mL}$ GABA : 4.5 $\mu\text{g/mL}$ AVA) were mixed, and this mixture was analyzed.

Brains were extracted at three-hour intervals over a 24-hour period, and GABA levels were determined for six brains from each time period in order to probe for the presence of a daily rhythm. After the presence of a daily rhythm was established, seven brains from light/dark adapted animals were extracted at 1200 hours and 1800 hours, the times at which GABA levels were highest and lowest respectively, and seven brains were extracted from animals subjected to total darkness (DD) at corresponding circadian times.

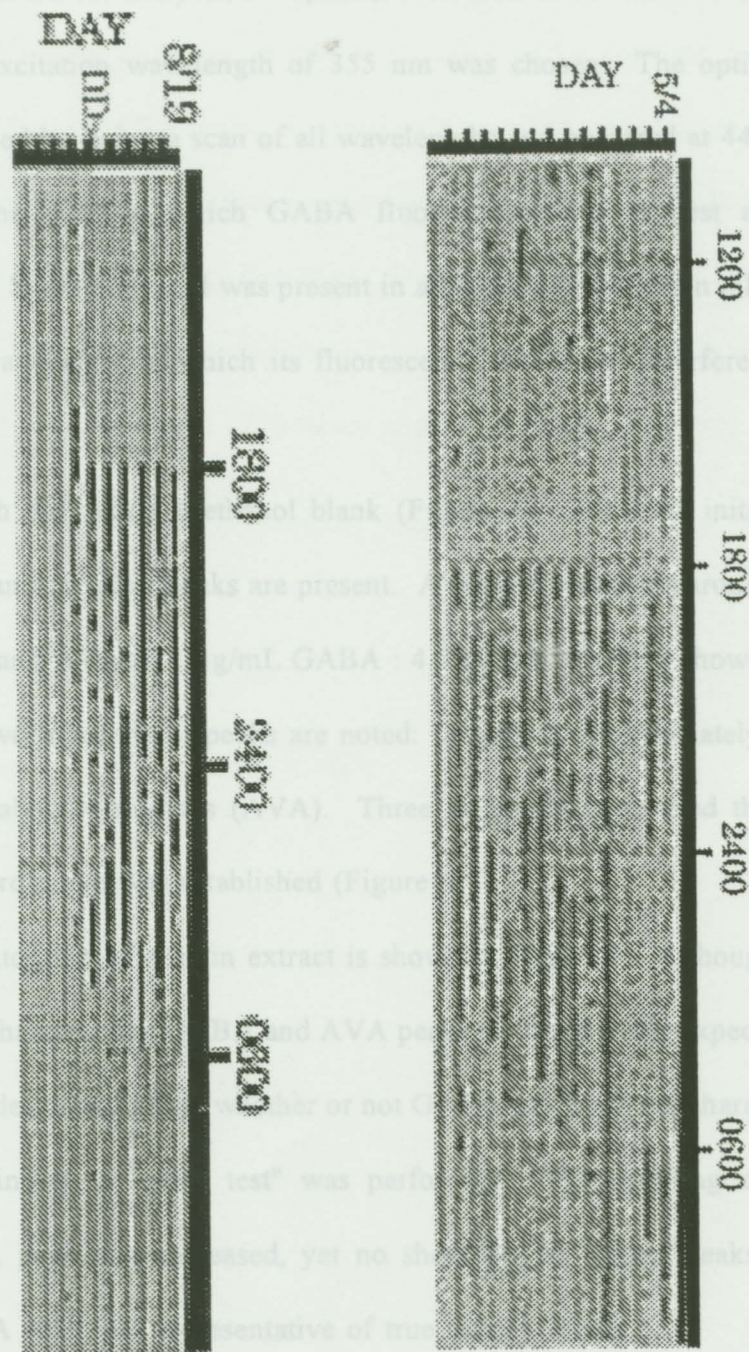
Activity levels of the cockroaches had to be monitored throughout the experiment using activity charts (Figure 2). When the cockroaches were entrained to an LD cycle, onset of activity occurred when the lights were extinguished (1800 h), and activity ceased prior to the time when

lights were turned on (0600 h). Brains could not be removed until the animals exhibited a predictable, consistent locomotor activity pattern, which indicated that the animals were entrained to the photoperiod cycle. Under circadian (DD) conditions, the animals were allowed to freerun, and the onset of activity occurred at earlier times each successive day. This was an indication of the animals' perception of 1800 h, the time lights would normally be extinguished (i.e. CT 1800). This shift in onset of activity required a shift in time of removal of the brains. Under DD conditions, brains were extracted on the second day after lights were turned off for animals which had previously exhibited a predictable activity rhythm.

Data was analyzed using two different statistical tests: Analysis of Variance (ANOVA) and Student-Newman-Keul's range test. In addition, standard deviations and standard errors were calculated.

FIGURE 2. Locomotor activity recording of two cockroaches. The first recording is from a cockroach maintained under LD conditions (12 hours light/ 12 hours dark cycle) at $25 \pm 2^\circ \text{C}$, and the second recording is from a cockroach maintained under LD conditions for a time and subsequently subjected to DD conditions (total darkness) on the day indicated. For LD conditions, lights were turned on at 0600 hours and were extinguished at 1800 hours.

RESULTS



After analyzing all brains from each time interval, a daily pattern of GABA was detected (Table I and Figure 8). GABA levels were found to be highest at 1200 hours and lowest at 1800

RESULTS

Before using the HPLC for analysis, the optimal excitation and emission wavelengths had to be determined. An excitation wavelength of 355 nm was chosen. The optimal emission wavelength was determined by doing a scan of all wavelengths and occurred at 440 nm (Figure 3). This represented the peak in which GABA fluorescence was highest and methanol fluorescence was lowest. Since methanol was present in all standards and brain dilutions, it was important to choose a wavelength in which its fluorescence would not interfere with GABA fluorescence.

The first run each day was a methanol blank (Figure 4). After the initial peaks, the baseline becomes stable and no other peaks are present. A sample standard chromatogram with known values of GABA and AVA (0.5 $\mu\text{g/mL}$ GABA : 4.5 $\mu\text{g/mL}$ AVA) is shown in Figure 5. After the initial peaks, two other major peaks are noted: a peak at approximately 4.5 minutes (GABA) and a peak at about 6 minutes (AVA). Three standards were used throughout the experiment, and a standard curve was established (Figure 6).

A sample chromatogram of a brain extract is shown in Figure 7. Although there were other peaks present, the characteristic GABA and AVA peaks occurred at the expected times and could be analyzed. In order to determine whether or not GABA produced the characteristic peak at approximately 4.5 minutes, a "spike test" was performed. The resulting chromatogram revealed that the GABA peak was increased, yet no shoulders or hidden peaks were noted, indicating that the GABA peak was representative of true GABA levels.

After analyzing all brains from each time interval, a daily pattern of GABA was detected (Table I and Figure 8). GABA levels were found to be highest at 1200 hours and lowest at 1800

hours. Once the daily pattern of GABA was detected, more brains were removed at 1200 hours and 1800 hours from light/dark adapted roaches and from animals kept in total darkness. It was determined that the rhythm was circadian in that it persisted under constant dark conditions (Table II and Figure 9). Brain GABA levels at circadian time 1200 were higher and similar to levels at 1200 hours under light/dark conditions, and levels at circadian time 1800 were lower and similar to levels at 1800 hours under light/dark conditions. In addition, GABA levels at circadian time 1800 and 1800 hours under light/dark conditions were significantly lower than GABA levels at circadian time 1200 and 1200 hours under light/dark conditions.

FIGURE 3. Preliminary scan to determine optimal emission wavelength to use in detecting GABA. Since GABA and methanol occur in all brain samples and standards, the optimal wavelength is represented by the peak in which GABA relative fluorescence is highest and methanol relative fluorescence is lowest. An emission wavelength of 440 nm was chosen to use for the fluorometric detection of GABA.

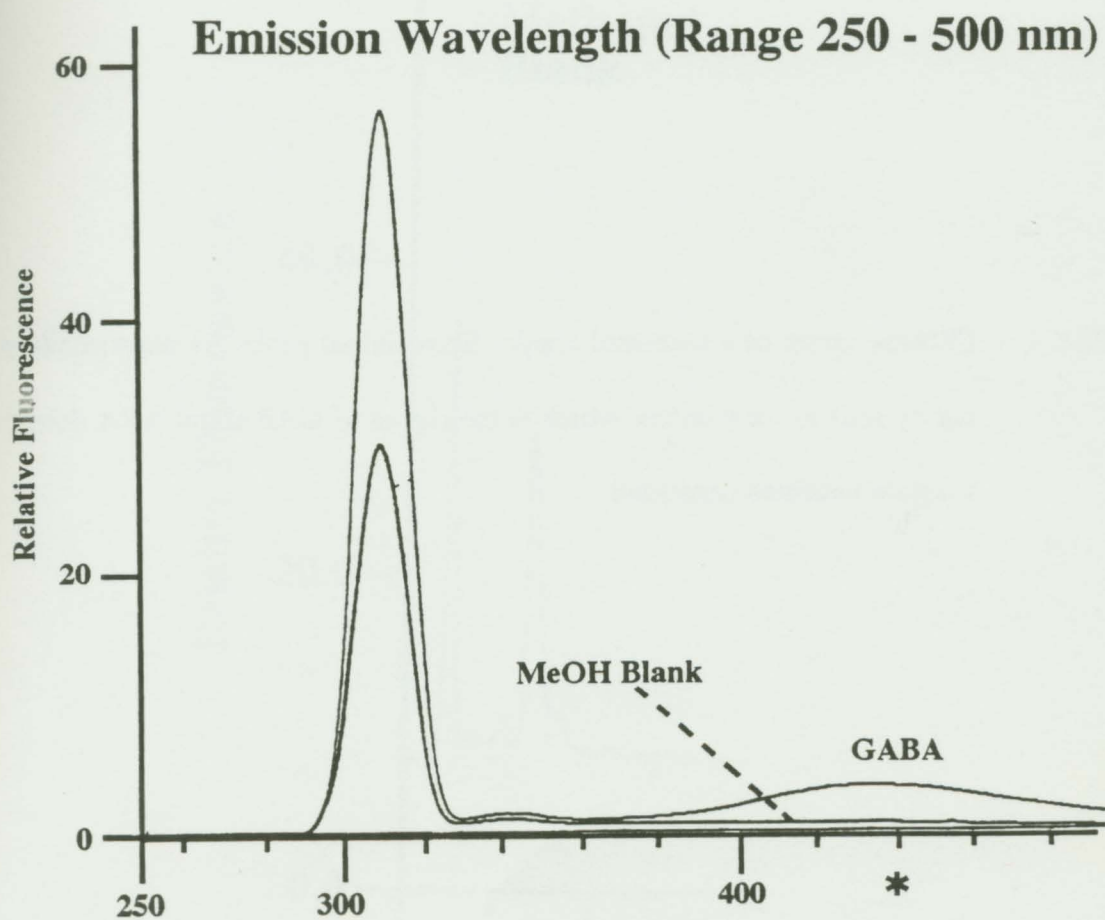
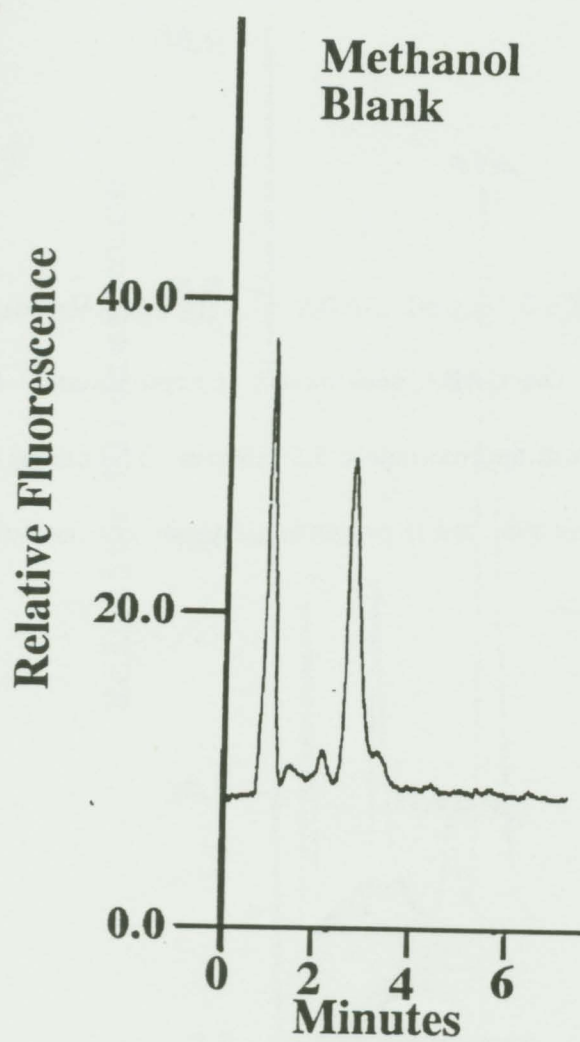


FIGURE 4. Chromatogram of a methanol blank. Some initial peaks are consistently present, but by four to six minutes, which is the region of GABA and AVA detection, the baseline becomes consistent.



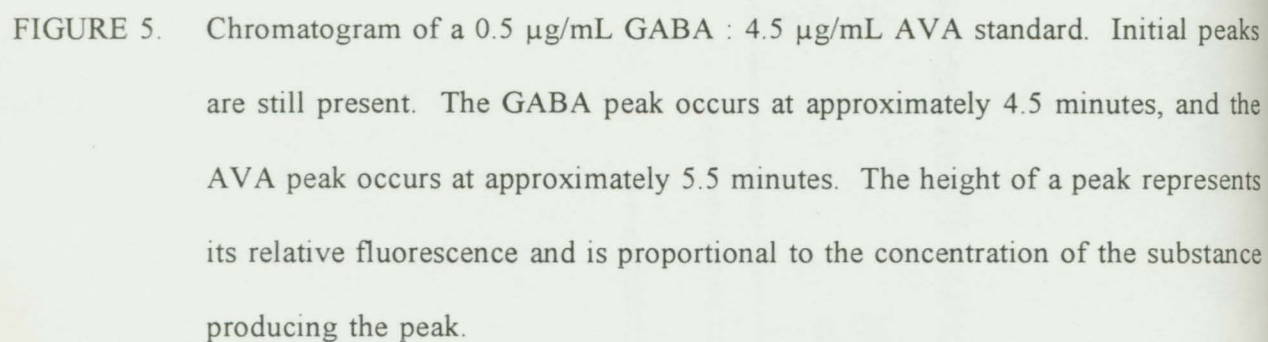


FIGURE 5. Chromatogram of a 0.5 $\mu\text{g/mL}$ GABA : 4.5 $\mu\text{g/mL}$ AVA standard. Initial peaks are still present. The GABA peak occurs at approximately 4.5 minutes, and the AVA peak occurs at approximately 5.5 minutes. The height of a peak represents its relative fluorescence and is proportional to the concentration of the substance producing the peak.

0.5 $\mu\text{g/mL}$ GABA:4.5 $\mu\text{g/mL}$ AVA
Standard

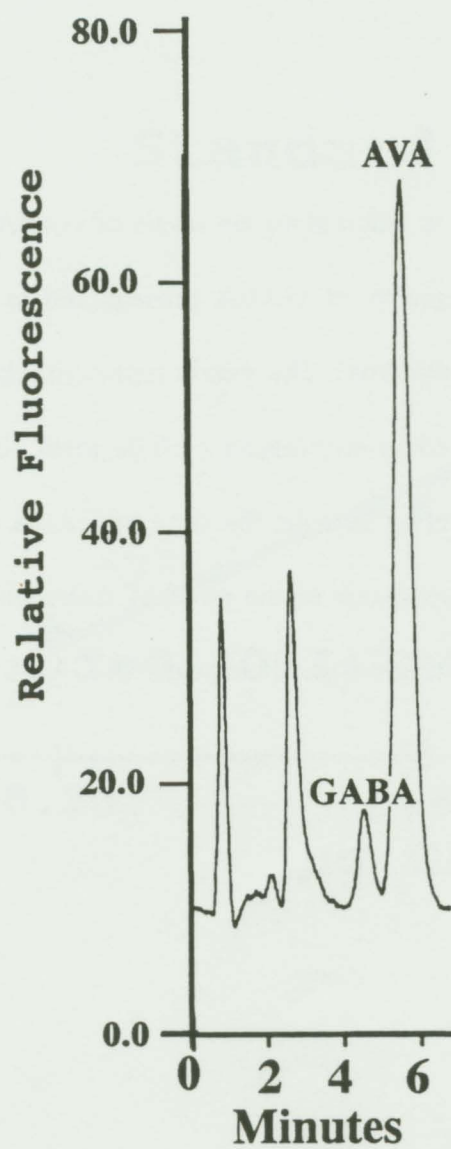


FIGURE 6. Standard curve used in calculating the levels of GABA in brain samples. The x-axis indicates micrograms of GABA present and is based on average values obtained for three standards. The y-axis represents the GABA to AVA ratio of peak heights measured in millimeters (± 0.01 mm). To determine the levels of GABA present in a brain sample, the ratio of GABA to AVA peak heights was calculated. The x coordinate of the point of intersection of the GABA to AVA ratio with the standard curve line represents the GABA levels present in the brain.

Standard Curve

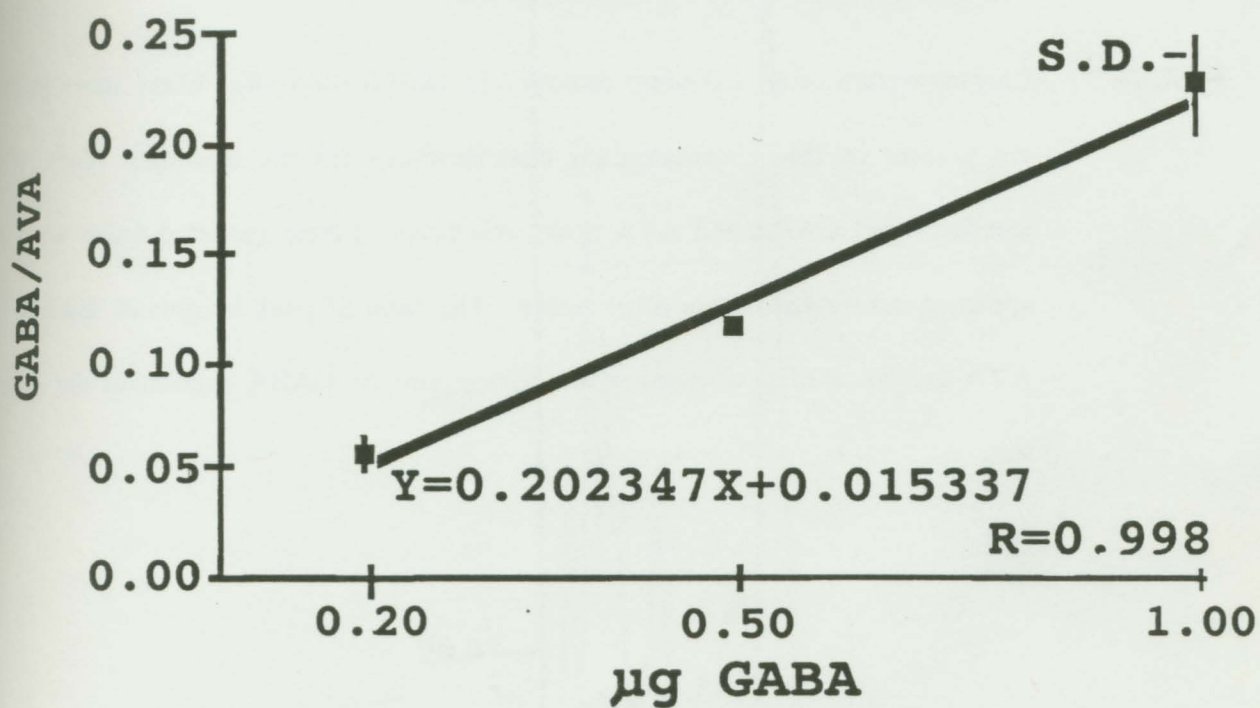


FIGURE 7. Chromatogram of an LD brain removed at 0600 h (0600-8). Many more peaks are present in this chromatogram than in those for the methanol blank and standards, but GABA and AVA peaks still occur at their expected times without apparent interference from other peaks. The ratio of peak heights of GABA to AVA can be used to determine the micrograms of GABA present in the brain sample.

Mean GABA levels determined for six roaches at each three-hour interval over a 24-hour time period.

TIME (h)

GABA LEVELS (ng/brain)

BRAIN 0600-8

0300

635 ± 25^a

0600

554 ± 93^a

0900

564 ± 42^a

1200

579 ± 36^a

1500

595 ± 52^a

1800

544 ± 13^a

2100

689 ± 71^b

2400

634 ± 31^b

80.0

AVA

60.0

40.0

20.0

GABA

0.0

TIME

Values reported are mean values \pm standard error.

Values with different letters are significantly different at the 95% confidence level.

determined by analysis of variance (ANOVA) and Student-Newman-Keuls test.

(SNK), $P \leq 0.05$.

TABLE I. Brain GABA levels determined for six roaches at each three-hour interval over a 24-hour time period.

TIME (h)	GABA LEVELS (ng/brain) ¹
0300	635 \pm 25 ^{2b}
0600	554 \pm 93 ^b
0900	564 \pm 42 ^b
1200	896 \pm 36 ^a
1500	595 \pm 52 ^b
1800	544 \pm 13 ^b
2100	689 \pm 71 ^b
2400	634 \pm 31 ^b

¹ Values reported are mean values \pm one standard error.

² Values with different letters are significantly different at the 95% confidence level as determined by analysis of variance (ANOVA) and Student-Newman-Keul's range test (SNK). $P \leq 0.05$.

FIGURE 8. Daily Pattern of Brain GABA Levels. The bar above the x-axis indicates the hours of light (unshaded) and dark (shaded) conditions. GABA levels were found to be highest at 1200 hours and lowest at 1800 hours.

TABLE II. Brain GABA levels determined at 1200 h and 1800 h in seven rats subjected to a LD 12:12 (LD) photoperiod schedule or during the second day of constant darkness (DD) at circadian times 1200 h and 1800 h.

Daily Pattern of Brain GABA Levels

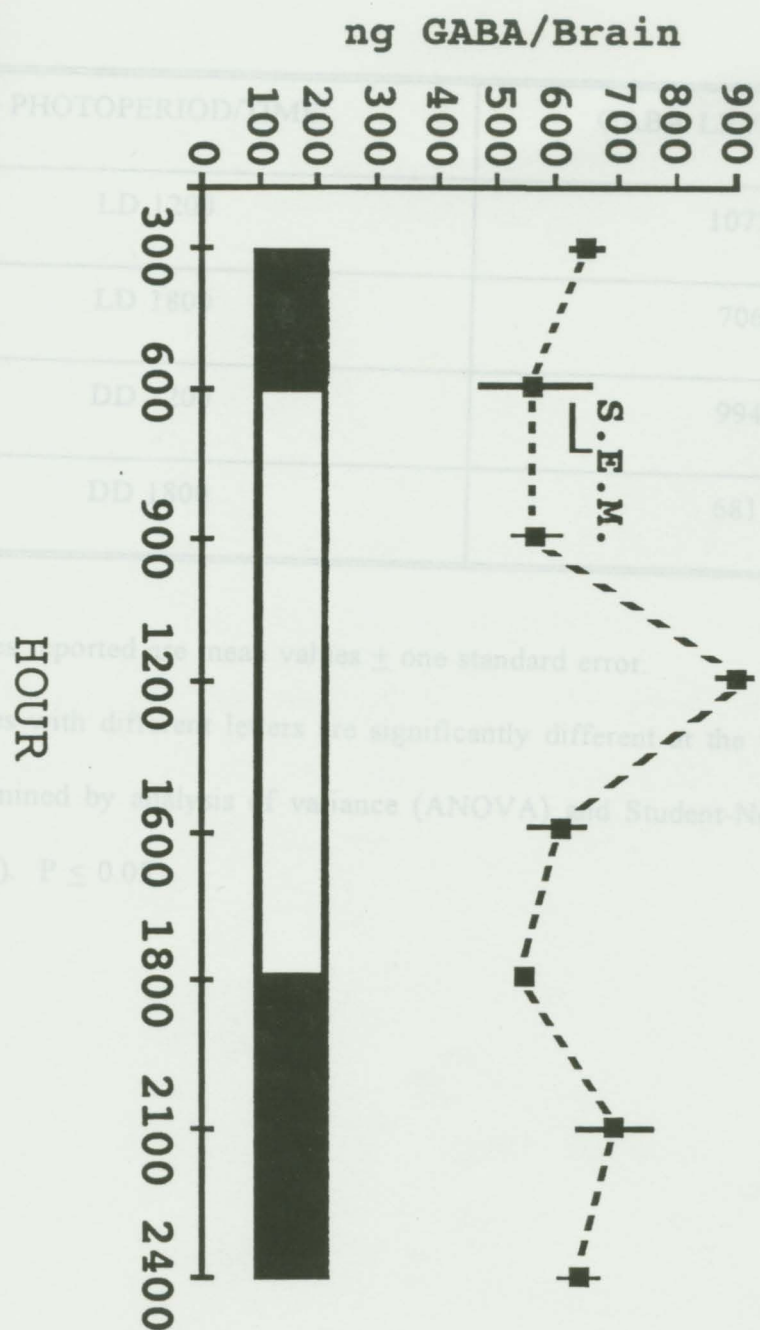


TABLE II. Brain GABA levels determined at 1200 h and 1800 h in seven roaches acclimated to a LD 12:12 (LD) photoperiod schedule or during the second day of constant darkness (DD) at circadian times 1200 h and 1800 h.

PHOTOPERIOD/TIME	GABA LEVELS (ng/brain) ¹
LD 1200	1073 \pm 63 ^{2a}
LD 1800	706 \pm 27 ^b
DD 1200	994 \pm 42 ^a
DD 1800	681 \pm 56 ^b

¹ Values reported are mean values \pm one standard error.

² Values with different letters are significantly different at the 95% confidence level as determined by analysis of variance (ANOVA) and Student-Newman-Keul's range test (SNK). $P \leq 0.05$.

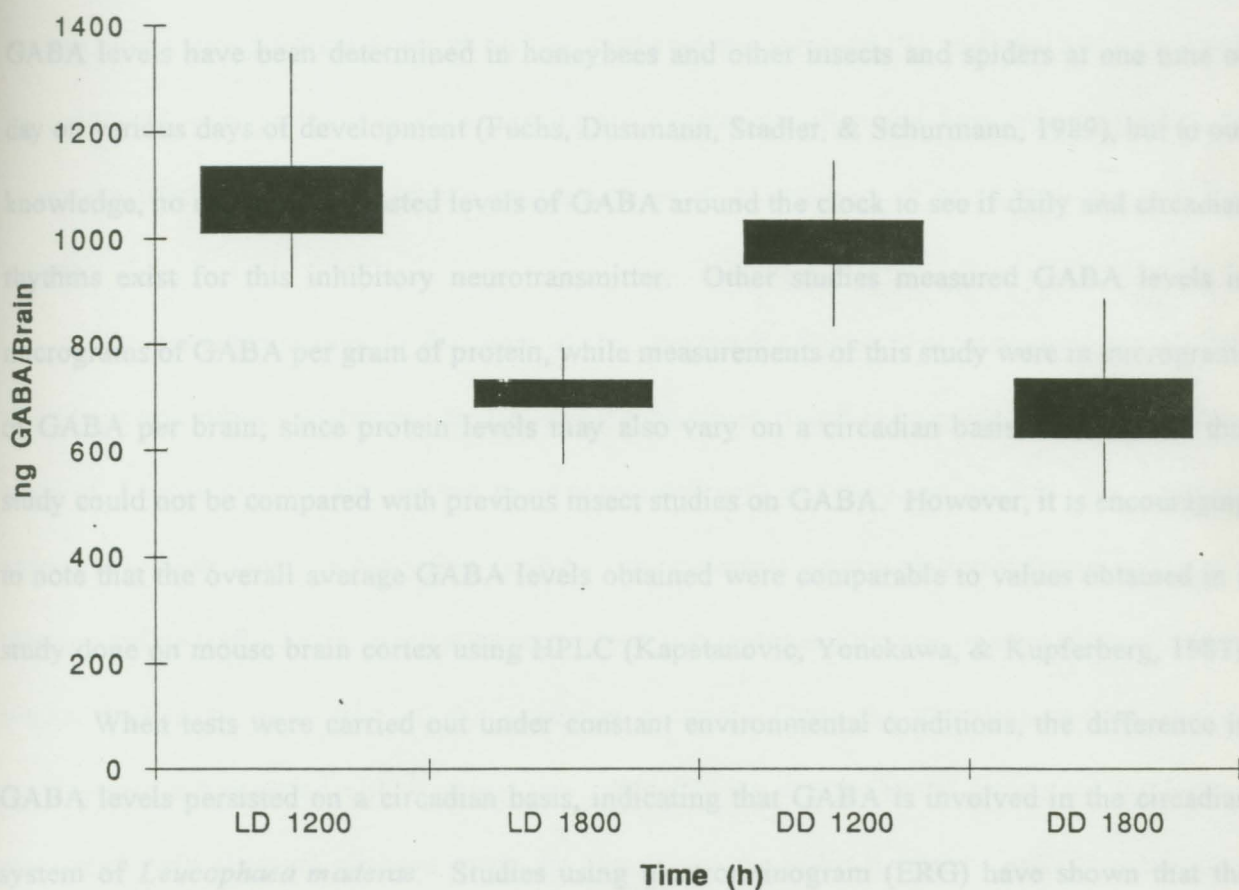
FIGURE 9. Brain GABA levels determined during times of high and low levels under LD and DD conditions. A difference was noted in GABA levels at 1200 hours and 1800 hours in cockroaches acclimated to a LD cycle similar to that in the previous experiment. This difference in GABA levels persisted for two days in animals subjected to DD conditions at circadian times 1200 and 1800 hours.

DISCUSSION

In this study, a daily pattern of GABA levels was detected with highest GABA levels at 1200 hours and lowest levels at 1800 hours. Levels at 1800 hours were similar to levels at other times of day, but 1800 hours was chosen for the circadian study since data from the LD portion of the experiment had the least standard deviation at this time of day. The fact that GABA levels were highest at a time of day of low activity (1200 h) corresponds with the fact that GABA is inhibitory. Prior to this study, GABA levels had not been determined in *Leucophaea maderae*.

GABA levels have been determined in honeybees and other insects and spiders at one time of day. In honeybees, GABA levels were determined at 1200 hours (Fachs, Dammann, Stadler, & Schürmann, 1989), but to our knowledge, no other studies have determined levels of GABA around the clock to see if daily and circadian rhythms exist for this inhibitory neurotransmitter. Other studies measured GABA levels in *Drosophila* (Fachs, 1990) and *Leucophaea maderae* (this study) in terms of GABA per gram of protein, while measurements of this study were in terms of GABA per brain, since protein levels may also vary on a circadian basis. This study could not be compared with previous insect studies on GABA. However, it is encouraging to note that the overall average GABA levels obtained were comparable to values obtained in a study of mouse brain cortex using HPLC (Kopelman, Yonckawa, & Kupferberg, 1987).

When tests were carried out under constant environmental conditions, the difference in GABA levels persisted on a circadian basis, indicating that GABA is involved in the circadian system of *Leucophaea maderae*. Studies using electroretinogram (ERG) have shown that the sensitivity of the compound eye to light exhibits circadian variation and that the highest off-transient peak amplitude occurs during the "subjective day" (i.e., circadian times of day 0000 h - 1800 h) (Willis, Page, & Colwell, 1986). Off-transient peak amplitude changes are regulated by the optic lobe pacemaker, which is suspected of generating inhibitory output. Since its levels of



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When tests were carried out under constant environmental conditions, the difference in GABA levels persisted on a circadian basis, indicating that GABA is involved in the circadian system of *Leucophaea maderae*. Studies using electroretinogram (ERG) have shown that the sensitivity of the compound eye to light exhibits circadian variation and that the highest off-transient peak amplitude occurs during the "subjective day" (i.e., circadian times of day 0600 h - 1800 h) (Wills, Page, & Colwell, 1986). Off-transient peak amplitude changes are regulated by the optic lobe pacemaker, which is suspected of generating inhibitory output. Since its levels of

highest output occur during the animal's perceived daylight hours under circadian conditions, results from this study are further corroborated since GABA levels were found to be highest at 1200 hours circadian time.

Through this research, the daily pattern of GABA in the brains of *Leucophaea maderae* was discovered, and this rhythm was determined to exist on a circadian basis. The implications of this study are widespread. Now that GABA has been found to be involved in the circadian system, further research can be conducted to determine if only one cell type determines the rhythm or if many different types of cells that interact are involved. In addition, research could be conducted with glutamic acid, an excitatory neurotransmitter and precursor to GABA, to determine whether or not these two compounds work in conjunction to control the circadian system.

The circadian system of the brain of the cockroach (Figure 1) is very analogous to that in humans and other vertebrates. In fact, GABA has been found to be present in high levels in areas of the vertebrate brain known to be associated with the circadian system. For instance, Ezzeil, C. (1993). This could be your brain on booze. *Science News*, 143 (1), 70. Moore and Speh found in 1993 that GABA is the principal neurotransmitter found in the rat's intergeniculate leaflet and suprachiasmatic nucleus, two regions of the mammalian central nervous system known to be a part of the circadian system. Once the circadian system and its mechanisms are mapped out, humans could benefit: the symptoms of jet lag or depression associated with seasonal changes might be eased, and the optimal times of day to administer prescription drugs might be determined. Also, since impaired GABA transmission has been implicated in certain neurological and psychological disorders (Gottlieb, 1988), the more that is understood about this neurotransmitter, the closer we are to understanding ailments as diverse as epilepsy, Alzheimer's disease, and alcoholism.

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