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Waterborne Lead Affects Circadian Variations of Brain Neurotransmitters in Fathead Minnows

R. E. Spieler,[1] A. C. Russo,[2] D. N. Weber[3]

[1]Oceanographic Center, Nova Southeastern University, Dania, Florida 33004, USA
[2]Department of Physical Therapy, California State University, Long Beach, California 90840, USA
[3]NIEHS Marine and Freshwater Biomedical Research Center, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201, USA

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Lead is a potent neurotoxin affecting brain levels of a number of vertebrate neurotransmitters. Reports on these effects are, however, not consistent either among or within species. For example, with lead-intoxicated rats there are reports of decreased acetylcholine (ACh) release and decreased ACh brain levels as well as reports of increased levels or no change in levels. Also, with rats there are reports of increased levels, decreased levels, or no change in brain catecholamines, with lead producing similar changes in both norephinephrine (NE) and dopamine (DA) in some cases and differences in response between the two in others. Although most early reports dealt with whole brain levels, reports on neurotransmitter levels in specific brain regions can be equally conflicting (for references see: Shih and Hanin 1978, Winder 1982, Shellenberger 1984). Similar sorts of discrepancies exist among studies with fishes (Katti and Sathyanesan 1986, Weber et al. 1991).

Much of the variation among studies on lead effects on neurotransmitters is, no doubt, due to differences among the studies in variables such as: species, age, dosage and duration, route of administration. However, lead can apparently affect circadian locomotor rhythms of both rats and fishes (Collins et al. 1984, Shafig-ur-Rehman et al. 1986, Weber et al. 1991). Therefore, another possible cause for the variation among studies is that there is an interaction among dosage, sampling time and endogenous rhythms. A lead-produced phase shift or disruption in endogenous neurotransmitter rhythms could in turn elicit a host of varying results and interpretations depending on the circadian time of sampling (Spieler 1992). We elected to examine this possibility in the fathead minnow, *Pimephales promelas*, a freshwater species widely used for toxicity studies.

Correspondence to: R. E. Spieler

MATERIALS AND METHODS

Fathead minnows of mixed sexes (2-3 years old) were obtained from a commercial dealer and placed in 16 60-L aquaria, 15-18 fish per aquarium. The aquaria were divided into two treatment groups of eight. One group received no lead (control) and the other was dosed with a stock solution of lead acetate so that the final concentration of lead in each aquarium was 1.0 ppm. Lead concentrations in both control (not detectable) and dosed aquaria were confirmed by atomic absorption spectrophotometry. Once a week the aquaria received a 10% water renewal and were dosed appropriately to reestablish the proper Pb concentration. The photoperiod was maintained at LD 12:12 with light onset at 0600 CST; water temperature was held at 15°C. The fish were fed once daily a commercial flake food (TetraMin SLM). Although the feeding time was not randomized per se, feedings were spaced throughout the light period to avoid entrainment by a fixed feeding time (Spieler and Noeske 1984). There were no deaths in either group during the period of treatment.

After 28 days of continuous dosing the fish were sampled at one of six times of day: 1000, 1400, 1800, 2200, 0200, 0600. Replicate aquaria for each time point were not feasible due to limited holding facilities. Two sets of replicate aquaria were, however, sampled at 2200 and at 1000 (the 1000 replicate was taken 24 hr after the first 1000 sample). At each sampling interval all the fish from a single aquarium were netted, with a single sweep of a dip net, and placed in an ice bath. Brains from eight fish were rapidly removed, weighed and immediately placed into capped tubes containing 100 μl 0.05 M perchloric acid, to prevent oxidation of the neurotransmitters, and frozen (-80^{0}C) until later HPLC analysis. Total elapsed time, for each group of eight fish, was less then 5 min from initial disturbance to freezing. Serotonin (5-HT), dopamine (DA), norephinephrine (NE), hydroxyindole-acetic acid (5-HIAA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and vanillylmandelic acid (VMA) were determined chromatographically (HPLC) with a two channel electrochemical detector (Saltzman et al. 1985). The limits of detection for this method are approximately 10-15 pg per sample. Due to holding facility and sample size limitations, brain lead levels were not determined. Neurotransmitters and metabolite levels were converted to per gram wet brain-weight for statistical analysis (there was no significant difference in brain-weights between treatments: control fish 19.8+/-0.5 mg, lead-exposed 20.8+/-0.6). Data analysis was aided by Statistical Analysis System (SAS)(Cary, NC) TTEST and GLM programs.

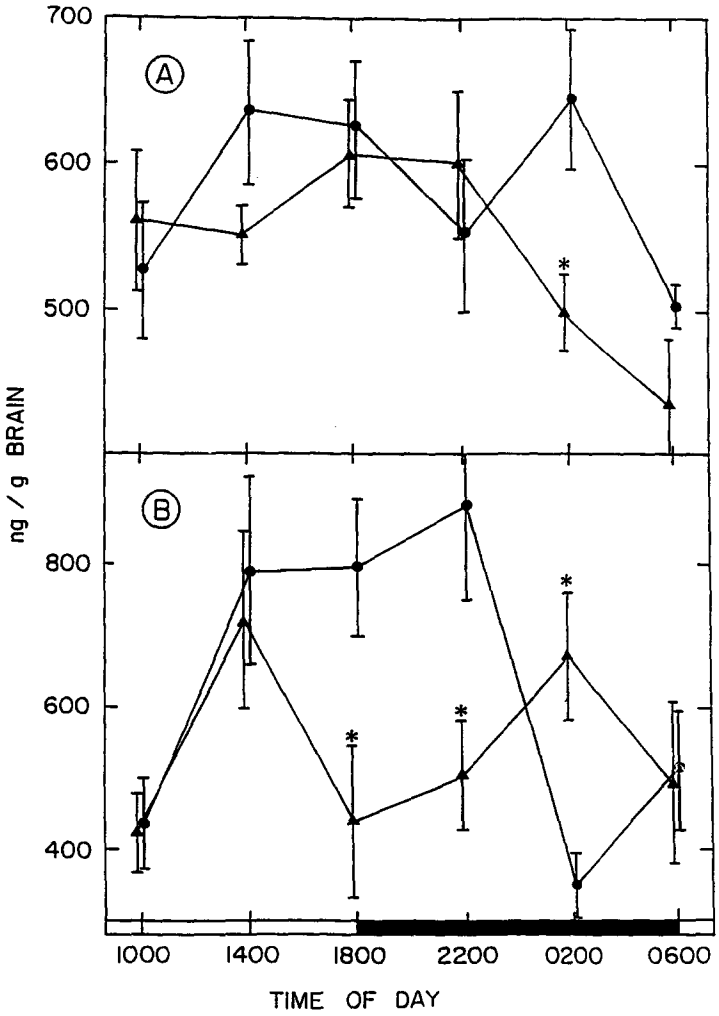

Figure 1. Brain levels (X ± SE) of norepinephrine (graph A), and VMA (B) of fathead minnows held in water containing 0 (nd., ●) or 1 ppm lead (▲) and sampled at one of six times of day. Asterisks indicate times of day when lead treated and control means differed (t-test, $P<0.05$).

RESULTS AND DISCUSSION

Initial statistics consisted of testing for differences between replicate aquaria sampled at 1000 vs. 24 h later and between replicate aquaria sampled at 2200. There was no significant difference ($P>0.05$, Bonferroni modified t-test) for any of the variables between replicate tanks (tanks receiving the same treatment and sampled at the same time-of-day) and the replicates were combined for further statistical analysis.

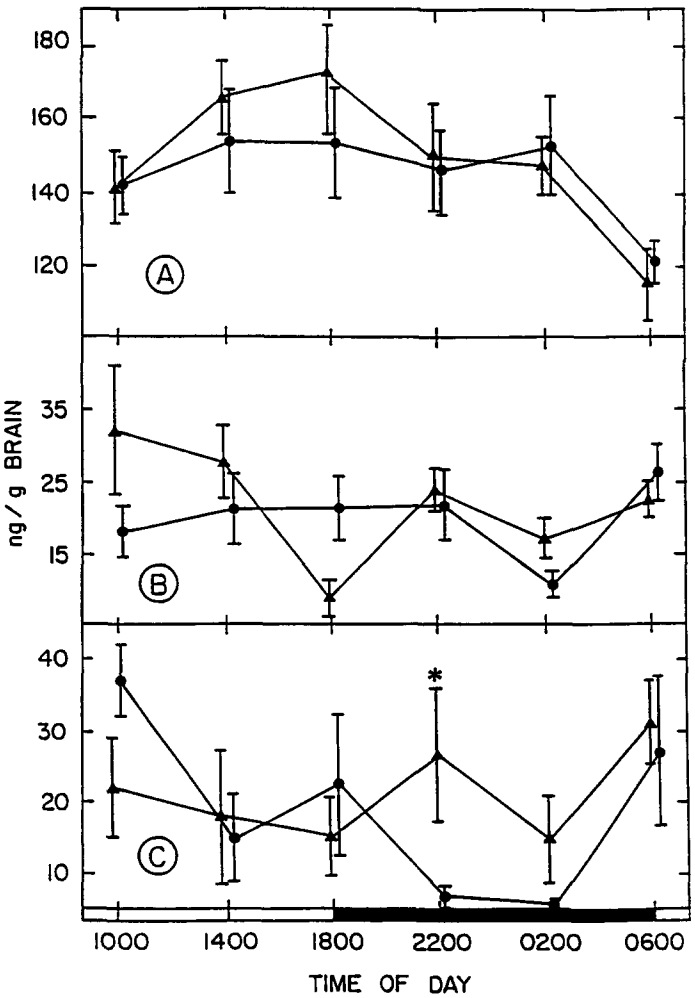

Figure 2. Brain levels (X ± SE) of dopamine (graph A), DOPAC (B) and HVA (C) of fathead minnows held in water containing 0 (nd.,●) or 1 ppm lead (▲) and sampled at one of six times of day. (* = t-test, $P<0.05$).

Although none of the neurotransmitters had a significant circadian variation among the six sampling times on either treatment (ANOVA, $P>0.05$), some of the metabolites did. Control fish exhibited a significant circadian variation of VMA and HVA; with lead-exposed fish 5-HIAA also varied (ANOVA, $P<0.05$) (Fig. 1-3). The fact that replicate aquaria were not different supports the conclusions that the variations noted are dependent on the time of sampling (not due to between tank differences - 1000 and 2200 replicates) and that these variations are circadian, eg. reproducible from day to day (1000 replicate).

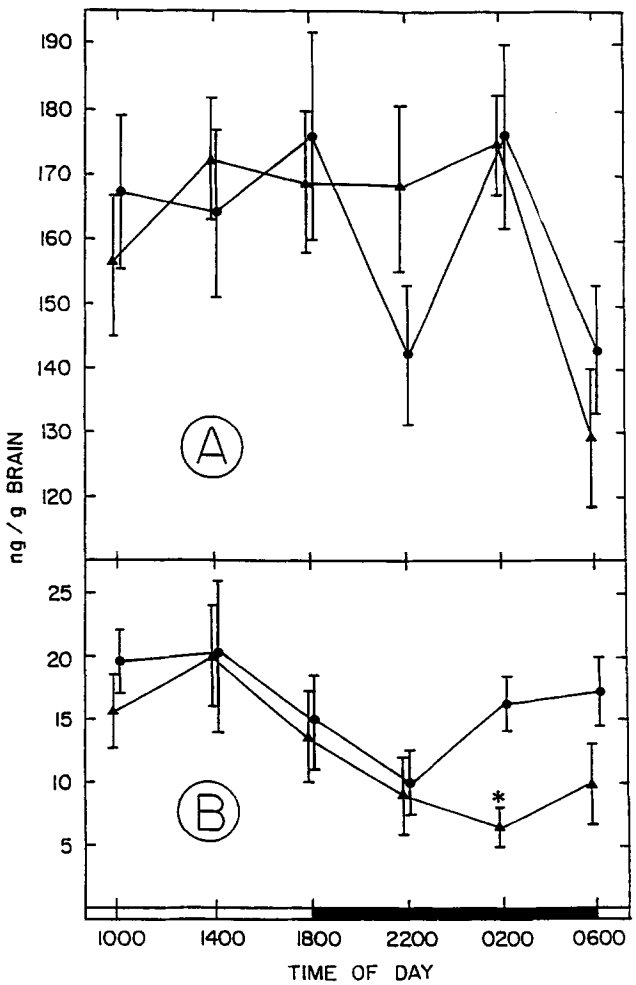

Figure3. Brain levels (X ± SE) of serotonin (graph A), and 5-HIAA (B) of fathead minnows held in water containing 0 (nd,●) or 1 ppm lead (▲) and sampled at one of six times of day. (* = t-test, $P<0.05$).

There was no difference in daily mean levels (mean of all samples throughout the day) between treatments. In contrast to the results with daily means, however, there were a number of times during the day when one or more of the variables differed between treatments (t-Test, $P<0.05$): 1800, VMA; 2200, VMA, HVA; 0200 NE, VMA, 5-HIAA (Fig 1-3).

The results of this study clearly demonstrate that lead does affect circadian variations of some neurotransmitters and their metabolites. Our experimental design, with a four hour interval between samples, was not intended to fully define the pattern of circadian changes in any of the variables we examined. Thus, we can not determine if the effects we noted are restricted to a certain portion of the day or if they represent full phase shifts in

endogenous rhythms. The possibility that these changes may be restricted to some times of day does, however, receive support from a visual examination of the skeletal outline provided by the six sampling times.

There does not appear to be an overall phase shift in any rhythm and the differences between treatments all occurred during one aspect of the photoperiod, the scotophase. Also, in a preliminary study with goldfish, lead produced increased activity during the scotophase (*authors, unpublished*).

The circadian effects of lead, noted in this study, may be responsible, in part, for the extreme variation noted among studies on lead effects on the CNS of vertebrates (see introduction). Researchers sampling at a single time of day could reach different conclusions on the effects of lead depending on the time of sampling. For example, with VMA, despite the fact that there was no significant differences in mean levels throughout the day, lead would appear to cause an increase if the sampling time was at 0200, and a decrease at 1800 or 2200. Similar conflicting conclusions could be reached with NE, HVA, and 5-HIAA (see Figs. 1-3).

In conclusion, the results of our study obviously highlight the need to take into account potential circadian changes when examining the effects of lead on neurotransmitter systems (and, by extension, on other variables affected by these neurotransmitters). We caution, however, in addition to lead, there are a multitude of aquatic contaminants that are both established neurotoxins as well as dyschronogens on a variety of rhythms outside the CNS. These compounds include other heavy metals, as well as solvents, pesticides, and herbicides. (Bengtsson and Larson 1981, Ikeda et al. 1981, Arito et al. 1982, Nicolau 1983a, b). A conservative assumption would be that these materials also affect neurotransmitter rhythms. Researchers working with such materials are counseled to take circadian variations and potential changes in these variations into account in their experimental designs.

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REFERENCES

Arito H, Sudo A, Hara N, Nakagaki K, Tori S (1982) Changes in circadian sleep-waking rhythms of rats following administration of methyl-mercury chloride. Industr Health 20:55-65

Bengtsson B-E, Larsson A (1981) Hyperactivity and changed diurnal activity in flounders, *Platichthys flesus*, exposed to DDT. Mar Pollut Bull 12:100-102
Collins MF, Hrdina PD, Whittle E, Singhal RL (1984) The effects of low-level lead exposure in developing rats: changes in circadian locomotor activity and hippocampal noradrenaline turnover. Can J Physiol Pharmacol 62:430-435
Ikeda T, Maehara N, Sadamoto I, Harabuchi K, Yamaura K, Miyake H (1981) Effects of toluene exposure on the rest-activity cycle of rats. Toxicol Lett 9:255-265
Katti SR, Sathyanesan AG (1986) Lead nitrate induced changes in the brain constituents of the freshwater fish *Clarias batrachus* (L). Neurotoxicology 7:47-52
Nicolau GY (1983a) Circadian rhythms of RNA, DNA and protein content in the rat thyroid, adrenal and testis in chronic pesticide exposure. II. Effects of the herbicides Aminotriazole and Arochlor. Endocrinologie 21:105-112
Nicolau GY (1983b) Circadian rhythms of RNA, DNA and protein content in the rat thyroid, adrenal and testis in chronic pesticide exposure. III. Effects of a insecticides (Dichlorvos and Trichlorphon). Rev Roum Morphol Embryol Physiol 20:93-101
Saltzman S, Llados-Eckman C, Hirofuji E (1985) Determination of spinal cord monoamines and metabolites using three micro columns and dual electrode detection. J Liquid Chromatogr 8:342-360
Shafig-ur-Rehman, Khushnood-ur-Reham, Kabir-ud-Din, Chandra O (1986) Differential effects of chronic lead intoxication on circadian rhythm of ambulatory activity and on regional brain norepinephrine levels in rats. Bull Environ Contam Toxicol 36:81-91
Shellenberger MK (1984) Effects of early lead exposure on neurotransmitter systems in the brain. A review with commentary. Neurotoxicol 5:177-212
Shih T-M, Hanin I (1978) Chronic lead exposure in immature animals: neurochemical correlates. Life Sci 23:877-888
Spieler RE, Noeske TA (1984) Effects of photoperiod and feeding schedule on diel variations of locomotor activity, cortisol, and thyroxine in goldfish. Trans Am Fish Soc 113:528-539
Spieler RE (1992) Circadian rhythms in fishes and their implications for research. IN: Schaeffer DO, Kleinow KM, Krulisch L (ed) The Care and Use of Amphibians, Reptiles and Fish in Research, Scientists Center for Animal Welfare. Bethesda, Maryland, p 161
Weber DN, Russo AC, Seale DB, Spieler RE (1991) Waterborne lead affects feeding abilities and neurotransmitter levels of juvenile fathead minnows (*Pimephales promelas*). Aquat Tox 21:71-80
Winder C (1982) The interaction between lead and catecholaminergic function. Biochem Pharmacol 31:3717-3721