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Chronic Exposure to Caffeine During Early Development Modifies Spatial Behavior in Juvenile Jewel Fish Schools

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BURGESS, J. W. *Chronic exposure to caffeine during early development modifies spatial behavior in juvenile jewel fish schools.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 137–140, 1982.—Thirty sibling African jewel fish (*Hemichromis bimaculatus*) were either chronically exposed to caffeine between 50 to 100 days of age (at a concentration of 14 mg/l) or reared without drug in a control environment. After caffeine was withdrawn, fish in each group were videotaped for 30 minutes while schooling in a large tank. From the video record, mean distances and coefficients of variation of spacing were computed for each fish and its 1st to 9th nearest neighbors in all observations. Spacing distances were nearly identical for the two groups, but the caffeine fish exhibited much more variability in their spacing behavior ($p<0.00001$). This study demonstrates an effect on behavior of moderate caffeine levels present throughout the early developmental period, and illustrates a quantitative method to test for such effects.

Jewel fish Caffeine Spatial behavior Early development

PREVIOUS studies have demonstrated that the presence of large doses of caffeine during development can cause birth defects [5, 19, 30, 35], fetal resorbtion [21] and increased mutation rates in cell culture [43]; but little is known about caffeine's effects on the development of behavior or the central nervous system in general. In this study we illustrate effects on a species-specific spatial behavior of chronic doses of caffeine administered during early development.

In order to avoid the use of large drug doses and to test for subtle drug effects, we tried to choose a sensitive measure of behavioral development. The locomotor behavior of fish is commonly used in assaying the toxicological effects of chemical agents [1, 2, 15, 29, 31, 40, 48] and environmental contaminants [2, 18, 25, 32, 34]. The species-specific geometrical patterns of fish schools, which result from synchronous locomotion and spacing responses, seem particularly sensitive to physiological manipulation in adult fish [16, 20, 38].

METHOD

Thirty jewel fish (*Hemichromis bimaculatus* Gill) from two spawns were reared with parents in standard 1.5-liter aquaria. At 50 days of age, 15 fry (standard length=2.09 cm ±0.55 SD) were placed into each of two 3.6-liter experimental aquaria where they remained for 50 days. These groups were maintained in aerated and filtered water at 27 degrees C, in a long-day (16:8 hr) light cycle, and provided twice daily with frozen brine shrimp for ad lib feeding.

The drug group was administered caffeine, U.S.P., at a dose level of 14 mg/liter, dissolved in aquarium water. This approximates the caffeine concentration in human plasma after about 4 cups of coffee (350 mg caffeine produces a plasma concentration of about 14 μg/ml; see [22]). A chronic, early dosage was used in order to model the worst expected condition of fetal exposure (see Discussion). The drug solution was changed every two days for the duration of the experiment.

Behavioral observations were noted during the course of the experiment. At 100 days of age (standard length=3.02 cm ±0.30 SD), fish from both groups were taken from the experimental aquaria and permitted to equilibrate for 24 hours in test aquaria containing 1000 liters of fresh water (aquarium dimensions=150 cm diameter × 57 cm). At that time, fish were videotaped from overhead for 30 minutes. Afterwards, the videotape was sampled at 1-minute intervals and the position of each individually identified fish was traced from the monitor onto a map of the test aquarium (see [8–14, 16]).

Measurement

Distances were measured between each individual and its 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th and 9th nearest neighbors on the paper maps of each observation. Animal positions were entered with a digitizing magnetic tablet (Texas Instruments Hi-Pad) into a microcomputer (Apple II) which calculated distances with the help of a computer program (available on request). The measuring precision of the tablet was 0.01 cm; overall error in the method was estimated to be less than 3% from measurements of a calibration grid.

[1]This research was conducted while the author held a position at the Department of Psychology, University of California, Davis, CA.

Analysis

Two behavioral variables were analyzed. As a measure of species-specific spacing, mean distances maintained by each animal to its 1st through 9th nearest neighbors were computed for every observation. These were averaged to provide one score for each animal. As a measure of individual spacing variability during the testing session, coefficients of variation (CV) of 1st to 9th nearest neighbor distances were calculated from the observations of each animal (coefficient of variation=SD/mean × 100, [39]). These provide a measure of variability that is independent of the magnitude of a measure (e.g., a CV=10 would demonstrate little variability, regardless of the quantity measured). Coefficients of variation were averaged to provide one score for each animal. Mean distances and coefficients of variation were compared by 2-factor repeated-measures analyses of variance (drug × 1st–9th neighbors, [7]).

RESULTS

The mean distances maintained by the caffeine and control groups to their 1st through 9th nearest neighbors were virtually identical (Fig. 1); $F(1,20)=0.746$; $p=0.40$. However, the spacing variability of the caffeine group, measured by coefficients of variation, far exceeded the controls for all 1st through 9th nearest neighbor distances (Fig. 2); $F(1,20)=126.817$; $p<0.00001$. Moreover, the coefficients of variation differed across 1st to 9th nearest neighbor distances, $F(8,160)=3.15$; $p=0.002$, with the greatest variability shown in 1st nearest neighbor distance.

Behavioral observations made during the experiment revealed expected acute caffeine responses in the drug group including increased respiration rate and extreme avoidance of the experimenter during feeding and tank cleaning periods; this persisted throughout the study. Both groups fed consistently; although the control fish grew slightly longer than the caffeine fish, subsequent measures of tectal thickness revealed no differences in the size of their brains, $F(1,8)=0.14$; $p=0.72$, nor in the relative thickness of layers within the brain (Burgess and Monachello, in press). Moreover, because the caffeine animals were allowed to acclimate and feed normally in the absence of caffeine before behavioral testing, it is unlikely that our behavioral results could stem from transient drug effects on activity or appetite.

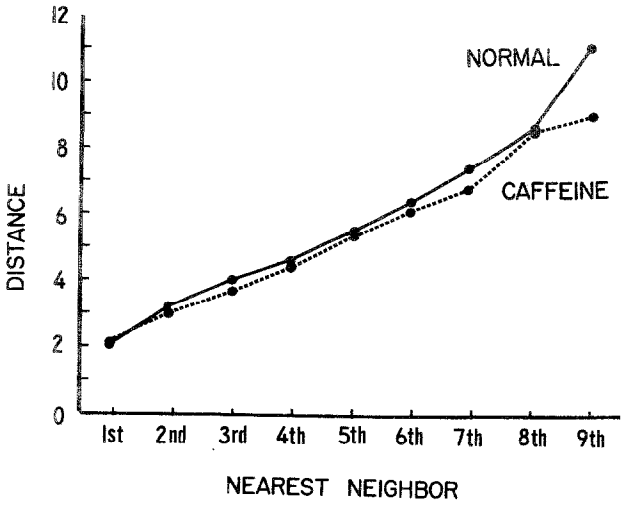

FIG. 1. Distance (cm) between fish and their 1st through 9th nearest neighbors while schooling in a large tank. In one group, 15 fish were exposed to caffeine (14 mg/l) between 50 to 100 days of age (dotted line), while fish in the other groups were controls reared without caffeine (solid line). Note the characteristic species-specific spacing distances exhibited by both groups.

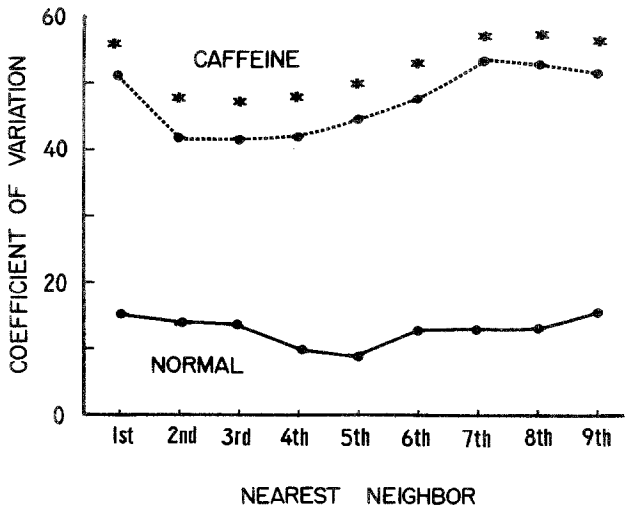

FIG. 2. Spacing variability (measured by coefficients of variation: %C.V.) between 1st through 9th nearest neighbors in drugged and control fish groups, corresponding to the distance measures in Fig. 1. Note that the fish exposed to caffeine during early development (dotted line) exhibit much more behavioral variability than the normal controls (solid line). These differences are statistically significant (*=$p<0.01$, Tukey's all pairs test).

DISCUSSION

Chronic adult effects of many pharmacological agents have been successfully evaluated by behavioral assays using complex, innate or species-specific behaviors in many animals (see reviews in [27, 36, 46]). However, even high, chronic doses of chemical agents may be remarkably nontoxic to the adult but highly dangerous for the developing organism (as witnessed in the thalidomide disaster of the 1960's [44]). Most of our information on developmental toxicity comes from animal screening studies, particularly those using behavioral assays [26]. Here we present such a technique, based on a species-specific behavior in fish, which was used to test for effects of moderate doses of caffeine administered during development.

Our results show that the variability of juvenile schooling behavior (measured by coefficients of variation of 1st through 9th nearest neighbor distances), increased after chronic administration of caffeine during the period of central nervous system development, although no drug was present at the time of the test. These behavioral measures, along with parallel evidence of changes in the maturation of brain neurons (Burgess and Monachello, in press), offer evidence that caffeine can alter the expression of early behaviors in at least this vertebrate species.

In this experiment we test for effects of chronic caffeine

exposure on the spatial behavior of juvenile fish during development. This method offers several advantages for the experimenter, since many fish mature rapidly, are convenient to house and maintain, and develop externally in constant contact with chemical agents dissolved in their environment. Schooling behavior occurs in 80% of known fish species under natural conditions and spacing relationships within schools are quite predictable and characteristic for each schooling species (see reviews in [12, 13, 16, 38]). Similar behavioral measures from a variety of other vertebrate species including primates are also available for comparison (e.g., [8–11, 14, 28, 42]).

Our measures of species-typical spacing behavior reflect many complex locomotor, orientation, and attentional responses. In this species, early spacing initially takes the form of aggregated schooling, first centering around the parents and later among siblings. In previous studies we have documented progressive changes in our distance measures during development [16] and as a result of early biological stressors like crowding or social deprivation [13,16]. These experimental manipulations changed the form of the spacing response, but not its variability; while caffeine changed variability but not spatial pattern. These studies suggest that caffeine effects are something quite different from biologically relevant changes in the rate of schooling development, which may reflect inabilities of the fish to execute the schooling behavioral program.

Our technique is not without its limitations. Fish are evolutionarily distant from most mammalian pharmacological subjects, and extrapolation to other species is difficult. Nevertheless, characteristic patterns of spacing to successive nearest neighbors are also known in primates, rodents, and bird [8, 9, 28, 41, 42], and spacing behavior has been found in other species to change in response to drugs. For example, when members of rhesus monkey troops received acute doses of THC, the daily variability of 1st nearest neighbor distances in the troop increased [14]. Chronic exposure to the drug, however, resulted in a decrease in daily variability of both distances to 1st nearest neighbors and mean distances between all neighbors. Acute doses of chlorpromazine, adrenaline or methamphetamine have also been found to decrease distances between nearest neighbors in rats [28,41], but variability of spacing was not measured in these studies.

The early effects of methylxanthines, including caffeine, theophylline and aminophylline, are of particular interest since these drugs are so commonly available to the central nervous system of the human during development. Caffeine can readily cross the placental barrier from the mother [23], in whom plasma levels may be as high as 3–30 μm/ml from the consumption of caffeinated beverages [22]. Moreover, methylxanthines (typically theophylline or aminophylline) are commonly administered to premature infants to combat episodes of respiratory failure (preterm apnea), thought possibly related to sudden infant death syndrome [6, 17, 33, 37]. The presence of caffeine may be more important in young children since they eliminate methylxanthines slower than do adults; for example, caffeine's half-life may be 10–40 times greater in preterm infants [4,24] than in adults. Thus, methylxanthines are seen to be present in significant amounts during early human development. Combined with previous evidence of developmental teratogeny of caffeine in large doses [5, 19, 21, 24, 35, 43, 45], our study indicates a need to know more about the effects of caffeine on biological processes during the critical developmental period.

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REFERENCES

1. Abramson, H. A. and L. T. Evans. Lysergic acid diethylamide (LSD 25): II. Psychobiological effects on the Siamese Fighting Fish. *Science* **120:** 990–991, 1954.
2. Abramson, H. A., A. Rolo, B. Sklarofsky and J. Stache. Production of cross-tolerance to psychosis-producing doses of lysergic acid diethylamide and psilocybin. *J. Psychol.* **49:** 151–154, 1960.
3. Anderson, J. W. Laboratory studies on the effects of oil on marine organisms: An overview. *Q. Am. Petrol. Inst.* **4249,** 1–70, 1975.
4. Aranda, J. V., D. S. Sitar, W. D. Parsons, P. M. Loughman and A. H. Neims. Pharmacokinetic aspects of theophylline in premature newborns. *New Engl. J. Med.* **295:** 413–146, 1976.
5. Bertrand, M., J. Girod and M. F. Rigaud. Ectrodactylie provoquee par la cafiene chez les rongeurs. Role des facteurs specifiques et genetiques. *C. r. Séanc. Soc. Biol.* **164:** 1488–1489, 1970.
6. Boutroy, M. J., P. Monin, M. Andre and P. Vert. Treatment with theophylline in near-miss sudden infant death. *Lancet* **1:** 1257, 1978.
7. Bruning, J. L. and B. L. Kintz. *Computational Handbook of Statistics.* Glenview, IL: Scott Foresman, 1977.
8. Burgess, J. W. Measurement of spatial behavior. *Behav. Neural Biol.* **26:** 132–160, 1979.
9. Burgess, J. W. Social group spacing of rhesus macaque troops (Macaca mulatta) in outdoor enclosures: Environmental effects. *Behav. Neural Biol.* **30:** 49–55, 1980.
10. Burgess, J. W. Development of social spacing in normal and mentally retarded children. *J. Nonverb. Behav.* **6:** 89–95, 1981.
11. Burgess, J. W. and D. Raulston. Territorial aggregation: An ecological strategy in acorn woodpeckers. *Ecology* **63:** 575–578, 1982.
12. Burgess, J. W. and E. Shaw. Development and ecology of fish schooling. *Oceanus* **22:** 11–17, 1979.
13. Burgess, J. W. and E. Shaw. Effects of acousticolateralis denervation in a facultative schooling fish: A nearest neighbor matrix analysis. *Behav. Neural Biol.,* **33:** 488–497, 1981.
14. Burgess, J. W., P. N. Witt, E. Phoebus and C. Weisbard. The spacing of rhesus monkey troops changes when a few group members receive Δ9THC or d-amphetamine. *Pharmac. Biochem. Behav.* **13:** 121–124, 1980.
15. Chessick, R. D., J. Kronholm, M. Beck and G. Maier. Effect of pretreatment with tryptamine, tryptophan and DOPA on LSD reaction in tropical fish. *Psychopharmacologia* **5:** 390–392, 1964.
16. Coss, R. G. and J. W. Burgess. Jewel fish retain juvenile schooling pattern after crowded development. *Devl Psychobiol.* **14:** 451–457, 1981.
17. Dietrich, J., A. N. Krauss, M. Reidenberg, D. E. Drayer and P. A. M. Auld. Alterations in state in apneic preterm infants receiving theophylline. *Clin. Pharmac. Ther.* **24:** 474–478, 1978.
18. Dixit, D. and J. W. Anderson. Distribution of napthalenes within exposed Fundulus simulus and correlations with stress behavior. In: *Proceedings of the 1977 Oil Spill Conference.* Washington, DC: American Petroleum Institute, 1977, pp. 633–636.

19. Fujii, T. and H. Nishimura. Adverse effects of prolonged administration of caffeine on rat fetus. *Toxic. appl. Pharmac.* **22:** 449–457, 1972.
20. Gardner, G. R. Chemically induced lesions in estuarine or marine teleosts. In: *The Pathology of Fishes,* edited by W. Ribelin and G. Migaki. Madison: University of Wisconsin Press, 1974, pp. 657–693.
21. Gilbert, E. F. and W. R. Pistey. Effect on the offspring of repeated caffeine administration on pregnant rats. *J. Reprod. Fert.* **34:** 495–499, 1973.
22. Gilman, A. G., L. S. Goodman and A. Gilman. *The Pharmacological Basis of Therapeutics.* New York: MacMillan, 1980.
23. Goldstein, A. and R. Warren. Passage of caffeine into human gonadal and fetal tissue. *Biochem. Pharmac.* **11:** 166–168, 1962.
24. Hendeles, L., M. Weinberger and L. Bighley. Disposition of theophylline after a single intravenous infusion of aminophylline. *Am. Rev. resp. Dis.* **118:** 97–103, 1978.
25. Hiatt, R. W., W. Naughton and D. C. Matthews. Effects of chemicals on a schooling fish, Kuhlia sandvicensis. *Biol. Bull. mar. biol. Lab.,* **104:** 28–44, 1953.
26. Hutchings, D. E. Behavioral teratology: Embryopathic and behavioral effects of drugs during pregnancy. In: *Early Influences,* vol. 4, edited by G. Gottleib. New York: Academic Press, 1978, pp. 7–35.
27. Joffe, J. M. *Prenatal Determinants of Behavior.* Oxford: Pergamon, 1969.
28. Joy, V. and B. Latané. Anatomic arousal and affiliation in rats. *Psychon. Sci.* **25:** 299–300, 1971.
29. Keller, D. L. and W. W. Umbreit. Chemically altered "permanent" behavior patterns in fish and their cure by reserpine. *Science* **124:** 407, 1956.
30. Knoche, C. and J. Konig. Zur pranatalen Toxizitat von Diphenyl-pyralin-8-chlor-theophyllinat unter Berucksichtingung von Erfahrungen mit Thalidomid und Coffein. *Arzneimittel-Forsch.* **14:** 415–424.
31. Kristoffersson, R., W. Broberg and A. Oikari. Physiological effects of a sublethal concentration of phenol in the pike (Esox lucius L.) in pure brackish water. *Annls zool. fenn.* **10:** 392–397, 1973.
32. Kuhnhold, W. W. The influence of crude oils on fish fry. In: *FAO Technical Conference on Marine Pollution and Its Effects on Living Resources and Fishing,* MP/70/E-64. Rome: Food and Agriculture Organization of the United Nations, 1970, pp. 1–10.
33. Kuzemko, J. A. and J. Paala. Apnoeic attacks in the newborn treated with aminophylline. *Archs Dis. Childh.* **48:** 404–406, 1973.
34. Morrow, J. E. Oil-induced mortalities in juvenile coho and sockeye salmon. *J. mar. Res.* **31:** 135–143, 1973.
35. Palm, P. E., E. P. Arnold, P. C. Rachwall, J. C. Leyczek, K. W. Teague and C. J. Kensler. Evaluation of the teratogenic potential of fresh-brewed coffee and caffeine in the rat. *Toxic. appl. Pharmac.* **44:** 1–16, 1978.
36. Poshivalov, V. P. *Ethological Atlas for Pharmacological Research in Laboratory Rodents.* Moscow: VINITI, 1978.
37. Shannon, D. C., F. Gotay, I. M. Stein, M. C. Rogers, I. D. Todres and F. M. B. Moylan. Prevention of apnea and bradycardia in low-birthweight infants. *Pediatrics* **55:** 589–594, 1975.
38. Shaw, E. Schooling in fishes: Critique and review. In: *Development and Evolution of Behavior,* edited by L. R. Aronsen, E. Tobach, D. S. Lehrman and J. S. Rosenblatt. San Francisco: W. H. Freeman, 1970, pp. 452–480.
39. Sokal, R. R. and F. J. Rohlf. *Biometry.* San Francisco: W. H. Freeman, 1969.
40. Struhasker, J. W., M. B. Eldridge and T. Echeverria. Effects of benzene (a water-soluble component of crude oil) on eggs and larvae of Pacific herring and northern anchovy. In: *Pollution and Physiology of Marine Organisms,* edited by F. J. Vernberg and W. B. Vernberg. New York: Academic Press, 1974, pp. 253–284.
41. Syme, L. A. and G. J. Syme. Effects of chlorpromazine and metamphetamine on sociability in rats. *Psychopharmacology* **32:** 81–84, 1973.
42. Syme, L. A. and G. J. Syme. The role of sex and novelty in determining the social response to lithium chloride. *Psychopharmacology* **40:** 91–100, 1974.
43. Timson, J. Caffeine. *Mutat. Res.* **47:** 1–52, 1977.
44. Warkany, J. and H. Kalter. Etiology and prevention of congenital malformations. *Proceedings of the Biregional Institute of Maternity Care-Primary Prevention.* Berkeley: University of California School of Public Health, 1964, pp. 102–121.
45. Weathersbee, P. S., L. K. Olsen and J. R. Lodge. Caffeine and pregnancy: A retrospective study. *Postgrad. Med.* **62:** 64–69, 1977.
46. Witt, P. N. Drugs alter web-building of spiders. *Behav. Sci.* **16:** 98–113, 1971.
47. Young, R. D. Developmental psychopharmacology: A beginning. *Psychol. Bull.* **67:** 73–86, 1967.
48. Waluga, D. Phenol effects on the anatomico-histopathological changes in bream (Abramis brama L.). *Acta hydrobiol.* **8:** 55–78, 1966.