Pharmacology Biochemistry & Behavior, Vol. 30, pp. 199–203. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

BRIEF COMMUNICATION

A New Device for Monitoring Early Motor Development: Prenatal Nicotine-Induced Changes

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Received 13 July 1987

SCHLUMPF, M., M. GÄHWILER, U. RIBARY AND W. LICHTENSTEIGER. *A new device for monitoring early motor development: Prenatal nicotine-induced changes.* PHARMACOL BIOCHEM BEHAV **30**(1) 199–203, 1988.—A new type of activity meter has been designed especially for young rats. It consists of a warmed platform for the animal, a TV camera with monitor and a microprocessor. The TV camera detects the animal as a black figure on a light background. This picture is digitalized and stored in a Z80 microprocessor. Every 200 msec a new image is compared to the foregoing one. The total number of black points that are changing from black to white and vice versa provides a measure for motor activity of the animal. Prenatally nicotine-treated rat pups were tested on the activity meter. The developmental pattern of motor activity was different for male and female pups. Motor activity of nicotine-treated male pups differed significantly from controls at postnatal days 7 and 15 while this drug effect was not seen in females.

Activity meter Motor activity Nicotine Prenatal drug treatment

MOTOR activity is one of the prominent manifestations of a normally functioning nervous system. It is known to be very sensitive to many kinds of environmental influences especially during pre- and postnatal development of the brain. Testing motor activity has become a useful diagnostic tool in order to evaluate adverse developmental impacts.

Disturbed motor patterns in pups have been reported after prenatal exposure to several drugs: benzodiazepines [2], opiates [8], alcohol [9], nicotine [6,7] and environmental pollutants such as lead [5]. Some of these studies clearly show that developmental profiles of motor activity provide better information on behavioral deficiencies in the offspring than single activity measurements at individual postnatal stages. Yet, currently available activity meters still lack sufficient resolution for evaluating motor activity patterns in single neonate pups. In the following, we describe a newly designed activity meter with a high resolution capacity suitable also for detection of minute motor activity in the neonate and very young rat pup.

METHOD

The activity meter consists of an activity platform, a TV camera with monitor and a microprocessor. TV camera and platform are shielded against scattering light by a dark screen with an open slit on the operating side (ca. 1/2 platform width, Figs. 1 and 2). The Plexiglas platform is built as an illuminated table with light bulbs (Philips 220 V PF 712 E) emitting red light of 760 nm wave length. The surface temperature of the platform is set at 30°C (±2°C). The TV camera (Bosch TYK 9A) equipped with a Heimann (Siemens) multidiodic tube is fixed in a vertical position above the platform. It detects the animal as a black figure on a light background. This picture is digitalized to 64 lines with 64 points=4096 points by a comparator and stored in a Z80 processor. During measurements, the monitor shows the digitalized image. For focussing of the image, the camera can be switched to the monitor.

Movements are detected as follows: Two video signals are digitalized and stored at an interval of 200 msec (equal to 4 complete pictures of the camera). The computer then scores for differences (changed bits) between the second and the first picture by comparing the total number of black points at the two time points. Every 200 msec a new image is compared with the preceding one. The system provides 3 types of data: (1) The total number of black points is determined before measurements start. It is a measure of the projection surface and hence, of the size of the animal. (2) Activity is expressed as the mean number of points exhibiting a change. This value is newly calculated every 200 msec for the period starting with the onset of measurements. (3) Ac-

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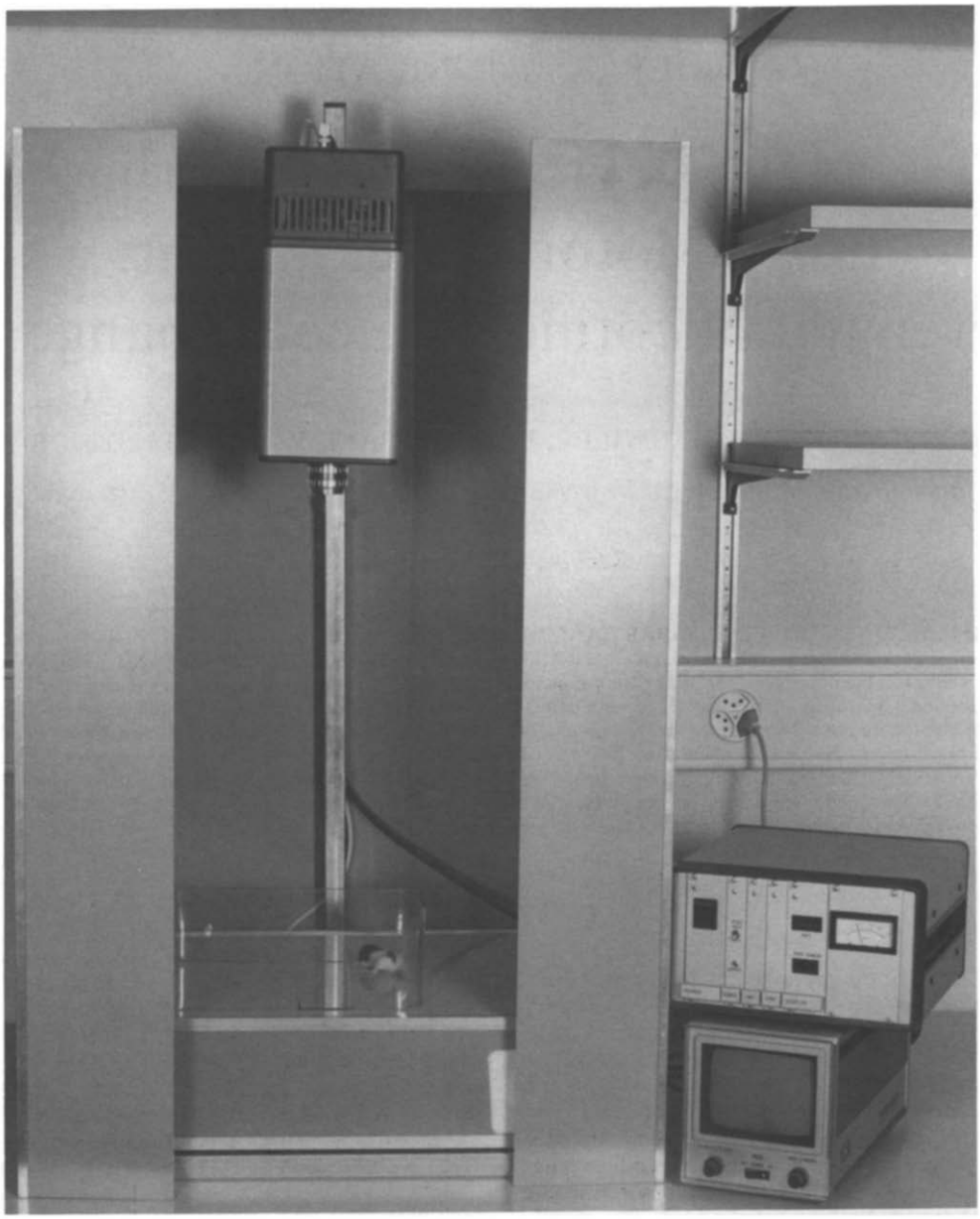

FIG. 1. Activity meter with TV camera and monitor, warmed Plexiglas platform, shielded from bright daylight, and the Z80 microprocessor.

tivity readings can be continuously plotted on a Y-T chart recorder through an analog output. The voltage is proportional to the number of points changed within 200 msec (0–5 V per 0–100 points).

Examination of Prenatally Nicotine-Exposed Rat Pups

The system was tested in prenatally nicotine-treated pups. Drug treatment was done by implanting time pregnant Long-Evans rats with an osmotic minipump (Alzet 2001) in light ether anesthesia at gestational day (GD) 12 [3]. The pump was filled with either nicotine hydrogen tartrate (BDH Chemicals Ltd., Poole, England) or tartaric acid dissolved in water. Nicotine was delivered at a rate of 25 μg/100 g $\times$ hour from GD 12 to 19. No difference in weight gain between nicotine-treated and control dams was seen. At birth, litter size was reduced to 8–10 pups. The developmental pattern of motor activity was studied in pups raised by their own mothers. No significant changes in motor patterns between these pups and cross-fostered pups (raised by control mothers) could be detected (Table 1). Also weight gain did not differ significantly between nicotine-treated and control pups. Motor activity was measured 2 to 3 hours after onset of the dark period (lights on 02.00–16.00). The litter was separated from the dam for 20 to 30 minutes in order to avoid differences resulting from the pups condition (rest-

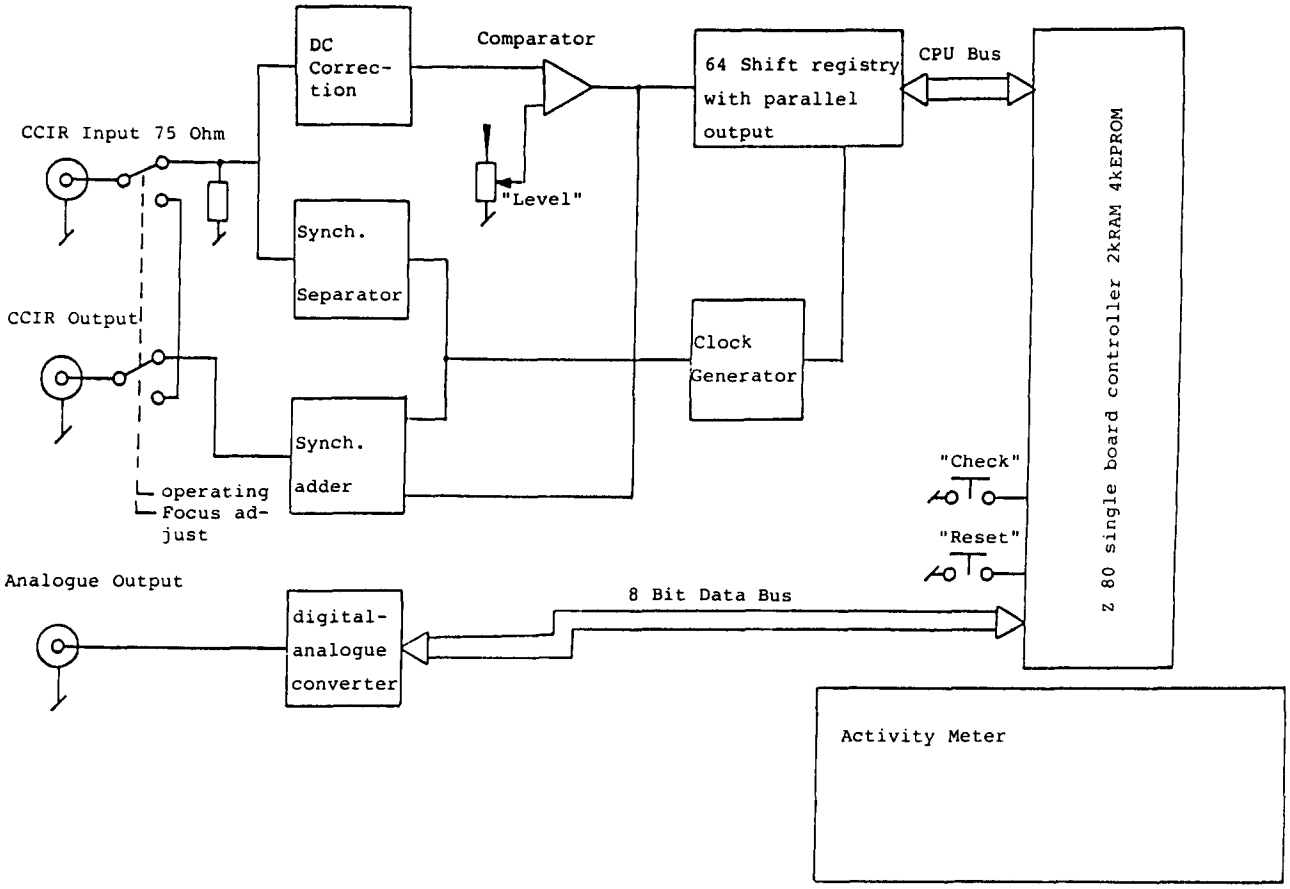

FIG. 2. Diagram of the activity meter.

ing/lactating) immediately before the testing. Thereafter, individual pups were placed onto the platform and activity was recorded during 3 min for periods of 1 min.

RESULTS AND DISCUSSION

Between PN 4 and 11 (day of birth=PN 1), a 5- to 7-fold increase in motor activity was noted in all groups (Fig. 3), then activity plateaued in controls of both sexes. In line with earlier studies [8], we observed in additional experiments a decrease of activity between the second and third week of life (M. Schlumpf, unpublished). Prenatally nicotine-exposed male pups displayed reduced values of activity at PN 7, i.e., a delayed rise in motor activity, and values above control levels at PN 15. In contrast, motor activity of female pups did not appear to be influenced by prenatal nicotine treatment. The stage of PN 7 was reexamined in cross-fostered pups (Table 1). The results were the same irrespective of whether the pups had been raised by previously nicotine- or tartaric acid-treated dams. Prenatal nicotine exposure has previously been found to affect spontaneous activity in adult offspring [6]. In a more recent study, Peters and Tang [7] noted a sex difference in the drug effect, with only male offspring being affected. Since their report refers to later developmental stages ($3^1/_2$ weeks until adulthood), their observation of a reduction in some parameters of spontaneous activity cannot be directly compared with our data. However, both sets of data indicate a preferential involvement of male offspring, which, according to our own data, can be detected already at an early prepubertal stage. In this context, it is interesting to note that the alterations seen in central catecholamine systems after prenatal nicotine exposure also differ between sexes; in adulthood, male offspring again appear to be more affected [4]. These data illustrate that the activity meter described here is well suited to study developmental profiles of motor activity in single rat pups. Since the apparatus is based on an analysis of the outlines of a black and white picture continuously recorded by the television camera, it allows the detection of small movements of the body parts of very young rat pups. The principle of analyzing the outlines of a black figure against a bright background renders the analysis independent of the color of the animal (e.g., albino vs. colored rats). Red light was combined with a red-sensitive TV camera for two reasons: (1) This combination allows for measurements during the dark period. (2) At the same time, the translucent platform is warmed; this helps to avoid a reduction of body temperature in young, furless animals separated from their mother. The short time-span needed for individual measurements further reduces the stress to which the pup is exposed. An important point to be considered are differences in the size of the animal which produces differences in both total count of

TABLE 1

MOTOR ACTIVITY IN CROSS-FOSTERED OFFSPRING OF NICOTINE OR TARTARIC ACID TREATED LONG EVANS RATS AT POSTNATAL DAY 7

Sex	Treatment in Pregnancy	Treatment in Pregnancy of Dam Raising the Pups	Activity Units	N
Male	nicotine	nicotine dam	0.8383 ± 0.1274	12
	nicotine	control dam	0.7586 ± 0.1311†	7
	control	nicotine dam	1.2326 ± 0.0693*	8
	control	control dam	1.2222 ± 0.1171*†	11
Female	nicotine	nicotine dam	0.8729 ± 0.0768	12
	nicotine	control dam	0.7474 ± 0.070†	9
	control	nicotine dam	0.9544 ± 0.0852	8
	control	control dam	0.8476 ± 0.0983†	10

*Significant differences ($p<0.05$) exist between nicotine-control dam and control-control dam, and between nicotine-nicotine dam and control-nicotine dam groups in male pups.

†Not significantly different from corresponding group raised by dam treated with nicotine during pregnancy.

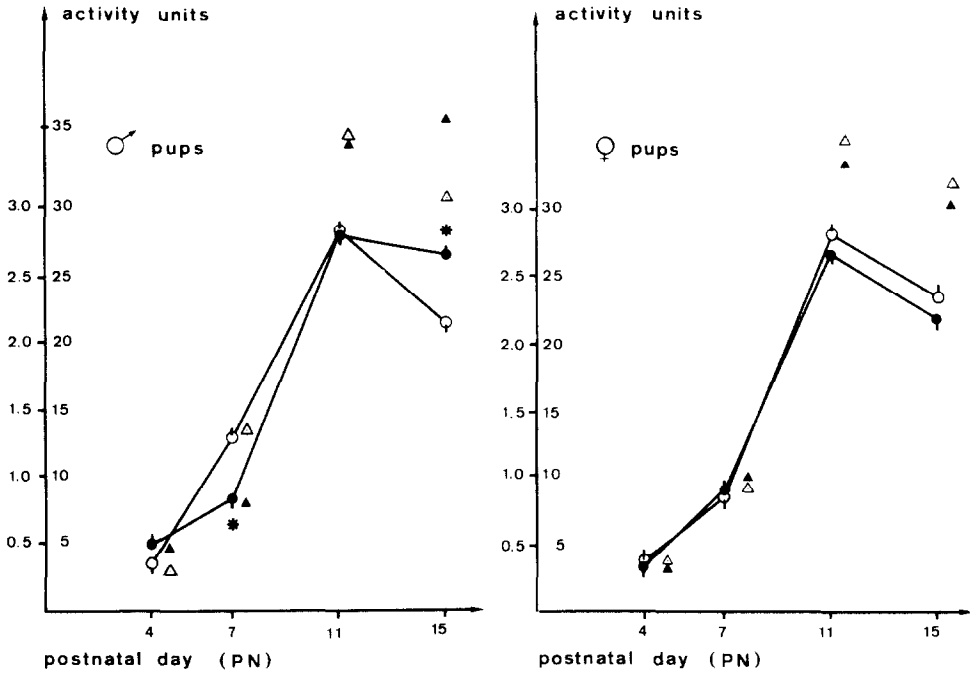

FIG. 3. Effect of prenatal nicotine treatment on motor activity of male and female offspring at PN (postnatal day) 4, 7, 11 and 15. Left: males, right: females; circles=values corrected for body surface (see text); triangles=uncorrected values; solid symbols=nicotine-treated; empty symbols=control pups. Motor activity of prenatally nicotine-treated male pups was different from control at PN 7 and 15 (Mean±S.D., n=10–16 pups; *=$p<0.05$, Student's t-test).

black points and number of points changed. If the animals are of comparable size, the analysis can be based on absolute values. In order to account for differences in size, the mean number of points exhibiting a change is related to the circumference of the black area. This can be approximated by the square root of the black area (=total numbers of black points) as done in our experiments. Alternatively, the activity may be related to the surface area=total number of black points covered by the animal (Fig. 3).

Development of early postnatal motor behavior is complex. It involves several different tasks, i.e., elevation of the head, shoulder and pelvis. Elevation of the shoulder is asso-

ciated with the functional maturation of the forelimbs supporting the weight of the anterior part of the body. Raising head and anterior part of the body is in part responsible for the increase in motor activity seen from PN 4 to PN 7. Within this time period additional spontaneous forelimb movements are seen with the pelvis remaining on the platform and the hindlegs not yet capable to support the body. This kind of circular movements by the forepaws is also called "pivoting" [1]. The steep increase in motor activity seen from PN 7 to PN 11 results from the developing support of the pelvis by the hindlegs. The quadruped position eventually attained allows the animal to crawl and walk. Quantification of all these separate movements would involve close observation of single animals during a prolonged period of time. Such a procedure is quite strainful especially to the very young animal. Multiple observation sessions necessary to obtain developmental profiles would represent a considerable experimental stress factor. While such detailed qualitative information is lost with the use of the activity meter, the system allows to establish developmental profiles of motor activity with comparatively little stress to the young animal.

ACKNOWLEDGEMENT

The study was supported by Swiss National Foundation grant 3.519-0.83.

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