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# Spontaneous Motor Activity Increases After Portacaval Anastomosis in Rats[1]

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CAMPBELL, A , B. JEPPSSON, J H. JAMES, V. ZIPARO AND J E FISCHER *Spontaneous motor activity increases after portacaval anastomosis in rats* PHARMACOL BIOCHEM BEHAV **20**(6) 875–878, 1984 —Spontaneous motor activity was monitored in rats at various times, up to 14 weeks, after portacaval anastomosis (PCA) or sham operation Total 24 hour activity scores rose significantly after PCA and remained significantly higher than those of sham operated rats for twelve weeks After PCA, activity during the twelve hour lighted period was greatly increased, whereas dark period activity was unchanged Increased activity in the light period was found to be well correlated with the degree of elevation in brain concentrations of tryptophan, tyrosine, phenylalanine and glutamine These results are consistent with previously reported EEG studies and suggest that motor-activity measurement may facilitate the correlation of biochemical changes with behaviour in rats with portal systemic shunting.

Portacaval anastomosis Hepatic encephalopathy Locomotor activity Serotonin
Behaviour after portacaval anastomosis

THE rat with a surgically created portacaval anastomosis (PCA) is widely used as an experimental model of chronic liver disease with portal-systemic shunting of the circulation. The use of this model has permitted detailed study of several neurochemical consequences of portal-systemic shunting, such as altered ammonia metabolism [3], increased brain serotonin turnover [4,5] and altered blood-brain transport of amino acids [10]. In the rat with PCA, behavioral disturbances comparable to human portal-systemic encephalopathy are not obvious Tricklebank reported decreased ambulation in an open field after PCA but no change in other motor activities such as grooming or rearing [18]. He also reported positive correlation between ambulation in an open air field and brain tryptophan concentration [19]. Encephalographic studies in rats after PCA have shown significant reduction in sleep-associated electrical activity of cortical and subcortical structures [2,15]. In order to identify behavioral abnormalities which could be correlated with neurochemical changes, we measured the spontaneous locomotor activity of rats, both before and after PCA or sham-operation as well as brain amino acids at sacrifice. We show here that after PCA, spontaneous motor activity is greatly increased during the normal sleep period and this increase parallels the increase in some brain neutral amino acids.

## METHOD

Adult male Sprague-Dawley rats weighing 175–225 grams (Charles River Laboratories, Wilmington, MA) were used. Experimental animals were anaesthetized with ether and a portacaval anastomosis was performed by a non-suture technique in 15 animals [7]. Using clean, but not sterile technique, the abdominal wall was opened on the midline; the portal vein was exposed, dissected and ligated. The clamped porta vein was then cut free and fed through a small piece of Teflon tubing previously sculptured to form a button of the type used for vascular anastomoses. The vein was everted over the button and secured with a silk ligature; the vein-button assembly was then anastomosed to the partially clamped vena cava with a purse string suture. After removal of the clamps, the patency of the shunt was checked by the rapid return of normal color to the bowel. Ten sham operated controls received laparotomy and exposure and manipulation of the splanchnic viscera for approximately ten minutes (Experiment 1).

Activity was measured using a Stoelting six-channel electronic activity monitor (EAM, Stoelting Company, Chicago, IL). The six activity sensors were calibrated and standardized using a rotating bar magnet. The sensors were housed in an electrically shielded and grounded enclosure to eliminate external electromagnetic artifacts, and were spaced at least 50 cm apart to prevent radio frequency coupling. Monitor gains were set at 0.7 mA and activity levels were set at a level which permitted the recording of locomotion plus a wide range of motor activities such as grooming, but not resting activities such as breathing or spontaneous artifactual discharge from the sensor.

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The animals were tested individually in plastic cages (42×20×15 cm) with food and water freely available Between experiments the animals were housed in groups of four in cages, identical to the testcages. Continuous activity scores were automatically printed at one hour time intervals over a 24 hour period and the light cycle was the same as the home cages, 07 00 hour, on—19 00 hour, off. Preoperative recording for each animal was made on three consecutive days. After either PCA or sham operation, 24 hour recordings were made at weekly intervals for one month and then at two week intervals thereafter. In addition, at 19 days postoperation, recording were made for three consecutive days in five animals from each group selected at random.

In a separate experiment (Experiment 2) spontaneous motor activity was correlated with brain neutral amino acids A group of 15 rats underwent PCA and their activity was monitored as above When, by the tenth week, the activity of seven of the 15 rats had returned to within two standard deviations of the normal mean, all 15 rats were sacrificed and plasma and brains taken for amino acid analyses. Free amino acids of plasma and brain of each animal were analyzed using a Beckman 121-MB automatic amino acid analyzer. Plasma was deproteinized by mixing with an equal volume of 7 5% sulfosalicylic acid (SSA) pH 1 7, containing an interval standard. The brains were blotted to remove adhering blood and frozen on dry ice. Each brain was homogenized in three volumes (3× weight) of 5% SSA, pH 1.4 containing an internal standard.

Data were subjected to analysis of variance and the Newman-Keuls test. Analysis of correlation was performed according to Pearson product-moment.

## RESULTS

Food intake after PCA was depressed for 5–7 days, by the tenth day, food intake achieved or surpassed preoperative levels and animals had regained their preoperative weight. This depression of food intake was much less in sham operated animals. At sacrifice 16 weeks after operation in Experiment 1, body weight were larger in sham operated animals than in rats with PCA (395±23 vs. 370±30). In Experiment 2 the body weights were at sacrifice ten weeks postoperatively 330±18 for sham operated rats and 312±20 for animals with PCA.

After PCA, total 24 hour spontaneous motor activity scores increased significantly over preoperative scores (Fig. 1). The maximum increase was seen at postoperative day 28 Thereafter, activity declined slowly until 98 days after the operation, after which time no difference between rats with PCA and sham-operated rats was observed The level of activity after 98 days approximated the preoperative scores.

The daily pattern of activity was investigated in detail in a group of five PCA animals and five sham-operated controls by making continuous hourly recordings for 72 hours. Figure 2 shows the daily activity pattern of these two groups from day 19 to day 21, postoperatively. Each point on the graph represents the mean hourly activity for five animals for three days. During nine consecutive hourly intervals (9.00 hour to 18 00 hour), the rats with PCA were more active. This period of activity contrasted with the behavior of the sham-operated rats, which were relatively inactive during this period. Thus in PCA animals, the normal day/night activity rhythm was lost, since these animals had up to 50% of their total activity during the light period. We have expressed this as the ratio of activity during the light period over the total 24 hour activity

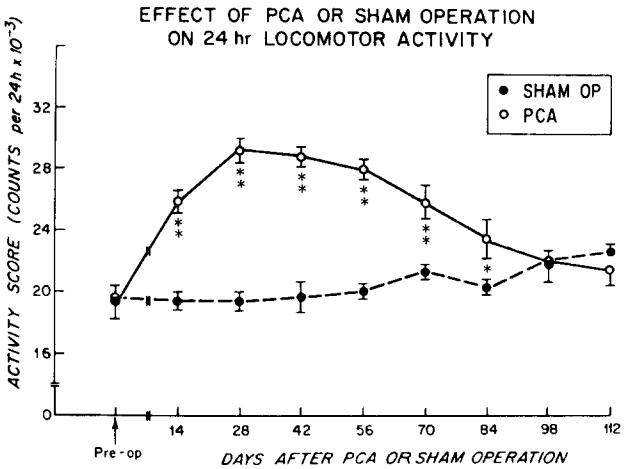


FIG 1 Cumulative 24 hr motor activity scores of rats before operation and at various times after portacaval anastomosis or sham operation Each point with error bars represents the mean±S E M of the activity scores of ten rats (Experiment 1) *$p<0.01$, **$p<0.001$ compared to sham operation

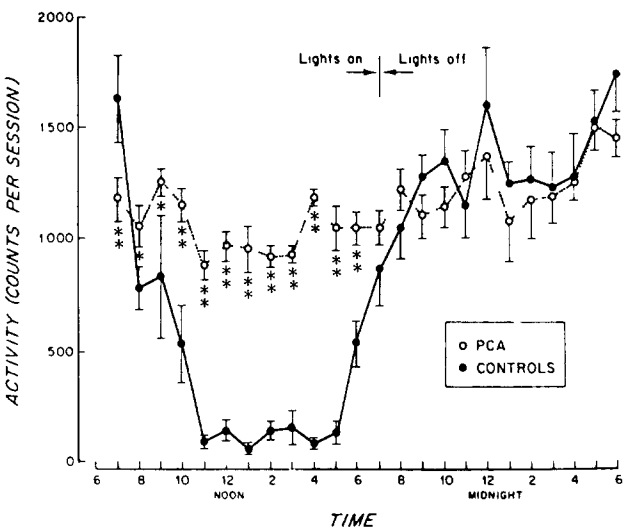


FIG 2 Hourly (60 min) activity scores of rats with PCA or sham operation Each point represents the activity during the previous 60 minutes of five rats The activity of each rat was monitored on three consecutive days (day 12 to 21 postoperation) and the scores for the same time of day were averaged Thus, each point with error bar represents the mean±S E M of 15 activity scores (Experiment 1) *$p<0.01$, **$p<0.001$ compared to sham operation

(Fig 3) In the dark (normally active) period, there was no significant difference between the two groups.

The 15 rats of the separate experiment (Experiment 2) all showed increased total activity with a maximum increase of the ratio of light activity over total activity at 24 to 26 days (Fig 3) Thereafter, the activity slowly decreased and by the time of sacrifice at day 70 to 72, seven of the animals had regained normal activity patterns. At this time, the light period to total activity ratio correlated well with the brain

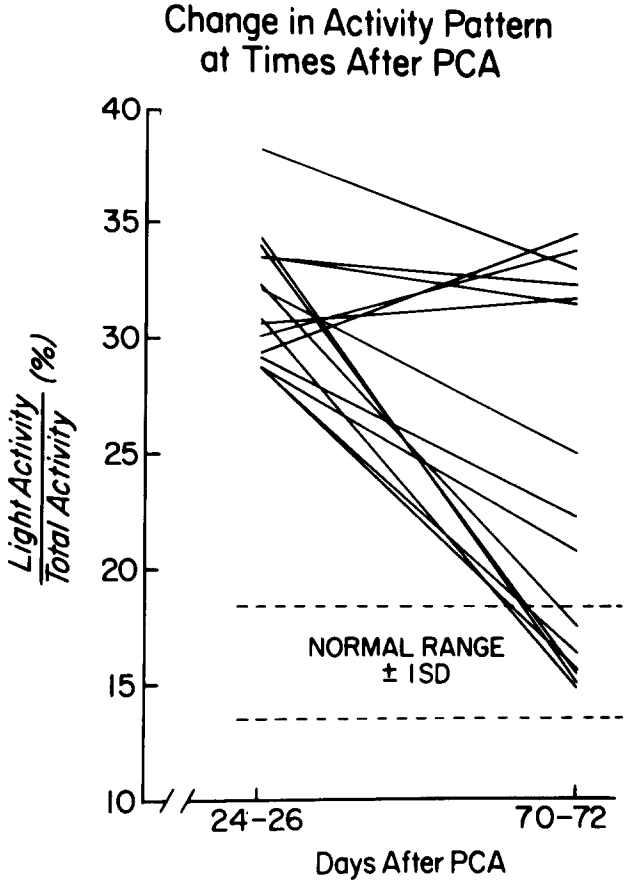


FIG. 3 Change in activity pattern at times after PCA in 15 rats used for amino acid analyses (Experiment 2 )

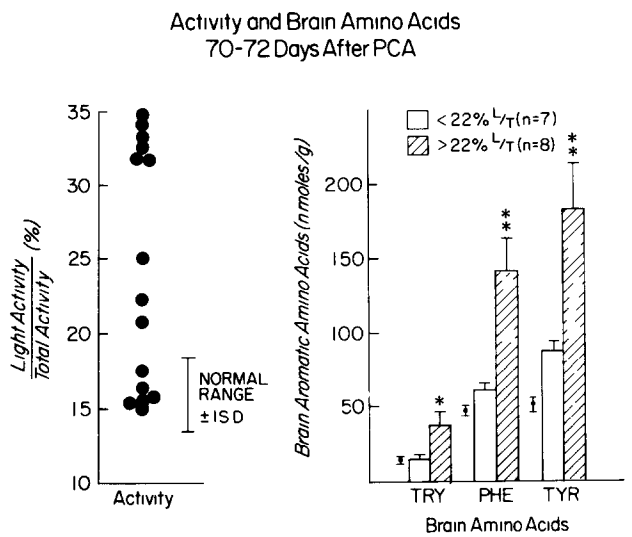


FIG 4 Activity and brain amino acids 70 to 72 days after PCA Each bar represents the mean±S E M of brain aromatic amino acids of 15 rats. The black points represent normal brain concentrations of tryptophan, phenylalanine and tyrosine from Reference 4. (Experiment 2 ) *$p<0.001$, **$p<0.001$, compared to L/T<22%

TABLE 1

CORRELATION BETWEEN PERCENT LIGHT ACTIVITY/ TOTAL ACTIVITY AND BRAIN AMINO ACIDS

| Percent L/T Activity | Spearman RHO | Significance |
|---|---|---|
| Brain Tryptophan | 0 652 | $p<0.01$ |
| Brain Tyrosine | 0 739 | $p<0.01$ |
| Brain Phenylalanine | 0 877 | $p<0.001$ |
| Brain Glutamine | 0 906 | $p<0.001$ |

concentrations of tryptophan, phenylalanine and tyrosine so that animals with increased activity had the highest brain concentrations of these amino acids (Fig 4, Table 1). The activity ratio also showed an excellent correlation with brain glutamine.

## DISCUSSION

These results demonstrate clearly that rats with PCA were significantly more active than control rats during the light period when rats normally sleep. However, no significant difference in the activity of the PCA and control rats was observed in these studies during the dark period, which is normally the time of maximum activity Monmaur showed a significant decrease during daylight hours both in total duration of electroencephalographic slow wave sleep activity and of paradoxical sleep activity in rats up to 63 days after PCA [15]. This is consistent with the present results since we observed increased motor activity over a comparable postoperative period of time. Since the increase in activity occurred only during the normal sleep period, it is likely the increased activity scores reflected the increased wakefulness demonstrated in encephalographic studies [2,15].

Tricklebank reported decreased ambulation in rats after PCA which is in apparent conflict with the present results [18]. It might, however, not be expected that our results should agree with these open-field experiments Their open-field studies in which ambulation was monitored were of shorter duration and might not reveal an increase in total motor activity; i.e., it is possible that our higher activity levels are due to an increase in small movements such as eating, head movements and grooming. Besides this, in the study by Tricklebank an Animex activity meter was used and the activity of paired rats were tested. These differences might well account for the different effects of PCA on gross activity. Furthermore, both studies agree in as much as the percent of total activity which occurred during the light period was increased. In our studies, the activity scores of rats with PCA returned to normal levels by the 14th postoperative week. This was consistent with the biochemical changes in that the normalization of activity correlated well with the normalization of brain neutral amino acids. This may reflect a gradual improvement in hepatic function over time due to increased hepatic arterial blood flow or to establishment of compensatory collateral circulation from the splanchnic viscera to the liver surface. The 24 hour cumulative scores of the shamoperated group increased slightly over the 112 day testing period. This was probably due to the normal growth of the animal resulting in increased electrical impedance and correspondingly higher activity scores.

Several neurochemical abnormalities have been identified in the rat with PCA. The increased brain concentrations of tyrosine and phenylalanine may result in an accumulation of so called false neurotransmitters, e.g., octopamine and B-phenylethanolamine [9] In dogs with PCA and encephalopathy depletion of norepinephrine and dopamine has been found in cerebrospinal fluid and the brain besides the accumulation of false neurotransmitters [6,17]. Brain tryptophan is two to three times normal in rats after PCA; serotonin is significantly elevated in the midbrain and in the medulla/pons region, and brain 5-HIAA is also significantly elevated [4] This was also noted by Tricklebank *et al* and they found positive correlation between ambulation and indices of brain 5-hydroxytryptamine metabolism [19]. The elevation of brain tyrosine, tryptophan, phenylalanine, histidine and methionine coincides with increased activity of the blood-brain neutral amino acid transport system [10].

Brain ammonia metabolism is enhanced in the rat with PCA with resulting accumulation in the brain of glutamine [3]. Since glutamine can compete with glutamate for uptake into nerve endings and be released, it has been suggested that glutamine could function as a false or inactive neurotransmitter [1]. Recently, high brain levels of glutamine have been suggested to raise brain concentrations of other NAA by stimulating exchange transport across the blood-brain barrier [11] Cerebrospinal fluid glutamine has previously been shown to correlate well with grade of encephalopathy in patients [8], and in this study brain glutamine together with brain phenylalanine, tyrosine and tryptophan correlate well with the disturbance in activity (Table 1).

Previous studies have shown that alterations in serotonin metabolism can alter the state of wakefulness [12,13] However, our results are paradoxical in that we have shown increased activity levels at a time when brain serotonin is also increased We have no explanation for this contradiction However, rats with PCA have chronically elevated brain serotonin as well as elevated octopamine, glutamine and ammonia, the interaction of which may have additional effects on sleep patterns It is of interest to note that electrically stimulating the raphe nuclei of rats at physiological frequencies increases brain serotonin and makes the animals somnolent, however, stimulation of these nuclei at a frequency five to ten times this rate produces a state of hyperalertness similar to that which follows LSD administration [13]

Patients with liver cirrhosis and portal-systemic shunting have markedly elevated cerebrospinal fluid tryptophan concentrations [19] and, in the early stages of hepatic encephalopathy, the sleep patterns of such patients are disturbed and the duration of sleep decreased as well as sensitive tests of psychomotor function and visual perception [14,16] The present results suggest that the motor activity of rats with PCA could be utilized as a model of sleep and other disturbances in early hepatic encephalopathy

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