Pharmacology Biochemistry & Behavior, Vol. 9, pp. 181–189. Printed in the U.S.A.

Effects of Chronic Experimental Liver Dysfunction and L-Tryptophan on Behaviour in the Rat

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(Received 24 February 1978)

TRICKLEBANK, M. D., J. L. SMART, D. L. BLOXAM AND G. CURZON. *Effects of chronic experimental liver dysfunction and L-tryptophan on behaviour in the rat.* PHARMAC. BIOCHEM. BEHAV. 9(2) 181–189, 1978.—Rats with chronic experimental portocaval anastomosis were hypoactive as indicated by diminished activity in the home cage, during habituation in red light to an observation box and during exposure in white light to an open-field. Food intake and responsiveness to electric shock were also decreased. However, there was an abnormally high frequency of social activity when anastomosed rats were paired together after having been caged singly for 3 weeks. Also, sham-operated rats interacted more with anastomosed rats than they did with other sham-operated animals. Anastomosis also raised brain concentrations of tryptophan, 5-hydroxytryptamine and 5-hydroxyindoleacetic acid. Administration of tryptophan to sham-operated rats increased shock threshold and decreased ambulation in an open-field. Thus, while anastomosed rats are not comatose they do have considerable behavioural abnormalities for which brain tryptophan changes may be in part responsible.

Activity Brain 5-Hydroxytryptamine Liver disease Open-field Shock threshold Social behaviour Tryptophan

CHRONIC liver dysfunction in man is often associated with hepatic encephalopathy, a neuropsychiatric syndrome usually commencing with changes of mood and signs of intellectual impairment and leading to confusion, slurred speech, drowsiness, hypersomnia, stupor and coma as the condition worsens [12,35]. Liver dysfunction is also characterized by raised concentrations of aromatic amino acids in plasma and cerebrospinal fluid [13, 19, 20, 24, 34]. These changes include an increase of plasma free tryptophan and are associated with raised CSF levels of tryptophan and 5-hydroxyindoleacetic acid (5-HIAA), a precursor and the terminal metabolite respectively of 5-hydroxytryptamine (5-HT) [24, 33, 42]. The concentration of tyrosine, the precursor of the catecholamines and the concentration of homovanillic acid, the major metabolite of dopamine are also increased in the CSF [24,33]. However, while evidence suggests that increased tryptophan availability increases human brain 5-HT turnover, increased availability of tyrosine does not appear to alter dopamine turnover appreciably [10]. Such metabolic disturbances among others, have been suggested to be involved in the development of hepatic encephalopathy [37]. Altered tryptophan metabolism has been particularly implicated since liver damage in both the dog and man increases the central toxicity of this amino acid [8,32].

Possible relationships between the behavioural and biochemical disturbances in liver disease can only be studied to a limited extent in man. However, many of the biochemical abnormalities of human liver disease also occur in the rat after surgical diversion to the vena cava of the portal vein which normally supplies the liver (portocaval anastomosis) [2, 3, 9, 21]. These animals remain in good condition for

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weeks and do not exhibit obvious encephalopathic signs. While they are reported to be less active than sham-operated rats they are said to react normally to auditory or tactile stimulation [18]. However, these conclusions were not based on a systematic behavioural study.

In this present work, some aspects of the behaviour of anastomosed rats have been examined. Measurements were made of motor activity in the home cage, behaviour in an open-field, threshold of response to electric shock and social behaviour. The effects of L-tryptophan on open-field behaviour and shock threshold were also studied. Results indicate that anastomosed rats show a wide range of behavioural abnormalities and that some of these may be mediated by elevated brain tryptophan and/or 5–HT metabolism. Some of these results have been presented in preliminary form elsewhere [5].

METHOD

Animals

Male Sprague-Dawley rats born in the Animal Unit of the University of Manchester Medical School were obtained at weaning and housed in littermate groups of four at 22 ± 1°C, 45 ± 5% relative humidity. A white/red light cycle was employed with deep red light imposed between 1230 hr and 0030 hr. Animals were fed ALGH Standard Rodent Diet (Grain Harvesters Ltd., Canterbury, England).

Surgical Procedures

Portocaval anastomosis or sham-operation was performed [14] under ether anaesthesia on 28 ten week old rats from 13 litters. Mean body weight was 341 ± 27(SD)g. In a second experiment, two anastomosed and two sham-operated rats were prepared from each of 12 litters. Mean body weight was 288 ± 30 g. The procedure involved exposure, ligation and cutting free of the portal vein. This was then everted over a Teflon button, secured with a silk ligature and anastomosed with the partially clamped vena cava using a purse string suture. Sham-operation involved all aspects of the operation (including partial clamping of the vena cava) except the cutting of the portal vein and anastomosis with the vena cava. In the first experiment, animals were housed in like-treatment groups of two for 17 days after the operation and singly thereafter. In the second experiment, animals were housed in similarly-treated littermate pairs for 10 days and then singly. These rats were used for the determination of thresholds of response to electric shock only.

Food and Water Intake and Body Weight

Food and water consumed per 24 hr (1100 hr–1100 hr) and body weight were measured daily for 14 days after the operation and then at weekly intervals. Intake was taken to be the loss in weight of water from the water bottle and of food from the food basket. Loss of water by dripping, and evaporation and of food by spillage and non-nutritive gnawing was small.

Activity in the Home Cage

Two Animex D.S.E. activity meters (L.K.B. Instruments Ltd., Surrey, England) were used with sensitivity and tuning set at 25 μA and 40μA, respectively, so that the instrument responded to locomotion, rearing and head movements but not to fine movements such as sniffing [30]. Nine or ten days after operation, cages containing two anastomosed or two sham-operated animals were transferred to an adjacent room (with identical environmental conditions to those of the home room except that no other animal was present) between 1045 hr and 1115 hr for measurement of activity over the following 24 hr. Meters were alternated between the two treatment groups over seven successive 24 hr periods in order to control for any small differences in responsiveness. The number of counts per hr was recorded on counters in another room.

Behaviour in the Open-Field

The open-field consisted of a brightly illuminated circular arena 740 mm dia., divided into 13 segments of approximately equal area and bounded by a wall 300 mm high. The animals were observed between 1300 hr and 2000 hr, 7 to 8 weeks after operation. Immediately before being placed in the open-field, they were given 0.9% NaCl or L-tryptophan (0.098 mmole/Kg IP) in 0.9% NaCl in a volume of 10 ml/kg body weight. Each animal was exposed to the open-field for a total of 95 min and behaviour was observed remotely by closed-circuit television (Shibadan Electric Co.) during seven 5 min periods at 10 min intervals commencing at time zero. The following behavioural parameters were scored: ambulation (number of areas entered with all four feet); frequency of rearing; number of head-lifts and frequency of grooming. Faecal pellets were removed from the open-field and the floor wiped with clean damp tissue after every occupation.

Social behaviour

Interactions between pairs of animals were observed remotely using closed-circuit television. The investigation took place during a period of 11 days commencing 4–5 weeks after the operation and when the animals had been housed singly for between 14 and 18 days. All observations were made in red light between 1300 hr and 1800 hr using an observation box 330×600×300 mm high with a clear Perspex front and a floor divided into six equal rectangles.

Rats were habituated to the box by placing each one in it alone for 10 min on six successive days. The animals were observed on the first four days by closed-circuit television and scored as described under 'Behaviour in the open-field' except that grooming was not scored. Starting two days later, pairs of animals were put together in the box for 10 min on each of four consecutive days (i.e., the 8th to the 11th day). Animals were paired in like-treatment groups on the 8th and 9th days and in different-treatment groups on the 10th and 11th days. The same pair of rats was never tested twice. Littermates were never paired. The frequency of occurrence of the following behaviours was scored: *sniffing* (the head of one animal orientated towards and close to any part of the body of its partner); *allogrooming* (burying of the snout in the fur of its partner); *mounting* (with or without pelvic thrusts); *boxing* (standing on hind legs facing and close to one another with forepaws raised); *fighting* (grappling and wrestling). The above behavioural terminology was adapted from Whatson *et al.* [41]. The duration of these activities was noted on the 8th and 9th days only. Faecal pellets were removed from the observation box and the floor wiped with clean, damp tissue after every occupation.

Threshold of Response to Electric Shock

Rats were tested 20–24 days after operation between 1300

hr and 1800 hr in white light in an aluminium box 240×210×90 mm high with a front wall of clear Perspex. The roof was low enough to prevent the animals from minimizing shock by rearing. The floor was a grid of 44 mm dia. stainless steel bars spaced 14 mm between centres through which shocks of variable voltage were delivered via a constant power shock source with scrambler through a source resistance of 150 kΩ.

After 5 min habituation to the test box, six series of unavoidable shocks were delivered. Each series consisted of seven shocks of between 0 and 30 V with 5 V steps. Shocks of 1 sec duration were presented in random order with 30 sec intervals between shocks and 2 min intervals between series. The delivery of shock was controlled by an operator in another room and its imminence indicated to the observer by a light which was not visible to the animal. Removal of one or more feet from the grid in response to shock onset was defined as a jump. Voltages at which this occurred were recorded. Responses to the first three series of shocks could not be scored accurately because of spontaneous motor activity and were therefore excluded from the analysis. Jump threshold was defined as the lowest voltage at which rats jumped, averaged over the three scored series. Animals not responding to the highest voltage (30 V) were assigned a threshold of 35 V.

In a second experiment with different animals, anastomosed or sham-operated animals were taken 14 days after operation and injected with either L-tryptophan (0.375 mmole/kg × 3 at 80 min intervals) in 0.9% NaCl or with 0.9% NaCl (× 3 at 80 min intervals) and shock responsiveness determined 40 min after the last injection exactly as before except that voltages used were 0, 15, 20, 25, 30, 35 and 40 V. One week later threshold was redetermined using the same animals 40 min after a single injection of tryptophan (0.375 mmole/kg). No further measures, behavioural or otherwise, were made on these animals.

Removal and Dissection of Brain

Rats were killed by guillotine immediately after removal from the open-field. Brains without the olfactory lobes were removed and placed on a glass tile embedded in CO_2-ice mixture and the following brain regions dissected out essentially as described by Glowinski and Iversen [15]; hypothalamus, hippocampus, midbrain, pons/medulla, striatum, rest of brain. All samples were rapidly frozen over CO_2 and stored at -20°C until analysis.

Biochemical Determinations

5-HT and 5-hydroxyindoleacetic acid were determined as described by Knott and Curzon [25]. Tryptophan was determined by the method of Bloxam and Warren [4] in the aqueous extract containing 5-HT. The reliability of these methods in this laboratory has been indicated previously [25].

RESULTS

Food and Water Intake

On the first day after operation both the anastomosed and sham-operated rats ate little (2–3 g, Fig. 1). Food intake of the sham-operated animals rose to 25 g/day on the 5th day and 30 g/day at 8 weeks. Food intakes of the anastomosed rats after the first post-operative day were consistently about

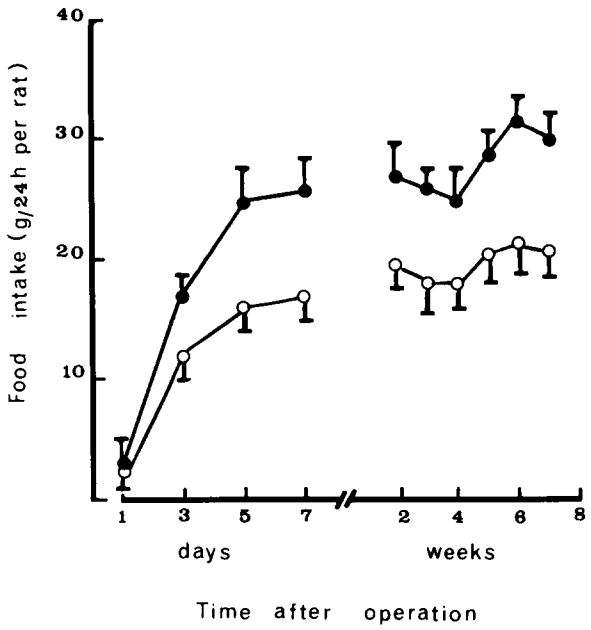

FIG. 1. Mean(+ or − SEM) food intake per 24 hr of sham-operated rats (●) and rats with portocaval anastomosis (○). Differences between the two groups were significant at two weeks after operation and subsequently ($p<0.01$, t test). N/group=7 (pairs) for 1–7 days. N=14 (singly caged) subsequently.

30% lower than those of the sham-operated animals. Anastomosed rats consumed more water than sham-operated animals during the first week after operation (Fig. 2) especially towards the end of this period, but consumed about 25% less during the latter five weeks of the investigation.

Body Weight

Both groups lost weight after operation, but while sham-operated rats began to gain weight during the second week and then returned to a normal growth trajectory, the anastomosed animals did not begin to gain appreciably until the 5th week and showed a deficit of 22% at seven weeks (Fig. 3) roughly in proportion with the deficits of food and water intake.

Activity in the Home Cage

Both groups showed normal diurnal patterns of motor activity [7], i.e., greater activity counts during red light than during white light and two separate periods of particularly high activity during red light (Fig. 4). The anastomosed rats were significantly less active than the sham-operated animals during the red light period but did not differ from them in white light.

Activity in the Open-Field

During the first 5 min exposure to the open-field in white light, both sham-operated and anastomosed animals showed similar behavioural patterns and were comparably active (results not shown). All activities measured declined markedly during the next 10 min but sporadic activity continued throughout the duration of the experiment. Excluding the

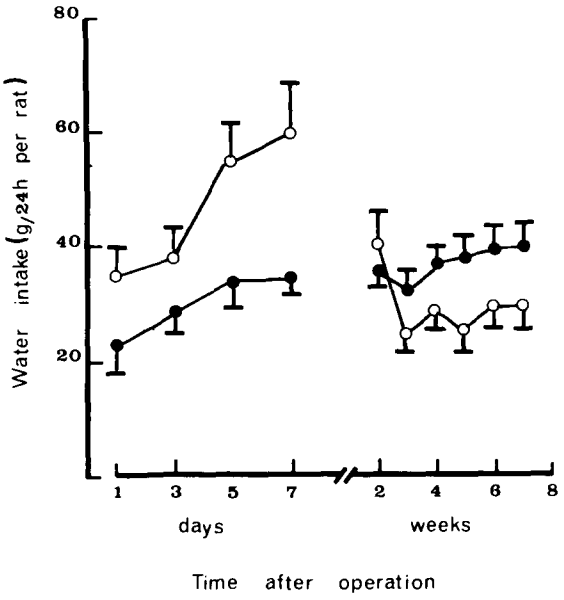

FIG. 2. Mean (+ or − SEM) water intake per 24 hr of sham-operated (●) and rats with portocaval anastomosis (○). Differences between the two groups were significant (*t* test) at 5 and 7 days ($p<0.05$), and at three weeks after operation ($p<0.05$) and subsequently ($p<0.01$). Other details as Fig. 1.

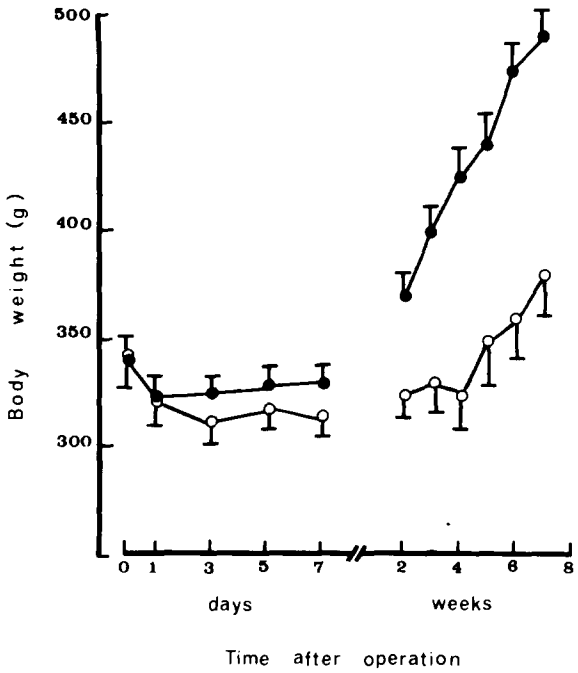

FIG. 3. Mean (+ or − SEM) body weight of sham-operated rats (●) and rats with portocaval ○) at various times after operation. Differences between the two groups were significant at two weeks after operation and subsequently ($p<0.001$, *t* test). N/group=14.

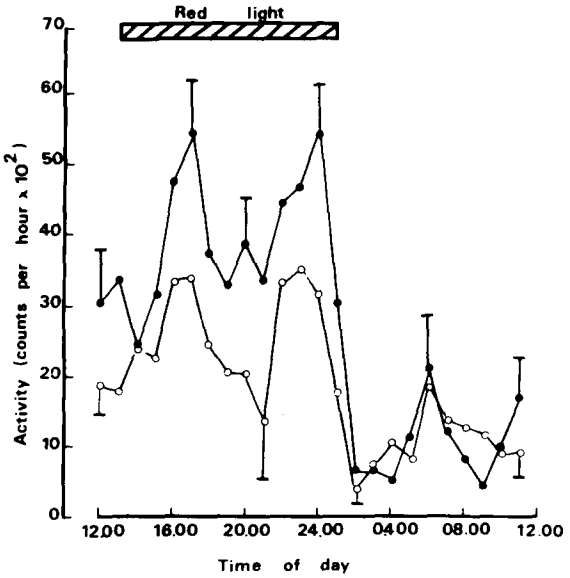

FIG. 4. Mean hourly active score (+ or − SEM) of sham-operated rats (●) and rats with portocaval anastomosis (○) in the home cage over 7 successive 24 hr periods as described under METHOD. Results were analyzed by repeated measures analysis of variance. There was a significant effect of anastomosis, $F(1,12)=4.785$, $p<0.05$, and of repeated measure, $F(23,276)=14.726$, $p<0.001$. The difference between the two groups occurred entirely within the red light period, $F(1,12)=6.367$, $p<0.05$. N/group=7 pairs.

initial 5 min observation period, the cumulative ambulation score of saline-treated anastomosed rats over 15–80 min after being placed in the open-field was considerably and significantly lower than that of similarly-treated, sham-operated rats (Fig. 5), but there were no significant differences between the two groups in the incidence of grooming, head-lifting, rearing or turning. The administration of tryptophan (0.098 mmole/kg) markedly decreased ambulation in the sham-operated but not in the less active anastomosed rats and did not significantly alter grooming, head-lifting, rearing and turning in either group.

Activity During Habituation to an Observation Box

During the four habituation sessions, during which rats were observed singly in the box later used for observation of social activity, the anastomosed rats lifted their heads and reared as frequently as sham-operated animals (Fig. 6). However, analysis of variance showed that there was a significant interaction between treatment and repeated exposure to the box with respect to ambulation. Anastomosed rats ambulated significantly less than sham-operated animals on Days 2 and 3.

Social Activity: Introduction of A Partner of the Same Treatment Group

Pairs of anastomosed rats spent significantly more time in the social activities recorded than did pairs of sham-operated animals (Table 1). Most of this difference was due to the anastomosed rats sniffing and allogrooming their partners

TABLE 1

EFFECT OF PORTOCAVAL ANASTOMOSIS ON SOCIAL BEHAVIOUR

Rat observed	Partner	Number of pairs	Behavioural scores Sniffing	Allogrooming	Mounting	Time in social activity (sec)	Boxing§	Fighting§
(a) Sham-operated	Sham-operated	14	35 ± 3	2.3 ± 0.4	1.9 ± 0.8	142 ± 19 (N=7)	4.5/7	4/7
(b) Anastomosed	Anastomosed	14	59 ± 4*	7.7 ± 1.2*	4.1 ± 1.0	290 ± 14*(N=7)	1/7	1/7
(c) Sham-operated	Anastomosed	13	48 ± 5†	8.8 ± 1.3‡	4.0 ± 0.7			
(d) Anastomosed	Sham-operated	13	63 ± 6	6.9 ± 1.3	2.9 ± 1.3			

§Number of pairs boxing or fighting averaged over two observation sessions/total number of pairs observed. Behaviour was scored as described under Method. Means ± SEM were calculated from the average score achieved by each animal when tested on two days.

* (a) v. (b). $p<0.002$ (Mann Whitney U test)

† (a) v. (c). $p<0.005$ (Wilcoxon Matched-Pairs Signed Rank Test)

‡ (a) v. (c). $p<0.001$ (Wilcoxon Matched-Pairs Signed Rank Test)

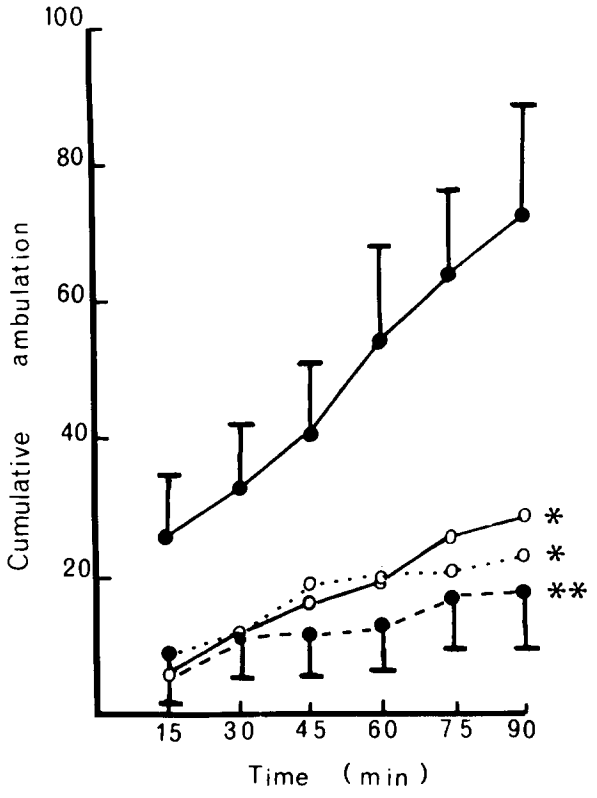

FIG. 5. Mean cumulative ambulation socres in open-field of sham-operated rats given saline (●–●), or L-tryptophan (0.098 mmole/kg IP) (●- - -●) and rats with portocaval anastomosis given saline (○–○) or L-tryptophan (○- - -○). Rats were observed as described under METHOD. For legibility, standard errors are given for sham-operated groups only. They were comparable to those of anastomosed groups. Scorings were over 5 min periods starting at times shown. Terminal scores were compared by analysis of variance followed by Mann-Whitney U test. Significantly different from saline-treated, sham-operated rats: *$p<0.05$; **$p<0.005$. N=7/group.

significantly more frequently than did sham-operated rats. Some anastomosed rats repeatedly attempted to mount their partners, but the difference in the mean number of mounts between groups did not achieve statistical significance. Anastomosed rats tended to box and fight less than did the sham-operated animals Ambulation was not scored but anastomosed rats appeared to ambulate at least as much as, and probably more than sham-operated animals, in contrast to their response to other test situations.

Social Activity: Introduction of a Partner of Different Treatment Group

No significant differences in any of the parameters of social behaviour recorded were apparent between the two groups when anastomosed rats were paired with sham-operated animals (Table 1:c,d). In order to investigate the effect of the type of partner the mean sniffing, allogrooming and mounting score for each rat with another rat of its own treatment group (Days 8 and 9) was compared with its mean score with a rat of the other treatment group (Days 10 and 11). This showed that anastomosed rats sniffed and allogroomed sham-operated or anastomosed rats with similar frequency and that sham-operated rats sniffed and groomed anastomosed rats significantly more than other sham-operated rats.

Thresholds of Response to Electric Shock

Eight anastomosed, but only one sham-operated rat, had jump thresholds greater than 30 V and were assigned thresholds of 35 V (i.e., the next step up). Mean jump thresholds were significantly greater in anastomosed rats than in sham-operated animals and administration of tryptophan consistently raised mean shock thresholds in both groups although the changes were not always statistically significant (Table 2). Thus 0.375 mmole/kg × 3 caused significant changes only in sham-operated rats while 0.375 mmole/kg × 1 had significant effects only in the anastomosed group.

Brain Tryptophan Metabolism

Anastomosed rats killed at the end of the open-field experiment had markedly and significantly higher tryptophan concentrations than sham-operated animals in all regions of

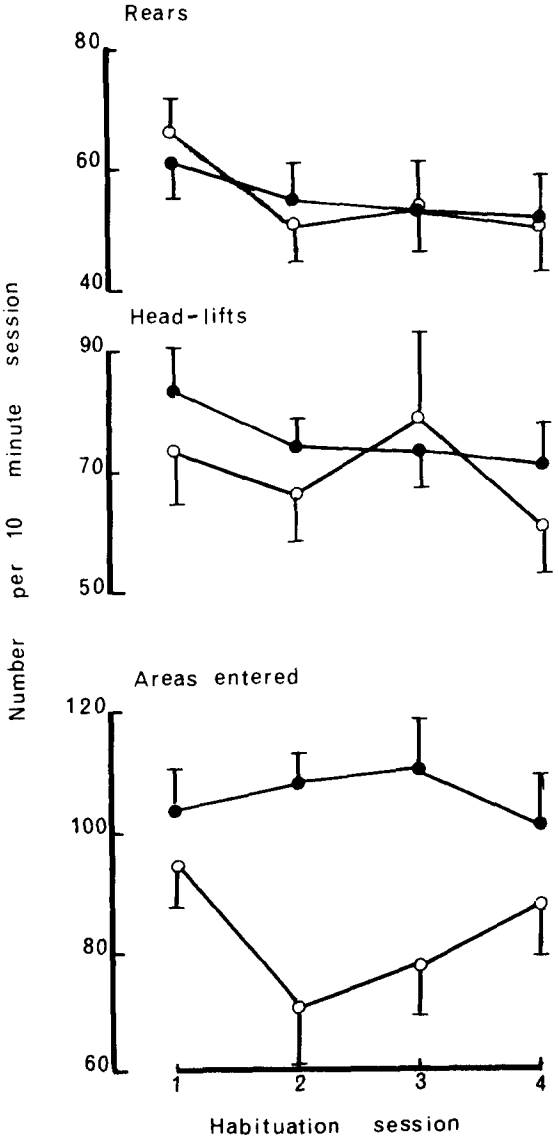

FIG. 6. Mean activity scores (+ or – SEM) of sham-operated rats (●) and rats with portocaval anastomosis (○) during habituation sessions in box subsequently used for observation of social activity. Rats were observed as described under METHOD and results analyzed by repeated measures analysis of variance. There was a significant interaction between treatment and repeated exposure to the box with respect to ambulation, $F(3,54)=3.228$, $p<0.05$. Anastomosed rats entered significantly fewer areas of the box than did sham-operated animals, $F(1,18)=8.206$, $p<0.025$, on the second and third days session ($p<0.01$, Mann-Whitney U test). N=14/group.

brain studied. Brain 5–HIAA was significantly raised in all regions except pons/medulla and striatum while 5–HT showed smaller increases which were only significant in the hippocampus, mid-brain and hypothalamus. Results are shown for two regions (mid-brain and hypothalamus) (Table 3). Tryptophan administration (0.098 mmole/kg) significantly increased tryptophan concentration 95 min later in both of these brain regions of sham-operated and anastomosed rats, but caused relatively small increases in the concentration of 5–HT, a significant rise being attained only in the mid-brain of the anastomosed rats. 5-Hydroxyindoleacetic acid (5–HIAA) rose significantly after tryptophan administration in the hypothalami of the sham-operated rats.

DISCUSSION

Experimental portocaval anastomosis in the rat causes a number of metabolic changes which also occur in chronic liver dysfunction in man. Thus, plasma amino acid changes are similar to those found in human cirrhotics [3, 9, 13, 19, 20, 21]. Also the increased brain concentrations of tryptophan, 5–HT and 5–HIAA found in the present study seven weeks after operation and in other studies at various times after operation [2, 3, 9] are consistent with results of determinations on brain autopsy material from patients with hepatic encephalopathy [10].

Anastomosed rats showed no obvious behavioural abnormalities after recovery from operation and although they weighed about 30% less than sham-operated animals they appeared to be in good condition throughout the course of the study. However, closer observation and monitoring showed that anastomosis led to hypoactivity in various situations as indicated by diminished activity in the home cage, during habituation in red light to an observation box and during exposure in white light to an open field. Responsiveness to electric shock and food intake were also decreased.

As rats eat and drink mostly at night, decreased locomotion associated with diminished food intake may have contributed to the decrease of nocturnal activity in the home cage, although results in Fig. 2 suggest that increased locomotion associated with increased water intake during the first two weeks after anastomosis could have affected these results in the opposite direction. However, altered food and water intake cannot explain the hypoactivity during habituation to the social activity chamber as food and water were not available. It is possible that changes in food intake and locomotion are related to some other common factor.

In view of the increased concentrations of tryptophan, 5–HT and 5–HIAA in brain following anastomosis [2, 3, 9] it is of interest that decreased ambulation after habituation to the open-field resulted not only from anastomosis, but also from administration of tryptophan (0.098 mmole/kg) to sham-operated rats. This latter finding is consistent with the results Taylor obtained using normal animals [39]. The decreased nocturnal activity of anastomosed rats in the home cage is also qualitatively consistent with the reported effects of tryptophan on the motor activity of caged mice [6,31].

The increase of brain tryptophan following anastomosis may also have some responsibility for the raised threshold of response to electric shock as giving a larger dose of tryptophan to sham-operated rats had similar effects. Although this finding disagrees with previous work in which tryptophan was given to normal animals and a different test procedure was used [17], it is consistent with the increased responsiveness to electric shock of rats on a tryptophan deficient diet [28] or after inhibition of 5–HT synthesis [40].

Another behavioural change which could be related to the increase of brain tryptophan after anastomosis is the decreased food intake throughout the seven week period of observation after anastomosis. This was probably a cause rather than a consequence of the lower body weight of the anastomosed rats since their food intake was lower than that of younger sham-operated rats of comparable weight (see

TABLE 2

EFFECTS OF PORTOCAVAL ANASTOMOSIS AND TRYPTOPHAN ON THRESHOLDS OF RESPONSE TO ELECTRIC SHOCK

	Threshold voltage (Sham-operated)	*p*	(Portocaval anastomosed)
Experiment 1	25.1 ± 1.5	<0.005	32.2 ± 0.9
Experiment 2			
0.9% saline	26.6 ± 1.6	<0.01	33.2 ± 1.5
p	<0.001		NS
L-tryptophan (0.375 mmole/kg × 3)	35.1 ± 2.2	NS	36.2 ± 1.9
0.9% saline	27.3 ± 1.8	<0.02	35.1 ± 1.8
p	NS		<0.02
L-tryptophan (0.375 mmole/kg × 1)	29.9 ± 1.9	<0.02	39.9 ± 1.3

Values are means ± SEM.
Shock threshold were determined as described under Methods.
Groups contained one rat from each of 13 (Experiment 1) or 12 (Experiment 2) littermate groups.
Significances are shown between groups compared and were calculated by paired-comparisons *t* test following analysis of variance where appropriate.

TABLE 3

BRAIN TRYPTOPHAN, 5HT AND 5HIAA OF ANASTOMOSED AND SHAM-OPERATED RATS AFTER SALINE OR TRYPTOPHAN INJECTION

	Given saline				Given tryptophan			
	Portocaval anastomosed	*p*	Sham-operated	*p*	Sham-operated	*p*	Portocaval anastomosed	*p**
Tryptophan (μg/g)								
Hypothalamus	12.76 ± 1.48 (6)	<0.001	5.20 ± 0.18 (7)	<0.01	7.99 ± 0.62 (7)	<0.01	19.36 ± 2.49 (7)	=0.05
Midbrain	9.76 ± 1.14 (6)	<0.001	4.28 ± 0.26 (7)	<0.05	5.73 ± 0.41 (7)	<0.001	16.23 ± 1.92 (7)	<0.05
5-Hydroxytryptamine (μg/g)								
Hypothalamus	1.19 ± 0.08 (6)	NS	1.03 ± 0.06 (7)	NS	1.22 ± 0.07 (7)	NS	1.24 ± 0.06 (7)	NS
Midbrain	1.37 ± 0.04 (6)	<0.01	1.15 ± 0.04 (7)	NS	1.26 ± 0.03 (7)	<0.01	1.59 ± 0.06 (7)	<0.05
5-Hydroxyindoleacetic acid (μg/g)								
Hypothalamus	1.93 ± 0.27 (6)	<0.01	0.99 ± 0.05 (7)	<0.01	1.29 ± 0.07 (7)	<0.01	1.89 ± 0.17 (7)	NS
Midbrain	3.37 ± 0.28 (6)	<0.001	1.25 ± 0.14 (7)	NS	1.63 ± 0.16 (7)	<0.001	4.58 ± 0.58 (7)	NS

Rats were killed 95 min after giving L-tryptophan (0.098 mmole/kg=20 mg/kg, IP). Open-field experiment: see Method.)
Results are given as means ± SEM. Significance of differences were calculated by *t* tests. Nos of rats shown in brackets.
*Values v those for corresponding saline-treated rats.

results in Figs. 1 and 3 for rats 6–7 weeks after anastomosis). These results are consistent with the significant inverse correlations between brain tryptophan and food intake and between brain 5–HIAA and food intake found in rats with liver damage due to carbon tetrachloride poisoning [26]. Also it has been reported that rats with free access to various diets showed an inverse relationship between protein intake and the ratio of plasma tryptophan to the neutral amino acids that compete with it for transport into the brain [1].

Thus anastomosis results in a number of behavioural changes involving hypoactivity or diminished response to stimuli which may at least partly reflect increased brain tryptophan and/or its metabolism. However, altered tryptophan metabolism is only a part of a complex of biochemical changes with potential behavioural consequences in anastomosed rats and in human subjects with liver disease (see [44] for review). For example, the false neurotransmitter octopamine is elevated in the brains of rats with experimental liver disease and is associated with decreased brain noradrenaline [21]. Various observations suggest that tryptophan changes alone are insufficient to explain the central effects of liver disease in man. For example, CSF tryptophan concentrations are similarily elevated in cirrhotic patients whether they are comatose or not [43]. Furthermore, very high plasma tryptophan levels can be attained acutely when normal human subjects are given tryptophan whilst consciousness is only slightly impaired [16].

Hypoactivity and decreased responsiveness was not shown by anastomosed rats in all test situations or for all behaviours tested. For example, ambulation was normal during both the initial 5 min in the open-field and the first habituation period in the social activity test chamber and various motor behaviours other than ambulation (e.g., rearing and head-lifing) also occurred with normal frequency. It is relevant that though tryptophan decreased ambulation in sham-operated rats it did not significantly affect rearing, head-lifting, turning or grooming and behavioural components were normal in anastomosed rats.

The abnormally high frequency of social activity shown by the anastomosed rats when paired together after being caged singly for three weeks was a striking and somewhat unexpected finding in view of the nature of the other behavioural abnormalities. Not only was sniffing and allogrooming by anastomosed rats paired with anatomosed animals more frequent than in sham-operated rats paired with sham-operated animals but also the latter animals exhibited these behaviours more frequently when paired with anastomosed rats.

These results suggest either that anastomosed rats transmit an attractant signal or fail to transmit a normal aversive signal. The latter is transmitted by male mice who excrete material in their urine with an aversive effect on other males [23]. This material is not produced by castrates [22] and as anastomosed rats show testicular atrophy [27] it may be that they do not emit normal male aversive signals. The suggestions of decreased aggressive behaviours (i.e., boxing and fighting) by anastomosed rats is also consistent with this assumption. Disturbed gonadal function often occurs in human liver disease where it may be related to defective hepatic destruction of oestrogens [11]. However the finding that anastomosed rats not only provoke more social behaviour in sham-operated animals but also display more social behaviour cannot be explained wholly by the absence of a normal aversive signal. Neither can it be explained in terms of increased 5-HT turnover. On the contrary, much evidence suggests that decreased 5-HT synthesis leads to increased social behaviour [29, 36, 38].

Results in general show that while portocaval anastomosis in the rat does not lead to hepatic coma there are considerable biochemical similarities between these animals and humans with liver disease and it may be that the biochemical abnormalities have some responsibility for the behavioural deficits which occur in both the experimental and clinical situations. Findings suggest that the anastomosed rat may provide a model of some use in relation to human liver disease and its treatment.

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