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The Level of GABA in the Brain and Locomotor Behavior

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GRIMM, V., Z. GOTTESFELD, I. WASSERMANN AND D. SAMUEL. *The level of GABA in the brain and locomotor behavior.* PHARMAC. BIOCHEM. BEHAV. 3(4) 573–578, 1975. – GABA content was measured in the brains of animals injected with AOAA, DPA or Saline. Significant increases in GABA were found in the motor cortex and cerebellum after treatment with both drugs as compared to saline injected controls. Increased GABA levels were associated with interference with the smooth execution of learned locomotor acts, especially where balancing and coordination of the hind limbs were necessary.

Gamma-aminobutyric acid Cerebellum Aminooxyacetic acid di-n-Propylacetate Locomotor behavior Balance Motor coordination

THERE is by now considerable physiological and biochemical evidence that gamma-aminobutyric acid (GABA) is a neurotransmitter involved in synaptic inhibition in the CNS (for review see [2,9]). However, the role of GABA in behavior in general, and in learning in particular, is still far from clear. Investigations in this area are faced with many methodological difficulties – among them being the difficulty of manipulating GABA concentrations independently of other chemical constituents of the brain, and the problem of whether a change in GABA level can be directly correlated with a specific and well-defined behavioral process.

In the present work, the possible relation between levels of GABA and some complex locomotor behaviors were investigated. The behaviors chosen were those that were considered to reflect the interaction of cerebellar and vestibular functions. Purkinje cells in the cerebellum are known to inhibit cells in lateral vestibular (Deiters' nucleus) [8]. This effect has been attributed to GABA which is present in high concentrations in the Purkinje cells' axon terminals [5, 8, 11]. Furthermore, the vestibular system sends afferent pathways amongst others, to the cerebellum. Thus it was expected that increasing GABA above its normal level, would cause changes in behavior dependent on vestibular-cerebellar interaction. It is rather difficult to isolate behavioral tasks that would reflect only the functions of the cerebellum or those of the vestibular system. The cerebellum is not in itself a motor system but a modifier of motor sequences. Vestibular information is just one of the various sensory inputs that the cerebellum utilizes in its modulating function. However, when an animal orients itself to given aspects of its environment, aims to jump at a target, or maintains its balance on various surfaces, the vestibular organs supply information to the CNS regarding acceleration, linear motion and inclination of the body. Upward running on narrow inclined surfaces necessitates activation of antigravity responses, added propulsion with the hind limbs, balance and generally increased postural control. As the need for these precise adjustments in movement increases, the function of the vestibular system and the modulating role of the cerebellum are thought to increase [1,3].

The purpose of this study was to increase GABA concentrations in the brain by injecting drugs that prevent its break down. Concomitantly the effects of increased GABA levels on some learned motor coordinations thought to be dependent upon cerebellar and vestibular interactions were also examined. It was expected that high GABA concentration in the cerebellum would interfere with the smooth execution of locomotor tasks, especially in cases where balancing and precise aiming in space were required.

METHOD

Animals and Apparatus

To test this hypothesis, male Wistar rats (ca. 115 g weight, 41–45 days old at the start of training) were trained to perform two consecutive tasks. One consisted of jumping from one to the other of 7 platforms to a goal platform (20 X 20 cm) for food and water reward. Five of the platforms were 14 cm and two were 17 cm in height, each with a surface area of 15 X 10 cm. At the end of the training period and in all the subsequent tests, the platforms were separated from each other by 11 cm gaps. The test required a total of 6 jumps including one jump to a higher platform, and one to a lower one (see Fig. 1). The

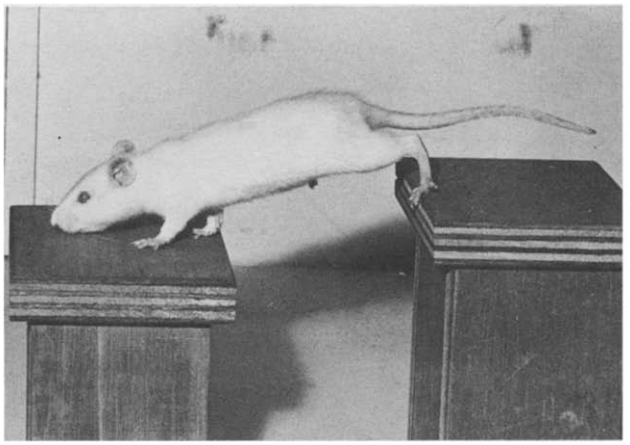

FIG. 1. Performance on the jumping platforms before treatment.

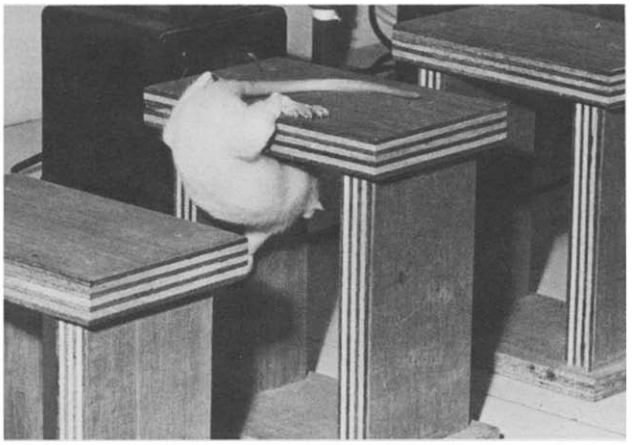

FIG. 3. Rat falling between platforms after injection of DPA.

FIG. 2. Running on the balancing bar before treatment.

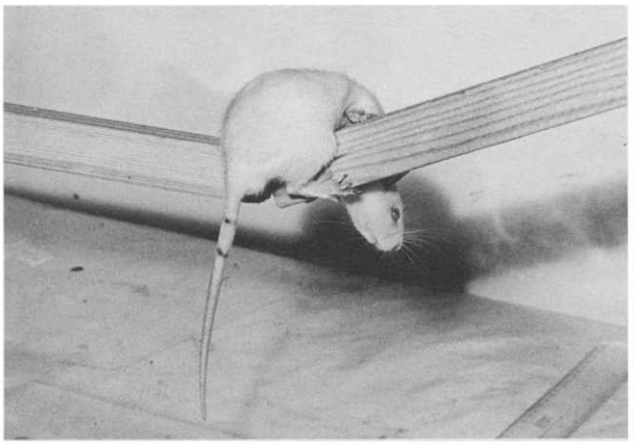

FIG. 4. Animal losing balance on the bar after injection of AOAA or DPA.

second task consisted of running along a narrow elevated bar (1.4 cm wide) one meter of which was horizontal and one meter inclined upward at a 30° angle. At the end of the inclined part of the bar the animal reached a goal platform (20 X 20 cm) for food and water reward (see Fig. 2).

Procedure

All animals were run on a 23 hr food and water deprivation-schedule. They first received 3 days of handling and familiarization with the apparatus. Then a daily training session on the jumping platforms and balancing bar for 7 consecutive days at approximately the same time of day. On the eighth day the experimental animals were injected intraperitoneally (IP). with either amino-oxiacetic acid (AOAA) or di-n-propyl-acetate (DPA), both compounds known to elevate GABA levels in the brain [6, 12, 14, 15]. AOAA and DPA were dissolved in isotonic saline solution and administered at the concentrations indicated in the Tables. Control animals were injected IP with equal volumes of saline.

The behavior of these animals was tested at various intervals of time following the injections. The first test was made at a time when it was assumed that the levels of GABA reached a peak. Subsequent tests were made at later intervals when GABA was thought to have returned to its preinjection levels. The following behavioral measures were taken: (1) time required to traverse the jumping platforms, (2) number of falls between jumping platforms, (3) time of running on the balancing bar, (4) number of slips and falls from the balancing bar (see Figs. 3 and 4).

After each test, randomly selected experimental and control animals were killed by decapitation and the actual GABA concentrations in the cerebellum and motor cortex were measured. The dissection was carried out at 0°C. The tissue parts were rapidly removed, homogenised in ice-cold 80% ethanol and the homogenate centrifuged at 15,000 g for 20 min at 4°C. GABA was determined in aliquots of the supernatant after chromatographic separation and ninhydrin staining [13].

RESULTS

The significance of the differences between treated and control groups was tested by means of Student's *t*-test in

TABLE 1

GABA CONCENTRATION IN TWO REGIONS OF RAT BRAIN AFTER TREATMENT WITH AMINOOXYACETIC ACID (AOAA) OR DI-N-PROPYLACETATE (DPA)

Treatment	Time After Treatment (hr)	Motor Cortex (μ moles/g)	Cerebellum (μ moles/g)
Saline		2.0 ± 0.1 (8)	1.6 ± 0.0 (14)
AOAA (30 mg/kg)	4	8.2 ± 1.8 (4)‡	7.8 ± 0.4 (3)§
	28	3.6 ± 0.8 (4)*	2.4 ± 0.4 (4)†
DPA (400 mg/kg)	1	3.1 ± 0.2 (8)‡	3.1 ± 0.4 (8)†
	25	2.4 ± 0.1 (3)*	2.2 ± 0.2 (3)*
	48	2.0 ± 0.3 (3)	1.8 ± 0.1 (3)

Results are expressed as Means ± SEM. Number of rats in parentheses.
*$p<0.05$ †$p<0.02$ ‡$p<0.01$ §$p<0.001$ (Student's t-test)

case of the biochemical data; while nonparametric statistics, Fisher's Exact Probability test and the Mann Whitney U test were used for the behavioral measures. These last two tests yielded similar results.

Table 1 shows the changes in GABA concentration in the cortex and cerebellum at various time intervals following treatments with AOAA, DPA and saline (control). It appears that AOAA treatment caused a significant increase in GABA in both the cortex and cerebellum ($p<0.01$) as compared to controls. These high levels still persisted 28 hr later, contrary to previous findings [7]. Significant elevation of GABA was also found 1 hr after injection of DPA and persisted above control levels 24 hr later.

Table 2 shows that running time and number of falls on the balancing bar and the level of GABA in the cerebellum 4 hr after AOAA treatment were all significantly increased ($p<0.005$). In this condition the behavioural measures reverted to control levels 24 hr later while the GABA remained slightly higher than control. The performance of these same animals on the jumping platforms was not affected by the drug treatment. The difference between the mean jumping time of AOAA and saline injected groups (13.4 and 12.9 sec respectively) was not found statistically significant, and of the 10 experimental animals only one evidenced falling between platforms.

The optimum dose of AOAA used throughout the behavioural tests was 20 mg/kg. It was found that 30 mg/kg AOAA caused toxic effects that prevented testing, since the animals became completely hypotonic, unable to stand or move, lethargic with watering eyes closed to slits, with piloerection and a reddish-blue flush in the exposed parts of the face. The animals did not recover completely from this condition even after 24 hr.

Tables 3 and 4 show that, in all instances, behavioural tests closely paralleled changes in GABA concentration.

DISCUSSION

Injections of both AOAA and DPA appear to result in the elevation of GABA concentration in both the cerebellum and motor-cortex of experimental animals. In general, high GABA levels were associated with a characteristic interference with the smooth execution of learned motor responses which require balancing, time sequencing and coordination of movement. The most striking effect seemed to be the lack of coordination in the animal's hind limbs. This is of interest, in view of the reports that the vestibular system affects output to motor neurons in the lumbar region of the spinal cord [16]. The total behavioural picture in many ways resembled what is clinically described as "cerebellar ataxia" or vestibular malfunction [4].

The one discordant result is the AOAA (20 mg/kg) group which reverted to normal behaviour 24 hr after injection, whilst still having significantly elevated GABA levels. This indicates that these behavioural changes are not simply a direct result of elevated GABA levels. McKearney and

TABLE 2

PERFORMANCE (RUNNING TIME AND FALLS) ON BALANCING BAR IN RELATION TO GABA CONCENTRATION IN RAT CEREBELLUM AFTER AMINOOXYACETIC ACID (AOAA) TREATMENT

Treatment	Time After Treatment (hr)	GABA (μ moles/g)	Running Time (sec)	Number of Falls
Saline		1.6 ± 0.0 (14)	6.9 ± 0.6 (10)	1.0 ± 0.3 (10)
AOAA (20 mg/kg)	4	2.5 ± 0.0 (3)†	10.0 ± 1.1 (10)‡	3.5 ± 0.3 (10)‡
	28	2.3 ± 0.0 (3)*	6.4 ± 0.8 (5)	1.8 ± 0.9 (5)

Results are expressed as Means ± SEM. Number of rats in parentheses.
*$p<0.02$ †$p<0.01$ (Student's t-test) ‡$p<0.005$ (Fisher's Exact Probability test)

TABLE 3

PERFORMANCE (RUNNING TIME AND FALLS) ON BALANCING BAR IN RELATION TO GABA CONCENTRATION IN RAT CEREBELLUM AFTER DI-N-PROPYLACETATE (DPA) TREATMENT

Treatment	Time After Treatment (hr)	GABA (μ moles/g)	Running Time (sec)	Number of Falls
Saline		1.6 ± 0.0 (14)	11.8 ± 1.5 (15)	2.0 ± 0.3 (15)
DPA (400 mg/kg)	1	3.1 ± 0.4 (8)*‡	53.4 ± 7.9 (13)‡	11.3 ± 1.2 (15)‡
	25	2.2 ± 0.2 (3)*‡	45.2 ± 21.0 (5)‡	7.0 ± 2.1 (5)‡
	48	1.8 ± 0.1 (3)	16.0 ± 7.0 (3)	4.3 ± 1.8 (3)

Results are expressed as Means ± SEM. Number of rats in parentheses.
*$p<0.05$ †$p<0.01$ (Student's t-test) ‡$p<0.01$ (Fisher's Exact Probability test)

Patton found similar apparent dissociation between GABA level and the disruption in FI-FR operant responding after AOAA (50 and 25 mg/kg) treatment. They found that 16 hr after AOAA treatment the performance of their animals was essentially normal while, according to published results, the level of GABA should have been still quite supranormal.

Kuryama *et al.* [10] reported similar findings with respect to the time course of changes in brain content of GABA and sensitivity to electroconvulsive shock in mice after administration of AOAA (25 mg/kg). They found that only during the first 1.5 hr period following AOAA treatment was there a strong correlation between the decrease in

TABLE 4

PERFORMANCE (JUMPING TIME AND FALLS) ON PLATFORMS IN RELATION TO GABA CONCENTRATION IN RAT CEREBELLUM AFTER DI-N-PROPYLACETATE (DPA) TREATMENT

Treatment	Time After Treatment (min)	GABA (μ moles/g)	Jumping Time (sec)	Number of Falls
Saline		1.6 ± 0.0 (14)	8.4 ± 1.6 (15)	0.0 ± 0.0 (15)
DPA (400 mg/kg)	60	3.11 ± 0.4 (3)*	33.7 ± 6.6 (15)†	2.3 ± 0.4 (15)†
DPA (200 mg/kg)	40	2.0 ± 0.2 (5)	12.5 ± 3.7 (10)	0.7 ± 0.3 (10)

Results expressed as Means ± SEM. Number of rats in parentheses.
*$p<0.01$ (Student's t-test) †$p<0.005$ (Fisher's Exact Probability test)

seizure susceptibility and the increase in GABA content. They suggest that one interpretation of these results may be that soon after GABA-T is blocked with AOAA, the increased levels of GABA may indeed decrease neuronal excitability. However, compensatory increases in excitation or decreases in inhibition due to processes not involving GABA may also take place with consequent restoration of normal neuronal sensitivity even during time when a marked elevation in GABA concentration exists.

Since this discrepancy between GABA elevation and behaviour was found only in the AOAA and not in the DPA treated groups, another possible explanation may be that AOAA may affect other neurochemical changes in addition to elevating GABA levels [17].

In summary, in the present study elevated GABA levels were found to be associated with interference with the execution of learned locomotor acts, and with decrease in the coordination of the animals' hind limbs. Whether these behavioural effects can be ascribed solely to the influence of GABA is not yet entirely clear. AOAA and DPA may affect other endogenous chemical systems, which also interact with GABA to produce these effects. Further investigations are planned in order to examine the relationship of other neurotransmitters and their interactions with the GABA system.

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