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The Effects of an Enriched Environment on the Histology of the Rat Cerebral Cortex

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Recently, it has been reported by Rosenzweig, Krech, Bennett, and Diamond ('62) that increasing the environmental complexity and training of rats resulted in measureable changes in brain chemistry and in brain weight. More specificially, some of the results have demonstrated that the cerebral cortex from rats subjected to training showed not only an increase in acetylcholinesterase but also an increase in weight. It is this weight increase which concerns us at present. Though small, 7.6% in the samples from the visual cortex and 3.3% in the samples from the somatosensory cortex, the increase was consistent and repeatable since the cortical weights from trained animals exceeded those from untrained and isolated rats in 79% of the cases for the visual area (53 of 67 pairs) and in 64% for the somatosensory area (43 of 67 pairs) (Rosenzweig, Krech and Bennett, unpublished data). In contrast to the cortical weight increase, there were no changes in weight in the subcortex. Also it might be pointed out that the body weights of the isolated rats were greater than those of the trained rats by approximately 7%.

Heretofore the brain has generally been considered incapable of macroscopic physical changes as a consequence of alterations in experience. Because of the findings cited above, anatomical examinations were considered essential to determine possible causes for this increase in cortical weight.

METHODS

Behavioral conditions

In Experiment I, 11 pairs of male littermate rats from the S_1, maze-bright strain (U. C. Psychology Colony) were used. The entire experiment was replicated (Experiment II) with nine pairs of animals of the S_1 strain. The behavioral procedure has been described in detail by Krech et al. (Krech, Rosenzweig and Bennett, '60), but will be mentioned briefly here.

At 25 days post partum one animal of each pair, chosen at random, was placed in the ECT (Environmental Complexity and Training) group, the littermate being assigned to the IC (Isolated Condition) group. The ECT animals were housed together in a large cage (25″ by 25″ by 18″). Two different wooden "toys" from a set of seven were put in the cage each day. There was also a small wooden maze in the cage which the rats used as a nesting box. For 30 minutes each day the rats were allowed to explore the Hebb-Williams maze, with the pattern of barriers changed daily. At about 50 days of age formal maze training began in the Lashley III maze, the Dashiell maze, and the Krech Hypothesis Apparatus. Glucose pellet rewards were given. Food and water were normally available *ad libitum* in the home cage.

At 25 days of age the littermate of the ECT rat was placed in the IC group, consisting of an individual cage (11″ by 8″ by 8″) with solid metal walls on three sides. Food and water were available *ad libitum*, and a glucose pellet was given each time an ECT animal was so rewarded. The animals did not see or touch another animal. Neither were they handled during cleaning of the cages. All of the animals in both groups were weighed at weekly intervals. Both the ECT and IC groups remained

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about 80 days in their respective experimental conditions.

Anatomical methods

At the end of the experimental period, the animals, now about 105 days of age, were brought to the histology laboratory for sacrifice. The previous treatment of each individual animal was not made known to the histologists; only animal numbers were given. The animals were decapitated, the brains removed and placed in 10% formol-saline. Transverse frozen sections were cut at 25 μ. To be certain that cortical sections were taken from a similar region of the brain from each animal, subcortical landmarks were used to identify the specific areas. In removing a sample from the somatosensory cortex, the anterior commissure served as a guide. Where the right and left anterior commissures decussated in the midline, four sections were taken. Two were stained with Windle's modified Nissl stain (Windle, Rhines and Rankin, '43), and the others with hematoxylin and eosin.

The appearance of the cisterna magna separated from the aqueduct of Sylvius by the posterior commissure was the landmark at which four visual cortical sections were taken. These tissues were stained in a manner identical with the somatosensory samples.

Sections stained by Windle's buffered thionine solution were used for measuring the cortical depths and for differential cell counts. In calculating the depth of the cortex, eight readings with an optical micrometer were taken on one hemisphere from the dorsal aspect of Layer II to the underlying white matter. The first reading was consistently taken immediately lateral to the elevation of the corpus callosum. The succeeding readings were made one micrometer width (150 μ) lateral to each previous measurement until eight consecutive measures were completed (fig. 1). Also on additional sections cortical

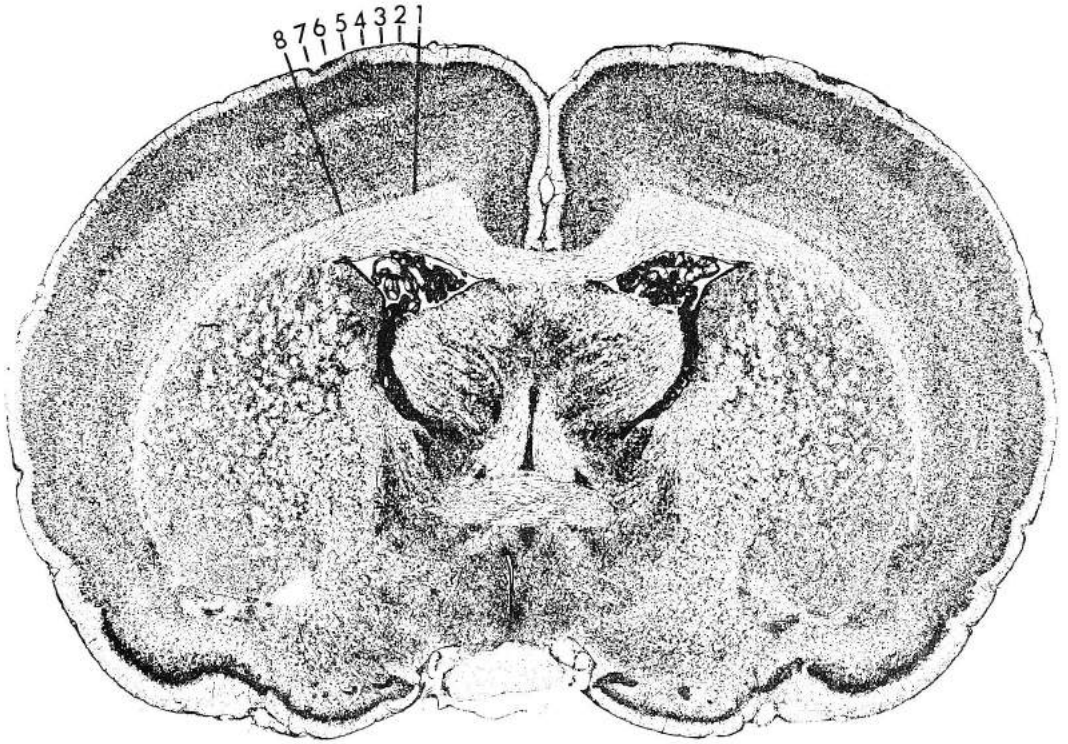

Fig. 1 Transverse section through the rat somatosensory cortex. Numbered lines indicate positions of micrometer readings through the cortical layers to measure the cortical depth.

depths were measured on both right and left hemispheres. On these latter sections, Layer I was included in the depth measurements and was also measured separately.

Differential cell counts were read with an oil immersion lens on the same slides which were used for measuring the cortical depths on one hemisphere. The cell counts were taken at the same position as was the first micrometer reading, i.e. immediately lateral to the elevation of the corpus callosum. The counts included only those neurons with nucleoli. Differential glial counts were made from the nuclei of oligodendrocytes, astrocytes, and microglia.

Blood vessel counts were made under high dry magnification (400 ×). All sizes of blood vessels were clearly defined with hematoxylin and eosin, for even if, at times, no endothelial cells were present, red blood cells in a continuous line indicated the presence of a vessel. An India ink-gelatin mixture was used in a preliminary attempt to demonstrate blood vessels. This method was abandoned, for it was felt that under the pressure of the injection, some vessels were being expanded beyond a normal picture. Also the pressure of the injections could not be standardized to the point where all animals were receiving identical treatment.

For counting, the vessels were divided into two groups, a diameter of 5 μ being the demarcation line. This division was chosen in agreement with the work of Eayrs ('54) who arbitrarily selected 5 μ to be the upper limit of size for capillaries in the rat. The first reading was taken from the surface of the cortex through Layer VI at the position immediately lateral to the elevation of the corpus callosum. Three additional readings were made one microscopic field lateral to each previous reading.

RESULTS

Cortical depth

The mean depth of the visual cortex was greater by 6.2% in the ECT than in the IC animals in both experiments I and II (table 1). Within each experiment, significance was calculated by t-test for correlated data. An analysis of variance revealed that the results of the two experiments did not differ from each other significantly and that the overall ECT-IC difference of 6.2% was highly significant ($P > 0.001$). The ECT effect was somewhat greater in the right than in the left hemisphere in both experiments (table 2), but this interhemispheric difference was not significant.

Layer I was included in some measurements (table 2) and not in others (table 1), for the pial surface on some sections was at times distorted, making measurements inaccurate on these tissues. However, with the pial surface intact, the depth of Layer I was measured with high accuracy. It was found that for Layer I the IC mean actually exceeded the ECT mean, but only by 1.7%, and the IC animal exceeded its ECT littermate on this measure in 11 cases out of 20. We may, therefore, conclude that Layer I was not concerned in the depth increase in the visual area.

In the measurements of *weight* differences, the somatosensory cortex had not shown as large or clear cut results as the visual region, and this was found again in the present measurements of cortical depth. There was greater variability in somatosensory depths, both among animals in a single experiment and between experiments, than in the visual depths. In the first experiment the ECT depths were greater than the IC depths; in the second experiment, the reverse was true.

TABLE 1

Depths (μ) of visual cortex from ECT and IC rats (excluding layer I)

Experiment	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
I	11	1332	17	1271	22	1.048	< 0.001	7/11
II	9	1404	29	1298	27	1.082	< 0.001	9/9
I+II	20	1364	16	1284	17	1.062	< 0.001	16/20

TABLE 2

Depths (μ) of visual cortex from ECT and IC rats indicating differences between right and left hemispheres (including layer I)

Experiment	N	Hemisphere	ECT Mean	IC Mean	P		
					ECT/IC	ECT vs. IC	ECT > IC
I	11	R	1323	1270	1.042	< 0.05	8/11
I	11	L	1321	1280	1.032	< 0.01	9/11
I	11	$\frac{R+L}{2}$	1322	1275	1.037	< 0.01	9/11
II	9	R	1300	1238	1.049	< 0.02	7/9
II	9	L	1304	1250	1.042	< 0.05	7/9
II	9	$\frac{R+L}{2}$	1302	1244	1.046	< 0.02	7/9
I+II	20	$\frac{R+L}{2}$	1313	1260	1.041	< 0.001	16/20

TABLE 3

Depths (μ) of somatosensory cortex from ECT and IC rats (excluding layer I)

Experiment	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
I	10	1962	18	1825	22	1.075	< 0.001	10/10
II	8	1975	40	1985	38	0.995	NS	3/8
I+II	18	1968	20	1896	21	1.038	< 0.01	13/18

TABLE 4

Mean numbers of neurons per miscroscopic field in visual cortex in ECT and IC groups

Experiment	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
I	10	3.68	0.22	4.45	0.25	0.826	< 0.05	2/10
II	8	3.18	0.26	3.79	0.24	0.839	NS	1/8
I+II	18	3.45	0.17	4.15	0.18	0.831	< 0.01	3/18

Combining experiments I and II showed the ECT mean greater than the IC mean by 3.8% ($P < 0.01$) (table 3). Here there was no consistent difference between the depths of cortex of the right and left hemispheres. The depth of Layer I was measured in the somatosensory area, but the inconsistent results prevent a definite report at this time.

Cell counts

The average number of cells was counted in each microscopic field, reading vertically from the pial surface to the underlying white matter.

In the visual area (table 4), the neurons per microscopic field were less numerous in ECT than in IC animals in both experiments. The overall difference for both experiments amounted to 17% and was statistically significant ($P < 0.01$). In the somatosensory area, the ECT group had 7% less neurons per microscopic field for experiments I and II combined ($P < 0.05$) (table 5). The decreased numbers of neurons *per field* in the ECT animals were to be expected in terms of their increased depth of cortex, but the magnitudes of the differences in neuronal density were several times as large as the differences in cortical

TABLE 5

Mean numbers of neurons per microscopic field in somatosensory cortex in ECT and IC groups

Experiment	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
I	11	3.71	0.30	3.97	0.28	0.934	NS	4.5/11
II	8	3.20	0.20	3.50	0.18	0.914	NS	3.0/8
I+II	19	3.49	0.20	3.77	0.18	0.926	< 0.05	7.5/19

depth. Again the differences were larger in the visual than in the somatosensory area.

In examining the distribution of neurons with nucleoli in the visual area, the IC animals showed more cells per field than ECT animals in Layers II, III, IVb, c, and Va. In Layers IVa and Vb the ECT counts were equal or more numerous than the IC counts (figs. 2 and 3).

In the somatosensory region the IC animals had more neurons per field than the ECTs in Layers II, IV and V. Layers III and VI were less consistent, with ECTs and ICs reversing positions throughout these layers (figs. 4 and 5).

The mean number of glia per microscopic field in the visual area was less by 6.7% in the ECT than in the IC animals for experiments I and II combined (means of 2.8 and 3.0 glia per field for the two groups, respectively). In the somatosensory area they were less by 5.9% in the ECT (3.2 glia per field) than in the IC group (3.4 glia per field). None of the glial differences was significant.

In the cortical layers, the difference in distribution of glia was so small that there was a continual reversal between ECT and IC groups, but with the overall mean for the ECT group being slightly less. It is to be noted that the somatosensory area had

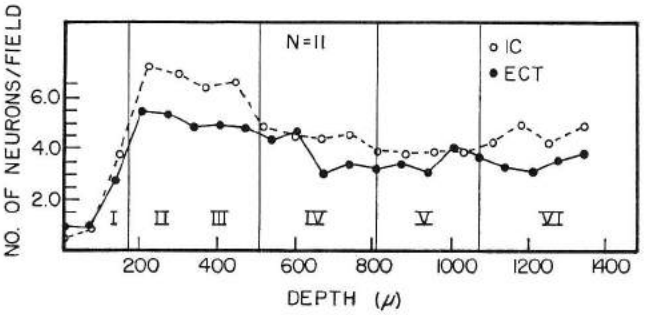

Fig. 2 Mean distribution of neurons in rat visual cortex, Experiment I.

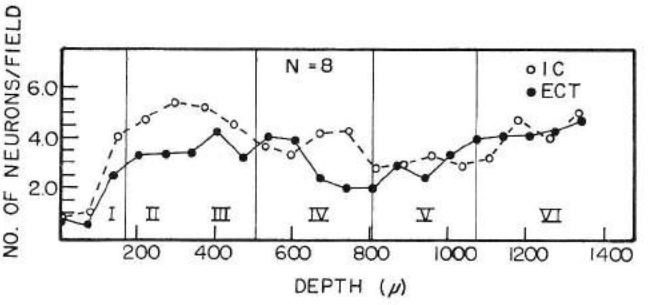

Fig. 3 Mean distribution of neurons in rat visual cortex, Experiment II.

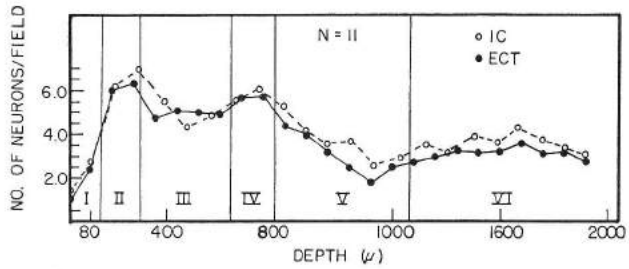

Fig. 4 Mean distribution of neurons in rat somatosensory cortex, Experiment I.

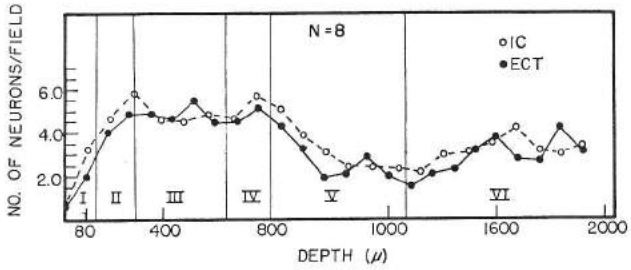

Fig. 5 Mean distribution of neurons in rat somatosensory cortex, Experiment II.

more glia per field than did the visual area by 14%; the ECT and IC groups did not differ in this respect.

In the S_1 strain of rats the ratio of glia to neurons in the visual and somatosensory areas of the cortex was approximately one to one. The ECT group has a smaller neuron/glial ratio than does the IC group in both the visual and somatosensory samples in experiments I and II (tables 6 and 7). Since the total number of neurons is presumed to be fixed, this indicates an increase in the total number of glia in the ECT group.

Capillary counts

The number of capillaries per field was less in the ECT group than in the IC group by 12% ($P < 0.05$) in the visual area and by 8% in the somatosensory area. The

TABLE 6

Neuron/glial ratios per microscopic field in visual cortex in ECT and IC groups

Experiment	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
I	10	1.28	0.09	1.53	0.18	0.837	NS	2/10
II	8	1.26	0.11	1.33	0.06	0.947	NS	2/8
I+II	18	1.27	0.07	1.44	0.10	0.882	NS	4/18

TABLE 7

Neuron/glial ratios per microscopic field in somatosensory cortex in ECT and IC groups

Experiment	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
I	11	1.18	0.10	1.23	0.10	0.959	NS	5.5/11
II	8	0.97	0.04	1.01	0.04	0.960	NS	3/8
I+II	19	1.09	0.06	1.11	0.06	0.956	NS	8.5/19

TABLE 8

Blood vessels per field in the visual cortex from ECT and IC rats in experiment I

Type of vessel	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
Capillary	9	6.17	0.28	7.07	0.32	0.873	< 0.05	1/9
Over 5 μ	9	2.39	0.35	1.90	0.22	1.258	< 0.05	7/9

TABLE 9

Blood vessels per field in the somatosensory cortex from ECT and IC rats in experiment I

Type of vessel	N	ECT		IC		P		
		Mean	S.E.	Mean	S.E.	ECT/IC	ECT vs. IC	ECT > IC
Capillary	7	8.00	0.54	8.67	0.72	0.923	NS	2/7
Over 5 μ	8	2.89	0.36	2.83	0.29	1.021	NS	6/8

number of vessels over 5 μ in diameter in the ECT group was increased greatly over the IC group in the visual area — 26% ($P < 0.05$) — and only slightly in the somatosensory area — 2%. The overall vascularity of the somatosensory area was greater than in the visual area (tables 8 and 9).

DISCUSSION

These results indicate that a complex environment leads to measureable histological changes in the rat cerebral cortex. The depth of the cortex increases with enriched experience, and the number of cells per field decreases. These changes are more marked in the visual area than in the somatosensory area. Since the depth increase in the visual area amounts to 6% and the neuron density decrease to 17%, it is not unreasonable to assume that the cortex of the visual region is expanding in volume and not in one direction only. (An increase of 6% in linear dimension would be expected to yield an increase of 19% in volume.) In the more variable somatosensory area the correlation is not as good. The depth increases by 4%, and the density of neurons decreases by 7%.

The expansion of the cortex might be due in part to further development of dendritic branching, a possibility supported by the following reports of other investigators. Allen ('12) and Sugita ('17) reported that the rat cerebral cortex has a full complement of neurons 20 days after birth. Any subsequent development is by peripheral extension of dendrites and increased amount of branching. Eayrs and Goodhead ('59) showed that in the rat the cortex has attained adult characteristics by 18 days, and the average number of dendrites arising from the perikaryon has reached the adult figure as early as the twelfth day of age, further development being due to dendritic branching. In favor of dendritic ramifications being responsible for increases in brain weight are the reports of Erp Taalman Kip ('38), Horn ('55), and Tower (Tower and Elliott, '52) who found a highly significant correlation between brain weight and fiber count, indicating that brain weight is directly related to fiber density. However, Layer I, being rich in dendritic branches, is not affected in the visual area by the enriched environment. According to Eayrs and Goodhead ('59), Layer I shows very little change during the growth of the rat cerebral cortex.

Studies are under way at present to determine by the Golgi-Cox method whether increased dendritic ramifications in the ECT group are the major components responsible for the depth increase. This work is being carried out by Dr. P. Coleman at the University of Maryland.

The greatest difference between cell counts in the ECT and IC groups is apparent in Layers II and III. According to Krieg ('46), the main mass of intercellular material in the supragranular layers is dendritic, again giving support to the hy-

pothesis that the ECT cortical increase is due in part to dendritic branching.

The indication of an increase in total number of glia with ECT is consistent with recent measures of acetylcholinesterase (AChE) and cholinesterase (ChE) in the cortex of the ECT and IC rats (Bennett, Krech and Rosenzweig, '63). AChE, which occurs chiefly in neurons, decreases per unit of tissue weight with ECT, while ChE, which occurs chiefly in glia, increases per unit of weight with ECT.

The present results indicate that the somatosensory area has a greater vascularity than does the visual area, a finding in accord with that of Craigie ('21). That the ECT group shows less capillaries per field, is in agreement with our findings of a smaller number of neurons per microscopic field, indicating again that the increase in the depth of the cortex is due likely to an increase in the dendritic branching between the cells and capillaries. The ECT group shows an increase in larger vessels, indicating a demand for a greater blood supply. Since the larger vessels (those over 5 μ) are running radially for the most part, their increase in number would not affect the cortical depth as measured. In order that measures of cell density would not be affected by the presence of blood vessels, the area to be included for cell counts was first examined under low power magnification to make certain no large vessels would be encountered during the final cell counts under oil of immersion.

That there are more larger vessels in the ECT group and less smaller ones might be explained in the following way. Perhaps all of the blood vessels in the ECT group increase in size, but due to the thinness of the small capillary walls these vessels collapse after death and leave no indication of previous size. This statement is in agreement with de Vries ('34) who reported that brain tissue exerts pressure on capillaries to compress them after death. On the other hand, the larger vessels with a thicker wall remain distended and more of them can be accounted for in the experimental group. Thus, the ECT group shows more larger vessels and fewer smaller vessels than their IC littermates. Without considering the possibility of vessels collapsing, these results could be explained by the hypothesis that all vessels increase in size with the ECT condition. Thus, more vessels are counted in the large vessel (over 5 μ) category, and less in the small vessel category.

It is well known that the degree of vascularity corresponds directly with the degree of functional activity, Craigie ('38), Mott ('14), and Scharrer ('45). Furthermore, with specific reference to the nervous system, Dunning and Wolff ('37) have reported that the vascularity of tissues varied with the number of synaptic structures and not with quantitative differences in nerve cell bodies. Since we have argued that the increase in cortical depth may be due to additional dendritic ramifications, then an increased blood supply is not unlikely in the ECT brains.

It is necessary to mention that increased dendritic ramifications are, undoubtedly, not the only factors to be considered as possible causes for the cortical depth increase. The possibility of changes in such factors as perikaryon size, glial volume, cortical water, sodium, potassium, and chloride content, should not be disregarded.

SUMMARY

1. An enriched environment increases the depth of the cortex in the rat brain.

2. The depth increase is greater in the visual cortex than in the somatosensory cortex.

3. The effect of the enriched environment is evident in both the right and left hemispheres in the visual area. In the somatosensory area the differences are not as consistent.

4. Layer I does not appear to be involved in the depth increase in the visual cortex. Layers II and III show a greater increase in the ECT group than do the other layers in the visual area.

5. The neurons, glia, and capillaries are less numerous *per field* in the animals experiencing the enriched condition, indicating a greater amount of intercellular and intervascular substance. It is suggested that increased dendritic branching may partially account for this substance.

6. There are more large vessels in the ECT animals and less smaller ones; a possible explanation for this result is offered.

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