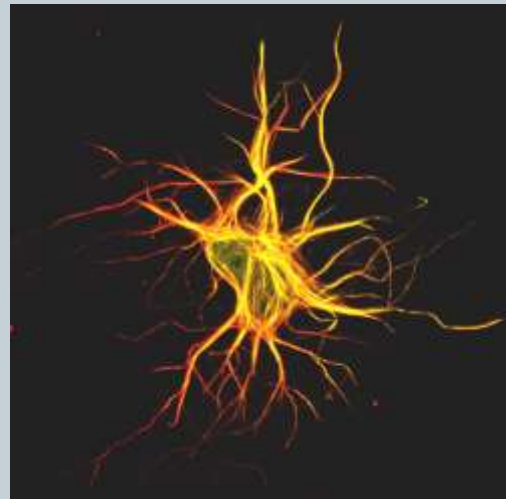


Cytoarchitectonic and Immunohistological Profile of GABAergic and Glutamatergic Neurons in the Posterior Piriform Cortex in Fragile X Mice

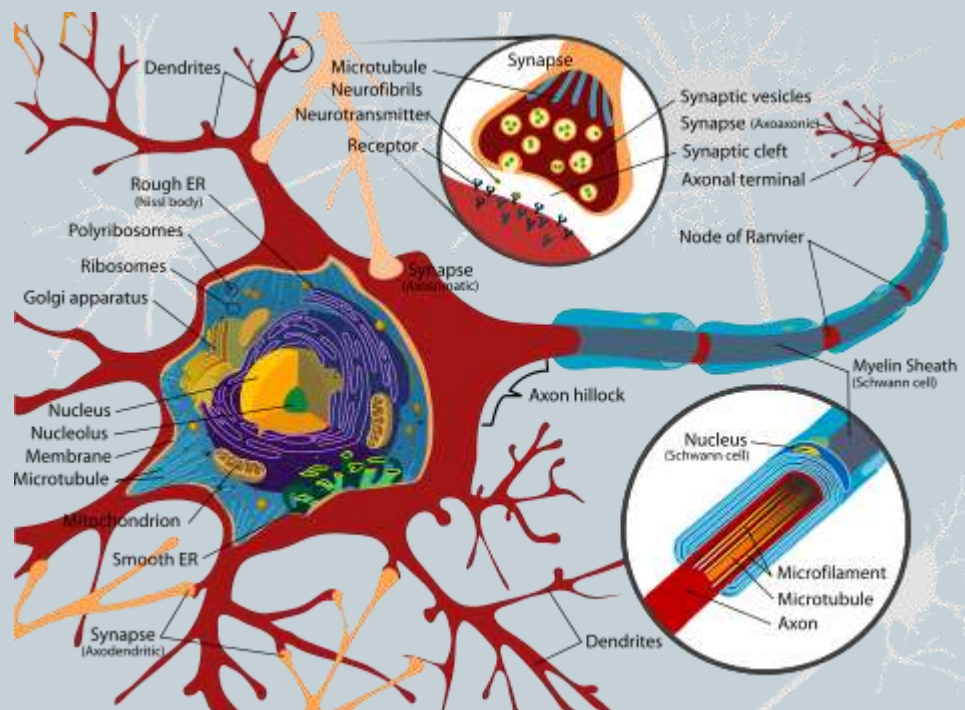


AN EXAMINATION OF THE CELL DENSITIES
IN FMR1KO MICE

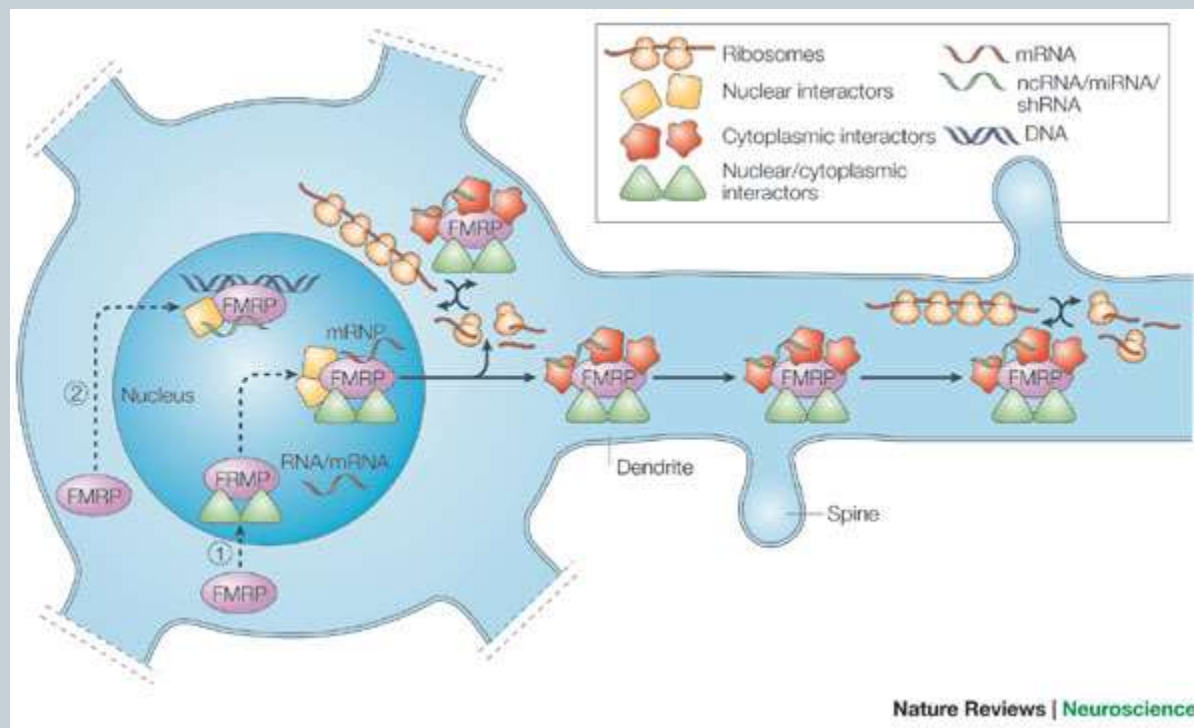
TYLER FELTON



Basic Cell Physiology



Basic Understanding of Fragile X Protein



Fragile X syndrome



- X-linked disorder caused by a repeat of a triplet of DNA nucleotides causing inactivation of the FMR-1 gene.
- Fragile X syndrome is the most common cause of inherited mental retardation occurring in 1 of 4,000 male births and 1 of 8,000 female births.
- Inactivation of the FMR-1 gene is thought to adversely affect synaptic maturation and brain circuitry. With notable hyperactivity hypersensitivity.
- FMR1 knockout mice were used in this experiment.

GABAergic Neurons



- GABA-releasing interneuron's are composed of multiple subtypes with combinatory expression of different neuronal markers.
- GABAergic Neurons show a unique easily identifiable multilayering in the posterior piriform cortex.

Posterior Piriform Cortex



- The PPC is a phylogenetically old region of the brain and has been extensively studied in an attempt to elucidate its intrinsic circuitry.
- Neural circuits of the PPC mediate complex functions related to integrating odor cues with behavior, affective states, and multisensory processing.
- Relatively simple three cortical layers, convenient segregation of afferent and associative inputs, and that the understanding of the PPC microcircuit may provide a model example of associative memory processes are some reasons for the examination of this region.
- Also, interneurons of the PPC are implicated as important participants in cortical processes including: epileptogenesis, and feedback inhibition to pyramidal cells.

Posterior Piriform Cortex



Methods



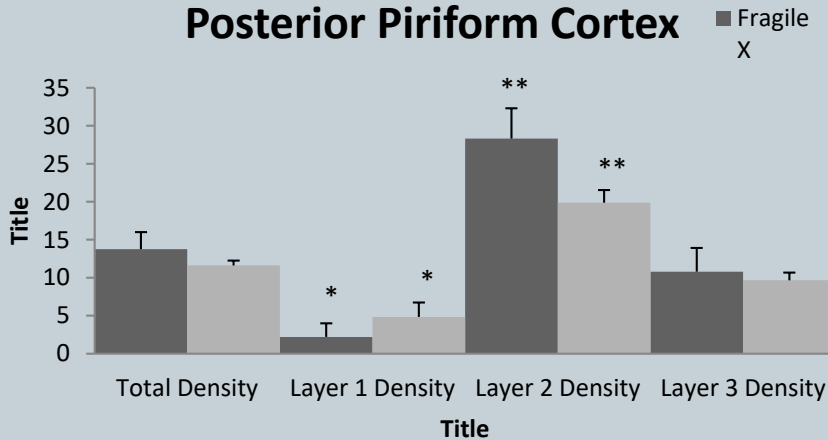
- Five groups of mice aged 1 year and 47 days were used including the wild-type GAD67-GFP mice as a control group and FMR1-Knock out mice.
- The mice were transcardially perfused and the brain was removed and cut into 40 μm sagittal cuts.
- The brain slices were then stained with Glur1, Glur2, GAD67, and PV fluorescent antibodies.
- The brain Slices were then mounted and examined under 10x and 20x through an epifluorescent microscope.
- Using Neurolucida®[®], we were able to obtain cell densities in each of the brain slices.

Results

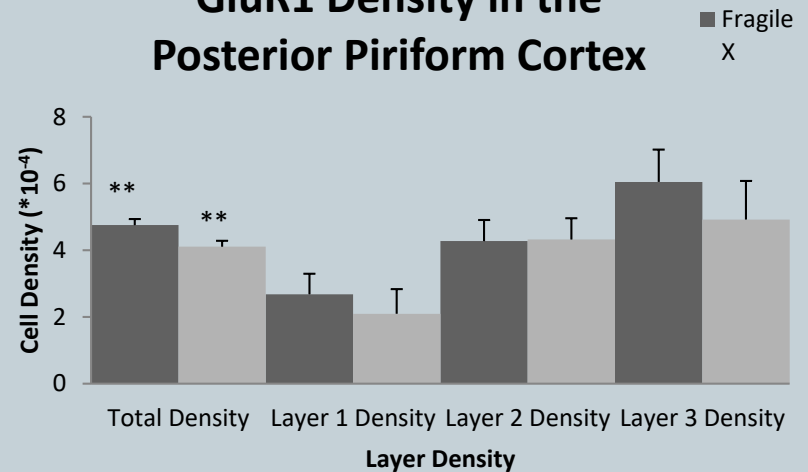


- The results showed a significant difference between the cell densities of GluR2 and GluR1 of the wild-type and FMR1KO mice, but not PV in region 1 of the PPC.

GluR2 Cell Density in the Posterior Piriform Cortex



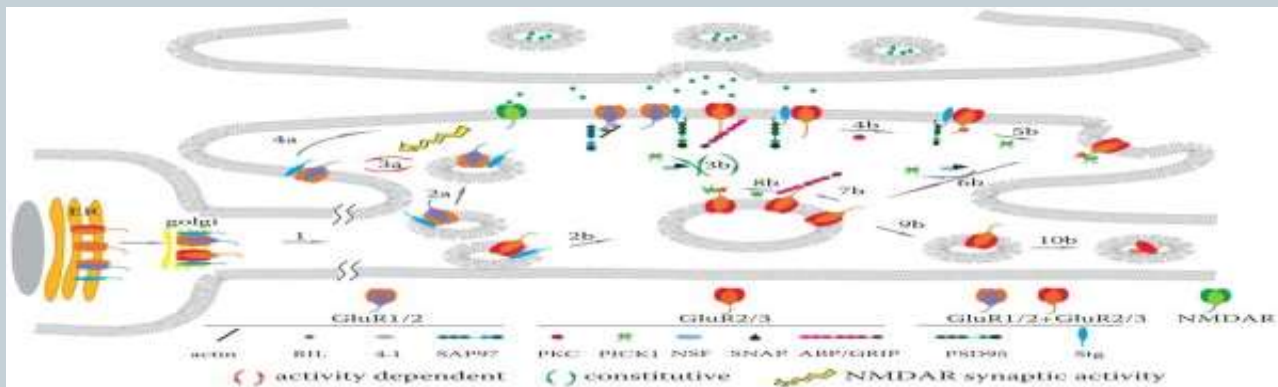
GluR1 Density in the Posterior Piriform Cortex



GluR2 and Glutamate



- Glutamate is the major excitatory neurotransmitter in the CNS and as such the glutamate receptors play a vital role in the mediation of excitatory synaptic transmission.
- GluR2 is an AMPA receptor ligand gated ion channel that functions to regulate the permeability of calcium through the plasma membrane



Glutamate Receptor 2 Structure

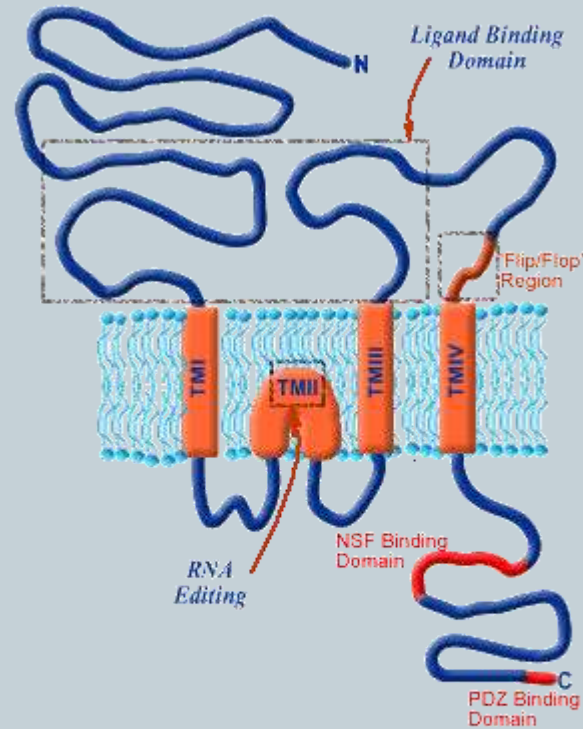
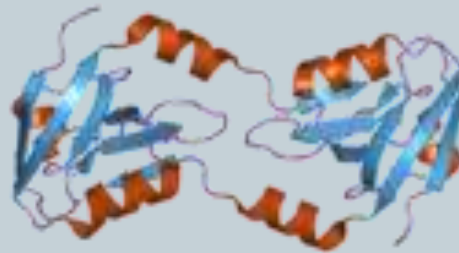


Figure 5. Structure of GluR2. The GluR2 subunit is used here to illustrate the nature of the AMPA receptor subunits. The N-terminus is extracellular and the C-terminus is intracellular. Splice variation is within the 'Flip/Flop' region, giving two variants for each gene sequence. The C-terminus possesses a binding site for NSF and a PDZ motif for the binding of proteins such as PICK1. In addition, GluR2 undergoes RNA editing at the Q/R site, resulting in the expression of Ca^{2+} impermeable receptors

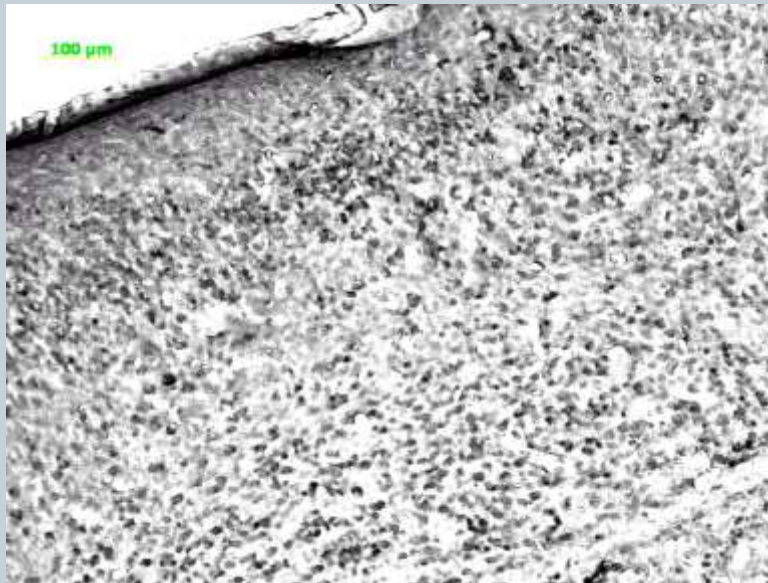
Glutamate 1 Receptor Structure



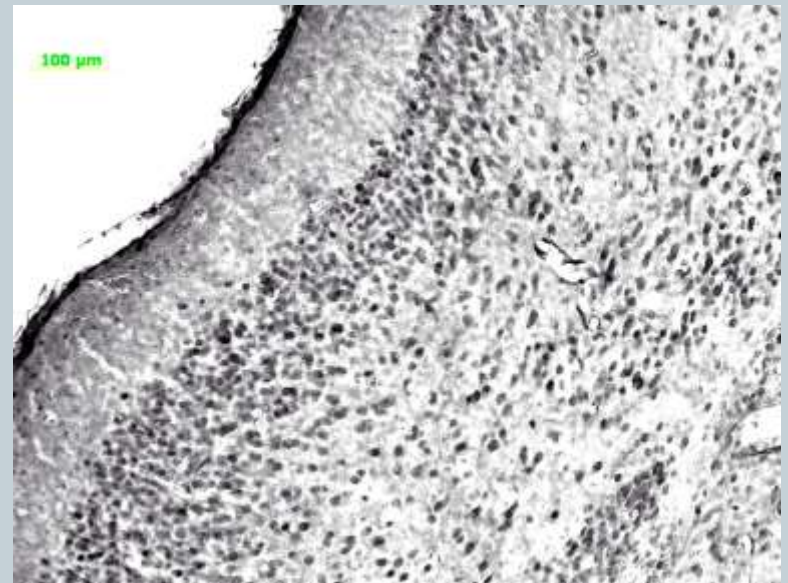
GluR2 in the Posterior Piriform Cortex



Wild-Type
GluR2



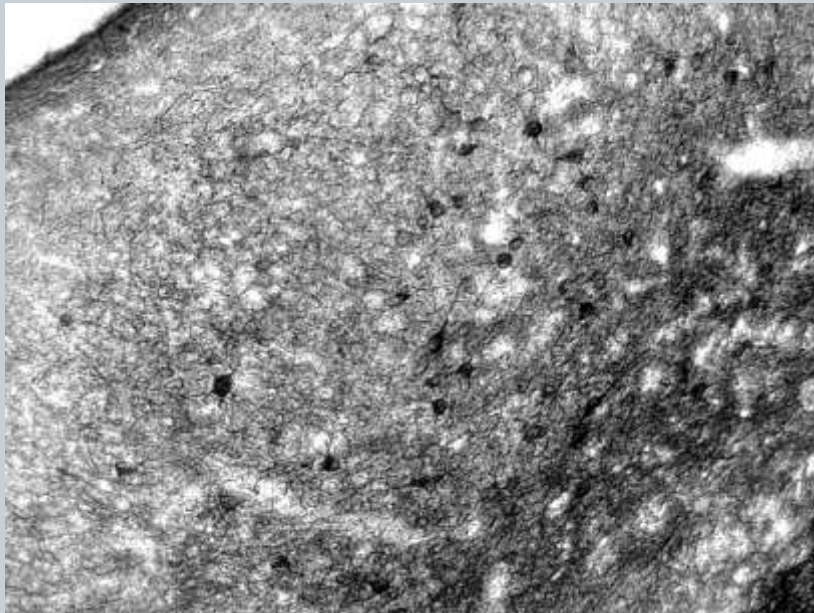
FMR1KO
GluR2



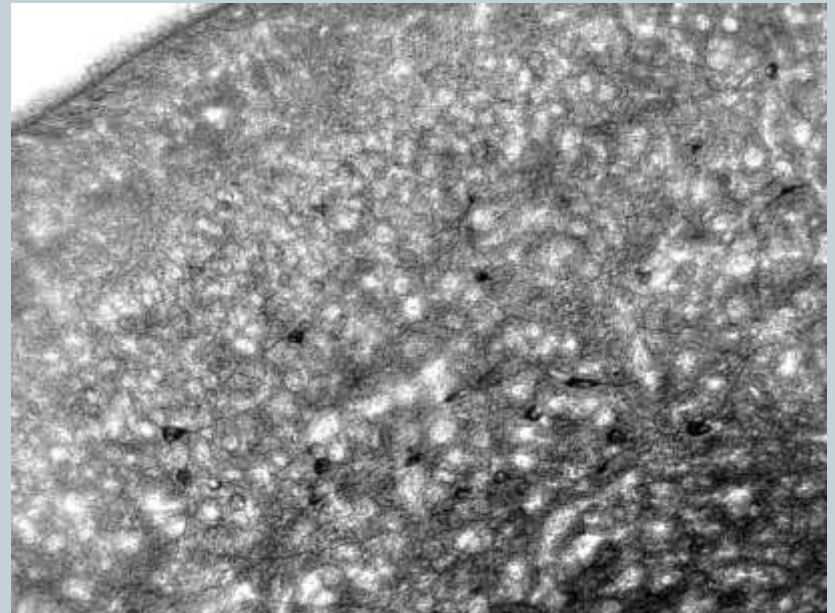
GluR1 in the Posterior Piriform Cortex



Fragile X Brain



Wild Type Brain



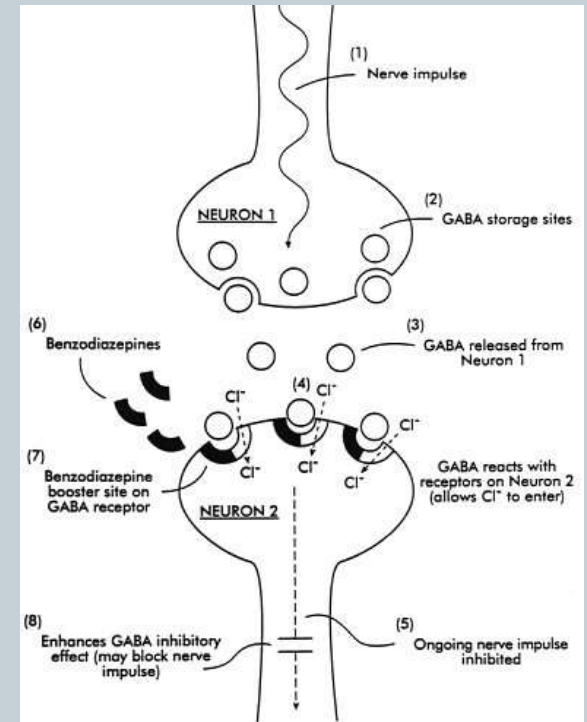
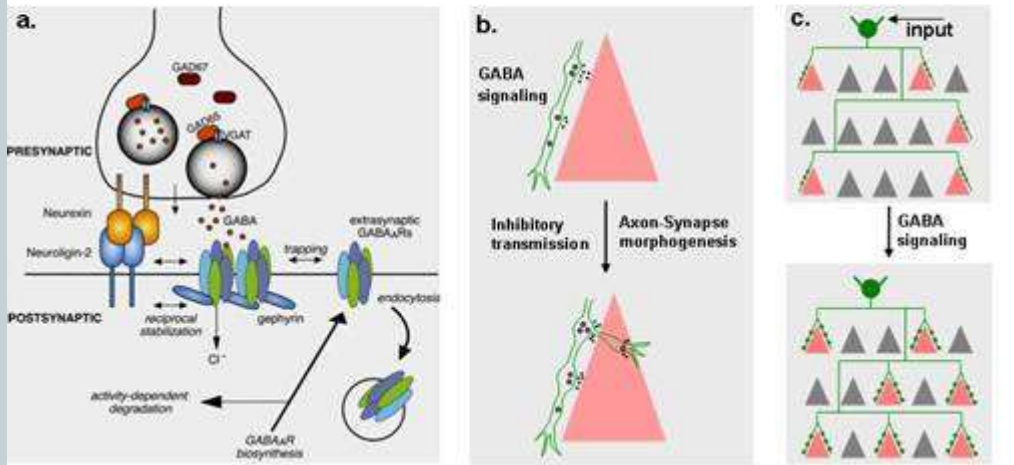
GAD67 Enzyme Function



GABA IS CONVERTED FROM GLUTAMIC ACID BY THE ACTION OF GLUTAMIC ACID DECARBOXYLASE (GAD). THERE ARE TWO FORMS OF GAD IN THE BRAIN, GAD67 AND GAD67, REFERRING TO A MOLECULAR WEIGHT, RESPECTIVELY.

GAD67

GABA signaling regulates activity-dependent development of inhibitory synapses and innervation patterns



Conclusion



- These results provide important details into the mechanisms of the Fragile X syndrome and consequences of the inactivation of the FMR1 gene.
- They provide a stepping ground for further understanding the mechanisms of the GluR2 receptor and provide consequences of the overproduction of GluR2 in the PPC.
- Research in this area is vital in providing potential treatment or even a cure for the #1 cause of inherited mental retardation.

Acknowledgements



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