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Analysis of BDNF protein expression

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Tamiris Silva[1], Sandra Kalil Bussadori[1], Raquel Agnelli Mesquita-Ferrari[1]

[1]Department of Postgraduate Program in Rehabilitation Sciences, Universidade Nove de Julho, UNINOVE, São Paulo, SP, Brazil

Tamiris Silva

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We are still developing and optimizing this protocol

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Abstract

Neuroplasticity regards physiological and/or structural changes in circuits in response to changes in patterns caused by an injury and/or environmental influences, resulting in an increase in angiogenesis and synapses. Although neuroplasticity is more commonly associated with the cerebral cortex, all parts of the nervous system, including the spinal cord, demonstrate plasticity, such as synaptic learning and memory mechanisms, dendritic pruning, collateral sprouting and axonal regeneration.Another factor that influences and regulates neuroplasticity is brain-derived neurotrophic factor (BDNF), the most important functions of which are the regulation of synaptogenesis, neuroprotection and increased dendritic branching. BDNF also exerts an influence on the functional and structural aspects of synaptic transmission. Rehabilitation induces neuroplasticity, which is demonstrated by improved functional performance and an increase in BDNF. Protein expression of BDNF will be quantified from the saliva samples using the ELISA technique. A DELUXE HUMAN commercial kit (BioLegend®) will be used and the analysis will be performed following to the manufacturer's instructions.

Attachments

DOCX

BDNF.docx

15KB

Materials

MATERIALS

LEGEND MAX™ Human BDNF ELISA Kit **Catalog** #446607

• Deionized water

Troubleshooting

Before start

Saliva samples will be collected on Day 1 and after 24 sessions between 10 and 11 am. For such, specific instructions will be given to participants, such as avoid brushing, using salivary stimulants and consuming a main meal one hour prior to the collection, avoid consuming acidic or sugary foods 20 minutes before the collection.

Centrifuge for 10 minutes in a refrigerated centrifuge at 1,000 X g. Separate 300 µl into 1.5 mL microtubes and store saliva samples at <-80 ° C.

After all collections are performed (pre and post treatment), begin preparing the assay.

LEGEND MAX™ Human BDNF ELISA Kit

Protein expression of BDNF will be quantified from the saliva samples using the ELISA technique. A DELUXE HUMAN commercial kit (BioLegend®) will be used and the analysis will be performed following to the manufacturer's instructions.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 1,000 µL of the 10,000 pg/mL top standard by adding 50 µL of the standard stock in 950 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 10,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the Human BDNF standard concentrations in the tubes are 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 312.5 pg/mL, and 156.3 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).
4. Wash the plate 4 times with 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on clean, absorbent paper. All subsequent washes should be performed similarly.
5. Add 50 µL of Assay Diluent C to each well that will contain the standard dilutions or samples.
6. Add 50 µL of standard dilutions or samples to the appropriate wells.
7. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with shaking.
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
9. Add 100 µL of Human BDNF Detection Antibody solution to each well, seal th10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP solution to each well, seal and incubate the plate at room temperature for 30 minutes with shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash

Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 15 to 20 minutes in the dark. Wells containing human BDNF should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
15. Read absorbance at 450 nm immediately. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nme plate and incubate at room temperature for 1 hour with shaking.