**Methods**

**according to the ARRIVE Guidelines (**<https://doi.org/10.1371/journal.pbio.1000412>**)**

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| Ethical statement | All procedures were conducted in conformity with the Brazilian Council for Control of Animal Experimentation (COBEA) for the care and use of laboratory animals, which complies with international laws and politics. The protocols described have been approved by the local Ethical Committee (CEUA 12.1.235.53.2) and all efforts were made to minimize animal suffering. |
| Study design | *Group randomization and analyses:*Animals were distributed to the different treatment groups by random individual sorting, each cage containing a pair of animals was randomly chosen from the shelfs to receive the treatments. Each animal of each cage was assigned as number 1 or 2 (tail mark) in order to stablish the treatment order and time. The animals from the same cage received the same treatments. Behavioral analysis was conducted by an observer blind to the treatments. Each experimental session was numbered and recorded four animals, the blind evaluator received the videos in a random order. *Groups controls:*All behavioral experiments had a negative (vehicle) control group. In the experiments with double injection of drugs a double negative group (vehicle + vehicle) was included, and all drugs had their own appropriate vehicle control group (vehicle + drug). Vehicles used were isotonic saline or 50%:50% Polyethylenglycol:isotonic saline. |
| Experimental procedures | *Stereotaxic surgery:*Coordinates for cannula implantation into the PL (AP = +3.3mm from bregma; L = +1.9mm from the medial suture, V =−2.4mm from the skull with a lateral inclination of 22◦) were selected from the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 2007). The cannulae tips were placed 1mm above the site of the injection, and the cannulae were attached to the skull bone with stainless steel screws and acrylic cement. An obturator inside the guide cannulae prevented obstruction. After surgery, the animals received a poly-antibiotic (Pentabiotico®, Fort Dodge, Brazil) and a nonsteroidal anti-inflammatory, flunixine meglumine (Banamine®, Schering Plough, Brazil), for post-operation analgesia. The needles (33G, Small Parts, Miami Lakes, FL, USA) used for microinjections were 1mm longer than the guide cannulae and were connected to a 10µL syringe (7002-H, Hamilton Co., Reno, NV, USA) through PE-10 tubing.*WB analyses:* Protein extraction: The animals were deeply anesthetized with chloral hydrate 5% (10 mL/Kg) and the brains were extracted. The mPFC was dissected with a punch needle (4 mm) and homogeneized with a lysis buffer (NaCl 137 mM; Tris-HCl 20mM pH 7,6; glicerol 10% e sodium ortovanadate 0.05%) complemented with a protease inhibitor cocktail (Sigma-Aldrich, Cat#P2714, USA), tyrosine phosphatase inhibitor and serine phosphatase inhibitor. The homogenate was centrifuged at 9000 x g for 15 min, the supernatant was collected and kept at -80 ºC until use. The amount of total protein was determinated by Bradford’s method (Bradford 1976) and used to normalize the amount of each sample to be used on the WB assay. *Measurement of TrkB and mTOR expression by WB:*Thirty micrograms of total protein of each sample was separated on an 8% (TrkB) or 12% (mTOR) polyacrylamide gel and transferred to a PVDF membrane. After the blockade with BSA 5% on TBST buffer (150mM NaCl, 20mM Tris-HCl, 0.05% Tween80, pH 7.6), the membranes we incubated overnight at 4 ºC with a primary antibody (ab) against TrkB (Dilution 1:1000, 80E3 - #4603 – Cell Signaling), pTrkB (Dilution 1:1000, Tyr 706/707 – C50F3 - #4621 – Cell Signaling), mTOR (Dilution 1:1000, 7C10 - #2983 – Cell Signaling) or pmTOR (Dilution 1:1000, Ser 2448 – D9C2 - #5546 – Cell Signaling). Then, the membranes were incubated for one hour at room temperature with a secondary ab conjugated to a peroxidase (Dillution 1:2000, #SC2317, Santa Cruz), which was suitable for the species where the primary ab was originated. After washing with TBST, the staining obtained with a chromogenic reagent was scanned and digitalized. The expression of pTrkB and mTOR was normalized using the total level of TrkB or mTOR in each sample. To enable this process, the membranes went through a mild stripping procedure with a solution containing β-mercaptoethanol (0.8% on TBS buffer, during 30 min at 50 ºC). Then, the membranes were washed, with TBS and TBST 0.05%, and incubated again with BSA 5% on TBST buffer. Next, the membranes were incubated with an ab against TrkB or mTOR (overnight, 4 ºC), followed by the secondary ab conjugated to a peroxidase. Finally, the membranes were revealed as described above.*Experimental conditions:* For all the experiments, animals were purchased one week prior to the start of the experiments. After the stereotaxic surgery, they were housed in pairs (cage area: 570 cm2) and returned to the animal keeping room where they remained until the experiments started one week later. Their cages were cleaned three times a week. On the experimental day, they were brought to the experimental area and allowed to acclimatize for 1h before starting the injections. The injections were performed in the acclimation room where the animals were kept until testing. The behavioral procedures (FST and OFT) were performed in specific experimental rooms next to the acclimation room with similar lightening and temperature conditions (25 ± 1◦C). All animals were kept in pairs throughout the experiments. Regarding drug choices, drug doses and route of administration, our choices were based on previously published papers and experience from our research group in order to test our hypothesis. The references provided show that the drugs, doses and route of administration chosen agree with scientific consensus as the appropriate approach to study the neurobiology of depression. |
| Experimental animals | *Animals source:*All animal used came from a breeding facility from the University of São Paulo – Campus Ribeirão Preto. |
| Housing and husbandry | *Facilities and nutrition:*The animals were kept in a non-SPF facility in an animal keeping room of the Pharmacology Discipline (housed in pairs - cage area: 570 cm2). The housing cages had no environmental enrichment. The animals had access to tap water and the food was delivered in processed chew pellets (Nuvilab, CR-1, Brazil) containing (as informed by the provider): SODIUM CHLORIDE (SALT COMMON), VITAMIN A, VITAMIN A, VITAMIN D3, VITAMIN E, VITAMIN K3, VITAMIN B1, VITAMIN B2, VITAMIN B6, VITAMIN B12 , NIACINE, CALCIUM PANTOTENATE, FOLIC ACID, BIOTINE, HYDROCHLORIDE CHLORIDE, MANGANESE MONOXIDE, ZINC OXIDE, COPPER SULFATE, CALCIUM IODATE, SODIUM SELENITE, COBALT SULFATE, LYSINE, METHIONINE, BHT. GUARANTEE LEVELS BY PRODUCT KILOGRAM: HUMIDITY (MAX) 125 G / KG; MINERAL MATERIAL (MAX) 90 G / KG; CALCIUM (MIN-MAX) 10-14 G / KG; GROSS PROTEIN (MIN) 220 G / KG; GROSS FIBER (MAX) 70 G / KG; PHOSPHORUS (MIN) 8,000MG / KG; EXTRACT ETEREO (MIN) 40 G / KG; VITAMINS: VITAMIN A (MIN) 13,000 IU / KG; VITAMIN D3 (MIN) 2,000 IU / KG; VITAMIN E (MIN) 34 IU / KG; VITAMIN K3 (MIN) 3 MG / KG; VITAMIN B1 (MIN) 5 MG / KG; VITAMIN B2 (MIN) 6 MG / KG; VITAMIN B6 (MIN) 7 MG / KG; VITAMIN B12 (MIN) 22 MCG / KG; NIACINE (MIN) 60 MG / KG; CALCIUM PANTOTENATE (MIN) 20 MG / KG; FOLLIC ACID (MIN) 1 MG / KG; BIOTINE (MIN) 0.05 MG / KG; HILL (MIN) 1,900 MG / KG. MINERALS: SODIUM (MIN) 2,700 MG / KG; IRON (MIN) 50 MG / KG; MANGANES (MIN) 60 MG / KG; ZINC (MIN) 60 MG / KG; COPPER (MIN) 10 MG / KG; IODO (MIN) 2 MG / KG; SELENIUM (MIN) 0.05 MG / KG; COBALT (MIN) 1,5 MG / KG; FLUOR (MAX) 80 MG / KG. AMINO ACIDS: LYSINE (MIN) 12 G / KG; METHIONINE (MIN) 4,000 MG / KG. ADDITIVES: BHT 100 MG / KG. 3.485 Kcal/g.*Welfare assessments:*The welfare of the animals was assessed daily. The cages and bedding were changed every two days as well as the food and water. During the surgery procedures, the breathing rate and tail and paw pinch reflexes were checked frequently to confirm that the animals were properly anesthetized. After the surgery, the animals were inspected daily to evaluate recovery from the surgery. In order to do this, the behavior and fur of the animals were evaluated: if necessary an extra dose of a nonsteroidal anti-inflammatory was given. |
| Sample size | The total number of animals used was 295 male Wistar rats. Experiment 1(A + B) used 134 rats, whereas experiment 2 (A + B) used 161 rats. Regarding the FST, sample size was calculated considering an error type a 5% and β = 20%, considering a minimal immobility time difference of 50 seconds and a standard deviation of 30 seconds (Joca & Guimarães, 2006), the estimated n was 6-8 animals/group. It is important to highlight that the stereotaxic surgery procedure leads to unexpected loss of animals due to different reasons; i.e. surgery complications/injections, guide cannula obstruction by blood clot, misplacement of the guide cannula leading to the wrong injection site. Consequently, the number of animals was smaller than expected in some experimental groups. For the OFT and WB, a similar number of animals/group was used. |
| Allocating animals to experimental groups | Animals were distributed to the different treatment groups by random individual sorting, each cage containing a pair of animals was randomly chosen from the shelfs to receive the treatments. Each animal of each cage was assigned as number 1 or 2 (tail mark) in order to stablish the treatment order and time. The animals from the same cage received the same treatments. Behavioral analysis was conducted by an observer blind to the treatments. Each experimental session was numbered and recorded four animals, the blind evaluator received the videos in a random order. All behavioral experiments had a negative (vehicle) control group. In the experiments with double injection of drugs a double negative group (vehicle + vehicle) was included, and all drugs had their own appropriate vehicle control group (vehicle + drug). |
| Experimental outcomes | Depressive-like state (FST), molecular and proteomic changes as stated in the manuscript. |
| Statistical methods | All statistical analyses were performed with GraphPad Prism version 5.01 for Windows (GraphPad software, San Diego, CA, USA). The results were analyzed as group means by one-way ANOVA, followed by Dunnett’s post-hoc test. Differences with p < 0.05 were considered significant. Bartlett’s test for equal variances was applied to verify normality and homogeneity of variances. Grubb's test was carried out to detect outliers. |