**Methods**

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

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| Ethical statement | All animal procedures were carried out under the approval of the Danish National Committee for Ethics in Animal Experimentation (2012-15-2934-00254), which comply with international laws and politics. 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). |
| Experimental procedures | *Experimental conditions:* For all the experiments, animals were purchased one week prior to the start of the experiments. The rats were housed in pairs (cage area: 570 cm2). 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 (22 ± 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 animals used came from Taconic, Copenhagen – DK. Animals arrived in the laboratory one week before the beginning of each experiment. |
| 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 apart from shelter, nesting material and chewing stick. The animals had access to tap water and the food was delivered in processed chew pellets Altromin, 1324, rat/mouse) containing (as informed by the provider): Crude nutrients [%]: crude protein 19.2, crude fat 4.1, crude fiber 6.1, crude ash 6.9, moisture 11.3, monosaccharides 0.0, disaccharides 4.9, polysaccharides 35.9. Amino acids [%]: alanine 0.9, lysine 0.8, arginine 1.2, methionine 0.2, aspartic acid 1.6, phenylalanine 0.8, cystine 0.3, proline 1.2, glutamic acid 3.8, serine 0.9, glycine 0.8, theronine 0.6, histidine 0.4, tryptophan 0.2, isoleucine 0.8, tyrosine 0.6, leucine 1.3, valine 0.9. Metabolic energy 3188 kcal/kg; kcal from protein 24 %, kcal from fat 12 %, kcal from carbohydrates 64 %. Trace elements [mg/kg]: iron 198, manganese 97, zinc 94, copper 13, iodine 1.6, selenium 0.3, cobalt 0.4. Minerals [%]: calcium 0.7, phosphorus 0.5, magnesium 0.2, sodium 0.2, potassium 0.9. Additive vitamins per kg: vitamin A 15000 IU, vitamin D3 600 IU, vitamin B1 18 mg, vitamin B2 12 mg, vitamin B6 9 mg, vitamin B12 24 µg, vitamin C 36 mg, vitamin K3 3 mg, vitamin E 75 mg, folic acid 2 mg, biotine 60 µg, nicotinic acid 36 mg, pantothenic acid 21 mg, choline chloride 600 mg.*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.  |
| Sample size | The total number of animals use was 114 male Sprague-Dawley rats. Experiment 1 used 77 rats, experiment 2 35 rats, experiment 3 and 4 used 37 rats. Regarding the FST, The 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. However, some animals showed abnormal behavior related to welfare changes before the experiments and were exclude from the measurements, thus leading a few experimental groups to be composed of five animals. Regarding the OFT, the sample size was based on the current scientific practice as observed in several published papers. |
| 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. 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) and blood corticosterone/ACTH 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. |