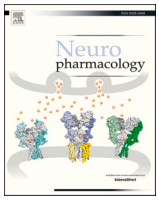
Neuropharmacology 242 (2024) 109767

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Temporal dynamics of BDNF signaling recruitment in the rat prefrontal cortex and hippocampus following a single infusion of a translational dose of ketamine

Lucia Caffino [a,1], Francesca Mottarlini [a,1], Alessandro Piva [b,1], Beatrice Rizzi [a], Fabio Fumagalli [a,2], Cristiano Chiamulera [b,*,2]

[a] Department of Pharmacological and Biomolecular Sciences 'Rodolfo Paoletti', Università degli Studi di Milano, Via Balzaretti 9, 20133, Milan, Italy
[b] Neuropsychopharmacology Lab, Section Pharmacology, Dept Diagnostic & Public Health, P.le Scuro 10, University of Verona, Verona, Italy

ARTICLE INFO

Handling Editor: Prof. J.F. Cryan

Keywords:
Subanaesthetic ketamine
Intravenous infusion
Prefrontal cortex
Hippocampus
BDNF
Rats

ABSTRACT

Despite several decades of investigations, the mechanisms underlying the rapid action of ketamine as antidepressant are still far from being completely understood. Several studies indicated Brain-Derived Neurotrophic Factor (BDNF) as critical for the fast antidepressant action of ketamine, due to its contribution in early and rapid synaptic adaptations. However, previous reports have been essentially based on ketamine dosing modes that differ from the clinical route of administration (slow intravenous infusion). In this report, we investigated the effects of a ketamine dosing mode in male Sprague-Dawley rats showed to be translational to the clinically effective mode in patients. We focused on the first 24 h after infusion to finely dissect potential differences in the contribution of BDNF signaling pathway in prefrontal cortex and hippocampus, two brain regions involved in the antidepressant effects of ketamine.

Our data show that the slow ketamine infusion activates the BDNF-mTOR-S6 pathway in prefrontal cortex as early as 2 h and remains on until at least 6 h after the infusion. At the 12 h timepoint, this pathway is turned off in prefrontal cortex while it becomes activated in hippocampus. Interestingly, this pathway appears to be activated in both brain regions at 24 h through a BDNF-independent mechanism adding complexity to the early action of ketamine.

We have captured previously unknown dynamics of the early effects of ketamine showing rapid activation/deactivation of BDNF and its downstream signaling in prefrontal cortex and hippocampus, following a precise temporal profile.

1. Introduction

The discovery that a single intravenous infusion of ketamine induces a rapid antidepressant response in patients with Major Depression (Berman et al., 2000; Trujillo and Iniguez, 2020; Zarate et al., 2006) brought to Food and Drug Administration approval of nasal spray S-enantiomer ketamine (esketamine) for treatment resistant depression (FDA, 2019). The recommendation is the result of several years of clinical and preclinical research showing that the anti-depressant effect was causally related to the ability of ketamine to rapidly trigger neuroadaptive arrangement of neuronal circuits involved in mood control (Maeng and Zarate, 2007; Zanos and Gould, 2018). The molecular events associated to this fast antidepressant effect have been extensively investigated pointing to Brain-Derived Neurotrophic Factor (BDNF) and its intracellular signalling pathway activation in hippocampus, cerebral cortex, and midbrain (Autry et al., 2011; Duman and Monteggia, 2006; Zhang et al., 2019) as causally associated to early and rapid synaptic adaptations.

It must be said that most preclinical manuscripts published in the literature about the effects of ketamine as antidepressant drug rely on intraperitoneal (IP) injection to mice or rats, raising some concerns about their translational value given that, in humans, ketamine is

* Corresponding author.
E-mail address: Cristiano.Chiamulera@univr.it (C. Chiamulera).
[1] Lucia Caffino, Francesca Mottarlini and Alessandro Piva contributed equally to the manuscript and can be all considered first author.
[2] Cristiano Chiamulera and Fabio Fumagalli share the senior authorship.

https://doi.org/10.1016/j.neuropharm.2023.109767
Received 18 June 2023; Received in revised form 25 September 2023; Accepted 15 October 2023
Available online 17 October 2023

infused intravenously. The benefits of an intravenous (IV) infusion of ketamine mainly consist in a steady-state maintenance of drug concentration in the plasma. In addition, the different modality of ketamine administration may also reflect different behavioural effects as it is shown by opposite effects on fear behaviours (Radford et al., 2018). For instance, we showed that also the intravenous infusion may induce opposite effects whether single injected vs. chronic self-administered, with the former increasing while the latter decreasing BDNF levels in the hippocampus (Caffino et al., 2016).

Therefore, in the present study we aimed at investigating the effects of a continuous intravenous (IV) ketamine infusion in freely moving rats according to a dosing modality showed by pharmacokinetic/pharmacodynamic (PK/PD) studies to be translational to the one clinically effective in patients (Shaffer, 2018; Shaffer et al., 2014). Moreover, we assess the temporal dynamics of BDNF and its signalling cascade, including tropomyosin receptor kinase B (TrkB), in the hippocampus and prefrontal cortex (PFC), two brain areas relevant for mood modulation and disorders, at 2, 6, 12, and 24 h after the slow intravenous infusion. Our prediction was that BDNF signalling dynamics differently changed over time in the two brain areas.

2. Material and methods

2.1. Animals

Fifty-six adult male Sprague Dawley (SD) rats (Charles River, Italy) were individually housed in a temperature- and humidity-controlled environment (19–23 °C, 60 ± 20 %) on a 12h light–dark cycle with light ON at 07.30 p.m. All the experimental procedures were conducted during the dark phase of the light–dark cycle. Rats were food restricted to achieve a reduction of 85% of their baseline weight (daily checked) and food was made available after each experimental session, water was given ad libitum except during experimental session. All animal care and experimental procedures are reported in compliance with Principle of laboratory animal care (NIH publication No. 85–23, revised 1985), and with the European Union regulations and the Directive 2010/63/EU, and were approved by the ethical committee (OPBA) of the University of Verona and by the Ministry of Health. All efforts were made to minimise animal suffering and to keep the lowest number of animals used.

2.2. Surgery

After 1 week of acclimatization and handling, chronic indwelling jugular catheters were implanted in all rats. Rats were anesthetized with 5% isoflurane (Iso-Vet®, Piramal Critical Care B·V., Netherlands) in oxygen, with oxygen flow rate at 1 L/min. Once the animal has lost its righting reflex and the breathing pattern has become deeper and slower, isoflurane was turn to 2% for maintenance. Then, rats were treated with 5 mg/kg/mL subcutaneous carprophene (Rimadyl®, Pfizer, Italy) and subsequently implanted with a home-made silastic catheter (inner diameter 0.30 mm, outer diameter 0.63 mm) in the right jugular vein. Immediately after surgery, animals were medicated with neomycin 99,5% + sulphathiazole 0,5% skin powder (Streptosil®, ITC Farma, Italy) and treated with 5 mg/mL/kg subcutaneous enrofloxacin (Baytril®, Bayer, Italy). After the surgery, during a 5-day recovery period, animals were treated with intravenous injection of 5 mg/mL/kg enrofloxacin (Baytril®, Bayer, Italy) and with 0.1 mL of heparin solution (30 IU/mL heparin sodium, Sigma, Italy). Every day after the recovery, and before and after the experimental session, rats received an intravenous injection of 0.1 mL of heparin solution (30 IU/mL heparin sodium, Sigma, Italy). Rats (N = 3) whose catheters showed leakage or loss of patency were removed from the study.

2.3. Apparatus

Ketamine or vehicle infusions were delivered in cages encased in sound-insulated cubicles, equipped with ventilation fans (Med Associates Inc., Georgia Regional Industrial Park, Fairfax, VT, USA). Each chamber was equipped with an infusion pump for the intravenous infusion (Razen A-99, Ugo Basile, Varese, Italy).

2.4. Drug

Ketamine hydrochloride (LGC Standards, Sesto San Giovanni, Italy) was freshly prepared immediately before the infusion. Ketamine was diluted in heparinized bacteriostatic saline (0,9% NaCl + 0,9% benzyl alcohol + 1 IU/mL heparin), and pH was adjusted to 7,4 with NaOH. Ketamine unit doses were expressed as mg/kg of bodyweight/infusion. Ketamine solution was administered via the infusion pump at a volume of 1,90 mL during a 40-min period.

2.5. Treatment groups and procedures

After recovery (10–11 weeks old), rats were divided into eight groups, four groups receiving ketamine 1,47 mg/kg infusion during a 40-min period (n = 27 rats) and 4 groups receiving heparinized bacteriostatic saline as vehicle during a 40-min period (n = 26 rats). Ketamine was infused between day 8 and 16 after surgery. During the 2 days before the infusion, rats were habituated to the infusion cage for 40 min to avoid possible neophobia influence on ketamine effects. After the infusion, rats were placed back into their home cages. Ketamine dose was chosen according to Shaffer's study: the 1,47 mg/kg over 40 min dose is the most similar, in terms of receptor occupancy, to the 0,5 mg/kg over 40 min clinical antidepressant dose (Shaffer et al., 2014). Rats from both ketamine and vehicle-treated groups were sacrificed at 2h (n = 7 for ketamine and vehicle), at 6h (n = 7 for ketamine and vehicle), at 12h (n = 7 for ketamine and vehicle) and at 24h (n = 6 for ketamine, n = 5 for vehicle) following the beginning of the treatment. Experiments were performed over 2 days for groups sacrificed at 2, 6, and 12h, and over 4 days for the 24h-time point (Fig. S1). Three or four pairs of rats, each one consisting of 1 vehicle- and 1 ketamine-treated animal, were treated on day 1, and the remaining on day 2 (or on day 3 for the 24h-time point). Animals were treated in pairs in order to have a control for every ketamine-treated subject, and distributed over 2 days (or 4 for 24h-time point) to limit the daily time window during which treatments were performed (so to control for variability due to the circadian rhythm). Before and after the ketamine infusion, rat's catheter was flushed with heparinized bacteriostatic saline solution.

Following decapitation, brains were rapidly removed, and medial prefrontal cortices (bregma +3.20 mm) and hippocampi (−3.30 mm) were dissected from 2 mm-thick slices using a Coronal Brain Matrix (RBMS-600C, World Precision Instruments, Germany) previously stored overnight at −20 °C. Brain extraction and dissection were performed over cold stainless-steel plate into a stainless-steel tray full of ice and previously stored overnight at −20 °C. The average duration of the procedure was 15 min. The areas from both the hemispheres were collected and stored in the same vials, immediately frozen on dry ice and stored at −80 °C. Blood from each rat was collected immediately after decapitation, promptly diluted with EDTA (0.5M, pH 8) as anticoagulant agent and centrifuged at 3000 g for 20 min at 4 °C to isolate plasma for quantification of BDNF levels.

2.6. Protein extraction and western blot analyses

The right and the left hemispheres of the PFC or Hip of each rat have been pooled together and homogenized using a cold buffer containing 0,32M sucrose, 0,1 mM PMSF, 1 mM HEPES, 0,1 mM EGTA pH 7,4 in presence of commercial cocktails of protease (Roche), phosphatase (Sigma-Aldrich) and RNAse (Euroclone) inhibitors. After this first homogenization step, we divided the total volume obtained to proceed with separate extractions: an aliquot has been dedicated to the protein isolation for the WB analysis and another one to the RNA isolation for

RT-PCR, as described below.

Crude membrane fraction prepared as previously described (Caffino et al., 2018). The biochemical fractionation was confirmed by the use of protein markers for specific subcellular compartments (Fig. S2). Total amount of proteins in the crude membrane fraction and in the homogenate was quantified according to the Bradford Protein Assay procedure (Bio-Rad, Milan, Italy), with bovine serum albumin as calibration standard. Samples were stored at −20 °C until molecular analysis.

Eight micrograms of proteins for each sample were run on a sodium dodecyl sulfate-14% polyacrylamide gel under reducing conditions and electrophoretically transferred onto nitrocellulose membrane (Bio-Rad Laboratories). Blots were blocked 1 h at room temperature (RT) with I-Block solution (Life Technologies Italia) in TBS + 0.1% Tween-20 buffer, incubated with antibodies against the phosphorylated forms of the proteins and then stripped and reprobed with the antibodies against corresponding total proteins.

Results were standardized to β-actin control protein detected at 43 kDa. Immunocomplexes were visualized by chemiluminescence using the Chemidoc MP Imaging System (Bio-Rad Laboratories) and analyzed with Image LabTM software (Bio-Rad) by evaluating the band density. Example of full-size cropped immunoblots are presented in Supplementary Materials (Figs. S3–S10).

Since gels were run in duplicate, the results from the two gels were averaged with a correction factor: correction factor gel B = average of (OD protein of interest/OD β-actin for each sample loaded in gel A)/(OD protein of interest/OD β-actin for the same sample loaded in gel B) (Caffino et al., 2020).

The conditions of the primary antibodies were the following: anti-BDNF (1:500, Icosagen, cod.327–100); anti-pTrkB Tyr706 (1:200, Novus Biologicals, cod.NBP2-54764); anti-TrkB (1:1000 Cell Signaling Technology cod.4606, RRID:AB_2267470); anti-pAkt Ser473 (1:1000, Cell Signaling Technology cod.4060, RRID: AB_2315049); anti-Akt (1:1000, Cell Signaling Technology, cod.9272, RRID:AB_329827); anti-pmTOR Ser2448 (1:1000 Cell Signaling Technology Inc., Antibody cod. 2971, RRID: AB_330970), anti-mTOR (1:1000 Cell Signaling Technology Inc., Antibody cod. 2972, RRID: AB_330978), anti-pS6 Ser240/244 (1:1000 Cell Signaling Technology Inc., Antibody cod. 9468, RRID: AB_2716873), anti-S6 (1:1000 Cell Signaling Technology Inc., Antibody cod. 2217, RRID: AB_331355), anti-peEF2 Thr56 (1:1000 Cell Signaling Technology Inc., Antibody cod. 9468, RRID: AB_2716873), anti-eEF2 (1:1000 Cell Signaling Technology Inc., Antibody cod. 2217, RRID: AB_331355), and anti-β-actin (1:5000, Sigma-Aldrich, cod. A5441, RRID: AB_476744).

2.7. mRNA extraction and real-time PCR analysis

Total RNA from prefrontal cortex and hippocampi of rats treated with vehicle or ketamine at each time point were isolated by single step guanidinium isothiocyanate/phenol extraction using PureZol RNA isolation reagent (Bio-Rad Laboratories, Italy) according to the manufacturer's instructions, quantified by means of the Nanodrop spectrophotometric analysis and stored at −20 °C until further processing.

After total RNA extraction, the samples were processed for real-time reverse transcription polymerase chain reaction (real time RT-PCR) to assess mRNA levels, as previously described (Caffino et al., 2019). In brief, an aliquot of each sample was treated with DNase (DNase I, RNase-free buffer di MnCl2-Thermo Scientific) to remove genomic DNA residues (30 min, 37 °C), afterwards, incubated with EDTA (10 min, 65 °C) to block DNase action. Expression levels of *cfos* were analyzed by TaqMan qRT-PCR 48 thermal cycler (CFX384 real time system, Bio-Rad Laboratories) using the iScriptTM one-step RT-PCR kit for probes (Bio-Rad Laboratories), and each sample was run in triplicate in a 384-wells plate. The first step of thermal cycling started with an incubation at 50 °C for 10 min (RNA retro-transcription) followed by 5 min at 95 °C (TaqMan polymerase activation). Afterwards, 39 cycles of PCR were performed, and each PCR cycle consisted of samples heating at 95 °C for 10 s to enable the melting process and then 30 s at 60 °C for annealing and extension reactions. Data were analyzed with the comparative threshold cycle (ΔΔCt) using *36B4* as internal standard (Caffino et al., 2015).

RT-PCR analysis were performed to evaluate the expression *cfos* gene. Primers and probe for *cfos* and *36B4* were purchased from Eurofins MWG-Operon and the primer efficiency was experimentally set up to identify the optimal concentration. Their sequences are shown below.

- *cfos*: forward primer 5′-TCCTTACGGACTCCCCAC-3′, reverse primer 5′-CTCCGTTTCTCTTCCTCTTCAG-3′, probe 5′-TGCTCTACTT TGCCCCTTCTGCC-3′;
- *36B4*: forward primer 5′-TTCCCACTGGCTGAAAAGGT-3′, reverse primer 5′-CGCAGCCGCAAATGC-3′, probe 5′-AAGGCCTTCCTGGCC GATCCATC-3′.

2.8. Plasma collection and BDNF ELISA assay

The total amount of blood was centrifuged at 3000 g for 20 min at 4 °C to precipitate and remove the cellular fraction. The supernatant, corresponding to the plasma fraction, was stored at −80 °C until the analysis. BDNF levels were determined by an enzyme-linked immunosorbent assay (ELISA) using commercial kits, according to the manufacturer's instructions (AbCam, RRID: AB_2924770). Calibration curve was run in duplicate together with control and samples, using the same procedure. BDNF concentration of each sample was calculated related to the standard calibration curve.

2.9. Statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2018). All animals tested were treated as independent values, there were no technical replicates.

Molecular changes produced by pharmacological treatment at each time point were tested with the Shapiro-Wilk test to determine normality of residuals. Data with a normal distribution were analyzed by unpaired Student's t-test, while data with a non-normal distribution with the Mann Whitney test (U).

Data were collected in individual animals (independent determinations) and are presented as means and standard errors (S.E.M.). Prism 9.0 (GraphPad) was used to analyse all the data. Statistical significance was assumed at $p < 0.05$.

3. Results

The main aim of our work was to investigate the temporal modulation of BDNF and its downstream signalling in two brain regions known to be critical for the action of ketamine, i.e., prefrontal cortex and hippocampus. We decided to employ a very low dose of ketamine (1.47 mg/kg/40 min), through a slow intravenous infusion, to be translational to studies in humans (Berman et al., 2000; Shaffer et al., 2014; Zarate et al., 2006).

3.1. Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathway in the prefrontal cortex (PFC) and hippocampus 2 h after treatment

Our data show that 2 h after a single ketamine infusion BDNF protein levels in homogenate and membrane fraction are increased in the PFC (Fig. 1a: BDNF homogenate +20% $t = 2.53$, $p = 0.0263$; BDNF membrane +21% $t = 6.71$, $p < 0.0001$) whereas decreased in the hippocampus (Fig. 1b: BDNF homogenate −13% $t = 2.56$, $p = 0.0250$; BDNF membrane −30% $t = 3.39$, $p = 0.0054$) compared to vehicle infusion. We then measured the activation of proteins involved in the BDNF

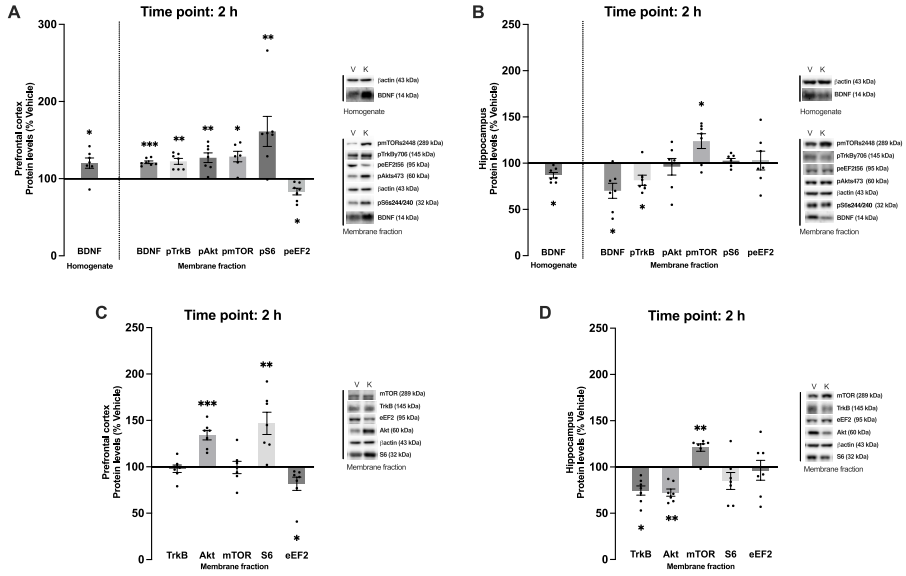


Fig. 1. Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathways in the prefrontal cortex (PFC) and hippocampus 2 h after treatment.

Rats were treated with a single intravenous (IV) infusion of ketamine at the dose of 1.47 mg/kg administered in 40 min and sacrificed 2 h after treatment. Protein expression of BDNF was measured in the whole homogenate and in the membrane fraction of the PFC **(A)** and hippocampus **(B).** Phosphorylation levels (p) of its downstream targets pTrkB (Tyrosine 706), pAkt (Serine 473), pmTOR (Serine 2448), pS6 (Serine 240/244) and peEF2 (Threonine 56) were measured in the membrane fraction of the PFC **(A)** and hippocampus **(B)**. The total expression of TrkB, Akt, mTOR, S6 and eEF2 is represented for the PFC and hippocampus in panel **C** and **D**, respectively.

Data are expressed as percentages of vehicle-treated rats and bar graphs represent the mean ± SEM from at least six independent determinations. Representative immunoblots for all the measured targets are shown in panel **E**. Unpaired Student's t-test or Mann Whitney test (*p < 0.05, **p < 0.01, ***p < 0.01 vs vehicle).

downstream signalling, such as the high affinity receptor TrkB phosphorylated in Tyr(Y)706 and the Akt effector phosphorylated in serine (S)473. In the membrane fraction of the PFC, the single ketamine infusion significantly increased the levels of both pTrkBY706 and pAktS473 2 h after treatment (Fig. 1a: pTrkB +22% t = 3.493, p = 0.0044; pAkt +27% t = 3.62, p = 0.0035); conversely in the hippocampus pTrkBY706 levels are reduced (Fig. 1b: −19% U = 6, p = 0.0175) while no changes were observed in pAktS473 (Fig. 1b: −4% t = 0.318, p = 0.7563). Although Akt is a well-known downstream effector of the BDNF-TrkB system, it is also an upstream regulator of mTOR, a key protein that regulates the initiation of protein translation, integrating both intracellular and extracellular signals fundamental in the control of protein synthesis required for many different physiological processes and critical also in mediating pathological processes. Moreover, S6 ribosomal protein phosphorylated in serine(S)240/244 and the eukaryotic elongation factor 2 (eEF2) in threonine(T)56, two of mTOR main downstream effectors involved in the processes of protein synthesis initiation and elongation, have been involved in fast antidepressant ketamine action (Autry et al., 2011; Tedesco et al., 2013). Of note, we first measured the levels of mTOR phosphorylation in serine(S)2448 and we found that pmTORS2448 is increased 2 h after treatment in both PFC and hippocampus (Fig. 1a: +29% t = 2.68, p = 0.0213; Fig. 1b: +24% t = 2.54, p = 0.0261). Downstream mTOR, we observed that, while in the PFC the levels of pS6S240/244 are significantly increased and peEF2T56 reduced (Fig. 1a: pS6 +61% U = 4, p = 0.007; peEF2 -17% t = 3.09, p = 0.0094), suggesting that the pathway of protein synthesis is boosted, in the hippocampus no effects were instead observed on the activation of these effectors (Fig. 1b: pS6 +3% t = 0.447, p = 0.6628; peEF2 +3% t = 0.284, p = 0.8106).

Together with the evaluation of the phosphorylation levels of BDNF- and mTOR-related downstream targets, we also measured the levels of expression of the total, non-phosphorylated form of these proteins in the membrane fraction of both PFC and hippocampus. The analysis of BDNF downstream targets revealed that TrkB protein levels are unchanged in the PFC 2 h after treatment whereas its levels are significantly reduced in the hippocampus at the same time point (Fig. 1c: −2% t = 0.227, p = 0.8243; Fig. 1d: −25% t = 2.80, p = 0.0160). Moreover, we found that the total form of Akt is increased in the PFC, while decreased in the hippocampus (Fig. 1c: +34% t = 5.69, p < 0.0001; Fig. 1d: −28% t = 3.91, p = 0.0021), and despite no changes are present for mTOR in the PFC, we found that mTOR protein levels are significantly increased in the hippocampus (Fig. 1c: −1% U = 24, p > 0.9999; Fig. 1d: +2% U = 5, p = 0.0111). Of note, when considering the mTOR effectors, S6 and eEF2, we observed that the single ketamine infusion increased the levels of S6 and decreased the levels of eEF2 in the PFC (Fig. 1c: S6 +47% t = 3.12, p = 0.0088; eEF2 -18% U = 4, p = 0.007), while it did not alter their expression in the hippocampus (Fig. 1d: S6 -15% t = 1.45, p = 0.1737; eEF2 -4% t = 0.245 p = 0.8106) 2 h after the single ketamine infusion.

3.2. *Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathway in the prefrontal cortex (PFC) and hippocampus 6 h after treatment*

Six hours after treatment BDNF is unchanged in the PFC homogenate while reduced in the hippocampus (Fig. 2a: +4% t = 1.21, p = 0.2527; Fig. 2b: −21% t = 2.79, p = 0.0164) whereas, in the membrane fraction, BDNF levels were unchanged in both brain regions (Fig. 2a: −6% t = 0.561, p = 0.5854; Fig. 2b: +2% t = 0.179, p = 0.8468). The analysis of the activation of BDNF pathway revealed a clear-cut difference between the two brain regions. In the PFC, we observed increased levels of pTrkB, pAkt, pmTOR and pS6 while peEF2 levels were reduced (Fig. 2a: pTrkB +18% t = 2.40, p = 0.0332; pAkt +21% t = 2.46, p = 0.0299; pmTOR +35% t = 2.70, p = 0.0193; pS6 +37% t = 2.47, p = 0.0296; peEF2 -24% t = 2.42, p = 0.0323), suggesting an overall activation of the pathway and consequently the activation of the protein synthesis machinery. Conversely, in the hippocampus, the BDNF-dependent signaling is switched off as shown by a significant reduction of pTrkB

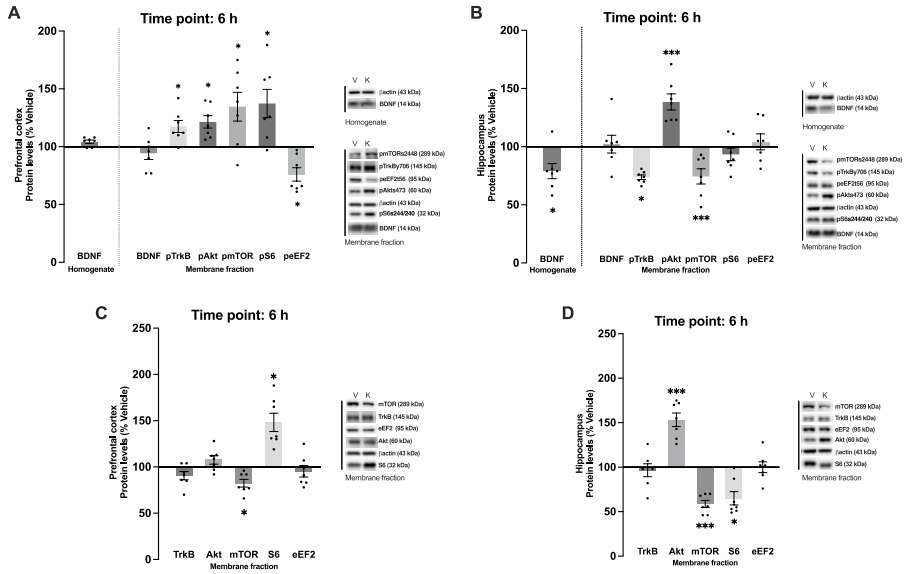


Fig. 2. Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathways in the PFC and hippocampus 6 h after treatment.
Rats were treated with a single IV infusion of ketamine at the dose of 1.47 mg/kg administered in 40 min and sacrificed 6 h after treatment. Protein expression of BDNF was measured in the whole homogenate and in the membrane fraction of the PFC **(A)** and hippocampus **(B).** Phosphorylation levels (p) of its downstream targets pTrkB (Tyrosine 706), pAkt (Serine 473), pmTOR (Serine 2448), pS6 (Serine 240/244) and peEF2 (Threonine 56) were measured in the membrane fraction of the PFC **(A)** and hippocampus **(B)**. The total expression of TrkB, Akt, mTOR, S6 and eEF2 is represented for the PFC and hippocampus in panel **C** and **D**, respectively. Data are expressed as percentages of vehicle-treated rats and bar graphs represent the mean ± SEM from at least six independent determinations. Representative immunoblots for all the measured targets are shown in panel **E**. Unpaired Student's t-test or Mann Whitney test (*p < 0.05, **p < 0.01, ***p < 0.01 vs vehicle).

and pmTOR and by unchanged levels of pS6 and peEF2 (Fig. 2b: pTrkB −26% t = 2.45, p = 0.0308; pmTOR −25% t = 3.21 p = 0.0075; pS6 -7% t = 0.911, p = 0.3804; peEF2 +4% t = 0.387, p = 0.7055) with the exception of pAkt that is significantly up-regulated (Fig. 2b: +38% t = 4.94, p = 0.0003). The analysis of the total levels of these targets did not show any changes for TrkB protein levels in both PFC and hippocampus (Fig. 2c: −9% t = 1.24, p = 0.2374; Fig. 2d: −3% t = 0.371, p = 0.7168) whereas Akt is unchanged in the PFC while increased in the hippocampus (Fig. 2c: +8% t = 0.964, p = 0.2374; Fig. 2d: +53% t = 4.74, p = 0.0005). At the same time point mTOR is decreased both in the PFC and hippocampus (Fig. 2c: −18% U = 6, p = 0.0175; Fig. 2d: −41% U = 0, p = 0.0006), whereas S6 is increased in the PFC and decreased in the

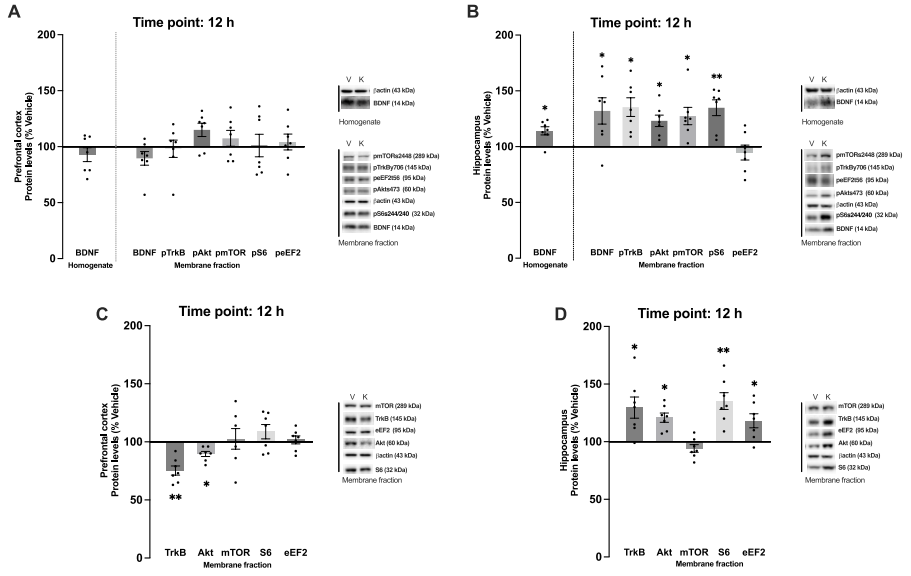


Fig. 3. Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathways in the PFC and hippocampus 12 h after treatment.
Rats were treated with a single IV infusion of ketamine at the dose of 1.47 mg/kg administered in 40 min and sacrificed 12 h after treatment. Protein expression of BDNF was measured in the whole homogenate and in the membrane fraction of the PFC **(A)** and hippocampus **(B).** Phosphorylation levels (p) of its downstream targets pTrkB (Tyrosine 706), pAkt (Serine 473), pmTOR (Serine 2448), pS6 (Serine 240/244) and peEF2 (Threonine 56) were measured in the membrane fraction of the PFC **(A)** and hippocampus **(B)**. The total expression of TrkB, Akt, mTOR, S6 and eEF2 is represented for the PFC and hippocampus in panel **C** and **D**, respectively. Data are expressed as percentages of vehicle-treated rats and bar graphs represent the mean ± SEM from at least six independent determinations. Representative immunoblots for all the measured targets are shown in panel **E**. Unpaired Student's t-test or Mann Whitney test (*p < 0.05, **p < 0.01, ***p < 0.01 vs vehicle).

hippocampus (Fig. 2c: +48% t = 2.73, p = 0.0182; Fig. 2d: −35% t = 2.71 p = 0.0190); eEF2 is unaltered in both areas (Fig. 2c: −4% t = 0.459, p = 0.6543; Fig. 2d: +0% t = 0.0092 p = 0.9928).

3.3. Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathway in the prefrontal cortex (PFC) and hippocampus 12 h after treatment

Again, a different picture is observed 12 h post-infusion. Interestingly, it appears that BDNF levels and the activation of the BDNF/mTOR pathway is blunted in the PFC showing no changes when compared to saline-infused rats while (Fig. 3a: BDNF homogenate −6% t = 0.594, p = 0.5633; BDNF membrane −11% U = 13 p = 0.1649; pTrkB −2% t = 0.171 p = 0.8667; pAkt +15% t = 1.62 p = 0.1322; pmTOR +7% t = 0.814 p = 0.4317; pS6 +1% t = 0.747 p = 0.9417; peEF2 +4% t = 0.387 p = 0.7057), instead, BDNF and its pathway is significantly activated in the hippocampus as shown by the increased levels of BDNF, pTrkB, pAkt, pmTOR and pS6 with the only exception is peEF2 whose levels were unchanged (Fig. 3b: BDNF homogenate +14% t = 2.76, p = 0.0172; BDNF membrane +32 % t = 2.59 p = 0.0237; pTrkB +35% t = 3.04 p = 0.0102; pAkt +23% t = 2.32 p = 0.0387; pmTOR +27% t = 2.63 p = 0.0221; pS6 +35% t = 3.34 p = 0.0059; peEF2 -5% t = 0.440 p = 0.6677). The analysis of the total level of TrkB and Akt were significantly reduced in the PFC whereas, on the contrary, in the hippocampus both protein levels were significantly increased (Fig. 3c TrkB −25% t = 3.48 p = 0.0045; Akt −10% t = 2.39 p = 0.0342; Fig. 3d TrkB +29% t = 2.55 p = 0.0254; Akt +21% t = 2.82 p = 0.0155). mTOR levels were unchanged in both areas (Fig. 3c +3% t = 0.243 p = 0.8125; Figs.. 3d−6% t = 0.842 p = 0.4164); in the PFC, S6 and eEF2 were unaltered whereas in the hippocampus both protein levels were significantly increased (Fig. 3c S6 +9% U = 14 p = 0.2086; eEF2 +2% t = 0.361 p = 0.7241; Fig. 3d S6 +35% t = 4.16 p = 0.0013; eEF2 +18% t = 2.73 p = 0.0184).

3.4. Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathway in the prefrontal cortex (PFC) and hippocampus 24 h after treatment

At the last time point considered after the single ketamine infusion, i.e., 24 h, no differences on BDNF expression were observed in the homogenate of the PFC while membrane BDNF, pTrkB, pmTOR and peEF2 levels were reduced and pAkt and pS6 were significantly increased (Fig. 4a: BDNF homogenate +5 % t = 0.656, p = 0.5282; BDNF membrane −25% t = 3.06 p = 0.0135; pTrkB −14 % t = 6.05 p = 0.0002; pAkt +45% t = 5.52 p = 0.0004; pmTOR −19% U = 4 p = 0.05194; pS6 +31% t = 2.47 p = 0.0366; peEF2 -34% t = 3.02 p = 0.0146). In the hippocampus, at the same time point, BDNF levels were increased in the homogenate but reduced in the membrane (Fig. 4b: BDNF homogenate +35% t = 3.64, p = 0.0054; BDNF membrane −24% t = 3.89 p = 0.0037). Such reduction was accompanied by a decreased activation of TrkB and Akt (Fig. 4b: pTrkB −20% t = 5.12, p = 0.0006; pAkt −16% t = 2.87 p = 0.0185). Interestingly, despite the BDNF-dependent pathway appears to be downregulated, the protein synthesis is sustained, since pmTOR and pS6 protein levels were significantly increased and peEF2 reduced (Fig. 4b: pmTOR +60% t = 3.63, p = 0.0055; pS6 +45% t = 2.78 p = 0.0213; peEF2 -25% t = 3.19 p = 0.0111). Interestingly, while the total levels of all the targets considered, TrkB, Akt, mTOR, S6 and eEF2, in the PFC were significantly reduced (Fig. 4c: TrkB −16% U = 0 p = 0.0043; Akt −11% t = 4.046 p = 0.0029; mTOR −23% t = 5.91 p = 0.0002; S6 -17% U = 3 p = 0.0303; eEF2 -18% t = 2.66 p = 0.0262), in the hippocampus we found a different patter of expression. TrkB and Akt were reduced (Fig. 4d TrkB −27% t = 4.49 p = 0.0015; Akt −40% t = 8.89 p < 0.0001), mTOR tended toward an increase (Fig. 4d mTOR +30% U = 4 p = 0.0519) while S6 were increased and eEF2 reduced (Fig. 4d S6 +59% U = 0 p = 0.0043; eEF2 -27% t = 3.827 p = 0.004), similarly to their respective phosphorylation levels.

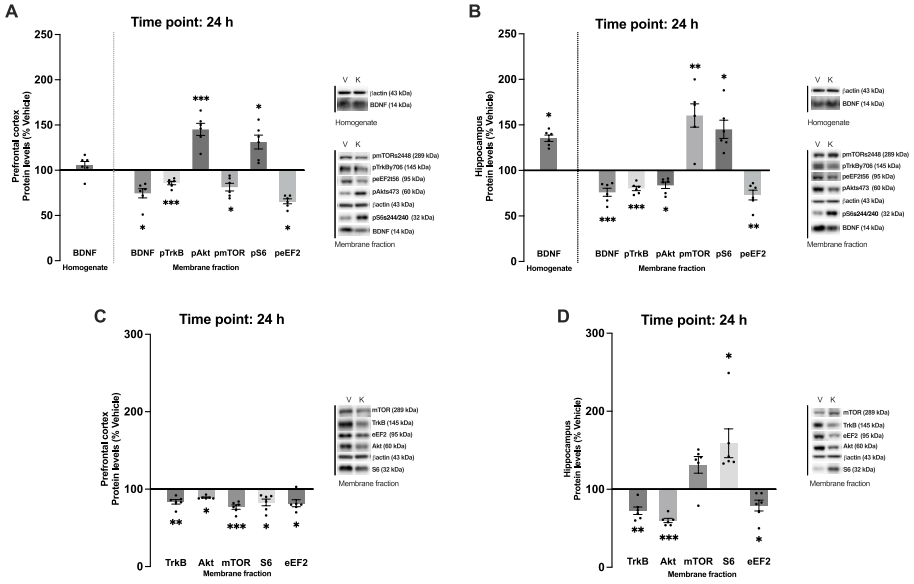


Fig. 4. Effect of a single ketamine infusion on the BDNF-mediated intracellular signaling pathways in the PFC and hippocampus 24 h after treatment.
Rats were treated with a single IV infusion of ketamine at the dose of 1.47 mg/kg administered in 40 min and sacrificed 24 h after treatment. Protein expression of BDNF was measured in the whole homogenate and in the membrane fraction of the PFC **(A)** and hippocampus **(B).** Phosphorylation levels (p) of its downstream targets pTrkB (Tyrosine 706), pAkt (Serine 473), pmTOR (Serine 2448), pS6 (Serine 240/244) and peEF2 (Threonine 56) were measured in the membrane fraction of the PFC **(A)** and hippocampus **(B)**. The total expression of TrkB, Akt, mTOR, S6 and eEF2 is represented for the PFC and hippocampus in panel **C** and **D**, respectively. Data are expressed as percentages of vehicle-treated rats and bar graphs represent the mean ± SEM from at least six independent determinations. Representative immunoblots for all the measured targets are shown in panel **E**. Unpaired Student's t-test or Mann Whitney test (*p < 0.05, **p < 0.01, ***p < 0.01 vs vehicle).

3.5. Effects of a single ketamine infusion on circulating levels of BDNF at the different time points examined

Finally, since in clinics it has been found that patients with MDD show reduced levels of circulating BDNF (Kim et al., 2007; Lee et al., 2007), thus suggesting BDNF as a potential biomarker of depression, we assessed BDNF levels in the plasma of rats treated with a single ketamine infusion. While, at the earliest time point investigated, we found no changes after treatment, a significant decrease was observed at 6 h followed by an up-regulation 12 and 24 h post-infusions (Fig. 5a: 2h: −31 pg/ml $t = 0.458$ $p = 0.6553$; 6h: −113 pg/ml $t = 2.30$ $p = 0.0403$; 12h: +60 pg/ml $t = 2.94$ $p = 0.0123$; 24h: +126 pg/ml $t = 2.58$ $p = 0.0296$).

3.6. Effects of a single ketamine infusion on the gene expression levels of the immediate early gene (IEG) cfos in the PFC and hippocampus measured at the different time points examined

Further, to assess whether these area-specific differences in the temporal modulation of BDNF/mTOR signaling activation could be ascribed to a variation of neuronal activation in the two brain areas considered, we measured *cfos* mRNA levels as an index of brain area responsiveness/activation. Interestingly, in the PFC, *cfos* undergoes a biphasic activation with a first peak at 2 h post-infusion that subsides at 6 and 12 h and a second peak 24 h later (Fig. 5b: 2h: +100% $t = 11.7$ $p < 0.0001$; 6h: +7% $U = 22$ $p = 0.8048$; 12h: −22% $U = 12$ $p = 0.1282$; 24h: +58% $t = 3.28$ $p = 0.0095$). Of note, in the hippocampus, *cfos* mRNA levels are unchanged (2 h) or reduced (6 and 12 h) and then increase at 24 h-post-infusion (Fig. 5c: 2h: +1% $t = 0.0734$ $p = 0.9427$; 6h: −15% $t = 2.52$ $p = 0.0271$; 12h: −32% $t = 6.75$ $p < 0.0001$; 24h: +39% $t = 5.66$ $p = 0.0003$).

4. Discussion

We decided to focus on the first 24 h following a single, slow infusion of low dose-ketamine to finely dissect potential differences in the contribution of BDNF-mediated signaling pathway to the early acute effects in the PFC and hippocampus, two brain regions known to be largely involved in the antidepressant outcome of ketamine.

Our data are the first to show that a slow infusion of low dose-ketamine, which has been shown to match the receptor occupancy-time profile of the clinically relevant antidepressant dose of ketamine (Berman et al., 2000; Shaffer et al., 2014; Zarate et al., 2006), is sufficient to activate the BDNF-mTOR-S6 pathway with different temporal dynamics in these two brain regions. Based on our results, we can state that a single, slow infusion of ketamine, after the initial activation of BDNF signaling in the PFC, has progressively de-recruited (starting from 6 to 12 h post-infusion) BDNF-mediated downstream signaling from the PFC while leading to an increased hippocampal activation of the same pathway (from 12 to 24 h post-infusion). Indeed, if the BDNF-dependent signaling is one of the major pathways supporting the antidepressant action of ketamine, then our data show that, during this early phase of action, the contribution of the PFC comes first (2–6 h), to be replaced later by hippocampus (12–24 h). Noteworthy, optogenetic evidence showed that hippocampal TrkB signalling plays a role of initiator of the sustained temporal component of the antidepressant effect of ketamine followed by prefrontal cortex, suggesting a change of 'direction' between these two brain areas for early and sustained effects (Carreno et al., 2016).

Our data highlight the critical importance of the very early activation of the BDNF system in the PFC since most of the lines of evidence so far accumulated had strongly suggested the hippocampal BDNF-mTOR-S6 pathway as a critical mediator for rapid antidepressant effects of ketamine (Autry et al., 2011; Kim and Monteggia, 2020). Our experimental paradigm allowed us to highlight that the BDNF-mTOR-S6 pathway turns on in the PFC as early as 2 h after the infusion of the drug and remains activated until at least 6 h after the infusion. These results suggest that the capacity of ketamine to early trigger a cascade of molecular events eventually leading to an antidepressant effect may depend upon its ability to modulate PFC function. Essentially, ketamine appears to increase cortical excitability during its acute pharmacological effects.

Notably, at the earlier time points investigated, in the hippocampus this pathway is either turned off or reduced. It is interesting to note how some signs of the forthcoming switching off of the signal in the PFC can be detected when the pathway is still activated; in fact, BDNF expression in the membrane is back to control levels at 6 h whereas the downstream pathway is still significantly increased. This suggests that increased translation and trafficking of the neurotrophin to the membrane is necessary very early after drug exposure to fuel the downstream pathway, but it also suggests that such BDNF-mediated functions are no longer necessary as early as 6 h post ketamine exposure. This could indicate that the activation of the pathway that we see at 6 h is a consequence of the activation that occurs before, while this time-point could represent the beginning of the signal switching off in the PFC given that the reduced trafficking in the membrane may not subsequently sustain the activation of the pathway at the later time points investigated (12 and 24 h). It is intriguing to observe how this hypothesis can be supported by the evidence that in the hippocampus, at 6 h, we continue to observe a reduction in BDNF expression in homogenate but no longer at the membrane thus suggesting that, at this time point, the BDNF is less synthesized but the one produced is normally transported to the membrane, presumably to start the set of the machinery necessary to activate the BDNF pathway at the following time points investigated. The analysis of the two brain areas 12 h after ketamine

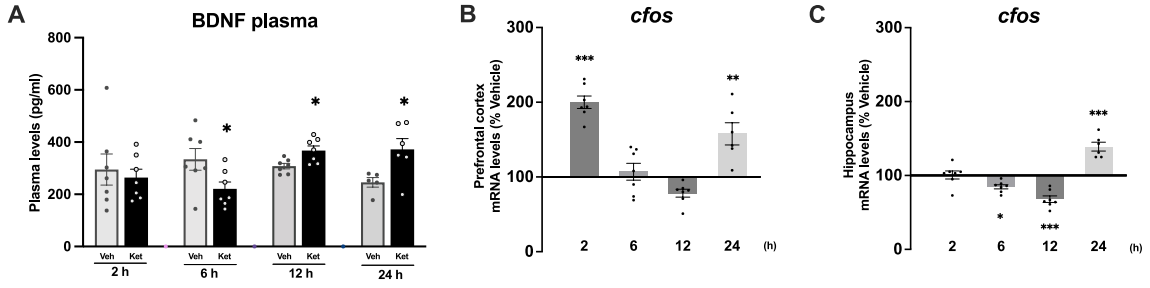


Fig. 5. Time course evaluation of the effects of a single ketamine infusion on circulating levels of BDNF and on the gene expression levels of the immediate early gene (IEG) *cfos* in the PFC and hippocampus measured after 2, 6, 12 and 24 h from the treatment.
Rats were treated with a single IV infusion of ketamine at the dose of 1.47 mg/kg administered in 40 min and sacrificed 2, 6, 12 and 24 h after treatment. BDNF plasma levels, mRNA levels of *cfos* in PFC and in the hippocampus are shown in panel **(A)**, **(B)** and **(C),** respectively.
Data are expressed as percentages of vehicle-treated rats and bar graphs represent the mean ± SEM from at least six independent determinations. Unpaired Student's t-test or Mann Whitney test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.01$ vs vehicle).

infusion revealed that the whole pathway is completely switched off in the PFC while the hippocampal BDNF signaling is, on the other hand, completely activated. Interestingly, at 24 h, in the hippocampus we find that the mTOR-S6-eEF2 pathway is still activated, an effect that appears to be dependent on the total level of these protein; notably, BDNF synthesis is still up-regulated perhaps in attempt to perpetuating its downstream activation. Based on our findings, we propose a cohesive hypothesis that suggests two phases in the very rapid action of ketamine, implying first an increased cortical excitability, presumably as result of an acute pharmacological effect, followed by a subsequent hippocampal activation. However, we must pay attention to the evidence that, whereas at 2 h for the PFC and 12 h for hippocampus the activation of the mTOR-S6-eEF2 pathway appears to be strongly dependent upon BDNF, at 24 h such activation appears to be independent from the neurotrophin. The downregulation of eEF2 phosphorylation in hippocampus at 24 h may disinhibit BDNF expression and activate mTOR (Lepack et al., 2014; Scotton et al., 2022) suggesting that, at this time point, such pathway is still activated, leading to increased translation of downstream effectors. Interestingly, the downregulation of eEF2 has been proposed as a key mechanism underlying a process of homeostatic synaptic plasticity that – activated by ketamine – induces 'a novel form of synaptic plasticity' underlying rapid ketamine effects (Kim et al., 2023). This may also occur in the PFC, where even though the phosphorylation of mTOR is reduced, pS6 and peEF2, which are the main mTOR effectors, are activated, thus suggesting that despite a different temporal profile of activation/deactivation of BDNF -trkB-Akt downstream pathway, there is a simultaneous activation of protein synthesis in both brain regions, which might sustain synaptic plasticity and is presumably responsible for the long-lasting effect of a single injection of ketamine.

Notably, the modulation of BDNF-dependent pathway is associated with neuronal activation in the PFC, as *cfos* mRNA levels are up-regulated at 2 h and then become reactivated at 24 h, when eEF2 is dephosphorylated to sustain protein synthesis. In the hippocampus, neuronal activity is unchanged at 2 h, reduced 6 and 12 h post-infusion and up-regulated at 24 h when BDNF downstream pathway is indeed activated. These results suggest that at 12 h, when the switch between PFC and hippocampus occurs, neuronal activity does not contribute to such switch. To this end, it must be noted that ketamine, besides acting at NMDA receptors, interacts with several other receptor systems (opioid, monoaminergic, muscarinic receptors) that could, ultimately, contribute to explain some effects. Nonetheless, to the best of our knowledge, this is the first time that *cfos* mRNA is reported as increased 24 h after ketamine treatment. A similar effect has been previously showed for the protein levels and suggested as essential for the long-term maintenance of a hippocampus-dependent memory trace (Katche et al., 2010). Considering that *cfos* increase is usually transient and back to baseline after few hours, the second wave of *cfos* mRNA observed both in the PFC and hippocampus after ketamine could be involved in its sustained antidepressant effects. (Autry et al., 2011). Moreover, we should consider that previous evidence already showed long-lasting effects of ketamine, even at different doses and route of administration, and these effects were ascribed to the metaplasticity phenomenon (Burgdorf et al., 2013; Chiamulera et al., 2021; Graef et al., 2015). Metaplasticity, i.e., the plasticity of the synaptic plasticity (Abraham and Bear, 1996), has been conceptualized as the dependency of synaptic functionality on previous synaptic activity. Thus, the second increase of *cfos* at 24 h, long after the infusion, could reflect the metaplastic properties of ketamine recently suggested as a possible therapeutic tool against mood disorders (Fattore et al., 2018).

We have also analyzed the plasma level of BDNF since peripheral measure of BDNF have been suggested as a potential biomarker of depression and, also, because it has been suggested that plasma BDNF levels reflect brain-tissue BDNF levels (Klein et al., 2011). Notably, the analysis of BDNF in the plasma of saline- and ketamine-infused rats revealed that BDNF modulation is faster in the brain than in the plasma since no effects were observed on BDNF levels 2 h after the infusion. Six hours later BDNF plasma levels were decreased whereas they raised significantly above control levels at both 12 and 24 h revealing a slower pattern of induction, which was more similar with that observed in the hippocampus than in the PFC.

We are aware that our study suffers from some limitations. First, we chose to perform this study in normal rats not exposed to experimental manipulation of mood. Therefore, we cannot report here the behavioral antidepressant correlates of the molecular data obtained with the present translational mode of ketamine administration. Further studies will be conducted in rodent models of depression, in order to improve the translational value of our present finding to the clinical condition. Second, our molecular analyses are limited to the first 24 h after infusion and, therefore, we do not know the molecular response of the system at later time points. Third, ketamine is metabolized mainly to norketamine, which is subsequently metabolized to hydroxynorketamine, and these compounds exhibit antidepressant properties in preclinical settings (Yang et al., 2019; Zanos et al., 2016). Even if it has been demonstrated that ketamine and norketamine enantiomers own short half-lives after an intravenous infusion in rats (Le Nedelec et al., 2018), it may still be the case that, at least in part, our results are mediated also by the activity of these metabolites. Fourth, we have used only male rats, despite some studies have shown sex-related differences in the metabolism of ketamine and its antidepressant effects (Carrier and Kabbaj, 2013; Wright et al., 2019). Accordingly, we cannot broaden our data to female rats. Finally, we did not analyse the possible ketamine effects on circadian rhythms and on core body temperature. In literature, only one report suggested a possible effect of subanaesthetic ketamine on the circadian clock (Orozco-Solis et al., 2017); thus, even if evidence is limited, we cannot exclude possible effects of our sub-anesthetic ketamine on circadian rhythms. We also exclude a significant induction of hypothermia by our sub-anesthetic ketamine dose, considering the results reported by Vučković and colleagues indicating no significant effects of ketamine on body temperature below 10 mg/kg i.p. (Vuckovic et al., 2014).

In conclusion, we here provide evidence of patterns of central translational networks, coupled with peripheral molecular measures, which may be potentially informative for the rapid action of ketamine when using an IV infusion similar with that used in humans. We believe that, having performed a longitudinal analysis within the first 24 h after ketamine infusion, we have captured previously unknown dynamics of the acute effects of ketamine showing rapid activation/deactivation of BDNF and its downstream signaling in PFC and hippocampus, following a precise temporal profile, yielding unique insights into the mechanism that might contribute to the rapid action of this drug.

Funding

This work was supported by grants from MIUR Progetto Eccellenza 2023–2027.

CRediT authorship contribution statement

Lucia Caffino: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. **Francesca Mottarlini:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Alessandro Piva:** Conceptualization, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Beatrice Rizzi:** Visualization. **Fabio Fumagalli:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Cristiano Chiamulera:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision,

Visualization, Writing – original draft, Writing – review & editing, All authors discussed the results and contributed to the final manuscript.

Declaration of competing interest

Declarations of interest: none.

Data availability

Data will be made available on request.

Acknowledgments

We thank the help of Nicholas Pintori, Claudio Marzo and Susanna Parolaro.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2023.109767.

References

Abraham, W.C., Bear, M.F., 1996. Metaplasticity: the plasticity of synaptic plasticity. Trends Neurosci. 19, 126–130.
Autry, A.E., Adachi, M., Nosyreva, E., Na, E.S., Los, M.F., Cheng, P.F., Kavalali, E.T., Monteggia, L.M., 2011. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. Nature 475, 91–95.
Berman, R.M., Cappiello, A., Anand, A., Oren, D.A., Heninger, G.R., Charney, D.S., Krystal, J.H., 2000. Antidepressant effects of ketamine in depressed patients. Biol. Psychiatr. 47, 351–354.
Burgdorf, J., Zhang, X.L., Nicholson, K.L., Balster, R.L., Leander, J.D., Stanton, P.K., Gross, A.L., Kroes, R.A., Moskal, J.R., 2013. GLYX-13, a NMDA receptor glycine-site functional partial agonist, induces antidepressant-like effects without ketamine-like side effects. Neuropsychopharmacology 38, 729–742.
Caffino, L., Calabrese, F., Giannotti, G., Barbon, A., Verheij, M.M., Racagni, G., Fumagalli, F., 2015. Stress rapidly dysregulates the glutamatergic synapse in the prefrontal cortex of cocaine-withdrawn adolescent rats. Addiction Biol. 20, 158–169.
Caffino, L., Di Chio, M., Giannotti, G., Venniro, M., Mutti, A., Padovani, L., Cheung, D., Fumagalli, G.F., Yew, D.T., Fumagalli, F., Chiamulera, C., 2016. The modulation of BDNF expression and signalling dissects the antidepressant from the reinforcing properties of ketamine: effects of single infusion vs. chronic self-administration in rats. Pharmacol. Res. 104, 22–30.
Caffino, L., Giannotti, G., Messa, G., Mottarlini, F., Fumagalli, F., 2019. Repeated cocaine exposure dysregulates BDNF expression and signaling in the mesocorticolimbic pathway of the adolescent rat. World J. Biol. Psychiatr. 20, 531–544.
Caffino, L., Piva, A., Mottarlini, F., Di Chio, M., Giannotti, G., Chiamulera, C., Fumagalli, F., 2018. Ketamine self-administration elevates alphaCaMKII autophosphorylation in mood and reward-related brain regions in rats. Mol. Neurobiol. 55, 5453–5461.
Caffino, L., Verheij, M.M.M., Roversi, K., Targa, G., Mottarlini, F., Popik, P., Nikiforuk, A., Golebiowska, J., Fumagalli, F., Homberg, J.R., 2020. Hypersensitivity to amphetamine's psychomotor and reinforcing effects in serotonin transporter knockout rats: glutamate in the nucleus accumbens. Br. J. Pharmacol. 177, 4532–4547.
Carreno, F.R., Donegan, J.J., Boley, A.M., Shah, A., DeGuzman, M., Frazer, A., Lodge, D. J., 2016. Activation of a ventral hippocampus-medial prefrontal cortex pathway is both necessary and sufficient for an antidepressant response to ketamine. Mol. Psychiatr. 21, 1298–1308.
Carrier, N., Kabbaj, M., 2013. Sex differences in the antidepressant-like effects of ketamine. Neuropharmacology 70, 27–34.
Chiamulera, C., Piva, A., Abraham, W.C., 2021. Glutamate receptors and metaplasticity in addiction. Curr. Opin. Pharmacol. 56, 39–45.
Curtis, M.J., Alexander, S., Cirino, G., Docherty, J.R., George, C.H., Giembycz, M.A., Hoyer, D., Insel, P.A., Izzo, A.A., Ji, Y., MacEwan, D.J., Sobey, C.G., Stanford, S.C., Teixeira, M.M., Wonnacott, S., Ahluwalia, A., 2018. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. Br. J. Pharmacol. 175, 987–993.
Duman, R.S., Monteggia, L.M., 2006. A neurotrophic model for stress-related mood disorders. Biol. Psychiatr. 59, 1116–1127.
Fattore, L., Piva, A., Zanda, M.T., Fumagalli, G., Chiamulera, C., 2018. Psychedelics and reconsolidation of traumatic and appetitive maladaptive memories: focus on cannabinoids and ketamine. Psychopharmacology (Berl) 235, 433–445.
FDA, U. S. F. a. D. A, 2019. FDA Approves New Nasal Spray Medication for Treatment-Resistant Depression; Available Only at a Certified Doctor's Office or Clinic. FDA News Release.
Graef, J.D., Newberry, K., Newton, A., Pieschl, R., Shields, E., Luan, F.N., Simmermacher, J., Luchetti, D., Schaeffer, E., Li, Y.W., Kiss, L., Bristow, L.J., 2015. Effect of acute NR2B antagonist treatment on long-term potentiation in the rat hippocampus. Brain Res. 1609, 31–39.
Katche, C., Bekinschtein, P., Slipczuk, L., Goldin, A., Izquierdo, I.A., Cammarota, M., Medina, J.H., 2010. Delayed wave of c-Fos expression in the dorsal hippocampus involved specifically in persistence of long-term memory storage. Proc. Natl. Acad. Sci. U. S. A. 107, 349–354.
Kim, J.W., Monteggia, L.M., 2020. Increasing doses of ketamine curtail antidepressant responses and suppress associated synaptic signaling pathways. Behav. Brain Res. 380, 112378.
Kim, J.W., Suzuki, K., Kavalali, E.T., Monteggia, L.M., 2023. Bridging rapid and sustained antidepressant effects of ketamine. Trends Mol. Med. 29, 364–375.
Kim, Y.K., Lee, H.P., Won, S.D., Park, E.Y., Lee, H.Y., Lee, B.H., Lee, S.W., Yoon, D., Han, C., Kim, D.J., Choi, S.H., 2007. Low plasma BDNF is associated with suicidal behavior in major depression. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 31, 78–85.
Klein, A.B., Williamson, R., Santini, M.A., Clemmensen, C., Ettrup, A., Rios, M., Knudsen, G.M., Aznar, S., 2011. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. Int. J. Neuropsychopharmacol. 14, 347–353.
Le Nedelec, M., Glue, P., Winter, H., Goulton, C., Medlicott, N.J., 2018. The effect of route of administration on the enantioselective pharmacokinetics of ketamine and norketamine in rats. J. Psychopharmacol. 32, 1127–1132.
Lee, B.H., Kim, H., Park, S.H., Kim, Y.K., 2007. Decreased plasma BDNF level in depressive patients. J. Affect. Disord. 101, 239–244.
Lepack, A.E., Fuchikami, M., Dwyer, J.M., Banasr, M., Duman, R.S., 2014. BDNF release is required for the behavioral actions of ketamine. Int. J. Neuropsychopharmacol. 18.
Maeng, S., Zarate Jr., C.A., 2007. The role of glutamate in mood disorders: results from the ketamine in major depression study and the presumed cellular mechanism underlying its antidepressant effects. Curr. Psychiatr. Rep. 9, 467–474.
Orozco-Solis, R., Montellier, E., Aguilar-Arnal, L., Sato, S., Vawter, M.P., Bunney, B.G., Bunney, W.E., Sassone-Corsi, P., 2017. A circadian genomic signature common to ketamine and sleep deprivation in the anterior cingulate cortex. Biol. Psychiatr. 82, 351–360.
Radford, K.D., Park, T.Y., Jaiswal, S., Pan, H., Knutsen, A., Zhang, M., Driscoll, M., Osborne-Smith, L.A., Dardzinski, B.J., Choi, K.H., 2018. Enhanced fear memories and brain glucose metabolism ((18)F-FDG-PET) following sub-anesthetic intravenous ketamine infusion in Sprague-Dawley rats. Transl. Psychiatry 8, 263.
Scotton, E., Antqueviezc, B., Vasconcelos, M.F., Dalpiaz, G., Paul Géa, L., Ferraz Goularte, J., Colombo, R., Ribeiro Rosa, A., 2022. Is (R)-ketamine a potential therapeutic agent for treatment-resistant depression with less detrimental side effects? A review of molecular mechanisms underlying ketamine and its enantiomers. Biochem. Pharmacol. 198, 114963.
Shaffer, C.L., 2018. Reverse and forward translational neuropharmacology in psychiatric drug discovery. Clin. Pharmacol. Ther. 103, 193–195.
Shaffer, C.L., Osgood, S.M., Smith, D.L., Liu, J., Trapa, P.E., 2014. Enhancing ketamine translational pharmacology via receptor occupancy normalization. Neuropharmacology 86, 174–180.
Tedesco, V., Ravagnani, C., Bertoglio, D., Chiamulera, C., 2013. Acute ketamine-induced neuroplasticity: ribosomal protein S6 phosphorylation expression in drug addiction-related rat brain areas. Neuroreport 24, 388–393.
Trujillo, K.A., Iniguez, S.D., 2020. Ketamine beyond anesthesia: antidepressant effects and abuse potential. Behav. Brain Res. 394, 112841.
Vuckovic, S.M., Savic Vujovic, K.R., Srebro, D.P., Medic, B.M., Vucetic, C.S., Prostran, M. S., Prostran, M.S., 2014. Synergistic interaction between ketamine and magnesium in lowering body temperature in rats. Physiol. Behav. 127, 45–53.
Wright, K.N., Hagarty, D.P., Strong, C.E., Schoepfer, K.J., Kabbaj, M., 2019. Sex-Dependent Ketamine Addiction-like Behavior Profile Following Exposure to Chronic Mild Stress. Chronic Stress. Thousand Oaks) 3.
Yang, C., Yang, J., Luo, A., Hashimoto, K., 2019. Molecular and cellular mechanisms underlying the antidepressant effects of ketamine enantiomers and its metabolites. Transl. Psychiatry 9, 280.
Zanos, P., Gould, T.D., 2018. Mechanisms of ketamine action as an antidepressant. Mol. Psychiatr. 23, 801–811.
Zanos, P., Moaddel, R., Morris, P.J., Georgiou, P., Fischell, J., Elmer, G.I., Alkondon, M., Yuan, P., Pribut, H.J., Singh, N.S., Dossou, K.S., Fang, Y., Huang, X.P., Mayo, C.L., Wainer, I.W., Albuquerque, E.X., Thompson, S.M., Thomas, C.J., Zarate Jr., C.A., Gould, T.D., 2016. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 533, 481–486.
Zarate Jr., C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch. Gen. Psychiatr. 63, 856–864.
Zhang, M., Radford, K.D., Driscoll, M., Purnomo, S., Kim, J., Choi, K.H., 2019. Effects of subanesthetic intravenous ketamine infusion on neuroplasticity-related proteins in the prefrontal cortex, amygdala, and hippocampus of Sprague-Dawley rats. IBRO Rep 6, 87–94.