EFFECT OF mTOR SILENCING IN MOUSE INFRALIMBIC CORTEX ON DEPRESSIVE-LIKE BEHAVIOUR

E. Garro-Martinez[1,2,3], N. Fullana[3,4], V. Paz[3,4], E. Ruiz[3,4], A. Bortolozzi[3,4], A. Pazos[1,2,3], F. PilarCuellar[1,2,3]

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[1]Institute of Biomedicine & Biotechnology of Cantabria (IBBTEC) (UC-CSIC-SODERCAN), Santander, Spain.
[2]University of Cantabria, Department of Physiology and Pharmacology, Spain.
[3]CIBERSAM, Biomedical Research Networking Center Consortium, Mental Health, Carlos III Institute, Spain.
[4]Department of Neurochemistry and Neuropharmacology, Institut d'Investigacions Biomèdiques de Barcelona, CSIC – IDIBAPS.

INTRODUCTION

Among the hypotheses postulated with the aim of clarifying the neurobiological basis of major depressive disorder (MDD), the neurotrophic hypothesis is one of the most accepted [1]. The extremely important role of trophic factors such as the brain derived neurotrophic factor (BNDF) in both, the etiopatology and the antidepressant effect, is well known [2]. However, in recent decades, the intracellular pathways related to neural proliferation and plasticity have gained strength in the field [3]. In this regard, increased activation of mammalian target of rapamycin (mTOR) pathway in the medial prefrontal cortex (mPFC), is necessary for the rapid antidepressant effect of the N-Methyl-D-aspartate (NMDA) antagonist ketamine [4].

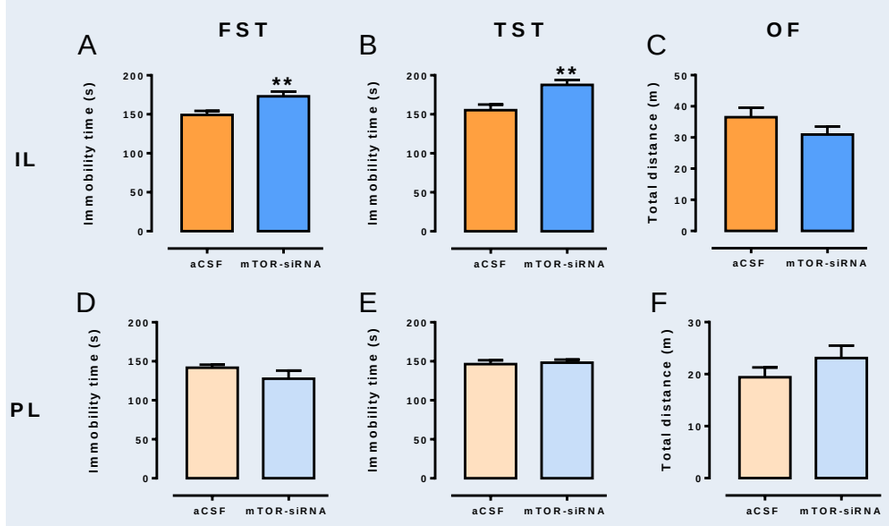
AIM OF THE STUDY

In an attempt to characterize the role of mTOR pathway in the neurobiology of this mood disorder, we silenced the expression of mTOR in mPFC, specifically in the infralimbic and prelimbic cortices (IL and PL), since the modulation of neuronal activity in the IL underlies the antidepressant and anxiolytic actions of ketamine [5].

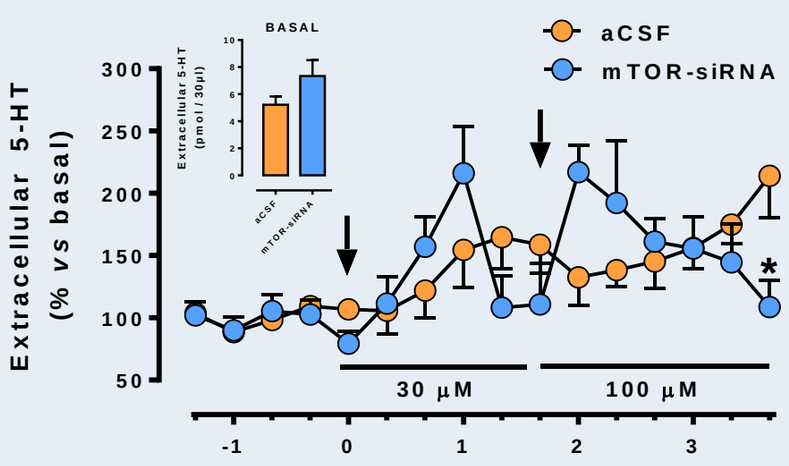
METHODS

Animals. Adult male C57Bl/6J mice of 8-10 weeks of age.
siRNAs. A pool of two different small interfering RNA molecules specifically designed against mTOR were used (mTOR-siRNA).
Intracerebral siRNA infusion. Mice were anesthetized with pentobarbital (40 mg·kg^{-1}) and artificial cerebrospinal fluid (aCSF) or mTOR-siRNA were stereotaxically infused unilaterally into IL cortex (AP: +2.2, ML: -0.2 and DV: -3.4), and PL cortex (AP: +2.0, ML: -0.2 and DV: -2.0). Mice were sacrificed 48 h post-infusion.
Forced swimming test (FST). Mice were placed in swimming tanks (12 cm diameter x 24 cm tall), filled with water at 25–27°C. Each mouse was placed individually in the swimming tank for a single 6 min session. The immobility time was scored using a video camera system.
Tail suspension test (TST). Mice were suspended 30 cm above the bench by the tip of the tail during a 6 min session in which the total duration of immobility was measured.
Open field test (OF). Mice were placed in open field boxes (35x35x40 cm) indirectly illuminated (25-40 luxes) during 15 min. The motor activity was recorded (Videotrack, View Point, France) and the total distance (m), and percentage time in the centre of the arena were analysed.
Intracerebral microdialysis. Extracellular serotonin (5-HT) levels were analysed by *in vivo* microdialysis 24 h post siRNA infusion. The aCSF+1µM Citalopram, 50µM veratridine and 100µM bicuculine were pumped (WPI model, SP220i) at 1.5 µl·min^{-1}and samples were collected every 20 min. 5-HT perisomatic levels were analysed by high-performance liquid chromatography.
***In situ* hybridization (ISH).** Mice were sacrificed and brains rapidly removed and frozen in dry-ice. 14 µm thick coronal tissue sections were cut and mounted onto 3-aminopropyltriethoxysilane pre-treated slides. Mouse mTOR and BDNF oligoprobes used were individually labelled (2 pmol) at the 3' end with [^{33}P]-dATP (DuPont-NEN) using terminal TdT (Calbiochem). Tissue sections were hybridized as previously described [6] and exposed to Biomax-MR film (Kodak, Madrid), during 7days for mTOR and 2 days for BDNF. Optical densities were obtained using a computer assisted image analyser.
Statistical analysis. Groups were statistically compared by Student *t*-test or two-way ANOVA followed by Newman-Keuls *post-hoc* test. Data are expressed as mean±S.E.M.

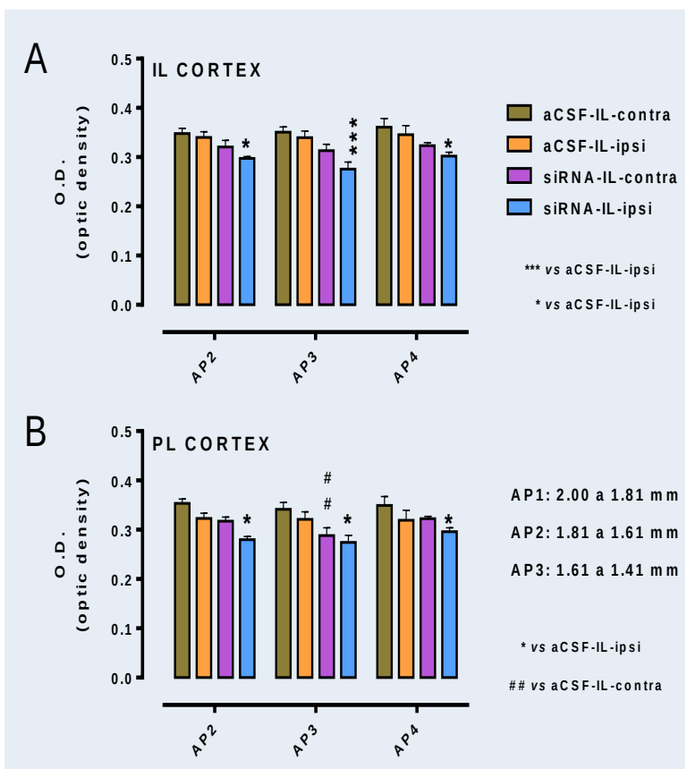
RESULTS



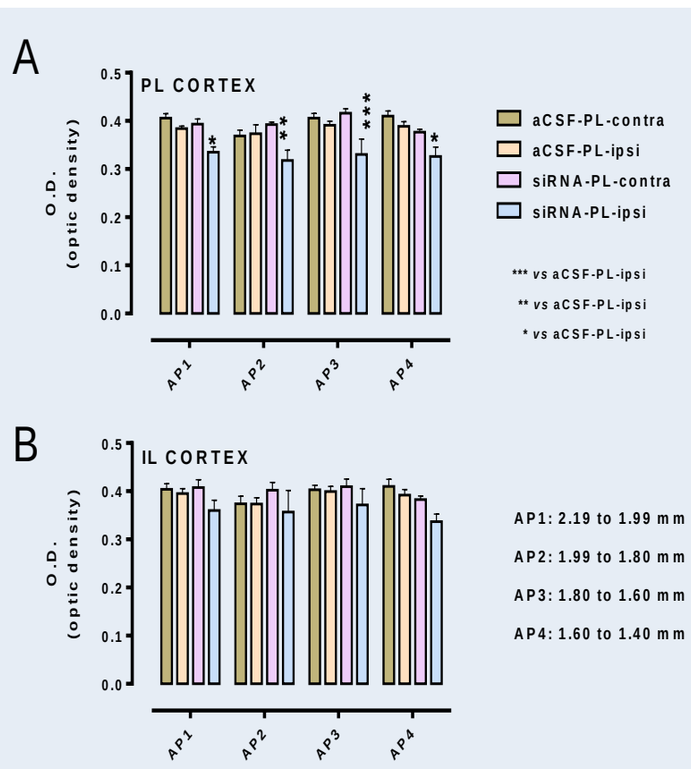
Depressive-like behaviour in mice unilaterally infused intra-IL cortex with mTOR siRNAs. FST (A) and TST (C) (24 and 48 hours respectively after silencing). In contrast animals infused in PL, did not show alterations in depressive-like behaviour (B and D). Silenced animals did not show alterations in locomotor activity (E and F). Student's t-test; **p<0.01. n=6-9 animals/group.



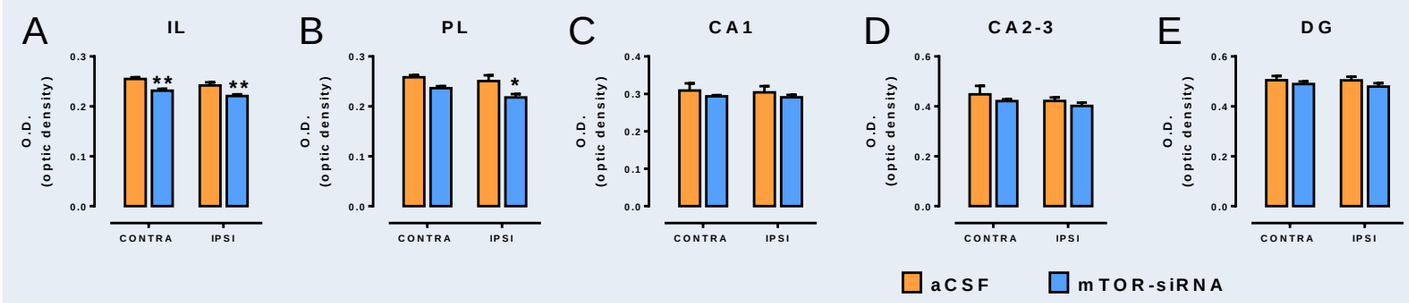
Differential control of 5-HT release after acute silencing of mTOR in the IL cortex. Values obtained after the administration of the $GABA_A$ antagonist bicuculine *versus* time. Basal 5-HT levels after acute mTOR silencing in the IL cortex (insert). Two-way ANOVA followed by Newman-Keuls *post-hoc* test; *p<0.05 vs control group. n=4-5 animals per group.



Reduction in mTOR mRNA expression in IL and PL cortices after the unilateral administration of mTOR-siRNAs into IL cortex. IL (A) and PL cortex (B). Two-way ANOVA followed by Newman-Keuls *post-hoc* test; *p < 0.05, ***p < 0.001 *vs* aCSF-IL-ipsi; ##p<0.01 *vs* aCSF-IL-contra. n=5 animals per group.



Reduction in mTOR mRNA expression in PL cortex after the unilateral administration of mTOR-siRNAs into PL cortex. PL cortex (C). Two-way ANOVA followed by Newman-Keuls *post-hoc* test; *p < 0.05, **p < 0.01, ***p < 0.001 *vs* aCSF-IL-ipsi. n=5 animals per group.



Reduction in BDNF mRNA expression in mPFC after the unilateral administration of mTOR-siRNAs into IL cortex. The downregulation of mTOR mRNA expression caused a reduction in BDNF mRNA levels in both IL and PL cortex (A and B) while no changes were observed in the hippocampus (C: CA1 area of the hippocampus; D: CA2-3 area of the hippocampus; and E: *dentate gyrus* (DG) of the hippocampus). Student's *t*-test; *p<0.05, **p<0.01 *vs* aCSF. n=5 animals per group.

CONCLUSIONS

The present study suggests that the mTOR pathway in IL cortex, but not in PL cortex, plays a major role in the depressive-like behaviour. This allows a better understanding of the biological basis of MDD and new approaches to antidepressant drug discovery.

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Disclosure

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