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FACILITATION OF DOPAMINE-DEPENDENT LTP IN THE MEDIAL PREFRONTAL CORTEX OF MALE RATS FOLLOWS THE BEHAVIORAL EFFECTS OF STRESS

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ABSTRACT

The effect of stress on animal behavior and brain activity has been attracting growing attention in the last decades. Stress dramatically affects aspects of animal behavior, including motivation and cognitive functioning, and has been used to model human pathologies such as post-traumatic stress disorder. A key question is whether stress alters the plastic potential of synaptic circuits. In this work, we evaluated if stress affects dopamine (DA) dependent synaptic plasticity in the medial prefrontal cortex (mPFC). On male adolescent rats, we characterized anxiety- and depressive-like behaviors using behavioral testing before and after exposure to a mild stress (elevated platform). After the behavioral protocols, we investigated DA-dependent long-term potentiation (DA-LTP) and depression (DA-LTD) on acute slices of mPFC and evaluated the activation of DA-producing brain regions by western and dot blot analysis. We show that exposure to the elevated platform stress enhances DA-LTP and that desipramine (DMI) treatment abolishes this effect. We also found that DA-LTD is not affected by elevated platform stress unless when this is followed by DMI treatment. In addition, elevated platform stress reduces anxiety, an effect abolished by both DMI and ketamine, while motivation is promoted by previous exposure to elevated platform stress independently of pharmacological treatments. Finally, this form of stress reduces the expression of the early gene cFOS in the ventral tegmental area. These findings support the idea that mild stressors can promote synaptic plasticity in PFC through a dopaminergic mechanism, an effect that might increase the sensitivity of mPFC to subsequent stressful experiences.

SIGNIFICANCE

Stress affects animal behavior and the homeostasis of the nervous system. Stress-induced alterations of synaptic plasticity might exert dramatically effects on the sensitivity of crucial brain regions, such as prefrontal cortex (PFC), to subsequent stressful experiences. Lamanna et al. show evidence for facilitation of long-term synaptic potentiation in PFC by a mild stress, likely involving the activation of the dopamine system. The authors also show potential interactions of traditional and novel pharmacological treatments with these phenomena. These findings shed light on the effects of stress on synaptic plasticity and suggest a key role played by the dopamine system.

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1 | INTRODUCTION

The effect of stress on animal behavior and brain activity has been attracting growing attention in the last decades among many fields of neurosciences. Some of the key questions about this topic relate to the effects of stress on cognitive performance (e.g. working memory (Yuen et al., 2012) and decision-making (Shafiei et al., 2012)), the differences in the effects produced by stress on synaptic transmission (for review, see (Popoli et al., 2012)), as well as the potential of stress to induce phenotypical changes in the animal model which mimic human pathologies such as post-traumatic stress disorder (PTSD) (Fenster et al., 2018) and depression (Willner, 2017).

Besides having shown how stress affects animal behavior, previous studies evidenced that single exposure to a stressor can exert enduring effects on synaptic transmission and plasticity in brain regions that are pivotal for memory processes and cognition such as the hippocampus (HC) and the prefrontal cortex (PFC). For example, glutamate release (Yan et al., 2011) as well as spontaneous synaptic transmission (Musazzi et al., 2010) are promoted by acute stress in the PFC, while long-term potentiation (LTP) is inhibited by previous exposure to acute stress both *in vitro* and *in vivo* at HC-to-mPFC synapses (D M Diamond & Rose, 1994; David M. Diamond et al., 1992; Richter-Levin & Maroun, 2010; Rocher et al., 2004; Shakesby et al., 2002).

As for the behavioral effects, these were initially thought as a direct consequence of the hypothalamus-pituitary-adrenal (HPA) axis activation and the associated release of corticosteroids having a broad action on the central nervous system (CNS; see (De Kloet et al., 2005; Joëls & Baram, 2009; Popoli et al., 2012) for review). More recently, a new player centralized the attention about this issue: the neurotransmitter dopamine (DA). This was also due to a parallel revision about the role of DA from signaling pleasure ('liking') to encoding incentive salience ('wanting') (Olney et al., 2018). Indeed, DA is involved in motivational processes and profoundly affects behavior. Several studies assessed DA response to aversive stimuli and stressors in terms of both electrical activation of nuclei such as the ventral tegmental area (VTA) (Anstrom & Woodward, 2005; Bettelheim, K A, Carlile, 1976; Enrico et al., 1998; Jahng et al., 2010; Moriya et al., 2018) and changes in the extrasynaptic levels of DA in target brain regions such as the *nucleus accumbens* (nACC) and the medial PFC (mPFC; for review, see (Grace, 2016; Vaessen et al., 2015)). From these studies a complex scenario emerges, where DA release is essentially promoted by stress, albeit the degree of such promotion depends on the specific stressor (Holly & Miczek, 2016; Valenti

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et al., 2012). Furthermore, DA can modulate synaptic plasticity in several brain areas, from the striatum (Kreitzer & Malenka, 2008) to the PFC (S. Otani et al., 2003; Satoru Otani et al., 2015).

Since PFC is a central player in cognitive control and motivational processes (Kouneiher et al., 2009), the effects of stress on its plastic potential are of great interest and might represent the target of psychoactive drugs modulating stress-related behaviors: desipramine (DMI), a tricyclic antidepressant which is also effective for the treatment of PTSD (Aga-Mizrachi et al., 2014; Sofuoglu et al., 2014), is known to alter DA levels in medial PFC (mPFC) (Bongiovanni et al., 2008; Pan et al., 2004); also ketamine (KET), at sub-anesthetic doses, exerts anti-depressive actions (Zanos et al., 2016; Zarate et al., 2006) which are rapid, contrary to DMI, but the mechanism of action is still unknown. Interestingly, KET has been previously evidenced to affect both local synaptic transmission (G. M. I. Chowdhury et al., 2016; Ferro et al., 2017) and dopamine release (Kokkinou et al., 2018; Lorrain et al., 2003) in the mPFC, as well as to induce changes in synaptic plasticity at mesolimbic circuits (Yao et al., 2018).

To our knowledge, there is no study available addressing the effects of acute stressors on DA-dependent LTP or LTD at mPFC local circuits. In this work, we investigated whether the exposure to the elevated platform can induce alterations in DA-LTP and depression (DA-LTD) in the mPFC of male adolescent rats, and if these are prevented by two pharmacological treatments, DMI and KET. In addition, we evaluated if these effects are correlated to changes in the animal motivational and anxiety-like behaviors and we characterized the involvement of the DA system.

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2 | METHODS

2.1 | Animal experimental procedures

Experiments were performed on 24 Sprague–Dawley rats (175-200 g at arrival, 5-6 weeks of age; Charles River, Italy, RRID: RGD_734476), maintained under a 12h/12h light/dark cycle, with food and water *ad libitum* and constant 23 °C temperature. Only male rats were selected in order to minimize variability in stress response (due to both sexual differences and estrous cycle). All experiments were performed during light phase. The number of animals was kept at minimum (see statistical analysis section) and 6 animals were randomly assigned to each of the four groups (control, stress only, S+KET and S+DMI); all efforts were made to minimize distress. Procedures were approved by our Institutional Animal Care and Use Committee in accordance with Italian Ministry of Health (IACUC 735/905). Ketamine (10 mg/kg, Ketavet 100, Intervet Production, Aprilia, Italy) and desipramine (20 mg/kg, dissolved in saline, Sigma-Aldrich) were used as pharmacological manipulation of rats, always intraperitoneally administered after induction of anesthesia (sevoflurane, Abbvie, North Chicago, IL, USA). Only the experimenter performing the injection was not blinded to the pharmacological treatment.

2.2 | Electrophysiological recordings on acute brain slices

At the end of behavioral tests, rats were deeply anesthetized with sevoflurane and then injected intraperitoneally with a lethal Thiopental dose (50 mg; RotexMedica, GMBH, Germany). The experimenter performing brain slicing and recording was blinded to the experimental group. Rats were then transcardially perfused with ice-cold carbonated ACSF mixed with 5000 IU l^{-1} heparin (Pharmatex, Milano, Italy), containing (in mM) 119 NaCl, 2.5 KCl, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 1 $CaCl_2$, 3 $MgCl_2$, 11 D-glucose, while the head was cooled by means of ice application. After decapitation, brains were quickly removed and transferred to an ice-cold cutting solution. The caudal part of the brain was cut in order to dissect SN and VTA in parallel to mPFC slice cutting (see western blot analysis section). Coronal slices containing mPFC were obtained using a Vibratome (2.5-3.5 mm anterior to bregma, slice thickness 400 µm; Vibratome Series 1000, TPI, St Louis, MO, USA). Slices were submerged in the extracellular recording solution, containing (in mM) 119 NaCl, 2.5 KCl, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 2 $CaCl_2$, 1.3 $MgCl_2$, 11 D-glucose, bubbled with 95% O_2 and 5% CO_2 and warmed at 33.6 °C for 1 h. Slices were then maintained at room temperature up to 6h before being used for electrophysiological experiments. Slices were then

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transferred to the recording chamber and constantly perfused with ACSF (room temperature for LTD experiments and 32° for LTP experiments) at a rate of 2 ml/min and bubbled with 95% O_2/5% CO_2 gas mixture. Extracellular recording was obtained by a glass electrode (resistance 0.3-0.7 MΩ) filled with ACSF and placed on layer V of the prelimbic mPFC, where the basal dendrites and cell body of pyramidal neuron are located. Voltage signals were amplified with an Axoclamp 2B amplifier (bridge mode, Axon Instruments, Foster City, CA, USA), then low-pass filtered (total gain = 1000; f_{LP} = 1 kHz) and digitalized at 20 kHz (16 bit) using the ITC-18 data acquisition interface (HEKA Elektronik, Lambrecht, Germany) controlled by a custom software developed in LabVIEW (http://www.ni.com/labview/, RRID: SCR_014325). Analysis of fEPSP for the extraction of amplitude was performed using custom algorithms developed in MATLAB (RRID: SCR_001622). Constant current stimuli (50-110 μA) were delivered by tungsten bipolar electrodes positioned on layer II-III of the same area to evoke fEPSPs. The testing stimuli for the basal synaptic response was delivered at 0.01 Hz (50 μs pulse duration). To induce long-term potentiation (LTP), 5 trains of 300 Hz tetanus (0.5 s train duration, 50 μs pulse duration, 3 min inter-train interval) were used, after bath application of dopamine (Sigma-Aldrich; 50 μM for 10 min). To induce long-term depression (LTD), 3 Hz stimulation for 15 min was applied in the presence of dopamine (200 μM, applied 10 min before start of stimulation and till 5 min after its end). Dopamine (always mixed with ascorbic acid 5-20 μM to prevent oxidation) was locally applied by the means of a thin cannula at a rate of ~1mL/min located above the layer V. For quantification of both LTP and LTD, final fEPSP amplitude was averaged 35-45 min after the end of electrical stimulation.

2.3 | Behavioral procedures

To control for additional stress due to behavioral tests, all rats were subjected to the same sequence of tests: the open field test (OFT), the elevated plus maze (EPM) and the forced swim test (FST). After 7 days of acclimation in the animal house (with daily 5-min sessions of acclimation to the behavioral experimenter for the initial 4 days), rats were subjected to a first sequence of tests on day 0 (‘pretest’; see next sections for durations). This allowed both to verify homogeneous behavior among rats before random group assignment, and to exclude novelty exposure effects in the next testing phase. This was performed the day after (day 1): rats were first treated with the stressor, *i.e.* the elevated platform (EP), then injected with saline or drugs, and then subjected to the same battery of tests (OFT, EPM and FST). Rightly at the end of day 1 procedures, rats were sacrificed to

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produce both acute brain slices and SN/VTA samples. A scheme of the experimental protocol is depicted in Fig. 1A.

Open Field Test

Test consisted in placing the rat into an open-field arena (70x70 cm^2; 10 min duration for both pretest and test) and filming the entire session, later analyzed using EthoVision XT14 by an experimenter blind to the experimental condition, to measure general locomotion, center-crossings and time spent in the center.

Elevated Plus Maze

Each rat was then placed onto the elevated maze facing one of the open arms. The rat was free to move and explore the maze (5 min duration for both pretest and test) and session was videotaped and analyzed with EthoVision XT14 by an experimenter blind to the experimental condition, to measure the total time spent in open arms and the time spent moving in open arms as a percentage of the total time spent in these arms.

Forced Swim Test

The FST was conducted as previously described (Porsolt et al., 1977). Briefly, rats were forced to swim (10 min duration for pretest, 5 min for test) in a transparent plexiglass cylinder (60 cm high, 25 cm diameter) filled with 25°C water, with no escape chance, then warmed/dried. After the pretest, rats were left undisturbed in their home cage till the following day (test day). The entire session was videotaped and immobility and struggling behavior evaluated using EthoVision XT14 (https://www.noldus.com/ethovision, RRID:SCR_000441) by an experimenter blind to the experimental condition. Rats were considered immobile when no movement were done except those aiming to keep the nose above water; struggling was classified as a highly active state (both when trying to climb walls and moving actively with high strength in the water).

Elevated Platform

For the stress treatment, on day 1 rats were placed on an elevated and unsteady transparent platform (30x30 cm^2, 1 m above ground) for 30 min, directly before drugs or saline injection and before behavioral tests.

2.4 | Western blot and dot blot analysis

The caudal part of the rats' brain was separated from the rest of the brain used to prepare acute slices (see electrophysiological recordings on acute brain slices). To collect VTA and SN samples as

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accurately as possible, we performed a dissection procedure based on the one described in (Salvatore et al., 2012), with some modifications: maintaining the caudal brain wet, we rapidly collected 1 mm thick slices using a mechanical tissue chopper, then transferred the slices in ice-cold cutting solution (the same used for acute slices) under a dissection microscope (20-40x magnification, Leica Microsystems). Then, we identified bilaterally SN according to position in the coronal plane, morphology, and color (Salvatore et al., 2012), and cut it using tweezers and a scalpel. VTA was identified as the small region medial to SN on each hemisphere. We successfully obtained tissue from 2-3 slices per brain. Samples were then frozen with liquid nitrogen and kept at -80 °C for later WB and dot blot analysis. Tissues were homogenated with dounce in 0.32 M sucrose and 5 mM Hepes (pH 7.4), with protease and phosphatase inhibitors added, and then solubilized with 0.5% Triton X-100 (Sigma-Aldrich) for 15 min at 4°C. After centrifugation (5 min, 1000 g, 4°C), protein concentration of the samples was determined by the BCA assay (BCA, Thermofisher). Fixed amounts of proteins (40 µg/lane) were boiled for 5 min at 95°C in Laemmli buffer, loaded on 12% polyacrylamide gels and transferred to nitrocellulose filters (0.22 µm). The following antibodies were used: rabbit polyclonal anti-cFOS (Abcam, #cat Ab7963, RRID: AB_306177), rabbit polyclonal anti-TH (Abcam, #cat Ab112, RRID: AB_297840), rabbit polyclonal anti-phosphoTH (Biovision, #cat 3612, RRID: AB_10987327) and rabbit monoclonal anti-vinculin (Abcam, #cat Ab129002 - EPR8185, RRID: AB_2810877). Specific antibody information is provided in Table 1.

Antibody	**Immunogen structure**	**Manufacturer**	**#cat / RRID**	**Species**	**Concentration**
cFOS	Synthetic peptide: MMFSGFNADYEASS (1-14 N-term amino acids of human c-FOS)	Abcam	Ab7963 / AB_306177	Rabbit polyclonal	1:200
Tyrosine Hydroxylase	Full length SDS denatured protein (purified from rat pheochromocytoma)	Abcam	Ab112 / AB_297840	Rabbit polyclonal	1:200
Phospho-(Ser19)	Phosphopeptide of aa surrounding the phospho-	Biovision	3612 / AB_10987327	Rabbit polyclonal	1:500

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Tyrosine Hydroxylase	Ser19 of rat tyrosine hydroxylase				
Vinculin	Synthetic peptide within human vinculin aa 1000-1100 (exact sequence is proprietary)	Abcam	Ab129002-EPR8185 / AB_2810877	Rabbit polyclonal	1:2000

Table 1. Information on the antibodies used in this study.

Blots were developed by chemiluminescence, using the enhanced chemiluminescence Western Blotting Detection reagent (Immobilon, Millipore). Signals were acquired and quantified by the Chemidoc Imaging System and program (Bio-rad). To minimize the amount of lysate used, we performed dot-blots for the analysis of TH/pTH expression: 1.5 µg of the same lysate was blotted (1 µl/sample/dot) on nitrocellulose membrane. After drying, membrane was processed with blocking and antibodies incubation, followed by development and acquisition as in western blot, while quantification was performed using ImageJ (https://imagej.net/, RRID: SCR_003070). Specificity of the antibodies against vinculin, TH and pTH was verified using whole gels, which are shown in Figure S3 and S4 of Supporting Information, then we opted for the dot-blot analysis to limit the amount of sample needed.

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2.5 | Statistical analysis

Experiments have been conducted on each subject on a separate day (behavioral pretests performed the day before). All subjects were independent and randomly assigned to a single treatment level (ctrl, stress only, S+KET, S+DMI; treatment as between-subjects factor). All treatments have been tested on each experimental 'block' (one rat for group), starting 4 days after rats' arrival (habituation phase), and the order of treatments was randomized on each block. Sample size was selected based on preliminary electrophysiological data: in order to detect the appearance of significant LTP or an appreciable modulation of LTD, we computed an hypothetical effect size of about 1.67 in our conditions (±25% starting from no LTP or a typical LTD of 0.75%, with $\sigma \simeq 15\%$), which suggests a minimum of $n = 6$ with two-samples, two-tailed t-tests comparisons, power = 0.8 and $\alpha = 0.05$. This minimum sample size was selected due to ethical considerations. In a few cases a single value was lacking ($n = 5$) due to technical problems during data collection. Normal distribution of data was verified on Q-Q plots. Homoscedasticity was verified with the Fligner-Killeen test. A linear mixed-model was fitted, which includes a random effect term (experimental block) and a fixed-effect term indicating the treatment. ANOVA was performed on the fitted model, followed by Dunnett's post-hoc tests. Data processing and graphical representation were performed using MATLAB (RRID: SCR_001622). Statistical modeling and analysis were performed in R (http://www.r-project.org/, RRID: SCR_001905; nmle package for model fitting). All data in the text are provided as mean ± SD. Threshold for statistical significance was set to 0.05 and indicated on graphs as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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3 | RESULTS

In order to simultaneously test the modulatory effects of the elevated platform (EP) stress on both animal behavior and DA-dependent synaptic plasticity in the PFC, our experimental design followed the scheme in Fig. 1A. We randomly assigned rats to four groups: all rats were subjected to the same sequence of behavioral tests, the OFT, the EPM and the FST, both the day before ('pretest') and immediately after the exposure to the EP stress, consisting in placing the rat on an EP for 30 min (with the exclusion of control rats, kept in their home cage for the same period of time). To evaluate if the modulatory effects of the EP stress can be blocked or enhanced by acute pharmacological treatments, two groups of rats where intraperitoneally injected rightly after the EP episode with: KET 10 mg/Kg ('S+KET' group) and DMI 20 mg/Kg ('S+DMI' group). Controls ('ctrl' group) and stress-only rats ('stress' group) were injected with the vehicle (saline) after the home-cage/EP epoch. At the end of the last behavioral test (FST), all rats were sacrificed to produce brain slices.

3.1 | Exposure to stress enhances DA-LTP in the PFC while preserving LTD

fEPSPs were recorded from layer V of mPFC slices while stimulating the II-III layer. After the acquisition of a stable baseline, DA was bath applied in the recording region (50 μM; 10 min), followed by 5 tetani at 300 Hz (3 min inter-train). This protocol was shown to induce LTP in similar preparations (Huang et al., 2004). In our conditions (no GABA blockers) we were not able to detect a significant LTP in controls (ctrl = 93.3 ± 20.4 %, n = 5; Fig. 1B). On the contrary, when we tested the same protocol on mPFC slices from animals subjected to EP stress, enduring potentiation was observed till 50 min after first tetanus (stress = 128.5 ± 18.2 %, n = 6; $F_{3,13}$ = 4.508, p = 0.022; stress *vs* ctrl: p < 0.001; Dunnett's post-hoc test; Fig. 1C). Importantly, we can reasonably exclude that behavioral tests significantly affect, if not marginally, DA-LTP, since in separate experiments without behavioral tests we have never been able to induce potentiation, unless with a different protocol and experimental conditions (1 μM picrotoxin in bath and 25 μM DA before tetani; data not shown).

We then tested whether also DA-LTD could be affected by previous exposure of the rat to the same stress. We selected a protocol for the induction of DA-LTD in mPFC brain slices (Bai et al., 2014), consisting in the delivery of low-frequency stimulation (3 Hz) for 15 min during high-DA bath-application (200 μM). This protocol reliably induced >20 % depression in the amplitude of

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fEPSPs that was dependent upon D2-receptor activation, as previously shown by others (Bai et al., 2014) (our validation data are shown in Figure S1 of Supporting Information). When tested on mPFC slices from rats subjected to the experimental design (Fig. 1A), we obtained a comparable level of DA-LTD for control animals (ctrl = 79.5 ± 6.0 %, n = 6; Fig. 1D), suggesting that behavioral testing and vehicle injection was not compromising the robustness of the selected protocol. Importantly, the exposure to EP stress did not affect DA-LTD (stress = 82.4 ± 5.6 %, n = 6; $F_{3,15}$ = 4.614, p = 0.018; stress *vs* ctrl: n.s.; Dunnett's post-hoc test; Fig. 1E).

3.2 | DMI treatment prevents the enhancement of DA-LTP by stress while occluding LTD

Interestingly, both sub-anesthetic dose of KET (10 mg/kg) and DMI (20 mg/kg) treatments, when administered after the EP stress experience, reduce such potentiation of LTP, albeit the effect of DMI is more severe and statistically significant (S+KET = 111.2 ± 15.8 %, n = 5; S+DMI = 105.7 ± 18.5 %, n = 6; S+KET *vs* stress: n.s.; S+DMI *vs* stress: p = 0.025; Dunnett's post-hoc test; Fig. 2A,B). Fig. 3A provides a summary of LTP experiments, where we can appreciate that the exposure to the EP mild stress significantly enhances DA-LTP at mPFC local circuits and this enhancement is abolished by successive, acute DMI treatment.

Concerning the effects of the pharmacological treatments applied after stress on LTD, while KET left this result unchanged (S+KET = 81.9 ± 4.1 %, n = 6; S+KET *vs* stress: n.s.; Dunnett's post-hoc test; Fig. 2C), DMI dramatically compromises DA-LTD and this effect was significant (S+DMI = 90.2 ± 5.6 %, n = 6; S+DMI *vs* stress: p = 0.015; Dunnett's post-hoc test; Fig. 2D), with an extent similar to sulpiride (50 μM; Sigma-Aldrich) bath-application (Figure S1C of Supporting Information). Fig. 3C summarizes LTD experiments.

3.3 | Effects of stress on DA-LTP is not paralleled by a change in DA sensitivity or neuronal excitability.

To investigate the presence of alterations in the phase of induction of DA-LTP that might explain the results described above, we tested if treatments induced some change in the effect of DA on basal synaptic transmission, but no significant effect could be appreciated (amplitude after DA 50 μM: ctrl = 119.8 ± 62.1 %, stress = 112.1 ± 24.3 %, S+KET = 127.6 ± 34.1 %, S+DMI = 127.7 ± 65.2 %; no significant effect detected; Fig. 3B). In the same figure, we reported the fEPSP amplitude for all groups measured after each tetanus (high-frequency stimulus, HFS1-5), where no clear or significant effect could be detected (Fig. 3B), suggesting that the action of EP stress cannot

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be directly ascribed to a change in the synaptic response during the induction phase, such as a detectable increase in synaptic sensitivity to DA (D1/D2-receptors) or in neuronal excitability.

Also for LTD, no cue comes from analyzing the effect of either DA or 3-Hz-stimulation on basal transmission (amplitude post-DA: ctrl = 76.3 ± 6.7 %, stress = 76 ± 6.8 %, S+KET = 77.2 ± 6.6 %, S+DMI = 75 ± 12 %; n.s.; amplitude post-3-Hz: ctrl = 57.3 ± 9.7 %, stress = 53.9 ± 9.9 %, S+KET = 62.9 ± 11.4 %, S+DMI = 57.8 ± 13.6 %; no significant effect detected; Fig. 3D).

3.4 | Anxiety-like behavior is reduced by stress and KET abolishes this effect

Several lines of evidence suggest that stress as well as DA can affect both anxiety- and depressive-like behaviors (Grace, 2016; Suvrathan et al., 2010). On the contrary, a clear link between stress-induced alterations at PFC circuits and these behaviors is still missing (Holmes & Wellman, 2009). In order to investigate whether the observed effects of EP stress on PFC plasticity are paralleled by changes in animals' behavior, we analyzed anxiety-like and depressive-like behaviors by using a battery of well-established tests. Interestingly, our EPM test data show that, while propensity of rats to explore open arms was comparable among groups in the 'pretest' phase (day 0; entrances in open arms: ctrl = 14.5 ± 8.8 %, stress = 20.7 ± 9.93 %, S+K = 17.0 ± 7.21, S+D = 15.7 ± 10.7; n = 6 per group; no significant effect detected; Fig. 4B), EP stress exposure resulted in a significant increase of this behavior and KET significantly abolished this effect (entrances in open arms: ctrl = 5.8 ± 2.2, stress = 14.2 ± 12, S+KET = 5.2 ± 1.7, S+DMI = 7.2 ± 4.8; n = 6 per group; treatment overall effect not significant: $F_{3,15}$ = 2.476, p = 0.101; stress *vs* ctrl: p = 0.041; S+KET *vs* stress: p = 0.029; Dunnett's post-hoc test; no other significant comparison; Fig. 4C). On the contrary, we could not detect a significant effect of treatment on the time spent by rats in open arms (time in open arms: ctrl = 16.5 ± 9.1 %, stress = 25.3 ± 14.3 %, S+KET = 6.9 ± 3.3 %, S+DMI = 26.7 ± 32.6; n = 6 per group; no significant effect detected; Fig. 4D). As a control, we tested if treatments altered total time spent by rats with the whole body confined in closed arms, which was left unchanged, indicating that the observed anxiolytic effects can be related to the promotion of an already present approach behavior rather than a learned avoidance of the closed arms (time in closed arms: ctrl = 60.4 ± 21.0, stress = 54.9 ± 21.1, S+KET = 82.2 ± 13.6, S+DMI = 61.7 ± 34.6; n = 6 per group; no significant effect detected; Fig. 4E). In conclusion, from our data it emerges that stress produces a sort of anxiolytic effect by promoting exploration and approaching behavior of the animal, and that KET counteracts this effect. Since OFT is another popular approach for quantifying anxiety in rodents by monitoring unlearned approach/avoidance behavior (Carli et al., 1989; Shoji &

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Mizoguchi, 2010) we used it as a further evaluation of anxiety-like behavior and general locomotion, which resulted comparable among all groups in our testing conditions (Figure S2, Supporting Information).

3.5 | Stress promotes motivation and reduces depressive-like behavior in the FST also when followed by KET or DMI treatments

Several lines of evidence suggest that stress can affect motivated behavior, both in reward-related and threat-related contexts. In addition, the role of DA system in modulating motivation is nowadays well accepted and this might have an impact on DA-dependent synaptic plasticity.

To address this aspect, we tested whether exposure to the EP increases motivation for escape and/or reduces inactivity in the FST (Fig. 5A). From our data, it clearly emerges that the fraction of time rats spent in strong escaping attempts (*i.e.* wall-climbing and strong exploratory swimming/diving) is significantly increased by stress (high activity time: ctrl = 14.3 ± 3.6 %, stress = 25.3 ± 2.6 %, S+KET = 24.7 ± 5.3 %, S+DMI = 29.2 ± 1.86 %; n = 5/6 per group; $F_{3,12}$ = 4.650, $p < 0.001$; stress *vs* ctrl: $p < 0.001$; Dunnett's post-hoc test; no other significant comparison; Fig. 5C). As a further comparison, Fig 5B shows the same measure in the 'pretest' phase (day 0), when no significant difference was detected among groups (pretest high activity time: ctrl = 20.9 ± 4.29 %, stress = 18.9 ± 6.7 %, S+KET = 24.7 ± 11.9 %, S+DMI = 19.8 ± 9.7 %; n = 5/6 per group; n.s.; Fig. 5B). It is worth noting that, as described above, no difference was detected when pharmacological treatments were administered after the stress episode.

Parallel to the previous finding, the analysis of the time spent by the animal without any escape activity (minimal swimming to avoid sinking) shows a significant effect of stress which is not appreciably modulated by either KET or DMI (inactivity time: ctrl = 69.7 ± 3.5 %, stress = 60.3 ± 5.3 %, S+KET = 54.7 ± 4.7 %, S+DMI 56.6 ± 7.8 %; n = 5/6 per group; $F_{3,12}$ = 9.215, p = 0.002; stress *vs* ctrl: p = 0.003; Dunnett's post-hoc test; no other significant comparison; Fig. 5D). As a final step we tested if moderate level of activity is affected by our manipulations and, only when KET treatment was administered, the stress episode resulted in a significant increase of this level of activity albeit the overall effect of treatment was not significant (moderate activity time: ctrl = 6.7 ± 1.3 %, stress = 6.2 ± 1.9 %, S+KET = 9.9 ± 3.8 %, S+DMI = 5.1 ± 3.8 %; n = 5/6 per group; $F_{3,12}$ = 3.089, n.s.; S+KET *vs* stress: $p < 0.038$; Dunnett's post-hoc test; no other significant effect; Fig. 5E). Taken as a whole, the results shown in Fig. 5 support the idea that stress induces an enhancement of motivation in the animals, and that KET and DMI, both known as antidepressive

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agents, cannot further increase the positive effect (with the exclusion of KET for 'moderate' escape activity).

3.6 | Stress reduces global cFOS expression in the VTA but only ketamine affects TH signaling

The results of both DA-LTP experiments and FST encouraged us to investigate the activity of DA-related brain structures. We performed three biochemical analyses on the homogenates coming from samples collected (at the end of all behavioral tests), which included VTA and *substantia nigra* (SN), the key dopaminergic nucleus of basal ganglia. At first, we tested by western blot (WB) EP stress was able to alter the expression of cFOS (Fig. 6A; full gels in Figure S3 of Supporting Information), an immediate early gene known to be calcium regulated and thus representing an indirect measure of neuronal activity (A. Chowdhury & Caroni, 2018; Montesano et al., 2015). Expression of cFOS in the VTA was significantly reduced by stress, and this reduction was present with both KET and DMI treatments (VTA normalized-cFOS: ctrl = 1.532 ± 0.744, stress = 0.797 ± 0.211, S+KET = 0.553 ± 0.299, S+DMI = 0.500 ± 0.310; n = 4/5 per group; $F_{3,12}$ = 5.826, p = 0.011; stress *vs* ctrl: p = 0.011; Dunnett's post-hoc test; no other significant comparison; Fig. 6C). No significant effect on cFOS expression was detected for SN samples (SN normalized-cFOS: ctrl = 0.714 ± 0.652, stress = 0.752 ± 0.533, S+KET = 0.518 ± 0.343, S+DMI = 0.330 ± 0.253; n = 4/5 per group; n.s.; Fig. 6D).

We then tested if either the phosphorylated fraction of tyrosine hydroxylase (pTH; residue 'Ser19') or the total TH, a crucial enzyme in DA synthesis, was affected by our experimental manipulations using a dot-blot analysis in order to minimize the amount of lysate used (Fig. 6B shows exemplary dots; WB gels testing Abs with single-banded, clean lanes are shown in Figure S4, Supporting Information). From this analysis it emerges that the relative fraction of pTH protein is not changed by stress, rather it seems to be increased when stress is followed by KET treatment but the overall treatment was not significant (VTA pTH/TH: ctrl = 2.166 ± 0.508, stress = 1.386 ± 1.038, S+KET = 3.722 ± 3.110, S+DMI = 2.122 ± 0.580, n = 5 per group; $F_{3,12}$ = 1.701, n.s.; S+KET *vs* stress: p = 0.038; Dunnett's post-hoc test; no other significant effect; Fig. 6E). No significant difference was detected among the groups for SN samples (SN pTH/TH: ctrl = 1.69 ± 1.770, stress = 2.098 ± 1.970, S+KET = 1.574 ± 1.861, S+DMI = 3.113 ± 3.030, n = 5 per group; n.s; Fig. 6F). As for the total level of TH protein in VTA, our data suggest that stress can induce some increase in it, but we failed to detect a significant treatment effect; interestingly, KET significantly reduces the total expression of TH in VTA when compared to the stress-only condition

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(VTA normalized-TH: ctrl = 0.696 ± 0.230, stress = 1.097 ± 0.685, S+KET = 0.408 ± 0.148, S+DMI = 0.733 ± 0.426, n = 5 per group; $F_{3,12}$ = 2.216, n.s.; S+KET *vs* stress: p = 0.011; Dunnett's post-hoc test; no other significant effect; Fig. 6G). As previously, the analysis of SN samples did not show any significant difference among groups (SN normalized-TH: ctrl = 0.436 ± 0.258, stress = 0.354 ± 0.150, S+KET = 0.440 ± 0.219, S+DMI = 0.584 ± 0.506, n = 5 per group; n.s.; Fig. 6H). In conclusion, our data suggest a global dampening effect of stress on VTA neuronal activity and a possible action of KET in modulating local DA synthesis in the VTA, both aspects that deserve attention for future investigations.

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4 | DISCUSSION

Our data show that EP stress potentiates DA-dependent LTP at local mPFC circuits (Fig. 1), while DA-LTD in mPFC is not affected. In addition, different pharmacological treatments can dampen this potentiating effect of stress: DMI, a classical tricyclic antidepressant, abolishes the potentiation while KET reduces it but not significantly. We can reasonably say that potentiation of LTP should be regarded as a beneficial action, able to enhance cognitive capabilities in the short-term thus explaining findings by other authors (Shafiei et al., 2012; Yuen et al., 2009). Nevertheless, we should also take into account that, on the long-term, such effects might dangerously expose PFC to an excessive degree of structural remodeling (Belujon & Grace, 2014; Dias-ferreira et al., 2009; Goldwater et al., 2009; Li et al., 2011; Wilber et al., 2011; Yuen et al., 2012). In this context, both drugs might help reducing the long-term effects of stressors, with DMI having a stronger action. In our experiments, drugs were delivered after the EP epoch, since the idea was to test whether these pharmacological treatments could block or revert the expression of stress effects without altering the stressful experience itself. A further question that could be addressed by future investigations is whether these drugs are more or less effective when administered before the EP stress.

We obtained this evidence on a form of LTP that is dependent on the action of DA, a molecule whose activity is modulated by both acute and chronic stress (Grace, 2016; Vaessen et al., 2015). In particular, DA release is enhanced at VTA projection targets after acute stress, as reported by other authors (Anstrom & Woodward, 2005; Bettelheim, K A, Carlile, 1976; Enrico et al., 1998; Jahng et al., 2010; Moriya et al., 2018). Hence, DA system might be involved in this stress-induced LTP enhancement. Starting from the available evidence on the forms of synaptic plasticity investigated here, we can hypothesize that stress might produce moderate, physiological increases in tonic release of DA in mPFC, which is known to promote LTP (Satoru Otani et al., 2015). On the contrary, much higher levels of DA are known to reduce and even abolish LTP. As the matter of fact, both KET (Kokkinou et al., 2018; Lorrain et al., 2003) and DMI (Bongiovanni et al., 2008; Pan et al., 2004) increase DA levels, in very good agreement with our data (Fig. 2). Conversely, LTD is less sensitive to moderate changes in DA, but DMI might be able to perturb also this form of plasticity (Fig. 2D) by producing very high levels of DA (Bai et al., 2014; Pan et al., 2004). Of course, these changes in DA tonic release produced *in vivo* might have important, but difficult to predict, consequences on the *in vitro* preparation. As described in section 3.3, we were not able to detect a significant effect of treatments on synaptic responses during the LTP induction phase.

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Albeit we cannot exclude that such changes are too subtle to be detected, a possible explanation is that stress affected LTP expression mechanisms (*e.g.* the involved kinases), rather than the responsivity of D1/D2 receptors to DA, or neuronal excitability in general. The specificity of the form of synaptic plasticity investigated here can also explain how different forms of LTP have been shown to be inhibited by stress (D M Diamond & Rose, 1994; Rocher et al., 2004). An important aspect in this context is of course animal age. In this work, rats were 6-7 weeks old at the time of experiments, *i.e.* at the end of adolescence. In mammals, this period is characterized by enhanced synaptic plasticity and brain maturation, with dramatic structural and functional changes on PFC circuits and dopaminergic system (Lupien et al., 2009; Selemon, 2013). Hence, stress responses are likely to exert profound effects on dopamine-dependent forms of synaptic plasticity in the PFC of adolescent rats, as it was actually shown in this study. It would be of great interest to further evaluate if the effects of stress described here reduced or enhanced when the brain enters the adult phase.

KET and DMI were selected for this study for two main reasons: at first, they are likely to modulate, in different ways, dopamine release (with KET also acting on glutamate release); secondly, their action is of interest for stress-related mental disorders, such as depression and PTSD, in both adult and adolescent patients (Cipriani et al., 2016; Elmer et al., 2020; Kim et al., 2020; Naughton et al., 2014; Parise et al., 2013). Indeed, DA is also central to the expression of motivated behaviors (Bromberg-Martin et al., 2010; Lamanna et al., 2019; Lamontagne et al., 2018; Russo & Nestler, 2013) and the behavioral characterization reported here supports the view described above, since the same stress significantly reduced anxiety- and depressive- like behaviors (Fig. 4 and 5). Anxiety in the EPM seems reduced by EP stress (Fig. 4) and this effect can be seen as beneficial because it could improve animal's fight-or-flight reactions. In this view, facilitation of LTP at PFC local circuits might play a role, since PFC exerts an important top-down control on amygdala nuclei. Another beneficial or positive effect of EP stress on animal behavior is the observed increase in motivation (activity in the FST, Fig. 5), which might promote active coping strategies when animals are challenged with new stressful situations, at least in the case of mild intensity stressors (similarly to the effect observed by (Consoli et al., 2005)).

Regarding DA system activation, the level of expression of cFOS in the VTA of our rats resulted to be lower for the *stress* group, also when animals when treated with KET or DMI (Fig. 6C). Since cFOS protein level reflects neuronal activation occurred about 60 min before animal sacrifice (A. Chowdhury & Caroni, 2018), we can argue that most of VTA neurons reduced their

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firing during the EP epoch. As the matter of fact, at least two populations of DA neurons exist in the VTA, having opposite responses to stress (see (Holly & Miczek, 2016) for review), and those reducing their activity show a broader cells distribution (Holly & Miczek, 2016), thus being better sampled by whole-tissue expression analysis. Hence, our data suggest that an initial reduction of global VTA activity during the exposure to the EP stress might drive the promotion of motivated behavior later observed, e.g. by transiently modifying phasic responses of DA neurons to salient stimuli. Future studies should better address this issue.

On the other hand, data provided by our TH analysis might reflect the whole pattern of DA activation, and the total level of TH seems increased by stress, but not significantly (Fig. 6G). Although previous studies showed that stronger stressors (footshock) are not able to induce changes in total TH or pTH in VTA (Ong et al., 2014), milder stressors like the EP might be more effective (a phenomenon already observed with behavioral responses (Consoli et al., 2005)). Interestingly, KET seems to interfere with DA synthesis in the VTA by promoting TH activity through phosphorylation (Dunkley et al., 2004), at least when stress is delivered (Fig. 6E).

A limitation of this study relates to the design of the control condition. Unfortunately, the study of stress effects is difficult because most experimental procedures are experienced as stressful by the animals. Indeed, in our experiments, the behavioral tests (OFT, EPM, FST) presumably introduced an additional source of stress which added to the EP stress and whose effects, although mild, could not be exactly quantified. Clearly, these sources of stress affected also the control group, which therefore is not hundred percent devoid of stress effects. Our reasoning was that the additive effect of EP and behavioral tests would have induced some detectable changes above control in the physiological parameters. Actually, this was found as indicated in Figure 1. It would be important in the future to establish if different sources of stress induce additive or multiplicative effects to better isolate the individual contributions.

Such aspects of the experimental design can contribute to a more complex viewpoint on the results collected. As the matter of fact, our behavioral analysis shows that previous exposure to a mild stressor (the EP) can enhance motivation when rats are challenged with a new stress (the inescapable situation of the FST; see paragraph 3.5), thanks to a sort of ‘priming’ or ‘adaptation’ effect. Interestingly, data of cFOS expression in EP-stressed animals, indicating a lower activation of VTA in EP-stressed animals, suggest that such ‘priming’ and the following behavioral response can be mediated by the DA system. In this context, we can reasonably say that only a sufficient degree of stress (provided by the exposure to the EP), and not the behavioral tests alone, are indeed

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able to potentiate DA-LTP measured in our conditions; DMI, which is administered after the EP and before tests, might act either by neutralizing the 'priming' effect of EP exposures. Further studies could better address this issue, e.g. by testing DA-LTP *in vivo* at different time points.

5 | CONCLUSIONS

In summary, we provided evidence for the enhancement of DA-dependent LTP at mPFC local circuits by stress, a finding paralleled by positive effects on depressive- and anxiety-like behaviors. Furthermore, we showed modulation of these effects by both slowly- and rapid-acting antidepressants. Our findings support the idea that stress might increase the plastic potential of PFC, an effect that is likely to positively affect animal behavior in the short term but might also compromise resilience to future stressful experiences. Finally, our results might contribute to design future translational studies addressing the effects of stress on human behavior as well as the pharmacological treatment of stress-related mental disorders.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors read and approved the final manuscript. Conceptualization, J.L. and A.M; Data Curation, J.L., F.I., G.R. and L.A.; Formal Analysis, J.L. and F.I.; Investigation, J.L., F.I., M.F., G.R., L.A., D.R.; Methodology, J.L. and F.I.; Project Administration, J.L. and A.M.; Validation, J.L. and F.I.; Writing – Original Draft, J.L.; Writing – Review & Editing, J.L., F.I., and A.M.; Funding Acquisition, J.L. and A.M.; Resources, J.L. and A.M.; Software, J.L., Supervision, J.L. and A.M.

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DATA AVAILABILITY STATEMENT

Images of complete western blot gels are provided in additional files of this article. All other data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Aga-Mizrachi, S., Cymerblit-Sabba, A., Gurman, O., Balan, A., Shwam, G., Deshe, R., Miller, L., Gorodetsky, N., Heinrich, N., Tzezana, O., Zubedat, S., Grinstein, D., & Avital, A. (2014). Methylphenidate and desipramine combined treatment improves PTSD symptomatology in a rat model. *Translational Psychiatry*, *4*(9). https://doi.org/10.1038/tp.2014.82

Anstrom, K. K., & Woodward, D. J. (2005). Restraint increases dopaminergic burst firing in awake rats. *Neuropsychopharmacology*, *30*(10), 1832–1840. https://doi.org/10.1038/sj.npp.1300730

Bai, J., Blot, K., Tzavara, E., Nosten-Bertrand, M., Giros, B., & Otani, S. (2014). Inhibition of dopamine transporter activity impairs synaptic depression in rat prefrontal cortex through over-stimulation of D1 receptors. *Cerebral Cortex*, *24*(4), 945–955. https://doi.org/10.1093/cercor/bhs376

Belujon, P., & Grace, A. A. (2014). Restoring mood balance in depression: Ketamine reverses deficit in dopamine-dependent synaptic plasticity. *Biological Psychiatry*, *76*(12), 927–936. https://doi.org/10.1016/j.biopsych.2014.04.014

Bettelheim, K A, Carlile, M. J. (1976). Selective activation of the mesocortical DA system by stress. *Nature*, *262*, 774–776.

Bongiovanni, R., Newbould, E., & Jaskiw, G. E. (2008). Tyrosine depletion lowers dopamine synthesis and desipramine-induced prefrontal cortex catecholamine levels. *Brain Research*, *1190*(1), 39–48. https://doi.org/10.1016/j.brainres.2007.10.079

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in Motivational Control: Rewarding, Aversive, and Alerting. *Neuron*, *68*(5), 815–834. https://doi.org/10.1016/j.neuron.2010.11.022

Carli, M., Prontera, C., & Samanin, R. (1989). Effect of 5-HT1A agonists on stress-induced deficit in open field locomotor activity of rats: Evidence that this model identifies anxiolytic-like activity. *Neuropharmacology*, *28*(5), 471–476. https://doi.org/10.1016/0028-3908(89)90081-6

Chowdhury, A., & Caroni, P. (2018). Time units for learning involving maintenance of system-wide cFos expression in neuronal assemblies. *Nature Communications*, *9*(1), 1–11. https://doi.org/10.1038/s41467-018-06516-3

Chowdhury, G. M. I., Zhang, J., Thomas, M., Banasr, M., Ma, X., Pittman, B., Bristow, L., Schaeffer, E., Duman, R. S., Rothman, D. L., Behar, K. L., & Sanacora, G. (2016). Transiently increased glutamate cycling in rat PFC is associated with rapid onset of antidepressant-like

effects. *Molecular Psychiatry*, *February*, 1–7. https://doi.org/10.1038/mp.2016.34

Cipriani, A., Zhou, X., Del Giovane, C., Hetrick, S. E., Qin, B., Whittington, C., Coghill, D., Zhang, Y., Hazell, P., Leucht, S., Cuijpers, P., Pu, J., Cohen, D., Ravindran, A. V., Liu, Y., Michael, K. D., Yang, L., Liu, L., & Xie, P. (2016). Comparative efficacy and tolerability of antidepressants for major depressive disorder in children and adolescents: a network meta-analysis. *The Lancet*, *388*(10047), 881–890. https://doi.org/10.1016/S0140-6736(16)30385-3

Consoli, D., Fedotova, J., Micale, V., Sapronov, N. S., & Drago, F. (2005). Stressors affect the response of male and female rats to clomipramine in a model of behavioral despair (forced swim test). *European Journal of Pharmacology*, *520*(1–3), 100–107. https://doi.org/10.1016/j.ejphar.2005.08.012

De Kloet, E. R., Joëls, M., & Holsboer, F. (2005). Stress and the brain: From adaptation to disease. *Nature Reviews Neuroscience*, *6*(6), 463–475. https://doi.org/10.1038/nrn1683

Diamond, D M, & Rose, G. M. (1994). Stress impairs LTP and hippocampal-dependent memory. *Annals of the New York Academy of Sciences*, *746*, 411–414. https://doi.org/10.1111/j.1749-6632.1994.tb39271.x

Diamond, David M., Bennett, M. C., Fleshner, M., & Rose, G. M. (1992). Inverted-U relationship between the level of peripheral corticosterone and the magnitude of hippocampal primed burst potentiation. *Hippocampus*, *2*(4), 421–430. https://doi.org/10.1002/hipo.450020409

Dias-ferreira, E., Sousa, J. C., Melo, I., Morgado, P., Mesquita, A. R., Cerqueira, J. J., Costa, R. M., & Sousa, N. (2009). Chronic Stress Causes Frontostriatal Reorganization and Affects Decision-Making. *Science*, *325*(July), 621–625.

Dunkley, P. R., Bobrovskaya, L., Graham, M. E., Von Nagy-Felsobuki, E. I., & Dickson, P. W. (2004). Tyrosine hydroxylase phosphorylation: Regulation and consequences. *Journal of Neurochemistry*, *91*(5), 1025–1043. https://doi.org/10.1111/j.1471-4159.2004.02797.x

Elmer, G. I., Tapocik, J. D., Mayo, C. L., Zanos, P., & Gould, T. D. (2020). Ketamine metabolite (2R,6R)-hydroxynorketamine reverses behavioral despair produced by adolescent trauma. *Pharmacology, Biochemistry, and Behavior*, *196*(June), 172973. https://doi.org/10.1016/j.pbb.2020.172973

Enrico, P., Bouma, M., De Vries, J. B., & Westerink, B. H. C. (1998). The role of afferents to the ventral tegmental area in the handling stress-induced increase in the release of dopamine in the medial prefrontal cortex: A dual-probe microdialysis study in the rat brain. *Brain Research*, *779*(1–2), 205–213. https://doi.org/10.1016/S0006-8993(97)01132-3

The final article is available, upon publication, at: Lamanna, J., Isotti, F., Ferro, M., Racchetti, G., Anchora, L., Rucco, D., & Malgaroli, A. (2021). Facilitation of dopamine-dependent long-term potentiation in the medial prefrontal cortex of male rats follows the behavioral effects of stress. *Journal of Neuroscience Research, 99*(2), 662–678. https://doi.org/10.1002/jnr.24732

Fenster, R. J., Lebois, L. A. M., Ressler, K. J., & Suh, J. (2018). Brain circuit dysfunction in post-traumatic stress disorder: from mouse to man. *Nature Reviews Neuroscience*, *19*(9), 535–551. https://doi.org/10.1038/s41583-018-0039-7

Ferro, M., Lamanna, J., Ripamonti, M., Racchetti, G., Arena, A., Spadini, S., Montesano, G., Cortese, R., Zimarino, V., & Malgaroli, A. (2017). Functional mapping of brain synapses by the enriching activity-marker SynaptoZip. *Nature Communications*, *8*(1). https://doi.org/10.1038/s41467-017-01335-4

Goldwater, D. S., Pavlides, C., Hunter, R. G., Bloss, E. B., Hof, P. R., McEwen, B. S., & Morrison, J. H. (2009). Structural and functional alterations to rat medial prefrontal cortex following chronic restraint stress and recovery. *Neuroscience*, *164*(2), 798–808. https://doi.org/10.1016/j.neuroscience.2009.08.053

Grace, A. A. (2016). Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nature Publishing Group*, *17*(8), 524532. https://doi.org/10.1038/nrn.2016.57

Holly, E. N., & Miczek, K. A. (2016). Ventral tegmental area dopamine revisited: Effects of acute and repeated stress. *Psychopharmacology*, *233*(2), 163–186. https://doi.org/10.1007/s00213-015-4151-3

Holmes, A., & Wellman, C. L. (2009). Stress-induced prefrontal reorganization and executive dysfunction in rodents. *Neuroscience and Biobehavioral Reviews*, *33*(6), 773–783. https://doi.org/10.1016/j.neubiorev.2008.11.005

Huang, Y.-Y., Simpson, E., Kellendonk, C., & Kandel, E. R. (2004). Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(9), 3236–3241. https://doi.org/10.1073/pnas.0308280101

Jahng, J. W., Ryu, V., Yoo, S. B., Noh, S. J., Kim, J. Y., & Lee, J. H. (2010). Mesolimbic dopaminergic activity responding to acute stress is blunted in adolescent rats that experienced neonatal maternal separation. *Neuroscience*, *171*(1), 144–152. https://doi.org/10.1016/j.neuroscience.2010.08.063

Joëls, M., & Baram, T. Z. (2009). *The neuro-symphony of stress*. *10*(juNE), 459–466.

Kim, S., Rush, B. S., & Rice, T. R. (2020). A systematic review of therapeutic ketamine use in children and adolescents with treatment-resistant mood disorders. *European Child and Adolescent Psychiatry*, *0123456789*. https://doi.org/10.1007/s00787-020-01542-3

Kokkinou, M., Ashok, A. H., & Howes, O. D. (2018). The effects of ketamine on dopaminergic

The final article is available, upon publication, at: Lamanna, J., Isotti, F., Ferro, M., Racchetti, G., Anchora, L., Rucco, D., & Malgaroli, A. (2021). Facilitation of dopamine-dependent long-term potentiation in the medial prefrontal cortex of male rats follows the behavioral effects of stress. *Journal of Neuroscience Research, 99*(2), 662–678. https://doi.org/10.1002/jnr.24732

function: Meta-Analysis and review of the implications for neuropsychiatric disorders. *Molecular Psychiatry*, *23*(1), 59–69. https://doi.org/10.1038/mp.2017.190

Kouneiher, F., Charron, S., & Koechlin, E. (2009). Motivation and cognitive control in the human prefrontal cortex. *Nature Neuroscience*, *12*(7), 939–945. https://doi.org/10.1038/nn.2321

Kreitzer, A. C., & Malenka, R. C. (2008). Striatal Plasticity and Basal Ganglia Circuit Function. *Neuron*, *60*(4), 543–554. https://doi.org/10.1016/j.neuron.2008.11.005

Lamanna, J., Sulpizio, S., Ferro, M., Martoni, R., Abutalebi, J., & Malgaroli, A. (2019). Behavioral assessment of activity-based-anorexia: how cognition can become the drive wheel. *Physiology and Behavior*, *202*(November 2018), 1–7. https://doi.org/10.1016/j.physbeh.2019.01.016

Lamontagne, S. J., Melendez, S. I., & Olmstead, M. C. (2018). Investigating dopamine and glucocorticoid systems as underlying mechanisms of anhedonia. *Psychopharmacology*, *235*(11), 3103–3113. https://doi.org/10.1007/s00213-018-5007-4

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., Li, X. Y., Aghajanian, G., & Duman, R. S. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological Psychiatry*, *69*(8), 754–761. https://doi.org/10.1016/j.biopsych.2010.12.015

Lorrain, D. S., Baccei, C. S., Bristow, L. J., Anderson, J. J., & Varney, M. a. (2003). Effects of ketamine and N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: Modulation by a group II selective metabotropic glutamate receptor agonist LY379268. *Neuroscience*, *117*(3), 697–706. https://doi.org/10.1016/S0306-4522(02)00652-8

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Montesano, G., Belfiore, M., Ripamonti, M., Arena, A., Lamanna, J., Ferro, M., Zimarino, V., Ambrosi, A., & Malgaroli, A. (2015). Effects of the Concomitant Activation of ON and OFF Retinal Ganglion Cells on the Visual Thalamus: Evidence for an Enhanced Recruitment of GABAergic Cells. *Frontiers in Neural Circuits*, *9*(November), 1–19. https://doi.org/10.3389/fncir.2015.00077

Moriya, S., Yamashita, A., Kawashima, S., Nishi, R., Yamanaka, A., & Kuwaki, T. (2018). Acute Aversive Stimuli Rapidly Increase the Activity of Ventral Tegmental Area Dopamine Neurons in Awake Mice. *Neuroscience*, *386*, 16–23. https://doi.org/10.1016/j.neuroscience.2018.06.027

Musazzi, L., Milanese, M., Farisello, P., Zappettini, S., Tardito, D., Barbiero, V. S., Bonifacino, T.,

The final article is available, upon publication, at: Lamanna, J., Isotti, F., Ferro, M., Racchetti, G., Anchora, L., Rucco, D., & Malgaroli, A. (2021). Facilitation of dopamine-dependent long-term potentiation in the medial prefrontal cortex of male rats follows the behavioral effects of stress. *Journal of Neuroscience Research, 99*(2), 662–678. https://doi.org/10.1002/jnr.24732

Mallei, A., Baldelli, P., Racagni, G., Raiteri, M., Benfenati, F., Bonanno, G., & Popoli, M. (2010). Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants. *PloS One*, *5*(1), e8566. https://doi.org/10.1371/journal.pone.0008566

Naughton, M., Clarke, G., Oleary, O. F., Cryan, J. F., & Dinan, T. G. (2014). A review of ketamine in affective disorders: Current evidence of clinical efficacy, limitations of use and pre-clinical evidence on proposed mechanisms of action. *Journal of Affective Disorders*, *156*, 24–35. https://doi.org/10.1016/j.jad.2013.11.014

Olney, J. J., Warlow, S. M., Naffziger, E. E., & Berridge, K. C. (2018). Current perspectives on incentive salience and applications to clinical disorders. *Current Opinion in Behavioral Sciences*, *22*(Mdd), 59–69. https://doi.org/10.1016/j.cobeha.2018.01.007

Ong, L. K., Guan, L., Damanhuri, H., Goodchild, A. K., Bobrovskaya, L., Dickson, P. W., & Dunkley, P. R. (2014). Neurobiological consequences of acute footshock stress: Effects on tyrosine hydroxylase phosphorylation and activation in the rat brain and adrenal medulla. *Journal of Neurochemistry*, *128*(4), 547–560. https://doi.org/10.1111/jnc.12482

Otani, S., Daniel, H., Roisin, M.-P., & Crepel, F. (2003). Dopaminergic Modulation of Long-term Synaptic Plasticity in Rat Prefrontal Neurons. *Cerebral Cortex*, *13*(11), 1251–1256. https://doi.org/10.1093/cercor/bhg092

Otani, Satoru, Bai, J., & Blot, K. (2015). Dopaminergic modulation of synaptic plasticity in rat prefrontal neurons. *Neuroscience Bulletin*, *31*(2), 183–190. https://doi.org/10.1007/s12264-014-1507-3

Pan, W. H. T., Yang, S. Y., & Lin, S. K. (2004). Neurochemical interaction between dopaminergic and noradrenergic neurons in the medial prefrontal cortex. *Synapse*, *53*(1), 44–52. https://doi.org/10.1002/syn.20034

Parise, E. M., Alcantara, L. F., Warren, B. L., Wright, K. N., Hadad, R., Sial, O. K., Kroeck, K. G., Iñiguez, S. D., & Bolaños-Guzmán, C. A. (2013). Repeated ketamine exposure induces an enduring resilient phenotype in adolescent and adult rats. *Biological Psychiatry*, *74*(10), 750–759. https://doi.org/10.1016/j.biopsych.2013.04.027

Popoli, M., Yan, Z., McEwen, B. S., & Sanacora, G. (2012). The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nature Reviews. Neuroscience*, *13*(1), 22–37. https://doi.org/10.1038/nrn3138

Porsolt, R. D. ., Le Pichon, M., & Jalfre, M. (1977). Depression: a new animal model sensitive to

antidepressant treatments. *Nature*, *266*(April), 3082–3086.

Richter-Levin, G., & Maroun, M. (2010). Stress and amygdala suppression of metaplasticity in the medial prefrontal cortex. *Cerebral Cortex*, *20*(10), 2433–2441. https://doi.org/10.1093/cercor/bhp311

Rocher, C., Spedding, M., Munoz, C., & Jay, T. M. (2004). Acute Stress-induced Changes in Hippocampal/Prefrontal Circuits in Rats: Effects of Antidepressants. *Cerebral Cortex*, *14*(2), 224–229. https://doi.org/10.1093/cercor/bhg122

Russo, S., & Nestler, E. (2013). The brain reward circuitry in mood disorders. *Nature Reviews Neuroscience*, *14*(September), 609–625. https://doi.org/10.1038/nrn3381

Salvatore, M. F., Pruett, B. S., Dempsey, C., & Fields, V. (2012). Comprehensive profiling of dopamine regulation in substantia nigra and ventral tegmental area. *Journal of Visualized Experiments*. https://doi.org/10.3791/4171

Selemon, L. D. (2013). A role for synaptic plasticity in the adolescent development of executive function. *Translational Psychiatry*, *3*(January). https://doi.org/10.1038/tp.2013.7

Shafiei, N., Gray, M., Viau, V., & Floresco, S. B. (2012). Acute stress induces selective alterations in cost/benefit decision-making. *Neuropsychopharmacology*, *37*(10), 2194–2209. https://doi.org/10.1038/npp.2012.69

Shakesby, A. C., Anwyl, R., & Rowan, M. J. (2002). Overcoming the Effects of Stress on Synaptic Plasticity in the Intact Hippocampus: Rapid Actions of Serotonergic and Antidepressant Agents. *Journal of Neuroscience*, *22*(9), 3638–3644. https://doi.org/10.1523/jneurosci.22-09-03638.2002

Shoji, H., & Mizoguchi, K. (2010). Acute and repeated stress differentially regulates behavioral, endocrine, neural parameters relevant to emotional and stress response in young and aged rats. *Behavioural Brain Research*, *211*(2), 169–177. https://doi.org/10.1016/j.bbr.2010.03.025

Sofuoglu, M., Rosenheck, R., & Petrakis, I. (2014). Pharmacological treatment of comorbid PTSD and substance use disorder: Recent progress. *Addictive Behaviors*, *39*(2), 428–433. https://doi.org/10.1016/j.addbeh.2013.08.014

Suvrathan, A., Tomar, A., & Chattarji, S. (2010). Effects of chronic and acute stress on rat behaviour in the forced-swim test. *Stress*, *13*(6), 533–540. https://doi.org/10.3109/10253890.2010.489978

Vaessen, T., Hernaus, D., Myin-Germeys, I., & van Amelsvoort, T. (2015). The dopaminergic response to acute stress in health and psychopathology: A systematic review. *Neuroscience and*

Biobehavioral Reviews, *56*, 241–251. https://doi.org/10.1016/j.neubiorev.2015.07.008

Valenti, O., Gill, K. M., & Grace, A. A. (2012). Different stressors produce excitation or inhibition of mesolimbic dopamine neuron activity: Response alteration by stress pre-exposure. *European Journal of Neuroscience*, *35*(8), 1312–1321. https://doi.org/10.1111/j.1460-9568.2012.08038.x

Wilber, A. A., Walker, A. G., Southwood, C. J., Farrell, M. R., Lin, G. L., Rebec, G. V., & Wellman, C. L. (2011). Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction. *Neuroscience*, *174*, 115–131. https://doi.org/10.1016/j.neuroscience.2010.10.070

Willner, P. (2017). The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiology of Stress*, *6*, 78–93. https://doi.org/10.1016/j.ynstr.2016.08.002

Yan, Z., Mcewen, B. S., Karatsoreos, I. N., Yuen, E. Y., & Eunice Y. Yuen, Wenhua Liu, Ilia N. Karatsoreos, Yong Ren, Jian Feng, Bruce S. McEwen, and Z. Y. (2011). Mechanisms for Acute Stress-Induced Enhancement of Glutamatergic Transmission and Working Memory. *Molecular Psychiatry*, *16*(2), 156–170. https://doi.org/10.1038/mp.2010.50.Mechanisms

Yao, N., Skiteva, O., Zhang, X., Svenningsson, P., & Chergui, K. (2018). Ketamine and its metabolite (2R,6R)-hydroxynorketamine induce lasting alterations in glutamatergic synaptic plasticity in the mesolimbic circuit. *Molecular Psychiatry*, *23*(10), 2066–2077. https://doi.org/10.1038/mp.2017.239

Yuen, E. Y., Liu, W., Karatsoreos, I. N., Feng, J., MCEWEN, B. S., & Yan, Z. (2009). Acute stress enhances glutamatergic transmission in prefrontal cortex and facilitates working memory. *Proceedings of the National Academy of Sciences*, *106*(33), 14075–14079. https://doi.org/10.1073/pnas.0906791106

Yuen, E. Y., Wei, J., Liu, W., Zhong, P., Li, X., & Yan, Z. (2012). Repeated Stress Causes Cognitive Impairment by Suppressing Glutamate Receptor Expression and Function in Prefrontal Cortex. *Neuron*, *73*(5), 962–977. https://doi.org/10.1016/j.neuron.2011.12.033.Repeated

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. S., Fang, Y., Huang, X.-P., Mayo, C. L., Wainer, I. W., Albuquerque, E. X., Thompson, S. M., Thomas, C. J., Zarate, C. A., & Gould, T. D. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, 1–18. https://doi.org/10.1038/nature17998

Zarate, C. A., Singh, J., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., Charney, D. S., & Manji, H. (2006). A Randomized Trial of an N-methyl-D-aspartate Antagonist in

Treatment-Resistant Major Depression. *Archives of General Psychiatry*, *63*(8), 856–864. https://doi.org/10.1001/archpsyc.63.8.856

The final article is available, upon publication, at: Lamanna, J., Isotti, F., Ferro, M., Racchetti, G., Anchora, L., Rucco, D., & Malgaroli, A. (2021). Facilitation of dopamine-dependent long-term potentiation in the medial prefrontal cortex of male rats follows the behavioral effects of stress. *Journal of Neuroscience Research, 99*(2), 662–678. https://doi.org/10.1002/jnr.24732

FIGURE LEGENDS

Figure 1. DA-dependent LTP at prefrontal cortex local circuits is facilitated by stress while LTD is unaffected.

(A) Experimental timeline: elevated platform (stress) or home (control), followed by drugs treatment and then behavioral tests, Open Field Test, Elevated Plus Maze and Forced Swim Test. (B-C) The application of dopamine and HFS failed to produce, in our condition, a stable potentiation of fEPSP in control animals (n = 5), but produced a reliable DA-LTP in stressed animals (n = 6). (D-E) 3 Hz stimulation, simultaneous to a dopamine application, produced a stable DA-LTD both in control and stressed animals (n = 6 for each group). Insets show a typical fEPSP trace, before (blue) and at the end (green) of plasticity protocol (scale: 0.2 mv / 20 ms). Time series shown as mean ± SE.

Figure 2. DMI treatment administered after stress prevents facilitation of DA-dependent LTP while occluding LTD

(A-B) Both KET (10 mg/kg, n = 6) and DMI (20 mg/kg, n = 5) treatments, with different strength, prevented the stress ability to promote DA-LTP. (C-D) DA-LTD remains with stress and KET treatment while DMI reduced the magnitude of the DA-LTD in the presence of stress. Insets show a typical fEPSP trace, before (blue) and at the end (green) of plasticity protocol (scale: 0.2 mv / 20 ms). Time series shown as mean ± SE.

Figure 3. Summary data about DA-dependent synaptic plasticity and drug effects.

Population data showing group differences. (A) DA-LTP was significantly increased by stress and only DMI (20 mg/kg) significantly decreased the DA-LTP enhancement by stress ($F_{3,13}$ = 4.508, p = 0.022; stress *vs* ctrl: p < 0.001; S+DMI *vs* stress: p = 0.025; Dunnett's post-hoc test; n = 5/6 per group). (B) No significant effects of treatment on the modulation of fEPSP amplitude by either DA application alone or tetanic stimulation were detected, suggesting a similar degree of sensibility to DA and excitability. (C) EP stress was not able to modulate LTD with or without KET treatment (10 mg/kg), while DMI significantly occluded LTD when administered after EP stress ($F_{3,15}$ = 4.614, p = 0.018; stress *vs* ctrl: n.s.; S+DMI *vs* stress: p = 0.015; Dunnett's post-hoc test; n = 6 for each group). (D) No significant effects of treatment on the modulation of fEPSP amplitude by either DA

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application or 3 Hz stimulation were observed. Error bars show mean ± SD, squares are values from individual animals (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 4. Stress reduces anxiety-like behaviors in the EPM, an effect occluded by ketamine.

(A) Schematic of the EPM adopted, with heatmaps of the locomotor activity averaged over groups. (B) On the 'pretest' at day 0, no group differences were appreciable in rats entrances in open arms. (C) On the test day, entrance in open arms was significantly increased by stress and this effect seems to be abolished when EP is followed by KET (10 mg/kg) treatment (treatment overall effect not significant: $F_{3,15} = 2.476$, $p = 0.101$; stress *vs* ctrl: $p = 0.041$; S+KET *vs* stress: $p = 0.029$; Dunnett's post-hoc test). (D-E) No significant effect of treatment was detected on either the time spent by rats in open arms (D) or closed arms (E). Error bars show mean ± SD, squares are values from individual animals (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure 5. Stress reduces depressive-like behavior in the FST, an effect which is not affected by DMI and KET.

(A) An example of the cylinder used for the FST and averaged heatmaps of rats activity, showing a lower zone of maximal activity in control group, pointing the increased climbing activity promoted by stress both alone and with KET (10 mg/kg) and DMI (20 mg/kg) treatments. (B) Differences in FST pretest were not significant, showing a similar pattern of behavior between groups; (C) on the contrary, after the delivery of EP stress, with or without following KET/DMI treatment, rats were more likely to produce a highly active behavior ($F_{3,12} = 4.650$, $p < 0.001$; stress *vs* ctrl: $p < 0.001$; Dunnett's post-hoc test; n = 5/6 per group) and (D) less prone to immobility compared to controls ($F_{3,12} = 9.215$, $p = 0.002$; stress *vs* ctrl: $p = 0.003$; Dunnett's post-hoc test; no other significant comparison; n = 5/6 per group). (E) The moderately active behavior, *i.e.* when rats are not struggling or floating inactively, was increased by the stress episode only when KET treatment was administered (no significant overall effect: $F_{3,12} = 3.089$, n.s.; S+KET *vs* stress: $p < 0.038$; Dunnett's post-hoc test; n = 5/6 per group). Error bars show mean ± SD, squares are values from individual animals (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

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Figure 6. Stress reduces cFOS expression while ketamine promotes TH phosphorylation in the VTA.

(A) Exemplary western blots showing cFOS expression in VTA and SN, for each group (arrows indicate the quantified bands). (B) Exemplary dot blots for pTH, TH and the loading control vinculin in VTA and SN; (C-D) Normalized cFOS expression is significatively decreased by stress in VTA (C) but not in SN (D), and neither KET (10 mg/kg) nor DMI (20 mg/kg) treatment prevented this effect ($F_{3,12}$ = 5.826, p = 0.011; stress *vs* ctrl: p = 0.011; Dunnett's post-hoc test; no other significant comparison; n = 4/5 per group). (E-F) pTH/TH was increased by KET in the presence of stress in VTA (E), but remained unchanged in SN (F; overall treatment effect not significant: $F_{3,12}$ = 1.701, n.s.; S+KET *vs* stress: p = 0.038; Dunnett's post-hoc test; n = 5 per group); (G-H) level of TH (normalized by vinculin) was decreased by KET treatment in the presence of stress in VTA (G) while remained unchanged in SN (H; $F_{3,12}$ = 2.216, n.s.; S+KET *vs* stress: p = 0.011; Dunnett's post-hoc test; n = 5 per group). Error bars show mean ± SD, squares are values from individual animals (* p < 0.05; ** p < 0.01; *** p < 0.001).

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FIGURES

Figure 1

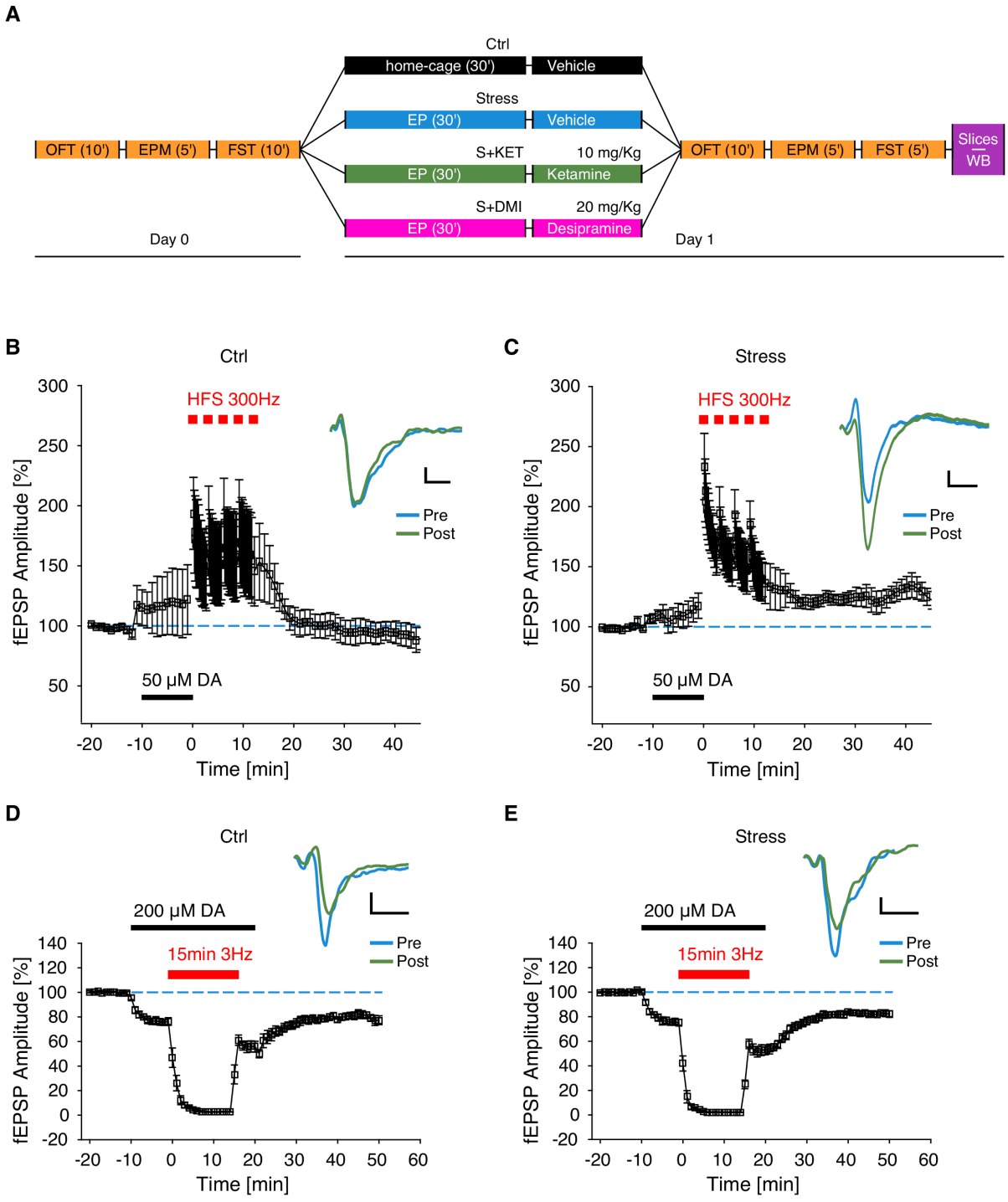

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Figure 2

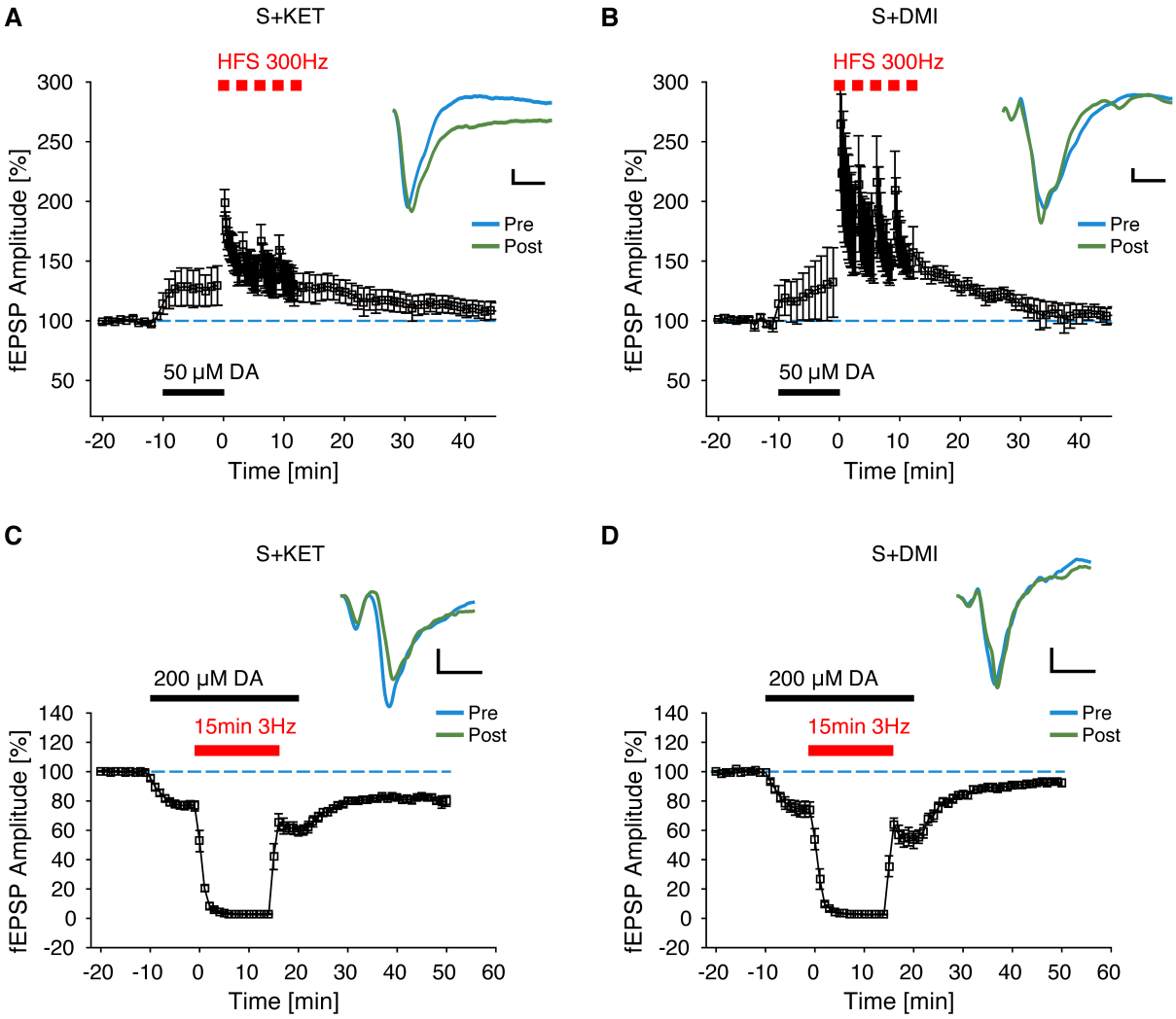

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Figure 3

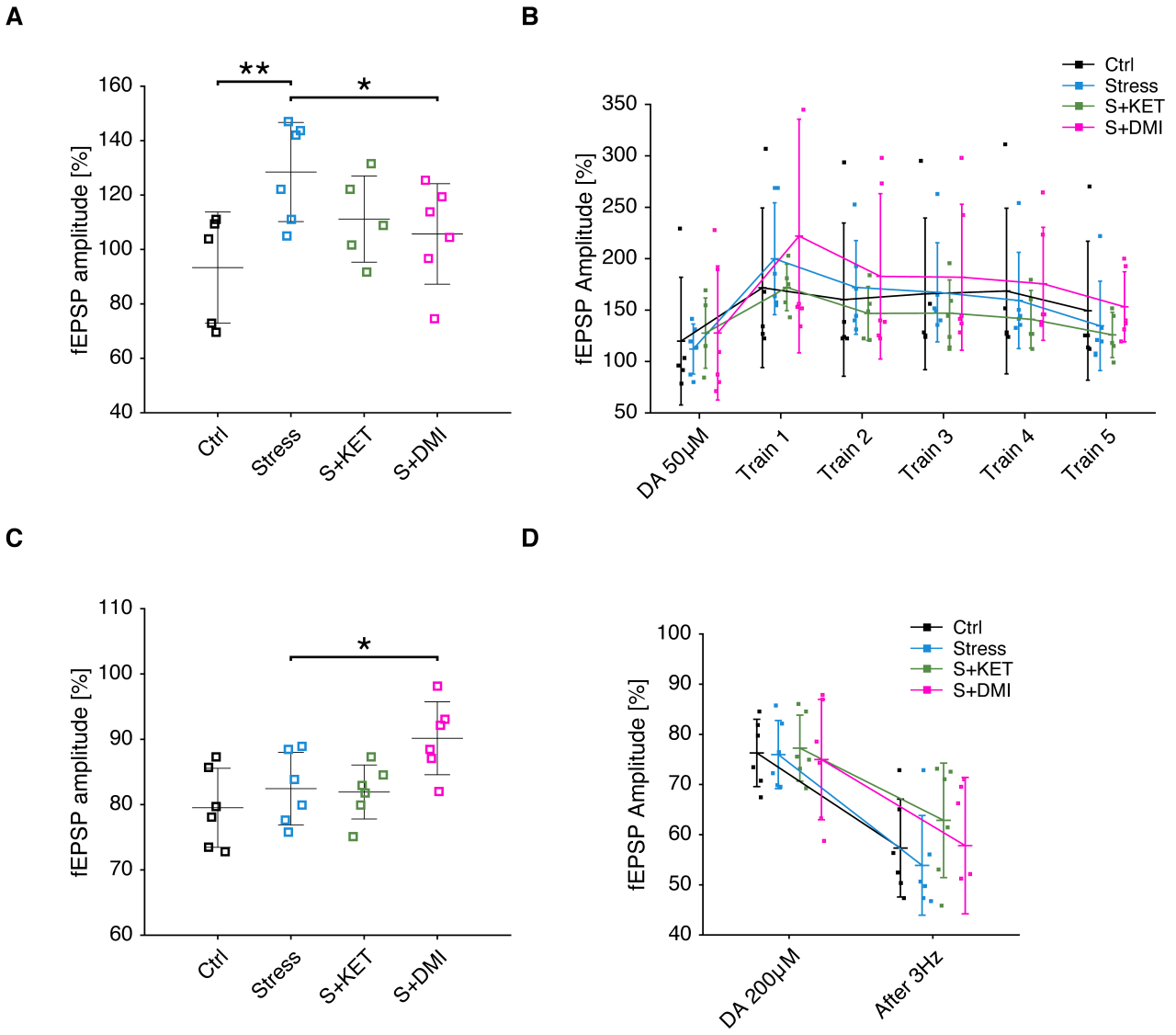

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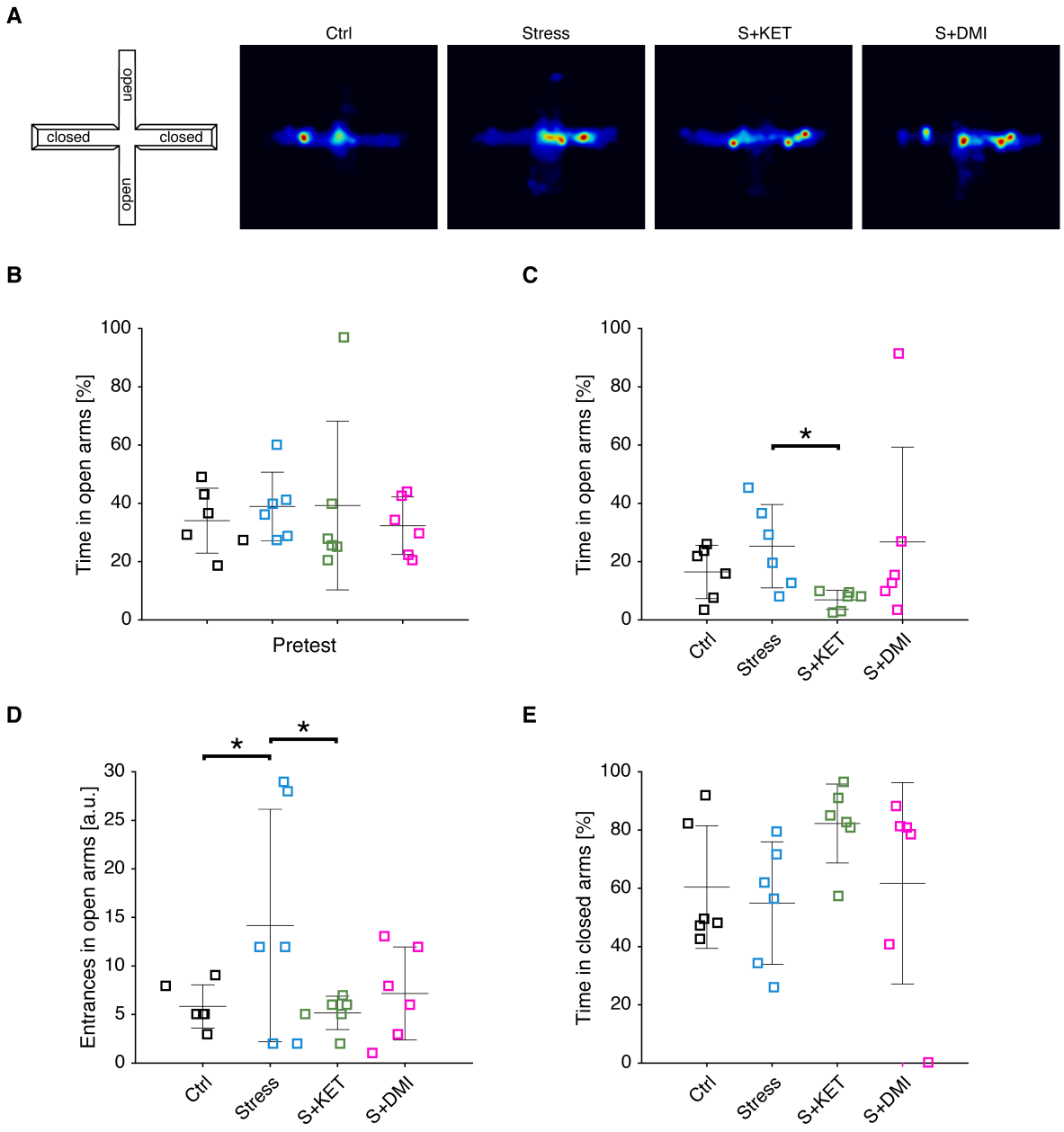
Figure 4



The final article is available, upon publication, at: Lamanna, J., Isotti, F., Ferro, M., Racchetti, G., Anchora, L., Rucco, D., & Malgaroli, A. (2021). Facilitation of dopamine-dependent long-term potentiation in the medial prefrontal cortex of male rats follows the behavioral effects of stress. *Journal of Neuroscience Research, 99*(2), 662–678. https://doi.org/10.1002/jnr.24732

Figure 5

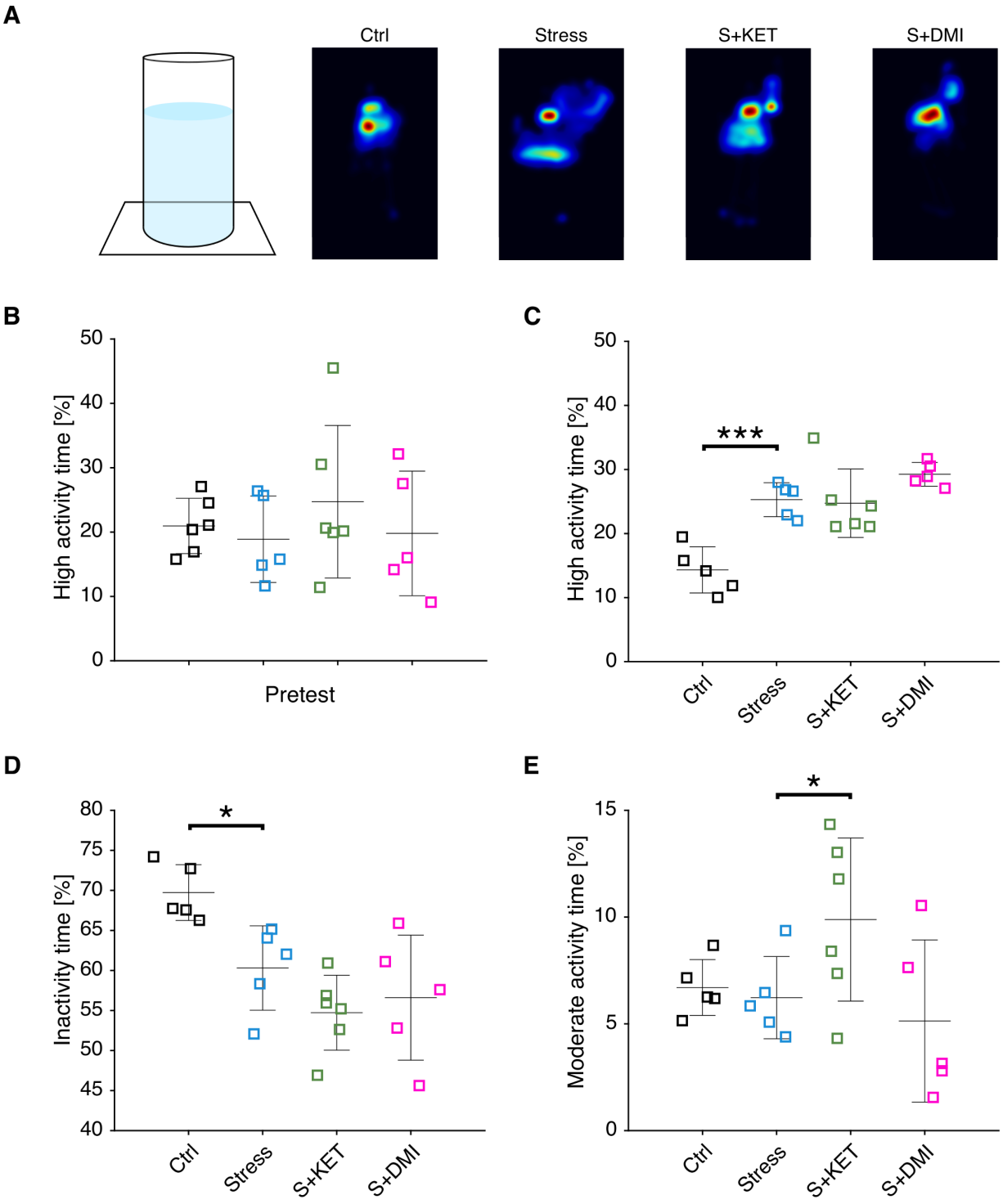


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Figure 6