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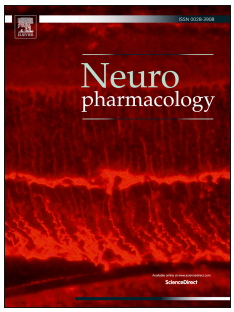

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AUTHORS CONTRIBUTIONS

T.C., L.B. and H.F. performed viral injections, behavioural experiments and histology. T.C. performed microscopy. P.V. provided intellectual and technical inputs. S.P.F. and J.B. designed the project. T.C. analysed data. S.P.F. and J.B. wrote the manuscript. All authors provided feedback on writing the manuscript.

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Thomas Contesse [1, 2], Loïc Broussot [1, 2], Hugo Fofo [1, 2], Peter Vanhoutte [3, 4, 5], Sebastian P. Fernandez [1, 2]* and Jacques Barik [1, 2]*.

1 Université Côte d'Azur, Nice, France.

2 Institut de Pharmacologie Moléculaire & Cellulaire, CNRS UMR7275, Valbonne, France.

3 CNRS, UMR 8246, Neuroscience Paris Seine, F-75005, Paris, France

4 INSERM, UMR-S 1130, Neuroscience Paris Seine, Institute of Biology Paris Seine, F-75005, Paris, France

5 Sorbonne Université, UPMC Université Paris 06, UM CR18, Neuroscience Paris Seine, F-75005, Paris, France

* These authors contributed equally to this work

Correspondence should be addressed to J.B. (barik@ipmc.cnrs.fr) and S.P.F. (fernandez@ipmc.cnrs.fr)

ABSTRACT

Stress has been acknowledged as one of the main risk factors for the onset of psychiatric disorders. Social stress is the most common type of stressor encountered in our daily lives. Uncovering the molecular determinants of the effect of stress on the brain would help understanding the complex maladaptations that contribute to pathological stress-related mental states. We examined molecular changes in the reward system following social defeat stress in mice, as increasing evidence implicates this system in sensing stressful stimuli. Following acute or chronic social defeat stress, the activation (i.e. phosphorylation) of extracellular signal-regulated kinases ERK1 and ERK2 (pERK1/2), markers of synaptic plasticity, was monitored in sub-regions of the reward system. We employed pharmacological antagonists and inhibitory DREADD to dissect the sequence of events controlling pERK1/2 dynamics. The nucleus accumbens (NAc) showed marked increases in pERK1/2 following both acute and chronic social stress compared to the dorsal striatum. Increases in pERK1/2 required dopamine D1 receptors and GluN2B-containing NMDA receptors. Paraventricular thalamic glutamatergic inputs to the NAc are required for social stress-induced pERK1/2. The molecular adaptations identified here could contribute to the long-lasting impact of stress on the brain and may be targeted to counteract stress-related psychopathologies.

Keywords: social stress, striatum, ERK1/2, D1 dopamine receptor, NMDA glutamate receptor, paraventricular thalamus.

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5 Sorbonne Université, UPMC Université Paris 06, UM CR18, Neuroscience Paris Seine, F-75005, Paris, France

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Stress has been acknowledged as one of the main risk factors for the onset of psychiatric disorders. Social stress is the most common type of stressor encountered in our daily lives. Uncovering the molecular determinants of the effect of stress on the brain would help understanding the complex maladaptations that contribute to pathological stress-related mental states. We examined molecular changes in the reward system following social defeat stress in mice, as increasing evidence implicates this system in sensing stressful stimuli. Following acute or chronic social defeat stress, the activation (i.e. phosphorylation) of extracellular signal-regulated kinases ERK1 and ERK2 (pERK1/2), markers of synaptic plasticity, was monitored in sub-regions of the reward system. We employed pharmacological antagonists and inhibitory DREADD to dissect the sequence of events controlling pERK1/2 dynamics. The nucleus accumbens (NAc) showed marked increases in pERK1/2 following both acute and chronic social stress compared to the dorsal striatum. Increases in pERK1/2 required dopamine D1 receptors and GluN2B-containing NMDA receptors. Paraventricular thalamic glutamatergic inputs to the NAc are required for social stress-induced pERK1/2. The molecular adaptations identified here could contribute to the long-lasting impact of stress on the brain and may be targeted to counteract stress-related psychopathologies.

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1. INTRODUCTION

The reward system has been remarkedly conserved amongst species. It promotes adaptive behaviours and therefore increases the likelihood of survival in complex environments. These responses rely on neural processes, which shape associative learning and motivated behaviours, to seek natural rewards such as food, sex and social rewards (Bromberg-Martin et al., 2010; Schultz, 2015). Within the reward system, midbrain dopamine (DA) neurons and the release of DA in targeted brain areas has by far received the most attention in the modulation of rewarding and motivational processes (Bromberg-Martin et al., 2010). Yet, accumulating evidence indicate that the activity of the reward system is sensitive to a large variety of stressors including aversive physical and psychological stimuli (Ungless et al., 2004; Marinelli and McCutcheon, 2014). Hence, DA neurons can react to both rewarding and aversive stimuli. Social cues are abundant in our daily lives and key to establish social and territorial relationships, as well as social hierarchies in order to limit fighting for resources (Sapolsky, 2005). Thus, social stress is the most frequent type of stressor experienced in our modern society. Social stress can initiate cellular maladaptations that can culminate in stress-related mental disorders such as depression (Russo and Nestler, 2013). Indeed, we and others demonstrated that chronic social stress triggers profound dysregulations of the activity of DA neurons from the ventral tegmental area (VTA) that project to the ventral striatum (nucleus accumbens, NAc) leading to the appearance of social withdrawal and anhedonia (Barik et al., 2013; Chaudhury et al., 2013). A single social defeat episode or exposure to a social threat, in previously defeated animals, trigger a transient but significant increase of DA in the NAc (Tidey and Miczek, 1996; Barik et al., 2013).

Released DA from midbrain DA neurons filters excitatory glutamatergic inputs converging onto the striatum (Bamford et al., 2004b). Recent findings indicate that social stress also affect glutamate (Glu) transmission at striatal excitatory synapses, which is associated with

social withdrawal (Christoffel et al., 2015). Hence, the NAc constitutes a key downstream interface that computes sensory, environmental contingencies and internal drives for both rewarding and aversive stimuli. Yet the molecular determinants of social stress outcomes are not fully understood. Key for the integration of rewarding signals are the extracellular signal-regulated kinases (ERK) 1 and 2 (ERK1/2) in medium-sized spiny neurons (MSNs), the most abundant (~95 %) striatal cell type (Pascoli et al., 2014). Phosphorylation on amino acids Threonine 202 and Tyrosine 204 promotes ERK1/2 activation and trigger dynamic changes of their subcellular localization (Berti and Seger, 2017). Phosphorylated ERK1/2 (pERK1/2) rapidly translocate to the nucleus and launch chromatin remodelling, gene transcription as well as long-lasting structural and synaptic plasticity and behavioural alterations (Girault et al., 2007; Salery et al., 2020). Hence, ERK1/2 is key to translate changes from molecular adaptations into deeply engrained altered behaviours. ERK1/2 dynamics have been intensively examined for many addictive rewards, but its modulation by social stress is poorly understood. Here, we combined pharmacological and pharmacogenetic approaches to dissect the impact of social stress on striatal pERK1/2. We show that acute and chronic stress differently shape pERK1/2 induction. Also, we demonstrate that stimulation of dopamine D1 receptors as well as different NMDA receptor subtypes are required for social stress-induced pERK1/2. Last, we found that silencing paraventricular thalamus glutamatergic inputs to NAc MSNs is sufficient to dampen stress outcomes. Overall, the molecular adaptations identified here could contribute to the long-lasting impact of stress on the brain and may have relevance for stress-related disorders.

2. MATERIAL AND METHODS

2.1 Animals

All procedures were in accordance with the recommendations of the European Commission (2010/63/EU) for care and use of laboratory animals and approved by the French National and Local Ethical Committees (*Comité Institutionnel d'Éthique Pour l'Animal de Laboratoire - AZUR*). 8-week-old Male C57Bl6J (25-30g, Janvier Labs France), and CD1 retired breeders (30-35g, Janvier Labs, France) were used. All the experiments were performed in accordance to the ARRIVE guidelines. Mice were kept on ventilated racks (cage size in cm: 30Lx 20l x 12H) on a 12/12 dark-light cycle (lights on at 8am). Mice had access to water and food ad libitum. Wood sticks, cotton and igloo were provided for environmental enrichment.

2.2 Drugs

Clozapine N-Oxyde (CNO) was purchased from Enzo Life (France), the GluN2A NMDA antagonist (PEAQX), the GluN2B NMDA antagonist (Ro 25 6981) and the DA D1 antagonist (SCH 23 390) from Tocris Cookson (UK), and Xylazine/Ketamine from Centravet (France). All drugs for *in vivo* administration were diluted in saline (0.9% NaCl) and administered intraperitoneally. Drugs (salt) were injected at the following doses: CNO (1 mg/kg), PEAQX (10 mg/kg), Ro 25 6981 (10 mg/kg), SCH 23 390 (0.1 mg/kg) and Xylazine/Ketamine (10 mg/kg and 150 mg/kg, respectively). Saline was administered at 10 ml/kg.

2.3 Stereotaxic injections

Stereotaxic injections were performed using a stereotaxic frame (Kopf Instruments) under general anesthesia with xylazine / ketamine (10 and 150 mg/kg respectively), as previously performed (Fernandez et al., 2018). Surgeries were performed on 5-week old mice, therefore anatomical coordinates and maps were adjusted from Paxinos (Paxinos and Franklin, 2019).

The injection rate was set at 100 nL/min. For specific manipulation of paraventricular thalamic (PVT) neurons to NAc glutamatergic projections, wild-type mice were injected bilaterally with CAV-2-CRE (Plateforme de Vectorologie de Montpellier; 400 nL/site; 2.5x10e12 pp/mL) in the NAc core (AP: + 1.2 mm, ML: ± 0.65 mm, DV: -4.4 mm from Bregma), and with AAV8-hSyn-DIO-hM4D-mCherry (400 nL; AddGene, USA; 7x10e12 pp/mL) in the PVT (AP: -1.2 mm, ML: 0 mm, DV: - 3.25 mm from Bregma). Animals received carprofen in their drinking for 1 week following surgery. They were then given a 2 weeks' recovery period to allow sufficient viral expression and fully recover from surgery.

2.4 Social defeat stress

The social defeat paradigm in mice was used to induce depressive-like symptoms (Krishnan et al., 2007). It was performed as previously published (Barik et al., 2013). Briefly, wild-type mice were subjected to either a single session of defeat (acute stress group) or a session of defeat repeated every day for 10 consecutive days (chronic stress group). The session(s) of social defeat consisted in 5 min exposition to a former CD1 breeder male mouse. Under chronic stress conditions, a new CD1 was used for the social defeat every day. Directly following the social defeat, mice were housed two per cage separated with a semi-permeable barrier allowing sensory, but not physical, contacts. Control mice (naive group) were not confronted with a dominant male but lived in similar housing conditions, i.e two per cage separated by a semi-permeable barrier. Mapping of pERK1/2-positive cells (see below) was performed on mice susceptible to stress, i.e. exhibiting social aversion (Krishnan et al., 2007). For pharmacological and pharmacogenetic manipulations all drugs (i.e. CNO, PEAQX, Ro 25 6981 and SCH 23 390) were administered 30 min before the social defeat episode. Vehicle (saline) was administered accordingly.

For the acute stress condition, mice were euthanized (see below) either 10 min (acute stress) or twenty four hours (24h post-acute stress) following the single defeat exposure. For the chronic stress condition, mice were stressed for 10 consecutive days. Then, on day 11 mice were either sacrificed (chronic stress group) or received an additional defeat session and sacrificed 10 min after (chronic stress + challenge). All experiments were conducted between 1pm and 4pm of the light cycle.

2.5 Immuno-histofluorescence and pERK1/2 mapping

Mice were deeply anaesthetized with xylazine/ketamine as described above and transcardially perfused with cold phosphate buffer (PB: 0.1 M, Na_2HPO_4/NaH_2PO_4, pH 7.4), followed by 4% paraformaldehyde (PFA) in cold PB 0.1 M. Brains were post-fixed overnight in 4% PFA-PB. Free-floating vibratome striatal coronal sections (30 µm) were obtained, and correct viral injections were confirmed for each animal when appropriate.

To identify pERK1/2-positive neurons, sections were incubated (30 min) in PBS-BT (phosphate-buffered saline (PBS), 0.5% bovine serum albumin (BSA), 0.1% Triton X-100 (T)) with 10% normal goat serum (NGS). Sections were then incubated (4°C) in PBS-BT, 1% NGS, with rabbit anti-pERK1/2 (1/500; Cell signaling #4370S) overnight. Sections were then rinsed (30 min) in PBS at room temperature and incubated (2 h) with goat anti-rabbit Alexa 488 secondary antibody (1:1000, A32733 Invitrogen) in PBS-BT, 1% NGS. Sections were finally rinsed (30 min) with PBS and incubated 5 min with DAPI in PB before mounting with Mowiol at 10%.

Counting of pERK1/2-positive neurons were performed on the following regions of interest: nucleus accumbens core (NAc Core), NAc medial Shell, NAc lateral shell, medial caudate putamen (CPu) and lateral CPu at a defined antero-posterior position (AP: + 1.2 mm). Images were acquired with an Olympus FV10 confocal microscope and a stitching of the images was

done with the microscope-associated software. Viral injection site images were acquired with an epifluorescent microscope (Axioplan2 Carl Zeiss) and stitched with Adobe photoshop CC 2018 Photomerge option. A visual threshold was set by the experimenter and maintained fixed for all images. The counting was manually done using ImageJ by a blind experimenter. The number of cells labelled for pERK1/2 was averaged over two brain slices per mouse.

2.6 Data analyses

Data are presented as means ± SEM and were analyzed using GraphPad Prism 6. Following a D'Agostino-Pearson's test, determining the normality of the distributions, statistical analyses were carried out using one-way (Fig1-2) or two-way analysis of variance (Fig3-5) followed by a post hoc Sidak's or Dunn's multi-comparison test. Statistical significance was set at $P < .05$.

3. RESULTS

3.1 Chronic social stress produces distinct pattern of pERK1/2 induction in striatal and accumbal subregions

Long exposure to social stress produces pronounced deficits in motivation and reward-learning, two functions associated with the striatum. We first evaluated the impact of social stress in mice on pERK1/2 pattern in this region. Considering that the caudate putamen (CPu) and NAc are large areas in which different subregions are largely associated with different anatomo-functional organizations, we focused our analyses on the NAc core, medial and lateral shell, as well as the lateral and medial CPu. Because protracted stress exposure can trigger enduring adaptations, and condition reactivity to future aversive stimuli, stressed mice were stressed for

10 consecutive days and then divided into two groups. On day 11 mice were either sacrificed (chronic stress group) to evaluate lasting effects, or received an additional defeat session and sacrificed 10 min after (chronic stress + challenge) to test for potential exacerbation of pERK1/2 induction (Figure 1a, left panel). These groups were compared to naive mice that were not exposed to dominant males and therefore not socially defeated, and pERK1/2-positive cells were quantified in different subregions of the striatum (Figure 1a, right panel). Representative pictograms show that chronic social stress differently increased the number of pERK1/2-positive neurons in the CPu and NAc (Figure 1b). Specifically, marked and significant increases in pERK1/2-positive neurons were observed after chronic stress in the NAc core and medial shell, but neither in the NAc lateral shell nor in any subdivisions of the CPu (Figure 1c). In the NAc core and medial shell, re-exposure to an acute stress challenge failed to further increase pERK1/2 positive neurons, suggesting a putative ceiling effect by chronic stress. Conversely, the lateral CPu was the only subregion analyzed that responded to chronic stress plus an acute stress challenge, but not to chronic stress per se (Figure 1c). To address the possibility of a ceiling effect, we quantified the fluorescence intensity in each of

the pERK1/2-positive cells included in Figure 1c using. We plotted the data as a frequency distribution, and as mean intensity for cells counted in the three experimental groups (Figure 1d). We found higher intensity in the chronic stress + challenge, suggesting that an extra defeat episode further increases pERK1/2 independently of changes in numbers of pERK1/2-positive cells. This first set of data already demonstrates the complex outcomes of chronic stress exposure on pERK1/2 patterns.

3.2 Acute social stress is sufficient to elicit ERK1/2 phosphorylation.

We next tested whether an acute exposure to social stress was sufficient to elicit pERK1/2 induction. Mice were subjected to an acute social stress and killed immediately after the stress episode, or 24h later to test for potential sustained pERK1/2 induction (Figure 2a). These groups were compared to naive non-stressed mice. As before, representative pictrograms show that acute stress has differential impact on pERK1/2 induction (Figure 2b). Robust and significant increases were only observed in the NAc core and medial shell (Figure 2c). No significant increase in pERK1/2-positive neurons was observed in the other subregions examined (Figure 2c). The ability of an acute episode of social defeat to induce pERK1/2 is transient as when analyses were carried out 24h post-stress exposure, numbers of pERK1/2-positive neurons did not differ from that of naive mice.

3.3 Dopamine D1 receptors are required for acute stress-induced ERK1/2 phosphorylation

So far, our data indicate that the NAc core and medial shell are the most sensitive subregions to social stress exposure. These parts of the NAc receive dense innervation from DA neurons located in the ventral tegmental area. In the case of addictive substances, activation of DA type 1 receptor (D1R) is key to affect intracellular signaling cascades. Hence, we tested

whether a similar mechanism of action could be shared by social stress exposure. Animals were treated with either saline or the DA D1R antagonist SCH23390, and 30min after being subjected to an acute social stress. The dose of SCH23390 was selected based on previous published protocols for *in vivo* antagonism (Valjent et al., 2000). Animals were killed rapidly after the stress (Figure 3a). As previously reported (Valjent et al., 2000), treatment with SCH23390 significantly decreased the number of pERK1/2-positive neurons in naive mice, indicative of a tonic tone of DA release that maintains basal phosphorylation of ERK1/2 (Figure 3b, c). Nevertheless, stress failed to increase numbers of pERK1/2 positive neurons (Figure 3b, c).

3.4 NMDA receptor subtypes mediate ERK1/2 activation after social stress

Released DA filters and selects sets of excitatory glutamatergic inputs to MSNs. Glutamate signaling involves binding to AMPA and NMDA receptors. Because activation of the latter by addictive rewards has been shown to induce strong ERK1/2 activation, we tested whether stress impact on pERK1/2 was mediated by NMDA receptors. Specifically, we used two selective antagonists: PEAQX, a selective antagonist for NMDA receptors containing the GluN2A subunit, and RO256981, a selective antagonist for NMDA receptors containing the GluN2B subunit. The doses of each drug for *in vivo* antagonism were selected based on previous reports (Bortolato et al., 2012; Kiselycznyk et al., 2015). Mice received an injection of saline, PEAQX or RO256981, and 30min after were submitted to an acute social stress and rapidly killed (Figure 4a). In naive conditions, NMDA antagonists did not significantly alter the number of accumbal pERK1/2-positive neurons (Figure 4b and c). However, both RO256981 and PEAQX significantly prevented the increase in pERK1/2-positive neurons promoted by an acute stress (Figure 4c). Overall these results unveiled a specific role of NMDA receptor subtypes in activating accumbal neurons after social stress exposure.

3.5 Thalamic paraventricular inputs mediate stress-induced activation of ERK1/2 in the NAc

The striatum receives multiple glutamatergic inputs from the amygdala, hippocampus, cortex and thalamus to regulate its activity. In particular, the paraventricular thalamus (PVT) has been shown to be sensitive to stressors (Hsu et al., 2014). To evaluate whether the source of glutamate controlling ERK1/2 following social stress arose from the PVT, we next used a pharmacogenetic approach (DREADD system) to specifically silence PVT neurons projecting to the NAc. To achieve projection-specific expression of inhibitory DREADD, mice were injected in the NAc with a canine adenovirus type 2 (CAV-2) with retrograde capacity, to express the Cre recombinase in regions projecting to this structure. We also injected a cre-dependent adeno-associated virus (AAV) in the PVT to express the inhibitory DREADD (hM4D) (Figure 5a). We achieved a transduction efficiency of 353.6 ± 16.2 cells/mm^2 within the PVT. This intersectional strategy allows us to selectively and timely silence PVT to NAc projections. Representative pictograms show the expression of hM4D fused with mCherry in cell bodies of the PVT, and dense presence of innervating axons into the NAc (Figure 5a. Mice were given saline or CNO (1 mg/kg) injections 30 min prior social stress exposure (Figure 5b). We observed that CNO treatment did not modify the number of pERK1/2-positive neurons in naive conditions (Figure 5c and d). However, the increase in pERK1/2-positive neurons by stress was significantly reduced by silencing PVT to NAc projections (Figure 5c and d). This result suggests that paraventricular glutamatergic inputs have a specific role in activating NAc MSNs in response to social stress.

4. DISCUSSION

In this manuscript we described how stress, specifically social stress by conspecifics, differently activates various subregions of the CPu and NAc using pERK1/2 expression as a marker of neuronal plasticity. We show that exposure to chronic social stress produces lasting activation of the NAc core and medial shell, while the lateral shell was not affected. In the CPu, only the lateral part show susceptibility to social stress. Molecular mechanisms of acute stress reactivity in striatal subregions was dissected using pharmacological tools revealing a prominent role for DA D1R and NMDA-GluN2B and NMDA-GluN2A glutamate receptors. Our results suggest that both receptor populations are important mediators of stress, but DA D1R also participate in maintaining basal levels of pERK1/2 expression. Finally, we uncover a glutamatergic thalamic input to the nucleus accumbens, which impinges on striatal cell substrates promoting pERK1/2 expression in response to stress. Overall, our study suggest that the striatum show region-specific reactivity to social stress, mediated by glutamatergic and dopaminergic signalling.

Stress has been acknowledged as one of the main risk factor for the development of psychiatric disorders (de Kloet et al., 2005). Because social interactions govern our everyday lives, the study of social stress and its effects on brain function is of great importance to understand the physiopathology of these disorders (McEwen, 2012). Here, we used a murine model of social stress, based on inter-male aggressivity that manifests most strongly on first encounters. Long term exposure to social stress has been shown to develop lasting behavioural and biochemical abnormalities in mice, including deficits in natural rewards and increased responses to drug reward (Krishnan et al., 2007; Der-Avakian et al., 2014; Morel et al., 2017; Newman et al., 2018). The striatum is important for reward processing and value-based decision-making, and therefore a primary target to understand associations between stress exposure and reward misprocessing. Functionally, the striatum is often divided into subregions thought to participate in different aspects of reward processing. The CPu is

involved in goal-directed behaviours that rely on stimulus-outcome associations, and the formation of habitual actions (Sousa and Almeida, 2012). The NAc is a limbic-motor interface, important for reinforcement and reward-motor learning (Morrison et al., 2017). Our first observation was that these striatal subregions exhibit different stress reactivity. In the NAc, a single social encounter triggered strong but transient activation of the core and medial shell regions, which is likely to initiate plastic changes that will favour maladaptive behaviours. In turn, the CPu showed high cellular resilience to single stress exposure. This agrees with a recent study that showed that pERK1/2 is not induced in the caudal part of the striatum (i.e. tail of the striatum) by a large variety of aversive stimuli that trigger innate avoidance (Gangarossa et al., 2019).

In contrast to an acute social stress episode, chronic exposure to social stress produced a lasting activation of ERK1/2, which has also been reported following western blot analyses on NAc punches in mice (Krishnan et al., 2007). Although not in the striatum, a recent study in depressed humans revealed alterations in pERK1/2 levels in the ventromedial prefrontal cortex (Labonté et al., 2017), highlighting the potential translational value of carefully examining pERK1/2 dynamics. Chronic stress prevented further enhancement of pERK1/2 induction in most striatal subregions examined when a new stress was encountered suggesting that repeated stress exposure could overcome homeostatic mechanisms, leading to sustained activation of cell substrates. The lateral CPu was the only subregion where a significant pERK1/2 induction was observed in chronic stressed mice challenged with a new intruder. This region has been heavily linked with habit formation, therefore this cell activation might be due to the repeated manipulation and the automation of motor responses in defensive behaviours (Sousa and Almeida, 2012).

The ERK1/2 signalling pathway plays a crucial role in regulating diverse neuronal processes in response to external stimuli. In particular, its expression in the striatum has been associated

with the effects of most drugs of abuse including psychostimulants, opiates and nicotine (Valjent et al., 2004; Pascoli et al., 2014). Blocking ERK1/2 phosphorylation or inhibiting its binding to its downstream molecular targets can prevent many behavioural consequences of drugs of abuse including their rewarding and locomotor sensitizing properties as well as incubation of craving (Valjent et al., 2000; Lu et al., 2005; Besnard et al., 2011). In turn, less is known about the involvement of the ERK1/2 cascade on stress adaptations. This is of high relevance for psychiatric disorders since stress exposure is a major risk factor for drug addiction (Sinha, 2008). Indeed, we and others have proposed that stress mal-adaptations may share some molecular and circuits mechanisms with those seen in drug addiction and depression, specifically on brain reward circuits (Russo and Nestler, 2013; Morel et al., 2017). We show here that social stress exposure strongly activates striatal ERK1/2 in a manner that resembles drugs of abuse. This could explain why stress exposure is a precipitating factor in drug compulsion and relapse (Sinha, 2008), but also in brain disorders where reward processing is affected such as depression (Russo and Nestler, 2013). Understanding the molecular mechanisms mediating ERK1/2 activation is thus of importance for the development of potential novel therapies.

The ventral striatum is under strict neuronal control from midbrain dopamine inputs from the ventral tegmental area, but also from cortical, thalamic and amygdalar excitatory inputs (Hunnicutt et al. 2016). We therefore studied the contribution of these neurotransmitter systems to stress activation of ERK1/2 by using specific antagonists. Our results showed that both DA and Glu receptor types participate in stress-induced striatal pERK1/2. This is in accordance with previous data showing that ERK1/2 activation by drugs of abuse depends on the coincident detection of both dopaminergic and glutamatergic signals, with a prominent role of D1R (Bertran-Gonzalez et al., 2008; Cahill et al., 2014b), as described here for stress. Hence, once again pointing a common ground between stress and drugs of abuse.

Nevertheless, our results showed that D1R antagonism abolished basal pERK1/2 levels. Hence, the failure of stress to activate ERK1/2 may not be specific to this aversive stimulus but rather reflect a generalized diminished response to any salient event. Conversely, antagonism of specific NMDA subtypes may be more promising targets as they block stress effects without affecting basal levels. Native NMDARs are assemblies composed of two GluN1 subunits and two GluN2 subunits, typically GluN2A and GluN2B (Paoletti et al., 2013). GluN2 subunits define the biophysical and pharmacological properties of the receptor. GluN2B-containing NMDARs display longer decay time constant and carry greater calcium current per unit charge (Cull-Candy and Leszkiewicz, 2004; Sobczyk et al., 2005), thus integrating excitatory post-synaptic synaptic currents across broader time intervals. Importantly, in adulthood the relative abundance of specific subunits is tightly regulated, and deviation from these native states has been associated with a number of physiological and pathological conditions (Paoletti et al., 2013). In the NAc, transient increases of GluN2B-containing NMDA receptors following repeated cocaine administration are important for subsequent long-term potentiation and behavioural abnormalities (Huang et al., 2009; Wolf, 2016). Using selective antagonist, we demonstrated that GluN2B-containing NMDA receptors significantly contribute to the induction of pERK1/2 by stress. Overall, our data shows that both dopamine and glutamate systems are involved in activating MSNs after a stressful experience, and underline the potential use of selective antagonists to prevent stress mal-adaptations that may contribute to psychiatric symptoms.

Which brain inputs contribute to stress-induced pERK activation in the striatum? Dopaminergic inputs to the CPu and NAc arise exclusively from midbrain neurons, and we have shown using microdialysis that an acute social defeat episode triggers a large dopamine transient release in the NAc (Barik et al., 2013). This is in accordance with our data showing that D1R antagonist prevents stress-induced ERK1/2 phosphorylation. Instead, glutamatergic

afferents to the striatum arise from diverse sources, including many regions of the cerebral cortex, thalamus and VTA (Hunnicutt et al.). In rats, neuronal activation has been observed in the medio-dorsal (MD) and the paraventricular thalamic nucleus (PVT) upon social interaction (Ahern et al., 2016). Recent work indicates that the thalamus can as well react to various stressors. Acute social defeat induces c-Fos expression in the MD and the PVT (Lkhagvasuren et al., 2014). The social avoidance acquired after repeated social defeat correlates with changes in thalamic volume (Anacker et al., 2016), and changes in synaptic strength at intralaminar thalamic-accumbens projections have been reported following chronic social stress (Christoffel et al., 2015). In light of these data, we tested whether excitatory PVT projections to the NAc could provide the source of glutamate that contributes to pERK1/2 induction. We used restricted DREADD expression in order to selectively silence this projection before exposure to social stress. This pharmacogenetic intervention was sufficient to block stress-induced ERK1/2 activation, while not affecting its basal levels in naive states. This suggest that the PVT provides an excitatory drive to the NAc carrying aversive information. Therefore, our data strengthen the PVT as a relay nucleus sensitive to stress.

Conclusions. Our results suggest that social stress exposure promote a prompt influx of both dopamine and glutamate release concluding in independent but eventually converging signalling cascades that promote phosphorylation of ERK1/2 (Figure 6). We have built our conclusive Figure 6 on different findings from our lab and others: (i) an acute social defeat episode triggers a significant increase of DA release measured by microdialysis within the NAc (Barik et al., 2013). This is likely to impact the activity of MSNs directly via DA receptors located on these neurons or indirectly by modulating the release of glutamate release within this region. Of note, D1 DA receptors are not expressed on accumbal glutamatergic terminals (Bamford et al., 2004a); (ii) direct application of glutamate on striatal brain slices induces ERK1/2 phosphorylation in MSNs (Vanhoutte et al., 1999); (iii) in striatal

cultures, stimulation of D1 DA receptors potentiates glutamate-induced pERK1/2 through activation of glutamate NMDA receptors in MSNs (Pascoli et al., 2011; Cahill et al., 2014a). Nevertheless, we cannot exclude that the systemic injections of DA and glutamate antagonists impact pERK1/2 induction by stress in the NAc via a polysynaptic pathway.

It remains to be determined whether chronic stress impact relies on transcriptional upregulation of ERK1/2, or post-translational modification, the two hypotheses not being mutually exclusive. The increased number of pERK1/2-positive cells following acute stress is likely to reflect a post-translational process due to short interval between the stimulus and its outcome. Importantly, the key point in ERK1/2 activity relies on its phosphorylation status. Hence, the fact that stress, either acute or chronic, increases its phosphorylation indicates that it should impact downstream targets of ERK1/2 and the launch of plastic changes likely impinging on the homeostasis of the NAc.

These results should be reproduced using female cohorts to evaluate for sex differences. Because social defeat stress is based on intermale aggression, this paradigm could not contemplate for sexual differences. Recent adapted protocols have been established however the basic cellular plasticity mechanisms linking stress to depressive-like behaviors are still missing (Krishnan et al., 2007; Barik et al., 2013). Could the circuit mechanism described here be exploited for the treatment of stress-related disorders? Antagonists to both NMDA and dopamine receptors have been studied, with the main disadvantage being the lack of specificity due to widespread expression of these receptors in the brain. Future refinements on circuit-based therapies such as deep brain stimulation and transcranial magnetic stimulation to achieve projection-specific modulation will surely represent an avenue to target the circuit mechanisms described here.

AUTHORS CONTRIBUTIONS

T.C., L.B. and H.F. performed viral injections, behavioural experiments and histology. T.C. performed microscopy. P.V. provided intellectual and technical inputs. S.P.F. and J.B. designed the project. T.C. analysed data. S.P.F. and J.B. wrote the manuscript. All authors provided feedback on writing the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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FIGURE LEGENDS

FIGURE 1 Chronic social stress produces distinct pattern of pERK1/2 induction in striatal and accumbal subregions. (a) Mice were submitted to a 10-day social defeat protocol, and on day 11th were sacrificed with or without an additional stress challenge. Number of pERK1/2+ neurons were quantified in five subregions. (b) Representative images showing ERK1/2 activation in striatal and accumbal regions after chronic stress. Arrowheads point pERK1/2+ neurons. (c) The number of pERK1/2+ neurons was significantly increased after chronic stress in the core and medial shell of the nucleus accumbens. In the caudate putamen only the lateral region responded with significantly to chronic stress with a challenge. (d) The cumulative frequency distribution of pERK1/2 fluorescence intensity in counted cells from naive, chronic stress and chronic stress + challenge groups (left panel). On the right panel, the same data is plotted as bar graph depicting pERK1/2 intensity. **P*<.05, ***P*<.01, ***P<0.01 Kruskal-Wallis test followed by Dunn's comparisons. Data are expressed as mean ± SEM. (NAc core: H(2) = 11.95, **; NAc medial shell: H(2) = 10.36, **; NAc lateral shell: H(2) = 1.246, P=0.5364; Medial caudate putamen: H(2) = 3.307, P=0.1914; Lateral caudate putamen: H(2) = 12.32, ***). Number of mice per group 7-8.

FIGURE 2 Acute social stress is sufficient to activate ERK1/2 phosphorylation. (a) Mice were subjected to a single defeat stress session and sacrificed immediately after or the next day (+24h). (b) Representative images showing the expression of pERK1/2 in the different brain regions analysed. (c) A single defeat stress significantly increased the number of pERK1/2+ neurons in the core and medial shell of the nucleus accumbens when analysed immediately but not 24h after. **P*<.05, ***P*<.01, ***P<0.01 Kruskal-Wallis test followed by Dunn's comparisons. Data are expressed as mean ± SEM. (NAc core: H(2) = 13.33, **; NAc

medial shell: H(2) = 10.91, **; NAc lateral shell: H(2) = 11.87, ***; Medial caudate putamen: H(2) = 17.28, ***; Lateral caudate putamen: H(2) = 13.98, ***). Number of mice per group 6-9.

FIGURE 3 Dopamine type 1 receptors contribute to stress-induced ERK1/2 activation. (a) Mice were injected ip with either saline or the D1R antagonist SCH23390 (0.1mg/kg), and 30 min after received a single defeat stress session. Naive mice only received ip injections without stress exposure. (b) Representative images showing the blockade of stress-induced pERK1/2+ increase by D1R antagonism. (c) Acute defeat was sufficient to increase the number of pERK1/2+ neurons in the both the core (left) and medial shell (right) of the nucleus accumbens. Pre-stress treatment with the D1R antagonist SCH23390 completely abolished this effect in both regions. ***P*<.01, ****P*<.001 two-way ANOVA followed by Sidak's comparisons. Data are expressed as mean ± SEM. (NAc core: interaction F(1, 26) = 7.744, **; NAc medial shell: interaction F(1, 28) = 3.980, P=0558; stress factor F(1, 28) = 18.96, ***; treatment factor F(1, 28) = 102.9, ***). # n.s. from naive SCH23390 in NAc core (*P*= 0.99) or in NAc medial shell (*P*= 0.33). Number of mice per group 6-10.

FIGURE 4 Glutamate NMDA receptor subtypes mediate ERK1/2 activation after social stress. (a) Mice were injected ip with either saline, the NMDA-GluN2A antagonist PEAQX (10 mg/kg) or the NMDA-GluN2B antagonist Ro256981 (10 mg/kg), and 30 min after received a single defeat stress session. Naive mice only received ip injections. (b) Representative images showing the blockade of stress-induced pERK1/2+ increase by NMDA antagonism. (c) Acute defeat was sufficient to increase the number of pERK1/2+ neurons in the nucleus accumbens core. Pre-stress treatment with Ro256981 and PEAQX blocked this effect. **P*<.05, ***P*<.01, ****P*<.001 two-way ANOVA followed by Sidak's comparisons.

Data are expressed as mean ± SEM. Interaction F(2, 77) = 0.9505, P=0.3911; stress factor F(1, 77) = 18.24, ***; treatment factor F(2, 77) = 7.908, ***); # n.s. from naive PEAQX (*P*=0.3453); δ n.s. from naive Ro256981 (*P*=0.9100). Number of mice per group 8-19.

FIGURE 5 Thalamic inputs mediate stress-induced activation of ERK1/2 in the NAc. (a) Intersectional viral strategy to express the inhibitory DREADD hM4 in PVT neurons projecting to the NAc. A retrograde CAV-2-CRE is injected in the NAc and an AAV is injected in the PVT to express hM4-mCherry in a CRE-dependent manner. Images show the expression of hM4-mCherry neurons correctly targeted in the PVT, and the presence of hM4-mCherry fibers in the NAc. (b) Mice were injected ip with either saline or the hM4 agonist CNO (1 mg/kg), 30 min before submitted to a single defeat stress, and sacrificed immediately after. (c) Representative images showing the blockade of stress-induced pERK1/2+ increase by silencing of the PVT to NAc neuronal pathway. (d) In saline treated mice, acute defeat was sufficient to increase the number of pERK1/2+ neurons in the NAc core an effect significantly blocked by CNO treatment. *P<.05, ***P<.001 two-way ANOVA followed by Sidak's comparisons. Data are expressed as mean ± SEM. Interaction F(1, 42) = 3.998, P=0.0521; stress factor F(1,42) = 28.01, ***; treatment factor F(1,42) = 4.548, *). Number of mice per group 9-14.

FIGURE 6 Schematic model of the impact of social stress on striatal ERK1/2 induction. Social stress by con-specifics produce glutamatergic and dopaminergic release, from the thalamic paraventricular nucleus and the ventral tegmental area respectively. These neurotransmitters activate NMDA and D1 postsynaptic receptors, respectively, and their intracellular signalling pathways lead to phosphorylation of ERK1/2.

Fig 1

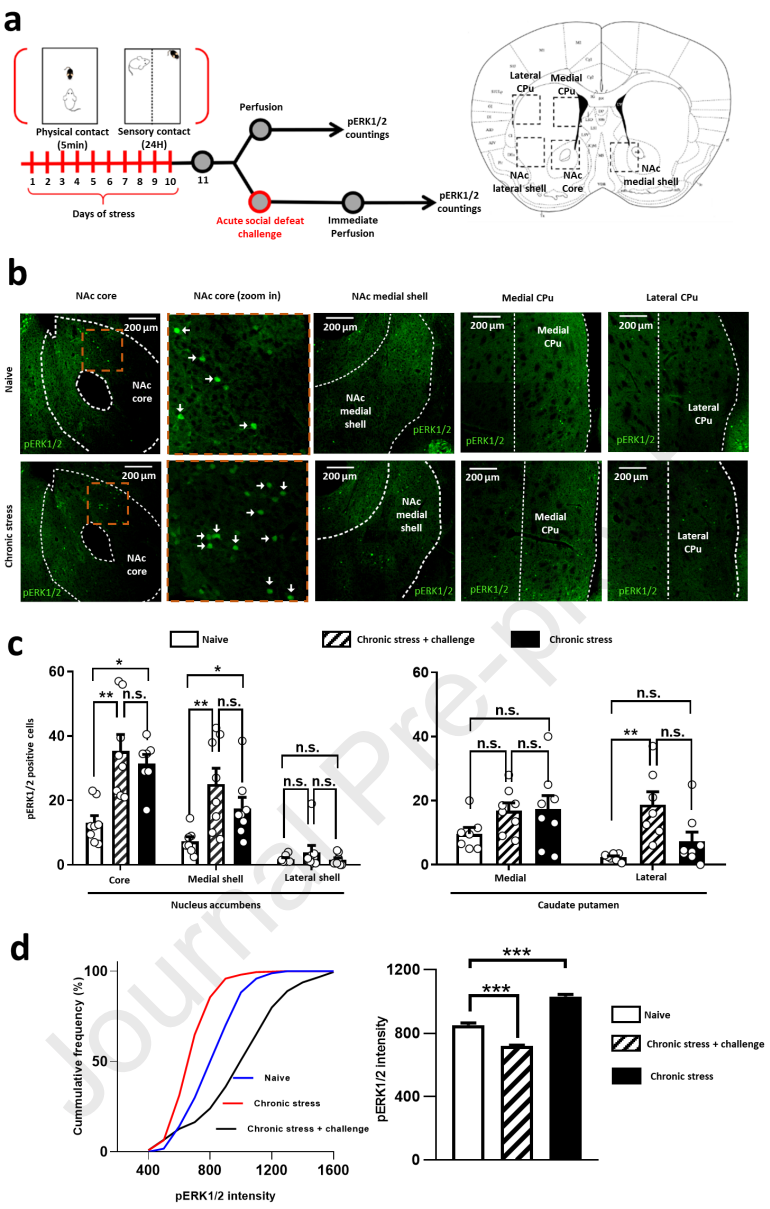


Fig 2

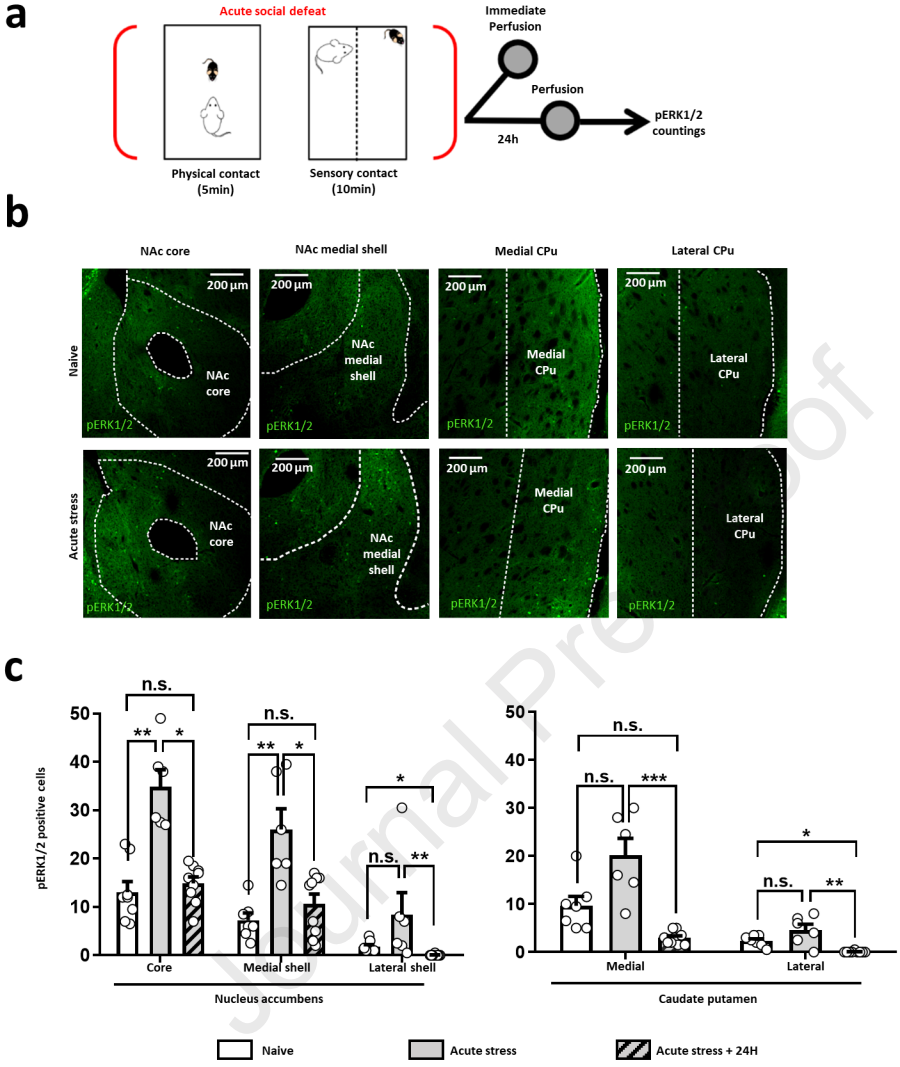


Fig 3

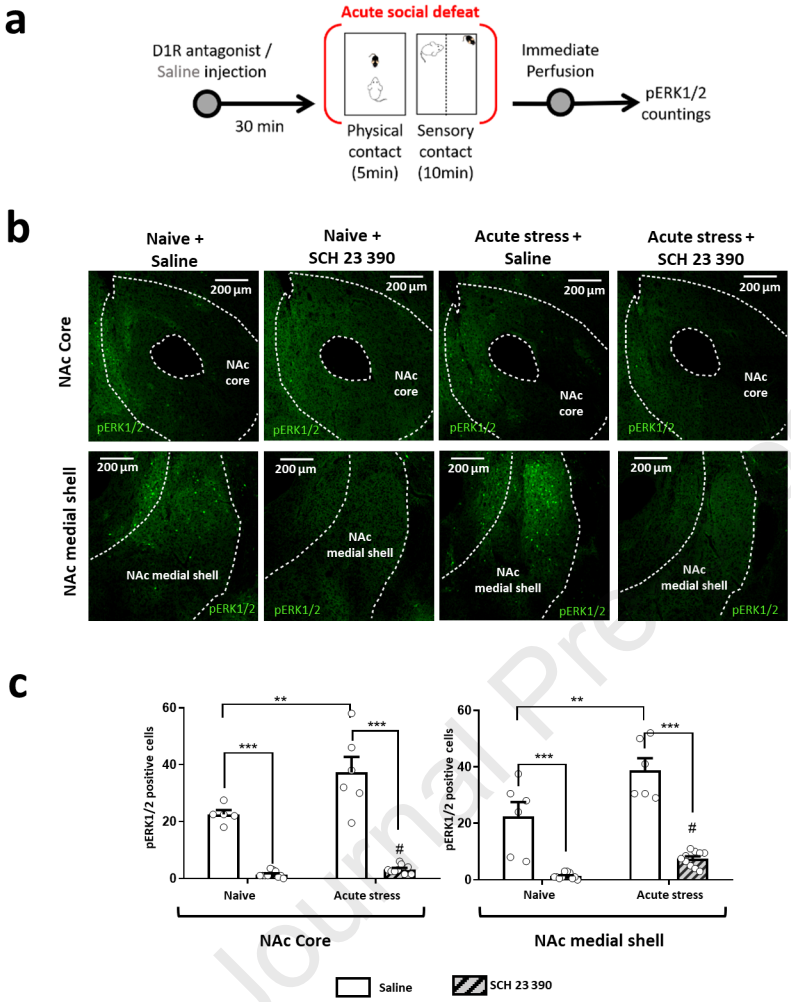


Fig 4

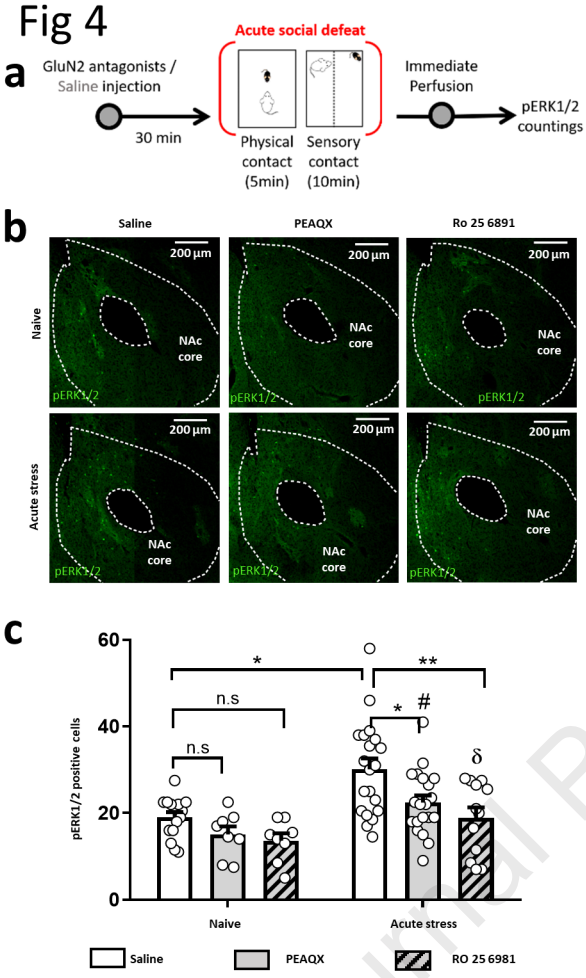
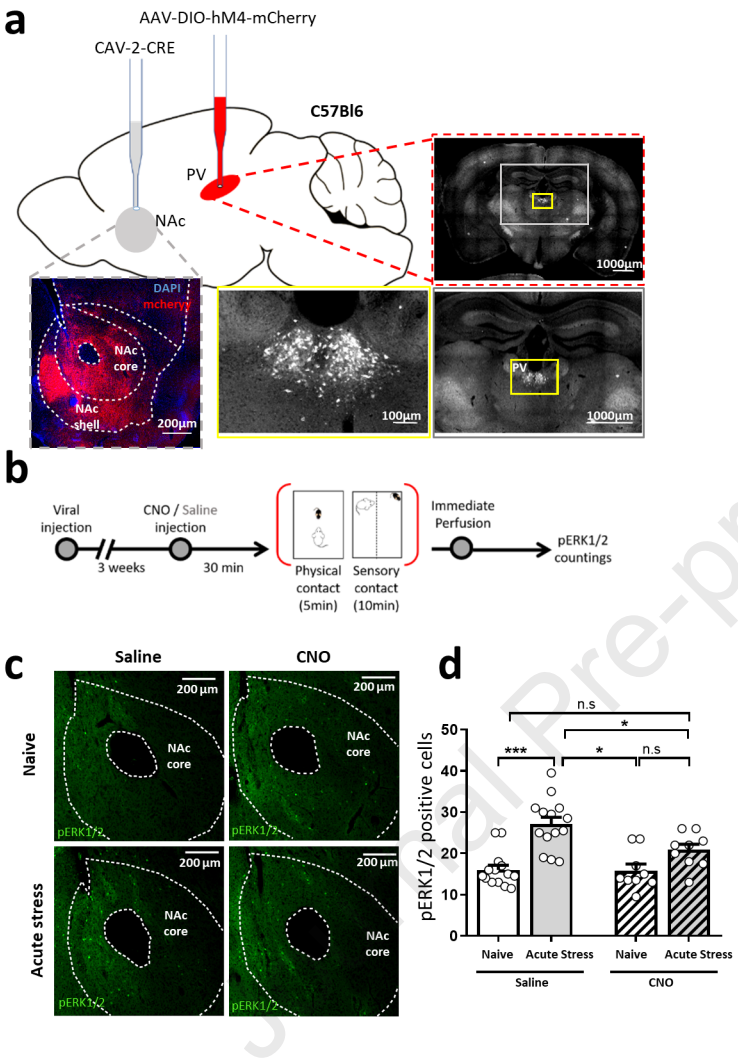


Fig 5


a
AAV-DIO-hM4-mCherry
CAV-2-CRE
C57Bl6
PV
NAc
DAPI
mcherry
NAc core
NAc shell
200µm
100µm
1000µm
1000µm
PV
b
Viral injection
CNO / Saline injection
3 weeks
30 min
Physical contact (5min)
Sensory contact (10min)
Immediate Perfusion
pERK1/2 countings
c
Saline
CNO
Naive
Acute stress
200 µm
NAc core
pERK1/2
d
pERK1/2 positive cells
n.s
*
***
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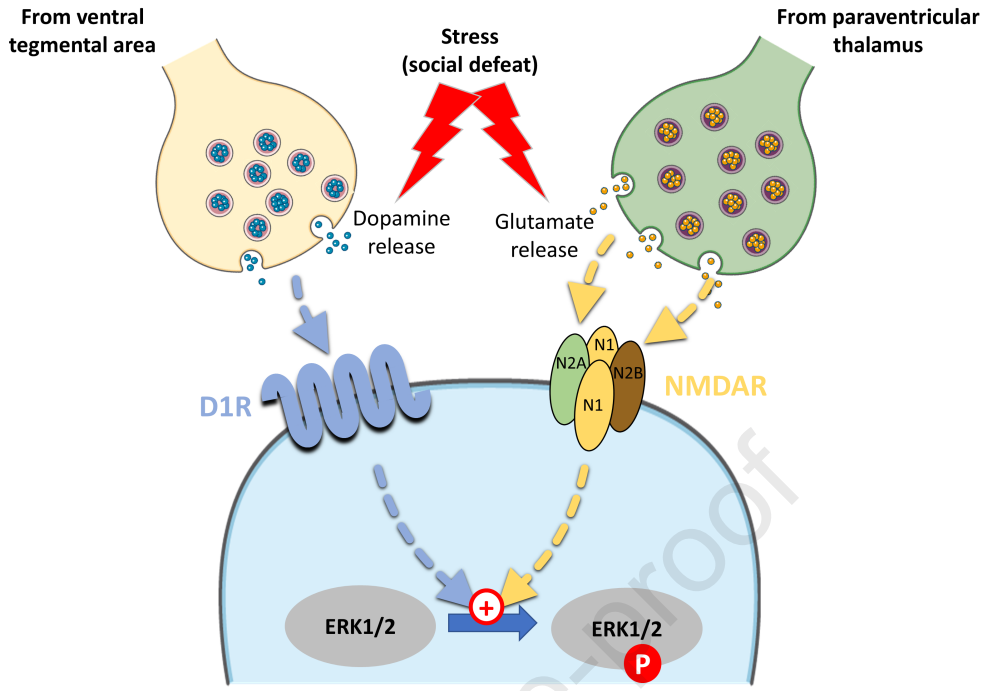
n.s
Naive
Acute Stress
Naive
Acute Stress
Saline
CNO



HIGHLIGHTS

- Social stress activates striatal ERK1/2 in the nucleus accumbens (NAc).
- Antagonism of dopamine D1.R prevents stress-induced ERK1/2 activation in the NAc.
- Blocking NMDA GluN2A or GluN2B receptors prevents activation of ERK1/2 by stress in the NAc.
- Pharmacogenetic silencing of paraventricular thalamic inputs to the NAc reduces stress impact.