Manuscript title: **Spatial object recognition memory formation under acute stress.**

Running title: **Acute stress and memory formation**

Authors:

Lopes da Cunha P[1,3], Villar ME[1,3], Ballarini F[1,3], Tintorelli R[1,3], Viola H [2,3]

[1]Universidad de Buenos Aires. Facultad de Medicina. Buenos Aires, Argentina.

[2]Universidad de Buenos Aires. Facultad de Ciencias Exactas y Naturales. Departamento de Fisiología, Biología Molecular y Celular "Dr. Hector Maldonado" (FBMC). Buenos Aires, Argentina.

[3]CONICET- Universidad de Buenos Aires. Instituto de Biología Celular y Neurociencias "Dr Eduardo De Robertis" (IBCN). Buenos Aires, Argentina.

Corresponding autor:
Dr. Haydée Ana María Viola

Address: Paraguay 2155 3rd floor (C1121ABG)- CABA, Argentina

Telephone and fax number: +54 (11) 5285-2800 (ext. 53179/53180)

E-mail: hviola@fmed.uba.ar

Keywords: Spatial Object Recognition, Elevated Platform, Long-Term Memory, Synaptic tagging, Hippocampus.

Text pages number: 17

Figures number: 5

Grant sponsor: National Agency of Scientific and Technological Promotion of Argentina (ANPCyT) and National Scientific and Technical Research Council (CONICET)

Grant number: PICT 2012-1035 and PIP 00366

List of acronyms:
behavioral tagging, BT
brain-derived neurotrophic factor, BDNF
elevated platform session, EP
glucocorticoid receptors, GR
long-term memory, LTM
mineralocorticoid receptors, MR
open field, OF
plasticity-related proteins, PRPs
short-term memory, STM
strong spatial object recognition training, sSOR
weak spatial object recognition training, wSOR

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/hipo.23037

Abstract

Stress is known to have a critical impact on memory processes. In the present work, we focus on the effects of an acute stress event closely associated to an unrelated learning task. Here we show that acute stress (elevated platform session, EP) experienced one hour after a weak spatial object recognition training (SOR), which only induces a short-term memory (STM), promoted the formation of SOR-long term memory (SOR-LTM) in rats. The effect induced by stress was dependent on the activation of glucocorticoid- and mineralocorticoid-receptors, brain-derived neurotrophic factor (BDNF) and protein synthesis in the dorsal hippocampus. In contrast, EP after a strong SOR impaired SOR-LTM probably by interfering with the use of necessary resources. Moreover, we show that the EP session before training induced anterograde interference, which it was not reversed by a subsequent exposure to an open field. Our findings provide novel insights into the impact of stress on LTM formation in rodents and they are discussed under the behavioral analogue of the synaptic tagging and capture hypothesis.

1. Introduction

Our daily lives are full of emotionally arousing experiences. Collectively, the potential threats of our bodily homeostasis are referred to as stress (Levine 2005). Stressors triggers hypothalamic-pituitary-adrenocortical axis activation leading to glucocorticoid release from the adrenal glands, accompanied by rapid sympathetic physiological responses, that influences neural structures that control emotion and cognition (Joëls et al 2006). When a situation is perceived as stressful, specific brain regions are activated, including hippocampus, amygdala and prefrontal cortex which are enriched with glucocorticoid receptors (Deppermann et al, 2014). These areas are also crucial in the formation of spatial memories and are involved in learning from stressful events and their surrounding context which is an essential mechanism to respond adaptively to similar demands in the future (Sandi and Pinelo-Nava, 2007).

Long- term memory (LTM) formation is a gradual process that requires new protein synthesis (McGaugh, 2000; Schafe *et al*, 1999). Learning tasks can induce the synthesis of proteins, if they are strong enough, or they can also use the proteins induced by other events close in time to them. However, synaptic plasticity and learning and memory require input specificity for the encoding and storage of the

information. In analogy to synaptic tagging and capture hypothesis (Frey and Morris, 1997) we postulated the behavioral tagging (BT) hypothesis (Moncada and Viola, 2007) proposing that a learning session sets a learning-tag indicating the place where the proteins will be captured to establish LTM (Redondo and Morris, 2011; Viola *et al*, 2014). In previous studies, we showed that the proteins provided by the exposure to a novel open field (OF) promote the formation of LTM of different learning tasks (Ballarini *et al*, 2009; Moncada *et al*, 2011). Then, several groups reproduced and extended our findings in rodents (for review see Moncada et al, 2015). Moreover, there are findings suggesting the existence of a similar process operating in LTM formation in humans (Ballarini *et al*, 2013; Dunsmoor *et al*, 2015). Besides OF, other experiences such as objects' exploration in a novel arena, a novel taste, a Morris water maze session, a contextual fear conditioning reminder session, contextual fear conditioning extinction session or a rewarded T-maze task were further described as protein supplier events that promote unrelated memories *(*Ballarini et al, 2009; Cassini et al, 2013; Dong et al, 2012; Salvetti et al, 2014). Considering the plethora of data about the modulatory effect on memory processes caused by stress or by the administration of glucocorticoids (Cadle and Zoladz 2015; Joëls *et al* 2006; Sandi and Pinelo-Nava, 2007) we considered that a stressful event could influence unrelated memory formation by providing or competing for protein resources.

The hypothesis of ''emotional tagging'' (Richter-Levin and Akirav, 2003) was introduced to characterize the relevance of affective factors in determining memory outcomes. It focuses on amygdala activation, resulting in modulation of neural plasticity in other brain regions involved in the emotional memory formation. Recently, it was proposed that stress enhances memory for other experiences using a mechanism of synaptic tagging and capture (Bergado et al*,* 2011). However, a detailed protocol to demonstrate that proposal still remains to be done.

Here, we show that acute stress experienced one hour after a weak spatial object recognition (wSOR) training, that only induces STM, promotes the formation of SOR-LTM in rats. This effect is impaired by drugs that antagonize the glucocorticoid receptors (GR) and mineralocorticoid receptors (MR), that inhibit protein synthesis, or that block BDNF function, which were administered into the dorsal hippocampus before the stressful event took place. However, the effect of one hour post-training stress depends on the strength of the learning session. If rats are trained in strong SOR (sSOR) task, the stressful experience impairs the SOR-LTM formation, probably by interfering with the use of the plasticity-

related proteins (PRPs). Also, we show that acute stress before sSOR training impairs its LTM formation. Our findings suggest that a stressful event affects LTM formation of an unrelated memory, and they are discussed under the conceptual framework of BT hypothesis.

2. Materials and Methods

2.1 Animals

Male adult Wistar rats (40-60 day old, 200-350 g), from Faculty of Exact and Naturals Sciences of Buenos Aires, were housed in groups of 5 per cage at 21ºC under 12h light/dark cycle. All rats had food and water available ad libitum. Animals were handled for two minutes for two consecutive days before the experiment. All procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publications No. 80-23, revised 1996) and were approved by the Animal Care and Use Committee of the University of Buenos Aires.

2.2 Drugs

All drugs used were purchased from Sigma, St. Louis, MO, USA. The protein synthesis inhibitors used were anisomycin (aniso, 80 µg/0,8 µl) and emetine (emet, 50 µg/µl). Aniso was dissolved in HCl, diluted in saline and adjusted to pH 7 with NaOH. Emet was dissolved in saline to reach the appropriate concentration. The MR antagonist spironolactone (spiro, 75 ng/µl) and GR antagonist mifepristone (mife, 20 ng/µl) was dissolved in DMSO and diluted in saline for a final concentration of DMSO 5%. The doses were determined from published studies (Korte *et al*, 1995; Moncada *et al*, 2011; Xing *et al*, 2014) and pilots experiments in our lab. The function-blocking anti-BDNF antibodies (Chemicon, Temecula, CA; AB1513P) were diluted to working concentration (0,5µg/0,8µl) with saline (Slipczuk *et al*, 2009).

2.3 Surgery and drugs administration

For the implantation of cannulas, rats were deeply anesthetized (70 mg/kg ketamine; 7 mg/kg xylazine). Cannulas were stereotaxically aimed to CA1 region of the dorsal hippocampus at coordinates A: -3.9 mm, L: ± 3.0 mm, D: -3.0 mm, from Bregma (Paxinos and Watson, 2007) and they were cemented to the skull with dental acrylic. To prevent clogging, a needle was placed in the cannula. During surgery, the

analgesic meloxicam (0.2 mg/kg) and the antibiotic gentamicin (3 mg/kg) were ip administered. Animals were allowed to recover from surgery at least for four days before the experiment.

To infuse the drugs, a 30-gauge needle with its tip protruding 1.0 mm beyond that of the guide was used. The infusion needles were linked by an acrylic tube to a Hamilton microsyringe. Drugs were infused 15 min before EP session. Rats were manually restrained during bilateral drug infusions delivered over 2 min. The needle was left in place for an additional minute after infusion to allow diffusion and to prevent reflux.

2.4 Histology

Histological examination of the cannulas' placement was performed after the experiments by the infusion of 0,8 µl of 4% methylene blue in saline solution (**Fig 1b**). Animals were killed by decapitation 15 minutes after and their brains were removed and sliced to check the infusion area (maximum spread of about 1.5 mm^3) (Villar *et al*, 2016). Only data from animals with correct cannulas implants (95% of the rats) was included in statistical analyses.

2.5 Spatial Object Recognition

Spatial object recognition memory (SOR) is the ability to detect the spatial displacement of previously encountered objects. If a familiar and a novel location of objects already encountered are presented to a rat, it will spend more time exploring the spatially displaced object (novel location) relative to the stationary one (Dere *et al*, 2005).

The objects were located in a 60 cm wide x 40 cm long x 50 cm high acrylic box. The frontal wall of the box is transparent and the back wall is hatched, while laterals walls are white with different visual clues. On the training day (TR), two identical objects (aluminum, glass or plastic objects of similar dimensions) were included in the arena in two adjacent corners and animals were left to explore the arena for 5 min in a weak training (wSOR) or 10 min in case of strong one (sSOR). The room where SOR training took place was dimly lighted. Exploration time for each object, defined as sniffing or touching it with the nose or forepaws, was measured using a hand stopwatch. Rats were excluded from the analysis if they explore one of the objects more than 65% of the total objects-exploration time during TR. In the test session (TS), performed 30 min later (STM) or 24 hours later (LTM), one of the objects was switched to a new position and exploration time was recorded again. Animals were allowed to explore for 2 min and

those with total objects-exploration time lower than 10 s were excluded. Results are expressed as a preference index: [Exploration time of new location (Tn)-Exploration time of familiar location (Tf)]/[Tn+Tf]. A positive and significantly different to zero score indicates memory. A representative mean ± SEM of the total exploration time during wSOR was 51.04 ±1.80 s and during TS was 17.63±0.59 s.

From rat to rat, the familiar or new position of the object in TS was counterbalanced. The box and the objects were thoroughly cleaned between trials.

2.6 Elevated Platform

Behavioral stress was evoked by placing the rat on an elevated platform (EP) made of white acrylic (20x 20 x 80 cm above ground level) for 30 min in a brightly lit room (Degroot *et al*, 2004). During this period, the animals show behavioral signs of stress (freezing immobility, piloerection, urination and defecation). We performed radioimmunoassay for measuring corticosterone plasma levels 5 min after EP and we confirmed high amount of this hormone (Control: 34.43 ±7.25 ng/ml, n=5; EP: 822.0 ± 77.30 ng/ml, n=6; p<0,001 Student´s t-test).

2.7 Open Field

The open field (OF) consisted of a square box of 50 x 50 x 39 cm, with black walls and floor, which is divided into nine quadrants by white stripes. Animals were left to explore for 5 min under normal lighting of the room (Moncada and Viola, 2007). Rats did not show any freezing signs; in contrast they displayed a typical spontaneous exploratory behavior. A representative mean (± SEM, n=21) for the number of quadrant crossings was 98.38 ± 3.11 and for the number of rearings was 49.19 ± 2.27.

2.8 Data Analysis

Statistical analysis of behavioral data was performed using Graph Pad Prism® software. Differences between the groups were determined using non-paired Student t-Test or one-way ANOVA. Post hoc comparisons were made using Newman-Keuls or Dunnett comparison test.

3. Results

3.1 Acute stress one hour after a weak SOR, that only induces STM, promotes SOR-LTM.

We trained rats to explore two identical objects inside a context (wSOR). In the test session, one of the objects was placed in a novel location in the same context and we measured the exploration to both objects. **Fig 1a** shows that the group of rats trained with wSOR and tested 30 min later showed SOR-STM; however, a parallel group of rats trained with the same wSOR but tested 24 h later did not show SOR-LTM ($p<0.01$ vs STM group). In contrast, when animals experienced a stressful event (30 min exposure in EP) 60 min after wSOR, SOR-LTM was established ($p<0.05$ vs LTM group).

3.2 The promoting effect of stress depends on the activity of MR and GR, BDNF and protein synthesis in the dorsal hippocampus.

Because SOR is a hippocampus-dependent task (Ballarini *et al*, 2009; Mumby *et al*, 2002), we wondered if the promoting effect of stress on SOR-LTM formation would be blocked by the administration of GR or MR antagonists in the CA1 of the dorsal hippocampus. Rats were trained with a wSOR and an EP stress session was given 60 min after. Animals received hippocampal infusions 15 min before EP, with vehicle, mifepristone or spironolactone (**Fig 1b** shows a representative schema of the infusion area). SOR-LTM was registered 24 h after training. **Fig 1c** shows that rats infused with veh expressed SOR-LTM ($p<0.01$ vs CTR); in contrast, rats infused with mife or spiro did not express it ($p<0.01$ vs veh). Moreover, the promotion of SOR-LTM formation induced by stress after wSOR ($p<0.001$ vs CTR), was blocked by the intra-hippocampal administration of the protein synthesis inhibitors anisomycin ($p<0.01$ vs veh) or emetine ($p<0.05$ vs veh), 15 min before EP exposure (**Fig 1d**). Because one effector protein with memory-enhancing action induced by GR activation is BDNF (Chen *et al*, 2012; Revest *et al*, 2014), here we blocked BDNF function by infusing anti-BDNF antibodies into the dorsal hippocampus 15 min before EP. We found that SOR-LTM promotion was blocked ($p<0.01$ vs veh, **Fig 1e**).

3.3 There is a narrow time window of efficacy of acute stress on SOR-LTM formation.

Next, we studied if the acute stress could have effects when it was experienced at other times around wSOR. Rats were exposed to EP 60 or 30 min before wSOR, or 30, 60 or 90 min after it. As

expected stress 60 min after wSOR promoted the formation of SOR-LTM ($p<0.05$ vs CTR, **Fig 2**). However, no other time interval either before or after wSOR was successful to induce SOR-LTM.

3.4 Stress one hour before weak SOR training has a detrimental effect on SOR-LTM.

Given that the EP exposure before wSOR did not induce the formation of SOR-LTM, we decided to expose rats to an OF session, an event widely demonstrated to promote LTM for different learning tasks by inducing protein synthesis (Moncada *et al*, 2014). **Fig 3a** shows that OF 60 min after wSOR induce SOR-LTM ($p<0.05$ vs CTR); however, in a parallel group of rats which also experienced EP session 60 min before wSOR, the SOR-LTM formation was prevented ($p<0.05$ vs OF). Moreover, rats trained in sSOR expressed SOR-LTM, but rats exposed to an EP 60 min before sSOR were amnesic ($p<0.05$ vs CTR), and this effect could not be reverted by the OF experienced 60 min after sSOR ($p<0.05$ vs CTR, **Fig 3b**). SOR-LTM was not affected by exposing rats to an OF 60 min after sSOR (preference index mean (±SEM), OF: 0.23 ± 0.07 (n=11) vs CTR: 0.21 ± 0.05 (n=14), $p>0.05$ after t-Test). Finally, we wondered if this negative effect of acute stress specifically affects SOR-LTM formation. To study this issue, rats were exposed to the EP 60 min before sSOR and SOR-STM was registered 30 min later. **Fig 3c** shows that animals can encode information and express STM ($p>0.05$ vs CTR), suggesting that the effect of EP is selective on memory consolidation.

3.5 Stress one hour after strong SOR training interfered with SOR-LTM formation.

In **Fig 4** we observed that sSOR induced SOR-LTM in rats; however, EP 60 min after sSOR impaired SOR-LTM expression ($p<0.05$ vs CTR). This amnesia was prevented by exposing animals to an OF session 60 min before sSOR ($p<0.05$ vs STR+60). OF session experienced 60 min before sSOR did not affect SOR-LTM formation (preference index mean (±SEM), OF: 0.17 ± 0.06 (n=19) vs CTR: 0.26 ± 0.06 (n=14), $p>0.05$ after t-Test). Therefore, EP 60 min after wSOR promoted SOR-LTM, but EP 60 min after sSOR blocks SOR-LTM, probably by providing or competing for resources, respectively.

4. Discussion

Most studies describe the effect of stress when it is intrinsic to a learning task, highlighting its involvement in memory consolidation (Lalumiere et al, 2017; McGaugh 2013). Also, increasing the stress

intensity at training accelerates systems consolidation and memory generalization in a process triggered by glucocorticoid and noradrenaline released into the hippocampus (Pedraza *et al*, 2016). However, an extrinsic stress experience affects the memory of any incidental learning temporally associated with it, and its impact depends on factors such as the intensity and the duration of the stress situation, the moment at which it was experienced and the type of the learning task involved (Joëls and Baram, 2009). A unifying theory states that stress acting around the time of the event to be remembered exerts its action on some overlapping circuits and facilitates learning and memory process (Joëls *et al*, 2006). Stress influences the acquisition of unrelated information in a time-dependent fashion, having an enhancing effect when is near to learning and an impairing one when it is temporally separated from it (Cadle and Zoladz, 2015).

We found that pre-training stress specifically impaired SOR-LTM without affecting SOR-STM. This effect was independent of the training strength and could not be reverted by OF exposure. In contrast, the effect of post-training stress was training strength-dependent. Thus, stress impaired SOR-LTM when the training was strong but promoted SOR-LTM when the training was weak. Such promoting effect was blocked by protein synthesis inhibitors, antibodies against BDNF and GR´s and MR´s antagonist, infused into the dorsal hippocampus before EP. Our findings are consistent with previous studies showing that post-training EP impaired a consolidated recognition LTM but it promoted SOR-LTM when rats were subjected to a weak training through a mechanism involving GR in the amygdala (Maroun and Akirav 2008; Segev et al*,* 2012). Also in line with our results, recent findings demonstrated that anisomycin-induced amnesia of SOR was reverted by infusing BDNF into the dorsal hippocampus, suggesting an important role of BDNF as a product of protein synthesis required for the consolidation for this task (Aarse et al*,* 2016; Ozawa et al, 2014). Here, we proposed that GC secreted by stress could be involved in the synthesis of PRPs in the hippocampus. It was reported that stress-increased GC secretion induces the expression of pro-BDNF and tissue-plasminogen activator proteins that allows further proteolytic processing of pro-BDNF into BDNF (Revest *et al*, 2014). Also, the experience of a short-time stress induced a significant increase in BDNF mRNA levels in the whole rat hippocampus, followed by an augmented BDNF protein level, probably due to glucocorticoids effects on glutamate release into the hippocampus (Marmigère *et al*, 2003). Moreover, several molecular pathways are proposed to be induced by GR activation in hippocampus including the release of glutamate, increases in synaptic GluA1 expression, phosphorylation of CaMKII, TrkB and CREB (Finsterwald and Alberini, 2014). Thus, given the effects of pCREB on gene transcription which include the increase of mRNA for BDNF and Arc (Barco *et*

al, 2005; Ying *et al*, 2002) and the transcriptional and translational effects of GR and MR in the hippocampus (Datson *et al*, 2001; Roozendaal *et al*, 2010), we suggest that stress could increase the expression of protein synthesis.

We have previously described that a novel OF exposure, 60 min before or after a wSOR, promoted SOR-LTM by protein synthesis-dependent mechanisms (Ballarini *et al*, 2009). Thus, either novel OF exposure as well as EP session 60 min post wSOR promoted SOR-LTM formation. In contrast, a EP experienced 60 min before a SOR had impairing effects on the SOR-LTM, which is consistent with previous findings using different stressors on spatial water maze LTM (Kim *et al*, 2005; Park *et al*, 2008). We observed that this negative effect of stress in SOR was specific on consolidation, leaving acquisition and SOR-STM expression intact. The detrimental effects of stress before learning on the formation of SOR-LTM considers the priming or preconditioning: a first synaptic plasticity primes or modifies a subsequent synaptic plasticity. This is referred as metaplasticity (Abraham and Bear, 1996). While metaplasticity was initially defined and studied at synaptic and cellular level, it was also useful to describe plasticity changes on a more global level referring as "behavioral metaplasticity" (Schmidt *et al*, 2013). Therefore, we suggest that the synaptic changes induced by exposure to EP could affect the following synaptic changes induced by SOR leading to the impairment in SOR-LTM formation.

Our results suggest that cellular and molecular mechanisms triggered by stress and SOR interact in the dorsal hippocampus and they may result in SOR-LTM. These results could be analyzed under the BT hypothesis, which postulates that a two-step cellular process is required to form a LTM: the setting of a tag induced by learning and the supply of PRPs. Under this view, stress post wSOR could positively affect this unrelated memory within specific time-lapse, providing PRPs into the hippocampus learning-tagged sites. Here, stress promoted the formation of another memory that per se was not induced; however, the classic view of memory modulation -implying the presence of a stimulus that increase or decrease the expression of a formed LTM- could also be mechanistically explained by the delivery of PRP to transient sites tagged by learning. It is interesting to highlight that BDNF receptor TrkB was postulated as a potential component of the behavioral tag, while BDNF is a good candidate for being one of the PRPs (Lu *et al*, 2011). In addition, there are results suggesting the role of Arc, GluA1 and Homer -1a as other molecules acting as PRPs (see Moncada et al, 2015 for revision). Moreover, our results also suggest the presence of a SOR learning-tag. We think that EP previous to SOR could prevent this tag setting

impairing the formation of SOR-LTM, and it is why OF exposure, a PRPs provider event (Ballarini *et al* 2009), did not rescue this impairment. An important aspect of the postulated learning-tag is its transient duration; so, if PRPs arrive when the tag has already decayed, the capture mechanism should not work (Viola et al, 2014). That is probably why EP 90 min after a wSOR did not promote SOR-LTM. Finally, the BT hypothesis predicts that the tags set by different tasks localized in a common population of neurons could compete for capturing the available PRPs (Moncada *et al*, 2015). In that sense, retroactive interference was observed when an event was experienced after a strong learning task (Martínez et al, 2012; Martinez et al 2014; Villar et al, 2016). We suggest that this is the case for the impairing effect of the EP experienced 60 min post sSOR, which is compatible with the fact that a source of PRPs, like OF 60 min before sSOR, prevents this impairing effect.

Taken as a whole, BT hypothesis offers a conceptual framework to analyze our present findings. However, we want to highlight that non-synaptic mechanisms, like changes in neuronal intrinsic excitability, were recently proposed as an alternative model (Korz, 2018). Moreover, although in our work we studied the effects of stress on a spatial memory focusing on the role of dorsal hippocampal area, this is a partial view of the complex phenomenon of memory formation that, in fact, involves the activation of other brain regions. Among them, the multiple effects of stress on amygdala and its impact on several brain areas, including the hippocampus, would affect different synaptic plasticity and memory processes (McGaugh, 2013; Richter-Levin and Maroun, 2010).

In conclusion, our results show that acute stress affects satellite SOR memory formation in rats. Stress one hour post SOR training is able to promote LTM if the training session is weak; however, stress impairs SOR-LTM when training is strong. Stress always prevents SOR-LTM when is experienced before a weak or strong SOR session (Fig 5). We discussed our results according to the BT hypothesis, which in connection to “synaptic tagging and capture” and “emotional tagging” hypothesis suggest a global physiological mechanism to explain promoting, enhancing or detrimental actions caused by stress on LTM-formation of temporally close unrelated learnings.

References

Aarse J, Herlitze S, Manahan-vaughan D (2016). The Requirement of BDNF for Hippocampal Synaptic Plasticity Is Experience-Dependent. *Hippocampus* 26: 739–751.

Abraham W. and Bear M. (1996). Metaplasticity : plasticity of synaptic. *Trends Neurosci* 19: 126–130.

Ballarini F, Martínez MC, Díaz Perez M, Moncada D, Viola H (2013). Memory in Elementary School Children Is Improved by an Unrelated Novel Experience. *PLoS One* 8: 1–7.

Ballarini F, Moncada D, Martinez MC, Alen N, Viola H (2009). Behavioral tagging is a general mechanism of long-term memory formation. *Proc Natl Acad Sci U S A* 106: 14599–604.

Barco A, Patterson S, Alarcon JM, Gromova P, Mata-Roig M, Morozov A, *et al* (2005). Gene expression profiling of facilitated L-LTP in VP16-CREB mice reveals that BDNF is critical for the maintenance of LTP and its synaptic capture. *Neuron* 48: 123–137.

Bergado JA, Lucas M, Richter-Levin G (2011). Emotional tagging-A simple hypothesis in a complex reality. *Prog Neurobiol* 94: 64–76.

Cadle CE, Zoladz PR (2015). Stress time-dependently influences the acquisition and retrieval of unrelated information by producing a memory of its own. *Front Psychol* 6: 1–11.

Cassini LF, Sierra RO, Haubrich J, Crestani AP, Santana F, Oliveira Alvares L de, *et al* (2013). Memory reconsolidation allows the consolidation of a concomitant weak learning through a synaptic tagging and capture mechanism. *Hippocampus* 23: 931–941.

Chen DY, Bambah-Mukku D, Pollonini G, Alberini CM (2012). Glucocorticoid receptors recruit the CaMKIIα-BDNF-CREB pathways to mediate memory consolidation. *Nat Neurosci* 15: 1707–14.

Datson NA, Perk J Van Der, Kloet ER De, Vreugdenhil E (2001). Identification of corticosteroid-responsive genes in rat hippocampus using serial analysis of gene expression. *Eur J Neurosci* 14: 675–689.

Degroot A, Wade M, Salhoff C, Davis RJ, Tzavara ET, Nomikos GG (2004). Exposure to an elevated platform increases plasma corticosterone and hippocampal acetylcholine in the rat : reversal by chlordiazepoxide. *Eur J Pharmacol* 493: 103–109.

Deppermann S, Storchak H, Fallgatter AJ, Ehlis A (2014). STRESS-INDUCED NEUROPLASTICITY : (MAL) ADAPTATION TO ADVERSE LIFE EVENTS IN PATIENTS WITH PTSD – A CRITICAL OVERVIEW. *Neuroscience* 283: 166–177.

Dere E, Huston JP, Souza Silva MA De (2005). Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. *Neurobiol Learn Mem* 84: 214–221.

Dong Z, Gong B, Li H, Bai Y, Wu X, Huang Y, *et al* (2012). Mechanisms of Hippocampal Long-Term Depression Are Required for Memory Enhancement by Novelty Exploration. *J Neurosci* 32: 11980–990.

Dunsmoor JE, Murty VP, Davachi L, Phelps EA (2015). Emotional learning selectively and retroactively strengthens memories for related events. *Nature* 520: 345–8.

Finsterwald C, Alberini CM (2014). Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: From adaptive responses to psychopathologies. *Neurobiol Learn Mem* 112: 17–29.

Frey U, Morris RGM (1997). Synaptic tagging and LTP. *Nature* 385: 533–536.

Joëls M, Baram TZ (2009). The neuro-symphony of stress. *Nat Rev Neurosci* 10: 459–466.

Joëls M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ (2006). Learning under stress : how does it work ? *Trends Cogn Sci* 10: 152–158.

Kim JJ, Koo JW, Lee HJ, Han J (2005). Amygdalar Inactivation Blocks Stress-Induced Impairments in Hippocampal Long-Term Potentiation and Spatial Memory. *J Neurosci* 25: 1532–1539.

Korte S, Boer S de, Kloet E de, Bohus B (1995). Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonist on fear-enhacenced behavior in the elevated plus-maze. *Psychoneuroendocrinology* 20: 385–394.

Lalumiere RT, Mcgaugh JL, Mcintyre CK (2017). Emotional Modulation of Learning and Memory :

Pharmacological Implications. *Pharmacol Rev* 69: 236–255.

Levine S. (2005) Stress: an historical perspective. In handbook of Stress and the Brain (Steckler, T. et al., eds), pp. 3–23, Elsevier

Lu Y, Ji Y, Ganesan S, Schloesser R, Martinowich K, Sun M, *et al* (2011). TrkB as a Potential Synaptic and Behavioral Tag. *J Neurosci* 31: 11762–771.

Marmigère F, Givalois L, Rage F, Arancibia S, Tapia-Arancibia L (2003). Rapid induction of BDNF expression in the hippocampus during immobilization stress challenge in adult rats. *Hippocampus* 13: 646–655.

Maroun M, Akirav I (2008). Arousal and Stress Effects on Consolidation and Reconsolidation of Recognition Memory. *Neuropharmacology* 33: 394–405.

Martínez MC, Alen N, Ballarini F, Moncada D, Viola H (2012). Memory traces compete under regimes of limited Arc protein synthesis: Implications for memory interference. *Neurobiol Learn Mem* 98: 165–173.

Martınez MC, Villar ME, Ballarini F and Viola H (2014). Retroactive interference of object-in-context long-term memory: role of dorsal hippocampus and medial prefrontal cortex. *Hippocampus* 24:1482–1492

McGaugh JL (2000). Memory — a Century of Consolidation. *Science (80-)* 287: 248–252.

McGaugh JL (2013). Making lasting memories : Remembering the significant. *Proc Natl Acad Sci* 110: 10402–10407.

Moncada D, Ballarini F, Martinez MC, Frey JU, Viola H (2011). Identification of transmitter systems and learning tag molecules involved in behavioral tagging during memory formation. *Proc Natl Acad Sci U S A* 108: 12931–6.

Moncada D, Ballarini F, Martinez MC, Viola H (2014). The Behavioral Tagging Hypothesis and its implications for Long-Term Memory Formation. *Synaptic Tagging Capture From Synapses to Behav* 231–259.

Moncada D, Ballarini F, Viola H (2015). Behavioral Tagging: A Translation of the Synaptic Tagging and Capture Hypothesis. *Neural Plast* 2015: 1–21.

Moncada D, Viola H (2007). Induction of Long-Term Memory by Exposure to Novelty Requires Protein Synthesis: Evidence for a Behavioral Tagging. *J Neurosci* 27: 7476–7481.

Mumby DG, Gaskin S, Glenn MJ, Schramek TE, Lehmann H (2002). Hippocampal Damage and Exploratory Preferences in Rats: Memory for Objects , Places , and Contexts. *Learn Mem* 9: 49–57.

Ozawa T, Yamada K, Ichitani Y (2014). Hippocampal BDNF treatment facilitates consolidation of spatial memory in spontaneous place recognition in rats. *Behav Brain Res* 263: 210–216.

Park CR, Zoladz PR, Conrad CD, Fleshner M, Diamond DM (2008). Acute predator stress impairs the consolidation and retrieval of hippocampus-dependent memory in male and female rats. *Learn Mem* 15: 271–280.

Paxinos G, Watson C (2007). The rat brain in stereotaxic coordinates (6th ed.). *Acad Press* .

Pedraza LK, Sierra RO, Boos Z, Quillfeldt JA, Alvares LDO (2016). The Dynamic Nature of Systems Consolidation: Stress During Learning as a Switch Guiding the Rate of the Hippocampal Dependency and Memory Quality. *Hippocampus* 26: 362–371.

Redondo RL, Morris RGM (2011). Making memories last: the synaptic tagging and capture hypothesis. *Nat Rev Neurosci* 12: 17–30.

Revest J-M, Roux A Le, Roullot-Lacarrière V, Kaouane N, Vallée M, Kasanetz F, *et al* (2014). BDNF-TrkB signaling through Erk1/2(MAPK) phosphorylation mediates the enhancement of fear memory induced by glucocorticoids. *Mol Psychiatry* 19: 1001–1009.

Richter-Levin G, Akirav I (2003). Emotional tagging of memory formation - In the search for neural mechanisms. *Brain Res Rev* 43: 247–256.

Richter-Levin G, Maroun M (2010). Stress and amygdala suppression of metaplasticity in the medial prefrontal cortex. *Cereb Cortex* 20: 2433–2441.

Roozendaal B, Hernandez A, Cabrera SM, Hagewoud R, Malvaez M, Stefanko DP, *et al* (2010). Membrane-Associated Glucocorticoid Activity Is Necessary for Modulation of Long-Term Memory via

Chromatin Modification. *J Neurosci* 30: 5037–5046.

Salvetti B, Morris RGM, Wang S-H (2014). The role of rewarding and novel events in facilitating memory persistence in a separate spatial memory task. *Learn Mem* 21: 61–72.

Sandi C. and Pinelo-Nava MT (2007). Stress and memory: Behavioral effects and neurobiological mechanisms. *Neural Plasticity* 2007:78970.

Schafe G, Nadel N, Sullivan G, Harris A, LeDoux J (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. *Learn Mem* 6: 97–110.

Schmidt et al (2013). Stress-induced metaplasticity : from synapses to behavior. *Neuroscience* 250: 112–120.

Segev A, Ramot A, Akirav I (2012). Stress Hormones Receptors in the Amygdala Mediate the Effects of Stress on the Consolidation , but Not the Retrieval , of a Non Aversive Spatial Task. *PLoS One* 7: 1–9.

Slipczuk L, Bekinschtein P, Katche C, Cammarota M, Izquierdo I, Medina JH (2009). BDNF activates mTOR to regulate GluR1 expression required for memory formation. *PLoS One* 4: 1–13.

Villar ME, Martinez MC, Lopes da Cunha P, Ballarini F, Viola H (2016). Memory consolidation and expression of object recognition are susceptible to retroactive interference. *Neurobiol Learn Mem* 138: 198–205.

Viola H, Ballarini F, Martínez MC, Moncada D (2014). *The tagging and capture hypothesis from synapse to memory*. *Prog Mol Biol Transl Sci* 122: 391-423.

Xing X, Wang H, Liang J, Bai Y, Liu Z, Zheng X (2014). Mineralocorticoid receptors in the ventral hippocampus are involved in extinction memory in rats. *PsyCh J* 3: 201–213.

Ying S, Futter M, Rosenblum K, Webber MJ, Hunt SP, Bliss TVP, *et al* (2002). Brain-Derived Neurotrophic Factor Induces Long-Term Potentiation in Intact Adult Hippocampus : Requirement for ERK Activation Coupled to CREB and Upregulation of Arc Synthesis. *J Neurosci* 22: 1532–1540.

Acknowledgements

We gratefully acknowledge Dr Jorge Medina for his comments to the manuscript. We thank Nadia Alen, Camila Martinez Calejman and Dr. Cora Cymeryng for plasma corticosterone levels measuring.

This work was supported by National Agency of Scientific and Technological Promotion of Argentina (ANPCyT, PICT 2012-1035) and National Scientific and Technical Research Council (CONICET, PIP 00366) to Haydee Viola.

The authors declare no conflict of interest.

Figure Legends

Fig 1. Acute stress 60 min after a weak SOR promotes SOR-LTM formation. The promoting effect of stress depends on GR and MR activity, BDNF and protein synthesis in the dorsal hippocampus. a) Rats were exposed to wSOR and independent groups were tested 30 min (STM, n=14) or 24 h (LTM, n=19) after. A third group of animals was exposed to an elevated platform (EP) 60 min after wSOR and tested 24 h after TR (LTM STR+60, n=12). Data are expressed as preference index mean (±SEM). Newman-Keuls analysis after one-way ANOVA, $F_{(2,42)}$= 5.838; *p<0,05 **p<0.01 b) Schematic representation of infusion area (top) and picture of infusion site (bottom). c) Rats were exposed to wSOR, injected with vehicle 45 min after and tested 23 h later (CTR, n=12). Independent animals were placed in an EP 60 min after wSOR and they received intra CA1 infusions of vehicle (STR +60 veh, n=15), MR antagonist spironolactone (STR+60 spiro, n=10) or GR antagonist mifepristone (STR+60 mife, n=11) 15 min before EP. SOR-LTM was tested 24 h after wSOR. Data are expressed as preference index mean (±SEM). Newman-Keuls analysis after one-way ANOVA, $F_{(3,44)}$= 7.074; **p<0.01 d) Rats were exposed to wSOR, injected with vehicle 45 min after and tested 23 h later (CTR, n= 18). Independent animals were placed in an EP 60 min after wSOR and they received intra CA-1 infusions of vehicle (STR+60 veh, n=25), anisomycin (STR+60 aniso, n=12) or emetine (STR+60 emet, n=11) 15 min before EP. SOR-LTM was tested 24 h after wSOR. Data are expressed as preference index mean (±SEM). Newman-Keuls analysis after one-way ANOVA, $F_{(3,62)=}$ 7.160; *p<0,05; **p<0.01; *** p<0.001 e) Rats were exposed to wSOR, injected with vehicle 45 min after and tested 23 h later (CTR, n=13) while another group of animals were placed in an EP 60 min after wSOR and received intra CA-1 infusions of vehicle (STR+60 veh, n=9) or anti-BDNF (STR+60 anti-BDNF, n=10) 15 min before EP. SOR-LTM was tested 24 h after wSOR. Data are expressed as preference index mean (±SEM). Newman-Keuls analysis after one-way ANOVA, $F_{(2,29)=}$ 12.12 ; **p<0.01; *** p<0.001.

Fig 2. There is a narrow time window of efficacy of acute stress on SOR-LTM formation: only stress 60 min after a weak SOR promotes formation of SOR-LTM. Rats were trained with wSOR and independent groups of rats were placed, or not (CTR, n= 23), in an EP at different times around training: 30 or 60 min before (STR PRE -30, n= 8 or STR PRE -60, n=13) or 30, 60 or 90 min after (STR POST +30, n=12; STR POST +60, n=20; STR POST +90, n=12). SOR-LTM was registered 24 h after wSOR.

Data are expressed as preference index mean (±SEM). Dunnett´s analysis after one-way ANOVA, $F_{(5,82)=}$ 4.07; *p<0,05 vs CTR.

Fig 3. Stress 60 min before SOR prevents SOR-LTM. a) Stress impaired the promoting effect of OF on SOR-LTM. Independent groups of rats were exposed, or not (CTR, n=7), to an OF session 60 min after wSOR (OF, n=10). A third group was exposed also to EP 60 min before wSOR (STR-60 OF, n=9). SOR-LTM was tested 24 h after wSOR. Data are expressed as preference index mean (±SEM). Newman-Keuls analysis after one-way ANOVA, $F_{(2,23)=}$ 5.187; *p<0.05. b) Stress prevented SOR-LTM formation induced by sSOR and this effect could not be reverted by OF. Control group of rats (CTR, n=19) was trained with sSOR. A second group was subjected to EP 60 min before training (STR-60, n=11) and a different group, besides EP, explored an OF 60 min after training (STR-60 OF, n= 13). SOR-LTM was tested 24 h after sSOR. All data are expressed as preference index mean (±SEM). Newman-Keuls analysis after one-way ANOVA, $F_{(2,40)=}$ 4.517; *p<0.05 c) Stress did not impair the SOR-STM. Rats were exposed to sSOR (CTR, n=9) and one group of animals was additionally placed in the EP 60 min before training (STR-60, n=14). SOR-STM was registered 30 min after sSOR. Data are expressed as preference index mean (±SEM). Student´s t-test. $t_{(21)=}$ 0.076; p>0.05.

Fig 4. Stress 60 min after strong SOR impairs SOR-LTM formation and this effect is rescued by OF. Control group of rats (CTR, n=18) was trained with sSOR. A second group was exposed to EP 60 min after training (STR+60, n=12) and a different group, besides EP, explored an OF 60 min before training (OF STR+60, n= 17). SOR-LTM was tested 24 h after sSOR. All data are expressed as preference index mean (±SEM). Newman-Keuls analysis after one-way ANOVA, $F_{(2,44)=}$ 3.540 ; *p<0.05.

Fig 5. Effect of stress on unrelated but temporally associated spatial memory: a possible interpretation based on BT hypothesis. Schema summarizes the present results. a) A weak SOR training (*Weak learning*, surrounded by a dotted circle) that does not induce per se SOR-LTM, can generate LTM if stress is experienced during a narrow time window. This is compatible with a postulated transient learning-tag setting (induced by SOR training session). b) This effect of stress on SOR-LTM

formation depends on protein synthesis, GR and MR activity and BDNF in the dorsal hippocampus. A main point in BT hypothesis is the requirement of PRPs provided by a strong experience, like stress c) Stress 60 min after strong SOR training (*Strong learning*, surrounded by continue line circle) impairs SOR-LTM formation, possibly due to the competition for PRPs. d) Stress 60 min before SOR training also prevents LTM formation, probably due to metaplastic changes that could hinder the setting of a putative learning-tag.

Fig 1

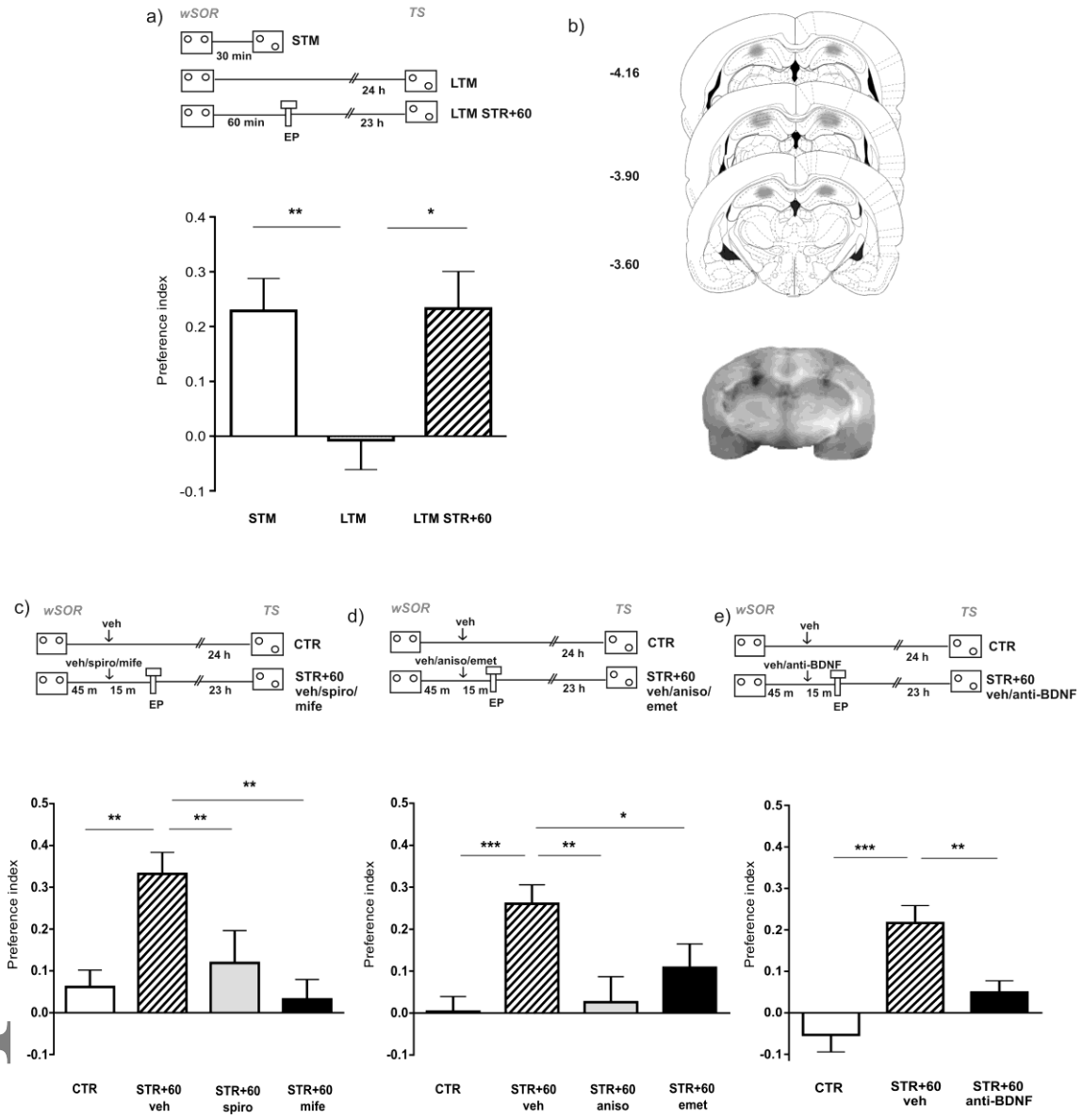

Fig 2

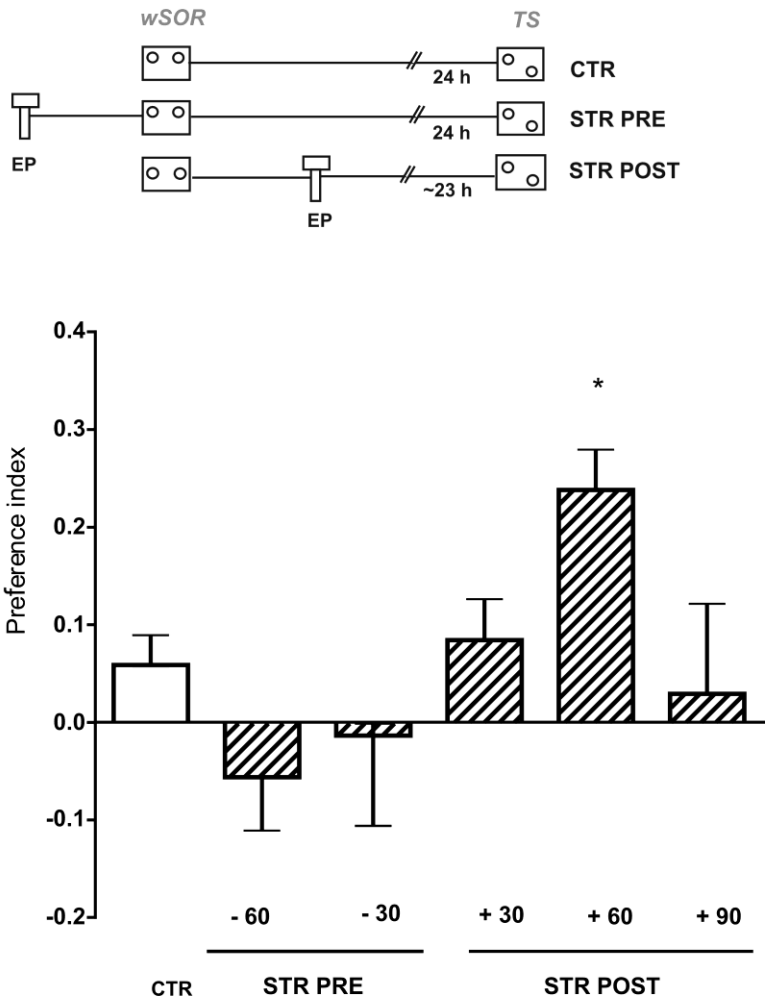

Fig 3

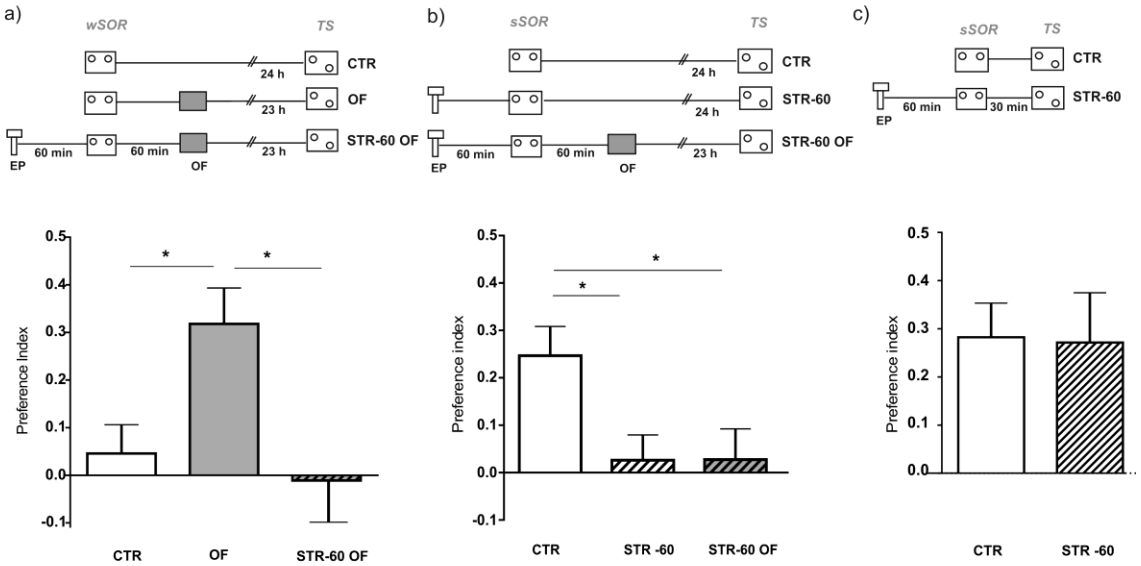

Fig 4