

# Evidence for a Role of Oxytocin Receptors in the Long-Term Establishment of Dominance Hierarchies

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Exposure to stress can affect the establishment of dominance hierarchies. In our model, a social hierarchy established by two male rats during a first encounter is not maintained 1 week later. If one of the two rats is stressed, the stressed rat becomes subordinate and the hierarchy that is formed is maintained. In this study, we investigated the changes in the expression of oxytocin (*Otr*) and vasopressin (*V1aR*) receptor genes in the medial amygdala (MeA) and the lateral septum (LS) in the hours following hierarchy establishment under both stressed and basal conditions. We found that the potentiation of a social hierarchy induced by stress is accompanied by social status- and region-specific changes in the expression of *Otr* mRNA in the MeA 3 h after the social encounter. At this time point, no evidence was found for the regulation of *V1aR* mRNA in any of the brain regions examined. Results from pharmacological experiments involving the microinfusion of a specific OTR antagonist immediately after the acquisition of a subordinate status under basal, non-stress conditions suggested a role for this receptor in the MeA on the long-term establishment of the subordinate status. Altogether, these findings highlight a role for the oxytocinergic system in the mechanisms through which stress facilitates the long-term establishment of a social hierarchy.

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## INTRODUCTION

In most animals that live in groups, males establish dominance hierarchies in which the dominant animal has priority access to limited resources, such as water, food, space, and females. One of the main functions of establishing dominance hierarchies is to eliminate fighting within a group, thus minimizing energy costs (Van Kreveld, 1970). In laboratory rats that are kept in a colony, a stable hierarchy usually develops within a few days (Blanchard *et al*, 1988).

Social recognition and social memory are critical for the establishment and maintenance of social relationships, including social attachment, sexual behavior, parental care, and dominance hierarchies (Bielsky and Young, 2004). Social memory for offspring and mates usually lasts for days, months or even years (Ferguson *et al*, 2002). However, recognition of an unfamiliar individual tends to be short lasting, in the range of minutes to hours. Because of the importance of social recognition, the hypothesis has been put forward that specific pathways in the brain have developed for this purpose. Among the brain areas

implicated in these pathways are the medial amygdala (MeA) and lateral septum (LS) (Ferguson *et al*, 2002; Bielsky and Young, 2004).

Oxytocin (OT) and arginine vasopressin (AVP) are two neuropeptides that have an important role in the regulation of social behavior (for a review, see Veenema and Neumann, 2008). These neuropeptides and their receptors, oxytocin receptor (OTR) and vasopressin receptor 1a (V1aR), are expressed in brain areas, such as MeA and LS, which are involved in social behavior (Ostrowski *et al*, 1994; Landgraf *et al*, 1998; Ostrowski, 1998; Newman, 1999; Engelmann *et al*, 2000; Gimpl and Fahrenholz, 2001; Goodson, 2005). OT or V1aR knockout mice are not able to form social memories (Ferguson *et al*, 2000, 2001; Bielsky *et al*, 2004; Crawley *et al*, 2007). The administration of OT to OT knockout mice before a social encounter or the re-expression of V1aR in V1aR knockout mice restores the ability to form social memories (Ferguson *et al*, 2000; Bielsky *et al*, 2005). In rats, administration of AVP or low, but not high, doses of OT facilitates social recognition (Popik and Van Ree, 1991; Popik and Vetulani, 1991; Popik *et al*, 1992; Benelli *et al*, 1995), whereas administration of OTR or V1aR antagonists blocks social recognition (Dantzer *et al*, 1987, 1988; Le Moal *et al*, 1987; Popik and Van Ree, 1991; Popik and Vetulani, 1991; Popik *et al*, 1992, 1996; Benelli *et al*, 1995; Engelmann *et al*, 1996, 1998; van Wimersma Greidanus and Maigret, 1996; Everts and

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**Table 1** Overview of the used Gene Expression Assays (Applied Biosystems)

Gene	Gene expression assay
Oxytocin receptor ( <i>Otr</i> )	Rn00563503_m1
Arginine vasopressin receptor 1a ( <i>V1ar</i> )	Rn00583910_m1
Ribosomal protein S18 ( <i>Rps18</i> )	Rn01428915_g1
Ribosomal protein S29 ( <i>Rps29</i> )	Rn00820645_g1
Eukaryotic elongation factor 1a1 ( <i>Eef1a1</i> )	Rn01639851_g1

Koolhaas, 1997, 1999; Landgraf *et al*, 2003; Choleris *et al*, 2007). In addition to its role in social recognition, AVP has also been implicated in aggressive behavior (Ferris *et al*, 1984; Irvin *et al*, 1990; Winslow and Insel, 1993; Delville *et al*, 1996).

In rats, exposure to different types of emotional and physical stressors has been reported to lead to OT and AVP release in different brain areas (Engelmann *et al*, 1999; Ebner *et al*, 2000; Wotjak *et al*, 1996, 1998; Neumann, 2007). Glucocorticoids were shown to modulate OTR- and V1aR-binding sites in different brain areas, including the amygdala and LS (Patchev *et al*, 1993; Watters *et al*, 1996). It has been suggested that interactions between OT, AVP, and glucocorticoids could provide a mechanism for dynamic changes in social behavior (Gimpl and Fahrenholz, 2001).

We have developed a rat model, in which stress experienced by one individual just before a first social encounter with another male equivalent in body weight and trait anxiety leads to submission in the stressed animal and the establishment of a dominance hierarchy that remains stable for at least 1 week (Cordero and Sandi, 2007). This finding contrasts with the short-lasting social hierarchy that develops in non-stressed rats, which is no longer present 1 week after a first encounter (Cordero and Sandi, 2007). Moreover, in unstressed dyads, a long-term hierarchy was facilitated when the subordinate, but not the dominant, rat received corticosterone post-encounter (Timmer and Sandi, 2010), suggesting that memory facilitation by stress is primarily retained by the subordinate rat. Here, we hypothesized a role for OTR and V1aR in the MeA and LS in the stress-induced facilitation of the established long-term dominance hierarchy. We investigated the changes in gene expression for the *Otr* and *V1aR* in each brain region following hierarchy establishment under stressed and basal conditions. We then studied the impact of pharmacologically antagonizing the receptors according to the gene expression data, and evaluating the effects on the establishment of a long-term hierarchy.

## MATERIALS AND METHODS

### Animals

Male naïve Wistar rats (Charles River Laboratories, Lyon, France) weighing 250–275 g on arrival were individually housed with *ad libitum* access to food and water. The rats were left undisturbed for 1 week before the start of the experiments. Animals were kept on a 12-h light/dark cycle with lights on at 0700 hours at a constant temperature of

22 ± 2 °C. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved through a license issued by the Cantonal Veterinary Authorities (Vaud, Switzerland).

All experiments took place between 0800 and 1400 hours, except for the water competition test, which took place between 1400 and 1900 hours after a water deprivation period of 6 h (from 0800 to 1400 hours). All rats were handled for 2 min per day for 3 days before exposure to the elevated plus maze (see below).

### Elevated Plus Maze (EPM)

Before the start of the social hierarchy experiment, the elevated plus maze (EPM) test was performed to measure anxiety-related behavior (Herrero *et al*, 2006). The EPM consists of two opposing open arms (45 × 10 cm) and two opposing closed arms (45 × 10 cm with walls 50 cm high) that extend from a central platform (10 × 10 cm) elevated 65 cm above the floor. The rats were individually placed on the central platform facing the same closed arm and were allowed to explore the EPM freely for 5 min. The behavior of each rat was video recorded and analyzed using a computerized tracking system (Ethovision 3.1.16, Noldus IT, The Netherlands). Time spent in the open and closed arms was measured.

### Dyads and Experimental Groups

Rats were distributed to dyads consisting of two rats that were matched for their body weight and anxiety level. The anxiety level was defined by the time spent in the open arms of the EPM. Thus, the rats in each dyad were considered equal in their probability to become dominant or subordinate during a first encounter. The fur of both rats in a dyad was marked on different body parts to help identify the animals. Rats were habituated to marking during the 3-day habituation period that took place before the first social encounter. Dyads were divided randomly over the different groups (non-stressed pairs or stressed pairs). Each group consisted of 7–14 dyads.

### Stress Delivery

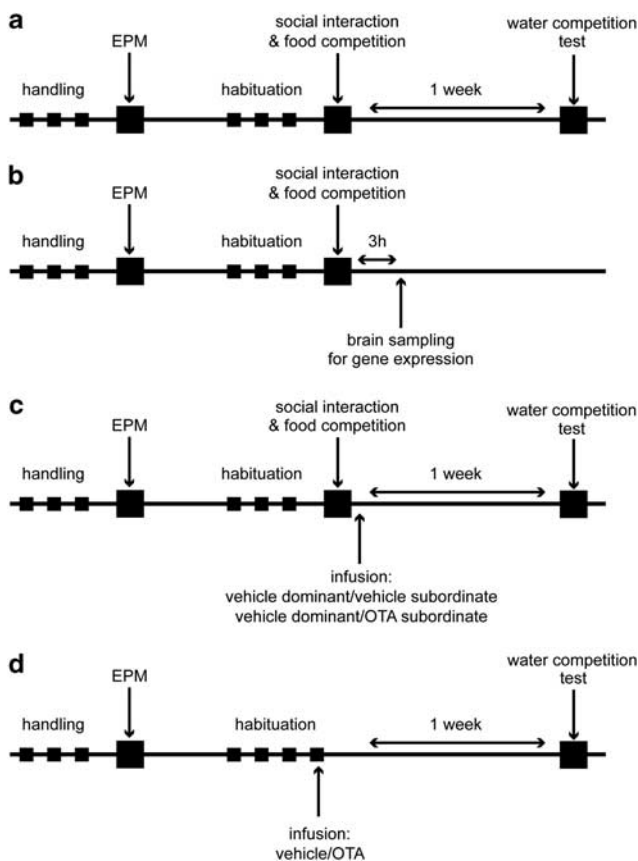
In non-stressed pairs (Pns), none of the rats was stressed before the social encounter. In stressed pairs (Ps), one rat of the pair was stressed just before the first social encounter. Stress consisted of exposure to three-foot shocks of 1 mA with an intershock interval of 1 min. At 30 sec after the last shock, the rat was taken out and immediately submitted to the first social encounter with an unfamiliar rat in an adjacent room. See Supplementary Materials and Methods for a detailed description of stress delivery.

### Food and Water Competition Test

The animals were given eight Chocopop flakes (Kellogg's, Switzerland) in their homecages daily for 2 days before the start of the habituation to the experimental setup to habituate them to the rewarding food used in the food competition test (FCT). The rats were habituated to the

experimental setup for 3 days, during which they were first placed individually in a clean housing cage for 20 min and then in a food competition box for 10 min. The food competition box is a 60 × 40 cm plastic box with a 32-cm high walls and a feeder containing eight Chocopops on one of the short sides. On day 1 of the experiment, the day after the last habituation day, the rats were submitted to a first encounter with an unfamiliar rat (a social interaction test) in a neutral housing cage without food and water for 20 min, followed by a FCT in which the rats had to compete for Chocopops for 10 min (see Supplementary Materials). After the tests, each rat was returned to its homepage. The group without an encounter on day 1 was exposed to an extra day of habituation.

The memory for the formed hierarchy was tested in a water competition test (WCT) that was performed on day 8 following a water deprivation period of 6 h. Rats were exposed to the same opponent as on day 1. The WCT was performed in a neutral homepage. After 2 min of habituation, a single bottle of water was presented and the behavior during the following 10 min was recorded. Figure 1a shows a scheme with the time line of the experiments.



**Figure 1** Scheme showing the time line of the experiments (a) The time line for the standard experiment. (b) The time line for the experiment including brain sampling 3 h after the first encounter for gene expression analyses. (c) The time line for the experiment, in which rats in each pair received infusions into the MeA immediately after the first encounter of either vehicle in both cases or vehicle to the dominant rat and OTA to the subordinate rat. (d) The time line for the control experiment, in which rats in each pair were, respectively, infused with either vehicle or OTA into the MeA 1 week before their social encounter.

Behavior was video recorded and scored blindly using The Observer software (v.5.0.25, Noldus IT, The Netherlands). The duration and frequency of offensive and defensive behaviors were scored. Offensive behaviors were attacks, keeping down, offensive upright, and lateral threat. Defensive behavior consisted of freezing, defensive upright, and submissive posture (as described in Timmer and Sandi, 2010). The rat that showed the most offensive behaviors during the interaction was considered the dominant rat, and the rat showing the least offensive behaviors and the most defensive behaviors was considered the subordinate rat.

Two control groups were added for the gene expression experiment. The first control group was submitted to habituation, but not to a social encounter. The stressed control group was submitted to habituation and to foot-shock stress (as described above) but not to a social encounter.

### Brain Sampling and Gene Expression

For the gene expression experiment rats were killed 3 h after the end of the FCT. The animals were decapitated, and the brains were quickly removed, frozen in isopentane at a temperature between  $-50$  and  $-40$  °C, and stored at  $-80$  °C until further processing. Figure 1b shows a scheme with the time line of the experiments. See Supplementary Materials and Methods for details on the processing of the samples for real-time PCR (Table 1).

To check the position of the cannulae in the OTA antagonist experiment, rats were decapitated, and the brains were removed, frozen in isopentane, and stored at  $-80$  °C until further processing. Coronal sections (40- $\mu$ m thick) were stained with thionin to detect the cannulae position. Animals in which the cannula was not in the right location were eliminated from the study.

### Drugs and Infusion Procedure

The selective OT antagonist (OTA) desGly-NH<sub>2</sub>-d(CH<sub>2</sub>)<sub>5</sub>[D-Tyr<sup>2</sup>,Thr<sup>4</sup>]OVT was used for infusions into the MeA (generous gift of Dr M Manning, Toledo, OH, USA). For details on the procedures followed for surgery and cannulation, see Supplementary Materials and Methods. The OTA antagonist was dissolved in saline. For MeA infusions, 11-mm internal injectors (extending 1 mm from the end of the guide cannula, 30 gauge, Plastics One) were used to administer the drug. The injectors were connected to 10  $\mu$ l microsyringes (SGE Analytical Science, Australia) on an automated infusion pump (Harvard Apparatus, Holliston, MA, USA) through polyethylene tubing. Drug dose was selected according to previous evidence in the literature. Thus, 1  $\mu$ g of OTA (Neumann *et al*, 2000) in 1  $\mu$ l of saline was infused into the MeA of each hemisphere at a rate of 0.5  $\mu$ l/min. After infusion, the injector was left in place for 1 min to allow diffusion of the drug. Saline was used as a vehicle. The OTA was infused into the subordinate rat immediately after the encounter or after the last habituation session in the group that was not exposed to a social encounter on day 1, which corresponds with the time when a change in OTR mRNA levels was found. The other rat of each pair and both rats from control pairs were infused with vehicle. In addition, a control experiment was

performed in which animals were injected with either vehicle or OTA without concomitant exposure to social encounter and their social rank was tested 1 week after injection. Figures 1c and d show the experimental time line of the drug experiments.

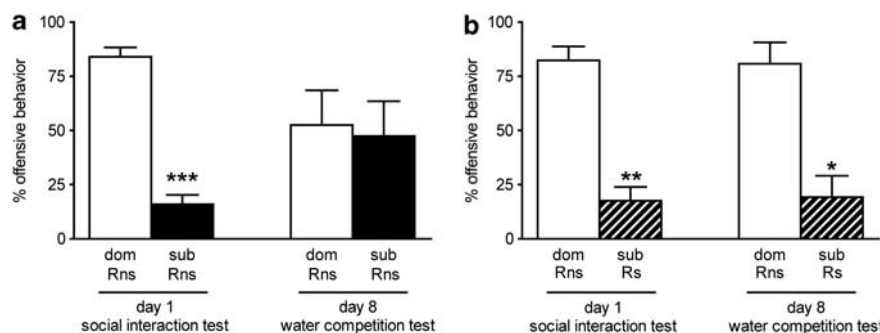
### Statistics

All results are expressed as the mean  $\pm$  SEM. For the behavioral experiments, the percentage of offensive behaviors for each rat within each dyad was calculated. A paired two-tailed Student's *t*-test was used to compare the percentage of offensive behaviors within a dyad of rats competing together. For statistical analyses of mRNA expression levels, data for experimental groups are expressed as fold change with regards to the non-stressed control group. ANOVA was performed to compare the mRNA expression levels across the different experimental groups (ie, dominant and subordinate rats from each dyad) and post hoc LSD tests performed when appropriate. Only significant results are indicated in the text. The computer software SPSS for Windows (v.13.0) was used for statistical analysis.  $P \leq 0.05$  was considered significant.

## RESULTS

### Exposure to Acute Stress Induces a Long-Term Memory for the Established Hierarchy

First, we confirmed our previous observation that exposing one rat of a dyad to acute stress before a first encounter induces a long-term memory for the establishment of a hierarchy that is not observed in non-stressed dyads (Cordero and Sandi, 2007). As expected, we found that under non-stress conditions (Pns, consisting of two non-stressed rats, Rns), rats form a hierarchy when exposed to a first social encounter on day 1 ( $t = 7.84$ ,  $df = 6$ ,  $p < 0.001$ ), but the hierarchy is not maintained on day 8 (Figure 2a). In the condition, in which one of the rats in each dyad is exposed to acute stress (Ps, consisting of one non-stressed rat Rns and one stressed rat Rs), the stressed rat becomes subordinate during the first encounter, and the hierarchy is still observed 1 week later ( $t = 5.08$ ,  $df = 5$ ,  $p < 0.01$  for day 1,  $t = 3.11$ ,  $df = 5$ ,  $p < 0.05$  for day 8, Figure 2b).

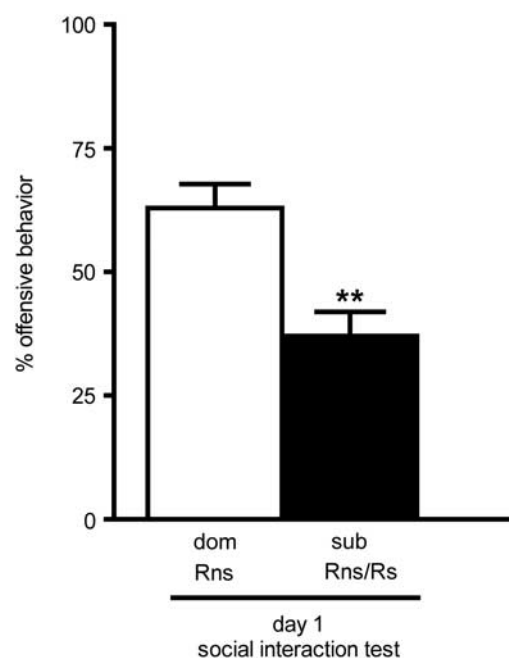


**Figure 2** Percentage of total offensive behaviors (mean  $\pm$  SEM) between two opponents in the social interaction test on day 1 and the water competition test on day 8. (a) The hierarchy of rats of non-stressed pairs ( $n = 7$ ). (b) The hierarchy of rats of the stressed pairs ( $n = 6$ ). The status 'dom' (dominant) or 'sub' (subordinate) refers to the status that was obtained during the social interaction test on day 1. Rns, non-stressed rat; Rs, stressed rat. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs the other rat of the dyad.

### OTR and V1ar mRNA Expression 3 h after a First Social Encounter

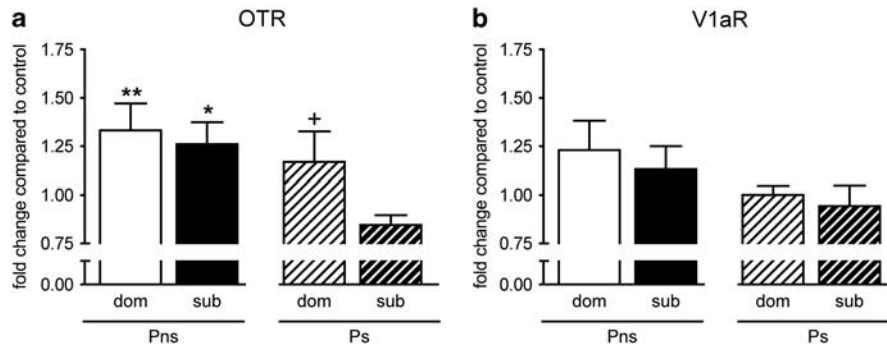
Next, we evaluated the effects of stress and hierarchy formation on the expression levels of *Otr* and *V1ar* mRNA in the MeA and LS occurring in the 3 h after the first social encounter. At the behavioral level, we confirmed that both Pns and Ps dyads established a dominance hierarchy during the first social encounter ( $t = 2.80$ ,  $df = 25$ ,  $p < 0.01$ , Figure 3). Rats from each experimental group were killed 3 h after the end of the first encounter.

ANOVA indicated a difference in *Otr* mRNA expression in the MeA across the different experimental groups ( $F_{3,48} = 2.87$ ,  $p < 0.05$ , Figure 4a). *Post hoc* tests show that animals from the Ps subordinate group displayed significantly lower *Otr* mRNA expression in MeA than each of the other experimental groups (vs Ps dominant rats:  $p < 0.01$ ;



**Figure 3** Percentage of total offensive behaviors (mean  $\pm$  SEM) between two opponents of non-stressed and stressed pairs in the social interaction test on day 1 ( $n = 20$ ). dom, dominant; sub, subordinate; Rns, non-stressed rat; Rs, stressed rat. \*\* $p < 0.01$  vs the other rat of the dyad.





**Figure 4** Expression of neuropeptide receptors in the MeA 3 h after the first social encounter (mean  $\pm$  SEM). (a) Expression of *Otr* mRNA. (b) Expression of *V1aR* mRNA. Dom, dominant; sub, subordinate; Pns, non-stressed pair; Ps, stressed pair.  $^+p < 0.08$ ,  $^*p > 0.05$ ,  $^{**}p > 0.01$  vs Ps subordinate. Each group consists of 12–14 animals.

vs Pns subordinate rats:  $p < 0.05$ ; vs Pns dominant rats:  $p < 0.08$ ; note that the latter only shows a trend towards significance). The stress control group did not differ from unstressed controls ( $0.95 \pm 0.1$  for stressed controls,  $1.00 \pm 0.1$  for unstressed controls, ns, Figure 4a). At this time point, no significant differences between groups were observed for *V1aR* mRNA levels in either the MeA (Figure 4b), not for *Otr* or *V1aR* mRNA expression levels in LS (data not shown).

#### Infusion of a Selective OTA into the MeA

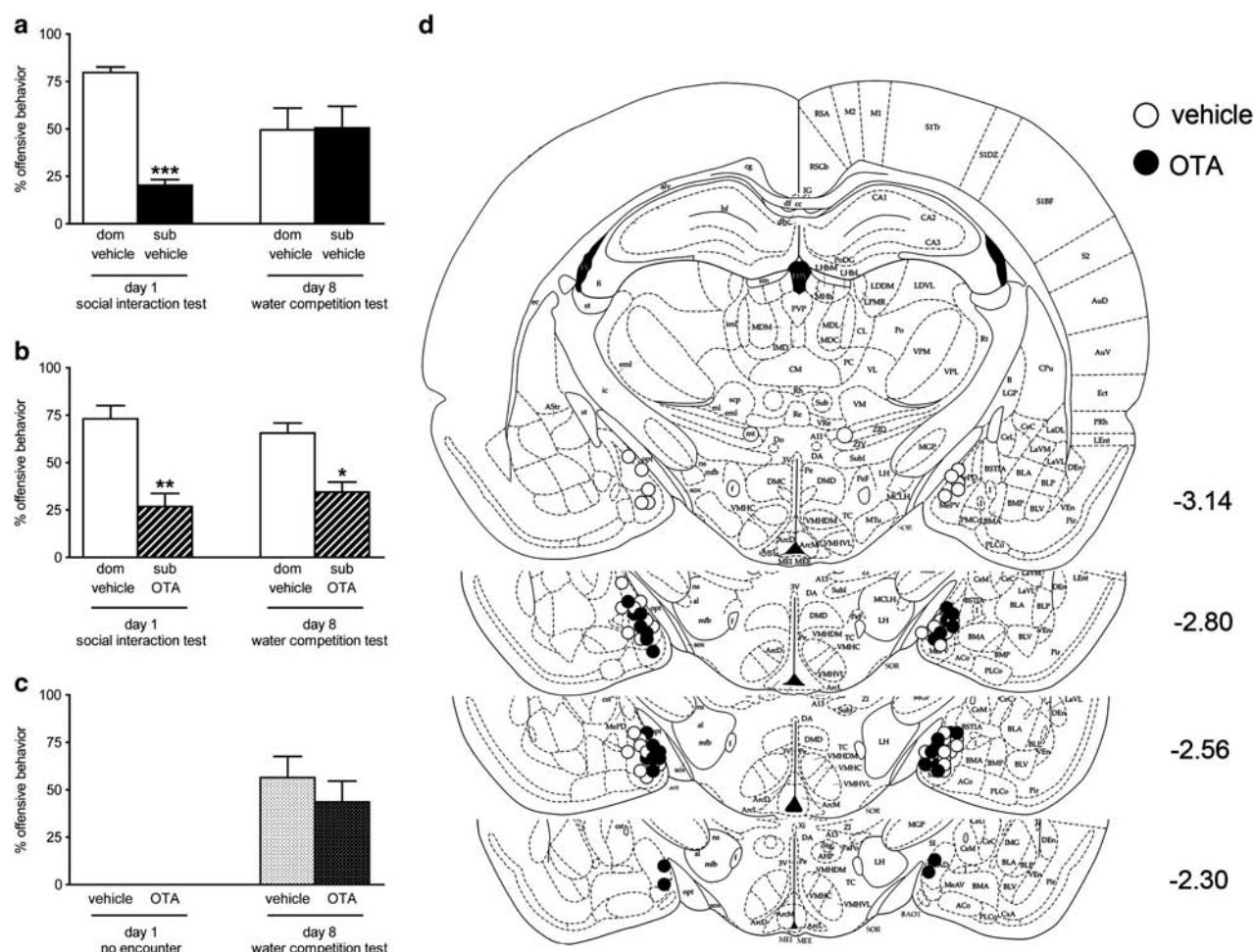
Given that the Ps subordinate group showed significantly lower *Otr* mRNA levels in the MeA than the other experimental groups 3 h after the first encounter, we reasoned that if this molecular change is involved in the long-term maintenance of the hierarchy, antagonizing OTRs in subordinate males that had not been previously stressed would facilitate a long-term establishment of the hierarchy. Therefore, in this experiment, we micro-infused a selective OTR antagonist (OTA), desGly-NH<sub>2</sub>-d(CH<sub>2</sub>)<sub>5</sub> [D-Tyr<sup>2</sup>,Thr<sup>4</sup>]OVT, 1  $\mu$ g/ $\mu$ l, into the MeA of non-stressed (Pns) subordinate rats immediately after the first encounter on day 1. The long-term establishment of the hierarchy was tested in a WCT 1 week after the first encounter. As a control, we verified that vehicle infusions given in the MeA immediately after hierarchy establishment on a first encounter ( $t = 10.07$ ,  $df = 7$ ,  $p < 0.001$ ) did not affect the outcome of the hierarchy normally observed in non-infused, unstressed animals when tested 1 week later. As shown in Figure 5a, no evidence for a memory of the hierarchy was observed at this later time point. As hypothesized, when OTA was infused into the subordinate rat immediately after the first encounter ( $t = 3.34$ ,  $df = 9$ ,  $p < 0.01$ ), the hierarchy is maintained 1 week later ( $t = 2.91$ ,  $df = 9$ ,  $p < 0.05$ , Figure 5b), suggesting a role for OTR in MeA in the long-term establishment of a hierarchy. In addition, to exclude a potential delayed effect of OTA infusion independent of its association with the formation of a social hierarchy, we infused OTA into the MeA in one rat per dyad, and we tested its effect on the establishment of a hierarchy 1 week later. As shown in Figure 5c, no effect was found in the establishment of a hierarchy during the WCT on day 8 when OTA was infused on day 1 in animals that did not receive an associated social encounter. Figure 5d shows a representative example of the injection site in the MeA.

#### DISCUSSION

This study shows that the potentiation of a stress-induced social hierarchy in male rats is accompanied by social status- and region-specific changes in the expression of *Otr* mRNA levels in the MeA during the hours after the hierarchy establishment. As previously shown (Cordero and Sandi, 2007), the dominance hierarchy established between a pair of rats confronted for the first time under our experimental conditions is not maintained during a second competition test 1 week later. However, when one animal from each dyad was stressed, the stressed rat became subordinate during the first encounter; the same hierarchy was observed in a WCT 1 week later. Thus, 3 h following the first social encounter, the Ps subordinate group showed lower *Otr* mRNA expression levels in the MeA than animals that become subordinate without having been submitted to former stress and whose established hierarchy is not maintained 1 week afterwards. No evidence was found for changes in the expression of *V1aR* mRNA in the MeA, or for the levels of any of these two receptor types in the LS.

In a pharmacological experiment designed to antagonize OTR in the MeA following the establishment of a subordinate status under unstressed conditions, a long-term facilitation of the subordinate status and social hierarchy was found. Specifically, a long-term expression of the dominance hierarchy was promoted by microinfusing rats in the Pns subordinate group (from the Pns dyads that do not form an enduring hierarchy), with the OTR antagonist into the MeA immediately after the first social encounter. Notably, further pharmacological experiments excluded potential effects of the oxytocin antagonist that were not associated to the social processes under study. Thus, when the antagonist was administered in the MeA in animals that were not exposed to a first social confrontation, no effect was observed in the WCT performed 1 week afterwards. These results support the hypothesis that the effects of antagonizing OTR in the MeA after a first encounter are dependent on their combination with the social experience.

Substantial evidence indicates that the neuropeptides OT and AVP have an important role in the regulation of social behaviors (Ferguson *et al*, 2000, 2001; Bielsky and Young, 2004; Crawley *et al*, 2007). The effect of OT on social recognition in males has been suggested to follow an inverted U-shape response, with administration of low doses of OT facilitating and high doses inhibiting



**Figure 5** Percentage of total offensive behaviors (mean  $\pm$  SEM) between two opponents in the social interaction test on day 1 and the water competition test on day 8. (a) The hierarchy of rats that were infused with vehicle ( $n = 8$ ). (b) Dyads in which the subordinate rat was infused with OTA immediately after the first encounter ( $n = 10$ ). (c) Rats that were infused with either vehicle or OTA on day 1 without exposure to an encounter until day 8 ( $n = 7$ ). (d) Schematic representation of the injection site into the MeA. The status 'dom' (dominant) or 'sub' (subordinate) refers to the status that was obtained during the social interaction test on day 1. OTA, oxytocin antagonist. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs the counterpart rat of each dyad.

recognition of a juvenile rat (Popik and Van Ree, 1991; Popik and Vetulani, 1991; Popik *et al*, 1992; Benelli *et al*, 1995). In females, infusions of either OTA (Engelmann *et al*, 1998) or OTR antisense DNA into the MeA (Choleris *et al*, 2007) interferes with social recognition. In all these cases, manipulations targeting the oxytocinergic system took place before the social interaction. In our study, OTA was infused after the encounter, and its effect was evaluated 1 week later. This result, along with the lower *Otr* mRNA levels found in the MeA of the 'stressed and subordinated' group than in the 'unstressed and subordinated', status of which is not maintained in the long term, indicates a role for this system in the MeA toward the potentiation of memory for the subordinate status after a first social encounter. Exposure to stressors was shown to increase the release of OT in different brain areas (Wotjak *et al*, 1998; Engelmann *et al*, 1999; Neumann, 2007; Ebner *et al*, 2000, 2005), and cell culture experiments showed that OT can lead to a downregulation of OTR (Insel *et al*, 1992; Di Scala-Guenot and Strosser, 1995). Although we could not measure neuropeptide release in the MeA during the social

encounter due to technical limitations, it is tempting to speculate that OT release in the MeA during the first encounter in the stressed animals differed from the other experimental groups. Moreover, OT release in the amygdala was shown to lead to a more passive coping style (Ebner *et al*, 2005). A passive coping style is associated with a lower aggression level (reviewed by Koolhaas *et al*, 2010). Thus, in our model, exposure to acute stress might lead to a more passive coping style through an increased OT release in the amygdala, and this, in turn, has a role in the stressed rat becoming subordinate.

Interestingly, the MeA is one of the first brain areas within the social information processing pathways to become activated after exposure to a conspecific mice (Bielsky and Young, 2004; Ferguson *et al*, 2001). Work in OT knockout mice has shown that OT in the MeA is essential for the processing or initial retention of social information. These mice can form a spatial memory and have normal smell, but cannot form social memories. Infusion of OT into the MeA is sufficient to restore the social memory (Ferguson *et al*, 2000, 2001). OT knockout

mice show normal social approach, social interaction and perception and recognition of social cues, which suggests that OT is selectively required for higher-order processing of social memory formation (Crawley *et al*, 2007). Therefore, it is plausible to hypothesize that the stress-induced enhancement of a memory for the subordinate status is linked to increased OT release in the MeA at the time of the first encounter and to the subsequent lower OTR expression.

Although we did not find a regulation of the V1aR, we cannot exclude the implication of these receptors in the phenomenon under study at other time points than the ones selected here for the gene expression analyses. Strong evidence in the literature links the vasopressinergic system and the lateral septum with social recognition. In rats, systemic or central AVP injections, as well as administration of *V1ar* overexpressing viral vectors in the LS, were shown to enhance social recognition (Le Moal *et al*, 1987; Dantzer *et al*, 1988; Popik *et al*, 1991; Popik and Van Ree, 1992; Engelmann and Landgraf, 1994), while converse effects (ie, disruption of social recognition) were found when V1aR antagonists were given or when V1aR were downregulated in the LS (Dantzer *et al*, 1987, 1988; Bluthé *et al*, 1990; Landgraf *et al*, 2003; van Wimersma Greidanus and Maigret, 1996; Everts and Koolhaas, 1997, 1999). V1aR knockout mice show impaired social recognition, with the LS, not the MeA, being particularly important for this effect (Bielsky *et al*, 2004). Increased levels of AVP release and V1aR activation have been suggested to have a role in increasing the duration of social memories (Bielsky *et al*, 2005). Thus, it is tempting to speculate that this receptor system might be involved in the modulation of the long-term expression of the subordinate status by stress. Further studies are in progress to evaluate this hypothesis.

The question arises as to which mechanisms could be engaged by the oxytocinergic system in its effect on the long-term establishment of subordinate social status. Although evidence is still scarce directly linking its memory-related effects and mediating mechanisms, there is increasing evidence supporting a potential link between the oxytocinergic system and glutamatergic transmission. Specifically, this link has been established in the amygdala in the context of coping with stress (Ebner *et al*, 2005; Bosch *et al*, 2007). Given the importance of the glutamatergic system in learning and memory processing, further studies are warranted to explore the potential role of this neurotransmitter system in the context of the behavioral effects described in this study.

In summary, we have presented evidence indicating that, along with the potentiation of a subordinate phenotype, stress triggers the OT neuropeptide pathway in the MeA, a brain region critically implicated in social recognition. Altogether, our findings suggest a role for the OTR in the MeA in the initial stages of the long-term establishment of a subordinate status within a dominance hierarchy.

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## DISCLOSURE

The authors declare no conflict of interest.

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