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The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rodents

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ABSTRACT AND KEYWORDS

Social avoidance and social phobia are core symptoms of various psychopathologies but their underlying aetiology remains poorly understood. Therefore, this study aims to reveal pro-social effects of the neuropeptide oxytocin, under both basal and stress-induced social avoidance conditions in rodents using a social-preference paradigm. We initially show that intracerebroventricular (icv) application of an oxytocin receptor antagonist (OTR-A) in naïve male rats (0.75µg/5µl) or mice (20µg/2µl) reduced social exploration of a novel con-specific indicative of attenuated social preference. Prior exposure of male rats to a single social defeat resulted in loss of their social preference and social avoidance, which could be restored by icv infusion of synthetic oxytocin (0.1µg/5µl) 10 min prior to the social preference test. While the amygdala has been implicated in both social and oxytocin-mediated actions, bilateral oxytocin receptor antagonist (0.1 µg/1µl) or oxytocin (0.01 µg/1µl) administration into various subnuclei of the amygdala did not affect basal or stress-induced social preference behavior, respectively. Finally, we demonstrate the social specificity of these oxytocin-mediated effects by showing that neither an arginine vasopressin V1a receptor antagonist (0.75µg/5µl, icv) nor the anxiogenic drug pentylenetetrazol (15mg/kg, i.p.) altered social preference, with oxytocin receptor antagonist not affecting state anxiety. Overall, the data indicate that the basal activity of the endogenous brain oxytocin system is sufficient to promote pro-social behavior in rodents while synthetic oxytocin shows potential to reverse stress-induced social avoidance and might thus be of use for treating social phobia and social dysfunction in humans.

Keywords: oxytocin, social interaction, social defeat, amygdala, vasopressin, anxiety

INTRODUCTION

Animal and human studies indicate a facilitatory role of the neuropeptide oxytocin (OT) in a broad variety of social interactions. Released within the brain (for reviews see Landgraf and Neumann, 2004; Neumann, 2009), OT promotes various aspects of social behavior in both females and males related to reproduction, including the onset and fine-tuned maintenance of maternal behavior in lactation (Bosch *et al.*, 2005; Kendrick, 2000; McCarthy, 1990; Pedersen *et al.*, 1982; van Leengoed *et al.*, 1987), receptive behavior of female rats (Schulze and Gorzalka, 1991; Witt and Insel, 1991), various aspects of sexual behavior in males (Argiolas and Melis, 2004), as well as pair bonding in female voles (Cho *et al.*, 1999; Williams *et al.*, 1994). Furthermore, chronic central OT administration increases social interactions of male with female rats (Witt *et al.*, 1992). Also, OT plays a role in social recognition in both male (Popik and van Ree, 1991) and female (Engelmann *et al.*, 1998) rats, which has been confirmed in OT and OT receptor knockout mice with impaired social memory (Ferguson *et al.*, 2000; Choleris *et al.*, 2003; Takayanagi *et al.*, 2005).

Intranasal OT administration has been shown to affect many aspects of human sociability ranging from social perception, increased gazing towards the eye region (Gamer *et al.*, 2010; Guastella *et al.*, 2008), and improved recognition of emotional facial expressions (Domes *et al.*, 2007; Savaskan *et al.*, 2008) to complex social behaviors like trust, social-risk taking, and empathy (Baumgartner *et al.*, 2008; Hurlemann *et al.*, 2010; Kosfeld *et al.*, 2005). The amygdala, a brain region strongly involved in social perception and emotional processing, has been implicated as one of the key regions mediating neuronal actions of OT on social behaviors in humans (Baumgartner *et al.*, 2008; Gamer *et al.*, 2010; Hurlemann *et al.*, 2010; Kirsch *et al.*, 2005) as well as in rodents (Choleris *et al.*, 2007; Ferguson *et al.*, 2001; Lee *et al.*, 2007).

In addition to these multiple effects on sociability, brain OT functions as an endogenous anxiolytic neuropeptide in females (Neumann *et al.*, 2000b) and males (Waldherr and Neumann, 2007), and acute or chronic administration of synthetic OT reduces anxiety-related behavior in rodents (Blume *et al.*, 2008; Ring *et al.*, 2006; Slattery and Neumann, 2010; Windle *et al.*, 1997). The anxiolytic effect of OT could be localized within both the amygdala of females (Bale *et al.*, 2001; Neumann, 2002) and the hypothalamic paraventricular nucleus (PVN) of males (Blume *et al.*, 2008). Based on these behavioral effects, we recently succeeded in showing that the prolonged anxiolysis observed following sexual activity in male rats is mediated by a mating-induced release of OT within the PVN (Waldherr and Neumann, 2007).

Based on the dual pro-social and anxiolytic effects of OT, it is anticipated that the endogenous OT system would also reduce social anxiety and increase social preference in a non-reproductive context. In support of this notion, OT homologues, such as isotocin and mesotocin, have been shown to promote sociability in fish and birds, respectively (Goodson *et al.*, 2009; Thompson and Walton, 2004). A transfer of this concept to rodents is strongly supported by data demonstrating that the prominent pro-social effect of 3,4-methylenedioxymethamphetamine (“ecstasy”) in rats is, at least partially, mediated via the endogenous OT system (Thompson *et al.*, 2007). Such facilitatory effects of OT on social preference are of particular importance in the context of social dysfunctions in humans, which represent core symptoms of various neuropsychiatric disorders including anxiety- and depression-related diseases (Merikangas and Angst, 1995) and autism spectrum disorders (Caronna *et al.*, 2008). Interestingly, intranasal OT has recently been shown to promote social approach and social comprehension in autistic patients (Andari *et al.*, 2010; Guastella *et al.*, 2010; Guastella *et al.*, 2009; Hollander *et al.*, 2007).

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Here, we hypothesize that brain OT promotes naturally occurring, i.e. normal, social preference and prevents social defeat-induced social avoidance in rodents. Specifically, we predict that blockade of the endogenous OT system by administration of a specific OT receptor antagonist (OTR-A), both icv and into various sub-regions of the amygdala, can reduce the preference for a social stimulus over a non-social stimulus. Further, we predict that central administration of synthetic OT will reverse social defeat-induced social avoidance. Finally, in order to exclude any direct interaction between non-social and social anxiety, effects of the anxiogenic compound pentylenetetrazol (PTZ) on social preference and of the OTR-A on non-social anxiety were tested.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (250-300 g) and adult male C57BL/6 mice (20-24 g) were purchased from Charles River (Sulzfeld, Germany) and group-housed in standard cages (rats: 55 × 22 × 18 cm, mice: 16 x 22 x 14 cm) for habituation to the laboratory facilities under standard laboratory conditions (12:12 h light:dark cycle, lights on at 6.00 h, 22 °C, 60 % humidity, food and water ad libitum). After surgery, subjects were transferred to observation cages (rats: 40 x 24 x 36 cm, mice: 16 x 22 x 14 cm) and single housed till the end of the experiment. For social defeat, male rats selectively bred for low anxiety-related behavior (LAB) with reliable high level of inter-male aggression (Beiderbeck *et al.*, 2007; Neumann *et al.*, 2010) were used as residents. Experiments were performed in the first 3 hours of the light or dark-phase and conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Oberpfalz and the guidelines of the NIH.

Social preference paradigm

The social preference paradigm in rats was based on the social approach-avoidance test previously described in mice (Berton *et al.*, 2006). In our study, the test has been modified and used both in rats and mice. The species-dependent durations of habituation, presence of object stimulus, presence of social stimulus and light conditions were determined based on a series of pilot experiments assessing the duration-dependent exploratory behavior in rats (arena and home cage) and mice (arena).

Social preference in novel arena

Rats and mice were placed in a novel arena (rat: 40 x 80 x 40 cm, red light; mouse: 28 x 46 x 27 cm, 100 lux; Fig. 1). After 30 sec of habituation an empty wire-mesh cage (object stimulus; rat: 20 x 9 x 9 cm; mouse: 11 x 9 x 6 cm) was placed at one side wall of the arena for 4 min (rats) or 2.5 min (mice). The empty cage was then exchanged by an identical cage containing an unknown male con-specific (social stimulus) for additional 4 min (rats) or 2.5 min (mice). Before each trial, the arena was cleaned with water containing a low concentration of detergent. In rats, all tests took place during the active phase starting one hour after lights off using an unknown Wistar rat as social stimulus. Mice were tested in the early light phase (one hour after lights on) using an unknown male CD1 mouse as social stimulus.

Social preference in home cage

In order to demonstrate that OT-induced changes in social preference are to a large extent independent of the environment, light conditions, and the quality of the social stimulus, rats were additionally tested for social preference in their home cage with slight modifications. Specifically, a red plastic ring (inner diameter 8.3 cm, outer diameter 9.4 cm, 2.8 cm wide)

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was used as object stimulus during the 4-min object stimulus period. Subsequently, the time investigating a freely moving 3-week-old male Wistar rat - used as social stimulus - was assessed for additional 4 min after removal of the ring. A juvenile rat was chosen as social stimulus because it doesn't elicit aggressive behavior in the experimental rats. The social preference test was performed in the light phase under standard laboratory illumination.

Each test procedure was videotaped and scored afterwards by an observer blind to the treatment using JWatcher behavioral observation software (V 1.0, Macquarie University and UCLA). Object and social stimulus times were scored by measuring the time the rat/mouse spent in active olfactory investigation. As total exposure time differs between rats and mice, data are presented as the percentage of time investigating the object stimulus and the percentage of time investigating the social stimulus, i.e. investigation time / total time [4 min / 2.5 min] \times 100 %. A significantly higher percentage of investigation of the social versus the non-social stimulus was considered social preference. Loss of this difference was defined as lack of social preference or accordingly social avoidance.

Elevated plus-maze

The elevated plus-maze was used to assess the effects of the OTR-A or of PTZ on non-social anxiety-related behavior in both rats and mice (Pellow *et al.*, 1985). It consisted of two opposing open arms (rat: 50 x 10 cm; mice: 6 x 30 cm; 100 lux) and two opposing closed arms (rat: 50 x 10 x 40 cm; mice: 6 x 30 x 17 cm; 20 lux) connected by a central area. The apparatus was made of dark grey plastics, elevated to a height of 80 cm (rats) or 35 cm (mice) above the floor, and surrounded by an opaque curtain to avoid external disturbance. Before each trial, the maze was cleaned with water containing a low concentration of detergent. Rats and mice were placed individually in the central area facing a closed arm and were allowed to explore the maze for 5 min. The percentage of time spent on the open arms was assessed as

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anxiety-related behavior (time on open arms/time on open and closed arms \times 100); the number of entries in the closed arms as measurement for locomotion was recorded by means of a video camera mounted above the platform and scored by a trained observer (Plus-maze version 2.0; E. Fricke). The elevated plus-maze testing was performed in the light phase.

Black-white box

To assess non-social anxiety-related behavior in an additional test, rats were monitored in the black-white box for 5 min (Waldherr and Neumann, 2007; Slattery and Neumann, 2010) (white compartment: 40 x 50 cm, 350 lux; black compartment: 40 x 30 cm, 70 lux). The black-white box was surrounded by an opaque curtain to avoid external disturbances. Rats were placed in the dark compartment, and the percentage time spent in the white compartment (time in white compartment / total time [5 min] \times 100 %) was assessed as anxiety-related behavior. Testing was performed during the early-dark phase.

Experimental design

Involvement of endogenous OT in social preference in rats and mice

To reveal the involvement of the brain OT system in basal social preference, the following experiments were carried out in male rats and mice (for group sizes see corresponding figures).

Rats: Rats were icv infused with either OTR-A (0.75 μ g/5 μ l) or vehicle 20 min (see Supplementary Materials and Methods) before testing in the social preference paradigm in the neutral arena. A separate group of rats received the same treatments and were tested for social preference in their home cage. In addition, another group of rats was icv infused with the selective vasopressin 1a receptor antagonist (V1aR-A; 0.75 μ g/5 μ l) or vehicle 20 min (see Supplementary Materials and Methods) prior to the social preference paradigm in the arena to

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test if the closely related brain neuropeptide vasopressin also influences social preference. Finally, to localize the positive effect of endogenous OT on social preference, additional rats were bilaterally infused with OTR-A at two different doses (0.1 μ g/ μ l and 1.0 μ g/ μ l) or vehicle into the central, medial, or basolateral amygdaloid nuclei (see Supplementary Materials and Methods) and tested in the social preference paradigm in the arena 10 min later.

Mice: Mice were icv infused with either OTR-A (20 μ g/2 μ l or 2 μ g/2 μ l) or vehicle (see Supplementary Materials and Methods) 20 min before testing in the social preference paradigm in the arena.

Social defeat-induced social avoidance and reversal by synthetic OT in rats.

First, we aimed to verify whether exposure to a single social defeat (see Supplementary Materials and Methods) induces social avoidance. Rats were tested once in the social preference paradigm 2 h after onset of the 30-min social defeat using either the former defeater rat or an unknown male rat as social stimulus. Second, in order to test the hypothesis that social defeat-induced social avoidance can be reversed by OT, male rats were socially defeated and OT (0.1 μ g/5 μ l) or vehicle were icv infused (see Supplementary Materials and Methods) 20 min before the social preference test using the former defeater rat as social stimulus. Third, to localize the positive effect of OT on social preference in defeated rats, socially defeated rats were bilaterally infused with either OT (0.01 μ g/ μ l) or vehicle into the central or medial nucleus of the amygdala (see Supplementary Materials and Methods) 10 min before being tested in the social preference paradigm (for group sizes see figures).

Involvement of non-social anxiety in the display of social preference in rats and mice

To test if the lack of social preference after blockade of brain OT receptors is specific for social anxiety, additional groups of rats and mice received the following treatments (for group sizes see figures).

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Rats: Rats were icv treated with OTR-A (0.75µg/5µl) or vehicle (see Supplementary Materials and Methods) 20 min before testing for non-social anxiety-related behavior in the black-white box.

Mice: Mice were icv treated with OTR-A (20µg/2µl) or vehicle (see Supplementary Materials and Methods) 20 min before testing non-social anxiety-related behavior on the elevated plus-maze.

Rats: To further test for a possible interaction between general state anxiety and social preference, rats were given a single injection of the anxiogenic agent PTZ (15mg/kg, ip) or vehicle (see Supplementary Materials and Methods) and tested 15 min later on the elevated plus-maze or in the social preference paradigm.

Statistics

For statistical analysis PASW/SPSS Statistics (Version 17.0) was used. Social preference was statistically analyzed using ANOVA for repeated measures (two-way: factors treatment × stimulus; three-way: factors treatment × stimulus × defeat) followed by a Bonferroni post-hoc test when appropriate. All relevant F-values are provided in Table 1. Non-social anxiety behavior was analyzed using the student's t-test. Significance was accepted at $p < 0.05$.

RESULTS

Involvement of endogenous OT in social preference

Rats: Icv OTR-A-treated rats showed a significant decrease in social investigation during the social preference test in the arena ($p < 0.01$ versus vehicle; Fig. 1). Still, both OTR-A- and vehicle-treated rats investigated the social stimulus significantly longer than the object stimulus ($p < 0.05$; Fig. 1), thereby demonstrating social preference. Central infusion of the

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V1aR-A did not change social investigation time, and thus, both vehicle- and V1aR-A-treated groups demonstrated a preference for the social compared to the object stimulus ($p < 0.01$, Fig 1).

In line with the results in the novel arena, when tested in their home cage, icv OTR-A-treated rats showed a decrease in social investigation ($p = 0.055$ versus vehicle; Fig 1). As a result, OTR-A-treated rats showed no social preference (stimulus versus object: $p = 0.673$, Fig. 1).

Bilateral infusion of either $0.1 \mu\text{g}/\mu\text{l}$ or $1.0 \mu\text{g}/\mu\text{l}$ OTR-A into either the central, medial, or basolateral amygdala did not result in altered social investigation times compared with respective vehicle-groups (Fig. 2). All rat groups independent of treatment showed social preference ($p < 0.05$ versus object stimulus, Fig. 2).

Mice: Similar to rats, icv OTR-A-treated mice showed a significant decrease in social investigation ($p < 0.05$ versus vehicle, Fig. 1). In contrast to rats, OTR-A ($20 \mu\text{g}/2 \mu\text{l}$) treatment induced social avoidance in mice, thereby completely preventing social preference ($p = 0.70$ versus object stimulus, Fig. 1). A ten-fold lower dose did not alter any behavior assessed in the social preference paradigm (data not shown).

Social defeat-induced social avoidance and reversal by icv OT infusion in rats

To demonstrate that a single exposure to social defeat induces social avoidance, male rats were defeated by an aggressive larger male rat and then tested in the social preference paradigm 2 h later. When an unknown male rat was used as social stimulus, both control and defeated rats showed a preference for the unknown social stimulus ($p < 0.05$ versus object stimulus, Fig. 3A). In contrast, exposure of the defeated males to their former defeater rat resulted in social avoidance, as demonstrated by a significant decrease in social stimulus investigation ($p < 0.05$ vs control rats, Fig. 3B) and a lack of social preference.

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To test whether OT can reverse defeat-induced social avoidance, defeated rats were icv or locally infused with either synthetic OT or vehicle. Whereas undefeated rats treated with either vehicle or OT showed social preference ($p < 0.05$, Fig. 4), vehicle-treated defeated rats lost their preference for the social stimulus (former defeater, $p = 0.468$ versus object stimulus, Fig. 4). The stress-induced social avoidance seen in defeated rats could be prevented by icv OT 20 min prior to the social preference test, resulting in reinstatement of social preference towards the former defeater ($p < 0.05$ social versus object stimulus, Fig. 4). Infusion of synthetic OT into either the central or medial amygdala of defeated rats failed to increase social investigation time towards the former defeater resulting in a similar lack of social preference as seen in vehicle-treated defeated rats (Fig. 4).

Involvement of general anxiety in the display of social preference in rats and mice

Rats: Icv OTR-A infusion in male rats did not change non-social anxiety-related behavior in the black-white box (percentage time spent in the white compartment of the black-white box; $t_{(14)} = 1.07$, $p = 0.302$) (Fig. 5A).

Mice: Similarly, non-social anxiety-related behavior on the elevated plus-maze following icv OTR-A infusion remained unchanged in mice ($t_{(9)} = -0.65$, $p = 0.531$, Fig. 5A).

Rats: As expected, injection of the anxiogenic drug PTZ in rats increased anxiety-related behavior on the elevated plus-maze as demonstrated by a reduction in the time spent on the open arms of PTZ-treated rats compared with vehicle-treated rats ($t_{(16)} = -3.36$, $p < 0.01$, Fig. 5B). Importantly, PTZ did not impair social preference. Here, both vehicle- and PTZ-treated rats spent more time investigating the social stimulus than the object stimulus ($p < 0.05$, Fig. 5C).

DISCUSSION

The present results provide the first direct evidence that the endogenous OT system facilitates social preference in both male rats and mice. More specifically, central blockade of OT receptors, using an OTR-A, significantly reduced normal social preference towards a novel con-specific in both species. Moreover, centrally-applied synthetic OT was found to rescue normal social preference that had been abolished by prior exposure to an acute social defeat. Furthermore, we were able to dissociate social avoidance from non-social anxiety, as the anxiogenic drug PTZ did not alter social preference in rats, while centrally-infused OTR-A did not alter non-social anxiety. Taken together, these findings highlight a role of the OT system in social preference both under basal conditions and during stress-induced social avoidance.

Most animal research investigating the pro-social properties of neuropeptides belonging to the oxytocin-vasopressin family has been performed either in relation to reproduction or in non-mammalian species (see Introduction). We are now able to demonstrate pro-social effects of the endogenous OT system in two rodent models of social preference as well as of synthetic OT in a rodent model of defeat-induced loss of social preference and social avoidance. Specifically, central application of an OTR-A and, thus, blockade of intracerebral OT receptors attenuated social preference in rats, and dose-dependently caused social avoidance in mice. Together, these results indicate the involvement of the brain OT system in normal social preference in two different species. Importantly, these results provide the first direct evidence that OT can have a pro-social effect on male-male interactions and not only on reproduction-related behaviors such as pair-bonding, maternal or sexual behaviors.

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Consequently, basal and/or social stimulus-induced release of OT within the brain (Landgraf and Neumann, 2004; Neumann, 2009) and subsequent OT-receptor interactions within discrete brain regions may underlie the motivation for social approach. Our finding of a facilitatory effect of endogenous OT on rodent social interaction is in line with a study showing that the high levels of sociability observed after 3,4-methylenedioxymethamphetamine (“ecstasy”) consumption are prevented by central infusion of an OTR-A (Thompson *et al.*, 2007). We further demonstrate that the promotion of social preference by the OT system is a robust effect as it could be observed both in rats and mice, thus underlining evolutionary conservation. Furthermore, OT-mediated social preference was observed under varying light conditions and environmental settings, i.e. in the novel, unknown arena and in the home cage. Although beyond the scope of the current studies, it would be interesting to determine whether blockade of the endogenous OT system would also result in social avoidance in females in our paradigm. This would seem likely in the light of studies showing alterations in social recognition and anxiety in female OT knockout mice (Crawley *et al.*, 2007) and OTR antisense DNA-treated female mice (Choleris *et al.*, 2007)

Intracerebral release of OT in specific brain regions was found to be increased in response to a variety of social stimuli, including mother-offspring interactions, maternal aggression, mating in males and females, and exposure to an adult con-specific during social defeat (reviewed in Neumann, 2009; Veenema and Neumann, 2008). In the context of social stimuli, increased OT release could be identified within the hypothalamic PVN (Neumann *et al.*, 1993; Waldherr and Neumann, 2007), the central amygdala (Bosch *et al.*, 2005), the olfactory bulb (Lévy *et al.*, 1995), the mediolateral septum (Ebner *et al.*, 2000) and the nucleus accumbens (Ross *et al.*, 2009). Together with the present findings, these results support the

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hypothesis that stimulation of the brain OT system by social cues reinforces appropriate social interactions most likely linked to the particular social cue.

In order to localize the pro-social effects of OT, we chose various subnuclei of the amygdala as the most promising target regions. Within the central amygdala, OT exerts anxiolytic effects and regulates social interactions after stress (Bale *et al.*, 2001; Lee *et al.*, 2007; Neumann, 2002). Moreover, the medial amygdala is prominent for its OT-dependent role in social recognition (Choleris *et al.*, 2007; Ferguson *et al.*, 2001) and social odor approach (Arakawa *et al.*, 2010), and OT receptors were described in relatively high density in the central, but also the medial, and basolateral amygdala (Lukas *et al.*, 2010; Tribollet *et al.*, 1988). Additionally, within the central amygdala, increased OT release has been demonstrated during social stimuli, i.e. during mother-offspring interactions (Bosch *et al.*, 2005). Likewise, human imaging studies suggest an involvement of amygdala subnuclei in oxytocinergic processing of social interactions and fear response (Gamer *et al.*, 2010; Hurlemann *et al.*, 2010; Kirsch *et al.*, 2005; Labuschagne *et al.*, 2010). However, whether OT exerts direct local effects or rather acts via multiple OT-responsive regions upstream of the amygdala after its intranasal application in humans needs to be shown; a question that could be approached using our rodent model. However, neither an acute effect of the OTR-A (0.1µg/µl and 1.0µg/µl) on social preference, nor of synthetic OT on social defeat-induced social avoidance could be localized within the amygdala. Thus, we suggest that social preference might require OT actions at yet to be identified brain regions where OT receptors are present, including olfactory bulb, prefrontal cortex, nucleus accumbens, lateral septum, and PVN (Barberis and Tribollet, 1996; Tribollet *et al.*, 1988; Young *et al.*, 1996) likely to participate in a complex network to orchestrate social approach behaviour. The extent to which diffusion processes after dendritic release, which have recently been discussed, impact on such a network and contribute to the behavioural effects of OT remains to be shown (Ludwig and Leng, 2006).

In accordance with previous studies demonstrating social avoidance after repeated or chronic exposure to social defeat (Berton *et al.*, 2006; Haller and Bakos, 2002; Meerlo *et al.*, 1996; Vidal *et al.*, 2007), we show here that a single social defeat exposure, prior to the social preference test, induces social avoidance in male rats. However, in our hands, loss of social preference could only be induced when the identical defeater rat was used as the social stimulus during the social preference test. This is in line with a study in golden hamsters that also reports specific social avoidance of the former defeater but not of an unfamiliar con-specific (Lai *et al.*, 2005). In our study, the social investigation time of defeated rats was even lower than object investigation time, indicating a high level of social anxiety. Synthetic OT was able to rescue social approach behavior under conditions of stress-induced social avoidance or social anxiety and, thus, to restore social preference. Despite the role of OT in recognition of familiar con-specifics (Bielsky and Young, 2004), it is unlikely that these effects are due to OT-mediated alterations in social recognition because the dose used in the current study was ineffective in social recognition (Benelli *et al.*, 1995) and, if anything, OT would have facilitated the recognition of the defeater rat, which would have promoted avoidance rather than the rescue of social avoidance as was found in the present study. We further found that exogenous OT did not further increase social preference under basal circumstances. Similarly, in human studies intranasal OT application reduced hyperresponsivity of the amygdala to facial cues in socially anxious patients, while not affecting the response observed in normal patients (Labuschagne *et al.*, 2010). Additionally, in male goldfish (*Carassius auratus*) icv-infused isotocin increased social approach behavior only in individuals with low sociability (Thompson and Walton, 2004). In this respect animal models of low-innate sociability, like the BTBR T+tf/J mice (McFarlane *et al.*, 2008), may be useful in investigating the possible facilitating properties of exogenous OT on social preference.

Remarkably, administration of the vasopressin 1a receptor antagonist was without effect on social preference indicating peptide-specific effects and high specificity of the antagonists used. This is an important finding because vasopressin is a key modulator of various aspects of social behavior in male rodents such as social memory (Engelmann *et al.*, 1994), inter-male aggression (Veenema *et al.*, 2010) and pair bonding (Wang *et al.*, 1998), but exerts rather anxiogenic effects (for reviews see Landgraf, 2006).

Due to the obvious close link between social and non-social anxiety, we assessed whether OT actions on social preference were in part due to an OT-mediated reduction in general anxiety. Although profound anxiolytic properties of synthetic OT have been described (Bale *et al.*, 2001; Blume *et al.*, 2008; Slattery and Neumann, 2010; Windle *et al.*, 1997), the role of the endogenous brain OT system in the regulation of anxiety-related behaviour needs particular attention. It has been repeatedly shown that anxiolytic effects of endogenous OT can only be identified under conditions of high brain OT activity, such as in lactating dams (Neumann *et al.*, 2000b) and after mating in males (Waldherr and Neumann, 2007). In contrast, blockade of OT receptors in virgin females or males under basal conditions failed to alter the level of anxiety (Neumann *et al.*, 2000a). This is in agreement with the present results in naïve male rats or mice demonstrating that icv infused OTR-A did not alter non-social anxiety-related behaviour (i) during the social preference test (investigation of the novel object), (ii) in the black-white box (rats) or (iii) on the elevated plus-maze (mice), further substantiating a dissociation between OT effects on social preference and non-social anxiety. In support, the anxiogenic drug PTZ did, as expected, reduce the time rats spent on the open arm of the elevated plus-maze without eliciting social avoidance. These results demonstrate the specificity of the social preference test for social facets of approach behavior, which can be distinguished from parameters mirroring general anxiety. They further demonstrate that the effects of OT on social preference are not due to direct interactions with non-social anxiety.

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In conclusion, our results provide direct evidence of an important involvement of the brain OT system in the natural occurrence of social preference in male rats and mice. Further, exposure to a single social defeat is efficient to induce social avoidance, which can be reversed by central administration of synthetic OT. However, these pro-social effects of OT are not likely to be mediated by direct actions of OT on the amygdala, but rather require additional regions of a potential network. Furthermore, these effects of OT are specific to social approach behavior as no alteration in non-social anxiety was observed, while - vice versa - increasing non-social anxiety did not alter social preference. Thus, the brain OT system is a promising therapeutic target for the treatment of social phobia or social avoidance as seen in social anxiety disorder and autism spectrum disorders, respectively.

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Disclosure/Conflict of Interests

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The authors declare no conflict of interest.

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Titles and legends to figures

Figure 1:

Effects of icv administration of an oxytocin receptor antagonist (OTR-A; rats: 0.75µg/5µl; mice 20µg/2µl) or vasopressin receptor antagonist (V1aR-A, rats: 0.75µg/5µl) on social preference in a novel arena (rats and mice; see schematic drawing) or in the home cage (rats). Social preference is reflected by the higher percentage of investigation time directed towards the *social* (black columns) versus the *object* (grey columns) stimulus during two sequential exposures. Numbers in parentheses reflect group size. Data are means + SEM, # $p < 0.05$ vs. object stimulus, * $p < 0.05$ vs vehicle, (*) $p = 0.055$ vs. vehicle; two-way ANOVA for repeated measures followed by Bonferroni post-hoc test.

Figure 2:

Effects of infusion of an oxytocin receptor antagonist (OTR-A, 0.1µg/1µl and 1.0 µg/µl) bilaterally into the central, medial, or basolateral amygdala on social preference of male rats. Social preference is reflected by the higher percentage of investigation time directed towards the *social* (black columns) versus the *object* (grey columns) stimulus during two sequential exposures. Numbers in parentheses reflect group size. Data are means + SEM, # $p < 0.05$ vs. object stimulus, two-way ANOVA for repeated measures followed by Bonferroni post-hoc test.

Figure 3:

Social preference behavior of socially defeated and control rats. (A) Control and defeated rats were exposed to an unknown male as social stimulus during the social preference test. (B) Socially defeated rats were exposed to their individual defeater rat as social stimulus during the social preference test. Social preference is reflected by a higher percentage of investigation time directed towards the *social* (black columns) versus the *object* (grey columns) stimulus during two sequential exposures. Numbers in parentheses reflect group size. Data are means + SEM, * $p < 0.05$ vs. non-defeated control, # $p < 0.05$ vs. object stimulus; two-way ANOVA for repeated measures followed by Bonferroni post-hoc test.

Figure 4:

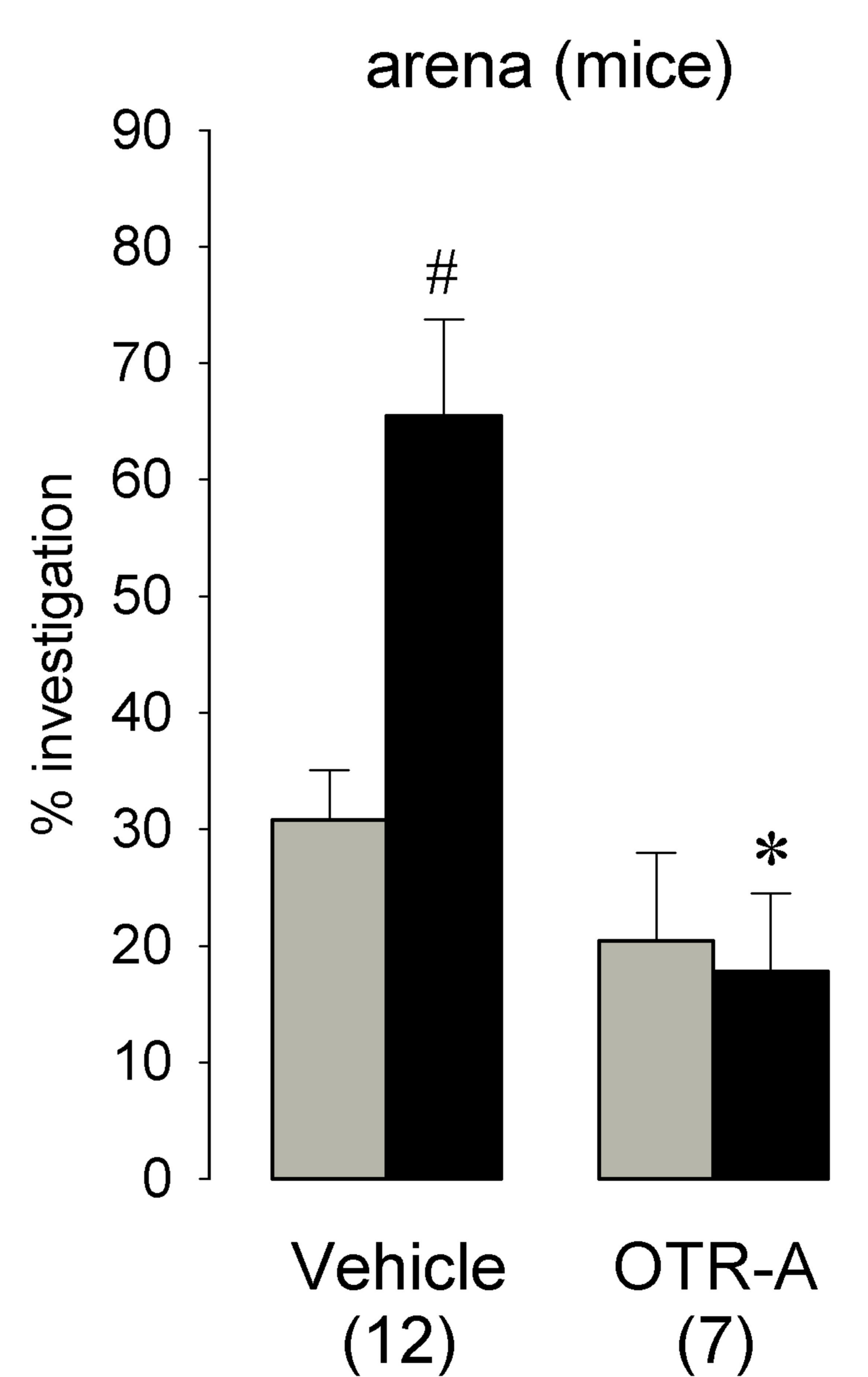
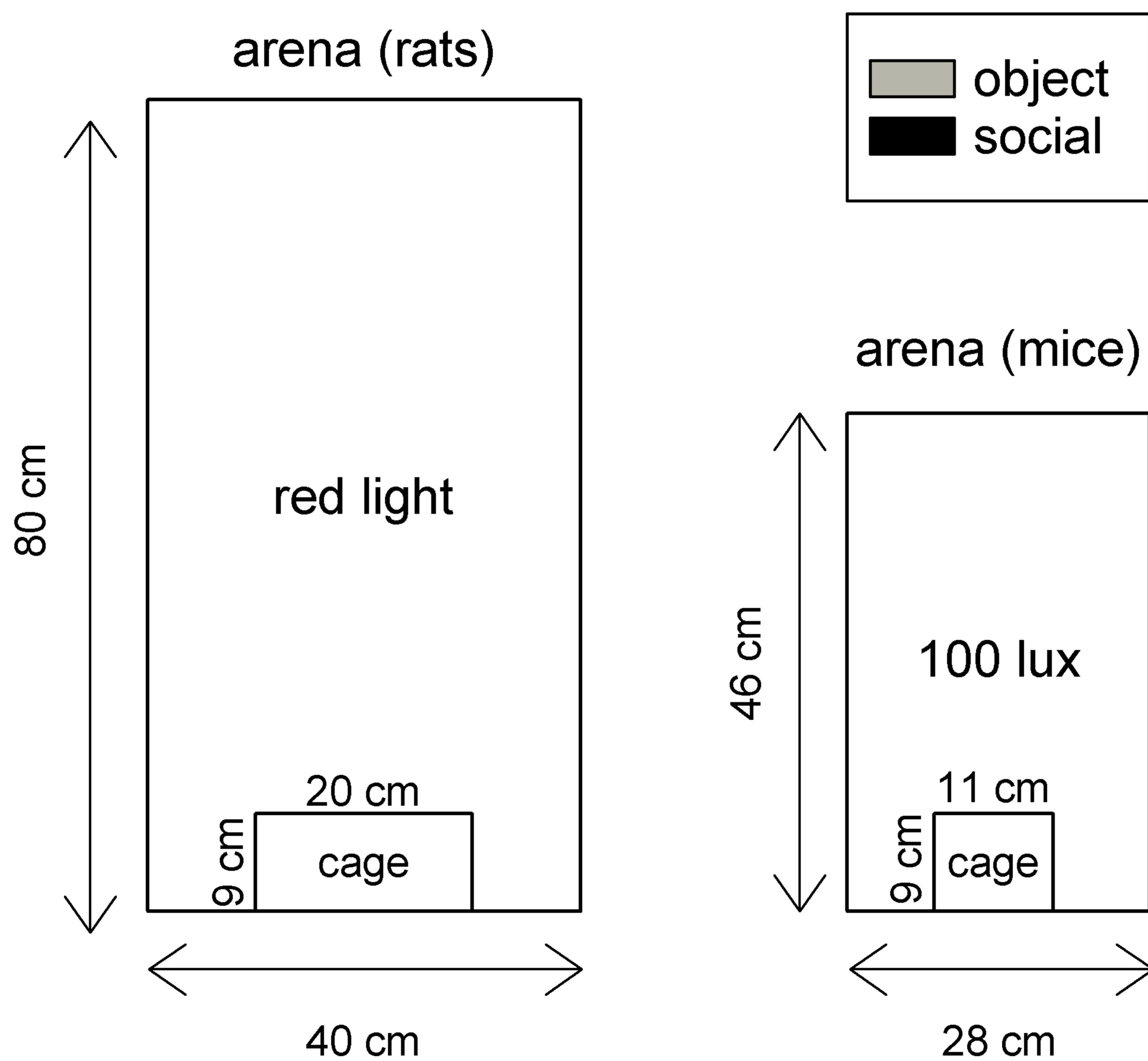
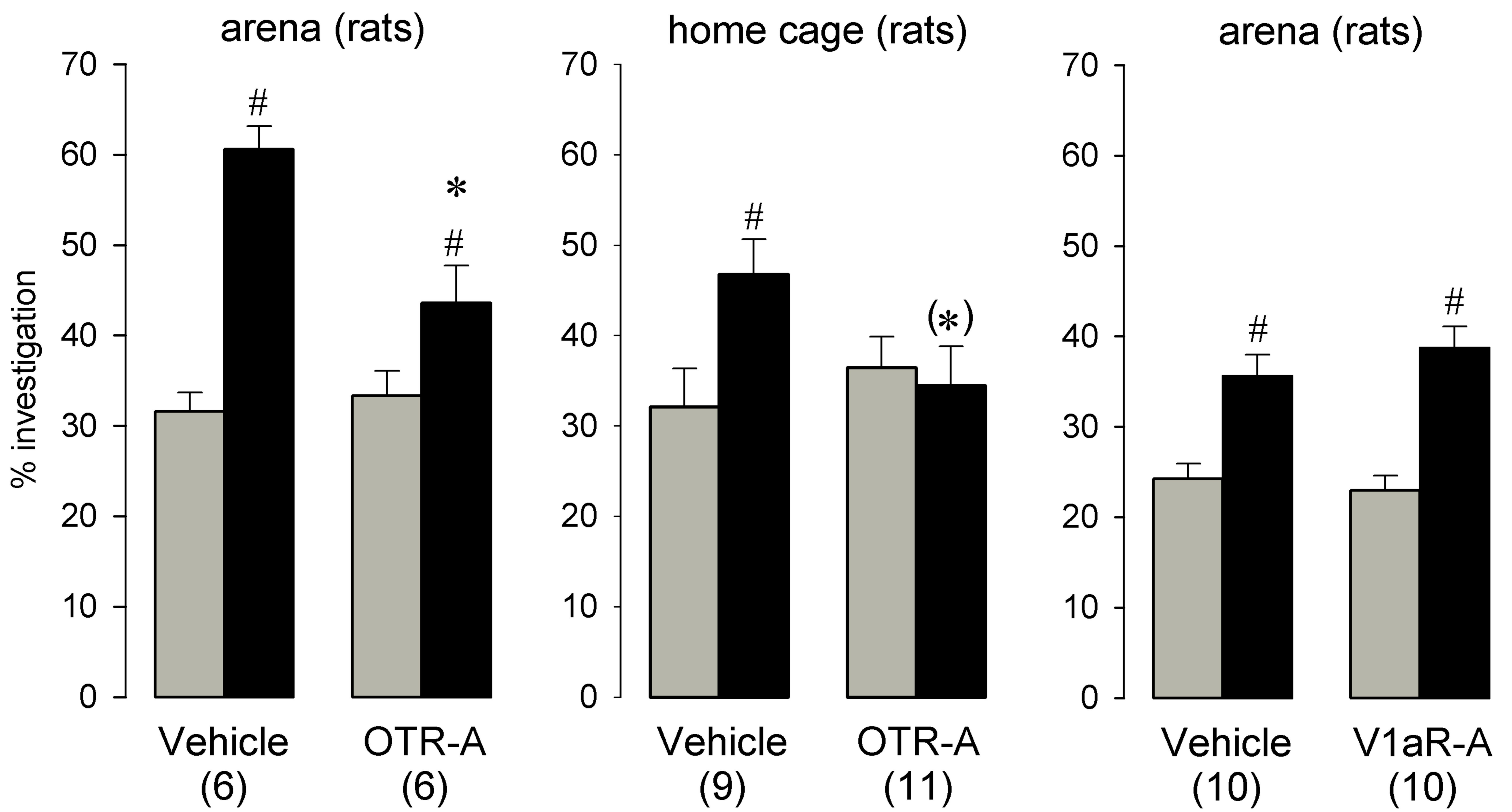
Social preference of socially defeated and control rats after either vehicle (veh) or oxytocin (OT) were applied icv (0.1µg/5µl) or bilaterally into the central (ceA) or medial (meA) amygdala (right) (0.01µg/1µl) 20 min prior to the social preference test. Social preference is reflected by the percentage of investigation time directed towards the *social* (black columns) versus the *object* (grey columns) stimulus during two sequential exposures. Numbers in parentheses reflect group size. Data are means + SEM, # $p < 0.05$ vs. object stimulus; three-way or two-way ANOVA for repeated measures followed by Bonferroni post-hoc test.

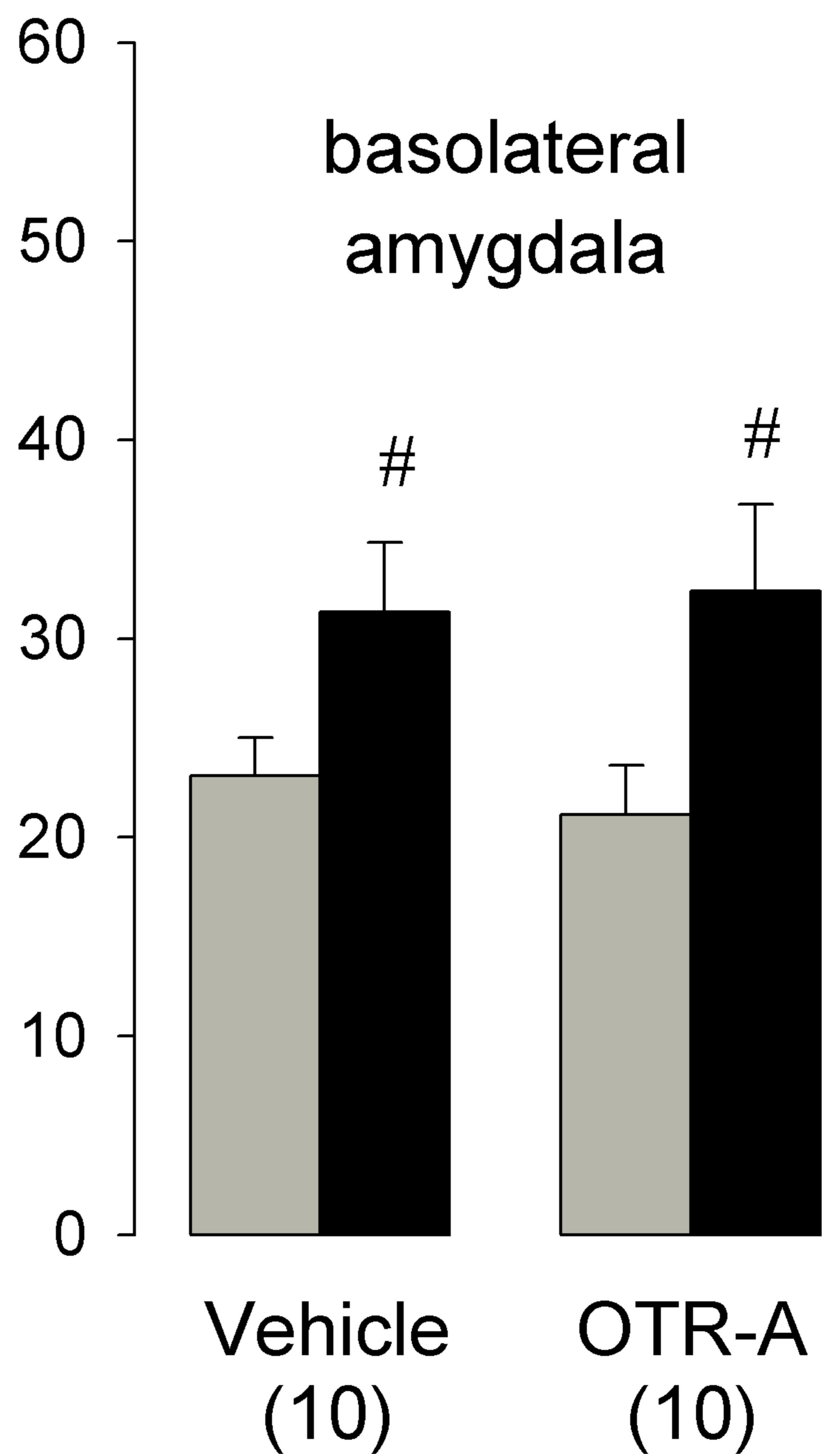
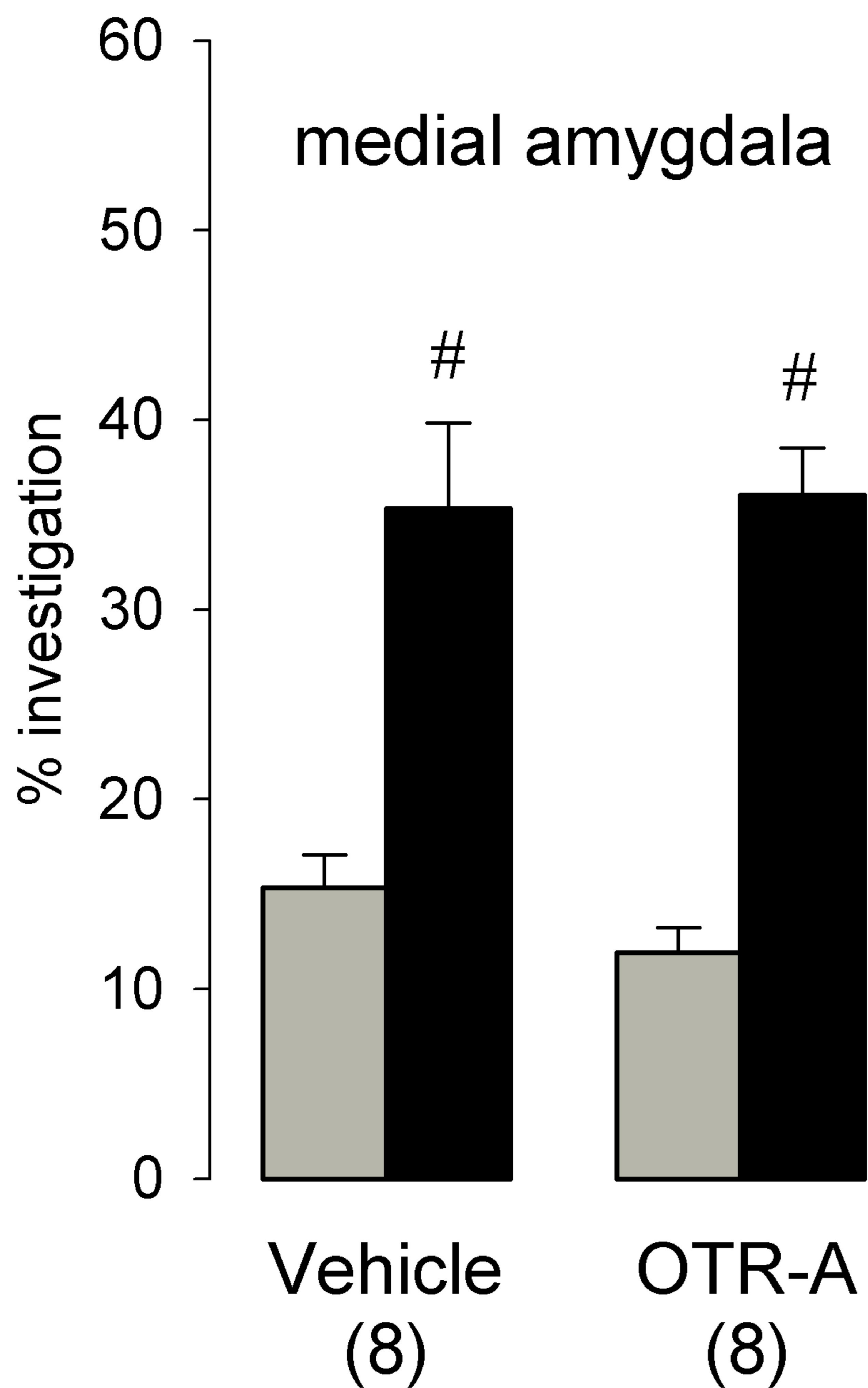
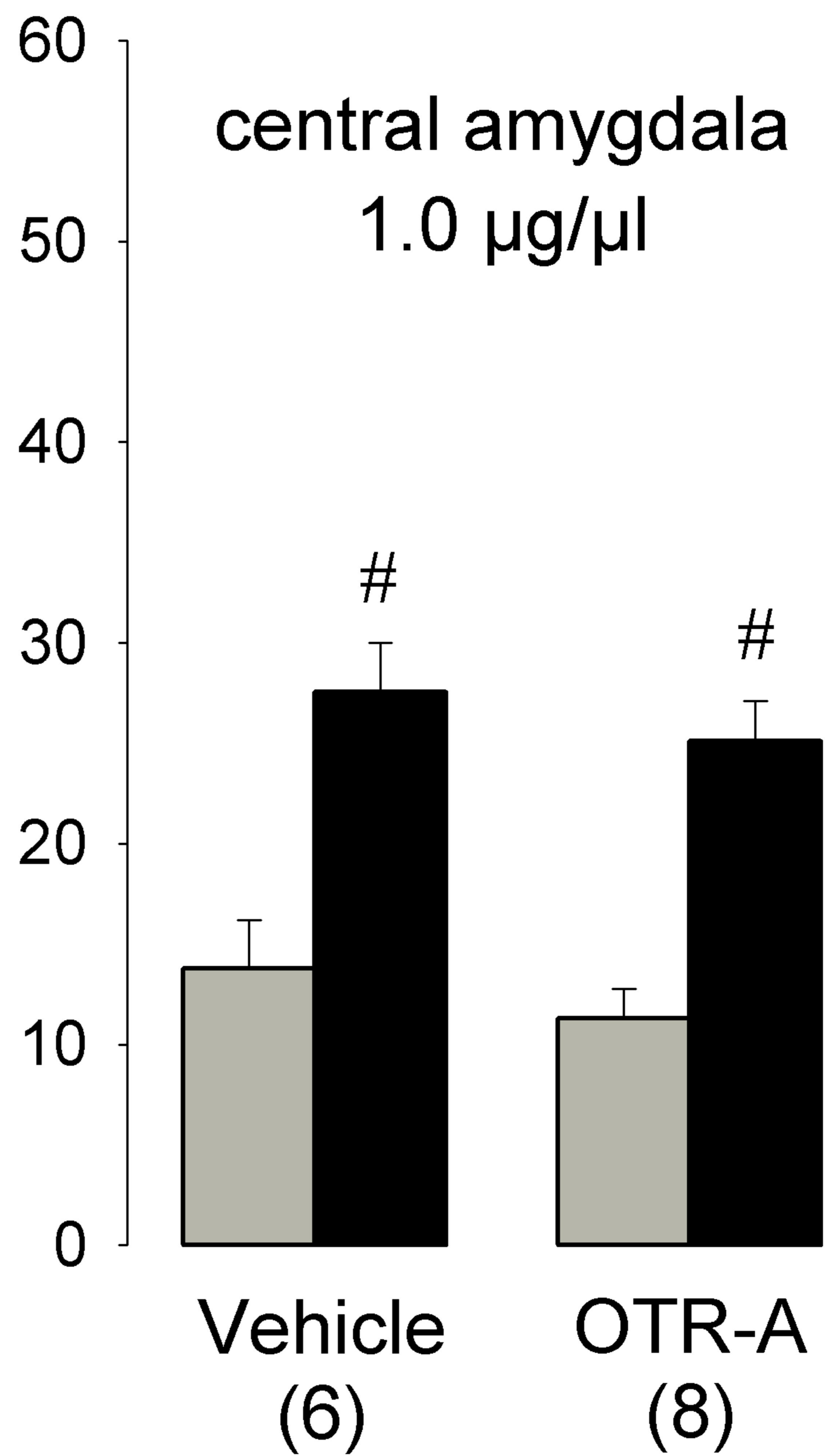
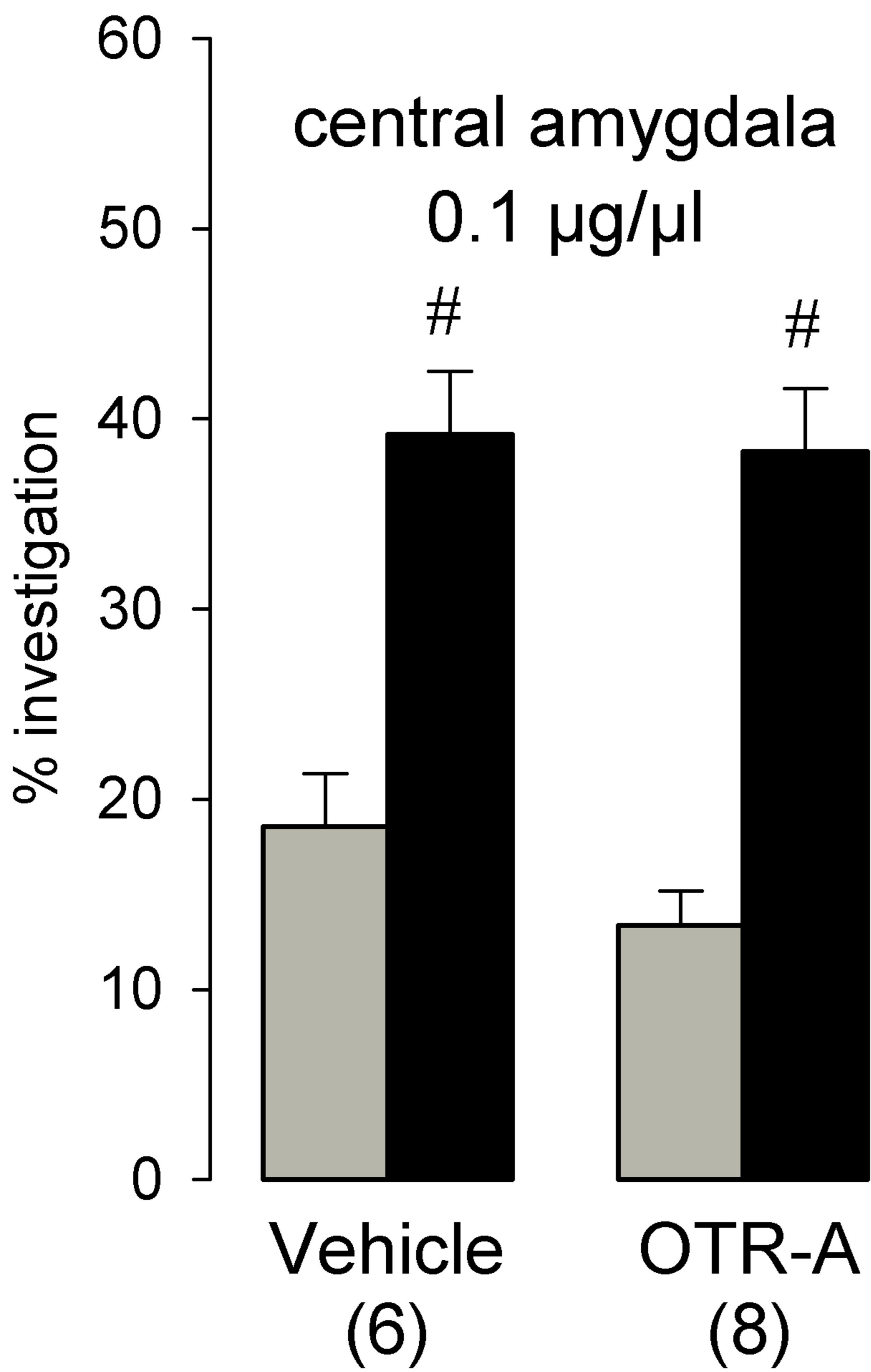
Figure 5:

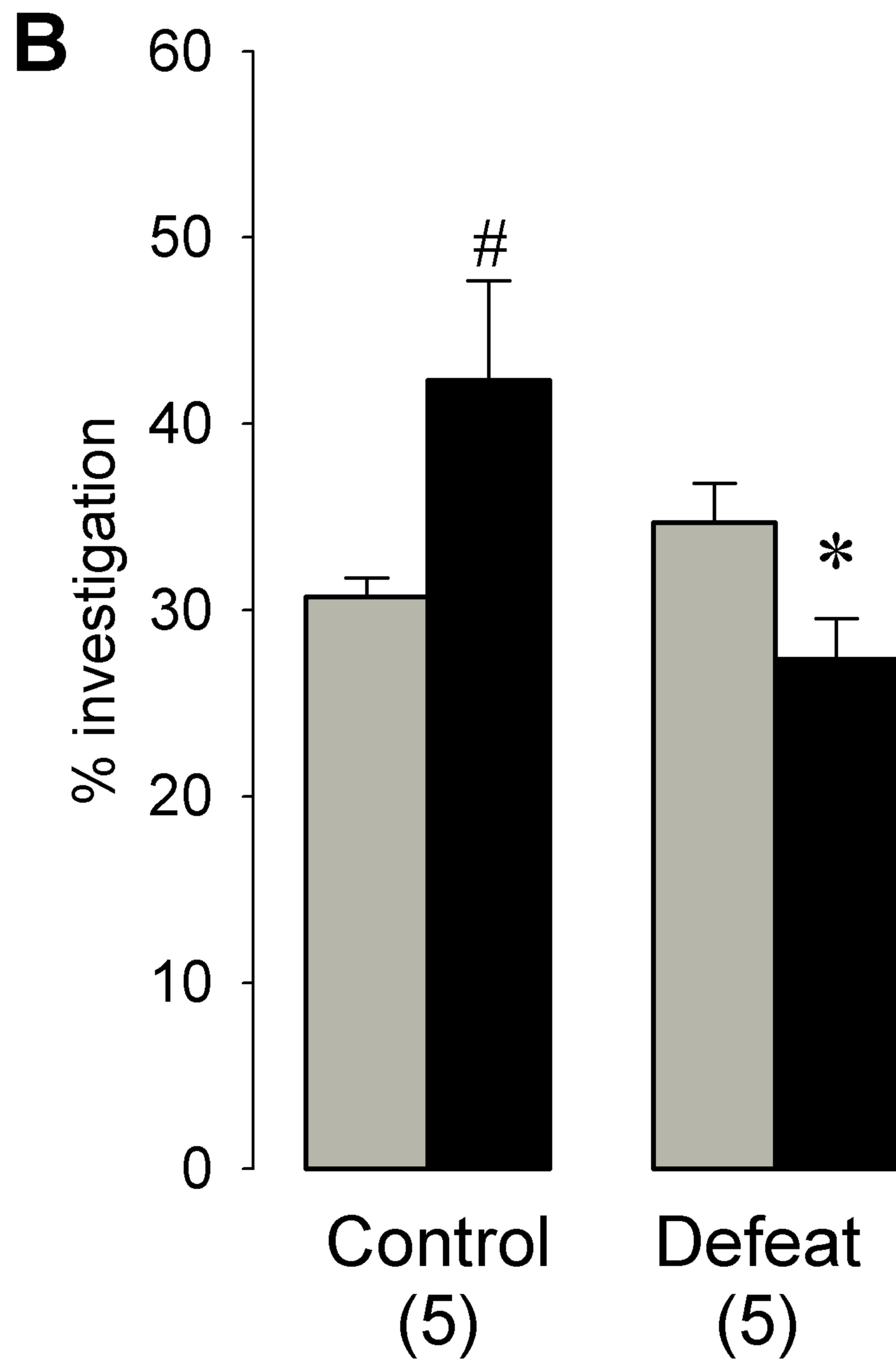
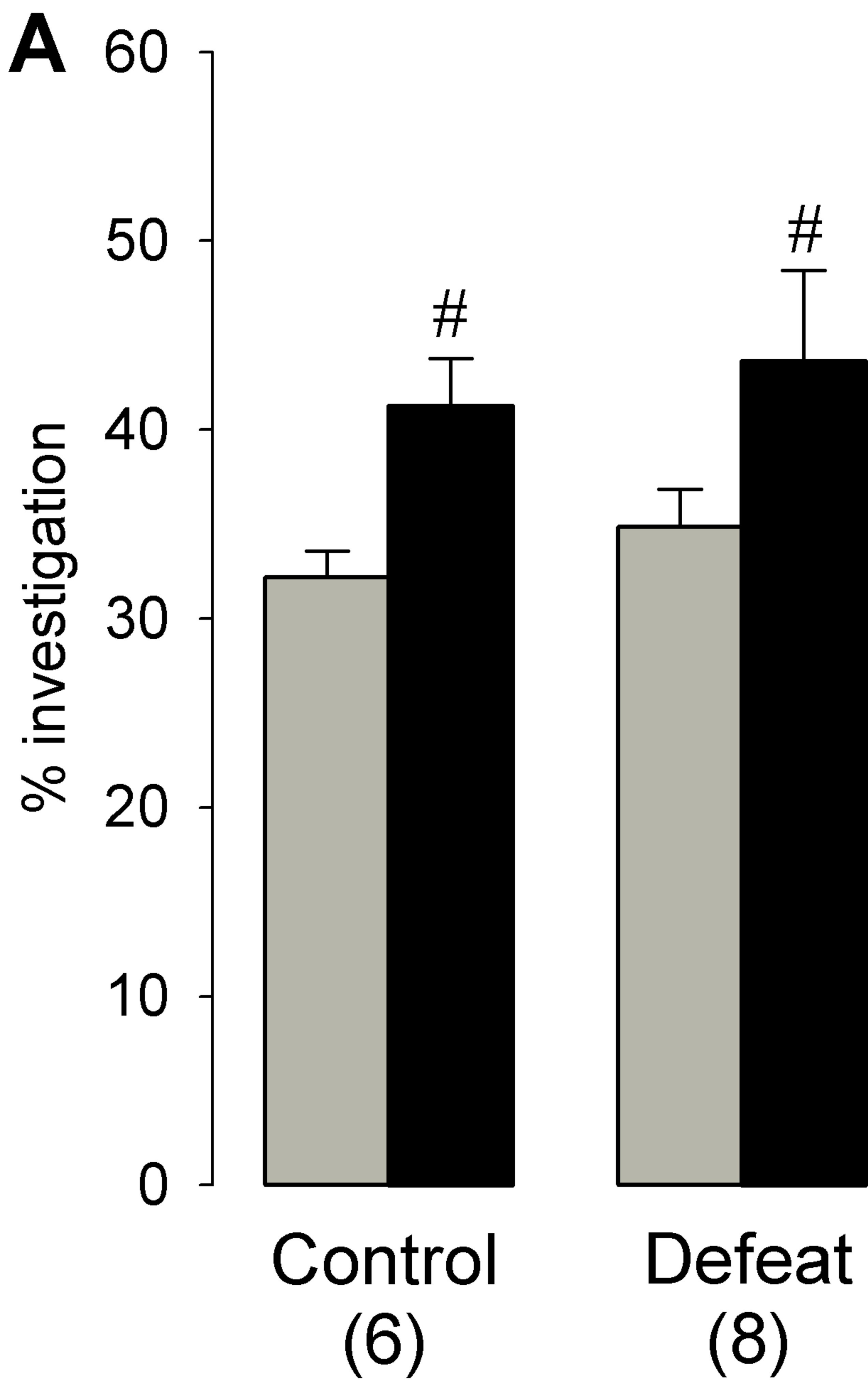
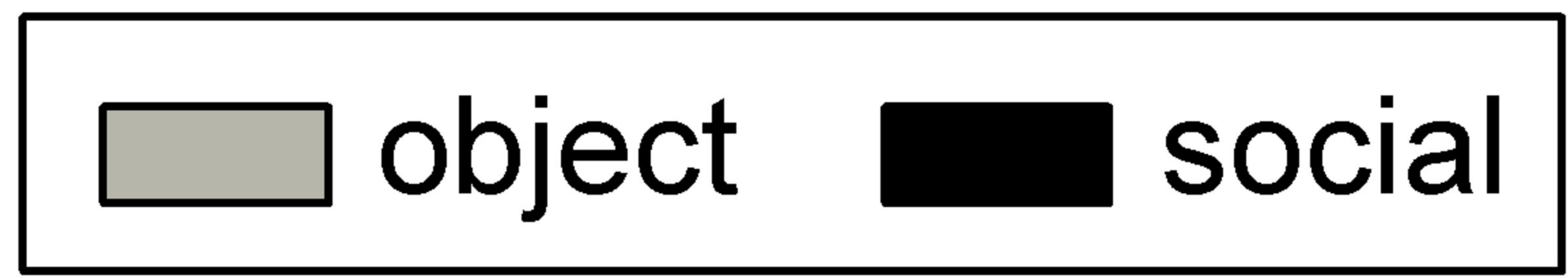
(A) Effects of infusion of an oxytocin receptor antagonist (OTR-A, icv) on non-social anxiety in the black-white-box in rats and on the elevated plus-maze in mice, respectively. Non-social anxiety is reflected by the percentage time from total time [5 min] spent in the white box of the black-white box and the percentage of time spent on the open arms to all arms in the elevated plus-maze. Data are means + SEM, Student's t-test. (B) Effects of PTZ (15 mg/kg, i.p.) on non-social anxiety and locomotion of male rats on the plus-maze. * $p < 0.05$ versus vehicle (C) Effects of PTZ on social preference in rats reflected by the higher percentage of investigation time directed towards the *social* versus the *object* stimulus. Data are means + SEM, # $p < 0.05$ vs. object stimulus, two-way ANOVA for repeated measures followed by Bonferroni post-hoc test.

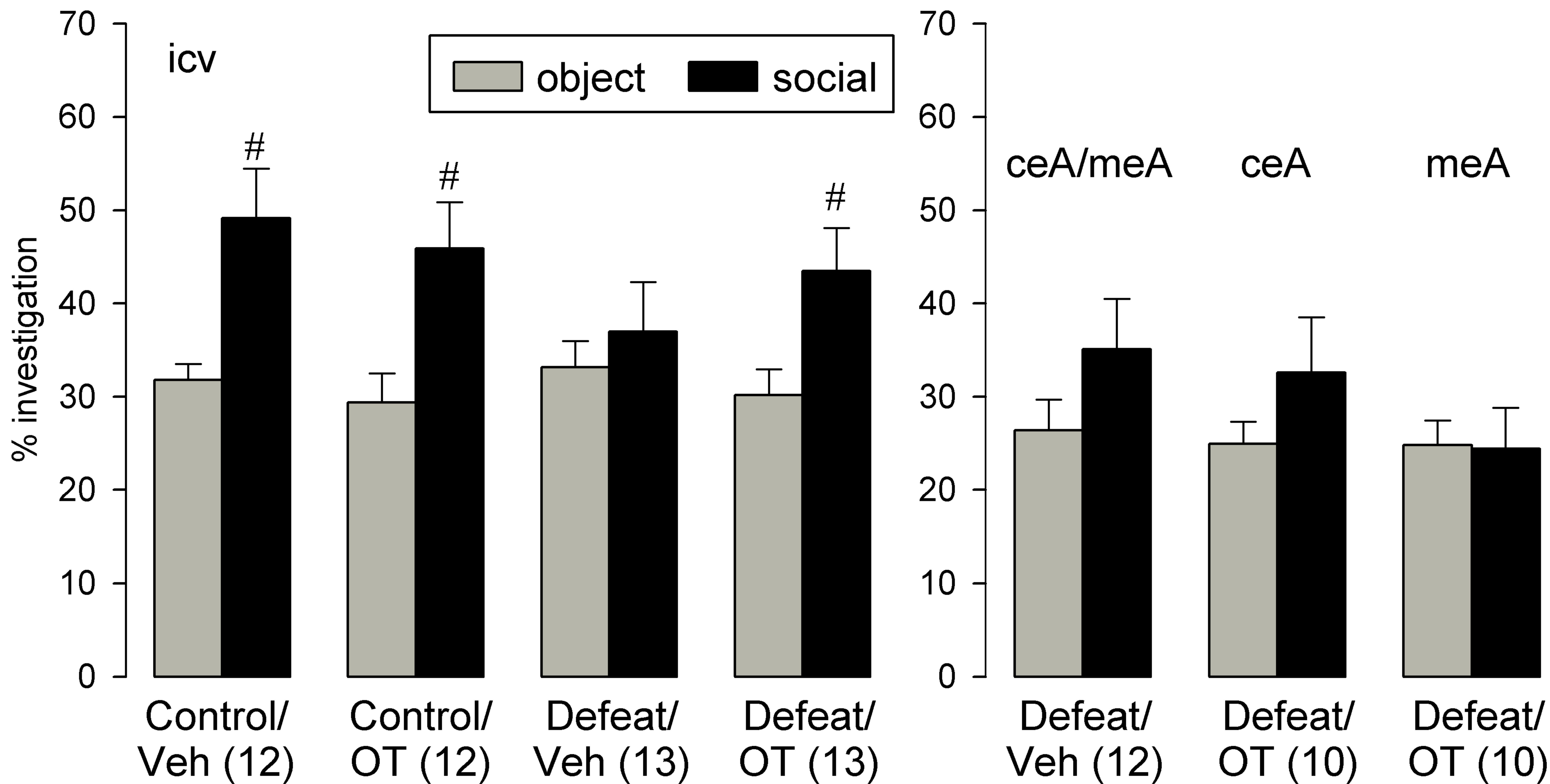
Table 1 Relevant overall effects for all social preference data (two-way or three-way ANOVA for repeated measures).

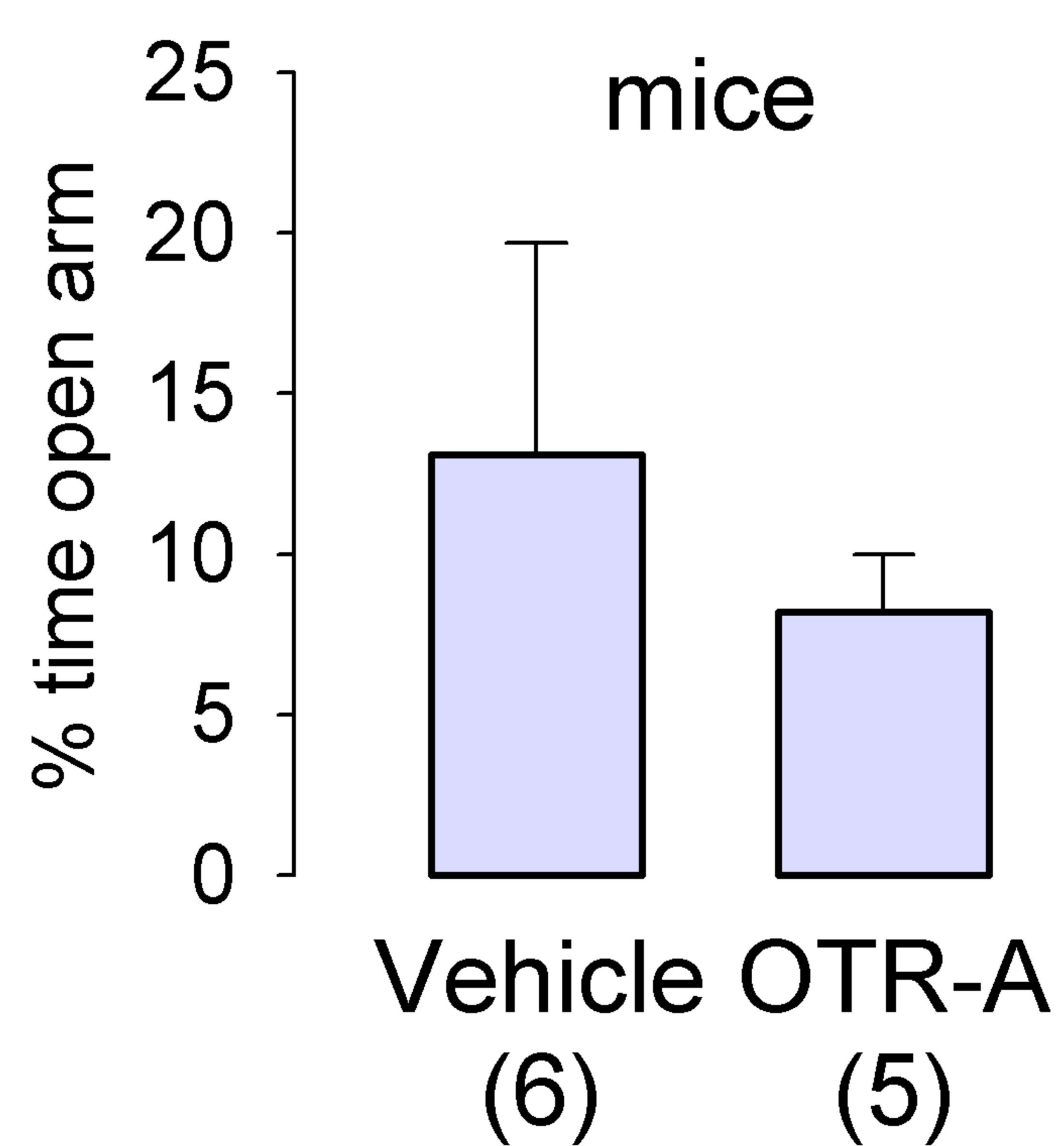
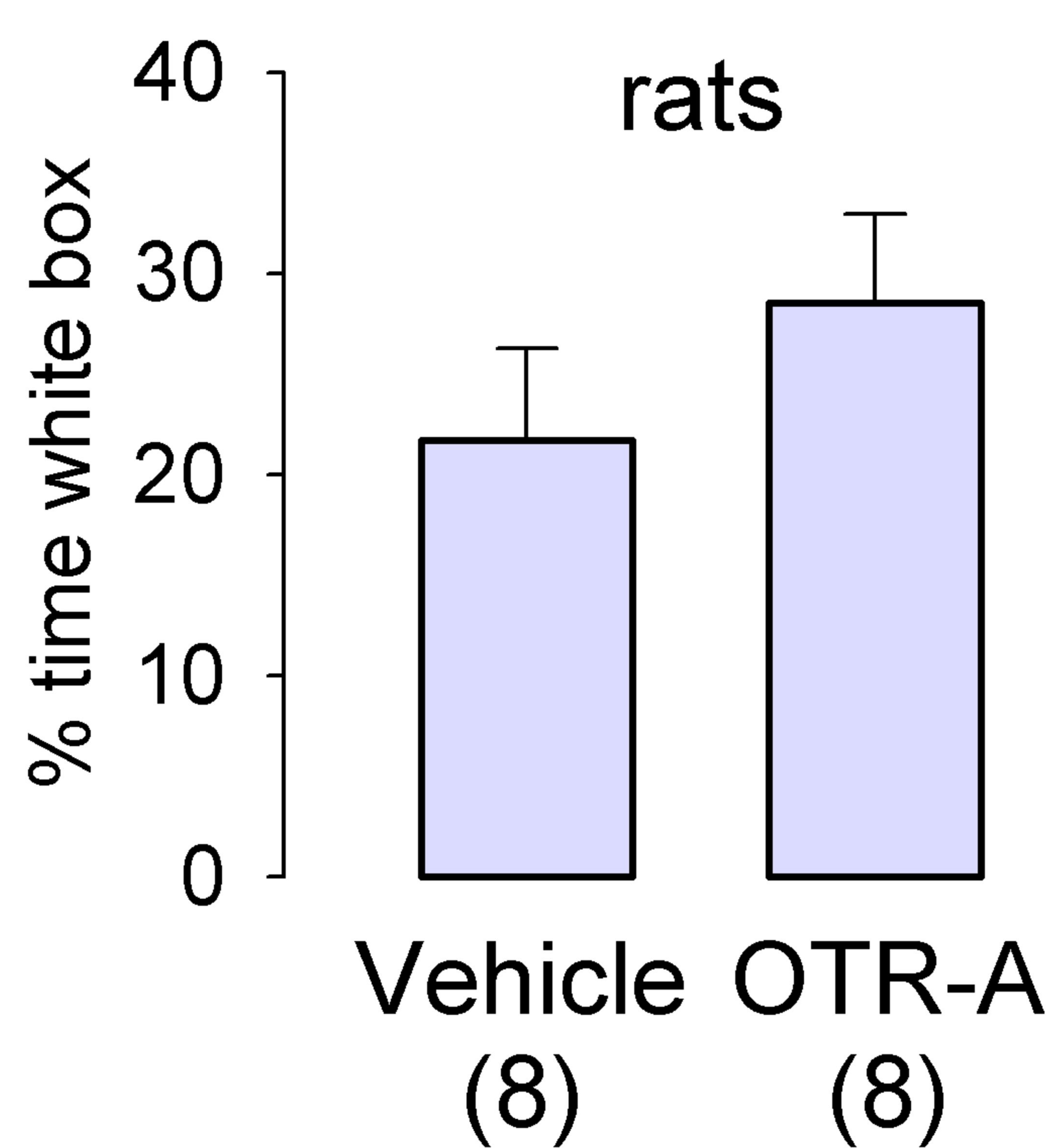
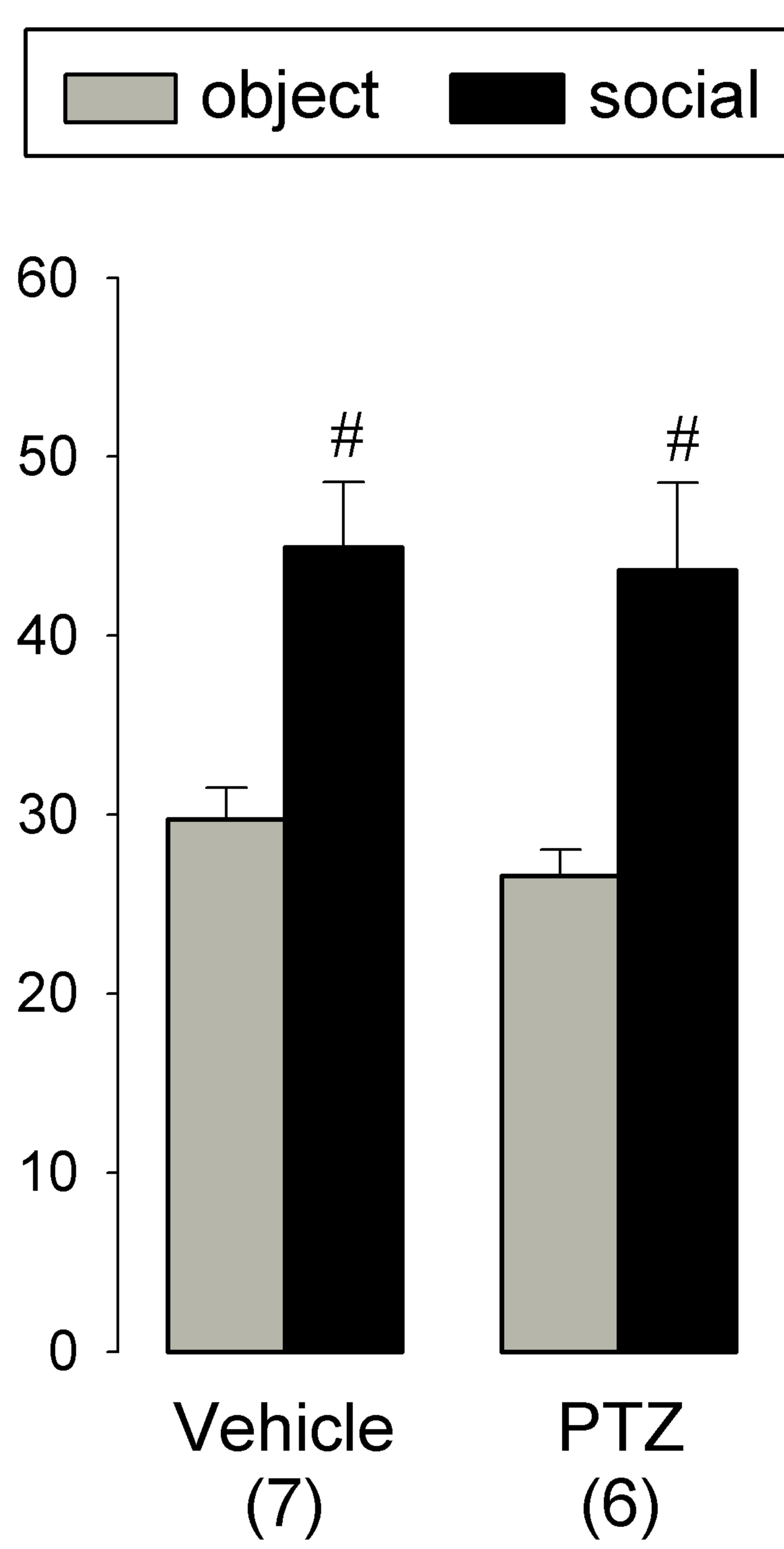
Overall effects for figure 1	factor stimulus	factor stimulus x treatment
<i>Arena (rats)</i>		
icv OTR-A	$F_{(1,10)}=58.5$; $p<0.001$	$F_{(1,10)}=11.9$; $p=0.006$
icv V1aR-A	$F_{(1,18)}=41.8$; $p<0.001$	$F_{(1,18)}=1.09$; $p=0.309$
<i>Home cage (rat)</i>		
icv OTR-A	$F_{(1,18)}=3.42$; $p=0.081$	$F_{(1,18)}=5.88$; $p=0.026$
<i>Arena (mice)</i>		
icv OTR-A	$F_{(1,17)}=15.3$; $p<0.001$	$F_{(1,17)}=20.7$; $p<0.001$
Overall effects for figure 2	factor stimulus	factor stimulus x treatment
central amygdala (0.1 $\mu\text{g}/\mu\text{l}$)	$F_{(1,12)}=61.5$; $p<0.001$	$F_{(1,12)}=0.54$; $p=0.476$
central amygdala (1.0 $\mu\text{g}/\mu\text{l}$)	$F_{(1,12)}=42.3$; $p<0.001$	$F_{(1,12)}<0.001$; $p=0.987$
medial amygdala	$F_{(1,14)}=37.3$; $p<0.001$	$F_{(1,14)}=0.64$; $p=0.439$
basolateral amygdala	$F_{(1,18)}=18.3$; $p<0.001$	$F_{(1,18)}=0.44$; $p=0.515$
Overall effects for figure 3	factor stimulus	factor stimulus x defeat
Unknown male (A)	$F_{(1,12)}=10.5$; $p=0.007$	$F_{(1,12)}=0.004$; $p=0.950$
Defeater male (B)	$F_{(1,8)}=0.52$; $p=0.490$	$F_{(1,8)}=10.9$; $p=0.013$
Overall effects for figure 4	factor stimulus	factor stimulus x treatment x defeat
icv OT (left)	$F_{(1,46)}=24.5$; $p<0.001$	$F_{(1,46)}=0.32$; $p=0.324$
	factor stimulus	factor stimulus x treatment
local OT (right)	$F_{(1,29)}=6.37$; $p=0.017$	$F_{(1,29)}=1.96$; $p=0.159$
Overall effects for figure 5	factor stimulus	factor stimulus x treatment
PTZ (C)	$F_{(1,11)}=23.7$; $p<0.001$	$F_{(1,11)}=0.08$; $p=0.785$









A**C****B**