ARCHIVAL REPORT

Long-Term Social Recognition Memory Is Mediated by Oxytocin-Dependent Synaptic Plasticity in the Medial Amygdala

Rotem Gur, Alex Tendler, and Shlomo Wagner

Background: Recognition of specific individuals is fundamental to mammalian social behavior and is mediated in most mammals by the main and accessory olfactory systems. Both these systems innervate the medial amygdala (MeA), where activity of the neuropeptide oxytocin is thought to mediate social recognition memory (SRM). The specific contribution of the MeA to SRM formation and the specific actions of oxytocin in the MeA are unknown.

Methods: We used the social discrimination test to evaluate short-term and long-term SRM in adult Sprague-Dawley male rats ($n = 38$). The role of protein synthesis in the MeA was investigated by local application of the protein synthesis blocker anisomycin ($n = 11$). Synaptic plasticity was assessed in vivo by recording the MeA evoked field potential responses to stimulation of the main ($n = 21$) and accessory ($n = 56$) olfactory bulbs before and after theta burst stimulation. Intracerebroventricular administration of saline, oxytocin, or oxytocin receptor antagonist was used to measure the effect of oxytocin on synaptic plasticity.

Results: Anisomycin application to the MeA prevented the formation of long-term SRM. In addition, the responses of MeA neurons underwent long-term depression (LTD) after theta burst stimulation of the accessory olfactory bulb, but not the main accessory bulb, in an oxytocin-dependent manner. No LTD was found in socially isolated rats, which are known to lack long-term SRM. Finally, accessory olfactory bulb stimulation before SRM acquisition blocked long-term SRM, supporting the involvement of LTD in the MeA in formation of long-term SRM.

Conclusions: Our results indicate that long-term SRM in rats involves protein synthesis and oxytocin-dependent LTD in the MeA.

Key Words: Long-term depression, long-term memory, medial amygdala, oxytocin, social recognition, synaptic plasticity

Mammalian social organizations require the ability of an individual to recognize and remember other individuals of the same species (conspecifics) (1). In rodents, this social recognition memory (SRM) is mediated mainly by chemical cues (semiochemicals) perceived via the main olfactory system and accessory olfactory system (AOS) (2). When semiochemicals bind to the receptors expressed by the sensory neurons of the main olfactory epithelium and vomeronasal organ, these neurons convey sensory information to the main olfactory bulb (MOB) and accessory olfactory bulb (AOB), respectively (3). Both the MOB and the AOB project to the medial amygdala (MeA), which transfers the information to the hippocampus through the lateral septum (4). The contribution of each of these brain stations to the processing of social information is still unclear.

The innate tendency of rodents to investigate novel conspecifics more persistently than familiar ones enables quantitative assessment of SRM (5). Using the social recognition paradigm, SRM was initially shown to be retained for <2 hours (6). However, we and others demonstrated that both mice and rats retain SRM for at least 1 week if housed in groups. In contrast, socially isolated animals lose their ability to acquire long-term SRM within 24 hours (7,8).

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Received Nov 6, 2013; revised and accepted Mar 19, 2014.

Long-term memory storage in the brain is mediated by a molecular process termed memory consolidation (9). This process is associated with protein synthesis that supports long-term synaptic plasticity, the most common forms of which are long-term potentiation (LTP) and long-term depression (LTD) of the synaptic current (10). These two types of synaptic plasticity were well studied in the CA3-CA1 synaptic pathway of the hippocampus, where LTP is induced by application of several brief high-frequency (50–100 Hz) stimuli, and LTD is induced by a prolonged (5–15 min) low-frequency (1–3 Hz) stimulation protocol (11).

The long-term SRM formation was found to be dependent on the hippocampus and mediated by protein synthesis (7). However, the brain regions where protein synthesis occurs during SRM consolidation have remained elusive. Also involved in SRM acquisition is the neuropeptide oxytocin, best known for its role in enhancing contractions of the uterus during labor and mediating milk release from mammary glands during suckling (12). Oxytocin activity in the brain plays an important role in mammalian social behavior (13). It acts through binding to the oxytocin receptor, a G protein–coupled receptor that is encoded in mammals by a single gene (14). In rodents, oxytocin activity in the MeA is crucial for SRM acquisition (15,16), but its mode of action is unknown. In the present study, we hypothesized that at least some of the molecular processes that are required for long-term SRM consolidation, such as protein synthesis, occur in the MeA and that they are augmented by the activity of oxytocin to enhance SRM consolidation.

Methods and Materials

Animals

Adult Sprague-Dawley male rats (8–10 wk, 270–340 g) were housed in groups of two to five animals per cage (60 cm × 40 cm × 20 cm) and handled daily for 2 weeks before behavioral testing.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2014.03.022

All animals were purchased from Harlan Laboratories (Rehovot, Israel) and maintained on a 12-hour light/dark cycle, 22 ± 2°C, with food and water available ad libitum under veterinary supervision in a specific pathogen free facility. Juveniles (30 g) of different strains (Wistar Hannover and Hola) served as social stimuli and were held in groups of two animals of the same strain. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Haifa.

Behavioral Procedures

Short-Term Social Discrimination. A Sprague-Dawley adult rat was exposed in its home cage to a juvenile (either Wistar Hannover or Wistar Hola) in a transparent plastic corral (9 cm in diameter), slotted to allow physical contact between the animals (five horizontal slots per side, each 1 cm × 13 cm) for 5 min. The adult rat was exposed 30 min later for 5 min simultaneously to the previous juvenile and to a novel juvenile of the other strain, each confined to a plastic corral.

Long-Term Social Discrimination. Following 30 min of habituation in the experimental cage (60 cm × 40 cm × 20 cm), the adult rat was exposed to a juvenile for 1 hour without restrictions. A second exposure as described for short-term social discrimination took place 1 day later.

Long-Term Odor Discrimination. The same protocol as used for long-term social discrimination was used but with spherical stainless steel tea strainers filled with odorized cotton wool as stimuli. Odors were chosen randomly from commercial odors regularly used in the olfactory learning maze (17).

Measurement of Investigation Time. Duration of investigatory behavior of the adult rat toward the corrals containing each juvenile or toward the tea strainers was blindly measured three times with a stopwatch, once during the experiment and twice off-line using video recording of the experiment, each time by a different observer. The final investigation time represents an average of all three measurements.

Surgery

The rats were tranquilized with isoflurane and anesthetized by injection of ketamine (10%, .09 mL/100 gr) and medetomidine (.1%, .055 mL/100 gr) subcutaneously. The body temperature was kept at ~37°C, using a closed-loop temperature controller (FHC, Bowdoin, Maine) connected to a rectal temperature probe and a heating pad placed under the rat. The animals were fixed in a stereotactic apparatus (Stoelting, Wood Dale, Illinois) with the head flat, and holes were drilled for bilateral implantation of cannula guides (inside diameter, .3 mm; outside diameter, .67 mm; RWD Life Science, ShenZhen, China). The cannulae were inserted to within 1 mm above the target location (MeA—anterior/posterior [A/P], −2.4 mm; lateral/medial [L/M], ±3.18 mm; dorsal/ventral [D/V], −8.5 mm) and secured with acrylic dental cement (Stoelting) stabilized by two skull screws. A dummy cannula (outside diameter, .28 mm; RWD Life Science) was placed in each of the guides to prevent clogging. Antibiotics (amoxicillin 15%, .07 mL/100 gr) and painkillers (metamizole .03 mL/100 gr) were administered immediately after surgery and 1 day later. Rats were allowed to recover for at least 7 days before experimentation started.

Electrophysiology

For investigation of synaptic plasticity in anesthetized animals, the same surgical procedures were followed. A bipolar 125-μm stimulating electrode (Better Hospital Equipment Corp., Weston, Florida) was implanted into the left AOB (A/P, +3.0 mm; L/M, +1.0 mm; D/V, −4.0 mm at 50°) or MOB (A/P, +7.08 mm; L/M, +1.0 mm; D/V, −5.5 mm), and a recording electrode (.010-inch tungsten 1-mm tip exposure; A-M Systems, Sequim, Washington) was placed in the left MeA (A/P, −2.4 mm; L/M, +3.18 mm; D/V, −8.5 mm). Additionally, a cannula guide was inserted into the right lateral ventricle (A/P, −1.0 mm; L/M, −1.5 mm; D/V, −3.5 mm) for intraventricular injection of drugs. All experiments started 30 min after the positioning of the electrodes and the cannula guide. Evoked responses were amplified (×1000) and filtered (.1–5 kHz) by an A-M systems microelectrode AC amplifier, digitized (10 kHz) and analyzed using a self-written MATLAB program. Amplitudes were measured as the mean of five successive postsynaptic field potential responses to test stimuli (monopolar pulses, 100 μsec duration) delivered at .1 Hz. Stimulus intensity (.5–2 mA) was selected to elicit a response representing 40%–50% of the maximal postsynaptic field potential response amplitude.

Synaptic plasticity was examined by applying theta burst stimulation (TBS) to either the AOB or the MOB. The TBS comprised three sets of 10 trains, each of 10 pulses (100-μsec pulse duration) at 100 Hz, with 200 msec between trains and 1 min between sets. The TBS was applied after 30 min of stable baseline recordings.

For long-term electrode implantation, stimulating and recording electrodes were prepared by twisting together two insulated stainless steel wires (A-M Systems) bare at the tip with bare diameter of .005-inch (coated diameter, .008-inch). These wires were implanted as described previously. A stainless steel wire attached to a skull screw served as a reference.

TBS in Behaving Rats

Rats were removed from the experiment cage into a round plastic box (diameter, 17 cm), and electrical wires were attached to the implanted stimulating and recording electrodes. Before applying TBS, the AOB was stimulated at .1 Hz at intensity of .5–2 mA to confirm normal evoked responses from the MeA. The TBS parameters and selection of stimulus intensity were as described previously for anesthetized animals. After TBS stimulation, rats were returned to the experimental cage for an additional 10–15 min before starting the behavioral test.

Microinjection and Pharmacology

Drugs were microinjected through an injection needle placed in the cannula guides after removal of the dummy. The needle (outside diameter, .28 mm; RWD Life Science) was connected via tubing to a microsyringe (.5 μL; ILS, Stutzerbach, Germany) driven by a microinfusion pump (Fusion 200; Chemyx, Inc). After injection, the needle remained in the guide cannula for an additional minute to avoid dragging the injected liquid out.

Anisomycin (50 μg/.5 μL/side; Sigma-Aldrich, St. Louis, Missouri), dissolved in hydrogen chloride 5N and brought to a final pH of 7.2 with sodium hydroxide 4N, was bilaterally infused over 2 min into the MeA of freely behaving rats. Oxytocin (American Peptide Company), dissolved in .9% saline to a final concentration of 250 nmol/L, was administered via the ventricles 5 min before TBS at a rate of 1 μL/min over 4 min. The oxytocin antagonist desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT (kindly donated by Dr. M. Manning, University of Toledo, Spain), dissolved in .9% saline to a final concentration of 153 μmol/L, was administered intracerebroventricularly 15 min before TBS at a rate of 1 μL/min over 5 min.

Histology

At the end of each experiment, rats were killed with an overdose of isoflurane, brains were removed and placed in paraformaldehyde 4% overnight, and sections of 200-μm slices were prepared using Vibroslice (Campden Instruments, Lafayette,

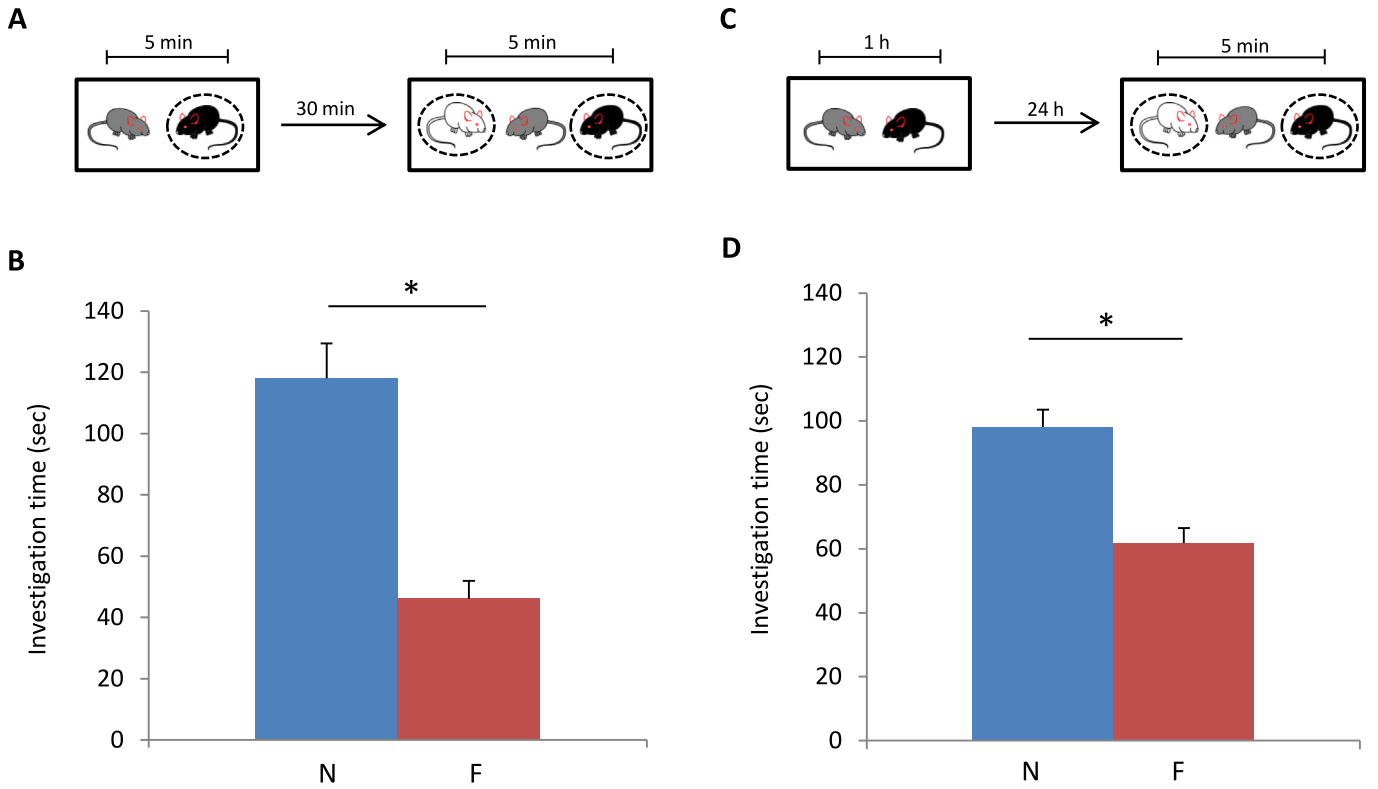


Figure 1. Short-term and long-term social discrimination paradigms. **(A)** Scheme of the short-term social discrimination paradigm. The examined adult subject is shown in gray, and the juveniles are shown in black and white. **(B)** The time the adult subject investigated the familiar (F) and the novel (N) juveniles when simultaneously presented in their corrals during the second exposure, 30 min after the first exposure. The familiar juvenile was investigated for significantly less time than the novel one (paired *t* test, $t = 7.504$, $df = 10$, $^*p < .01$, $n = 11$), reflecting a short-term social recognition memory. **(C)** Scheme of the long-term social discrimination paradigm, same as **(A)**. **(D)** Same as **(B)**. The significant difference between the investigation times reflects long-term social recognition memory (sign test, $^*p < .01$, $n = 27$).

Indiana). The locations of the target sites were identified using stereomicroscope and stereotactic coordinates in the *Rat Brain Atlas* (18) (Figure S1 in Supplement 1). Rats with misplaced cannula and electrode tip location were excluded from the data analyses (one rat for anisomycin injection and one rat for TBS administration).

Statistical Analysis

SPSS (SPSS 19; IBM, Armonk, New York) software was used throughout. Parametric *t* test and analysis of variance were used if data were found to be normally distributed (Kolmogorov-Smirnov); otherwise, nonparametric Mann-Whitney and sign tests were used. When multiple tests were used, α was corrected accordingly. In general, detailed statistical analyses of data shown in the figures are provided in the corresponding figure legend. All the statistical results are summarized in Table S1 in Supplement 1.

Results

We first assessed SRM using the short-term social discrimination paradigm (Figure 1A) (19). On average, the adult rat explored the novel juvenile two to three times more than the familiar juvenile (Figure 1B). This difference was not observed if the second exposure occurred 24 hours rather than 30 min after the first exposure (not shown, $p > .05$; sign test, $n = 7$), suggesting that the short-term memory was not consolidated into a long-term memory. To investigate long-term SRM, we used the long-term social discrimination paradigm (Figure 1C), which enabled unrestricted contact between the adult rat and the novel juvenile for 1 hour during the first exposure. When this rat was tested with the familiar juvenile and a novel juvenile 24 hours later, it clearly discriminated between the two and explored the familiar juvenile for only about 60% of the time it explored the novel juvenile (Figure 1D).

We examined the effect of blocking protein synthesis in the MeA on both the short-term and the long-term SRM. To do this, we compared the behaviors of rats administered either saline or the protein synthesis blocker anisomycin 10 min before the first exposure (Figure 2A). Application of either saline or anisomycin to the MeA did not affect short-term SRM acquisition, as reflected by the significant differences in investigation time of the novel and familiar juveniles during the second exposure (Figure 2B,C). There was no significant difference between saline and anisomycin injection in the ratio of investigation times measured for the two juveniles (Figure 2D).

In contrast, in the long-term SRM test, anisomycin injection to the MeA completely blocked long-term SRM, whereas saline injection had no effect (Figure 2E–H). In the only animal where the cannula tips were located outside the MeA (Figure 2I), anisomycin injection did not block the long-term memory (Figure 2J). These results show that local blockade of protein synthesis in the MeA before memory acquisition blocks the consolidation of long-term SRM, suggesting that the MeA harbors at least part of the molecular processes underlying long-term SRM.

To explore further the involvement of the MeA in long-term SRM formation, we examined whether MeA neurons show long-term

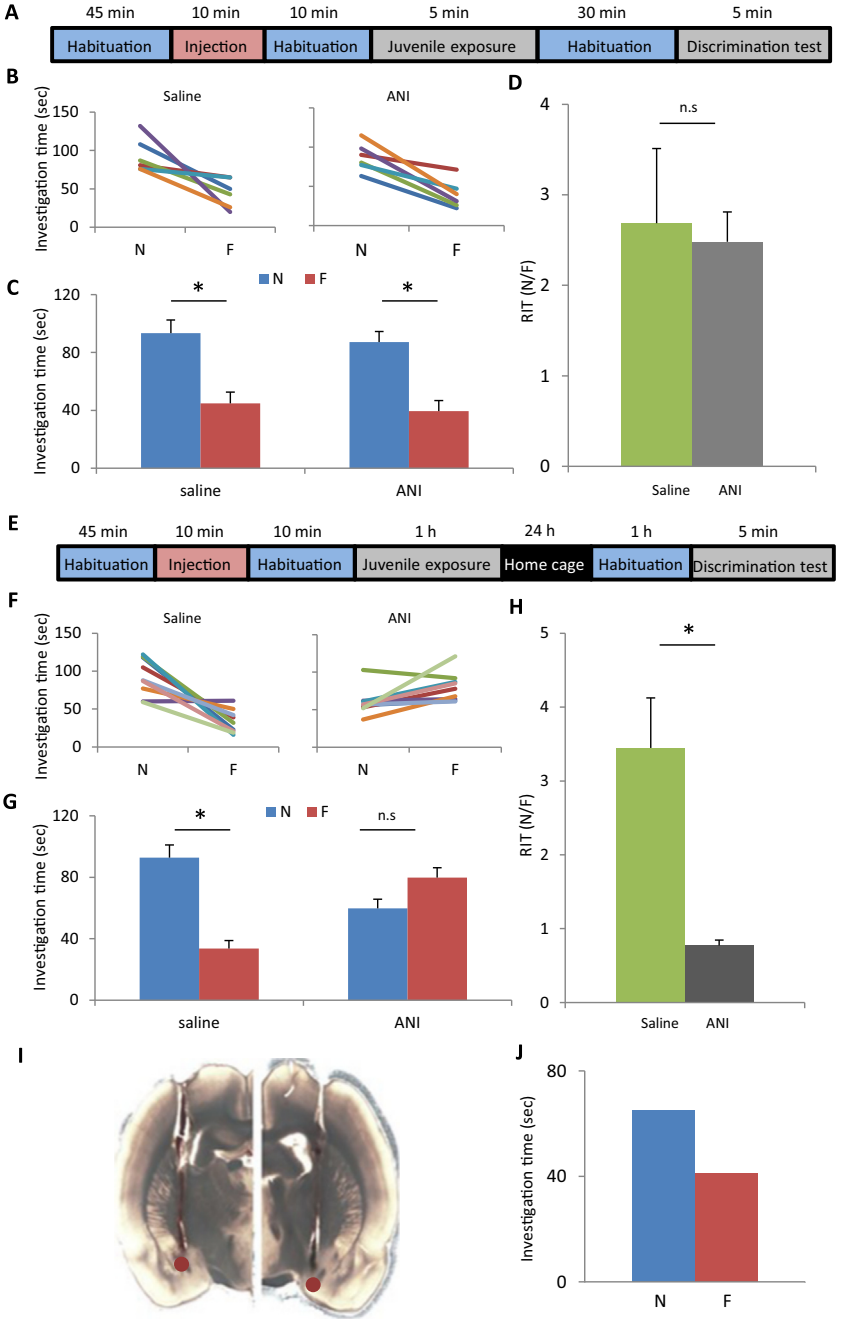


Figure 2. Local application of protein synthesis blocker to the medial amygdala prevented long-term social recognition memory, but not short-term social recognition memory. **(A)** Scheme describing the time course of the experiment using the short-term social discrimination paradigm. **(B)** Lines connecting the investigation times of the novel (N) and familiar (F) juveniles for each rat receiving bilateral injections of saline or the protein synthesis blocker anisomycin (ANI) into the medial amygdala. **(C)** In the short-term social discrimination paradigm, investigation times for the novel and familiar juveniles differed significantly, regardless of whether saline or anisomycin was injected. (sign test; saline, $p < .05$; anisomycin, $p < .05$; $n = 6$). **(D)** The ratio of investigation times (RIT) for the juveniles in the short-term social discrimination paradigm showed no significant difference between saline or anisomycin injection (Mann-Whitney test, $Z = -.714$, $U = 16$, $p > .05$). **(E)** Scheme for long-term social discrimination, same as **(A)**. **(F)** Same as **(B)**. **(G)** In the long-term social recognition memory test, anisomycin injection abolished the difference in investigation time for the novel and familiar juveniles, reflecting a blockade of long-term social recognition memory. Saline injection did not produce this effect (sign test; saline, $p < .05$, $n = 9$; anisomycin, $p > .05$, $n = 11$). **(H)** There was a significant difference in RIT between saline and anisomycin injections in the long-term social discrimination paradigm (Mann-Whitney test, $Z = -3.609$, $U = 2$, $p < .01$). **(I, J)** In one animal that still showed a preference for the novel juvenile after anisomycin injection (right), the cannula tips (red dots, 1 mm below the cannula guide tract) were found to be misplaced, located in the central amygdala (left) and piriform cortex (right). *Significant; n.s., not significant.

synaptic plasticity that is thought to play a major role in long-term memory (10,11). Stimulation of the sensory input pathways from the AOB and MOB evoked positive field potential responses in the MeA of anesthetized rats (Figure 3A,B). The initial phase of the response, which resembled the field potentials evoked in other amygdala regions by olfactory bulb stimulation (20), had a latency of ~2 msec, peaking at ~6 msec after the stimulus; this suggests a monosynaptic response. Responses to both AOB and MOB stimulation showed a similar course of input-output curves (Figure 3C). The typical response to AOB stimulation differed from the response to MOB stimulation by a second prolonged positive phase starting 15–20 msec after the stimulus.

We examined the effect of TBS, widely used for induction of LTP (21), on these field potential responses (Figure 3). A small but significant LTD, rather than LTP, was caused by TBS of the AOB, reducing the amplitude of the MeA response to AOB stimulation by about 15%. This LTD was strongly augmented on intraventricular administration of oxytocin 5 min before TBS. In the presence of oxytocin, the response was still depressed to <70% of its baseline value 120 min after TBS. This enhancement of LTD was most likely due to oxytocin receptor activation because the effect was completely blocked when an oxytocin receptor antagonist was applied 10 min before oxytocin administration (Figure 3D–F).

In contrast, when injected without TBS, oxytocin caused ~30% increase in the amplitude of the MeA response to AOB stimulation compared with saline injection. This effect of oxytocin administration was also probably mediated by oxytocin receptor

activation because it was blocked by prior injection of oxytocin receptor antagonist (Figure 3G–I).

In contrast to AOB stimulation, TBS given in the MOB with or without oxytocin or oxytocin receptor antagonist administration did not cause any change in the MeA response to MOB stimulation (Figure 3J–L). We conclude that only the synapses from AOB neurons to MeA neurons show TBS-mediated LTD and that this LTD is oxytocin-dependent.

Having discovered oxytocin-dependent LTD in the MeA, we examined whether this type of synaptic plasticity is involved in long-term SRM formation. We first checked whether oxytocin-dependent LTD can be detected in socially isolated rats, which we have previously shown to lack the ability to acquire long-term SRM (8). To that end, we recorded MeA responses to AOB stimulation in rats that were housed in solitary cages for 7 days and compared them with group-housed rats. In contrast to the group-housed rats, socially isolated rats did not show any oxytocin-dependent LTD after TBS administration (Figure 4A–C). The socially isolated rats also did not show the enhancement of the MeA response to AOB stimulation observed in group-housed rats after oxytocin administration without TBS (Figure 4D–F). Acute social isolation abolished the oxytocin-dependent synaptic plasticity in the AOB-MeA pathway, which correlated with the loss of long-term SRM, suggesting a link between these two phenomena.

We looked for evidence for a direct link between the oxytocin-dependent LTD in the MeA and long-term SRM. Seeking this evidence, we examined the effect of TBS applied to the AOB in rats performing in the social recognition tests, presuming that the TBS would cause nonspecific LTD in the AOB synapses with MeA neurons as in the anesthetized rats. Administration of TBS 10–20 min before memory acquisition (Figure 5A) completely abolished the difference in investigation time for the familiar and novel juveniles, in contrast to the control experiments performed with the same animals 4 days before (control 1) and 6 days later (control 2) (Figure 5B–D). Application of TBS caused the novel juvenile to be investigated for similar time as the familiar juvenile in the control 1 experiment (paired t test, $n = 6$; $t = 1.137$, $df = 5$, $p > .05$). These results suggest that TBS in the AOB causes the adult rat to treat both juveniles as familiar subjects, supporting the idea that stimulus-nonspecific long-term SRM is prompted by TBS-induced LTD in the MeA. In contrast to long-term SRM, short-term SRM (Figure 5E–H) and long-term odor recognition memory (Figure 5I–L) were not impaired by TBS in the AOB before memory acquisition.

Discussion

Mammals show an extremely broad range of social systems and organizations (22), most of which demand the memorization and recognition of specific individuals. This SRM is also the basis for the wide range of emotional relationships humans tend to develop with parents, friends, foes, and lovers. Despite its variability, social behavior of most mammalian species is mediated by a common network of brain regions comprising multiple limbic areas, including the MeA, the lateral septum, the nucleus accumbens, the bed nucleus of stria terminalis, and several hypothalamic areas such as the ventromedial hypothalamus (23). Neuronal activity in this network is thought to be regulated by several modulators, among which oxytocin and its closely related neuropeptide arginine-vasopressin seem to play a pivotal role (24). Oxytocin receptor is variably expressed in most regions of the network in a species-dependent manner, presumably enabling species-specific modulation of the network activity and social behavior (25).

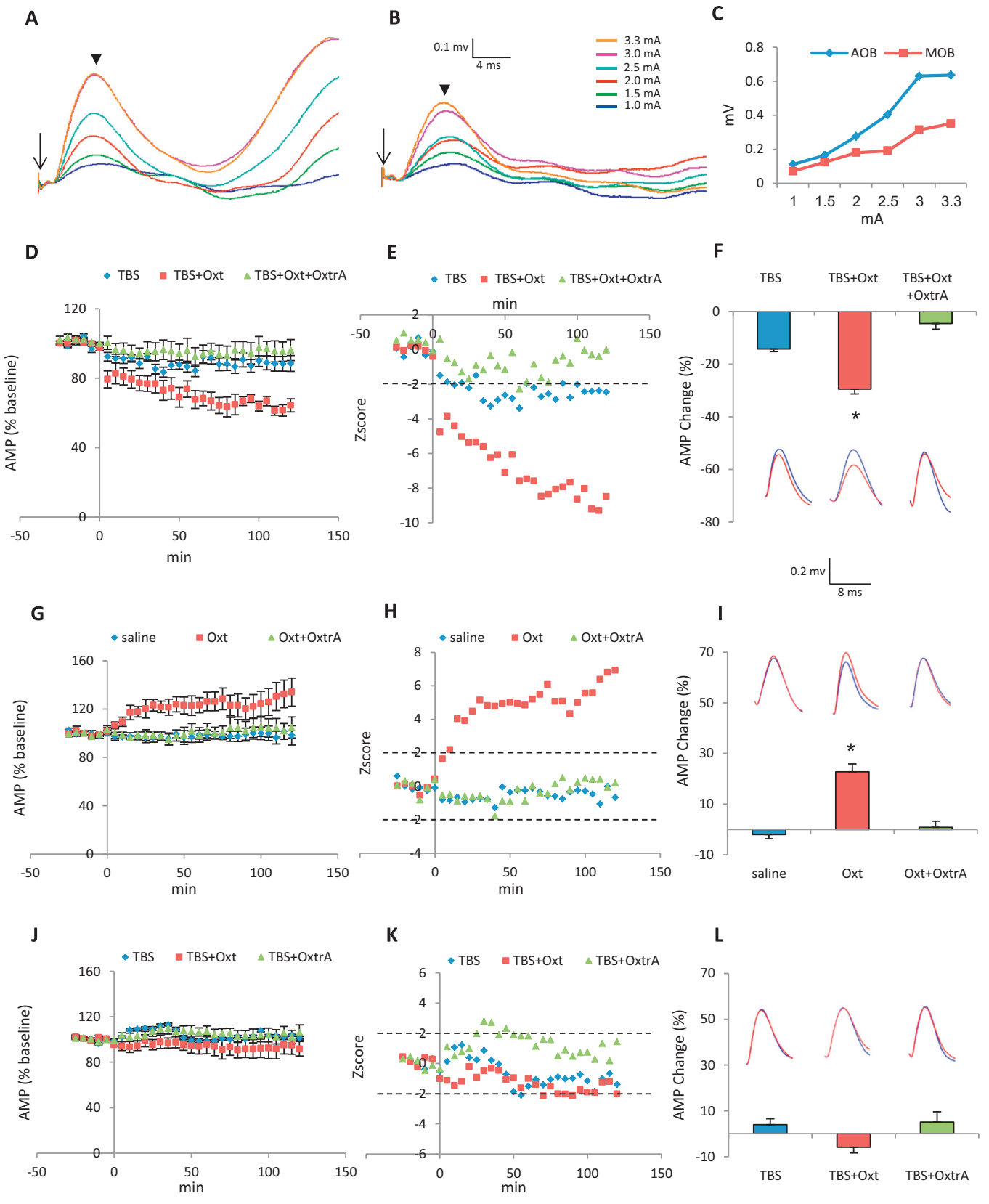
In the present study, we focused on the effect of oxytocin in the MeA and the role of this effect in the acquisition of long-term SRM. The MeA is an integral part of the AOS, receiving the main output of the AOB. It also receives both direct and indirect inputs from the MOB (26), so it is perfectly positioned to integrate information of semiochemicals detected by both olfactory systems. However, little is known about the exact role of the MeA in SRM. For example, it is unknown whether molecular processes, such as protein synthesis in this brain area, are associated with social memory consolidation. We have demonstrated in this study that local administration of the protein synthesis blocker anisomycin to the MeA completely blocks long-term SRM with no effect on short-term memory. This result proves that protein synthesis in the MeA is crucial for consolidation of long-term SRM.

One surprising finding of our study was that application of high-frequency (100 Hz) stimulation, commonly used to elicit LTP in the hippocampus and other brain regions (11), caused LTD rather than LTP in the AOB. This result suggests that at least one process related to long-term SRM is long-lasting depression in specific synaptic contacts of the AOB-MeA pathway. This conclusion is supported by the lack of LTD in socially isolated rats, previously shown by us to be impaired in long-term SRM consolidation (8). Application of TBS to the AOB before SRM acquisition blocked long-term SRM of a specific stimulus, presumably by saturating most synapses with LTD. We conclude that the oxytocin-mediated LTD in the AOB-MeA pathway is directly involved in long-term SRM formation.

The sense that familiarity with a specific social stimulus may be associated with depression of inputs arriving through the AOS fits more recent data showing that these stimuli are related to alertness or even aversion. A major portion of mouse vomeronasal receptors specifically bind heterospecific cues from sympatric competitors (27). In addition, AOS activation by pup cues blocks parental behavior and induces aggression toward pups in virgin males and females (28), whereas reduction of AOS activation caused by pup exposure inhibits this aggression and allows induction of parental behavior (29). The mate recognition memory associated with the Bruce effect in mice is also mediated by increasing the inhibition in the AOB (30). Several types of social learning seem to be mediated by depression of inputs arriving via the AOS. This conclusion is further supported by multiple lines of evidence showing that the prosocial effect of oxytocin in mammals involves the reduction of responses to aversive social stimuli detected at least partially by the AOS (31). For example, oxytocin is involved in the reduction in aggression toward pups and emergence of maternal behavior in rats (32). Our finding that oxytocin enhanced LTD induction in the AOB-MeA pathway suggests a mechanism by which oxytocin may attenuate AOS responses.

Multiple studies support the involvement of oxytocin activity in the MeA in SRM formation. The MeA expresses high levels of the oxytocin receptor (14), and an antisense-mediated down-regulation of oxytocin receptor expression in the murine MeA severely impairs SRM (33). Oxytocin-knockout mice do not show SRM at all, unless oxytocin is locally administered to the MeA (15). Similar requirement for oxytocin activity in the MeA for SRM acquisition was reported in rats, although some species-specific differences may apply (16). Nevertheless, the precise nature of the action of oxytocin in the MeA remains unknown.

Oxytocin was shown to modulate synaptic plasticity in several brain regions. In brain slices, it enhances long-lasting LTP induction in hippocampal CA1 pyramidal cells (34,35) as well as in layer V pyramidal neurons of the medial prefrontal cortex.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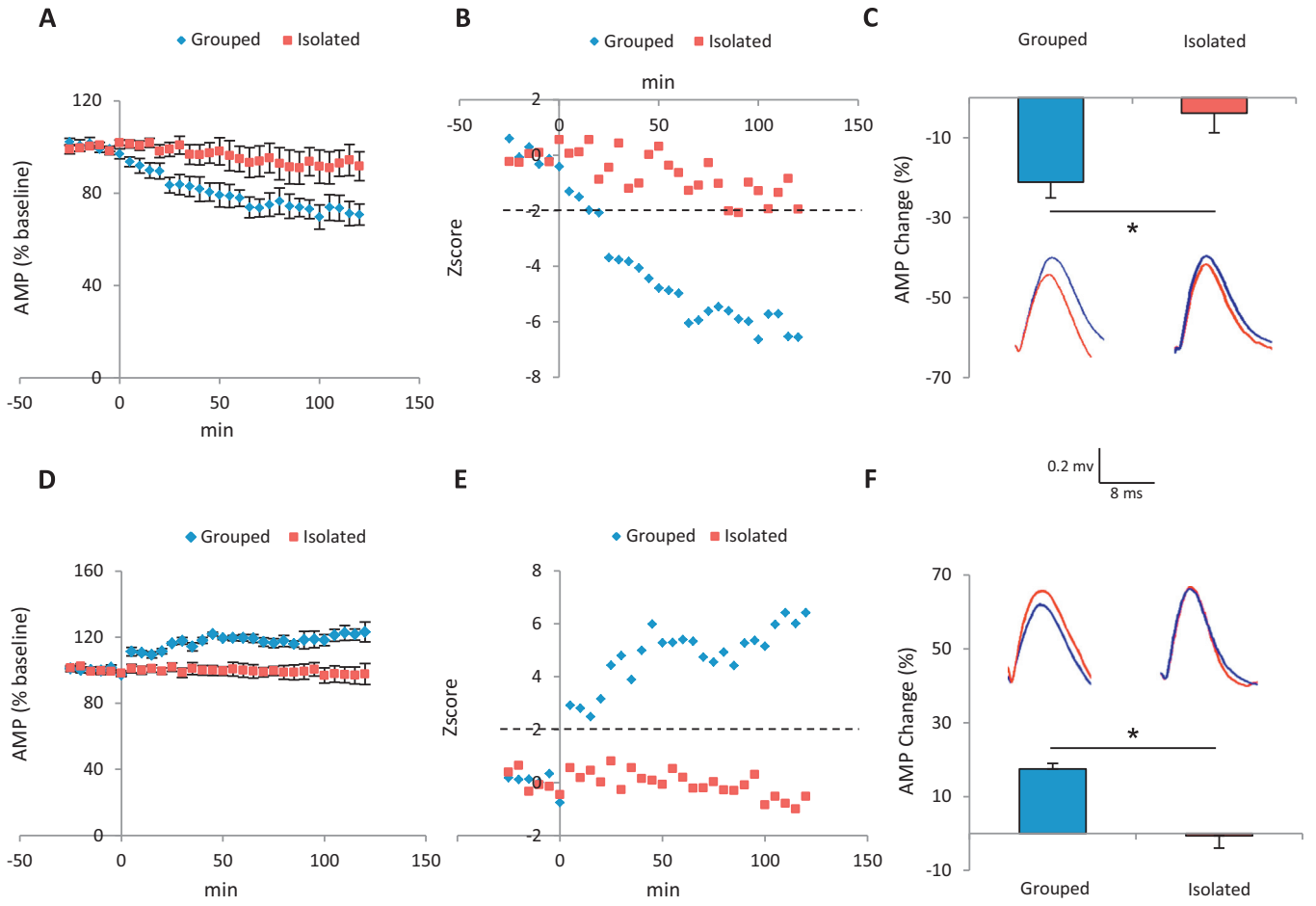

Figure 4. Lack of oxytocin-dependent long-term depression in socially isolated rats. **(A)** Mean amplitude (AMP; % of baseline) of the medial amygdala response to accessory olfactory bulb stimulation after theta burst stimulation at time 0 with oxytocin application to group-housed (blue, $n = 6$) and socially isolated (red, $n = 6$) rats. **(B)** Changes in Z score of the recordings shown in **(A)**. Dashed line marks the border of significance (2 SD from the baselines). No significant long-term depression was found in socially isolated rats, in contrast to group-housed rats. **(C)** Mean percent amplitude changes from baseline (± SEM) of the recording in **(A)** averaged over 120 min after theta burst stimulation. Representative traces of the response before (blue) and 120 min after (red) theta burst stimulation are shown superimposed below each bar. A statistically significant difference was found between the two groups (t test; $t = -2.646$, $df = 10$, $p < .05$). **(D)** Same as **(A)** but with no theta burst stimulation. Increase in amplitude was not observed after oxytocin application in socially isolated rats (red, $n = 6$), in contrast to group-housed rats (blue, $n = 4$). **(E)** Same as **(B)**, for the recordings in **(D)**. **(F)** Same as **(C)**, for the recordings in **(D)**. A statistically significant difference was found between both groups (t test; $t = 2$, $df = 8, 46$, $p < .01$). *Significant.

Oxytocin was shown to facilitate LTP induction in synapses of excitatory mitral cells on inhibitory interneurons in mouse AOB slices (36). This result aligns with our results to suggest that oxytocin acts to attenuate AOS responses at several stages of this system. In contrast to all of these examples of oxytocin-enhanced LTP in brain slices, in the only study that explored the effect of

Figure 3. Oxytocin-dependent, pathway-specific long-term depression in the medial amygdala. **(A)** Local field potential recorded in the medial amygdala of one animal in response to various intensities of accessory olfactory bulb (AOB) stimulation (arrow). The peak of the early positive phase (arrowhead) occurs ~6 msec after the stimulus, suggesting a monosynaptic response. **(B)** Same as **(A)** for the main olfactory bulb (MOB) of the same animal. Note the lack of the second positive phase, seen in the response to AOB stimulation **(A)**. **(C)** Input-output curves for the recordings in **(A, B)**. **(D)** Mean amplitude (% of baseline) of the medial amygdala response to AOB stimulation after administering theta burst stimulation (TBS) at time 0 with and without administration of oxytocin (TBS + Oxt, red, $n = 6$; TBS, blue, $n = 9$) or with administration of oxytocin together with oxytocin receptor antagonist (TBS + Oxt + OxtrA, green, $n = 5$). **(E)** Changes in Z score of the recordings shown in **(D)**. Dashed line marks the border of significance (2 SD from the baselines). Long-term depression after TBS alone (blue) lies mostly beyond the significance border. **(F)** Mean percent amplitude changes from baseline (±SEM) in the recording in **(D)** averaged over 120 min after TBS. Representative traces of the response before (blue) and 120 min after (red) stimulation are shown superimposed below each bar. Note the significant enhancement of long-term depression after oxytocin administration compared with no drug administration or with oxytocin and oxytocin receptor antagonist administration (analysis of variance; $F = 8.737$, $df = 2, 17$, $p < .05$; *$p < .05$, Tukey's post hoc). **(G)** Same as **(D)** but with no TBS. Application of oxytocin (red, $n = 5$) caused a gradual increase of 20%–30% in the local field potential response compared with application of either saline (blue, $n = 4$) or oxytocin and oxytocin receptor antagonist (green, $n = 5$). **(H)** Changes in Z score of the recordings in **(G)**. Only oxytocin application caused a significant change. **(I)** Same as **(F)**, for the recordings in **(G)**. An increase in amplitude was observed after oxytocin application compared with application of either saline or oxytocin and oxytocin receptor antagonist (analysis of variance; $F = 5.679$, $df = 2, 11$, $p < .05$; *$p < .05$, Tukey's post hoc). **(J–L)** Same as **(D–F)** for TBS in the MOB, without (red, $n = 6$) or with oxytocin (blue, $n = 9$) or oxytocin receptor antagonist (green, $n = 6$) application. As apparent from the Z score analysis **(K)**, no significant long-term change was observed using MOB stimulation, with no difference between the treatments (analysis of variance; $F = .177$, $df = 2, 18$, $p > .05$). AMP, amplitude.

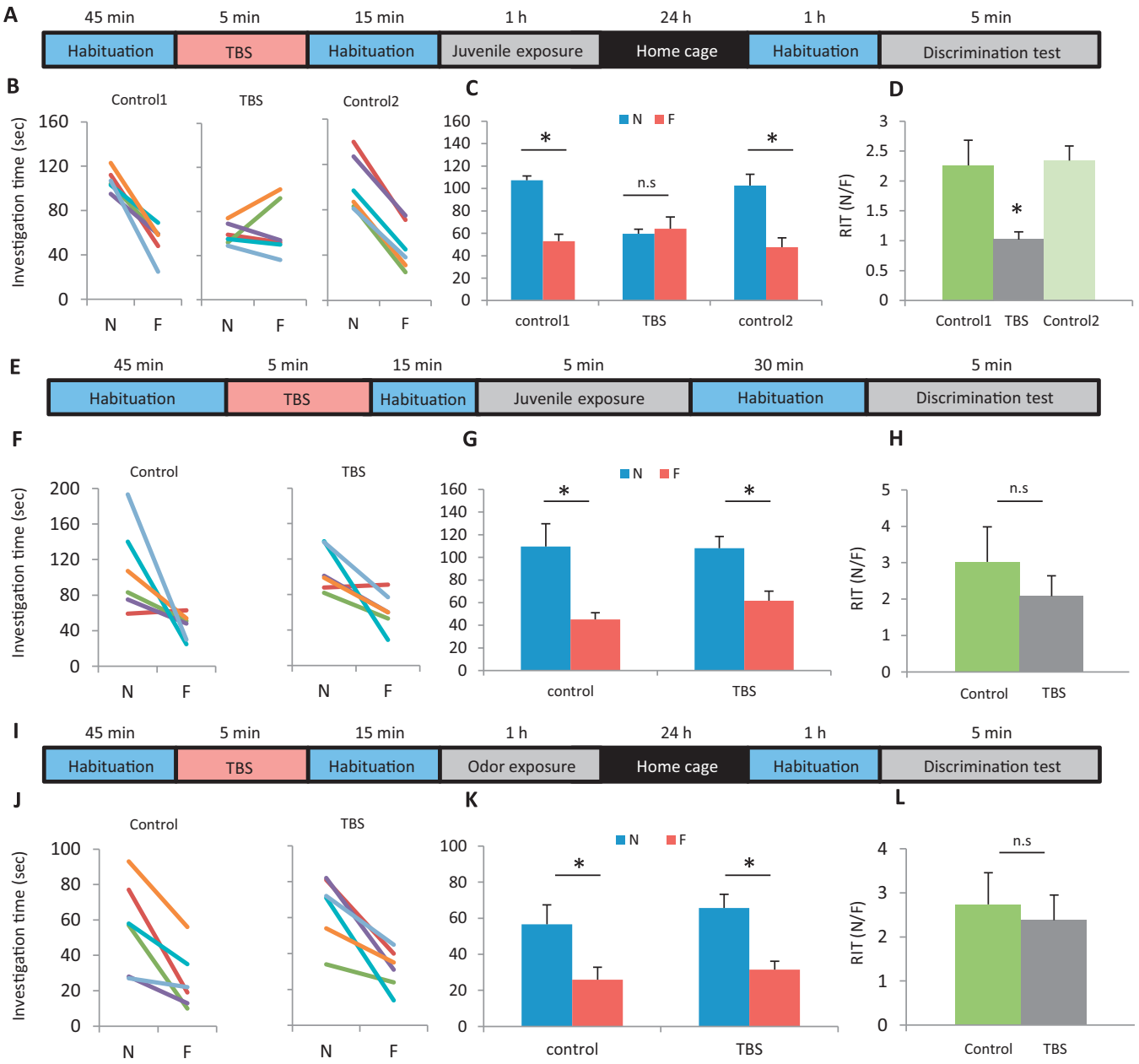

Figure 5. Blockade of stimulus-specific long-term social recognition memory by induction of stimulus-nonspecific long-term depression in the medial amygdala. **(A)** Scheme describing the time course of the experiment using the long-term social discrimination paradigm. **(B)** Lines connecting the investigation times of the novel (N) and familiar (F) juveniles for each rat receiving theta burst stimulation (TBS) or no stimulation (control) in the accessory olfactory bulb before the long-term social discrimination paradigm. The control tests were done, using the same animals and paradigm, 4 days before (control 1) and 6 days after (control 2) the experiment with TBS administration. **(C)** The TBS abolished the difference in investigation time for the novel and familiar juveniles (paired *t* test, $n = 6$; control 1, $t = 6.996$, $df = 5$, $p < .01$; TBS, $t = .463$, $df = 5$, $p > .05$; control 2, $t = 15.704$, $df = 5$, $p < .01$). Note the similarity between the investigation time for both juveniles with TBS and familiar juveniles in the control experiments, suggesting induction of juvenile-nonspecific long-term memory by the TBS administration. **(D)** There was a significant difference in ratio of investigation times (RIT) between TBS administration and the control experiments (paired *t* test; control 1–long-term potentiation, $t = 3.236$, $df = 5$, $p_{corr} < .05$; control 2–long-term potentiation, $t = 3.589$, $df = 5$, $p_{corr} < .05$). **(E)** Same as **(A)**, for the short-term social discrimination paradigm. **(F)** Lines connecting the investigation times of the novel and familiar juveniles for each rat receiving TBS or no stimulation (control) before the short-term social discrimination paradigm. **(G)** In contrast to the long-term paradigm, TBS administration did not affect the significant difference in the investigation time for both juveniles when the short-term social discrimination paradigm was used (paired *t* test, $n = 6$; control, $t = -2.521$, $df = 5$, $p < .05$; TBS, $t = -2.993$, $df = 5$, $p < .05$). **(H)** No significant difference in RIT was found with and without TBS in the short-term social discrimination paradigm (sign test, $p > .05$). **(I–L)** Similar to **(E–H)**, no change was found in a long-term odor discrimination test after TBS in the accessory olfactory bulb (paired *t* test, $n = 6$; control, $t = -3.761$, $df = 5$, $p < .05$; TBS, $t = 4.516$, $df = 5$, $p < .01$ **(K)**; paired *t* test, $n = 6$; $t = .338$, $df = 5$, $p > .05$ **(L)**). *Significant; n.s., not significant.

oxytocin on activity-induced synaptic plasticity in vivo, it was found to induce LTD in the dentate gyrus of anesthetized rats (37), similar to our results.

We demonstrated in our study that exogenous oxytocin administration strongly augmented the LTD after TBS in the AOB-MeA pathway. Application of specific oxytocin receptor

antagonist completely blocked this LTD, suggesting that it is supported by a low presence of endogenous oxytocin in the amygdala of the anesthetized rats (38). The mechanism by which oxytocin modulates LTD in the MeA may be related to our observation that oxytocin administration without TBS increases the MeA synaptic response to AOB stimulation, an effect that is expected to enhance the induction of synaptic plasticity by TBS applied later.

Our observation that the effect of oxytocin on synaptic plasticity is specific for the AOB-MeA pathway and was not present in the MOB-MeA pathway may be related to a synapse-specific mechanism but may also be explained if distinct neuronal populations in the MeA are targeted by AOB and MOB mitral cells. Further experiments using intracellular recordings are needed to determine the biochemical and biophysical basis of the effect of oxytocin on the synaptic responses of MeA neurons.

The relationship between the data presented here and human social memory is complicated by the minor role played by olfaction in general (39) and pheromones in particular (40) in human social interactions. Nevertheless, human social behavior is still highly influenced by the network of limbic areas described earlier (24,41) and mainly by the amygdala, which receives vast inputs from all sensory systems (42). A growing body of evidence (43–49) points to the role of oxytocin in attenuating human amygdala responses to aversive and fearful stimuli as a main mechanism for the prosocial effect of this neuropeptide (50). The oxytocin-dependent LTD in the amygdala shown in this study may be one of the mechanisms subserving oxytocin activity in the human brain.

In conclusion, our results reveal the pivotal role played by the MeA in long-term social memory formation and suggest a mechanism by which oxytocin activity in this region enhances social learning and supports prosocial behavior.

This work was supported by the Legacy Heritage Bio-Medical Program of the Israel Science Foundation (Grant No. 1901/08), by the Autism Speaks Foundation (Grant No. 3613), and by the Israel Science Foundation (Grant No. 1350/12). We thank Dr. M. Manning from the University of Toledo, Spain, for a generous gift of desGly-NH_2,d$(CH_2)_5$[D-Tyr^2,Thr^4]OVT.

The authors report no biomedical financial interests or potential conflicts of interest.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2014.03.022.

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