

Behavioral Tagging: Role of Neurotransmitter Receptor Systems in Novel Object Recognition Long-Term Memory

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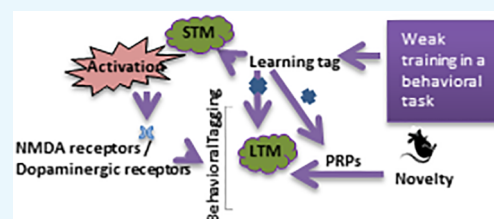
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ABSTRACT: Strong training is known to form long-term memory (LTM) as it is an inducer for both a learning tag (just like a synaptic tag/molecular tag) and plasticity-related proteins (PRPs), while weak training is an inducer of only a learning tag. However, weak training can also lead to LTM if paired with another behavioral task (open field in our study—a representative of a novel environment) around the time of PRP arrival. Weak behavioral training is a learning tag inducer, while the open field is a PRP inducer. The learning tag then captures these PRPs to form LTM. This is the basis of behavioral tagging (BT). BT is a well-known model for the evaluation of a few learning and memory

forms. In this work, we examined the role of glutamate and D1/D5 (dopamine) receptors in the synthesis of a novel object recognition (NOR) tag (learning) as well as in PRP arrival, which come together to form NOR-LTM. Employing antagonists and/or agonists preceding or proceeding the open field and/or NOR training, it was revealed that the activation/stimulation of D1/D5 (dopamine) receptors and glutamatergic NMDA receptors plays a critical part in PRP arrival. We found that the activation/stimulation of NMDA receptors also contributes to the setting of the learning tag. Moreover, changes in glutamate, dopamine, and GABA neurotransmitter levels were also analyzed. These findings thus demonstrate the critical time window required for NOR-LTM formation based on the process of BT along with the role of activation/stimulation of D1/D5 (dopamine) receptors and NMDA receptors in the arrival of PRPs and learning tags for NOR-LTM formation.



1. INTRODUCTION

Molecular machinery activation in neurons after a stimulus causes synapse-associated changes, and learning stimulations lead to memory.^{1,2} The memory consolidation process requires the stabilization of novel information through steps over time that make the memory stay for long.³ It has been now widely acknowledged that LTM needs PRP synthesis. The memory formation process requires some proteins to activate. These proteins activate during learning. This is a crucial step for the transformation of newly attained information into LTM. The Synaptic Tagging and Capture (STC) hypothesis focuses on late associativity and Long Term Potentiation.^{4,5} It states that when a synapse gets activated through a stimulus (which is weak in nature), a tag (synaptic) is generated, and this tag captures proteins that arrive in response to another stimulus (which is strong in nature).⁶ BT model derives its roots from the above statement. According to BT, LTM is dependent on the generation of a learning tag and the arrival of PRPs. The capture of PRPs by the learning tag at tagged sites (often synapses) around a certain time frame leads to LTM.^{7–10}

There is evidence that the medial prefrontal cortex (mPFC) plays a significant role in object recognition memory.¹¹ Glutamate, a type of an important excitatory neurotransmitter, acts postsynaptically on the following ionotropic receptors, the NMDA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainic acid receptor.¹² Ion flux through

NMDA is known to have associations with neuroplasticity, learning, and memory. Similarly, receptors like dopamine (catecholamines) are important in mediation of many functions of the CNS, such as cognition, emotion, memory processing, etc.¹³

Here, in the present work, we first demonstrated BT using NOR and open field at different time frames. This was done to find out the critical time frame during which PRPs arrive at tagging site/s for capture by the learning tag that would lead to NOR-LTM formation. Second, we investigated whether the activation of NMDA and D1/D5 (dopamine) receptors plays a role in inducing tag setting and PRP synthesis. The below work reveals that catecholamines like D1/D5 (dopamine) receptors are important for PRP arrival. Also, the activation/stimulation of NMDA (glutamatergic) receptors is important for the generation of a learning tag as well as PRP arrival.

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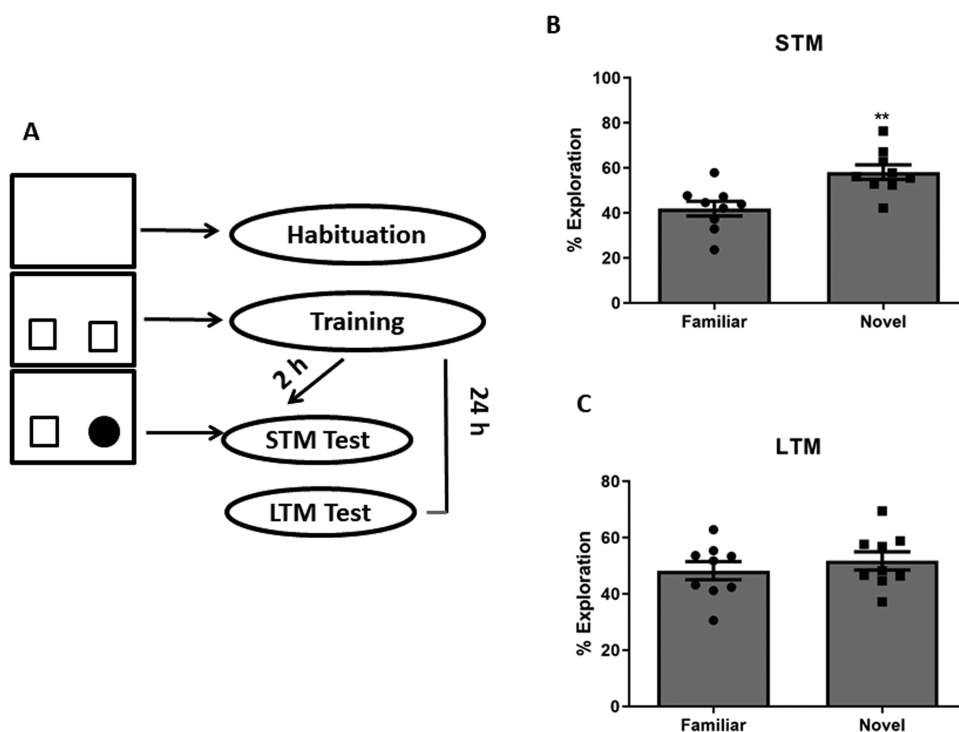


Figure 1. (A) Experimental outline for a novel object recognition test. (B) Significant difference was found between familiar and novel object exploration when the animals were subjected to STM after 2 h; $**P < 0.01$ versus familiar. (C) No significant difference was found when rats were tested for LTM 24 h following training. Time of exploration (%) for the novel object over time taken for both the objects is presented. Data were analyzed statistically using a *t*-test and are presented as the mean \pm SE ($n = 9$ per group).

2. RESULTS

2.1. Exposure to Open Field 1 h before and Not 2 h before Training Promotes the LTM of the Novel Object Recognition Task (NOR-LTM).

We subjected rats to NOR training and tested whether LTM formation occurred due to the exposure of rats to a novel environment provided before training. For NOR training, the animals were allowed to freely explore two similar objects. For the test, memory was evaluated in terms of the time rats took to explore a previously explored/familiar object (similar to the one during the training session) and a novel object. This training-induced STM was tested 2 h later ($P < 0.01$; Figure 1B). The training was unable to form LTM when tested after 24 h ($P = \text{n.s.}$; Figure 1C). For evaluating the result of novel environment exploration on NOR-LTM, the rats were subjected to a 5 min open field exploration prior to NOR training. Exploration of the open field 1 h and not 2 h prior to object recognition training (weak) induced LTM ($P < 0.01$; Figure 2B) ($P = \text{n.s.}$; Figure 2C). The results were analyzed using a *t*-test. Thus, it can be said that environment novelty plays a role in object recognition memory, restricted to a certain time frame.

2.2. Post-training Open Field Exposure Promotes Object Recognition LTM around a Restricted Time Frame.

The result of post-training novelty exposure in response to weak NOR training (after) was evaluated. Rats were made to undergo a 5 min exploration of the open field after NOR training. Exploration of the open field 0.25 h as well as 1 h after the training led to LTM ($P < 0.001$; Figure 3B) ($P < 0.01$; Figure 3C). The exposure to a novel environment 2 h after NOR training was unable to form LTM ($P = \text{n.s.}$; Figure 3D). Therefore, here also, it can be said that the role of

environment novelty after NOR training (weak) in LTM formation is confined to a restricted time frame.

2.3. Effect of Novel Open Field Exploration on Dopamine, GABA, and Glutamate Levels.

We evaluated the levels of dopamine, GABA, and glutamate neurotransmitters in control (animals not subjected to novelty/open field) and BT (animals subjected to novelty/open field) groups. We found significantly higher dopamine levels in the BT group compared to those in the control group ($P < 0.01$; Figure 4A), significantly lower GABA levels in BT as compared to those in the control group ($P < 0.01$; Figure 5A), while no significant difference was found in glutamate levels ($P = \text{n.s.}$; Figure 6A).

2.4. Exploration of Open Field Makes PRPs Available for BT.

The effect of novel environment exploration on NOR-LTM was reliant on proteins made available by open field exposure and the formation of learning tags by NOR training (weak). This is what BT is based on. Thus, to confirm that the exploration of open fields provides the necessary proteins for the formation of NOR-LTM, we infused the PRP inhibitor Ani into the mPFC just after the exploration of the open field 1 h before weak NOR training. It was observed that the inhibition of PRPs stopped the effect of novel environment exploration on LTM after NOR training (weak) ($P < 0.001$) (Figure 7A). However, Ani administered 1 h prior to NOR training (strong and capable of inducing both learning tag and PRPs) was unable to alter LTM, indicating that Ani was unable to affect LTM acquisition/consolidation (Figure 7B). Exploration time was evaluated in terms of the time exploring familiar/replaced new objects over the time (total) exploring both objects.

2.5. NMDA Receptors Play a Dual Role in Tagging and Protein Synthesis.

The effect of novel environment exploration on NOR-LTM was reliant on proteins made

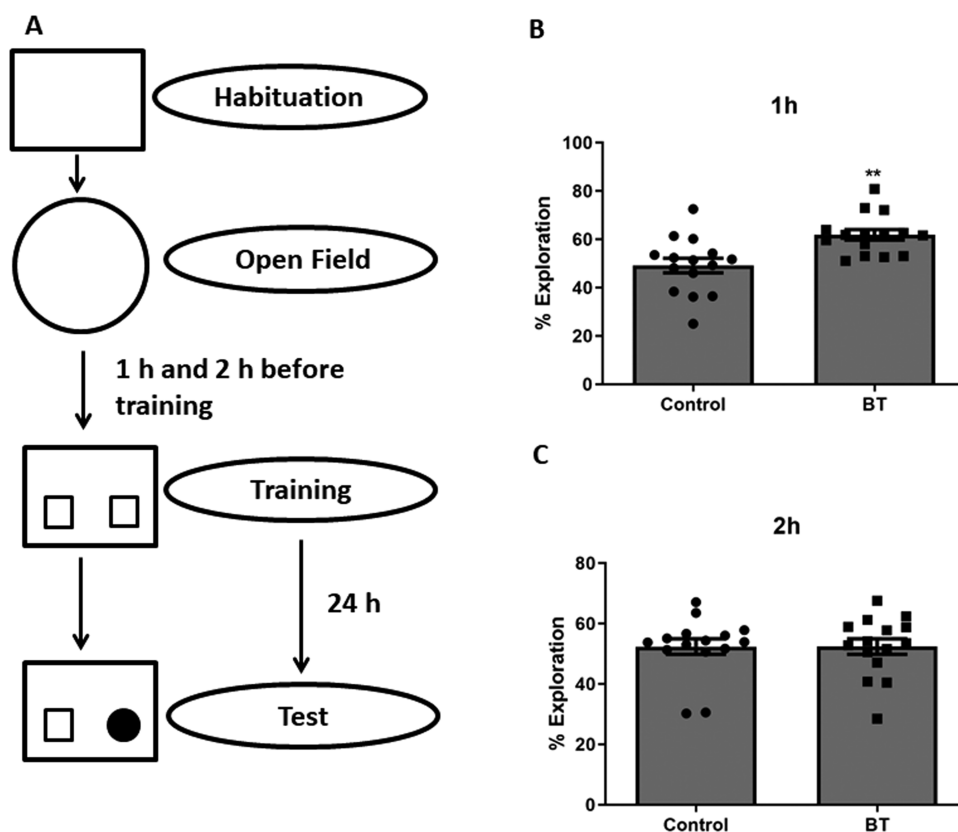


Figure 2. (A) Novel open field exploration promotes NOR-LTM 1 h before training. (A) The experimental design of the procedure followed (BT), (Control = BT - open field). (B) The animals explored the novel open field arena for 5 min before 1 h of NOR training. The animals were tested 24 h later for LTM ($n = 15$ per group). Time of exploration (%) for the novel object over time taken for both objects is presented. Data were analyzed statistically using a t -test and shown in the form of mean \pm SEM; $**P < 0.01$ versus control. (C) The animals explored the novel open field arena for 5 min before 2 h of NOR training. The animals were tested 24 h later for LTM ($n = 15$ per group). Time of exploration for the novel object over time taken for both the objects is presented. Data were analyzed statistically using a t -test and presented as mean \pm SE.

available by open field exposure and the formation of learning tags by NOR training (weak). To investigate this, the animals were trained in an NOR (weak) task and then tested after 24 h. A lower % exploration indicated that the training (weak) was insufficient for NOR-LTM (Figure 8A). But, when the animals explored a novel open field 1 h prior to training, NOR-LTM induction occurred ($P < 0.001$) (Figure 8A). For investigating the role of NMDA receptors in NOR learning tag formation, we administered NMDA antagonist MK-801 20 min prior to subjecting the animals to NOR training (weak). Inhibitions created by the NMDA receptor antagonist MK-801 administered prior to training remained unaffected by novel exploration. MK-801 impaired the formation of learning tags, thereby affecting NOR-LTM, as evident from the figure ($P < 0.001$) (Figure 8A). This prevention was supported by Ani administered just after open field exploration ($P < 0.001$) (Figure 8A). The failure of open fields to promote NOR-LTM indicates that the formation of an NOR learning tag is hindered in the case of receptor blockade. In addition, to reassure that NMDA receptor activation/stimulation is required for PRP arrival, MK-801 was administered before subjecting rats to novelty 1 h prior to training. NOR-LTM testing was done after 24 h. The administration of MK-801 impaired NOR-LTM ($P < 0.01$) (Figure 8A). This establishes that NMDA receptors are crucial for NOR-LTM. Therefore, our results indicate that the NMDA receptors are necessary for both the formation of learning tags and PRPs needed for NOR-LTM.

The animals were treated (i.p.) with or without the NMDA agonist DCS or vehicle 30 min prior to NOR training (weak), and testing of NOR-LTM was performed after 24 h ($P < 0.001$) (Figure 8B). Moreover, investigations were done to determine whether DCS is involved in NOR-LTM via influencing PRP arrival. For this, first, DCS was administered (i.p.) to the animals; second, Ani (PRP inhibitor) was administered into the mPFC 10 min after DCS. The animals were subjected to NOR training (weak) 30 min later. LTM was measured 24 h later. DCS promoted NOR-LTM ($P < 0.001$), but Ani administration in rats prevented NOR-LTM ($P < 0.001$) (Figure 8B). Exploration time was evaluated in terms of the time exploring familiar/replaced new objects over the time (total) exploring both objects.

2.6. Promotion of LTM Formation by Novel Open Field Exposure in Object Recognition Memory is Influenced by D1/D5 (Dopamine) Receptor Stimulation.

As mentioned above that the influence of novel environment on NOR-LTM involves PRP arrival (in response to open field exposure) and learning tag formation (in response to weak NOR training), investigation of the dopaminergic role in PRP arrival was performed. For this, the animals were provided NOR training (weak), and a test was performed after a delay of 24 h. Less % exploration indicated nonconsolidation of LTM in response to weak training (Figure 9). But, when the novel open field was explored 1 h prior to training, the formation of NOR-LTM was observed ($P < 0.001$) (Figure 9). As cell groups of dopamine from the ventral tegmental area (VTA)

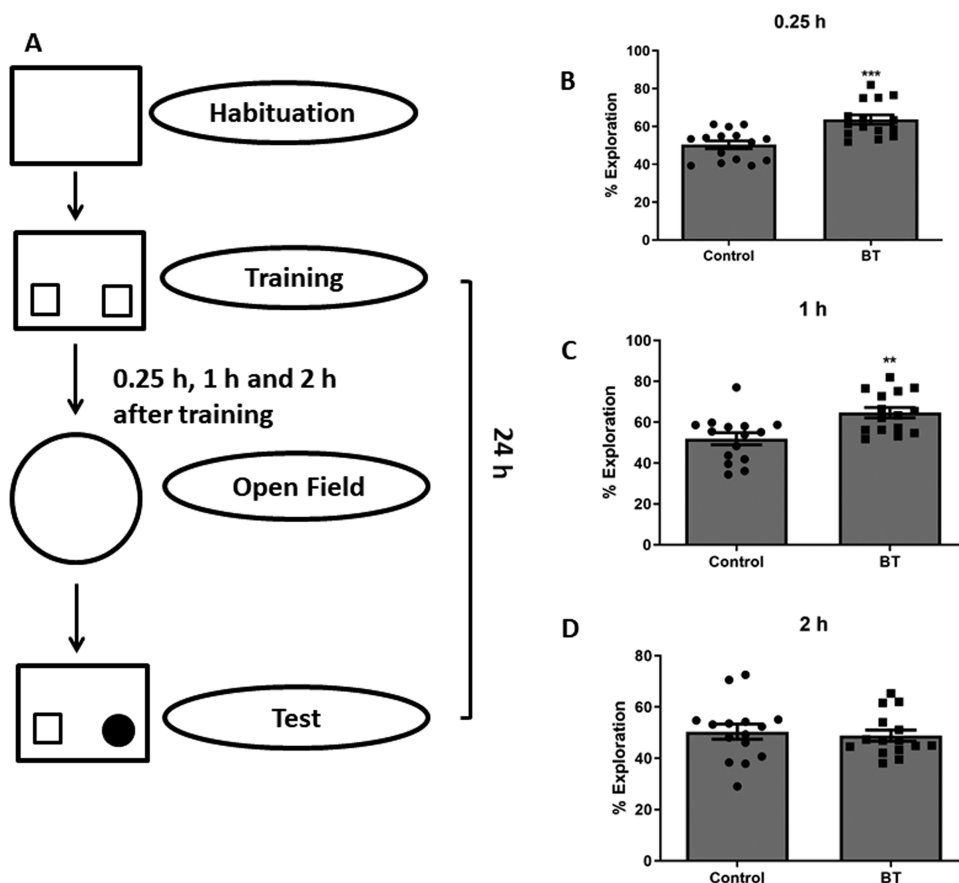


Figure 3. (A) Experimental design of the procedure followed (BT), (Control = BT - open field). The animals were exposed to novel open field exploration 2, 1, and 0.25 h after NOR training. (B) The animals explored the novel open field arena for 5 min after 0.25 h of NOR training. The animals were tested 24 h later for LTM ($n = 15$ per group); $***P < 0.001$ versus control. (C) The animals explored the novel open field arena for 5 min after 1 h of NOR training. The animals were tested 24 h later for LTM ($n = 15$ per group). $**P < 0.01$ versus control. (D) The animals explored the novel open field arena for 5 min after 2 h of NOR training. The animals were tested 24 h later for LTM ($n = 15$ per group). Exploration time for the novel object to the time taken for both objects is presented. Data were analyzed statistically using a t -test and are presented as the mean \pm SE.

innervate the mPFC and amygdala, effects of the D1/D5 (dopamine) receptors were investigated employing SCH (a dopamine antagonist). Open field exploration was successful in escaping the inhibitory effect of SCH, which was administered 10 min before training. Ani successfully altered this when administered just after open field exploration ($P < 0.001$) (Figure 9). SCH was infused into the mPFC 10 min prior to exposing rats to open field (which was 1 h pretraining). Testing of NOR-LTM was performed 24 h later. The administration of SCH impaired NOR-LTM ($P < 0.001$) (Figure 9), confirming that D1/D5 (dopamine) receptors have an important contribution toward promoting the effect of novelty on NOR-LTM. Therefore, this indicates that the stimulation of D1/D5 (dopamine) receptors is crucial for PRP arrival needed for NOR-LTM. Exploration time was evaluated in terms of the time exploring familiar/replaced new objects over the time (total) exploring both objects.

3. DISCUSSION

Here, in this work, we have shown how exposure to a novel environment (open field in this study) helps in NOR-LTM formation. Synaptic stimulation due to one stimulus (weak) forms a tag, which in turn pairs up with the PRPs arrived as a result of another stimulus (strong).^{7,8} Novelty has reinforcing properties that motivate the exploration of new environments.

The results from our study suggest that some of the neurons that get stimulated in NOR training get tagged and later on capture PRPs (in response to novel open field exploration) when this incident occurs around a critical time window of 1 h, indicating that this is the time frame needed for NOR-LTM. At 2 h, we did not find significant LTM formation. This might be due to the transient characteristics of the learning tag as well as the temporal course of proteins required. The memory trace might favorably be assigned to those neurons that are in highly excitable mode at the time of training.^{18,19} Here, the excitation ability of neurons gets amplified due to learning.^{20–22}

The neurotransmitter correlates of BT have not been investigated yet. The interactions of forebrain neurotransmitter systems during novelty have not yet been unraveled. There have been previous reports highlighting elevated dopamine levels with novelty detection in the mPFC.^{23,24} In accordance, we found here that object recognition BT is associated with increased dopamine levels. GABA levels have indicated significantly reduced levels of GABA on exposure to novelty and enhanced levels on exposure to familiarity.²⁵ Similar to this, we observed decreased GABA levels in BT as the latter is associated with novelty detection. No significant change has been found in glutamate tissue levels, consistent with a previous report demonstrating that in the case of novelty

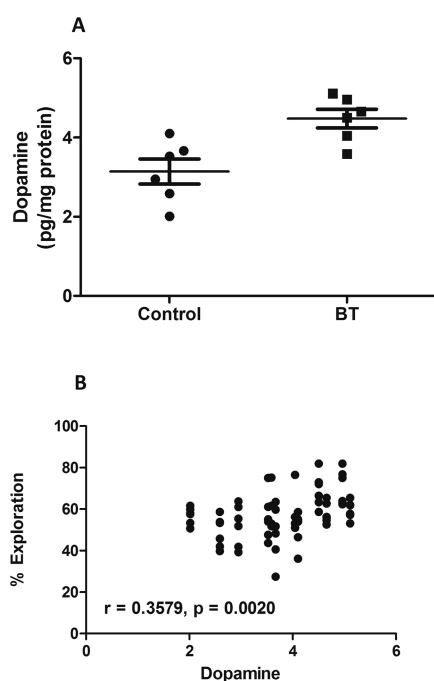


Figure 4. (A) Dopamine concentration evaluated in the PFC of animals with (BT) and without (Control) novel open field exploration. The animals were sacrificed immediately after the behavioral assessment. Dopamine (concⁿ) is presented in the form of pg/mg protein. The results were analyzed statistically using a *t*-test and presented as mean \pm SE; ***P* < 0.01 versus control. (B) Correlation between exploration (%) of novel objects versus dopamine. A significant positive correlation was present between the exploration (%) of novel objects versus dopamine levels (*n* = 6 per group).

exposure, no significant change was found in the glutamate levels in the hippocampus/cortex regions of rodents.^{6,26}

The D1/D5 (dopamine) receptor has been known to participate in the tagging of synapses and subsequent capture of proteins^{27–29} and also BT.³⁰ Suppression of the D1/D5 (dopamine) receptor affected the BT process. Studies on the formation of a loop by dopaminergic neurons (VTA), which penetrates the prefrontal cortex, have been reported. It also tells about the propagation of information (basically a type of signal in response to novelty) from the hippocampus toward VTA as soon as the hippocampus receives such information.³¹ These projections, which are in the prefrontal cortex, are essential for normal cognition. Novelty exposure is known to enhance the stimulation of VTA dopaminergic cell groups.³² Exposure to novelty increases dopamine levels,^{33,34} and similar findings have been found in the present study.

Moreover, the involvement of different neurotransmitter systems in both PRP synthesis and the learning tag formation process has also been investigated. We found that the administration of MK-801 around both open field exposure and NOR training impaired the NOR-LTM promotion. NMDA receptors have been known to be involved in different learning and memory tasks.^{14,35} Based on this, we studied whether NMDA receptors have a role to play in the formation of the NOR learning tag. The failure of open field in promoting NOR-LTM when MK-801 was administered before weak NOR training indicates that an NOR learning tag is affected by the blocking of NMDA receptors. This suggests that NMDA receptors are crucial for the formation of learning tags. There

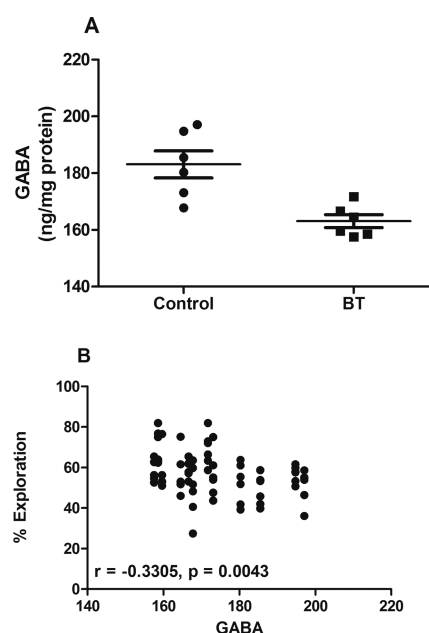


Figure 5. (A) GABA levels evaluated in the PFC of animals with (BT) and without (Control) novel open field exploration. The animals were sacrificed immediately after the behavioral assessment. Levels of GABA are presented as ng/mg protein. Data were analyzed statistically using a *t*-test and presented as mean \pm SE; ***P* < 0.01 versus control. (B) Correlation between exploration (%) of novel objects versus GABA. A significant negative correlation was present between the exploration (%) novel objects and GABA levels (*n* = 6 per group).

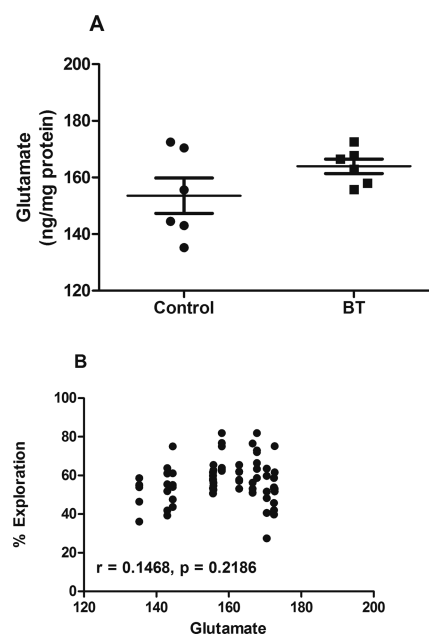


Figure 6. (A) Glutamate levels evaluated in the PFC of the animals with (BT) and without (Control) novel open field exploration. The animals were sacrificed immediately after the behavioral assessment. Levels of glutamate are presented as ng/mg protein. Data were analyzed statistically using a *t*-test and presented as mean \pm SE. No significant difference was observed. (B) Correlation between the exploration (%) of novel objects and glutamate levels (*n* = 6 per group).

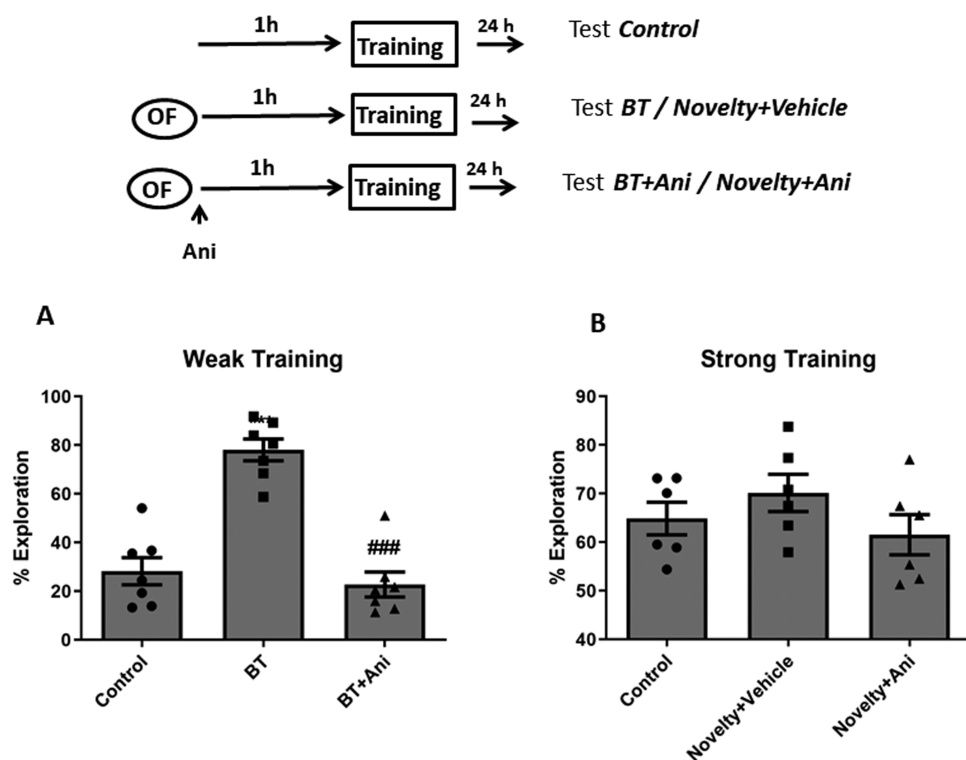


Figure 7. Obstruction to PRP arrival hinders NOR-LTM promotion. The effect of Ani infusion on the promotion of NOR-LTM is shown in rats treated under weak and strong training. Rats received bilateral intra-mPFC infusions of vehicle and Ani. The animals of the control group were administered with vehicle 1 h prior to NOR training and did not undergo novelty exposure. % Exploration was analyzed 24 h following NOR training. (A) PRP inhibitor Ani blocked NOR-LTM promotion $***P < 0.001$ versus control (weak training) ($n = 7$ per group), $###P < 0.001$ versus BT (weak training). (B) No significant difference was observed among different groups when subjected to strong training ($n = 6$ per group). Time of exploration (%) for the novel object over time for both objects is taken into consideration for data analysis. Data were analyzed statistically using ANOVA (one way) and Tukey's test and presented as mean \pm SE.

are studies that have demonstrated that NMDA receptor stimulation is crucial for PRP arrival.^{36,37} In accordance, we have found that the injection of MK-801 before open field exploration 1 h prior to NOR training (weak) also alters NOR-LTM. This suggests the role of NMDA receptors in PRP arrival. In addition, we also found that the influence of novel exploration on NOR-LTM was hindered when D1/D5 (dopamine) antagonist SCH was administered close to the open field exploration time. The outcome indicates that PRP arrival that occurred in response to open field exploration also relied on D1/D5 (dopamine) stimulation. This indicates that SCH plays a modulatory role in LTM formation.³⁵ When SCH was infused before training, it could not impair NOR-LTM consolidation. Therefore, the above results indicate that D1/D5 (dopamine) receptors play a role in PRP arrival without affecting the formation of NOR learning tags. A similar observation was made in an inhibitory avoidance study conducted by Moncada et al.¹ Moreover, the inhibition of D1/D5 (dopamine) receptors along with the delivery of Ani into the mPFC around open field exploration causes deficits in NOR-LTM promotion. The above data confirm that catecholaminergic inhibition-induced memory alteration might be associated with PRP arrival. This supports our previous findings, suggesting that the heterosynaptic activation via dopaminergic and NMDA receptors contributes to de novo protein synthesis.^{5,32} In support of the above findings, we also suggest that NMDA receptors are necessary for both learning tag formation as well as PRP arrival.

4. MATERIALS AND METHODS

4.1. Animals. Rats (Wistar) weighing 200–230 g were used. Four animals were kept in one cage. The temperature was maintained at 22 °C. Light was available for 12 h a day. Food and water were available *ad libitum*. The Animal Ethics Committee (173/GO/Re/S/2000 CPCSEA) of the institution approved all of the methods and experimental protocols. Rats were kept in an animal house (Central Animal House Facility, Jamia Hamdard, New Delhi), which was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals.

4.2. Novelty Exploration/Open Field. The apparatus is circular in shape painted in black color. The animals were allowed to explore a novel environment for 5 min. To familiarize rats with the arena, they were left for a 30 min session a day prior to the experiment.

4.3. Behavioral Experiment. NOR was performed similarly to that of Vishnoi et al. with required modifications.¹⁴ The animals were left to habituate to the test chamber for around 20 min. During training, the animals were returned to the same arena with two objects (similar in shape and size). The animals openly explored the objects for 5 min before returning to their respective cages. For the test, a pre-explored object was substituted with a new one. The animals were allowed to explore one new and one pre-explored (familiar) object for a 5 min session. There was a camera mounted over the chamber. The explored objects were assessed using ANY-maze software. The duration for which the animals were present around the object was recorded. The exploration time

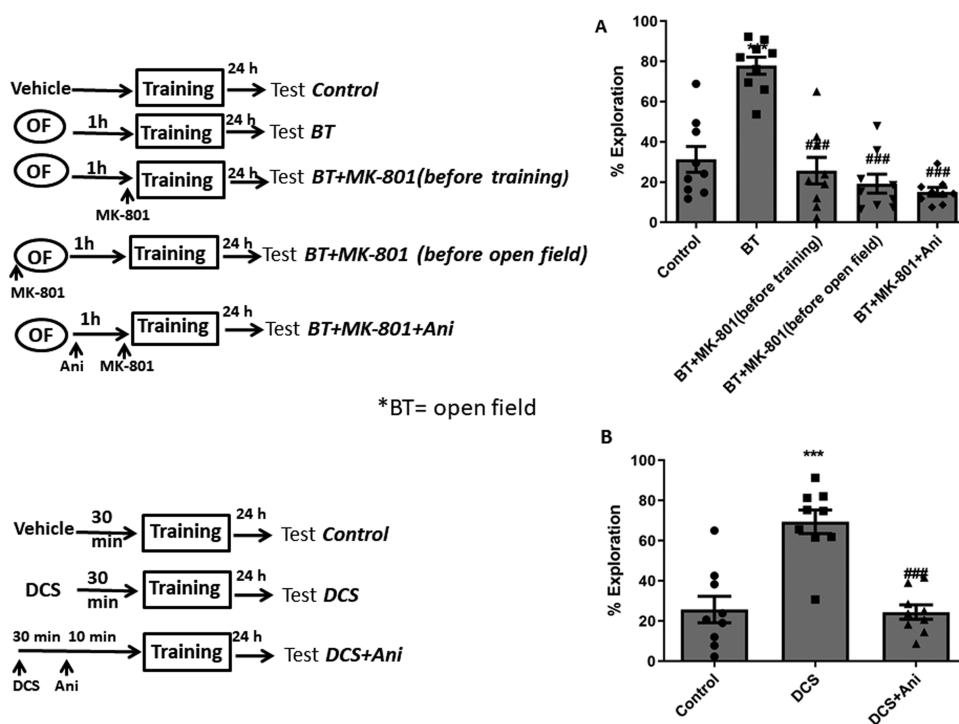


Figure 8. Effect of NMDA receptor activation on BT. (A) % Exploration in the BT group is significantly greater as compared to that in the control group. $***P < 0.001$ versus control. NOR-LTM was prevented by MK-801 administration both before weak NOR training as well as before open field, and the infusion of anisomycin supported this prevention. $####P < 0.001$ versus BT. (B) DCS injected before weak NOR training promoted NOR-LTM. $***P < 0.001$ versus control. DCS-induced NOR-LTM was hindered due to the administration of Ani in the mPFC 10 min following DCS administration. $####P < 0.001$ versus DCS. Time of exploration (%) for the novel object over time for both objects is taken into consideration for data analysis. Data were analyzed statistically using ANOVA (one way), followed by Tukey's test and shown in the form of mean \pm SE ($n = 9$ per group).

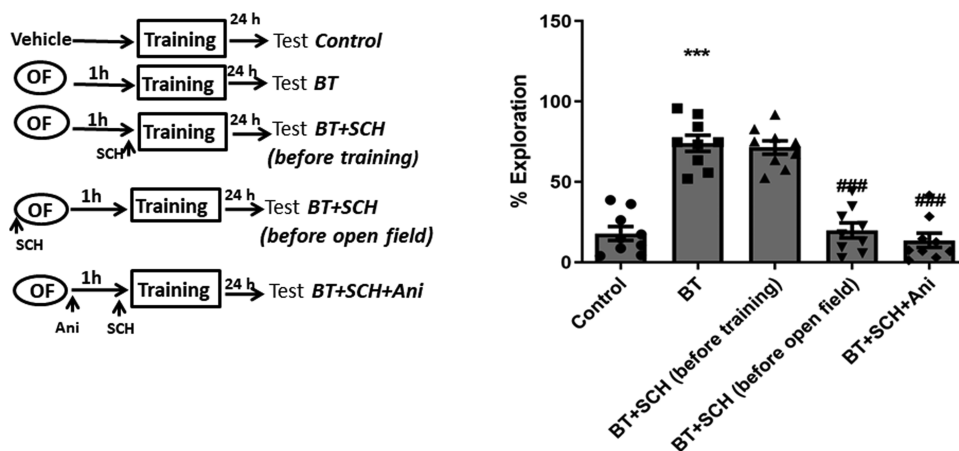


Figure 9. Effect of dopaminergic receptor activation on BT. % Exploration in the BT group is significantly greater as compared to that in the control group. $***P < 0.001$ versus control. Open field-induced NOR-LTM is not affected due to the administration of SCH into the mPFC before weak NOR training. Open field-induced NOR-LTM is hindered due to the administration of SCH into the mPFC before open field exposure. $####P < 0.001$ versus BT. Open field exposure hindered the inhibitory effect of SCH (administered before training). This effect was hindered upon the administration of Ani just after open field exposure. $####P < 0.001$ versus BT. Time of exploration (%) for the novel object over time for both objects is taken into consideration for data analysis. Data were analyzed statistically using ANOVA (one way), followed by Tukey's test, and presented as mean \pm SE ($n = 9$ per group).

was evaluated in terms of the time exploring the familiar/replaced new object over the time (total) exploring both objects.

4.4. Drugs and Surgery. (+)-MK-801 hydrogen maleate, bovine serum albumin (BSA), D-cycloserine (DCS), and D1/D5 receptor blocker SCH-23390 were purchased from Sigma. Perchloric acid (PCA) was procured from Merck. The NMDA

receptor antagonist MK-801, agonist DCS, and SCH-23390 were dissolved in saline. MK-801 was administered intraperitoneally (i.p.) (0.2 mg/kg/mL).¹⁵ DCS was also administered i.p. (15 mg/kg/2 mL).¹⁶ Two micrograms of SCH-23390 (SCH) was infused per side (0.8 μ L/side).¹ Eighty micrograms of anisomycin prepared in HCl and saline was infused per side (pH 7.4). The animals were anesthetized,

mounted into a stereotaxic apparatus, and their skulls were drilled. For cannula implantation, 22-gauge cannulas were used. mPFC stereotaxic coordinates from the bregma were AP +3.20 mm, ML \pm 0.75 mm, and DV $-$ 3.5 mm. Rats were left to recover after their surgery. Cannulated animals were infused with the drug for around 1 min. Cannulas were kept for an extra 1 min to control the backflow and to ensure drug delivery. The animals were sacrificed after a test for HPLC analysis.

4.5. Neurotransmitter Estimation through HPLC. After performing the test, rats were anesthetized using chloral hydrate at a dose of 400 mg/kg i.p. and sacrificed for the estimation of neurotransmitters using HPLC-ECD.¹⁷ The results were evaluated and processed using the Empower Pro Operating System. A mixture of sodium acetate (0.02 M), di-*n*-butyl amine (0.01%), EDTA (0.2 mM), heptane sulfonic acid (0.055%), and methanol (16%) (pH 3.92 adjusted using H₃PO₄) was used as the mobile phase. The mobile phase was filtered using a 0.2 μ m membrane, and a sonicator was used for degassing. The flow rate was maintained at 1 mL/min. For the sample, PFC was homogenized using 0.1 M PCA. It was then centrifuged for 30 min (10 000g at a temperature of 4 °C). The supernatant was filtered using a 0.2 μ m membrane. The same supernatant (filtered) was used for HPLC analysis. The results were obtained by comparing the retention times of neurotransmitter peaks in the sample. The concentration was evaluated using the area under the curve employing a straight line equation $y = mx + c$.

4.6. Protein Estimation. Protein evaluation was done in the homogenate as well as the supernatant using the Bradford method and BSA (standard).

4.7. Statistical Analysis. Statistical analysis was done with a *t*-test, analysis of variance (ANOVA), and Tukey's test. The results were expressed as mean \pm SE. The relation between various biochemical and behavioral parameters was evaluated using Pearson's correlation coefficient (*r*). Linear regression was done to evaluate the strength of the relationship among parameters. $P < 0.05$ was considered significant. Analysis of statistics was done employing GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA).

5. CONCLUSIONS

In conclusion, our results show that 1 h is the critical time window where PRPs arrive to form a tag-PRP complex that contributes to NOR-LTM formation. NMDA receptors, along with their role in the arrival of PRPs, are crucial for tagging specific sets of synapses, which require the storage of memory within certain time frames. D1/D5 (dopamine) receptors are crucial for the consolidation of NOR-LTM as they contribute to PRP arrival and hence are needed for NOR-LTM. Thus, the modulatory effect of SCH acting on D1/D5 (dopamine) receptors on memory strength certainly is a response connected to modulation at the level of PRP arrival. Together, it can be said that the activation of NMDA and D1/D5 (dopamine) receptor systems are important for NOR-LTM formation.

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Author Contributions

S.V. and S.P. designed the study. S.V. conducted the experiments. S.V., S.R., and S.P. analyzed the data. S.V. and S.P. wrote the manuscript. All authors have read and approved the final manuscript.

Notes

The authors declare no competing financial interest. All surgical and experimental procedures were performed in accordance with the Institutional Animal Ethics Committee of Jamia Hamdard, New Delhi, India.

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