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Proteins or RNA synthesis inhibitors suppressed induction of amnesia developing under impairment of memory reconsolidation by serotonin receptors antagonist

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ABSTRACT

Studies have shown that retrieval of long-term memory can cause memory reconsolidation, and impaired reconsolidation leads to amnesia development. However, the mechanisms of amnesia induction due to impaired memory reconsolidation remains poorly described. Using experiments involving grape snails trained to conditioned food aversion, we studied the role of translation and transcription processes and the role of serotonin receptors in the mechanisms of amnesia induction. We found that administration of a serotonin receptor antagonist or a protein synthesis inhibitor before the administration of a reminder using a conditioned food stimulus induced amnesia development, whereas injections of mRNA synthesis inhibitor did not affect memory safety. Moreover, combined injections of an antagonist of serotonin receptor and inhibitors of protein or mRNA synthesis before reminder administration completely prevented amnesia development. In addition, inhibitors of protein or mRNA synthesis prevented amnesia development 3 h but not 9 h after the administration of a serotonin receptor antagonist/reminder. We hypothesize that the mechanisms of amnesia induction caused by impaired memory reconsolidation depend on protein and mRNA syntheses within a certain time window, similar to the mechanisms of induction of other long-term plastic brain rearrangements.

1. Introduction

One of the fundamental problems in neurobiology is the mechanism of amnesia induction and development. Amnesia is usually understood as non-specific memory loss as a result of memory impairment (due to brain injury, disease, pharmacological interventions, brain ischemia, etc.), difficulties in learning new information, or difficulties in recalling the past (Kopelman, 2002; Shrager and Squire, 2008). The discovery of memory reconsolidation processes has been important in studying amnesia mechanisms. Upon presentation of a reminder, a previously consolidated long-term memory can be reactivated, transformed into a labile state (destabilized), and then consolidated again (reconsolidated) (Alberini, 2011; Besnard et al., 2012; Dudai, 2004; Finnie and Nader, 2012; Lee, 2009; Misanin et al., 1968). During memory reconsolidation, the action of amnestic agents, such as neurotransmitter receptor antagonists or protein synthesis inhibitors, leads to amnesia development (Anokhin et al., 2002; Besnard et al., 2012; Chen et al., 2014; Gainutdinova et al., 2005; Roesler, 2017; Romano et al., 2006; Sangha et al., 2003; Wideman et al., 2018). Amnesia resulting from impairment of memory consolidation or reconsolidation has a number of features (Dudai, 2004; Finnie and Nader, 2012; Gold, 2006; Lee, 2009; Roozendaal and McGaugh, 2011; Sara and Hars, 2006; Tronson and Taylor, 2007). In particular, amnesia is specific to the memory that is impaired during consolidation or reconsolidation. A number of amnestic agents used in experiments are natural modulators of molecular processes in neurons, and their action in the brain cannot be unambiguously defined as pathogenic in many cases. In addition, long-term memory impairment is possible only during memory reactivation within a certain time window. These features of amnesia after impairment of memory consolidation or reconsolidation are important because they allow the investigation of specific molecular and cellular mechanisms underlying amnesia.

Memory loss has long been assumed to be a passive process of destroying a memory trace or of impeding its retrieval. In particular, amnesia is interpreted as a result of the suppression of molecular processes necessary for memory consolidation or reconsolidation (Dudai,

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2004; Finnie and Nader, 2012; Miller and Sweatt, 2006; Sara and Hars, 2006; Tronson and Taylor, 2007). However, impairment of reconsolidation mechanisms does not explain why amnesia develops and what the mechanisms of its induction are. The complexity, diversity, and the gaps in research findings on amnesia mechanisms warrant the need to develop new research strategies that can shed light on ways to fill this knowledge gap (Nader, 2009). Earlier, we proposed a hypothesis stating that in case of impairment of memory reconsolidation, a new process is induced—active amnesia (Solntseva et al., 2007; Solntseva and Nikitin, 2010; Nikitin et al., 2018). This assumption is based on the results of studies on snails trained to conditioned food aversion, wherein memory reconsolidation was impaired by NMDA glutamate receptor antagonists or by protein synthesis inhibitors. It was found that amnesia develops over time. The early stage of amnesia lasted for less than 10 days and was characterized by a gradual decrease in the possibility of memory formation during a second training (Solntseva and Nikitin, 2010; Nikitin et al., 2018). At a later stage (10 days and beyond), amnesia was transformed into specific anterograde amnesia, which is characterized by the impaired ability of animals to form long-term memory during a second training. This resistance of amnesia to learning was specific to food, which was used as a reminder when investigating memory reconsolidation impairment (Kozyrev and Nikitin, 2010; Solntseva et al., 2007; Solntseva and Nikitin, 2008, 2010). The administration of amnestic substances without being combined with a reminder did not influence memory. These results suggest that amnesia is not characterized by "turning off" of memory; rather, it is an active, specific, long-lasting process of molecular and cellular changes in the brain (Solntseva and Nikitin, 2010; Nikitin et al., 2018).

Another process of memory loss is forgetting, which is considered a normal, time-dependent process of the loss or modification of information previously acquired and stored in the long-term memory; forgetting can also be an active process (Davis and Zhong, 2017; Hardt et al., 2013; Richards and Frankland, 2017; Parvez et al., 2006; Shrager and Squire, 2008). For example, mammalian and invertebrate studies have shown that forgetting may depend on the activity of some neurotransmitter receptors and intracellular signaling molecules, as well as on the translation and transcription processes (Davis and Zhong, 2017; Sachser et al., 2016; Berry et al., 2012; Knezevic et al., 2011; Hadziselimovic et al., 2014; Sangha et al., 2003; Shuai et al., 2010).

The key to our chosen amnesia research strategy is the identification of the neurochemical mechanisms of amnesia induction. Similar to the mechanisms of induction in other long-term plastic rearrangements, the mechanisms of amnesia induction possibly depend on protein and RNA syntheses. To test this hypothesis, we investigated the role of translation and transcription processes in the mechanisms of amnesia induction in snails trained to conditioned food aversion (Nikitin et al., 2016a, b; Solntseva and Nikitin, 2012). We found that injections of NMDA glutamate receptor antagonists or of protein synthesis inhibitors to the trained animals before the administration of a reminder using a conditioned food stimulus (CS) led to amnesia development, whereas injections of RNA synthesis inhibitors before reminder administration did not affect memory retention. Moreover, combined injections of glutamate receptor antagonists and protein or mRNA synthesis inhibitors before reminder administration completely prevented amnesia development. Significant differences in the time windows of the dependence of memory reconsolidation and amnesia induction processes on protein synthesis were also revealed, that is, approximately 3 and 9 h, respectively. We assumed that these processes are independent and that they involve specific molecular mechanisms.

In addition to glutamate receptors, many other neurotransmitter receptors, particularly serotonin receptors, are involved in memory reconsolidation and amnesia induction mechanisms (Ogren et al., 2008; Wideman et al., 2018). In mollusks, serotonin plays an important role in the regulation of defensive and feeding behaviors as well as in the mechanisms of acquisition of conditioned food aversion memory (Balaban et al., 2016; Hawkins and Byrne, 2015; Totani et al., 2019). Earlier, we showed that administration of the serotonin receptor antagonist methiothepin before a reminder involving a CS led to amnesia development (Solntseva and Nikitin, 2008; Nikitin et al., 2018).

In addition, we found significant differences in the participation of glutamatergic and serotonergic systems in the mechanisms of memory reconsolidation and amnesia development. In particular, blockade of NMDA glutamate receptors before reminder administration impaired the reconsolidation of the recent (2 days) but not of the remotely (10 days) formed conditioned food aversion memory. Interestingly, memory impairment was very persistent—memory could not be recovered by a subsequent training (Nikitin et al., 2016a, 2018; Solntseva and Nikitin, 2010). Conversely, inhibition of 5-HT receptors before a reminder impaired both the recently and remotely formed conditioned food aversion memories (Nikitin et al., 2016a). Unlike "NMDA-dependent" amnesia, "5-HT-dependent" amnesia did not impede recovery of the original memory through a subsequent training session (Nikitin et al., 2018).

In connection with the above, a question arises, as follows: are the mechanisms of amnesia induction, which we described for NMDA glutamate receptors, unique? Or is the induction of amnesia caused by impaired memory reconsolidation by serotonin receptor antagonists also dependent on protein and RNA syntheses?

To experimentally find answers to these questions, we used grape snails to investigate the effect of protein or RNA synthesis inhibitors on amnesia induction due to impairment of reconsolidation of conditioned food aversion memory caused by the serotonin receptor antagonist methiothepin. To reveal the time window of the dependence of amnesia induction on translation and transcription processes, we injected the inhibitors of translation and transcription processes into the snails 3 or 9 h after the action of methiothepin/reminder.

2. Material and methods

2.1. Object of the experiment

The experiments were carried out on grape snails (*Helix lucorum* L., Crimea population), which weighed 25–30 g, reared in "home" boxes, and fed with raw carrots for not less than 3 weeks prior to the experiments and during the intervals between the experimental procedures. The animals were deprived of food 3 days before the training.

2.2. Training

Conditioned food aversion training was performed as previously described (Balaban et al., 2016; Nikitin et al., 2018). This training model is widely used in studies on mollusks (Kiss et al., 2009; Takigami et al., 2013). The snails were fixed behind the sink to the bracket in such a way that they could move relatively freely on a plastic ball floating in water containing 0.01% NaCl. A piece of food weighing 2–3 g was placed 0.5 cm from a snail's head by using a mechanical manipulator. A banana was used as a CS+, and the reinforcing stimulus was an electric shock (50 Hz, 300 ms, 1.2 mA). Electric current was passed through the banana and through the body of the snail at the first "bites" (consummatory reactions). One electrode was connected to the food while the second electrode was placed in the water where a ball was floating. The plastic ball was lined with strips of metal foil. Electrical stimulation suppressed the eating behavior and induced a "withdrawal" reaction (pulling the head and body into the sink). In addition, a differentiating stimulus (CS-, boiled carrots) was presented to the snails, and it was not combined with electric shock. The latencies at the beginning of food eating were recorded using a video camera and a computer. Combined presentation of a banana and electric shock was carried out every 15–20 min. Three training sessions were conducted daily for 3 days. On the first training day, 2 CS+ and 2 CS- were presented to the animals. On the second day, 5 CS+ and 3 CS- were presented, whereas 5 CS+ and 3 CS- were presented on the third day. The

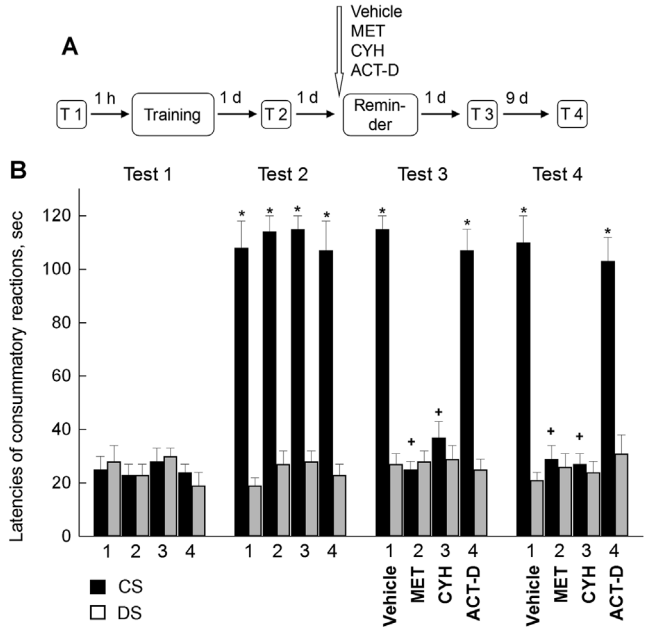

Fig. 1. Two days after injections of the serotonin receptor antagonist methiothepin or the protein synthesis inhibitor cycloheximide before reminder administration led to memory impairment and development of amnesia in the testing snails, whereas administration of the RNA synthesis inhibitor actinomycin D exerted no influence on memory. Fig. 1A shows the experimental scheme. T1, T2, T3, T4 – testing snails; h – hours; d – days. The bright arrow indicates the time of injection of vehicle, methiothepin (MET), cycloheximide (CYH), or actinomycin D (ACT-D). Fig. 1B shows the summary diagram. Dark and gray bars represent the snails' reactions to the conditioned stimulus (CS+, banana) and differentiation stimulus, which was not reinforced by electric shock (CS-, boiled carrots), respectively. Test 1: initial reactions to food stimuli 1 h before training. Test 2: reactions 1 day after training. Tests 3 and 4: 1 and 10 days after drug injections and reminder, respectively. The numbers indicated below the diagram represent the reactions of the animals that were injected 2 days after training and before reminder administration, as follows: 1 – vehicle (control group of animals); 2 – MET; 3 – CYH; and 4 – ACT-D. Vertical – latencies of consummatory reactions to food stimuli expressed in seconds (data are presented as mean ± S.E.M.). * $p < 0.001$, comparison of responses to CS + and CS- (Wilcoxon test); + $p < 0.001$, comparison of reactions to CS+ in experimental and control snails (group 1, Mann–Whitney test).

food was presented every 15–20 min, and 2–3 CS+ presentations were alternated with 1–2 CS- presentations. If the animals did not try to eat for 120 s, the food was removed, and electric shock was not applied. The criterion for the development of the aversive reaction to food was an increase in the latencies of consummatory reactions in intact animals before training from 20–35 s to 100–120 s or a complete refusal to eat a banana during its presentation within 2 min, as well as the differences in the latencies of consummatory reactions to CS+ and CS-.

2.3. Reminder administration

Two days after the training, the snails in a neutral context (i.e., on a glass plate) were injected with the substance solutions (Fig. 1). The animals were subsequently placed in a learning context (i.e., on plastic balls) and, after 20–30 min, a reminder procedure was carried out, as follows: CS+ (banana) was presented three times at 15 min interval. The latencies of the consummatory reactions were recorded for 120 s. If the animals tried to eat the food, it was removed. Reinforcing stimulus was not introduced during reminder administration. One hour after the reminder, the snails were transferred from the balls to the home boxes.

2.4. Testing

Responses to food stimuli were tested 1 h before the training (test 1), 1 day after the training (test 2), and 1 and 10 days (tests 3 and 4, respectively) after administration of substance injections/reminder (Fig. 1). To perform the tests, we placed the snails in a training context for 30 min, presented the CS+ and CS- at 15 min interval, and measured the latencies of consummatory reactions for 120 s. When the animals tried to eat food, the testing was stopped. Electroshock was not used during the testing.

2.5. Substances and injections

The effects of the protein synthesis inhibitor cycloheximide and the RNA synthesis inhibitor actinomycin D and those of the serotonin receptor antagonist methiothepin were studied (all drugs were obtained from Sigma-Aldrich, St. Louis, MO, USA). Methiothepin was diluted in saline. Inhibitors of translation and transcription were dissolved beforehand in dimethyl sulfoxide (DMSO) and then diluted in saline. Substance solutions (0.25 ml/snail) were injected into the body cavity through an insensitive part of the leg skin under the mantle roller by using a thin needle. Under combined substance administration, cycloheximide or actinomycin D were first injected before reminder administration; 5 min later, methiothepin was administrated. The final amount of DMSO in saline was 1%. Methiothepin, cycloheximide, and actinomycin D were administrated at 10, 100, and 1 mg/kg body weight. The doses of the substances effectively influenced the training processes in animals (Abramova et al., 2006; Barbas et al., 2003; Fulton et al., 2005; Kiss et al., 2009; Pedreira et al., 1996; Sangha et al., 2003; Watanabe et al., 2005). Saline solution containing 1% DMSO was injected into the control snails 2 days after the training and before the reminder. A “blind” method was used in this work, that is, solution injections into the snail and memory testing were carried out by different experimenters.

2.6. Animal group study

Each animal was used only in one series of experiments. Before the reminder administration, the animals in the four groups were injected with DMSO, methiothepin, cycloheximide, or actinomycin D. Two other groups of snails were injected with methiothepin + cycloheximide or methiothepin + actinomycin D before the reminder. Three or 9 h after the administration of methiothepin/reminder, two groups were injected with cycloheximide, and the two other groups were injected with actinomycin D in the same time intervals to study the dependence of the time window of amnesia induction mechanisms on translation and transcription processes. For the inhibitor injections, the animals were transferred from the home boxes to a neutral context, that is, on a glass plate.

2.7. Data analysis

For classic parametric tests to produce accurate results, the assumptions underlying them (e.g., normality and homoscedasticity) must be satisfied. We can't be sure that learning and memory parameters are normally distributed in the population of snails, and our samples are rather small to check this. In addition, we used semi-quantitative data (such as 120 s cut-off latency) which required exclusively non-parametric methods of analysis. Considering these facts, we used nonparametric criteria to analyze the results obtained. Data were averaged and the standard error of the mean (SEM) was calculated. The latencies of the consummatory responses to CS+ presentation in animals injected with substances before reminder were compared with the latencies of responses to CS- as well as with the latencies of responses to banana presentation before training in the same animal group and with the latencies of responses to CS+ in snails injected with

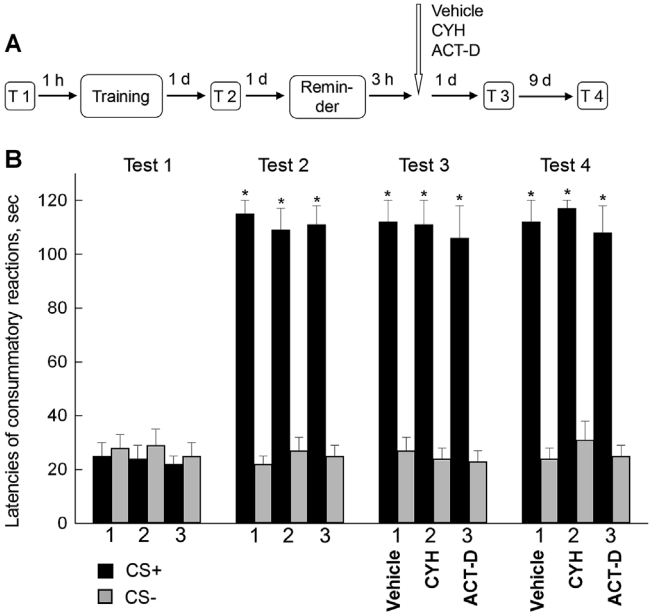

Fig. 2. The time window of the dependence of conditioned food aversion memory reconsolidation on protein synthesis was less than 3 h. Fig. 2A shows the experimental scheme. The bright arrow indicates the vehicle, CYH, or ACT-D injection 3 h after reminder administration. Fig. 2B shows the summary diagram.
The figures indicated below the diagram are the reactions of animals that received: 1 – vehicle; 2 – CYH, 3 – ACT-D. The other symbols are similar to those in Fig. 1.

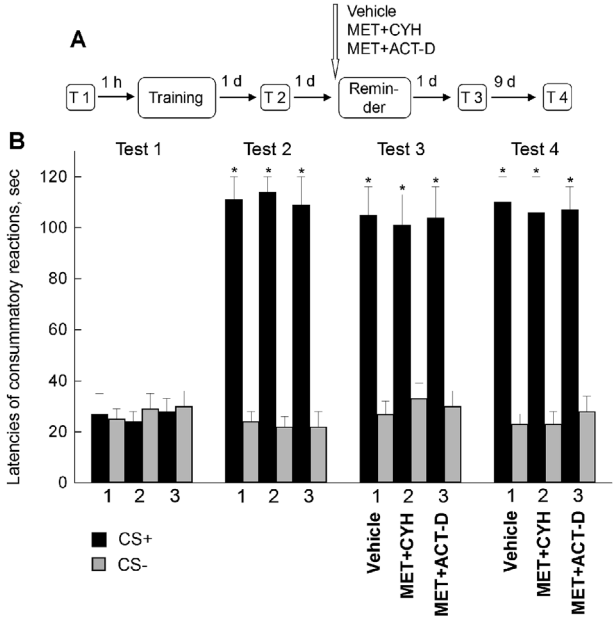

Fig. 3. Combined injections of a 5-HT receptor antagonist and a protein synthesis inhibitor or an RNA synthesis inhibitor before reminder prevented amnesia development. Fig. 3A shows the experimental scheme. Bright arrow represents the time of drug injection. The numbers under the diagram are the reactions of animals that received injections of: 1 — vehicle; 2 – MET + CYH, 3 – MET + ACT-D. The other symbols are similar to those in Fig. 1.

vehicle before the reminder. To compare the latencies of the reactions of two different animal groups, we used the nonparametric Mann–Whitney rank sum test. We used the Wilcoxon signed rank test to compare the data obtained from the same snails (CS+ vs CS-).

3. Results

In untrained snails, the average latencies of consummatory reactions to the presentation of a banana and boiled carrots were 20–35 s (Figs. 1–3, test 1). The results showed that in the testing snails in all studied groups (Figs. 1–3; Test 2), the latencies of consummatory responses to the presentation of CS+ (banana) 1 day after the training were significantly longer than the latencies of responses to the presentation of CS- (boiled carrots; $p < 0.001$; Wilcoxon test) and longer than the initial reactions to the banana presentation before training ($p < 0.001$; Wilcoxon test).

3.1. Injections of methiothepin or cycloheximide before a reminder led to memory impairment, whereas actinomycin D exerted no effect on memory

Two days after the training, methiothepin ($n = 14$) or cycloheximide ($n = 12$) were injected, and a reminder was presented to the snails. One and ten days after the administration of the substances/reminder, the latencies of reactions to CS+ were lower in the training animals (Fig. 1) than in the control animals ($n = 12$), which received vehicle injections before the reminder (for methiothepin and cycloheximide 1 and 10 days later; $p < 0.001$; Mann–Whitney test). Moreover, the latencies of reactions to CS+ did not differ from the latencies of responses to CS- (for methiothepin and cycloheximide 1 and 10 days later; $p > 0.6$; Wilcoxon test) or from the initial reactions to banana presentation before the training (for methiothepin and cycloheximide $p > 0.6$; Wilcoxon test).

As shown in Fig. 1, the latencies of reactions to CS+ of the testing snails ($n = 12$) 1 and 10 days after actinomycin D/reminder administration did not differ from those of the control animals (1 and 10 days later; $p > 0.8$; Mann–Whitney test) but were longer than the latencies of reactions to CS- (1 and 10 days later; $p < 0.001$; Wilcoxon test) and longer than the reactions to banana presentation before the training ($p < 0.001$; Wilcoxon test). The effects caused by the substances were similar to those described previously (Nikitin et al., 2016b, 2018; Solntseva and Nikitin, 2012).

Hence, the administration of a serotonin receptor antagonist or a protein synthesis inhibitor before the reminder induced amnesia development, whereas injections of RNA synthesis inhibitor before the reminder did not exert influence on memory.

3.2. Injections of translation or transcription inhibitors 3 h after the reminder presentation did not lead to memory impairment

Two days after the training, a reminder was presented; 3 h later, the substances were injected to snails. One and ten days after the administration of the reminder and cycloheximide ($n = 8$) or actinomycin D ($n = 8$) injections, the latencies of the reactions to CS+ of the control and trained snails did not differ (Fig. 2) ($p > 0.4$, both 1 and 10 days later; Mann–Whitney test) and were longer than the latencies of the reactions to CS- ($p < 0.001$; Wilcoxon test).

Thus, the time window of the dependence of memory reconsolidation processes on protein synthesis was less than 3 h.

3.3. Combined administration of methiothepin and cycloheximide or methiothepin and actinomycin D before the reminder suppressed amnesia induction

Two days after the training, the snails were injected with substances under investigation, and a reminder procedure was performed. One and ten days after the administration of methiothepin + cycloheximide/reminder ($n = 12$), the latencies of the reactions to CS+ of the testing snails did not differ from those of the control snails (Fig. 3) ($n = 12$; $z = 0.18$, $p = 0.85$ and $z = 0.13$, $p = 0.89$ at 1 and 10 days later, respectively; Mann–Whitney test) and were longer than the latencies of

the reactions to CS- ($z = 3.02$, $p < 0.001$ and $z = 2.95$, $p < 0.001$, respectively; Wilcoxon test).

At 1 and 10 days after the action of methiothepin + actinomycin D/reminder, the latencies of the reactions to CS + of the testing snails ($n = 12$) did not differ from those of the control snails (Fig. 3) ($z = 0.35$, $p = 0.72$ and $z = 0.04$, $p = 0.96$, respectively; Mann–Whitney test) and were longer than the latencies of the responses to CS- ($z = 3.03$, $p < 0.001$ and $z = 3.2$, $p = 0.001$, respectively; Wilcoxon test).

Hence, no impairment of conditioned food aversion memory was observed after the combined injections of methiothepin + cycloheximide or methiothepin + actinomycin D before the reminder.

3.4. Injections of cycloheximide or actinomycin D 3 h after administration of the methiothepin/reminder resulted in partial amnesia while injection inhibitors at 9 h was not effective in preventing amnesia

Two days after the training, the snails were injected with methiothepin, and a reminder procedure was performed. Three hours after the administration of methiothepin/reminder, the snails were injected with cycloheximide or actinomycin D. One day after the action of methiothepin/reminder and the subsequent injection of cycloheximide ($n = 12$) or actinomycin D ($n = 10$) 3 h later, we found that the latencies of the reactions to CS + of the testing snails (Fig. 4) were longer than the latencies of the responses to CS- (for cycloheximide: $z = 2.45$, $p = 0.008$; for actinomycin D: $z = 2.26$, $p = 0.016$; Wilcoxon test) but shorter than the latencies of the reactions to CS + of the control animals (for cycloheximide: $z = 2.19$, $p = 0.03$; for actinomycin D: $z = 2.41$, $p = 0.015$; Mann–Whitney test).

One day after the action of methiothepin/reminder and the subsequent administration of cycloheximide ($n = 10$) or actinomycin D

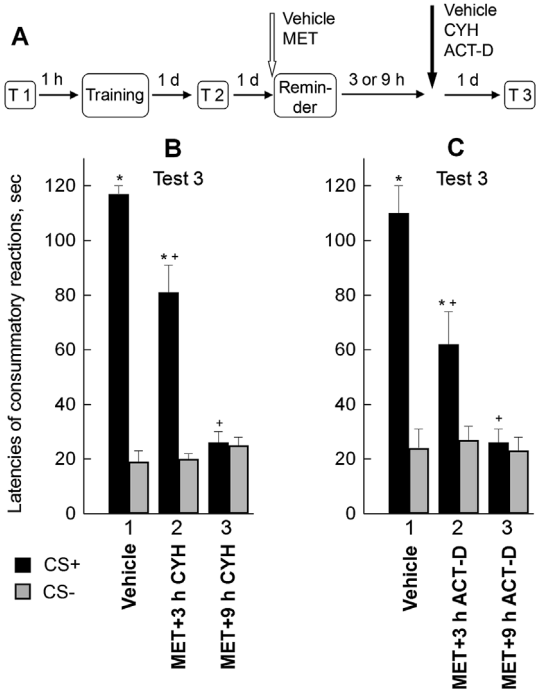

Fig. 4. The time windows of the dependence of amnesia induction on translation and transcription processes was 3–9 h. Fig. 4A shows the experimental scheme. Bright arrow represents the time of vehicle or MET injection before reminder administration; dark arrow represents the injections of CYH or ACT-D, that is, 3 or 9 h after MET + reminder administration. Below B and C – snails testing (T3) 1 day after administration of MET/reminder and injections of CYH (B) or ACT-D (C) following 3 or 9 h after. * $p < 0.05$, comparison of responses to CS + and CS-; + $p < 0.05$, comparison of the reactions to CS + of the experimental and control snails. The other symbols are similar to those in Fig. 1.

($n = 10$) 9 h later, the latencies of the reactions to CS + of the testing snails (Fig. 4) were lower than those of the control animals (for cycloheximide: $z = 3.37$, $p < 0.001$; for actinomycin D: $z = 3.39$, $p < 0.001$; Mann–Whitney test) and did not differ from the latencies of the responses to CS- (for cycloheximide: $z = 0.07$, $p = 0.94$; for actinomycin D: $z = 0.35$, $p = 0.73$; Wilcoxon test).

Thus, conditioned food aversion memory was partially impaired following cycloheximide or actinomycin D injections 3 h after administration the methiothepin/reminder action, whereas the administration of the substances 9 h after methiothepin/reminder administration led to the full development of amnesia.

4. Discussion

In previous experiments, we found that protein synthesis inhibitors and serotonin receptor antagonists did not affect memory retrieval in grape snails with conditioned food aversion training (Solntseva et al., 2007; Solntseva and Nikitin, 2012). At the same time, injections of a protein synthesis inhibitor or serotonin receptor antagonist before CS reminder caused impairment of long-term memory reconsolidation and amnesia development (Nikitin et al., 2018; Nikitin et al., 2016a,b; Solntseva and Nikitin, 2012). The time window of the dependence of memory reconsolidation on translation processes was less than 3 h; cycloheximide injections 3 h after the reminder were not effective. In addition, injections of mRNA synthesis inhibitors, either before or after a reminder, did not affect the snails' memory. Thus, inhibitors of protein synthesis impaired the reconsolidation of long-term memory for conditioned food aversion, whereas the inhibitors of mRNA synthesis did not affect this process. We assumed that proteins necessary for memory reconsolidation can be translated from mRNA that were previously synthesized and were in a deposited ("silent") state (Solntseva and Nikitin, 2012). "Deposited" mRNA are believed to be reactivated when synapses are stimulated, and the translated proteins are involved in specific structural and functional modifications of synaptic connections underlying the mechanisms of long-term memory preservation (Redondo and Morris, 2011). At the same time, we cannot exclude the possibility that memory reconsolidation processes sensitive to RNA synthesis can occur in time intervals beyond the limits studied by us.

The most important and interesting result of our research was that the combined injections of serotonin receptor antagonist and protein or RNA synthesis inhibitor before the reminder did not impair memory. In addition, injections of cycloheximide or actinomycin D 3 h after the administration of methiothepin/reminder resulted in partial memory suppression, whereas injections of inhibitors 9 h later did not affect the amnesia development.

As noted above, we obtained results similar to an earlier finding on the participation of translation and transcription processes in the mechanisms of amnesia induction due to the impairment of memory reconsolidation caused by NMDA glutamate receptor antagonist. In particular, we found that conditioned stimulus reminder during the action of the NMDA glutamate receptor antagonists MK-801 or APV in snails trained to conditioned food aversion led to the development of stable amnesia (Nikitin et al., 2018; Solntseva and Nikitin, 2010). At the same time, co-administration of NMDA receptor antagonist and protein or RNA synthesis inhibitor before the reminder completely prevented amnesia development (Nikitin et al., 2016b). The time windows of the sensitivity of the mechanisms of NMDA-dependent amnesia induction to the translation or transcription inhibitors was approximately 9 h.

Thus, we found for the first time the peculiarities of the mechanisms of reconsolidation of long-term memory and amnesia induced by impairment of reconsolidation processes in snails trained to conditioned food aversion. First, memory reconsolidation depended on protein synthesis but not on RNA synthesis, whereas the induction of amnesia depended on both protein and RNA syntheses. Second, the time windows of the dependence of memory reconsolidation on translation processes and amnesia induction differed significantly. These results

indicate the specificity of the molecular mechanisms of amnesia induction, which apparently differ from the mechanisms of memory reconsolidation.

What are the possible mechanisms of amnesia induction after memory reconsolidation impairment? According to a common view, amnesia is a consequence of the impairment of repeated memory consolidation after its activation caused by a reminder (Dudai, 2004; Finnie and Nader, 2012; Lee, 2009; Sara and Hars, 2006; Tronson and Taylor, 2007). However, this assumption does not explain the mechanisms of the elimination of complex and diverse molecular and structural changes formed during memory consolidation and the subsequent memory reorganization. We hypothesized that amnesia is an independent, active, time-developing process (Nikitin et al., 2018; Solntseva and Nikitin, 2010). Amnesia induction, as well as the induction of other plastic rearrangements in the brain, possibly involves the synthesis of proteins and mRNA as one of the main mechanisms. We suggested the following experimental approach to investigate the hypothetical mechanisms of amnesia induction. A reminder during inhibition of one receptor type (for example, serotonin receptors or NMDA glutamate receptors) changes the pattern of neurotransmitter receptors activated by the reminder, leading to a mismatch of the expected and incoming information. This mismatch induces molecular cascades of signals involved in the development of a new process—amnesia. One of the key stages in the induction of amnesia is possibly the synthesis of specific proteins and RNA. Translation or transcription inhibitors can suppress the synthesis of the proteins involved in amnesia processes and thus interrupt amnesia induction. Hence, our results can be explained by the fact that the combined administration of neurotransmitter receptor antagonists and transcription or translation inhibitors suppresses “amnestic” protein and mRNA syntheses and blocks amnesia development.

It is noteworthy that inhibitors of protein and RNA syntheses prevented the development of amnesia caused by the action of different neurotransmitter system receptor antagonists—glutamatergic and serotonergic. The similarity of the duration of the time windows (approximately 9 h) of the dependence of these amnesia induction mechanisms on transcription and translation processes can indicate the similarity of the mechanisms of amnesia caused by memory reconsolidation impairment by the antagonists of different neurotransmitter receptors. However, the facts we have obtained earlier testify against this assumption. In particular, as noted above, we found that NMDA glutamate receptor inhibition during memory reactivation led to a persistent memory impairment—memory could not be recovered by a subsequent training (Nikitin et al., 2016a, 2018; Solntseva and Nikitin, 2010). Unlike the NMDA-dependent amnesia, the 5-HT-dependent amnesia did not prevent the recovery of the original memory through a second training session (Nikitin et al., 2016a, 2018).

Thus, various types of amnesia exist, that is, sensitive and insensitive to second training at the late stage of their development. These results, in turn, suggest that the described amnesia types can be based on the peculiarities of their molecular mechanisms and, in particular, they may involve the synthesis of different proteins and mRNAs during their induction.

The presented views are indirectly consistent with the data showing that different neurotransmitter systems are characterized by a number of features linking their participation in the mechanisms of learning and memory. Thus, the functions of the glutamatergic system are believed to be associated primarily with the direct provision of cognitive processes in an animal's brain, whereas monoaminergic systems (including serotonergic) are mainly involved in the modulation of cognitive processes (Balaban et al., 2016; Nikitin et al., 2018; Roozendaal and McGaugh, 2011; Seyedabadi et al., 2014; Wideman et al., 2018).

However, the proposed concept does not “fit” the data on the protein synthesis inhibitor-induced impairment of memory reconsolidation. Our hypothesis suggests that protein synthesis is necessary for both memory reconsolidation and amnesia induction. This raises the question of how amnesia can be induced during the action of protein synthesis inhibitors. There are no direct answers to this question, but we believe that this problem can be clarified with the help of the following arguments. The data above suggest that memory reconsolidation depends on protein synthesis within a time window of less than 3 h. Moreover, the synthesis of proteins involved in amnesia mechanisms occurs much longer, approximately 9 h. By contrast, protein synthesis in the brain of animals, including mollusks, is effectively suppressed (by more than 90%) by inhibitors (e.g., cycloheximide) within 2–3 h (Fulton et al., 2005). Subsequently, the efficiency of translation inhibition decreased. Therefore, we cannot rule out the possibility that 2 h after the impairment of memory reconsolidation by protein synthesis inhibitors, the inhibition intensity for the translation processes weakened, possibly resulting in the synthesis of proteins involved in amnesia induction and consequently in the manifestation of amnesia. In addition, memory reconsolidation impairment can activate regulatory molecules (e.g., protein kinases, phosphatases, and proteinases) (Baumgärtel and Mansuy, 2012; Citri and Malenka, 2008; Di Prisco et al., 2014; Hell, 2016; Kennedy, 2013; Mansuy and Shenolikar, 2006; Salazar et al., 2016) that, first, can facilitate protein synthesis-independent plastic rearrangements of neurons, which are involved in amnesia induction and development for a time sufficient for the end of period of translation process inhibition; second, these regulatory molecules can induce the synthesis of “amnesia proteins.” Translation-dependent plastic transformations of neurons can occur many hours after training or its simulation at the cellular level (Abbas et al., 2015; Baumgärtel and Mansuy, 2012; Hell, 2016; Izquierdo et al., 2016; Kemenes et al., 2006; Kennedy, 2013; Kukushkin and Carew, 2017). Thus, in case of memory reconsolidation impairment by translation inhibitors, amnesia proteins can be synthesized in relatively late time intervals. Moreover, we must admit that the available data are insufficient to offer a satisfactory explanation of amnesia induction mechanisms after memory reconsolidation impairment by protein synthesis inhibitors. However, we cannot exclude the possibility that the mechanisms of amnesia induction due to memory reconsolidation impairment caused by neurotransmitter receptor antagonists or by protein synthesis inhibitors differ significantly.

5. Conclusions

Thus, for the first time we discovered specific mechanisms of reconsolidation of long-term memory and amnesia induced in case of reconsolidation processes impairment in snails trained to conditioned food aversion. The obtained results are experimental confirmation of our hypothesis, according to which memory reconsolidation and amnesia induction are separate processes and impairment of memory reconsolidation simultaneously initiates mechanisms of amnesia development. Amnesia induction, as well as the induction of other plastic rearrangements in the brain, involves the synthesis of specific proteins and mRNA as one of the main mechanisms. In addition, there is reason to suppose that the mechanisms of amnesia induction in case of memory reconsolidation impairment by NMDA glutamate receptors antagonists and 5-HT receptors antagonists involve the synthesis of specific proteins and mRNA. In our experiments, we used a CS that has biological significance for animals. In this regard, we cannot exclude the fact that the effects we have identified will differ from the effects found in the case of using neutral stimuli as a CS (such as tone, light, or context). Therefore, the solution to this problem requires further study. The results of the study have a certain practical significance, since the mechanisms of amnesia induction and development, being one of the manifestations of various neuropsychiatric diseases, remain poorly described.

Conflicts of interest

There are no conflicts of interest to declare.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2019.104520.

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