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Acute inhibition of mGluR5 disrupts behavioral flexibility

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Abstract

Conditioned cues can sometimes elicit maladaptive responses as seen in the post-traumatic stress disorder (PTSD). Lack of effective fear extinction, which involves a process of inhibitory learning, is hypothesized to associate with PTSD. In this study, we tested the effect of acute pharmacological inhibition of mGluR5 activity on the extinction of fear memory and spatial memory. Intraperitoneal injection of the mGluR5 (metabotropic glutamate receptor 5) antagonist MPEP [2-Methyl-6- (phenylethynyl) pyridine hydrochloride] allowed the retrieval but prevented the extinction of contextual fear memory in mice. Without altering locomotor activity, MPEP inhibited the acquisition but not the consolidation of contextual fear memory. Further, administration of MPEP blocked the extinction of spatial memory in the Morris water maze paradigm. Our data suggest a necessary role of mGluR5 in regulating certain aspects of behavioral flexibility.

Keywords

extinction; contextual fear memory; spatial memory; mGluR5; MPEP; PTSD

1. Introduction

While learning and memory consolidation are important for survival, extinction of the previously formed memory represents a significant aspect of adaptive brain function. Extinction is believed to be a process of new learning rather than an erasure of the old association (Gershman, Blei, and Niv, 2010; Gershman and Niv, 2012; Maren, 2011). This belief is supported by observations in humans as well as in animal models that fear responses can spontaneously recover following extinction (Bouton and Bolles, 1979; Rescorla, 2004). As extinction-based behavioral therapies are considered to treat patients with post-traumatic stress disorder (PTSD) or dysregulated fear (Kearns, Ressler, Zatzick, and Rothbaum, 2012; Parsons and Ressler, 2013; Quirk, Pare, Richardson, Herry, Monfils,

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Schiller, and Vicentic, 2010), investigation on mechanisms underlying the inhibitory learning during extinction may aid the development of new therapeutic strategy.

Since extinction is a process of active learning (Maren, 2011; Quirk et al., 2010), potentiating and inhibiting the glutamate-mediated signaling have been expected to facilitate and suppress extinction, respectively. Indeed, the function of NMDA type receptors (NMDAR, the ionotropic glutamate receptors), which are heavily involved in learning, memory and forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) has been implicated in the process of extinction (Baker and Azorlosa, 1996; Myers, Carlezon, and Davis, 2011; Tang, Shimizu, Dube, Rampon, Kerchner, Zhuo, Liu, and Tsien, 1999). Numerous studies have shown that a partial agonist of NMDAR, D-cycloserine (DCS), facilitates extinction (Bouton, Vurbic, and Woods, 2008; Ledgerwood, Richardson, and Cranney, 2005; Parnas, Weber, and Richardson, 2005). In contrast, inhibition of NMDAR blocks extinction (Baker and Azorlosa, 1996). Further, administration of an AMPA receptor (an ionotropic glutamate receptor) agonist, PEPA, also facilitates contextual fear memory extinction in mice (Yamada, Zushida, Wada, and Sekiguchi, 2009; Zushida, Sakurai, Wada, and Sekiguchi, 2007).

Although metabotropic glutamate receptors (mGluRs) can modulate the ionotropic GluRs and are also involved in regulating synaptic plasticity (Awad, Hubert, Smith, Levey, and Conn, 2000; Clewa and Olive, 2011; Huber, Kayser, and Bear, 2000), their function in memory extinction has only been indicated in few studies. The mGluR5 knockout mice show deficits in fear extinction (Xu, Zhu, Contractor, and Heinemann, 2009) and a previous study has found that acutely blocking mGluR5 prevents the recall of tone-conditioned fear extinction memory in rats (Fontanez-Nuin, Santini, Quirk, and Porter, 2011). These studies suggest that mGluR5 receptors may be crucial for fear extinction, but do not demonstrate whether blocking mGluR5 receptor function during the process of contextual fear memory extinction may affect this behavior. Further, it is not clear whether mGluR5 is required for other aspects of memory processes such as acquisition, consolidation, and retrieval.

To determine the necessary role of mGluR5, we examined the effects of acute mGluR5 inhibition on contextual fear memory formation and extinction. We found that acute inhibition of mGluR5 using antagonist MPEP prevented both the initial contextual fear learning during the conditioning session and the inhibitory learning during the extinction session. By using the Morris water maze paradigm, we found that MPEP blocked spatial memory extinction. Our data suggest that certain aspects of behavioral flexibility require mGluR5 activity.

2. Materials & Methods

2.1. Animals

All experiments were performed with 2- to 3-month old male C57BL6 mice obtained from the Jackson Laboratory and bred at Michigan State University. Animals were housed in the university laboratory animal research facility and all manipulations were in compliance with the guidelines of Institutional Animal Care and Use Committee at Michigan State

University. The mice had ad libitum access to water and food, and were housed under 12-h dark/light cycle.

2.2. Drugs

Mice were intraperitoneally (i.p.) injected with 10 mg/kg MPEP [2-Methyl-6-(phenylethynyl) pyridine hydrochloride], Tocris (Cat. No. 1212)] or saline as vehicle control.

2.3. Contextual fear conditioning training

As described in our previous study (Wang, Zhang, Moon, Hu, Wang, Martin, Sun, and Wang, 2009), the mice were placed in a contextual chamber with dimensions of 28 cm (length) × 28 cm (width) × 34 cm (height) for 2 min before receiving a mild foot shock (0.7 mA, 2 sec). Following the foot shock, the mice were left in the chamber for 1 min before returning to their home cage. The activity of the mice in the chamber was recorded by the TruScan Photo Beam Activity System (Coulbourn Instruments, Whitehall, PA). Percentage of freezing (defined as immobility) was also recorded.

2.4. Extinction of contextual fear memory

Mice were re-exposed to the contextual chamber 24 hours after training for 15 min. Freezing behavior at every 10-second interval was scored. Activity measurement using TruScan was also performed.

2.5. Measurement of consolidation of contextual memory or extinction

Mice were re-introduced into the chamber 24 hours post fear conditioning training or post extinction training. Freezing behavior was recorded at every 10-sec interval for 2 min. The activity was also recorded with TruScan.

2.6. Morris water maze

As described in our previous study (Zhang, Storm, and Wang, 2011), a circular pool with diameter of 125 cm was filled with opaque water and surrounded by extramaze cues. A 10-cm diameter escape platform was placed into the opaque water such that its surface was 1 cm below the water surface. The mice were tracked by a video-based tracking system (WaterMaze, Coulbourn Instruments). The mice were first trained to find the visible platform, which was marked with a flag. The mice were trained by 4 trials a day for 1 day with the inter-trial interval being 3 min. The position of the platform was randomly changed for each trial. Mice were introduced into the pool and allowed to explore until they found the platform. If a mouse did not find the platform within 60 sec, it was manually guided to the platform. Mice were allowed to stay on the platform for 30 sec, after which they were returned to their home cage.

Next, the mice were subjected to the hidden platform training, which was performed similarly as the visible platform training except that the visible cue was removed. The platform was placed at a constant position throughout the training; the mice were introduced into the pool randomly from one of six different designated start locations. Mice were trained by 4 trials a day (with a 3-min interval between trials) for 4 days.

Three extinction trials were performed after the last hidden platform training session. During the extinction trial, the platform was removed and mice were allowed to explore the pool for 60 sec. Time spent in each quadrant were recorded using the tracking system.

2.7. Statistical analysis

The data for extinction, locomotion during fear conditioning training, and Morris water maze were analyzed using repeated measures ANOVA followed by post-hoc Tukey's HSD test. The data from the acquisition and consolidation experiments were analyzed using t-test or one-way ANOVA and Tukey's HSD test. These analyses were performed using the SPSS software.

3. Results

3.1. Acute inhibition of mGluR5 prevents extinction of fear memory

We examined the effect of acute inhibition of mGluR5 activity on contextual fear memory extinction. Mice were trained by contextual fear conditioning on day1 (Fig. 1A). These mice were randomly assigned as the saline or MPEP group, which did not display behavioral difference during training [$t(13)=0.14$, $p=0.89$, Figure 1B; $t(13)=0.46$, $p=0.653$, Figure 1C]. Twenty-four hours later, the trained mice received i.p. injection of either saline or the mGluR5 antagonist MPEP. Thirty minutes after the injection, mice were exposed to the conditioned contextual chamber for 15 min (extinction session 1) (Fig. 1A). Compared to the preconditioning behavior, the saline- and MPEP-treated mice both showed significant increase in freezing [$t(6)=5.47$, $p<0.05$ for the saline group; $t(7)=7.33$, $p<0.05$, for the MPEP group; Figure 1B] and correspondingly reduced ambulatory movement [$t(6)=8.48$, $p<0.05$ for the saline group; $t(7)=8.93$, $p<0.05$, for the MPEP group; Figure 1C] during the first 3 min of extinction session 1. The t-test revealed no significant difference in freezing [$t(13)=0.75$, $p>0.05$, Fig. 1B] or ambulatory movement [$t(13)=0.59$, $p>0.05$, Fig. 1C] between the saline and MPEP group during the first 3 min of the extinction session. This demonstrates that inhibition of mGluR5 did not affect contextual memory retrieval. However, mice injected with MPEP showed significant slower decrease in freezing ($F_{1, 13} = 18.89$, $p<0.01$, treatment effect; $F_{4, 52} = 4.95$, $p<0.01$, time effect; $F_{4, 52} = 5.33$, $p<0.01$, treatment-time interaction; Fig. 1B) and increase in locomotor activity ($F_{1, 13} = 15.26$, $p<0.01$, treatment effect; $F_{4, 52} = 6.21$, $p<0.01$, time effect; $F_{4, 52} = 6.63$, $p<0.01$, treatment-time interaction; Fig. 1C) than the saline-injected animals during the 15-min extinction session 1.

To test the retention of the contextual memory after extinction session 1, we examined the conditioned fear response 24 hours later (retention test 1, Fig. 1A). Mice injected with saline prior to extinction session 1 showed less freezing [$t(13)=2.53$, $p<0.05$, Fig. 1B] and more movement [$t(13)=2.61$, $p<0.05$, Fig. 1C] than the MPEP-treated group.

As the saline group did not show significant reduction of fear [$t(6)=0.94$, $p=0.36$, degree of freezing, Fig 1B; $t(6)=0.91$, $p=0.37$, ambulatory movement, Fig 1C] and MPEP group showed increased fear [$t(7)=2.55$, $p<0.05$, degree of freezing, Fig 1B; $t(7)=2.42$, $p<0.05$, ambulatory movement, Fig 1C] following a single extinction session, we performed

extinction session 2 (Fig. 1A). During the first 3 min in extinction session 2, both groups showed similar freezing ($F_{1, 13} = 2.19$, $p=0.16$, Fig 1B) and movement ($F_{1, 13} = 2.67$, $p=0.12$, Fig 1C) to what was observed during the retention test 1. The MPEP-injected group showed less decay of freezing ($F_{1, 13} = 31.63$, $p<0.01$, treatment effect; $F_{4, 52} = 5.58$, $p<0.01$, time effect; $F_{4, 52} = 5.27$, $p<0.01$, treatment-time interaction; Fig. 1B) and less increase in movement ($F_{1, 13} = 11.38$, $p<0.01$, treatment effect; $F_{4, 52} = 4.96$, $p<0.01$, time effect; $F_{4, 52} = 4.55$, $p<0.01$, treatment-time interaction; Fig. 1C) during the 15-min extinction session 2.

To test the retention of the contextual memory after extinction session 2, mice were re-exposed to the conditioned contextual chamber 24 hours later (Fig. 1A). During retention test 2, the saline-treated group showed less freezing [$t(13)=2.99$, $p<0.05$, Fig. 1B] and more movement [$t(13)=2.31$, $p<0.05$, Fig. 1C] than the MPEP-treated group. After 2 extinction training sessions, only the saline- but not the MPEP-treated group showed significant reduction of contextual fear memory, when compared with the retrieved memory on day 2 during the initial 3 min in extinction session 1 ($F_{1, 13} = 7.87$, $p<0.05$, Fig. 1B; $F_{1, 13} = 6.3$, $p<0.05$, Fig. 1C). Collectively, these data demonstrate that effective contextual fear memory extinction requires mGluR5 activity.

3.2. Acute inhibition of mGluR5 impairs acquisition but not consolidation of fear memory

As extinction requires active learning, we tested whether blocking mGluR5 also impairs the initial learning of contextual fear memory (Fig. 2A). Mice injected with saline or MPEP showed comparable overall locomotion ($F_{1, 10} = 0.19$, $p=0.67$, Fig. 2B) and response to the foot shock, which stimulated running behavior leading to a momentary increase in movement ($F_{1, 10} = 0.67$, $p=0.53$, drug effect; $F_{2, 10} = 5.22$, $p<0.05$, time effect; $F_{2, 10} = 0.32$, $p=0.73$, interaction effect; analysis of movement data before, during, and after the delivery of foot-shock, Fig. 2B). This indicates that MPEP does not alter sensitivity to the shock-induced pain sensation. MPEP-treated mice showed less contextual memory, as indicated by lower freezing [$t(10)=2.34$, $p<0.05$, Fig. 2C] and more movement [$t(10)=2.42$, $p<0.05$, Fig. 2D] when tested 24 hours after the training session.

To test whether MPEP affected the acquisition or the consolidation of contextual fear memory, new cohorts of mice were injected with MPEP 10 minutes or 60-minutes after training (Fig. 3A). Post-training administration of MPEP did not impair fear memory when tested 24 hours after the training session ($F_{1, 15} = 0.57$, $p>0.05$, Fig. 3B; $F_{1, 15} = 0.13$, $p>0.05$, 3C). This shows that blocking mGluR5 impairs acquisition of fear memory, but does not affect consolidation. Together, our data indicate that MPEP suppresses both initial learning (Fig. 2) and extinction learning (Fig. 1).

3.3. Acute inhibition of mGluR5 impairs spatial memory extinction

Our results demonstrate that acute pharmacological modulation of mGluR5 activity affects the extinction of contextual fear memory. We further tested whether it would have impact on the extinction of other forms memory such as spatial memory. We first trained mice to acquire spatial information in the Morris water maze. Following visible and hidden platform training, we examined spatial memory by probe tests, before which animals were injected with either saline or MPEP (Fig. 4A). The extinction training consisted of 3 probe trials,

during which the animal would re-learn that there is no platform in the water maze. During the extinction training, the saline- and MPEP-treated animals showed similar swimming speed in all 3 probe tests ($F_{1, 18} = 0.72$, $p > 0.05$, treatment effect; $F_{2, 36} = 0.53$, $p > 0.05$, time effect; $F_{2, 36} = 0.11$, $p > 0.05$, treatment-time interaction; Fig. 4B). In the first probe test, both groups displayed strong and comparable preference to the target quadrant, indicating that MPEP does not affect memory retrieval [$t(18) = 0.17$, $p = 0.86$ Fig. 4C]. Saline-injected mice displayed progressive decrease (f value and p value for the main effect of probe sessions, Fig. 4C) in the time spent in the target quadrant during the 2nd ($p < 0.05$, repeated measures ANOVA followed by post hoc analysis, Fig. 4C) and 3rd probe test ($p < 0.05$, repeated measures ANOVA followed by post hoc analysis, Fig. 4C), thus showing extinction of the previously established spatial memory. In contrast, extinction was completely abolished in the MPEP-injected group ($F_{1, 18} = 5.39$, $p < 0.05$, treatment effect; $F_{2, 36} = 11.24$, $p < 0.01$, time effect; $F_{2, 36} = 9.15$, $p < 0.01$, treatment-time interaction; Fig. 4C).

4. Discussion

Glutamate neurotransmission plays a major role in regulating synaptic long-term potentiation and long-term depression, which are considered as potential cellular substrates of learning and memory. Partial NMDAR agonist D-cycloserine (DCS) has been found to enhance learning performance in rodents as well as facilitate extinction of cocaine place preference and fear memory. Spermine, another NMDAR positive allosteric modulator has been shown to enhance extinction of inhibitory avoidance response (Gomes, Mello, da Rosa, Bochi, Ferreira, Barron, and Rubin, 2010). N-acetylcysteine, which stabilizes extracellular glutamate levels has also been shown to enhance extinction (Zhou and Kalivas, 2008). An AMPA receptor agonist PEPA, has also been shown to facilitate fear extinction in mice (LaLumiere, Niehoff, and Kalivas, 2010). As mGluR5 shows interaction with NMDAR and potentiates NMDAR function [(Awad et al., 2000) but also see (Fontanez-Nuin et al., 2011)], it has been shown to regulate synaptic flexibility such as LTP and LTD (Ayala, Chen, Banko, Sheffler, Williams, Telk, Watson, Xiang, Zhang, Jones, Lindsley, Olive, and Conn, 2009; Lu, Jia, Janus, Henderson, Gerlai, Wojtowicz, and Roder, 1997). Further, mGluR5 is expressed in brain regions that regulate learning and memory. Previous studies found that positive allosteric modulators (PAMs) of mGluR5 facilitate the extinction of cocaine-induced place preference and methamphetamine seeking behavior (Cleva, Hicks, Gass, Wischerath, Plasters, Widholm, and Olive, 2011; Kufahl, Hood, Nemirovsky, Barabas, Halstengard, Villa, Moore, Watterson, and Olive, 2012).

The functional effect of mGluR5 inhibition on the hippocampus-dependent contextual fear memory extinction has been implicated and partially addressed. Genetic deletion of mGluR5 leads to less fear expression after receiving the amygdala-dependent cued and the hippocampus-dependent contextual conditioning (Lu et al., 1997; Xu et al., 2009). Depending on the training and testing paradigms, the effects of acute inhibition of mGluR5 may differ. MPEP blocks the acquisition but not the consolidation/expression of contextual and auditory fear memory [this study and (Rodrigues, Bauer, Farb, Schafe, and LeDoux, 2002)]. In the fear-potentiated startle paradigm, MPEP blocks both acquisition and expression (Schulz, Fendt, Gasparini, Lingenhoehl, Kuhn, and Koch, 2001). Strikingly, the weaker fear memory in the mGluR5 knockout mice is more resistant to extinction training

(Xu et al., 2009). Despite that the effects of mGluR5 deletion on neural development and signaling compensation are not known, the study strongly implicates that intact mGluR5 function is required for effective memory formation and extinction. In this study, we used acute pharmacological inhibition of mGluR5, which allows us to separate the effects on initial learning and inhibitory learning. In addition to avoiding potential developmental abnormalities caused by germ line mGluR5 deletion, the acute mGluR5 inhibition also allowed us to exclude the effects on locomotor activity. We found that the mGluR5 inhibitor MPEP disrupted both the initial contextual fear learning and the inhibitory learning during the extinction sessions. This is consistent with the recent finding showing that enhancement of mGluR5 function facilitates both initial learning and extinction learning of contextual fear memory [(Sethna and Wang, 2014) but also see (Xu, Zhu, Kraniotis, He, Marshall, Nomura, Stauffer, Lindsley, Conn, and Contractor, 2013)]. Interestingly, the mouse model of Fragile X syndrome shows abnormal hyperfunction of mGluR5 (Huber, Gallagher, Warren, and Bear, 2002) along with faster extinction of passive avoidance memory (Dolen, Osterweil, Rao, Smith, Auerbach, Chattarji, and Bear, 2007).

The effects of mGluR5 inhibition on fear memory extinction may depend on the feature of the conditioned stimuli (CS) and how CS is presented during the extinction sessions. MPEP suppressed within-session extinction when the CS (context) was continuously presented (this study). When animals are exposed to repeated but discontinuous CS (i.e. delivery of 20 episodes of tone), pre-extinction MPEP administration does not affect the within-session extinction but rather impairs the consolidation or the recall of the extinguished tone-associated fear memory (Fontanez-Nuin et al., 2011). Interestingly, when more repeated CS (30 episodes of tone) are delivered, MPEP does not affect either within-session extinction or extinction retention (Toth, Dietz, Peterlik, Huber, Fendt, Neumann, Flor, and Slattery, 2012). The effect of MPEP on the within-session extinction demonstrated by our study also suggests that the extinction of the conditioned fear associated with contextual spatial cues and sensory cues (e.g. tone or light) may be differentially regulated.

It has been observed that mGluR5 PAMs facilitate hippocampal LTP and LTD (Ayala et al., 2009), and they also enhance the performance in both the initial hidden platform learning and the reversal platform learning in Morris water maze (Ayala et al., 2009; Xu et al., 2013). However, these studies had a different training paradigm and studied reversal learning, and not necessarily spatial memory extinction. By using the repeated probe tests, we found that the spatial memory extinction was absent in mice injected with MPEP. Interestingly, previous studies show that inhibition of mGluR5 by MPEP has no effect on acquisition and reference memory following water maze training (Car, Stefaniuk, and Wisniewska, 2007).

Accumulating lines of evidence have suggested the function of mGluR5 in extinction of various forms of acquired behavioral changes. How mGluR5 regulates the extinction of fear and spatial memory remains largely unknown. It has been demonstrated that repeated extinction sessions over days trigger up-regulation of mGluR5 expression in the hippocampus (Riedel, Casabona, Platt, Macphail, and Nicoletti, 2000), which may lead to reduction in subsequent recall or spontaneous recovery of fear (Mao, Chang, Wu, Orejarena, Manzoni, and Gean, 2013). At the cellular level, it is suggested that mGluR5 activity supports the extinction-induced changes in AMPA receptor/NMDA receptor ratio and

intrinsic excitability in prefrontal cortex (Sepulveda-Orengo, Lopez, Soler-Cedeno, and Porter, 2013). As extinction may involve multiple brain regions including hippocampus, amygdala, and prefrontal cortex, future investigations implementing region-specific manipulation of mGluR5 paired with simultaneous neuronal recording and detection of signaling markers may help to better define neural substrates and molecular mechanisms.

5. Conclusions

By using acute administration of MPEP, our data support that mGluR5 activity is necessary for certain aspects of behavioral flexibility.

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Abbreviations

mGluR5	metabotropic glutamate receptor 5
MPEP	[2-Methyl-6-(phenylethynyl) pyridine hydrochloride]
PTSD	post-traumatic stress disorder

Highlights

- Acute inhibition of mGluR5 by MPEP suppresses the extinction of contextual fear memory.
- Acute inhibition of mGluR5 by MPEP suppresses acquisition but not consolidation of contextual fear memory.
- Acute inhibition of mGluR5 by MPEP suppresses the extinction of spatial memory.

Figure 1. MPEP administration inhibits contextual fear memory extinction. (A) Behavioral and MPEP administration procedure. (B) Mean +/- SEM of % freezing per min during contextual fear conditioning training, extinction session 1, retention test 1, extinction session 2, and retention test 2. (C) Mean +/- SEM of distance traveled per min during contextual fear conditioning training, extinction session 1, retention test 1, extinction session 2, and retention test 2. Repeated measures ANOVA shows significant difference between the saline- and MPEP-injected group during extinction sessions 1 and 2. *: $p < 0.05$, t-test. ns: not significant, t-test.

Figure 2.

Pre-training administration of MPEP impairs contextual fear memory. (A) Behavioral and MPEP administration procedure. (B) Mean $\pm$ SEM of distance traveled for each of the 5-sec bin during the 3-min contextual fear conditioning training. The arrow indicates the delivery of the foot shock (0.7 mA, 2 sec duration). (C) Mean $\pm$ SEM of % freezing per min during the 2-min memory test. (D) Mean $\pm$ SEM of distance traveled per min during the 2-min memory test. Repeated measures ANOVA shows no significant difference between the saline- and MPEP-injected group in B. *: $p < 0.05$, t-test.

Figure 3.

Post-training administration of MPEP does not affect contextual fear memory. (A) Behavioral and MPEP administration procedure. (B) Mean $\pm$ SEM of % freezing per min during the 2-min memory test. (C) Mean $\pm$ SEM of distance traveled per min during the 2-min memory test. ns: not significant, one-way ANOVA.

Figure 4.

MPEP administration inhibits the extinction of spatial memory in Morris Water Maze. (A) Behavioral and drug administration procedure. (B) Mean $\pm$ SEM of the swimming speed in the probe tests during the extinction training. There is no difference between the saline- and MPEP-injected mice, as determined by repeated measures ANOVA. (C) Mean $\pm$ SEM of the percentage of time spent in the target quadrant in the probe tests during the extinction training sessions. *: $p < 0.05$ between the saline- and MPEP-injected group, repeated measures ANOVA followed by post-hoc analysis.