Original Article

Amyloid β protein injection into medial septum impairs hippocampal long-term potentiation and cognitive behaviors in rats

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Abstract: The specific loss of cholinergic neurons and the progressive deficits of cognitive function are the most primary characteristics of Alzheimer's disease (AD). Although the neurotoxicity of amyloid β protein (Aβ) in AD has been investigated extensively, it is still unclear whether the Aβ aggregated in the medial septum (MS), a major cholinergic nucleus projecting to the hippocampus, could affect hippocampal synaptic plasticity and further impair the memory behaviors. The present study investigated the effects of Aβ injection into the MS on hippocampal long-term potentiation (LTP) and cognitive behaviors of rats by using Morris water maze (MWM), Y maze and *in vivo* hippocampal LTP recording. The effects of kainic acid (KA), an agent with specific neurotoxicity to GABAergic neurons, were also observed. The results showed that: (1) Intra-MS injection of Aβ$_{25-35}$, not KA, impaired spatial learning and memory of rats in classical and reversal MWM tests; (2) Both Aβ$_{25-35}$ and KA impaired novelty-seeking behavior of rats in Y maze; (3) Intra-MS injection of Aβ$_{25-35}$, not KA, suppressed *in vivo* hippocampal LTP in the CA1 region of rats; (4) Both Aβ$_{25-35}$ and KA did not affect the motor ability in behavioral tests and the hippocampal paired-pulse facilitation (PPF) in electrophysiological recording. These results indicate that intra-MS injection of Aβ could impair spatial memory, cognitive flexibility and exploratory motivation, as well as hippocampal LTP in rats, suggesting that the cholinergic neurons in the MS and the septo-hippocampal projection could be important targets of neurotoxic Aβ, and the specific damage of cholinergic neurons in the MS is likely responsible for the impairments of hippocampal synaptic plasticity and cognitive function in AD.

Key words: medial septum; amyloid β protein; kainic acid; Morris water maze; Y maze; long-term potentiation

内侧隔核注射淀粉样β蛋白损害大鼠的长时程增强和认知行为

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摘 要： 胆碱能神经元的逐渐丢失和进行性认知功能障碍是阿尔茨海默病(Alzheimer's disease, AD)的主要特征。脑内胆碱能神经元集中分布的区域之一是基底前脑的内侧隔核(medial septum, MS)，其发出投射纤维至海马。尽管AD患者和动物模型脑内淀粉样β蛋白(amyloid β protein, Aβ)的神经毒性包括特异性损伤胆碱能神经系统的作用已被广泛报道，但仍不清楚聚集在MS的Aβ是否会影响海马突触可塑性，进而影响学习记忆行为。本研究采用Morris水迷宫、Y型迷宫和在体海马长时程增强(long-term potentiation, LTP)记录，观察了MS注射Aβ对大鼠海马LTP及认知行为的影响，同时还以能特异性损伤γ氨基丁酸

(γ-aminobutyric acid, GABA)能神经元的海人藻酸(kainic acid, KA)作为对照，进行了效应比较。结果显示：(1) MS注射Aβ$_{25-35}$，而非KA，明显损伤了大鼠在经典Morris水迷宫和对位水迷宫中的空间学习记忆能力；(2) MS注射Aβ$_{25-35}$和KA均损害了大鼠在Y迷宫中的新异环境探索能力；(3) MS注射Aβ$_{25-35}$，而非KA，明显抑制了大鼠海马CA1区在体LTP；(4) Aβ$_{25-35}$和KA均未影响大鼠在行为学测试中的运动能力和电生理记录中的海马CA1区双脉冲易化(paired-pulse facilitation, PPF)。以上结果表明，MS注射Aβ能够损伤大鼠空间学习记忆能力、学习记忆灵活性和探索行为，并压抑海马LTP。结合以往研究，本研究提示：MS的胆碱能神经元及其海马投射可能是AD病程中受Aβ神经毒性作用损害的主要细胞和组织，选择性损伤MS中的胆碱能神经元会导致AD病程中的海马突触可塑性损伤和认知功能伤害。

关键词：内侧隔核；淀粉样β蛋白；海人藻酸；Morris水迷宫；Y迷宫；长时程增强
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Alzheimer's disease (AD) is an incurable, progressive, and devastating neurodegenerative disorder mainly manifested as the early cognitive function decline and the late cerebral atrophy and dementia. According to World Alzheimer's Disease Report 2016, more than 47 million people in the worldwide were afflicted dementia, and this number will increase to 131 million by 2050 [1]. High density of senile plaques primarily composed of amyloid β protein (Aβ) in the brain, most profoundly in the cortex and hippocampus, is an important neuropathological hallmark of AD [2]. In addition, intraneuronal Aβ accumulation is a relatively selective trait of basal forebrain (BF) cholinergic neurons early in adult life, which is a potential contributor to the degeneration of BF cholinergic neurons in AD [3]. According to previous reports, no matter *in vivo* or *in vitro*, the neurotoxicity of natural Aβ has been confirmed [4, 5]. In our previous experiments, we also testified that not only the full length of Aβ molecule such as Aβ$_{1-42}$ and Aβ$_{1-40}$ but also the Aβ fragments including Aβ$_{25-35}$ and Aβ$_{31-35}$ could significantly impair the spatial memory and hippocampal synaptic plasticity in normal rats [6, 7].

The loss of cholinergic neurons in the BF is another pathological event in the pathogenesis of AD [8]. In the medial septum (MS), a main part of BF cholinergic system, cholinergic and GABAergic neurons directly project to the hippocampal CA1 region, a central brain region associated with the process of learning and memory information [9]. According to the existing reports, cholinergic neurons in MS play an important role in spatial learning and memory [10], and the activated septal cholinergic input could induce different types of hippocampal Schaffer collateral to CA1 synaptic plasticity [11]. In addition, Aβ plays a crucial role in the cholinergic neuronal degeneration [12]; intra-hippocampal injection of Aβ$_{1-40}$ could induce injury of the septo-hippocampal projections, which lead to memory impair-

ments [13]; Aβ$_{1-40}$-treated animals showed significantly reduced number of MS cholinergic neurons, but not GABAergic neurons [21, 22]; the degree of dementia is positively related to the number of degenerated cholinergic neurons in the BF [14]. On the other hand, the vulnerability of GABAergic neurons to AD is also getting more attention [15]. It has been reported that the dysfunction of GABA signaling affected the homeostasis between excitation and inhibition in the brain and contributed to the pathogenesis of AD [16]; the activity of GABAergic interneuron played a critical role in controlling spatial learning and memory retrieval [17]; transgenic mice displayed significant reduction in GABAergic activity even at early phase of AD [18]. However, the exact roles of both types of neurons in MS and their projections to the hippocampus are still controversial. For example, some researchers did not find severe memory deficits in rats after depletion of cholinergic neurons in septo-hippocampus [19]; selective injury of GABAergic neurons in MS did not affect the spatial reference or working memory [20].

Considering the close structural connection between MS and hippocampus, the selective intraneuronal Aβ accumulation in the BF cholinergic neurons occurred early in adult life, and the crucial role of Aβ in the cholinergic neuronal degeneration, it will be interesting to clarify whether the aggregated Aβ in the MS could affect the synaptic plasticity in the hippocampus and impair the memory behaviors of rats, and whether the effects of Aβ were mainly dependent on the injury of cholinergic neurons. Therefore, the present study investigated the effects of Aβ injection into MS on the hippocampal long-term potentiation (LTP) in the CA1 region and different types of memory in rats. As a control, small amounts of kainic acid (KA) were used to damage non-cholinergic neurons, especially GABAergic neurons [23].

1 MATERIALS AND METHODS

1.1 *Animals and surgery*

Sprague-Dawley (SD) rats (230–250 g) were supplied by the Research Animal Center of Shanxi Medical University. All experiments were done with approval of the Shanxi Committee on Ethics of Animal Research. All rats were housed under controlled room temperature (20–24 °C) and humidity (60%–80%) and received food and water *ad libitum*. The rats were randomly divided into three groups based on the solutions injected: control, Aβ$_{25-35}$ and KA (n = 10 for each group). After adaptation for 3 days, 10% chloral hydrate (0.3 mL/100 g) was used to anesthetize rats by intraperitoneal (i.p.) injection, and then the rats were placed in a stereotaxic device for intra-MS injection, with the following injection coordinate on the cortex: anteriorposterior (AP): 0.7 mm, mediallateral (ML): 2.0 mm, from bregma [24]. To avoid the damage of blood vessels in the brain, the vertical arm of stereotaxic device was set up with an 18° angle toward middle line. The final injection target point was located at the center of both MS. Saline, 5 nmol Aβ$_{25-35}$ or 0.75 µg KA (purchased from Sigma) dissolved in 1 µL saline was administered to the MS by using a microinjection pump (KDS310 Plus, USA). Different behavioral tests and *in vivo* electrophysiological recording were performed 2 weeks later (Fig. 1).

1.2 *Morris water maze (MWM) test*

MWM test was used to evaluate the spatial learning and memory behavior of rats. As described before [6], the rats were trained to locate an escape underwater platform placed at the midpoint of the first quadrant in a large circular pool containing tape water at room temperature. The inner surface of the pool was painted black and hung with various prominent visual cues. A video tracking system (Ethovision 3.0, Noldus Information Technology, Wageningen, Netherlands) was used to collect animals' swimming activity. Each animal was trained four times per day for 5 consecutive days in hidden platform test, and the escape latency, escape distance and swimming speed of each rat were recorded. After hidden platform tests, a 120 s probe trial was given to evaluate the ability of memory retention of each animal on day 6.

1.3 *Reversal MWM test*

To test the cognitive flexibility of the animals, the reversal MWM task was performed after the classical MWM test on day 7–11. In the reversal MWM, the platform was moved to the opposite quadrant (third quadrant) of the pool. Then, the animal's ability to learn a novel platform location against interference from the previously acquired memory could be examined [25]. The experimental process of reversal MWM test was similar to MWM, with consecutive 4 days (days 7–10) of hidden platform tests, and one day (day 11) of probe trial. After the probe trial test, a visible platform test was used to examine the visual and motor ability of rats by recording the time when rats arrived at the target platform.

1.4 *Y maze spontaneous spatial novelty preference test*

The rats were subjected to the Y maze spontaneous spatial novelty preference test on the next day after reversal MWM test. The three arms of Y maze connected at angles of 120°; the length, height and width of each arm were 45 cm × 15 cm × 15 cm. Each arm could be blocked from the central triangle by a removable opaque barrier door at the entrance. As described before, the task consisted of 2 trials, exposure phase and test phase [26]. During exposure phase, the access to the novel arm was blocked. Then each rat was placed at the end of the start arm, facing the central triangle, and was allowed to explore both the start arm and the other arm freely for 5 min. When all paws of rat were placed inside an arm, it was defined as arm entering. Similarly,
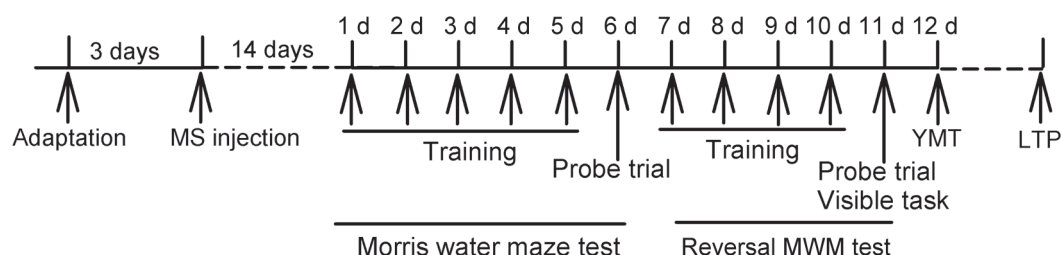

Fig. 1. Sequence of events of the experimental procedure. Two weeks after intra-MS injection of Aβ$_{25-35}$, KA or saline, different behavioral tests were performed, including classical Morris water maze (MWM) task, reversal MWM test, and Y maze spontaneous spatial novelty preference test (YMT). After behavioral tests, *in vivo* hippocampal LTP was recorded.

arm departure was considered as all 4 paws were outside the arm. In the test phase, the barrier door was removed, and the rats were placed again in the start arm, being allowed to explore all three arms freely for 5 min. The percentage of time spent in each arm was recorded and manually scored.

1.5 *In vivo hippocampal LTP recording*

The same rats used in behavioral tests were used for the electrophysiological recording after Y maze test. The *in vivo* hippocampal LTP was recorded as described before [6]. Each rat was anesthetized by i.p. injection of urethane (1.5 g/kg). Then the rat was placed in a stereotaxic device for acute surgery and LTP recording. A bound stimulating/recording electrode was inserted into the left hippocampal CA1 region (4.2 mm posterior to and 3.8 mm lateral to bregma). Field excitatory postsynaptic potentials (fEPSPs) were evoked by test stimuli (0.033 Hz) to the Schaffer-collateral/commissural pathway, and baseline fEPSPs were monitored for 30 min. Paired stimuli with an interval of 50 ms were used to observe the change of paired-pulse facilitation (PPF) ratio. Then, a high-frequency stimulation (HFS) protocol, consisted of three times of 20 pulses at 200 Hz at an interval of 30 s, was used for the LTP induction. After HFS, the change of fEPSPs amplitude was recorded for another 1 h by using test stimuli again.

1.6 *Statistics*

All values were expressed as mean ± SEM. The data of LTP and the escape latencies in MWM were analyzed using two-repeated measures analysis of variance (ANOVA) and other data were analyzed using one-way ANOVA. The statistical significance was defined as $P < 0.05$, and all statistical analyses were performed using SPSS 17.0.

2 RESULTS

2.1 Intra-MS injection of Aβ$_{25-35}$, not KA, impaired spatial learning and memory ability of rats

The MWM test was used to assess long-term spatial memory of rats. The learning ability of the rats to acquire spatial information was first evaluated by the hidden platform test for five consecutive days. As shown in Fig. 2*A* and 2*B*, the average escape latency and distance of the rats to find the hidden platform gradually decreased during the 5 training days. However, the escape latency and distance of rats in Aβ$_{25-35}$ group significantly increased compared with those in control

group on days 2–5, while no significant changes were shown in KA group. In order to assess the spatial memory ability of the rats, probe trial test was performed on day 6. As shown in Fig. 2*D*, the percentage of swimming time in target quadrant was (44.9 ± 1.5)%, (22.6 ± 1.0)% and (44.2 ± 1.3)% in control, Aβ$_{25-35}$ and KA groups, respectively, with a significant decrease in the Aβ$_{25-35}$ group ($P < 0.01$). In addition, there was no significant difference in the average swimming speed of rats among three groups in probe trial (Fig. 2*E*, $P > 0.05$). These results indicate that the spatial learning and memory ability of rats was significantly impaired by intra-MS injection of Aβ$_{25-35}$, but not KA.

2.2 Intra-MS injection of Aβ$_{25-35}$, not KA, impaired cognitive flexibility of rats

To test the cognitive flexibility of the rats, the platform was switched to the opposite region of the pool in the reversal MWM test. In hidden platform tests (Fig. 3*A*, 3*B*), compared with control group, the average escape latency and distance of the rats to find the hidden platform in Aβ$_{25-35}$ group on days 7–10 all significantly increased ($P < 0.01$). In probe trial, the percentages of time (Fig. 3*D*) of rats swimming in target quadrant was (21.9 ± 0.8)% in Aβ$_{25-35}$ group, significantly lower than (42.2 ± 1.3)% ($P < 0.01$) in control group. These results indicate that intra-MS injection of Aβ$_{25-35}$ impaired the relearning ability of rats in the reversal MWM. However, there were no statistical significant differences between KA group and control group in the average escape latency, distance and the percentage of time in target quadrant in probe trial (Fig. 3), indicating that KA injection into MS did not impair the cognitive flexibility to purge old memory and relearn new strategies.

After probe trial, a visible platform test was performed to test the vision and motor ability of the rats (Fig. 3*E*). There was no significant difference of escape latency of rats to arrive the visible platform among three groups ($P > 0.05$), indicating the vision and motor ability of rats were not affected by Aβ$_{25-35}$ and KA pretreatment.

2.3 Both Aβ$_{25-35}$ and KA impaired novelty-seeking behavior of rats

The exploring motivation of rats was examined using Y maze spatial novelty preference test. As shown in Fig. 4, the average time percentage of rats spent in the novel arm was (21.2 ± 1.2)% in Aβ$_{25-35}$ group, significantly lower than that of (40.1 ± 3.6)% in control group ($P < 0.01$). Similarly, the rats in KA group also showed
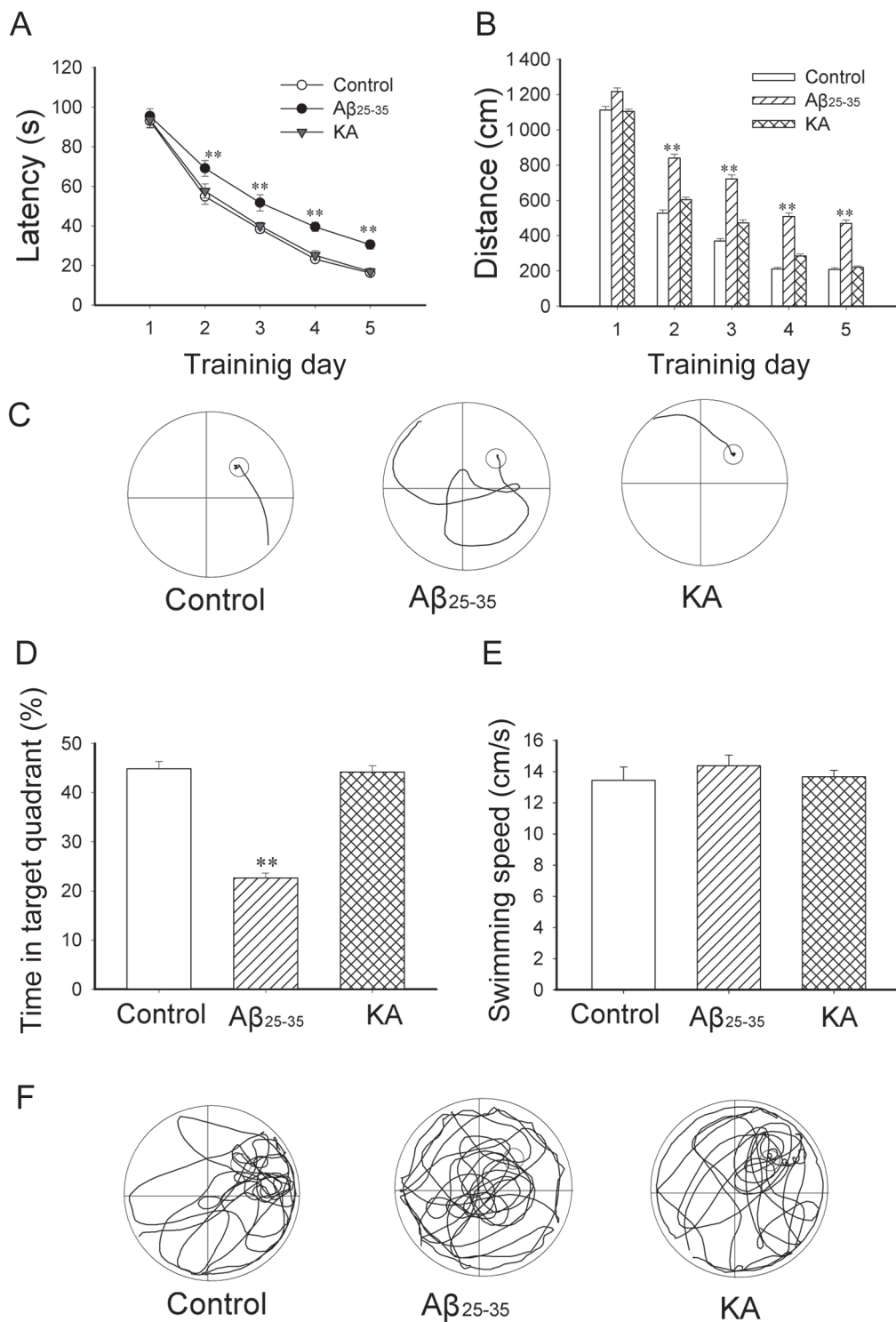
Fig. 2. Medial septum injection of Aβ$_{25-35}$ impaired spatial learning and reference memory ability of rats in classical MWM. *A*: Plots showing the average escape latency of rats searching for the hidden platform over five consecutive training days ($^{**}P < 0.01$ compared with the control group, $n = 10$ for each group). *B*: Histograms showing that the escape distance was significantly increased in the Aβ$_{25-35}$ group, but not in KA group, on training days 2–5 ($^{**}P < 0.01$ compared with the control group). *C*: Representative swimming traces of rats in three groups during the hidden platform test. *D*: Histograms showing the decrease in swimming time in the target quadrant in the Aβ$_{25-35}$ group during the probe trial test ($^{**}P < 0.01$ compared with the control group). *E*: Histograms showing no significant difference of swimming speed of rats among three groups in the probe trial. *F*: Representative swimming traces of rats in three groups during the probe trial.
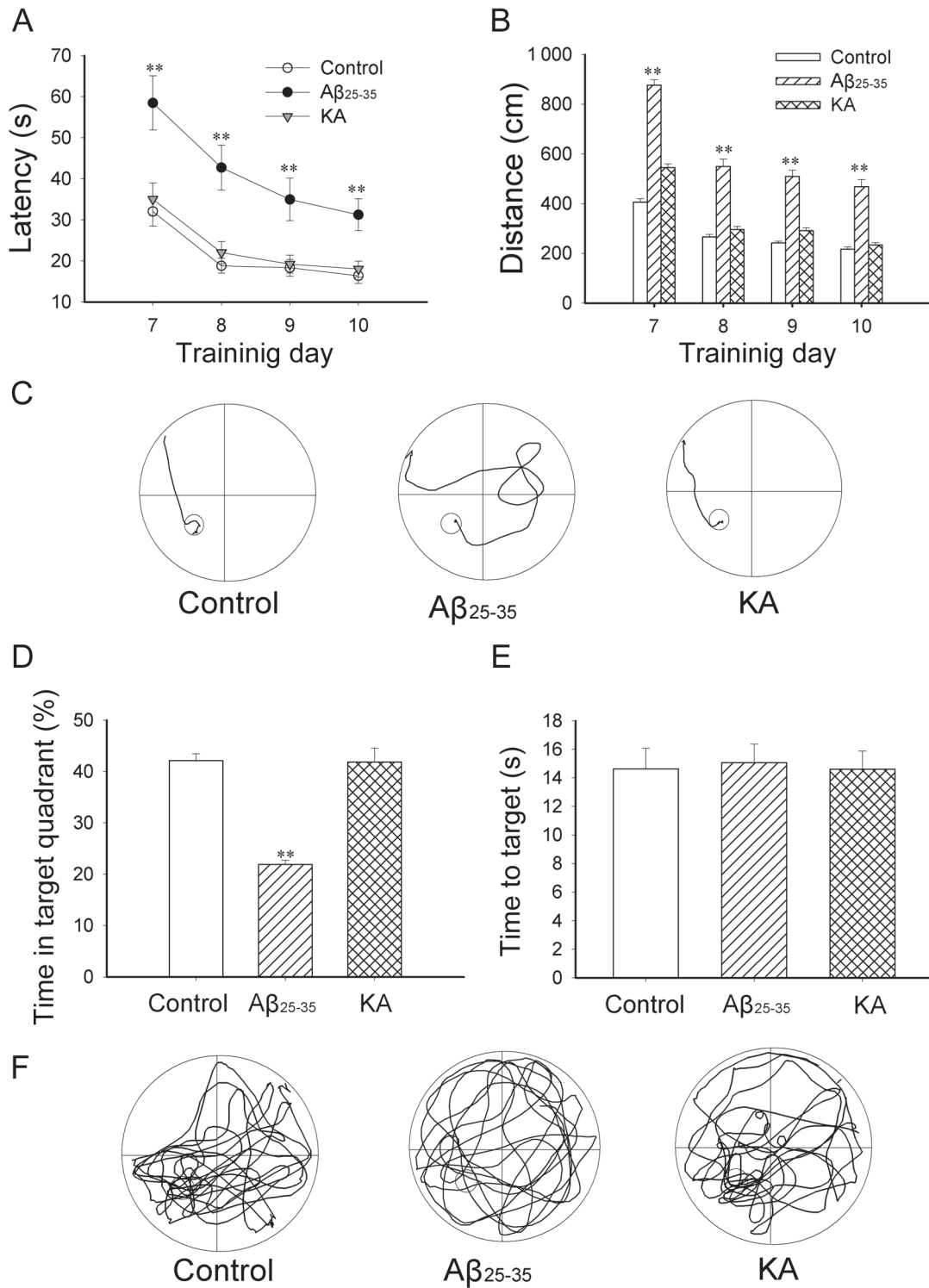
Fig. 3. Intra-MS injection of $A\beta_{25-35}$ impaired the relearning ability and cognitive flexibility of rats in reversal MWM. *A*: Plots showing the average escape latency of rats on training days 7–10 ($^{**}P < 0.01$ compared with the control group, $n = 10$ for each group). *B*: Histograms showing that the escape distance was significantly increased in the $A\beta_{25-35}$ group on training days 7–10 ($^{**}P < 0.01$ compared with the control group). *C*: Representative swimming traces of rats on the training day 10 in three groups. *D*: Histograms showing that the percentage of time of rats staying in the target quadrant during the probe trial test ($^{**}P < 0.01$ compared with the control group). *E*: Histograms showing the time when rats arrived at the target platform in all groups on the visible platform test. *F*: Representative swimming traces of rats in three groups during the probe trial.

decreased preference for novel arm, the average time percentage in the novel arm being only $(23.1 \pm 1.3)\%$ ($P < 0.01$). Correspondingly, both $Aβ_{25-35}$ and KA increased the time percentages in the familiar arms, including start arm and the other arm. In start arm, the average time percentages in $Aβ_{25-35}$ and KA groups were $(40.5 \pm 1.4)\%$ and $(37.9 \pm 2.4)\%$, respectively, both significantly larger than the value of $(27.6 \pm 1.3)\%$ in control group ($P < 0.01$). In the other familiar arm, the average time percentages in $Aβ_{25-35}$ and KA groups were $(36.2 \pm 1.4)\%$ and $(38.8 \pm 1.9)\%$, respectively, larger than the value of $(29.4 \pm 2.3)\%$ in control group ($P < 0.05$ for both), and there was no significant difference between $Aβ_{25-35}$ group and KA group. These results indicate that the rats pretreated with $Aβ_{25-35}$ or KA tended to prefer the familiar arms than the novel arm.

2.4 Intra-MS injection of $Aβ_{25-35}$, not KA, suppressed *in vivo* hippocampal LTP

In view of the close association between the spatial memory and the hippocampal synaptic plasticity, hippocampal LTP in the CA1 region was recorded after behavioral tests. Baseline fEPSPs were firstly recorded for 30 min. We did not find any significant difference among the three groups, indicating that $Aβ_{25-35}$ or KA injection did not affect baseline synaptic transmission. Then, HFS was applied to induce LTP. As shown in the Fig. 5*A* and Fig. 5*C*, the amplitude of fEPSPs abruptly

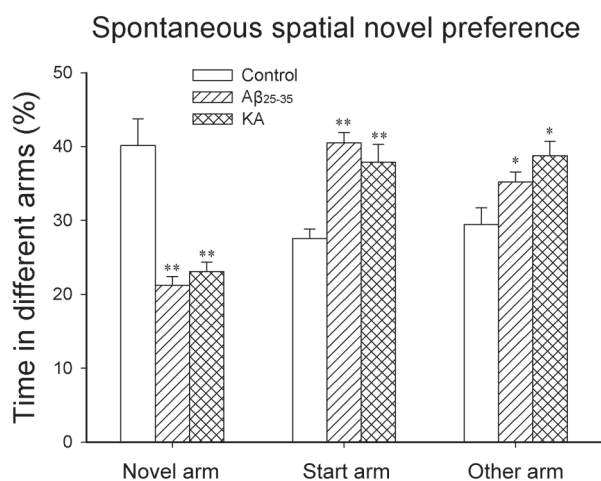Spontaneous spatial novel preference

Fig. 4. Intra-MS injection of $Aβ_{25-35}$ and KA both impaired novelty-seeking behavior of rats in the Y maze. The histograms showing the time percentages of rats staying in different arms. Compared with the control group, the time percentage of rats in exploring novel arm was significantly reduced in $Aβ_{25-35}$ and KA groups, respectively, along with a longer resident time in the start arm. *$P < 0.05$, **$P < 0.01$ compared with the control group.

increased to $(202.8 \pm 4.7)\%$, $(162.7 \pm 2.7)\%$ and $(207.9 \pm 4.3)\%$ immediately after delivering HFS in the control ($n = 7$), $Aβ_{25-35}$ ($n = 6$) and KA ($n = 6$) groups, respectively, indicating that LTP was successfully induced in the three groups. However, after HFS application, $Aβ_{25-35}$ injection produced a significant depression of LTP. Comparing the values at 0, 30 and 60 min after HFS, the average fEPSP amplitude from $(202.8 \pm 4.7)\%$, $(165.2 \pm 3.5)\%$ and $(158.8 \pm 4.4)\%$ in control group decreased to $(162.7 \pm 2.7)\%$ ($P < 0.01$), $(118.5 \pm 2.9)\%$ ($P < 0.01$) and $(111.4 \pm 2.5)\%$ ($P < 0.01$) in $Aβ_{25-35}$ group, respectively. On the contrary, the average fEPSP amplitude in KA group was $(207.9 \pm 4.3)\%$, $(164.4 \pm 3.1)\%$ and $(157.4 \pm 4.1)\%$ at 0, 30 and 60 min after HFS, respectively, without any significant difference compared with control group. These results indicate that intra-MS injection of $Aβ_{25-35}$, but not KA, suppressed *in vivo* hippocampal LTP.

To ascertain whether $Aβ_{25-35}$ and KA altered the presynaptic neurotransmitter release, PPF was examined prior to HFS. The PPF always appeared after applying paired pulses, and there was no significant difference among three groups (Fig. 5*D*, $P > 0.05$), suggesting that the presynaptic neurotransmitter release in the hippocampal CA1 region of rats was not affected by $Aβ_{25-35}$ and KA injection.

3 DISCUSSION

3.1 Septo-hippocampal projection is an important target of Aβ in AD

The neurotoxicity of Aβ, including different Aβ fragments such as $Aβ_{1-42}$, $Aβ_{1-40}$, $Aβ_{25-35}$, and even a shorter fragment $Aβ_{31-35}$, has been widely reported. For example, $Aβ_{1-42}$ induced cell death in cultured cortical neurons [27] and in septal cultured neurons in a time- and concentration-dependent manner [28]. Moreover, prolonged infusion of synthetic Aβ fragments, such as $Aβ_{25-35}$, into the brain caused learning and memory deficits in rats, including impairments of working memory, spatial memory and exploratory behavior in different behavioral tests [29, 30].

Because of the bidirectional connection between MS and hippocampus [9], the aggregated Aβ in hippocampus would affect and injure septal neurons. It has been reported that intrahippocampal $Aβ_{1-40}$ injection injured MS neurons most likely by Aβ interaction with septo-hippocampal axon terminals [13]. Vice versa, the aggregated Aβ in MS would also affect the neuronal activi-
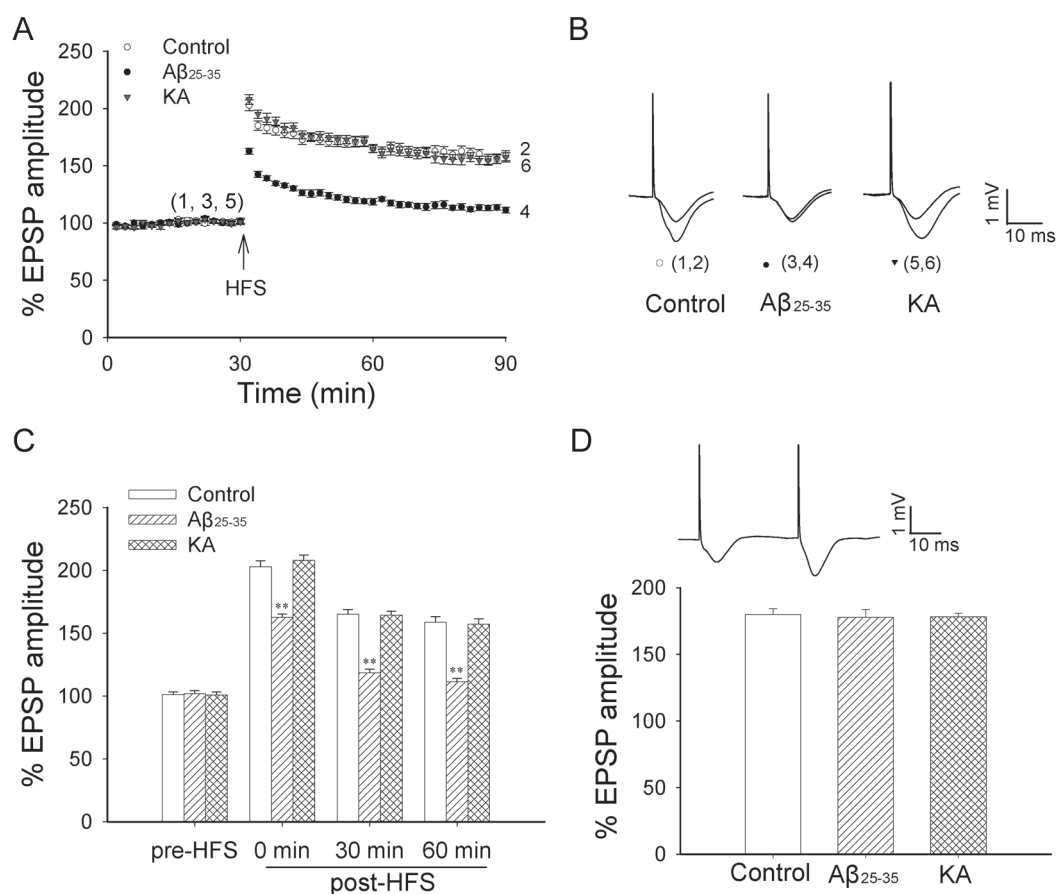
Fig. 5. Intra-MS injection of Aβ$_{25-35}$ suppressed *in vivo* LTP in the hippocampal CA1 region of rats. *A*: Time course of fEPSPs and LTP induced in the hippocampal CA1 region of rats in the control (*n* = 7), Aβ$_{25-35}$ (*n* = 6) and KA (*n* = 6) groups. *B*: Typical fEPSP traces before and after HFS recorded from rats in the three groups. Scale bars, 1 mV and 10 ms. *C*: Histograms showing the suppression of LTP at different time points after HFS (**$P < 0.01$ compared with the control group). *D*: PPF was not affected by Aβ$_{25-35}$ and KA injection. Inset, representative paired fEPSPs.

ties in the hippocampus, and thus impair various cognitive behaviors of rats [22]. In the present study, intra-MS injection of Aβ$_{25-35}$ resulted in remarkable deficits in spatial memory, cognitive flexibility and exploratory behavior. In our previous studies, we also found that intrahippocampal injection of Aβ$_{25-35}$ significantly impaired the spatial memory, hippocampal early-phase LTP (E-LTP) and late-phase (L-LTP) in normal rats [6, 29]. Therefore, we postulate that the Aβ accumulated in the MS, as well as in the hippocampus, could impair the advanced cognition of rats through injuring septo-hippocampal two-direction projections.

Intra-MS Aβ injection-induced cognitive impairments are associated with the suppression of hippocampal synaptic plasticity. Hippocampal LTP, a persistent increase of synaptic efficacy, has been thought as an important electrophysiological cellular model of learning

and memory. The cognitive behavior of animals may be encoded by modification of synaptic strength [31]. It is reported that enhancing or blocking hippocampal LTP is associated with the improvement or deficit of learning ability in animals, respectively [32]. As mentioned above, intrahippocampal injection of Aβ$_{25-35}$ impaired hippocampal E-LTP and L-LTP [6, 29]. In the present study, we examined the effects of intra-MS injection of Aβ$_{25-35}$ on hippocampal LTP in the same rats used in the spatial reference memory tests. We found that Aβ$_{25-35}$ injection into MS not only significantly impaired the spatial learning and memory of rats but also suppressed the hippocampal LTP. This electrophysiological result, in accordance with the results of MWM, suggested that intra-MS injection of Aβ-induced cognitive impairments may be closely associated with the suppression of hippocampal synaptic plasticity. It is well known

that cholinergic neurons in the MS could promote theta rhythms, one of the most thoroughly studied electroencephalogram (EEG) phenomena, and the mnemonic functions of the hippocampus may depend upon theta-related neuronal activity [33]. Interestingly, the septo-hippocampal connections are involved in theta rhythm production, i.e. septal neurons are the pace maker of the hippocampal theta rhythm [34]. Therefore, the Aβ injection into MS might disorder the hippocampal theta rhythm [22], just like what we observed in the previous study [35], and further impair hippocampal synaptic plasticity and animal cognitive behaviors.

3.2 Aβ-induced damage of cholinergic neurons in MS is responsible for the synaptic failure and cognitive impairments

The MS, as the main part of BF, exclusively contains cholinergic and GABAergic neurons [36]. Both cholinergic and GABAergic neurons in the MS were associated with spatial memory. The cholinergic neurons play an important role in the cognitive functions [14], and cholinergic neurotransmission promoted hippocampal synaptic plasticity [37] while cholinergic depletions affected cognitive flexibility [38]. It is reported that the loss of cholinergic neurons in the BF is one of the pathological events in the pathogenesis of AD [8]. The degeneration of cholinergic neurons in the septum was evident in the early stages of AD. Moreover, the neurotoxicity of Aβ on cholinergic neurons was widely reported. For example, selective intraneuronal Aβ accumulation was found in the cholinergic BF neurons in aging and AD patients [39]; $A\beta_{1-40}$ injection into the hippocampus and MS of rats specifically decreased septal cholinergic, but not GABAergic neurons [13, 21, 22, 40]; $A\beta_{1-42}$ could induce cytotoxicity in cultured rat primary BF cholinergic neurons [41]; the spatial learning and memory in APP/PS1 transgenic mice was impaired by decreasing cholinergic neurons in the MS [42]. Thus, the MS cholinergic neurons are more vulnerable to the Aβ toxicity. Accordingly, we postulate that the impairments observed in the present study, including hippocampal LTP suppression, spatial memory and exploratory behavior deficits, were mainly caused by the intra-MS Aβ injection-induced damage of cholinergic neurons. Similar to our results by intra-MS Aβ injection, Nell *et al.* found that i.c.v. injection of $A\beta_{25-35}$ also resulted in age-related reduction of BF cholinergic neurons, along with neuronal loss in the hippocampal CA3 subfield and impairments in long-term reference memory of MWM [43].

GABAergic neurons are the principal inhibitory neurons in the central nervous system, and the alteration of inhibitory neurotransmission might participate in the dysregulation of the balance between excitatory and inhibitory neurotransmission, so the septo-hippocampal GABAergic projections might have a main role in spatial memories as well as in modulating electrical rhythmic activity in the hippocampal formation [44]. Since KA could reduce septo-hippocampal GABAergic neurons and sparing cholinergic neurons [23], we also examined the effects of intra-MS injection of KA on hippocampal LTP and cognitive activities of rats. The results showed that intra-MS injection of KA diminished exploratory behavior, but did not influence the hippocampal LTP and spatial reference memory or cognitive flexibility. This result is consistent with Dashiani's report that KA preferentially reduced GABAergic neurons in the MS, but did not affect spatial short-term memory of rats [45]. However, some researchers showed different results, in which selective lesion of GABAergic neurons in MS impaired spatial learning of rats [46]; extensive damage of GABAergic neurons in MS did not impair spatial reference memory and avoidance learning of rats, but obviously impaired spatial working memory and extinction of the avoidance response [47]. So the effects of MS GABAergic neurons on different types of cognitive behaviors need to be further investigated.

In summary, the present study justified that intra-MS injection of Aβ could impair spatial memory, cognitive flexibility and exploratory motivation, as well as hippocampal LTP in rats, suggesting that the septo-hippocampal projection, especially the cholinergic neurons in MS, could be one of important targets of neurotoxic Aβ in AD, and the specific damage of cholinergic neurons in MS is probably responsible for the impairments of hippocampal synaptic plasticity and cognitive function in AD.

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