Research report

Apamin improves reference memory but not procedural memory in rats by blocking small conductance Ca\(^{2+}\)-activated K\(^{+}\) channels in an olfactory discrimination task

C. Fournier, S. Kourrich, B. Soumireu-Mourat, C. Mourre *

Laboratoire de Neurobiologie des Comportements, UMR 6562, CNRS-Université de Provence, IBHOP, Traverse Susini, 13388 Marseille, Cedex 13, France

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Abstract

Apamin blocks SK channels responsible for long-lasting hyperpolarization following the action potential. Using an olfactory associative task, the effect of an intracerebroventricular 0.3 ng apamin injection was tested on learning and memory. Apamin did not modify the learning of the procedure side of the task or the learning of the odor-reward association. To test reference memory specifically, the rats were trained on a new odor-association problem using the same procedure (acquisition session), and they were tested for retention 24 h later. Apamin injected before or after the acquisition session improved retention of the valence of a new odor pair. Apamin injected before the retention session did not affect the retrieval of the new valence. Thus, the results indicate that the blockage of apamin-sensitive SK channels facilitate consolidation on new-odor-reward association. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Potassium channels; Hippocampus; Afterhyperpolarization; Odor-reward association; Learning; Memory; Behavior; Calcium-activated potassium channel

1. Introduction

Potassium channels play a crucial role in regulating neuronal excitability by controlling factors such as the width of the action potential, the interspike interval, firing patterns, and neurotransmitter release. A wide variety of potassium channels have been described in the central nervous system (CNS), and specific K channel families may be involved in different central functions. Indeed, numerous studies have found differences in the distribution pattern of K channels, and high densities of K channels are often found in limbic structures, suggesting their role in learning and memory. However, there is limited information on the role of different K channel subtypes in cognition.

To date, voltage-dependent K channel blockers have been investigated in cognitive behavioral studies [1,39,47]. Antisense knockdown of Kv1.1 channels was shown to impair memory in a mouse passive avoidance task and a rat spatial memory task and K channel openers produced an amnesic effect in a mouse passive avoidance test [18,30]. Another K channel family, the small conductance Ca\(^{2+}\)-dependent K channels (SK channels), also seems to be involved in mnesic processes. Among the SK channels, some were specifically blocked by apamin, a neurotoxin extracted from bee venom. The blockage of the SK channels by apamin facilitated learning and memory in a test in which mice learned to press a lever in order to obtain a food reward [31]. In this study apamin that was intraperitoneally (ip) injected before training increased the animals' bar-pressing rates. Injected after the acquisition test, apamin improved the animals' performance on the retention session by suppressing the Kamin’s effect...
Apamin also improved learning in an object recognition task in rats. In this study, intraperitoneal (ip) injection of apamin before training was shown to be effective, but injection after training was ineffective [11]. Apamin does not seem to have any effects on spatial navigation, spontaneous alteration, inhibitory avoidance, and passive avoidance become behavior in rodents [10,23]. However, apamin alleviates the spatial navigation deficit and impairment of spatial reference memory in medial septal-lesioned mice, and the working memory deficit in hippocampal-lesioned mice [23,22,52]. These studies suggest that SK channels are involved in memory processes but only when the task does not implicate a spatial strategy or a stressful situation. However, in all these investigations, apamin (that only weakly goes through the blood-brain barrier) [19], was administrated by ip injection. A high density of apamin binding sites was present in adrenal medulla chromaffin cells [26]. SK channels involved in the control of the release rate of epinephrine, well known to modulate learning and memory [6,25,32,38,56,7]. Thus, the apamin effects observed on learning and memory processes could be caused by a central action, but also by an indirect peripheral action.

In order to investigate the central role of SK channels in memory, we studied the effects of intracerebroventricularly (icv) injected apamin on an olfactory associative task, for the following reasons. Experiments with rats suggest that olfactory stimuli provide better access to higher cognitive processes than stimuli in other sensory modalities [16,28]. Rats trained with olfactory stimuli can rapidly acquire learning-set performance comparable to that achieved by primates in response to visual stimuli [50]. Moreover, in the present study, the successive odor-association learning task paradigms used allowed us to distinguish different categories of memory in rats. Several dichotomies have been proposed to differentiate these categories, including procedural learning (concerned with the acquisition and expression of skilled performance) versus declarative learning (associations among items or events that can be accessed flexibly to guide memory expression in various situations, sometimes even new ones). Declarative memory, which may be related to reference memory in rodents, is thought to be more flexible than procedural memory [5,16,51].

We found that apamin improved the consolidation of successive-odor pair learning but did not alter odor-reward association learning and the long-term storage of the association.

2. Materials and methods

2.1. Animals and surgery

Male Sprague–Dawley rats (280–300 g) (Iffa-Credo Company, France) were housed individually and given ad libitum food and water at a constant temperature (22°C) under 12-h light:12-h dark cycle (lights on at 19:00 h). Before the behavioral experiments, a cannula was unilaterally implanted in the lateral ventricle of the rats, anaesthetized with ketamine (8 mg/100 g) (Imalgéne 1000, Rhône-Mérieux) and xylazine (0.8 mg/100 g) (Rompun 2%, Bayer). After 7 days of recovery, each animal was handled (10 min) for 1 day before the experiment. On the next 2 days, the rats were handled and put in the experimental cages for familiarization with the experimental environment. For the olfactory associative task, the rats were deprived of water for 48 h before the first training session. On the following days, the rats were given water ad libitum for 30 min per day at 17:30 h.

2.2. Drugs

Apamin (Sigma) (0.3 or 0.5 ng) was dissolved in physiological saline solution (vehicle). The toxin was administered through an icv injection in a volume of 1.2 μl, and the corresponding vehicle (1.2 μl of physiological saline solution) was used as a control injection.

2.3. Experiments 1–3: olfactory associative task

2.3.1. Apparatus

Discrimination training was conducted in a behavioral apparatus similar in design to those used by [41]. Briefly, the olfactory training apparatus was a rectangular box made of wire mesh (30 × 30 × 50 cm). A conical odor port (1.5 cm in diameter, 0.5 cm above the floor) was drilled horizontally through a triangular wedge of Plexiglas, which was mounted in one corner of the cage. A circular (1 cm diameter) water port in the shape of a well was placed directly above the odor port; water port responses were monitored by a photoelectric circuit. Two flashlight bulbs, which could be turned on and off as conditions required, were placed outside the cage, one on each side of the odor and water ports, 10 cm above the floor.

Individual odors were delivered by forcing clean air (0.7 bars) through one of two Erlenmeyer’s flasks that contained 500 ml of water mixed with one of the chemicals or natural odors (Lavandin de Grignan, France). A ‘positive’ odor (S+) flask was paired with a ‘negative’ odor (S−) flask. Non-odorized air was delivered by passing air through a flask that contained only water. Odorized and clean air streams were passed individually through tubes, which were put through the back of the sound attenuating chamber and attached to the odor port. Water was delivered using a gravity feed system and was passed through a valve which, when opened, allowed 0.1 ml to be released into the water port. All experiments were conducted with an apamin treated animal in one cage and a control animal in the other to allow for training under the same conditions at
the same time. All procedural and behavioral events were controlled and recorded by microcomputers.

2.3.2. Experiment 1: odor-reward training

Animals were trained to make two odor-reward associations. Each of the odors had to be associated with a specific reward; one odor (jasmine) was arbitrarily designated as positive and the other (strawberry) as negative, using a successive ‘go-no go’ paradigm. Rats had to approach the odor and water ports to interrupt the light beam in front of them only when the positive odor was ejected for 10 s. Response to the odor designated as negative resulted in a 10-s presentation of a non-aversive light. The water was only distributed with the response to the positive odor.

Individual trials were presented in a quasi-random fashion during which one of the odors was delivered for at most 10 s. A new trial was started only when the subject left the corner; if not the trial was delayed for 10 s (cumulative time). In no case did a new trial start earlier than 15 s after the end of either water or light delivery or no response. A daily session was made up of 60 trials with an inter-trial interval of 15 s. Animals were tested on 5 consecutive days between 08:00 h and 15:00 h.

Correct responses were ‘go’ for the positive odor and ‘no-go’ for the negative odor. Incorrect responses were ‘go’ for the negative odor and ‘no-go’ for the positive odor. The number of correct responses on both positive and negative odors was expressed as a percentage of the total number of odor presentations. Latencies for positive (S+) and negative (S−) odors were recorded, representing the time elapsed between the beginning of a trial and its end when the rat responded to the odor; if a rat did not respond, a latency of 10 s was scored. The percentage of correct responses and the latency were used to assess discriminative performance. In addition, as the subject could delay the trials by responding during the inter-trial interval, the cumulative time was considered. The cumulative time was the number of seconds that exceeded the fixed 15 s inter-trial interval divided by the number of inter-trial intervals in the experiment. The effect of apamin on procedural learning was evaluated by the cumulative time, and acquisition of the odor-reward association was evaluated by the percentage of correct responses and the latency on S+ and S− stimuli [42,43].

In the first part of experiment 1a, the injection was administered 30 min before the beginning of the second session, where rats generally make only few correct odor-reward associations. The effect of apamin was evaluated on this session because apamin could accelerate neuronal mechanisms implicated in memory processes [2]. In the second part, rats were given an icv injection 30 min before the beginning of the third training session. The third session was chosen because rats generally start to make correct olfactory cue-reward associations during the third session [42].

In experiment 1b, the effect of apamin on information consolidation in this task was investigated. We administered the injections immediately at the end of the second session, specifically to observe performance during the third session.

2.3.3. Experiment 2: successive odor-pair olfactory training

This paradigm was composed of two phases and was similar to paradigm used by [41]. The first phase was exactly the same as that used in experiment 1. At the end of this phase, an 80% of correct responses had generally been reached on the last session. The next day, a sixth session of 60 trials was used to stabilize performance. The rats that did not reach at least the 80% of correct responses were eliminated. In the second phase, the animals were submitted to a new association problem (i.e. a new odor pair) over 3 days. During this phase, the animals were trained on a new odor pair (lily of the valley and passion fruit) but with only 12 trials (acquisition session). Then they were tested for retention in 12 trials, 24 h later. On the second day, after the retention session, a second new odor pair (peach and violet) was used for acquiring a new association. On the third day, the retention session with the second new odor pair was performed.

Different injection times were used to analyze the effects of apamin on different phases of memory (experiments 2a, b and c). In experiment 2a, apamin or vehicle was injected 30 min before the first acquisition session. The percentages of correct responses and latencies were used to evaluate the potential effects of apamin on the acquisition and consolidation of the odor reward association. In experiment 2b, apamin or vehicle was injected immediately after the first acquisition session. The results of the retention session were used to estimate apamin effects on consolidation processes only. In experiment 2c, apamin or vehicle was injected 30 min before the retention session so as to evaluate the effects of apamin on information retrieval. In all three experiments, the second association and retention sessions were conducted to determine whether the potential effects of apamin were temporary or permanent.

2.3.4. Experiment 3: long-term retrieval training

This paradigm, used to test the effects of apamin on long-term retrieval of information, consisted of two phases. The first phase was an odor-reward training identical to that used in experiment 1 but without injection. At the end of the fifth session, rats that had not reached a 80% of correct responses were eliminated. The remaining rats were deprived for 48 h before the second phase held 4 weeks after the odor-reward train-
ing. During the second phase, all animals underwent a reversal test and apamin or vehicle was injected 30 min before the test. In the reversal test, the valence of the earlier learned odors was reversed. Thus, the odor that had served earlier as S+ stimulus was now an S− stimulus, and vice versa. As in the earlier training sessions, the reversal test consisted of 60 trials. To evaluate the effects of apamin on the retrieval of the earlier learned odors and not on the relearning of the new odor valence, we mainly examined performance on the first block of ten trials [43].

2.3.5. Experiment 4: locomotor and drinking activities

Since the odor-association learning task involved locomotor and drinking activities, it was necessary to test the effect of apamin on these behaviors because this toxin at high doses causes convulsions and affects movements [19,34,35]. We used an automatic system designed and built by A. Bouquerel in our laboratory. The system is placed inside the animal house and is composed of 12 wire netting cages (40 × 22 × 16 cm). Each cage was equipped with a trough, a bottle, and diodes (7 lengthways, 5 widthways) around the cage that were connected to a computer and recorded each move of a rat. The water bottle was daily weighed at 11:00 h to determine drinking activity. The parameters recorded were the moving and the volume of the water drunk frequency. The moving frequency was the number of rat moves detected every 5 s during the diurnal period. Each movement (walking, running, and jumping) was recorded as an interruption of one of the cell beams. Following canula implantation, rats recovered for 2 weeks. Two concentrations of apamin were used to test the effects of this toxin on locomotor and drinking behavior. In the first and second experiments, rats were icv-injected with 0.5 or 0.3 ng of apamin during the first hour of the diurnal period, respectively. Control groups were composed of rats injected with vehicle. In the third experiment, rats were deprived of water for 48 h, as in the olfactory associative task. On the following days, the rats were given water ad libitum for 30 min per day at 17:30 h. Four days after the beginning of water deprivation, apamin (0.3 ng) or vehicle was injected during the first hour of the diurnal period. In these experiments, the locomotor and drinking behaviors were recorded 2 days before injection (day 0) and 3 days after. Moreover, water consumption frequency (the number of drinks from the bottle) was recorded for 2 h following injection in the second and third experiments.

2.4. Statistical analysis

Statistical analysis was performed with the SPSS/PC statistics 4.0 software marketed by SPSS Inc. The data were analyzed in MANOVAs with repeated measure-

ments. Selected analyzes of variance (ANOVA) were then conducted.

3. Results

3.1. Experiment 1: effect of apamin on odor-reward learning

3.1.1. Experiment 1a: effect of apamin on acquisition of the odor-reward association

Apamin and control groups improved across sessions, reaching a percentage of correct responses of 80% or better on session 5. Before the injection, the comparison of the percentages of correct responses and latencies of the control and apamin groups indicated no difference across sessions. Thus, before the injection of apamin or vehicle, the groups exhibited similar learning performance. Two injection times were chosen to evaluate the effect of apamin on acquisition of the odor-reward association. In the first experiment, apamin was injected before session 2 when rats were not yet able to discriminate between the two odors, and in the second experiment, apamin was injected before session 3 when rats had begun to discriminate between the odors.

Table 1 shows that the apamin and control groups learned the odor-reward association in a very similar way. Regardless of the injection time, MANOVAs on the percentage of correct responses for S+ and S− stimuli revealed no difference between control and apamin groups and no group × session interaction across the sessions following the injection ($F_{1,16}$ ≤ 0.80, NS; $F_{2,32}$ ≤ 1.55, NS, respectively). For every group, a significant latency difference between S+ and S− stimuli was observed starting on session 3, as revealed by separate ANOVAs ($F_{1,17}$ ≥ 16.10, $P < 0.001$). The significant discrimination was due to a session-by-session decrease in the time taken to respond to the positive stimulus, and a gradual increase in response time to the negative stimulus (Fig. 1A and C). The S+ stimulus latencies, as well as the S− latencies, were similar for the control and apamin groups on the sessions after the injection (separate ANOVAs, $F_{1,17}$ ≤ 1.54, NS). The cumulative time decreased regularly from session to session for all groups, indicating that rats learned the procedure across sessions (Fig. 1B and D). There was no difference on this parameter between control and apamin groups, and no group × session interaction was observed at either injection time (MANOVAs, $F_{1,16}$ ≤ 0.45, NS; $F_{2,32}$ ≤ 0.42, NS, respectively).

3.1.2. Experiment 1b: effect of apamin on long-term storage of information during odor-reward training

As in experiment 1a, apamin and control groups improved across sessions, reaching a percentage of cor-
rect responses of at least 80% by session 5. The performance of the two groups on the first two learning sessions was the same, indicating that control and apamin groups had similar learning patterns. Apamin or vehicle was icv-injected immediately after session 2. A MANOVA on the percentage of correct responses for S+ and S− stimuli indicated no group difference and no group × session interaction across the last three sessions ($F_{1,16} \leq 0.02$, NS; $F_{2,32} \leq 2.35$, NS, respectively) (Table 1). On session 3, no difference in the percentage of correct responses was observed between the groups (ANOVA: $F_{1,17} \leq 0.33$, NS, Table 1). In the control and apamin groups, the time taken to respond to the

Table 1

| Experiment 1: odor-reward training mean correct response rate (± S.E.M.) |
|---------------------------------|-----------------|
| S+ stimulus                     | S− stimulus     |
| Control group                   | Apamin group    |
| Control group                   | Apamin group    |

**Experiment 1a:** effect of apamin on acquisition: first investigation

<table>
<thead>
<tr>
<th>Session 1</th>
<th>74.11 ± 4.92</th>
<th>93.11 ± 5.14</th>
<th>26.78 ± 4.56</th>
<th>29.11 ± 6.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 2</td>
<td>91.11 ± 2.00</td>
<td>95.89 ± 1.41</td>
<td>15.78 ± 4.98</td>
<td>15.00 ± 5.61</td>
</tr>
<tr>
<td>Session 3</td>
<td>94.33 ± 1.33</td>
<td>89.22 ± 3.83</td>
<td>54.00 ± 9.73</td>
<td>59.11 ± 6.71</td>
</tr>
<tr>
<td>Session 4</td>
<td>91.44 ± 3.20</td>
<td>96.89 ± 1.09</td>
<td>67.00 ± 8.97</td>
<td>74.44 ± 7.14</td>
</tr>
<tr>
<td>Session 5</td>
<td>96.11 ± 1.53</td>
<td>97.56 ± 1.18</td>
<td>70.44 ± 7.31</td>
<td>76.22 ± 5.89</td>
</tr>
</tbody>
</table>

**Experiment 1b:** effect of apamin on consolidation of information

<table>
<thead>
<tr>
<th>Session 1</th>
<th>66.22 ± 7.19</th>
<th>66.33 ± 7.89</th>
<th>31.44 ± 4.14</th>
<th>29.89 ± 6.86</th>
</tr>
</thead>
<tbody>
<tr>
<td>Session 2</td>
<td>89.00 ± 4.40</td>
<td>91.89 ± 2.09</td>
<td>18.78 ± 4.97</td>
<td>13.78 ± 2.98</td>
</tr>
<tr>
<td>Session 3</td>
<td>95.78 ± 1.79</td>
<td>94.78 ± 1.63</td>
<td>42.44 ± 9.17</td>
<td>41.78 ± 7.28</td>
</tr>
<tr>
<td>Session 4</td>
<td>97.56 ± 1.07</td>
<td>94.00 ± 1.68</td>
<td>67.11 ± 8.00</td>
<td>68.89 ± 9.68</td>
</tr>
<tr>
<td>Session 5</td>
<td>96.67 ± 1.05</td>
<td>97.22 ± 1.14</td>
<td>72.33 ± 5.98</td>
<td>70.11 ± 7.65</td>
</tr>
</tbody>
</table>

S+ stimulus decreased across sessions after the injection, whereas the response time for the S− stimulus increased gradually (Fig. 1E). From session 3 on both groups differentiated the odors, because a significant difference between the S+ and S− stimulus latencies was observed (ANOVA, $F_{1,17} \geq 14.10$, $P < 0.02$). There was no group difference in the S+ latency, and no group × session interaction across sessions after injection (MANOVA: $F_{1,16} = 0.40$, NS; $F_{2,32} = 0.13$, NS). On session 3, which followed the injection, the S+ latency was very similar for the two groups (Fig. 1E). From session 3 on, MANOVAs also indicated no group variation and no group × session interaction in the S− latency ($F_{1,16} = 0.18$, NS; $F_{2,32} = 1.99$, NS). On session 3, the animals in the two groups responded similarly to the S− stimulus (ANOVA: $F_{1,17} = 0.24$, NS). In the same way, the cumulative time was similar for the two groups (on session 3, ANOVA: $F_{1,17} = 1.40$, NS) (Fig. 1F).

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*a* Apamin (0.3 ng) or vehicle icv-inj before session 2.

*b* Apamin (0.3 ng) or vehicle icv-inj before session 3.

*c* Apamin (0.3 ng) or vehicle icv-inj after session 2.

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3.2. Experiment 2: effect of apamin on successive odor-pair learning

When on the first phase, the animals had learned the odor-reward association, on the second phase, they had to learn to discriminate a new odor pair in a few trials (acquisition session) and were then tested for retention (retention session) 24 h later. This paradigm was repeated once. Injections were performed at different times during the second phase to study the effect of apamin on different mnestic phases of the successive odor-pair learning. In all experiments, the overall percentage of correct responses showed that animals improved across the six sessions, and reached a percentage of correct responses of at least 80% on session 6 during the odor-reward training (Table 2, Fig. 2). Then they underwent the second phase of the new-successive odor-pair olfactory training.

Table 2
Experiment 2: new earlier olfactory learning mean correct response rate (± S.E.M.)

<table>
<thead>
<tr>
<th></th>
<th>S+ stimulus</th>
<th>S− stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control group</td>
<td>Apamin group</td>
</tr>
<tr>
<td><strong>Experiment 2a: effect of apamin on acquisition</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 6</td>
<td>97.10 ± 1.40</td>
<td>97.60 ± 1.50</td>
</tr>
<tr>
<td>Acquisition</td>
<td>88.89 ± 5.56</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>Retention</td>
<td>87.04 ± 5.40</td>
<td>94.44 ± 3.93</td>
</tr>
<tr>
<td><strong>Experiment 2b: effect of apamin on consolidation of information</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 6</td>
<td>94.40 ± 2.40</td>
<td>96.30 ± 0.70</td>
</tr>
<tr>
<td>Acquisition</td>
<td>85.42 ± 4.92</td>
<td>94.44 ± 2.77</td>
</tr>
<tr>
<td>Retention</td>
<td>95.83 ± 2.70</td>
<td>84.14 ± 5.39</td>
</tr>
<tr>
<td><strong>Experiment 2c: effect of apamin on information retrieval</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session 6</td>
<td>99.30 ± 0.40</td>
<td>97.60 ± 1.11</td>
</tr>
<tr>
<td>Acquisition</td>
<td>92.59 ± 4.04</td>
<td>92.59 ± 4.05</td>
</tr>
<tr>
<td>Retention</td>
<td>94.44 ± 2.78</td>
<td>92.59 ± 4.04</td>
</tr>
</tbody>
</table>

*a Session 6: sixth session of odor-reward training.
*b Apamin (0.3 ng) or vehicle icv-injected: before acquisition.
*c Apamin (0.3 ng) or vehicle icv-injected: after acquisition.
*d Apamin (0.3 ng) or vehicle icv-injected: before retention.
*P < 0.05, following ANOVAs comparing rats treated with vehicle or apamin.
3.2.1. Experiment 2a: effect of apamin on the acquisition of information

Apamin (0.3 ng) or vehicle was injected 30 min before the acquisition session. ANOVAs indicated no difference in the percentage of correct responses for the S+ and S− stimuli between the two groups on the acquisition session \( (F_{1,17} \leq 4.00, \text{NS}) \) (Table 2). Another ANOVAs revealed that the two groups had similar latencies for the S+ stimulus \( (F_{1,17} = 1.11, \text{NS}) \) and for the S− stimulus \( (F_{1,17} = 0.46, \text{NS}) \) (Fig. 2A). Neither group discriminated the odors because no statistically significant difference was found between the S+ and S− stimulus latencies (ANOVA: \( F_{1,17} \leq 4.45, \text{NS} \)).

On the retention session, the percentage of S− correct responses was higher for the apamin group than for the control group, contrary to the percentage of S+ correct responses (ANOVA: \( F_{1,17} = 4.97, P < 0.05 \)) (Table 2). ANOVAs revealed a significant difference between the S+ and S− stimulus latencies for the two groups, indicating that the groups discriminated between the odors \( (F_{1,17} = 11.67, P < 0.005) \). A difference in the S− latency was found between the control and apamin groups (ANOVA: \( F_{1,17} = 4.66, P < 0.05 \)) (Fig. 2A). Thus, in comparison to the controls, apamin rats inhibited a response to an S− stimulus for a longer time. A MANOVA indicated no group difference but revealed a group × session interaction across the acquisition and retention sessions on the percentage of S− correct responses and on the time taken to respond to the S− stimulus \( (F_{1,16} \leq 2.94, \text{NS}; F_{1,16} \geq 5.32, P < 0.05) \) (Table 2 and Fig. 2A). In contrast, a similar analysis, no difference was detected on the percentage of correct responses or latency for the S+ stimulus.

3.2.2. Experiment 2b: effect of apamin on long-term storage of information

In this successive odor-pair learning task, apamin or vehicle was injected immediately after the acquisition session. On the acquisition session, no correct response difference was observed between control and apamin rats (ANOVA: \( F_{1,17} \leq 0.45, \text{NS} \)) (Table 2). The two groups took a similar amount of time to respond to the S+ and S− stimuli (ANOVA: \( F_{1,17} \leq 3.23, \text{NS} \)) (Fig. 2B). A significant latency difference between S+ and S− stimuli was observed for the two groups (ANOVA: \( F_{1,17} \geq 5.33, P < 0.05 \)). Thus, before injection, all rats exhibited similar learning performance. On the retention session, the percentage of S− correct responses revealed a significant increase for the apamin group compared with the control group (ANOVA: \( F_{1,17} = 7.85, P < 0.02 \)) (Table 2). Concerning the S+ stimulus, no difference was detected between the two groups. An ANOVA indicated a significant difference between the groups in their S− latencies \( (F_{1,17} = 4.67, P < 0.05) \). Thus, in comparison to controls, apamin animals were more effective at inhibiting a prepotent response for the S− stimulus (Fig. 2B).

3.2.3. Experiment 2c: effect of apamin on information retrieval

In this paradigm, apamin or vehicle was injected 30 min before the retention session. On the acquisition session, ANOVAs indicated no difference in the percentage of S+ and S− correct responses or latencies \( (F_{1,17} \leq 0.20, \text{NS}) \) (Table 2 and Fig. 2C). Thus, the control and apamin groups were at the same level of learning before the retention session. On the retention session, the comparison of the percentages of correct responses indicated no difference between the control and apamin groups (ANOVA: \( F_{1,17} \leq 0.20, \text{NS} \)) (Table 2). Fig. 2C shows also no difference in the S+ and S− latencies for both groups.

In all successive odor-pair training experiments, after the retention session, the rats were given a second new odor pair in an acquisition session followed by a retention session 24 h later. This was aimed at determining whether the apamin effects were transient or permanent. When apamin was injected before the first acquisition of a new odor pair (experiment 2a), on the second acquisition session, the S− stimulus latency was lower for the apamin group \( (4.1 \pm 0.6 \text{ s}) \) in comparison to controls \( (5.8 \pm 0.5 \text{ s}) \) (ANOVA: \( F_{1,17} = 5.23, P < 0.05 \)). The other recorded parameters did not exhibit a group difference \( (F_{1,17} \leq 3.37, \text{NS}) \). On the retention session 24 h later, separate ANOVAs indicated no difference between groups for the two parameters considered, percentages of correct responses and S+ or S− stimulus latency \( (F_{1,17} \leq 3.26, \text{NS}) \). In experiments 2b and 2c, on the acquisition and retention sessions, no difference between the control group and the apamin group was found for any of the three parameters (data not shown).

3.3. Experiment 3: effect of apamin on long-term retrieval of the odor-reward association

Apamin or vehicle was injected 30 min before the reversal session held 30 days after odor-reward training. All animals improved similarly across the sessions of the training, as indicated by their percentages of correct responses and latencies on session 5 (Table 3 and Figs. 3 and 4). On the reversal session, no difference was found when the percentages of correct responses were compared between the control and apamin groups (ANOVA: \( F_{1,17} \leq 1.24, \text{NS} \)) (Table 3). A MANOVA across the six blocks of 10 trials on this parameter for the S+ and S− stimuli indicated no group difference and no group × block interaction \( (F_{1,16} \leq 0.89, \text{NS}; F_{3,80} \leq 0.64, \text{NS}) \), respectively. In the same way, no group × block interaction across the six blocks was found on the S+ latency \( (F_{3,80} \leq 0.76, \text{NS}) \). Separate
Table 3: Effect of apamin on long-term retrieval of odor-reward training mean correct response rate (± S.E.M.)

<table>
<thead>
<tr>
<th>S+ stimulus</th>
<th>Control group</th>
<th>Apamin group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 5</td>
<td>Global</td>
</tr>
<tr>
<td>Trials 10</td>
<td>61.11 ± 11.45</td>
<td>44.44 ± 11.78</td>
</tr>
<tr>
<td>20</td>
<td>69.4 ± 14.29</td>
<td>50.00 ± 16.66</td>
</tr>
<tr>
<td>30</td>
<td>88.88 ± 5.80</td>
<td>84.44 ± 11.43</td>
</tr>
<tr>
<td>40</td>
<td>100.00 ± 0.00</td>
<td>95.23 ± 4.76</td>
</tr>
<tr>
<td>50</td>
<td>100.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>60</td>
<td>100.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S− stimulus</th>
<th>Control group</th>
<th>Apamin group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 5</td>
<td>Global</td>
</tr>
</tbody>
</table>

Note: *Session 5: fifth session of odour-reward training. Apamin (0.3 ng) or vehicle injected before reversal test.

ANOVA indicated no significant difference in the two parameters considered, percentage of correct responses and S+ and S− stimulus latency during the first block of ten trials ($F_{1,17} = 2.78$, NS) (Table 3). The apamin group had a similar retrieval percentage on the earlier odors as the control group. Nevertheless, during the following blocks, apamin-treated rats, like control animals, exhibited a generalization phenomenon by responding on both odors.

3.4. Experiment 4: Effect of apamin on locomotor and drinking activity

Since apamin is a convulsing toxin, it was essential to first determine what dose of apamin does not alter the locomotor and drinking behaviors involved in the olfactory associative task. So, before using the olfactory associative task, we investigated the effect of apamin on locomotor and drinking activity in rats. Apamin or vehicle was injected during the first hour of the diurnal period. A dose of 0.5 ng of apamin slightly decreased the moving frequency of rats, but the difference between apamin and control groups was not significant (ANOVA, $F_{1,19} = 3.92$, NS) (Fig. 4A). In contrast, Fig. 4B shows that 0.5 ng of apamin significantly decreased water intake for 2 days following the injection. A MANOVA across days indicated a group × day interaction ($F_{3,54} = 3.95$, $P < 0.05$), and a separate ANOVA revealed a significant difference between groups on the injection day ($F_{1,19} = 7.21$, $P < 0.02$). Neither the locomotor behavior nor the drinking activity were altered following an 0.3 ng apamin injection (MANOVAs across days, $F_{1,54} = 0.36$, NS) (Fig. 4C and D). In the third experiment, we tested the effect of 0.3 ng apamin in rats deprived of water as in the memory experiments. Fig. 4E shows the similar moving frequency of rats during the diurnal period, before the injection, and 3 days after the injection. No difference in the moving frequency was found for 2 h after the injection (Fig. 4G). Thus, apamin did not disturb locomotor activity during the olfactory associative task. Water intake was very similar for the both groups after the injection of apamin or vehicle (Fig. 4F). Moreover, on the first day following 0.3 ng apamin injection, the water consumption frequency for rats not deprived of water was similar for the apamin group (39.20 ± 10.61) and the control group (21.20 ± 7.92) (ANOVA: $F_{1,19} = 1.85$, NS). Fig. 4H shows a similar water consumption frequency for at least 2 h following injection, in the two groups. There was no group difference and no group × block interaction was found across blocks of 10 min for 2 h after the injection (MANOVAs: $F_{1,18} = 0.85$, NS; $F_{12,216} = 1.56$, NS).

These results demonstrate that a dose of 0.3 ng of apamin does not affect locomotor or drinking activity.

4. Discussion

The central blockage of SK channels by icv-injected apamin (0.3 ng) improved learning and memory processes. More precisely, the present results showed that in an olfactory associative learning task, apamin improved the consolidation of the valence of a new odor pair in a successive odor-pair training task without affecting the odor-reward learning or the long-term
Fig. 4. Effect of apamin on locomotor and drinking activity. A–B, moving frequency and water intake (in ml) of apamin rats (n = 10) and control rats (n = 10). The dose of apamin was 0.5 ng. C–D, same conditions for a 0.3 ng apamin dose; E–F, moving frequency and water intake (in ml) of rats deprived of water, as in the olfactory associative learning task. The apamin dose was 0.3 ng. G, moving frequency for 120 min after the injection of 0.3 ng apamin (n = 6) or vehicle (n = 7) in rats deprived of water. H, frequency of water consumption for 120 min after the injection of 0.3 ng apamin (n = 10) or vehicle (n = 10) in rats not deprived of water. Injection was administered during the first hour of the diurnal period. The data were recorded during the diurnal period for 2 days before the injection and 3 days after the injection (A–F). The data on day 0 are the mean values recorded on 2 days before the injection.

retrieval of information. Apamin acted directly on memory mechanisms, because at the dose of 0.3 ng, it did not alter the locomotor or drinking activities involved in our mnesic paradigms.

In our experiments, the behavioral facilitation can be explained either by olfactory information efficacy or by memory improvement, or both. Due to the high density of apamin binding sites in the olfactory tubercules
facilitate the discrimination by an increase in olfactory system efficiency cannot be ruled out but seems nevertheless unlikely. Indeed, apamin did not change performance on the odor-reward learning. Moreover, in the successive odor-pair training the improvement in performance showed up in the increase in the percentage of correct responses and latency on the S— stimulus only and never on the S+ stimulus. It is unlikely that an alteration in the motivational state is involved in the improvement of memory because there was no difference in the consumption of water 2 h after the apamin injection compared with controls, and because the apamin injection did not alter the S+ stimulus rewarded with water, whatever paradigm was used. These results taken together suggest that apamin-sensitive SK channels are involved in memory processes.

Apamin improved the consolidation of information in a successive odor-pair training task. This paradigm was used to assess the learning of a new-odor valence and to dissociate information acquisition from its consolidation and retrieval in reference memory. In this experiment, performed once the animals had learned the odor-reward association and the corresponding procedure, the rats had to solve a new odor pair problem using the same rule in a few trials. This behavioral, non-spatial context can be likened to reference learning [51]. When apamin was injected before the acquisition session, the blockage of SK channels by apamin did not improve acquisition in comparison to the control group. In the same way, when the toxin was injected before the retention session, apamin did not affect the retrieval of the meaning of a new-odor pair, learned 24 h earlier. Conversely, on the retention session, the consolidation of the new valence was facilitated because the percentage of correct responses and the latency for the S— stimulus were significantly higher when apamin was injected before or after the acquisition session.

The odor-reward training task combined two aspects of memory — the association of an odor with its reinforcement, and a procedural attitude (go or don’t go to the water port). This paradigm can be considered to form a predictive relationship between each stimulus and its corresponding response. The procedural attitude was evaluated by analyzing the cumulative time. This parameter was found to decrease and become very low when rats began to acquire the procedure (our data; [42]). Regardless of the icv-injection time, neither the odor-reward association nor the procedural attitude was altered by apamin because the values of the parameters studied were statistically similar in the apamin and control groups. Moreover, apamin did not affect performance on the long-term retrieval of an odor-reward association during a reversal test. This test provides a good index of the strength of the memory trace, because the animals had trouble switching their responses to adjust to the new valence of earlier learned odors. So in our experimental conditions, the blockage of apamin-sensitive SK channels was ineffective in the procedure-learning situation and on learning and retrieval of an odor-reward association.

However, earlier results showed that apamin improved performance in an appetitively-motivated bar-pressing test in which a procedural attitude, as well as a bar pressing-reward association were required for mice [31]. In this study, pre-training injection of apamin (1 mg/kg) accelerated acquisition of a bar-pressing response, but also increased the animals’ bar-pressing rates, which suggests a generalized enhancement of behavioral activity. However, injection of apamin immediately after training where animals were tested under a 20 min continuous reinforcement schedule facilitated memory in a retention test 24 h after the initial acquisition. This apparent facilitation of consolidation was not observed when apamin was injected 2 h after the initial training. Thus, the results of this study and of our experiments seem to be contradictory. However, a major difference between the two studies is the injection route. In our paradigms, apamin was injected by the icv route, so apamin only acted in the central nervous system, whereas [31] used the ip route to administer apamin, so it could act in both the peripheral and central systems. The blockage of apamin-sensitive SK channels, present in high density in adrenal medulla chromaffin cells, may have induced peripheral effects that indirectly acted upon memory mechanisms. A similar action on memory storage was demonstrated with amphetamine. The ip administration of amphetamine was shown to enhance retention of an inhibitory avoidance response, whereas the icv injection did not [29]. Several studies have demonstrated that apamin-sensitive SK channels are involved in the control of the epinephrine release rate under physiological conditions [32,38,7]. And there is extensive evidence that epinephrine modulates learning and memory in different ways [56]; for a review, see [6,25]. Thus, the apamin facilitation of memory processes on a bar-pressing task for mice could be the result of peripheral effects of ip-injected apamin (0.2 mg/kg), whereas in our odor-reward training, free of any the peripheral effects of apamin, the blockage of SK channels did not alter the learning of the odor-reward association and the procedural side of the task.

The SK channels are responsible for the slow component of the after hyperpolarization phases (AHP) that follows the action potential [20,21]. So the blockage of a part of these SK channels by apamin induces a decrease in the slow AHP component, and then leads to an increase in neuronal excitability and changes in the firing of several types of neurons [53,15,48,49,4]. Thus, apamin could alter the cellular mechanisms of synaptic plasticity involved in memory processes. Several studies have shown in rabbits that the marked reduction in the
AHP response of hippocampal pyramidal cells is related to hippocampus-dependent classical conditioning. Indeed in CA1 pyramidal cells from rabbits well-trained with the nictitating membrane conditioning procedure, the duration and amplitude of AHP were much smaller than in pseudoconditioned or naive control animals [12,13,27,9,46]. This conditioning-specific decrease in AHP amplitude was also observed in CA3 cells but not in dentate gyrus cells, and concerned with the slow and medium component of AHP [14,55]. The AHP changes were time-dependent because the AHP was reduced the most 1–24 h after learning and then went back up to its basal level within 7 days, whereas behavioral performance remained asymptotic for months. The AHP reductions observed were highly specific to acquisition or to consolidation of the conditioned response and not to its long-term retrieval [37,55]. It was thus not surprising that apamin did not affect the retrieval of information in our olfactory association task. In an olfactory association task similar to our paradigms, extensive training induced a transient reduction in AHPs by 43% in pyramidal cells of the pyriform cortex (the primitive olfactory cortex) compared with rat pseudotraining. This extensive training consisted of learning rats to discriminate between 35 and 50 pairs of odors and could involve reference memory [44]. In another paradigm, rats were trained only until they demonstrated ‘rule learning’ that induced the odor-reward association and procedural attitude. In this experiment only a slight decrease in AHP (20%) was observed [44]. Therefore, the improvement of information consolidation induced by the apamin blockade of SK channels could be explained by the pharmacological enhancement of a physiological decrease in AHP related to learning of a new odor pair. However, the learning paradigm itself might have already modified the AHP of certain cells so that apamin might facilitate the successive odor-pair learning task only if new cell groups are involved. Conversely during the ‘rule learning’ related to a weak physiological decrease in AHP, apamin at the dose of 0.3 ng could not sufficiently reinforce the reduction of AHP to induce memory facilitation in our odor-reward training.

Recent results demonstrated that the decrease in AHP facilitated the induction of a long-term potentiation (LTP) in CA3-CA1 hippocampal cells [8,45]. Moreover, LTP induced by a single 100 Hz tetanization was enhanced by apamin at nanomolar concentrations in the CA1 field of hippocampal slices. The positive modulation of LTP induced by the apamin could be the result of an AHP decrease in hippocampal pyramidal cells but also in excitatory interneurons regulating the strength of pyramidal cell excitation during orthodromic stimulation [3]. Both cell populations contain a high density of apamin-sensitive SK channels [33,24,54]. Moreover, in an olfactory association task similar to ours, [40,42] recorded an LTP that remained unchanged for 24 h in pyriform cortex and hippocampus, and was correlated to behavioral responses in an associative context. We can speculate that the blockage of SK channels by apamin could improve the synaptic potentiation by enhancing of the reduction in AHP related to learning and memory. Another argument for this hypothesis is that a marked impairment was observed aged rats in a successive odor-pair olfactory task [43]. Compared with young adult, aged rats exhibited an AHP increase in hippocampal cells that could impair a cognitive process such as LTP [36,45].

In conclusion, all of these findings suggest that apamin reinforces the physiological reduction in AHP mediated by the activity of SK channels in a learning and memory context. The SK channel blockers could be a promising pharmacological route to understanding cognitive deficits.

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