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Short communication

Effects of apamin on memory processing of hippocampal-lesioned mice

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Abstract

We investigated the effects of acute i.p. injections of the Ca^{2+}-dependent K^+ channel blocker, apamin, on water maze spatial navigation and radial arm maze performance in mice with partial hippocampal-lesions. In the radial arm maze, apamin 0.06 and 0.2 mg/kg dose-dependently reversed the lesion-induced defect. In the water maze, apamin 0.2 mg/kg alleviated the defect, but a lower dose 0.06 mg/kg was ineffective. At a higher dose, 0.4 mg/kg, apamin impaired the water maze performance. These results suggest that Ca^{2+}-dependent K^+ channel blockers can alleviate the spatial reference memory and working memory impairment induced by partial hippocampal lesions.

Keywords: Hippocampus; Apamin; K^+ channel, Ca^{2+}-dependent; Spatial navigation; Learning; Memory; (Mouse)

1. Introduction

Apamin is a peptide extracted from bee venom that specifically blocks slow conductance Ca^{2+}-mediated K^+ channels. These channels are involved in the generation of the slow afterhyperpolarisation that occurs subsequent to the action potential in several cell types (Kawai and Watanabe, 1986). Blocking of these apamin-sensitive channels leads to increased activity of the neuron caused by an inability to desensitise after a period of intense activity. Apamin binding sites are present in several learning- and memory-related brain areas, such as the septum, the hippocampal formation, cingulate cortex and anteroventral thalamic nuclei (Mourre et al., 1986; Gehlert and Gackenheimer, 1993).

Recently, several studies have reported that apamin is effective in improving the memory performance of mice and rats. First, apamin was shown to facilitate memory processes in an appetitively-motivated bar-pressing response in mice (Messier et al., 1991). The dose 0.2 mg/kg i.p. before or after the training accelerated the acquisition of the bar-pressing response. Second, in another study (Deschaux et al., 1997), apamin improved learning in an object recognition task in rats. Apamin 0.4 mg/kg i.p. before the training was shown to be effective. Third, apamin increases the expression of immediate early genes c-*fos* and c-*jun* in the hippocampus (Heurteaux et al., 1993). These genes are thought to be involved in the initial activation of neurons during the memory process. Fourth, we observed that apamin reverses the spatial navigation defect induced by a medial septal lesion (Ikonen et al., 1998). A dose of 0.06 mg/kg i.p. before the training was effective. Finally, apamin was shown to facilitate the induction of long-term potentiation in the CA1 region of rat hippocampus in vitro (Behnisch and Reymann, 1998). However, apamin does not seem to have any effects on passive avoidance behavior (Deschaux and Bizot, 1997; Ikonen et al., 1998), delayed matching to position task (Poorheidari et al., 1998) or water maze performance (Ikonen et al., 1998) of intact mice or rats. Since apamin is effective in some of these examples, but fails to have an effect in others, it appears that apamin-sensitive channels affect only certain circuitries involved in memory processing.

In the light of these previous studies, it is unclear whether the effect of apamin is dependent on the presence of an intact hippocampus or involves some other memory-related areas. Therefore, we examined, whether apamin can alleviate the memory failure induced by partial hip-

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pocampal lesions. Hippocampal function can be assessed with tests that measure spatial reference memory, such as Morris water maze and radial arm maze. One advantage of using the radial arm maze is that it is possible to test both spatial reference memory and working memory at the same time. In mice, spatial reference memory measured in water maze is disrupted by electrolytical lesioning of the dorsal hippocampus (Ikonen and Riekkinen, 1999). This indicates that in mice, spatial navigation is dependent on the hippocampus. The purpose of this study was to characterise in detail the effect of apamin on memory functions that require normal function of the hippocampus. Therefore, we tested its effect on the performance of hippocampal-lesioned mice in the radial arm maze and water maze.

2. Materials and methods

2.1. Animals

Young (3–4-month-old; $n = 119$) female C57BL/6J//Kuo mice were used in the present study. The mice were housed one per cage after the operation. The environment conditions were controlled and constant (21°C ± 1°C, humidity at 50% ± 10%, light period 0700–1900 h). Food and water were available ad libitum, except during radial arm maze testing, when the body weight of the mice was controlled to be 85% of the original body weight. The study plan was approved by the provincial government of Kuopio.

2.2. Drugs

Apamin (Sigma) was dissolved in NaCl 0.9% and injected intraperitoneally (i.p.) at 0.06, 0.2 and 0.4 mg/kg (10 ml/kg). Controls received vehicle injections of equal volume. The groups received the injections 30 min before the water maze or radial arm maze training.

2.3. Surgery

Partial hippocampal (A: −2.3 mm, M: ±1.0 mm, D: −2.4 mm; A: −2.9 mm, M: ±1.8 mm, D: −2.2 mm relative to the bregma) lesions were made by passage of an anodal DC current (1 mA, 10 s) via tungsten electrodes (diameter 0.0625 mm, 0.5 mm tip uninsulated). Sham-lesioned mice were treated identically, but no current was applied. Mice were deeply anaesthetised with a 1:1 mixture of Dormicum (Roche) and Hypnorm (Janssen Pharmaceutica) (s.c.) during the operation, with analgesia achieved with a 0.1-mg/kg injection of buprenorphine (Temgesic; Reckitt and Colman) (s.c.) after the surgery. The mice were allowed to recover from the surgery for 2 weeks before starting the first experiments.

2.4. Radial arm maze

The radial-arm maze used a design similar to the one developed for rats (Olton et al., 1978) and later adapted for mouse (Crusio et al., 1987). Two days of pre-training (5 min per day) allowed the mice to explore the baited radial arm maze. During the experimental phase, four of the arms were baited in a semi-random manner, with a unique combination of baited arms for each mouse. After each return to the center from an arm, the doors were closed for 5 s. The training was continued until all the baits were consumed or 15 min had passed. After each day, the apparatus was cleaned and rotated by 90°. A working memory error was defined as a visit to an arm that had been visited before. A reference memory error was defined as a visit to an unbaited arm for the first time. Days 3–20 were used for the statistical analysis. The following treatment groups were used: (1) sham-lesioned: $n = 12$; (2) vehicle-treated lesioned: $n = 12$; (3) apamin 0.06 mg/kg-treated lesioned: $n = 11$; (4) apamin 0.2 mg/kg-treated lesioned: $n = 11$.

2.5. Water maze

The water maze was performed as described earlier (Ikonen et al., 1998). The following paradigm was used.

2.5.1. Experiment 1

The training schedule consisted of 15 days of testing divided into three 5-day blocks separated by 2 days. Four platform trials of 60 s were assessed per day during the first 10 training days. The platform location was kept constant during this period of training. During the 3rd 5-day block, the training was performed in another room with different appearance, visual cues and location of the platform relative to the experimenter. At this stage, the treatment groups were changed as shown below. The following treatment groups were used: (1) blocks 1–3 vehicle: $n = 12$; (2) blocks 1–2 apamin 0.06 mg/kg — block 3 vehicle: $n = 6$; (3) blocks 1–2 apamin 0.2 mg/kg — block 3 vehicle: $n = 12$; (4) blocks 1–2 vehicle — block 3 apamin 0.06 mg/kg: $n = 7$; (5) blocks 1–2 vehicle — block 3 apamin 0.2 mg/kg: $n = 12$. All groups were hippocampal-lesioned. During the training, escape length and swimming speed were measured.

2.5.2. Experiment 2

The training schedule consisted of 10 days of testing divided into two 5-day blocks separated by 2 days. Four platform trials of 60 s were assessed per day. The platform location was kept constant during this period of training. During the training, escape length and swimming speed were measured. The following treatment groups were used: (1) vehicle treated: $n = 12$, (2) apamin 0.4 mg/kg treated: $n = 12$. All groups were hippocampal-lesioned.

2.6. Histology

After the testing, the mice were decapitated. The brains were removed and immersed for 1–2 days in 4% formaldehyde solution. 50-μm sections were cut with a vibrating microtome and the sections of the dorsal hippocampal area were stained with cresyl fast violet to determine the position of the lesion.

2.7. Statistics

The effects of the drugs on water maze and radial arm maze behavior were evaluated using analysis of variance for repeated measurements followed by contrasts analysis.

3. Results

3.1. Radial arm maze

In the radial arm maze, analysis of the working memory errors (Fig. 1A) revealed a significant overall group effect ($F(3,42) = 18.519$, $P = 0.000$). Hippocampal lesions increased the number of working memory errors (contrast analysis, sham lesioned vs. vehicle treated lesioned: $P = 0.000$), and apamin treatment dose-dependently reversed this defect (contrast analysis, apamin 0.06 mg/kg vs. vehicle: $P = 0.004$; apamin 0.2 mg/kg vs. vehicle: $P = 0.000$). Analysis of the reference memory errors (Fig. 1B) revealed a significant overall group effect ($F(3,42) = 13.092$, $P = 0.000$). Hippocampal lesions increased the number of reference memory errors (contrast analysis, sham lesioned vs. vehicle treated lesioned: $P = 0.000$),

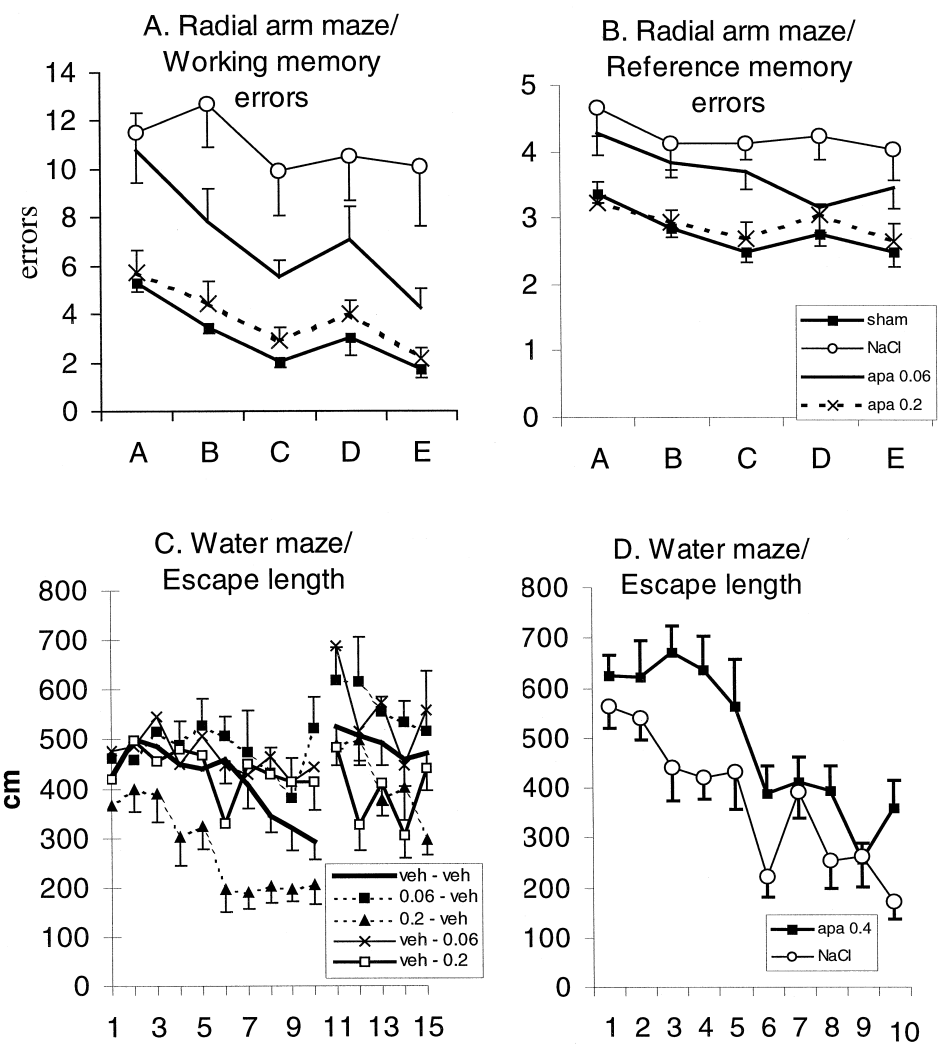

Fig. 1. The effects of apamin on the performance of hippocampal-lesioned mice in the radial arm maze (Parts A and B) and water maze (Parts C and D). In the radial arm maze, the training days were averaged for each parameter into four blocks of 4 days (*X*-axis: A–D) and one block of only 2 days (*X*-axis: E). Apamin dose-dependently reversed the effect of hippocampal lesioning. In the water maze, apamin 0.2 mg/kg improved the performance of hippocampal-lesioned mice. Values in the *X*-axis are training days. All values are group means ± S.E.M.

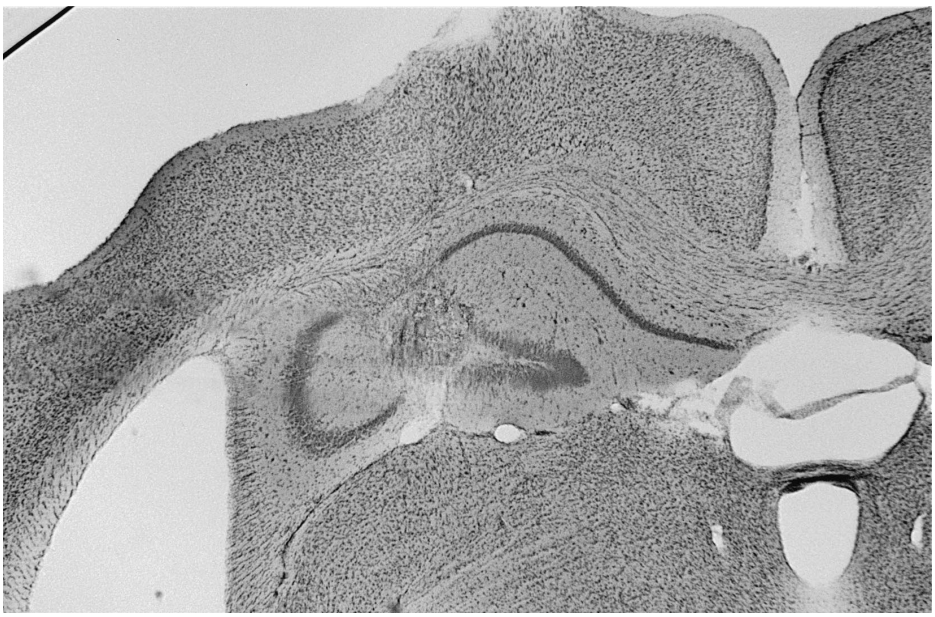

Fig. 2. Nissl staining of a coronal section containing hippocampal-lesioned site.

and again, apamin treatment dose-dependently reversed this defect (contrast analysis, apamin 0.06 mg/kg vs. vehicle: $P = 0.048$; apamin 0.2 mg/kg vs. vehicle: $P = 0.000$).

3.2. Water maze

In water maze experiment 1 (Fig. 1C), the escape length analysis revealed a significant overall group effect during days 1–10 ($F(4,43) = 7.603$, $P = 0.000$). The escape length of the group treated with apamin 0.2 mg/kg was shorter compared to the vehicle group (contrast analysis, $P = 0.001$), but the other groups did not differ significantly (contrast analysis, $P > 0.05$). During days 10–15, there was a significant group effect ($F(4,43) = 4.371$, $P = 0.005$) and the group that was now treated with apamin 0.2 mg/kg performed better than the vehicle treated group (contrast analysis, $P = 0.045$). The other groups did not differ from the vehicle group. The swimming speed analysis showed a significant overall group effect during days 1–10 (data not shown, $F(4,43) = 3.944$, $P = 0.008$). The swimming speed of the group treated with apamin 0.2 mg/kg was reduced compared to the vehicle group (contrast analysis, $P = 0.004$), but there were no differences between the other groups and the vehicle group (contrast analysis, $P > 0.05$). During days 10–15, there were no differences between the groups in swimming speed ($F(4,43) = 2.471$, $P > 0.05$).

In water maze experiment 2 (Fig. 1D), apamin 0.4 mg/kg increased the escape length compared to the vehicle group ($F(1,21) = 7.775$, $P = 0.011$), and it also increased the swimming speed (data not shown, $F(1,21) = 7.215$, $P = 0.014$).

3.3. Histology

The locations of the lesions were confirmed by studying the cresyl fast violet-stained sections. The lesioned area was located in the dorsal hippocampus and was approximately 1 mm wide and 1.5 mm long (Fig. 2).

4. Discussion

We observed that apamin alleviates the spatial reference memory and working memory deficit induced by a partial electrolytic hippocampal lesion. This effect was dose-dependent and it could be seen both in water maze and in radial arm maze behavior.

The water maze training was performed in two different environments in order to find out whether apamin improves learning per se, or if it merely affects a general search strategy. If a certain compound improves water maze learning per se, the following criteria should be fulfilled: a drug-treated group should perform better than controls in the first environment, but the same group should no longer perform better in the second environment without the drug administration. And vice versa: a group that has been treated with vehicle in the first environment should start to show improved performance if it is treated with the drug when it is being trained in the second, novel environment. On the contrary, if a certain compound merely helps the mice to develop a better search strategy, it should improve the performance not only in the first environment with the drug administration but also in the second environment even without the drug treatment. The reason for

this is that the mice should be able to use their more efficient search strategy also later, when the drug is no longer present.

In the water maze, apamin 0.2 mg/kg reduced the escape length of the hippocampal-lesioned mice during the initial training during days 1–10. During days 11–15, when the testing was continued in a different environment with different treatment groups, the group previously treated with 0.2 mg/kg, which now received saline, no longer differed from the vehicle group. In addition, the group that now received apamin 0.2 mg/kg instead of previous vehicle injections, now performed significantly better than the vehicle treated group. This suggests that apamin affects learning per se. The same effect can be seen in the swimming speed, which confirms the finding.

The swimming speed of the 0.2 mg/kg treated group was reduced (data not shown). This could be interpreted as a noxious effect of too high a dose, but this is not the case, since the dose 0.4 mg/kg in experiment 2 did not reduce the swimming speed. However, this dose (0.4 mg/kg) did not improve the performance of hippocampal-lesioned mice, in fact it tended to increase the escape length. This can be explained by the toxic effects of apamin described earlier (Lallement et al., 1995). These toxic effects are more likely to be caused by peripheral side effects than central effects since lethal doses of apamin do not induce any abnormalities in EEG (electroencephalogram) (Lallement et al., 1995).

In the radial arm maze, apamin alleviated the defect present in hippocampal-lesioned mice. A dose of 0.2 mg/kg reduced the amount of working- and reference memory errors to the level of sham-lesioned mice. The radial arm maze measures the same aspect of learning as the water maze, i.e., spatial reference memory. However, other factors, such as motivation and stress, differ in these two tests. Nonetheless, in this study, the results from the radial arm maze confirmed the findings from the water maze.

This is the first study reporting a favourable effect of apamin on partial hippocampal-lesioned mice. Previously, it has been shown that apamin does not improve the water maze performance of intact mice, but it does alleviate the defect induced by a medial septal lesion (Ikonen et al., 1998). This study demonstrates that apamin can alleviate the symptoms of a damaged septo-hippocampal axis, irrespective of whether the damage is in the septum or in the hippocampus. This is not true for example in the case of metrifonate, a cholinesterase inhibitor, which can reverse the effects of a lesion in the septum, but not in the hippocampus (Ikonen and Riekkinen, 1999).

The facilitating effect of apamin can be explained by its stimulating action on either the remaining intact parts of the hippocampus or on other brain areas critical for performance in the water maze or radial arm maze. There is evidence to suggest that the site of action is the hippocampus. First, apamin facilitates long-term potentiation in the CA1 area of rat hippocampus (Behnisch and Reymann, 1998). Apamin has also been shown to block Ca^{2+}-mediated K^+ currents in several cell types, including the CA1 hippocampal pyramidal neurons (Stocker et al., 1999) and the cholinergic cells of the medial septum-diagonal band region (Matthews and Lee, 1991), cells that project to the hippocampus. In addition, acquisition of trace eyeblink conditioning, a hippocampus-dependent task, induces a reduction of afterhyperpolarisation in CA1 pyramidal neurons of rabbits (De Jonge et al., 1990; Moyer et al., 1996). Furthermore, apamin has been shown to reverse the spatial navigation defect induced by medial septal-lesion (Ikonen et al., 1998) — a lesion used for modelling a cholinergic defect of the hippocampus. These results suggest that apamin acts by stimulating the function of hippocampus either directly or by affecting the cholinergic system that controls the hippocampus. However, apamin does not alleviate the memory defect induced by scopolamine in delayed matching to position test (Poorheidari et al., 1998), which suggests that the cholinergic system is not the most likely target. Therefore, it is probable that apamin has a broad range of effects which are not limited to a single transmitter system. This idea is supported by the fact that the tests in which apamin has been shown to facilitate memory processing have been heterogeneous, measuring different aspects of memory processing.

In conclusion, hippocampus is the principal site for spatial memory in mice and rats. Lesions of the hippocampus disrupt the ability of the animals to perform complex spatial tasks. Apamin reverses the memory defect caused by partial hippocampal lesions. This indicates that blockade of Ca^{2+}-mediated K^+ channels can alleviate the memory defect induced by a damaged septo-hippocampal axis.

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References

Behnisch, T., Reymann, K.G., 1998. Inhibition of apamin-sensitive calcium dependent potassium channels facilitate the induction of long-term potentiation in the CA1 region of rat hippocampus in vitro. Neurosci. Lett. 253, 91–94.

Crusio, W.E., Schwegler, H., Lipp, H.P., 1987. Radial-maze performance and structural variation of the hippocampus in mice: a correlation with mossy fibre distribution. Brain Res. 425, 182–185.

De Jonge, M.C., Black, J., Deyo, R.A., Disterhoft, J.F., 1990. Learning-induced afterhyperpolarization reductions in hippocampus are specific for cell type and potassium conductance. Exp. Brain Res. 80, 456–462.

Deschaux, O., Bizot, J.C., 1997. Effect of apamin, a selective blocker of Ca^{2+}-activated K+-channel, on habituation and passive avoidance responses in rats. Neurosci. Lett. 227, 57–60.

Deschaux, O., Bizot, J.C., Goyffon, M., 1997. Apamin improves learning in an object recognition task in rats. Neurosci. Lett. 222, 159–162.

Gehlert, D.R., Gackenheimer, S.L., 1993. Comparison of the distribution of binding sites for the potassium channel ligands [^{125}I]apamin, [^{125}I]charybdotoxin and [^{125}I]iodoglyburide in the rat brain. Neuroscience 52, 191–205.

Heurteaux, C., Messier, C., Destrade, C., Lazdunski, M., 1993. Memory processing and apamin induce immediate early gene expression in mouse brain. Brain Res. Mol. Brain Res. 18, 17–22.

Ikonen, S., Riekkinen, P. Jr., 1999. Characterization of learning and memory behaviors and the effects of metrifonate in the C57BL strain of mice. Eur. J. Pharmacol. 372, 117–126.

Ikonen, S., Schmidt, B., Riekkinen, P. Jr., 1998. Apamin improves spatial navigation in medial septal-lesioned mice. Eur. J. Pharmacol. 347, 13–21.

Kawai, T., Watanabe, M., 1986. Blockade of Ca-activated K conductance by apamin in rat sympathetic neurones. Br. J. Pharmacol. 87, 225–232.

Lallement, G., Fosbraey, P., Baille Le Crom, V., Tattersall, J.E., Blanchet, G., Wetherell, J.R., Rice, P., Passingham, S.L., Sentenac Roumanou, H., 1995. Compared toxicity of the potassium channel blockers, apamin and dendrotoxin. Toxicology 104, 47–52.

Matthews, R.T., Lee, W.L., 1991. A comparison of extracellular and intracellular recordings from medial septum/diagonal band neurons in vitro. Neuroscience 42, 451–462.

Messier, C., Mourre, C., Bontempi, B., Sif, J., Lazdunski, M., Destrade, C., 1991. Effect of apamin, a toxin that inhibits Ca^{2+}-dependent K+ channels, on learning and memory processes. Brain Res. 551, 322–326.

Mourre, C., Hugues, M., Lazdunski, M., 1986. Quantitative autoradiographic mapping in rat brain of the receptor of apamin, a polypeptide toxin specific for one class of Ca^{2+}-dependent K+ channels. Brain Res. 382, 239–249.

Moyer, J.R. Jr., Thompson, L.T., Disterhoft, J.F., 1996. Trace eyeblink conditioning increases CA1 excitability in a transient and learning-specific manner. J. Neurosci. 16, 5536–5546.

Olton, D.S., Walker, J.A., Gage, F.H., 1978. Hippocampal connections and spatial discrimination. Brain Res. 139, 295–308.

Poorheidari, G., Stanhope, K.J., Pratt, J.A., 1998. Effects of the potassium channel blockers, apamin and 4-aminopyridine, on scopolamine-induced deficits in the delayed matching to position task in rats: a comparison with the cholinesterase inhibitor E2020. Psychopharmacology 135, 242–255.

Stocker, M., Krause, M., Pedarzani, P., 1999. An apamin-sensitive Ca^{2+}-activated K+ current in hippocampal pyramidal neurons. Proc. Natl. Acad. Sci. U.S.A. 96, 4662–4667.