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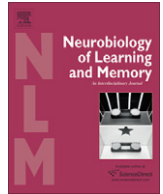
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## Inhibition of PKA anchoring to A-kinase anchoring proteins impairs consolidation and facilitates extinction of contextual fear memories

Ingrid M. Nijholt<sup>a,\*</sup>, Anghelus Ostroveanu<sup>a,1</sup>, Wouter A. Scheper<sup>a</sup>, Botond Penke<sup>b</sup>, Paul G.M. Luiten<sup>a</sup>, Eddy A. Van der Zee<sup>a</sup>, Ulrich L.M. Eisel<sup>a</sup>

<sup>a</sup> Department of Molecular Neurobiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

<sup>b</sup> Department of Medical Chemistry, University of Szeged, 8 Dóm tér, Szeged, H-6720, Hungary

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### ABSTRACT

Both genetic and pharmacological studies demonstrated that contextual fear conditioning is critically regulated by cyclic AMP-dependent protein kinase (PKA). Since PKA is a broad range protein kinase, a mechanism for confining its activity is required. It has been shown that intracellular spatial compartmentalization of PKA signaling is mediated by A-kinase anchoring proteins (AKAPs). Here, we investigated the role of PKA anchoring to AKAPs in different stages of the memory process (acquisition, consolidation, retrieval and extinction) using contextual fear conditioning, a hippocampus-dependent learning task. Mice were injected intracerebroventricularly or intrahippocampally with the membrane permeable PKA anchoring disrupting peptides St-Ht31 or St-superAKAP-IS at different time points during the memory process. Blocking PKA anchoring to AKAPs resulted in an impairment of fear memory consolidation. Moreover, disrupted PKA anchoring promoted contextual fear extinction in the mouse hippocampus. We conclude that the temporal and spatial compartmentalization of hippocampal PKA signaling pathways, as achieved by anchoring of PKA to AKAPs, is specifically instrumental in long-term contextual fear memory consolidation and extinction, but not in acquisition and retrieval.

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### 1. Introduction

Contextual fear conditioning is a form of associative learning in which animals learn to fear a new environment because of its temporal association with an aversive unconditioned stimulus (US), usually an electrical footshock. The neuroanatomical systems and neurochemical basis underlying conditioned fear have been extensively investigated. It affects multimodal sensory information processing of continuously present (tonic) stimuli and it depends on a time-limited function of the hippocampus (see for review e.g. Sanders, Wiltgen, & Fanselow, 2003).

Studies investigating the intracellular signal transduction pathways involved have shown a crucial role for cAMP-dependent protein kinase (PKA) in contextual fear conditioning. Abel and colleagues generated transgenic mice which express R(AB), an inhibitory form of the regulatory subunit of PKA, only in forebrain regions such as the hippocampus. In these mice hippocampal PKA activity is reduced, which is paralleled by behavioral deficits in long-term but not short-term memory for contextual fear conditioning (Abel et al., 1997). The time course of amnesia in these

transgenic mice is similar to the time course observed in mice treated with inhibitors of PKA (Bourtchouladze et al., 1998). Other studies using pharmacological approaches also reported that PKA inhibitors impair contextual fear conditioning (Ahi, Radulovic, & Spiess, 2004; Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999; Walenstein, Vago, & Walberer, 2002).

Although much is known about the mechanisms involved in the storage of contextual fear memories, the processes underlying the extinction of fear memories are far less understood. Recently, a role for PKA in fear extinction was proposed. Transgenic mice which express R(AB) show facilitated extinction of both recent and remote contextual fear memories (Isiegas, Park, Kandel, Abel, & Lattal, 2006) whereas increased PKA activity was found to impair extinction (McNally, Lee, Chiem, & Choi, 2005; Wang, Ferguson, Pineda, Cundiff, & Storm, 2004). In general these studies suggest that the PKA signal transduction pathway is important in the consolidation and extinction of contextual fear memories.

However, PKA is a multifunctional enzyme with a broad substrate specificity and thus coordinated control of PKA signaling is required. This is partly achieved by association of the enzyme with so called A-kinase anchoring proteins (AKAPs) (Rubin, 1994). AKAPs are a group of more than 50 identified functionally related proteins. Although they share little primary structure similarities, they all have the ability to bind the regulatory subunits of PKA,

\* Corresponding author. Fax: +31 50 3632331.

E-mail address: [i.m.nijholt@rug.nl](mailto:i.m.nijholt@rug.nl) (I.M. Nijholt).

<sup>1</sup> These authors contributed equally to this work.

and therefore to coordinate specific cAMP signaling pathways by sequestering PKA to a particular subcellular location (Beene & Scott, 2007; Wong & Scott, 2004). Up to 75% of the total cellular PKA is believed to be associated with some member of the AKAP family. Compartmentalization of individual AKAP–PKA complexes occurs through specialized targeting domains that are present on each anchoring protein.

Interestingly, several AKAPs bind more than one signaling enzyme simultaneously. These multivalent AKAPs serve as scaffolds for the assembly of signaling complexes consisting of several kinases and phosphatases. Compartmentalization of both kinases and phosphatases to the same location may provide a coordinated activity of two enzymes with opposite catalytic activities.

Previous studies mainly focused on the effect of changes in PKA activity on learning and memory processes. However, recent findings suggest that positioning of PKA at its proper subcellular location by AKAPs is crucial for its efficient catalytic activation and accurate substrate selection and may thus be important in learning and memory processes. Hitherto knowledge on the importance of PKA anchoring to AKAPs in learning and memory processes is limited. In an initial study Moita and colleagues showed that local inhibition of PKA anchoring in the rat lateral amygdala impaired memory consolidation of auditory fear conditioning (Moita, Lamprecht, Nader, & LeDoux, 2002). More recent studies in *Drosophila* reported an important role for AKAPs in olfactory memory processing (Lu, Lu et al., 2007; Schwaerzel, Jaekel, & Mueller, 2007). Furthermore, data from genetically modified mice that conditionally express Ht31, an inhibitor of PKA anchoring to AKAPs, showed that an anchored pool of PKA is important in theta-burst LTP and hippocampus-dependent spatial memory storage (Nie, McDonough, Huang, Nguyen, & Abel, 2007). In aplysia sensory neurons Ht31 was found to prevent both short- and long-term facilitation (Liu, Hu, Schacher, & Schwartz, 2004).

In the present study, we investigated the importance of PKA anchoring in the distinct stages of the memory process during contextual fear conditioning.

## 2. Materials and methods

### 2.1. Animals

All experiments were performed with 9–12 weeks old male C57BL/6J mice (Harlan, Horst, The Netherlands). Individually housed mice were maintained on a 12 h light/dark cycle (lights on at 7.00 a.m.) with food (Hopefarm® standard rodent pellets) and water ad libitum. A layer of sawdust served as bedding. The animals were allowed to adapt to the housing conditions for 1–2 weeks before the experiments started. The procedures concerning animal care and treatment were in accordance with the regulations of the Ethical Committee for the use of experimental animals of the University of Groningen (DEC4174C).

### 2.2. Fear conditioning

Fear conditioning was performed in a plexiglas cage (44 × 22 × 44 cm) with constant illumination (12 V, 10 W halogen lamp, 100–500 lux). The training (conditioning) consisted of a single trial. Before each individual mouse entered the box, the box was cleaned with 70% ethanol. The mouse was exposed to the conditioning context for 180 s followed by a footshock (0.7 mA, 2 s, constant current) delivered through a stainless steel grid floor. The mouse was removed from the fear conditioning box 30 s after shock termination to avoid an aversive association with the handling procedure. Memory tests were performed 1 or 24 h after fear conditioning. Contextual memory was tested in the fear conditioning box for 180 s without footshock presentation. Freezing, defined as the lack of movement except for respiration and heart beat, was assessed as the behavioral parameter of the defensive reaction of mice by a time-sampling procedure every 10 s throughout memory tests. In addition, mean activity of the animal during the training and retention test was measured with the Ethovision system (Noldus, The Netherlands). In some experiments, animals were exposed to an alternative context 24 h after the training session. This alternative context consisted of a white plastic chamber (39 × 29 × 19 cm) which was exposed to 500–1000 lux, did not have a rod floor and was washed with 1% acetic acid, before each individual mouse entered the chamber.

To assess fear extinction mice underwent a daily re-exposure to the conditioning chamber for 3 min after the retention test. During these extinction trials freezing behavior and mean activity was measured.

### 2.3. Animal surgery

Double guide cannulae (C235, Plastics One, Roanoke, VA) were implanted using a stereotaxic holder during 1.2% avertin anesthesia (0.02 ml/g, i.p.) under aseptic conditions as previously described (Nijholt et al., 2004) into both lateral brain ventricles (i.c.v.) with anteroposterior (AP) coordinates zeroed at Bregma AP 0 mm, lateral 1 mm, depth 3 mm or directed toward both dorsal hippocampi (i.h.), AP –1.5 mm, lateral 1 mm, depth 2 mm (Franklin & Paxinos, 1997). Each double guide cannula with inserted dummy cannula and dust cap was fixed to the skull with dental cement (3M ESPE AG, Germany). Administration of 1 mg/ml finadyne (0.005 ml/g i.p.) before the surgery served as pain killer. The animals were allowed to recover for 6–7 days before the behavioral experiments started.

### 2.4. Brain injections

Bilateral injections were performed during a short isoflurane anesthesia using a Hamilton microsyringe fitted to a syringe pump unit (TSE systems, Bad Homburg, Germany) at a constant rate of 0.5 µl/min (final volume: 1 µl per side) for the i.c.v. injections and 0.34 µl/min (final volume: 0.3 µl per side) for the i.h. injections.

PKA anchoring to AKAPs was inhibited by intracerebroventricular (i.c.v.) or intrahippocampal (i.h.) injection of the peptide Ht31 (InCELLect® AKAP St-Ht31 inhibitor peptide (Promega, Madison, WI)) or superAKAP-IS. These peptides inhibit the interaction between the regulatory subunits of PKA and AKAP (Gold et al., 2006; Vijayaraghavan, Goueli, Davey, & Carr, 1997). SuperAKAP-IS was synthesized by solid phase peptide synthesis using BOC-chemistry and purified after cleavage from the matrix by preparative HPLC. Purity was controlled by analytical HPLC and mass spectrometry. The steared form of Ht31 and superAKAP-IS was used to enhance the cellular uptake of the peptide through the membrane. St-Ht31 was injected in a final concentration of 10 mM (i.c.v. 20 nmol/mouse and i.h. 6 nmol/mouse) and St-superAKAP-IS in a final concentration of 5–500 µM (i.h. 0.003–0.3 nmol/mouse per injection). Unfortunately, it was not possible to prepare concentrations of St-superAKAP-IS higher than 500 µM. 50 mM Tris–HCl (pH 7.5) served as vehicle. To test the specificity of the observed effects another set of animals was injected with either InCELLect® St-Ht31P, a proline-substituted derivative which does not inhibit PKA anchoring (control peptide; final concentration 10 mM in 50 mM Tris–HCl, pH 7.5; i.c.v. 20 nmol/mouse and i.h. 6 nmol/mouse), or vehicle alone (50 mM Tris–HCl, pH 7.5). Untreated animals without cannula served as controls for possible cannulation and injection effects. The number of animals per group varied from 6 to 18.

### 2.5. Histology

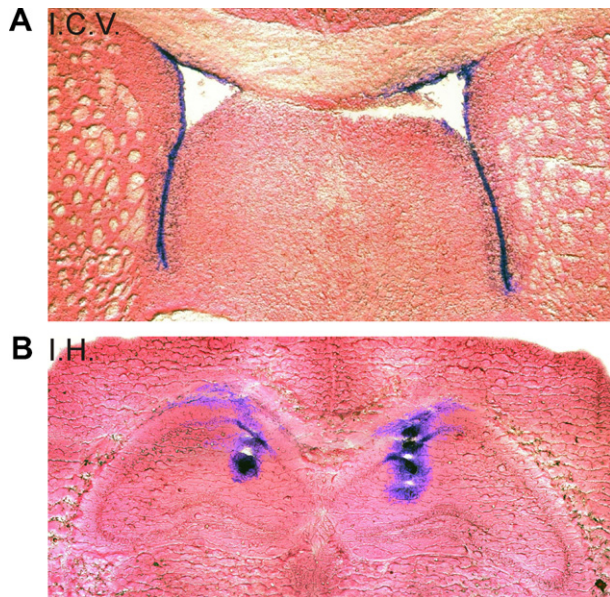
Immediately after the behavioral test mice were injected during 1.2% avertin anesthesia (0.02 ml/g, i.p.) with methylene blue solution i.c.v., or i.h. Brains were removed and serially sectioned at 50 µm, collecting the sections on glass slides. Sections were stained on glass for 5 min in 0.1% nuclear fast red solution. To identify the location of the injection, sections were analyzed using light microscopy (Fig. 1).

Only data from animals in which the exact site of injection was confirmed after the behavioral experiments were evaluated. The methylene blue injections in the dorsal hippocampus did not show a diffusion of the solution to other brain or hippocampal areas.

### 2.6. Immunoprecipitation

One hour after intrahippocampal injection of PKA anchoring disruptor peptide or vehicle solution, the dorsal hippocampus was excised and mechanically homogenized in 10 volumes of homogenization buffer [50 mM Hepes (pH 7.4), 150 mM NaCl, 0.2% NP-40, 4 mM EGTA, 10 mM EDTA, 15 mM sodium pyrophosphate, 100 mM β-glycerophosphate, 50 mM sodium fluoride, 5 mM sodium orthovanadate, 1 mM dithiothreitol, 1 mM PMSF, and Complete Mini Protease Inhibitor Cocktail (Roche)]. The homogenate was centrifuged at 20,000g for 10 min at 4 °C, and the resulting supernatant was used for AKAP150 immunoprecipitation.

Per sample 100 µl of Dynabeads protein A (DynaL Biotech) was washed twice with Na-phosphate buffer (0.1 M, pH 8.1). Ten micrograms of goat anti-AKAP150C-20 antibody (1:2500, sc-6445 Santa Cruz, CA, USA) was incubated with the beads for 10 min. Afterwards the beads were washed three times with Na-phosphate buffer (0.1 M, pH 8.1) and twice with triethanolamine (0.2 M). IgGs were crosslinked with dimethyl pimelimidate (20 mM in 0.2 M triethanolamine) for 30 min. The beads were washed for 15 min with Tris (50 mM, pH 7.5) and three times with phosphate buffered saline. Unbound IgG was removed by washing twice for 30 min with Na-citrate (0.1 M, pH 2–3). The dorsal hippocampus homogenate was incubated for 1 h with the beads. Bound proteins were eluted by denaturation at 95 °C for 5 min. The immunoprecipitated sample was stored at –80 °C until use. All the steps of the immunoprecipitation procedure were performed at room temperature.



**Fig. 1.** Representative coronal brain sections of bilateral (A) intracerebroventricular (i.c.v.) and (B) dorsal hippocampal (i.h.) injections with methylene blue injections after counterstaining with nuclear fast red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

### 2.7. Western blotting

AKAP150 immunoprecipitates were separated on a 10% SDS–polyacrylamide gel and transferred to PVDF membranes (Millipore, USA). The blots were blocked for 1 h in blocking buffer (0.2% I-Block (Tropix), 0.1% Tween 20) and then incubated overnight at 4 °C with goat anti-AKAP150 C-20 (1:2500, sc-6445, Santa Cruz) and mouse anti-PKA-RII $\beta$  (1:2,000, 610625, BD Biosciences). The blots were incubated with horse radish peroxidase-conjugated secondary antibodies [HRP-conjugated donkey anti-goat IgG (1:4,000)] (sc-2020 Santa Cruz, CA, USA) and HRP-conjugated donkey anti-mouse (1:4,000) (sc-2005 Santa Cruz, CA, USA). Western blots were developed using the chemiluminescence method (Pierce ECL, 32106). The immunoblots were digitized and quantified using a Leica DFC 320 image analysis system (Leica, Cambridge, UK).

### 2.8. Statistical analysis

Statistical comparisons were made by analysis of variance (ANOVA). For each significant *F* ratio, Fisher's protected least significant difference (PLSD) test was used to analyze the statistical significance of appropriate multiple comparisons. Data were expressed as means  $\pm$  sem. Significance was determined at the level of  $p < 0.05$ .

## 3. Results

### 3.1. Consolidation of contextual fear memory is impaired by i.c.v. St-Ht31 injection

To investigate the effect of inhibition of PKA anchoring to AKAPs on the acquisition and consolidation of fear memory, animals were injected i.c.v. with St-Ht31, control peptide or vehicle 1 h before training. Injection of none of these substances resulted in changes in mean activity during training or shock reactivity when compared to untreated animals without cannula (data not shown). However, injection of St-Ht31 caused a significant reduction in freezing behavior during the retention test 24 h after training in comparison to control peptide, vehicle-injected and untreated animals (one-way ANOVA:  $F(3,31) = 5.471$ ,  $p = 0.004$ , Fig. 2A).

Similarly, injection of St-Ht31 immediately after training significantly attenuated conditioned fear (one-way ANOVA:  $F(3,30) = 3.932$ ,  $p = 0.018$ , Fig. 2B). The learning deficit observed when St-Ht31 was injected immediately after training was similar

to the effect of St-Ht31 injected 1 h before training ( $43.8 \pm 8.1\%$ ,  $n = 9$  versus  $40.0 \pm 7.3\%$ ,  $n = 7$ , respectively). To be able to distinguish between acquisition and consolidation, we performed a retention test 1 h after training with mice that were injected 1 h before training. Overall, the contextual fear response was somewhat lower 1 h after training than 24 h after training (Fig. 2A versus Fig. 2C). This result is in full agreement with previous studies of Rudy and Morledge who investigated the time course of the expression of context-dependent fear (Rudy & Morledge, 1994). Interestingly, the performance of St-Ht31 injected animals did not differ from the control groups when the retention test was performed 1 h after training (one-way ANOVA:  $F(3,20) = 0.257$ ,  $p = 0.855$ , Fig. 2C). The finding that mice which received St-Ht31 1 h before training, showed unimpaired freezing 1 h after training but attenuated freezing 24 h after training, suggests that PKA anchoring onto AKAPs plays a specific role in the consolidation of contextual fear memories but not in acquisition.

The importance of PKA anchoring in the retrieval of memories was studied by injecting mice with St-Ht31 1 h before the retention test 24 h after training. There was no significant difference in freezing behavior between all groups (one-way ANOVA:  $F(3,25) = 0.071$ ,  $p = 0.975$ , Fig. 2D).

### 3.2. Intrahippocampal injection of PKA anchoring disrupting peptides impairs consolidation of contextual fear memory

We tested the subregion-specific contribution of the hippocampus by i.h. injection of St-Ht31, different concentrations of St-superAKAP-IS, control peptide or vehicle. When injected immediately after training, both St-Ht31 and St-superAKAP-IS caused an impairment of contextual fear memory when compared to the control groups (one-way ANOVA:  $F(7,69) = 4.219$ ,  $p = 0.001$ , Fig. 3A). The effect of St-superAKAP-IS on freezing behavior appeared to be dose-dependent (Fig. 3A).

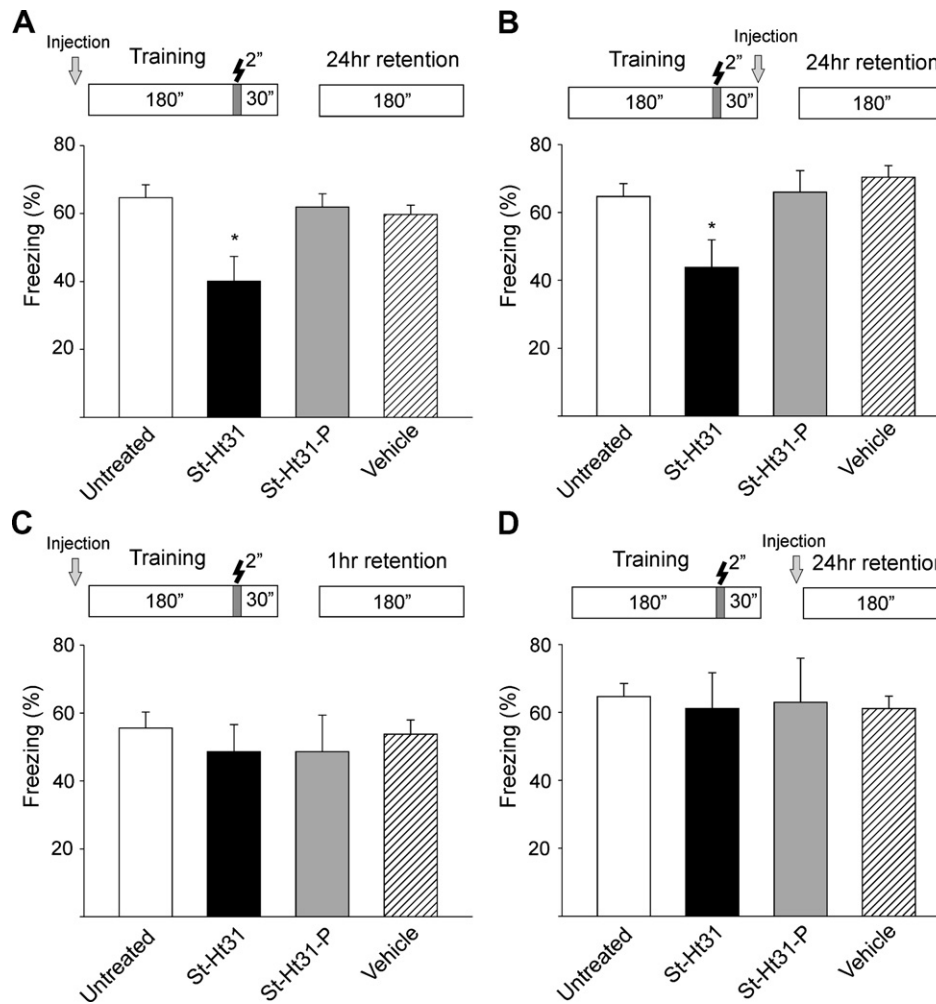
In addition, consistent with other studies (Radulovic, Kammermeier, & Spiess, 1998), mice showed contextual generalization of fear in an alternative context 24 h after the training session. However, freezing in this alternative context was much lower than in the conditioning context and was not affected by 500  $\mu$ M St-superAKAP-IS injection (one-way ANOVA:  $F(2,15) = 1.154$ ,  $p = 0.342$ , Fig. 3B), indicating that the non-associative component of the freezing response is not dependent on PKA anchoring.

Overall we can conclude that PKA anchoring to AKAPs located in the hippocampus is instrumental in associative memory consolidation. However, we cannot completely rule out the additional involvement of extrahippocampal PKA signaling pathways.

In all experiments, the injection procedure itself had no effect on conditioned fear as indicated by the finding that there was never a significant difference between vehicle-injected and non-injected animals (Figs. 2 and 3).

### 3.3. Intrahippocampal injection of St-superAKAP-IS promotes fear extinction

Next we assessed the role of PKA anchoring in the extinction of contextual fear memory. Mice underwent a single training trial and retention test and after the retention test mice were daily re-exposed to the conditioning chamber for 3 min. St-superAKAP-IS (500  $\mu$ M) or vehicle was injected i.h. immediately after each extinction trial. Inhibition of PKA anchoring by St-superAKAP-IS significantly facilitated fear extinction (Extinction 5, one-way ANOVA:  $F(1,10) = 7.836$ ,  $p = 0.019$ ; Extinction 6, one-way ANOVA:  $F(1,10) = 8.188$ ,  $p = 0.017$ ; Extinction 7, one-way ANOVA:  $F(1,10) = 10.152$ ,  $p = 0.010$ , Fig. 4).



**Fig. 2.** Intracerebroventricular injection of St-Ht31 impairs the consolidation of contextual fear memory. Mice were injected either one hour before training (A and C), immediately after training (B) or 1 h before the retention test (D) with St-Ht31, control peptide or vehicle. Untreated mice served as controls. The training consisted of a 180 s exposure to the fear conditioning box followed by a footshock (0.7 mA, 2 s). 30 s after the footshock mice were returned to their home cage. Freezing behavior was measured in the memory test 1 h (C) or 24 h (A, B, and D) after training. Error bars indicate standard error of the mean. Statistically significant differences:  $p < 0.05$  versus all control groups (vehicle, control peptide and untreated).

#### 3.4. Intrahippocampal injection of St-superAKAP-IS reduced PKA anchoring to AKAP150

Using immunoprecipitation we specifically assessed the amount of PKA anchored to AKAP150 in the dorsal hippocampus 1 h after intrahippocampal injection of vehicle or 500  $\mu$ M St-superAKAP-IS. The AKAP150 complex was immunoprecipitated with an antibody directed against AKAP150. Subsequent analysis of the amount of PKA bound to AKAP150 showed that St-superAKAP-IS reduced the amount of PKA anchored to AKAP150 in the dorsal hippocampus (one-way ANOVA:  $F(1,7) = 12.115$ ,  $p = 0.01$ , Fig. 5).

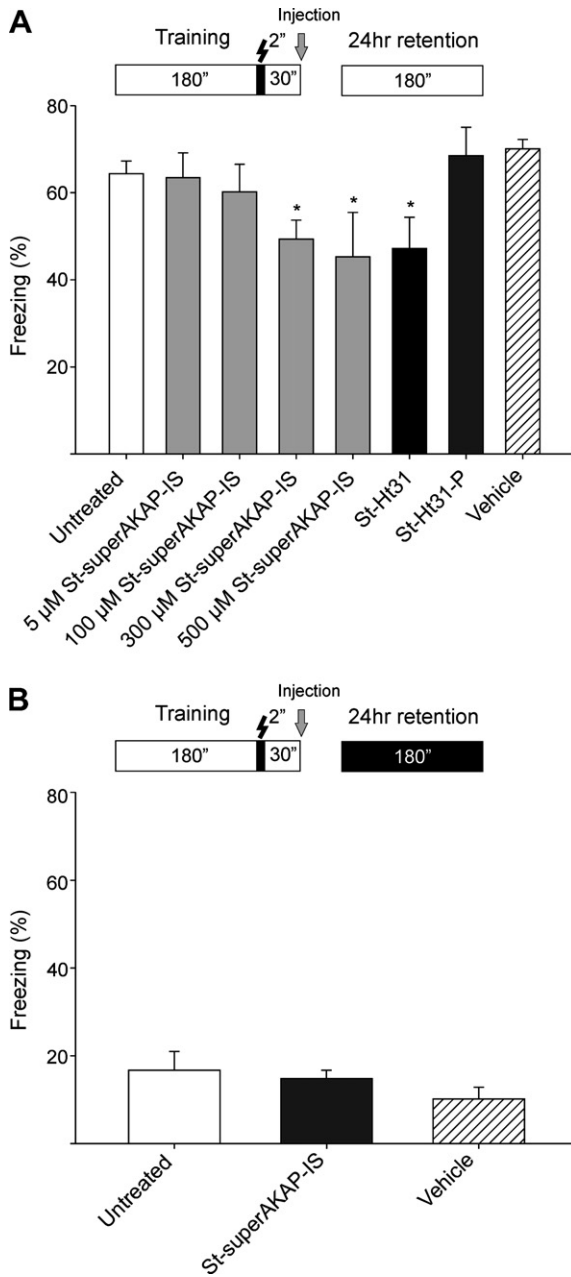
#### 4. Discussion

In summary, we conclude that hippocampal PKA anchoring to AKAPs is important for the consolidation and extinction of contextual fear memories whereas acquisition and retrieval are not affected.

These findings are consistent with earlier studies using genetic and pharmacological approaches to inhibit PKA activity. The genetic reduction of hippocampal PKA activity in mice that express PKA-R(AB) selectively impairs hippocampus-dependent long-term memory for contextual fear conditioning (Abel et al., 1997). To ex-

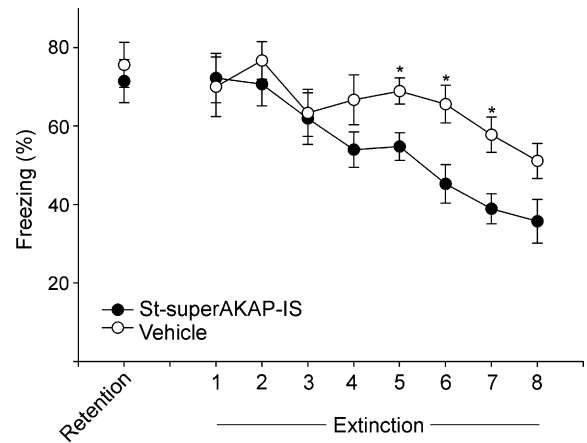
clude the developmental effects as a result of transgene expression Abel and colleagues confirmed their data via injection of a PKA inhibitor (Bourtchouladze et al., 1998). Both i.c.v. and i.h. injections of PKA or PKA/PKC inhibitors before or after training did not affect memory after 1 h but significantly impaired memory after 24 h (Bourtchouladze et al., 1998; Schafe et al., 1999; Wallenstein et al., 2002). Overall, these data suggest an important role for PKA signaling in the long-term consolidation of contextual fear memories. Besides PKA, extracellular regulated kinase/mitogen-activated protein (ERK/MAP) kinase is necessary for the consolidation of associative memories in the mammalian nervous system (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998). It is suggested that coactivation of PKA and MAPK signaling leads to the concurrent activation of CREB-dependent gene expression required for hippocampal long-term memory formation (Impey et al., 1998). From our data it can be concluded that not only PKA activity is necessary for proper consolidation of memories, but also the spatial and temporal compartmentalization of PKA achieved via anchoring to AKAPs.

Mammalian PKA includes four regulatory (RI $\alpha$ , RI $\beta$ , RII $\alpha$ , RII $\beta$ ) and three catalytic (C $\alpha$ , C $\beta$ , C $\gamma$ ) subunits, each encoded by a separate gene. PKA consists of an inactive heterotetramer of two catalytic subunits bound to two regulatory subunits (Taylor,

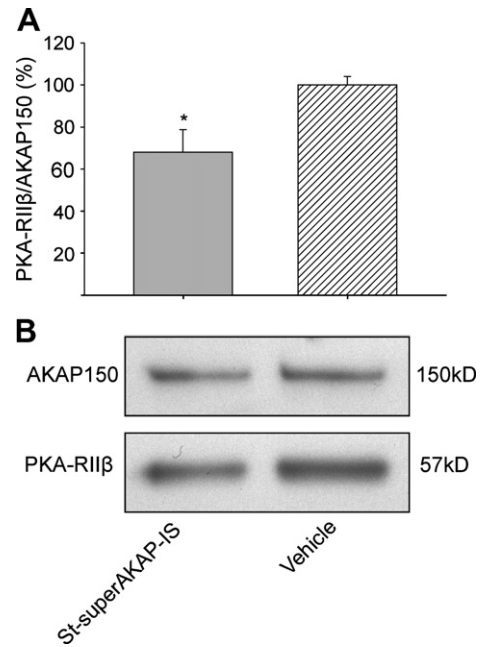


**Fig. 3.** Hippocampal PKA anchoring plays an important role in the consolidation of contextual fear memory. Mice were injected intrahippocampally with St-Ht31, St-superAKAP-IS, control peptide or vehicle immediately after training. Untreated mice served as controls. Freezing was measured in the memory test in the same context (A) or in an alternative context (B) 24 h after training. Error bars indicate standard error of the mean. Statistically significant differences:  $p < 0.05$  versus all control groups.

Buechler, & Yonemoto, 1990). PKA is associated to AKAPs with its regulatory subunits via an amphipathic helix binding motif (Herberg, Maleszka, Eide, Vossebein, & Tasken, 2000). In studies by Fink and colleagues inhibition of PKA anchoring by Ht31 resulted in redistribution of the regulatory subunits and decreased compartmentalization of PKA (Fink et al., 2001). Thus, disrupted spatial compartmentalization of PKA attenuates the specificity of the cAMP/PKA signaling pathway. This will affect downstream proteins such as the phosphorylation of CREB and may finally lead to impaired long-term memory consolidation. Our finding that only long-term memory consolidation is affected and not acquisi-



**Fig. 4.** Intrahippocampal injection of St-superAKAP-IS facilitates the extinction of contextual fear memory. Mice were injected intrahippocampally with St-superAKAP-IS and vehicle immediately after each extinction. Freezing was measured in the memory test performed 24 h after training and on 8 consecutive days, starting 24 h after the memory test. Error bars indicate standard error of the mean. Statistically significant differences:  $p < 0.05$  versus all control groups.



**Fig. 5.** Intrahippocampal injection of St-superAKAP-IS impairs PKA anchoring to AKAP150. Dorsal hippocampus was excised 1 h after St-superAKAP-IS or vehicle injection. AKAP150 was immunoprecipitated from the dorsal hippocampus. (A) Bar graph showing the ratio of PKA-RIIβ complexed to AKAP150. The ratio in the vehicle-injected group was set at 100% for each experiment. Results shown represent three separate experiments. Error bars indicate standard error of the mean. Statistically significant differences:  $p < 0.05$  versus the vehicle group. (B) Representative Western blot for AKAP150 and PKA-RIIβ.

tion or retrieval indicates that there is a critical time window in which PKA anchoring is essential in contextual fear memories.

The specific ways in which inhibition of PKA anchoring accelerates extinction remains to be determined. However our findings are in line with the facilitated extinction of contextual fear memories observed in mice with a transgenic inhibition of PKA (Isiegas et al., 2006) and the impaired extinction in mice with increased PKA activity (McNally et al., 2005; Wang et al., 2004).

It has been hypothesized recently that both memory formation as well as extinction are actively controlled by a tightly regulated

balance between PKA and protein phosphatase 2B (PP2B) in which the one opposes the activity of the other (Mansuy, 2003). In line with these findings it was reported that a reduction of PP2B signaling in forebrain neurons improves memory consolidation whereas it deteriorates fear extinction (Havekes, Nijholt, Visser, Eisel, & Van der Zee, 2008; Ikegami & Inokuchi, 2000; Lin et al., 2003). Our data showed that St-superAKAP-IS injection into the CA1 area of the dorsal hippocampus specifically reduced the amount of PKA bound to AKAP150 in this area. AKAP79/150 targets PKA to postsynaptic densities in neurons (Dell'Acqua et al., 2006) and is also able to bind PP2B (Dell'Acqua et al., 2002). In vitro studies using the peptide Ht31 showed that displacement of PKA from AKAP75/79/150 shifts the balance to PP2B activity (Snyder et al., 2005). Thus, AKAP79/150 might be an important coordinator of PKA and PP2B activity in memory consolidation and extinction. Recently, we and others provided additional evidence for an important role of AKAP79/150 in learning and memory. Electrophysiological measurements from hippocampal slices of mice with a stop codon inserted into the AKAP150 gene to truncate the last 36 residues, which constitute the PKA binding site, showed the importance of AKAP150-anchored PKA in LTP (Lu, Allen et al., 2007). We observed that AKAP150 is highly abundant in the mouse brain especially in those areas that are known to be involved in learning and memory (Ostroveanu et al., 2007). Moreover, the levels of hippocampal AKAP150 were elevated after exposure of animals to a novel context and during the consolidation phase of contextual fear conditioning, indicating that upregulated levels of AKAP150 contribute to processing the exposure to a novel context and the consolidation of associative learning (Nijholt et al., 2007). Although we cannot exclude the involvement of additional AKAPs, it thus seems likely that at least AKAP79/150 is important in the spatial compartmentalization of PKA signal transduction pathways that are active in the consolidation of contextual fear memories.

Both superAKAP-IS and Ht31 inhibit the anchoring of PKA to several AKAP species. However, whereas Ht31 has the potential to disrupt RII but also some RI mediated localization (Herberg et al., 2000), superAKAP-IS is a peptide that is 10,000-fold more selective for the RII isoform relative to RI (Gold et al., 2006). Our results show that RII anchoring is important in the consolidation and extinction of contextual fear memories. In future experiments the impact of the RI isoform-selective anchoring on learning and memory processes could be assessed using the RI anchoring disruptor (RIAD) (Carlson et al., 2006). To study in greater detail which specific AKAP is involved, it would be necessary to develop inhibitors that disrupt the interaction of PKA with one particular AKAP or to disrupt the interaction of PKA by introducing site-specific mutations in the PKA binding domain of a specific AKAP.

Overall, our data suggest that the temporal and spatial specificity of the hippocampal PKA signaling pathway, mediated by AKAPs, is critical to consolidate long-term contextual fear memory whereas PKA anchoring to AKAPs may put a constraint on extinction.

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