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Dynorphins regulate the strength of social memory

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ABSTRACT

Emotionally arousing events like encounter with an unfamiliar con-species produce strong and vivid memories, whereby the hippocampus and amygdala play a crucial role. It is less understood, however, which neurotransmitter systems regulate the strength of social memories, which have a strong emotional component. It was shown previously that dynorphin signalling is involved in the formation and extinction of fear memories, therefore we asked if it influences social memories as well. Mice with a genetic deletion of the prodynorphin gene Pdyn (Pdyn$^{-/-}$) showed a superior partner recognition ability, whereas their performance in the object recognition test was identical as in wild-type mice. Pharmacological blockade of kappa opioid receptors (KORs) led to an enhanced social memory in wild-type animals, whereas activation of KORs reduced the recognition ability of Pdyn$^{-/-}$ mice. Partner recognition test situation induced higher elevation in dynorphin A levels in the central and basolateral amygdala as well as in the hippocampus, and also higher dynorphin B levels in the hippocampus than the object recognition test situation. Our result suggests that dynorphin system activity is increased in emotionally arousing situation and it decreases the formation of social memories. Thus, dynorphin signalling is involved in the formation of social memories by diminishing the emotional component of the experience.

1. Introduction

During life new memories are continuously formed but most of them quickly fades and will be lost (Lansdale and Baguley, 2008). How long and how vivid a life episode is remembered – sometimes during the whole life – is strongly influenced by the intensity of emotions associated with the event. Highly emotional experiences produce longer lasting memories than emotionally neutral ones (Buchanan, 2007; LaBar and Cabeza, 2006). The forming of strong memories about emotionally arousing events has a clear evolutionary advantage: remembering a cue or environment associated with danger or reward helps to respond adequately to similar situations in the future. However, forming strong emotional memories could significantly contribute to the pathogenesis of anxiety and mood disorders in humans.

The emotional context has an important impact on the strength of social memories (Olson et al., 2013; Somerville et al., 2006). This special form of declarative memory is critical for many aspects of social behaviour like the formation of hierarchies (Terburg et al., 2012; van der Kooij and Sandi, 2012), pair bonding (Hostetler and Ryabinin, 2013), parental care (Neumann, 2008), and social learning (Choleris et al., 2009). Neuropeptides play a significant role in the regulation of social behaviours. It was shown that the central distribution of the V1a arginine/vasopressin receptor and the oxytocin receptor differs between the monogamous prairie voles and the polygamous montane voles (Winslow et al., 1993). The principal role of arginine/vasopressin in pair bonding was further supported testing genetically modified animals. Mice transgenic to prairie vole V1a receptor showed similar receptor distribution as in prairie vole and increased affiliative behaviour after injection with arginine/vasopressin (Young et al., 1999). Recently the contribution of dynorphin signalling in pair bonding maintenance was also suggested: Pharmacological manipulation of its receptor, the kappa opioid receptor (KOR), altered aversive social motivation in prairie vole (Resendez et al., 2012). A prerequisite for monogamous pair bonding is the recognition of the partner whereby oxytocin plays a major role both in animal (Arletti et al., 1995; Ferguson et al., 2000) and in human (Rimmele et al., 2009). Considering the influence of stress on social memory (Cordero and Sandi, 2007), it is not surprising that neuromodulators involved in stress reactivity like the corticotropin releasing hormone (Hostetler and Ryabinin, 2013) or cannabinoids (Bilkei-Gorzo et al., 2012a, 2005) also influence social memory.

Remembering a past event involves a partial reactivation of brain areas, which were active during the event (Buckner and Wheeler, 2001). The hippocampus plays an essential role in this process,

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because its activity is necessary for the recollection of memory traces stored in the separate brain areas (Danker and Anderson, 2010). Social memory, but not immediate partner recognition, is impaired in mice with hippocampal lesions suggesting that the hippocampus is necessary for social memory recall (Kogan et al., 2000). The crucial role of amygdala in the formation and storage of emotional memories was shown both in humans and animals. Functional brain imaging study in humans revealed that the amygdala was more activated by emotional contexts with negative or positive valence than neutral contexts at both encoding and retrieval (Smith et al., 2004). The amygdala is involved in the consolidation of memories of emotionally arousing experiences (McGaugh, 2004), like social interactions (Cassidy and Gutchess, 2012). In good agreement with the hypothesised central role of amygdala in social memory formation enhanced activity of amygdala during social information processing was shown in humans (Cassidy and Gutchess, 2012). Anatomical studies revealed that oxytocin expressing neurons within the medial amygdala mediate social memory and may encode the relevance of social stimulus (Ferguson et al., 2002; Lukas et al., 2013).

The endogenous opioid dynorphin and its Gi protein coupled receptor KOR are present both in the hippocampus and amygdala (Schwarzer, 2009). Within the hippocampus the granular cells in the dentate gyrus express prodynorphin. Dynorphin A and B proteins, the splice products of prodynorphin can be localised in the mossy fibre projection area (McGinty et al., 1983). When released, dynorphins modulate the information flow between the dentate gyrus and the CA3 region of the hippocampus by decreasing excitatory glutamatergic signalling (Simmons and Chavkin, 1996) and thus blunting hippocampal activity. Within the amygdala the central nucleus contains high level of prodynorphin whereas the basolateral nucleus has a high density of KOR (Schwarzer, 2009).

In stressful, aversive situations dynorphins are released (Christiansen et al., 2011; Palkovits, 2000; Shirayama et al., 2004) and besides mediating stress responses (Bilkei-Gorzo et al., 2008; McLaughlin et al., 2003; Suh et al., 2000) they also play an important role in the generation and extinction of fear memories. It was suggested that KOR signalling encodes the aversive emotional component of the stress-related event (Bruchas et al., 2007) and contribute to the stress-induced learning and memory deficits (Carey et al., 2009). We have recently shown that dynorphins and their receptors modulate the plastic changes in fear memories both in humans and animals (Bilkei-Gorzo et al., 2012b). We now ask, whether dynorphin signalling has a more general effect regulating also the strength of social memories or does it specifically affect fear memories. To answer this question we tested animals with genetically or pharmacologically altered dynorphin signalling in the object and partner recognition tests. These paradigms have a very similar logic and experimental setup, but the partner recognition test is used to assess social memory (van der Kooij and Sandi, 2012) whereas object recognition test is a more general test for episodic memory (Dere et al., 2005).

2. Materials and methods

2.1. Animals

The behavioural experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the *Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen*. For the experiments 42 wild-type ($Pdyn^{+/+}$) and 26 dynorphin null-mutant ($Pdyn^{-/-}$) (Zimmer et al., 2001) male mice were used. The animals were 3–5 month old and they were kept under a reversed light–dark cycle (lights on: 19:00; lights off: 9:00) in groups of 3–5.

2.2. Object recognition test

Animals were tested in an open-field arena (44 cm × 44 cm) in dimly lit, sound isolated environment. The floor of the arena was covered with sawdust saturated with the odour of mice. Animals were tested in 7 consecutive days. In the first four days mice were habituated for 5 min daily to the test environment. After habituation at day 5, 6 and 7 in the first session two objects were placed into the arena (identical plastic balls, diameter 2 cm, objects A) and mice were allowed to explore the area and the objects for five minutes. After an interval of 1 h (at day 5), 2 h (day 6) or 4 h (day 7) in the second session mice were put again for five minutes into the same box where one familiar and one novel object (similar size, different in shape (oval) and colour, object B) were placed. The behaviour of mice was video taped, time spent with inspections of each object (TA for the familiar object and TB for the novel object) was determined by an observer blind to genotype using "The Observer" software (Noldus, Netherlands). Groups were compared using three-way ANOVA (between effects: genotype and duration of interval; within effect: novelty). Novelty preference was calculated as TB/(TA + TB)*100 and plotted. Duration of recognition was additionally assessed as the longest interval between the sessions where significantly higher TB as TA was detected using Bonferroni's *t*-test (Reibaud et al., 1999).

2.3. Partner recognition test

The experiment was carried out similarly as the object recognition test with a different group of animals. The floor of the arena was covered with sawdust saturated with the odour of mice. Animals were tested in 11 consecutive days. In the first four days the animals were habituated for five minutes daily to the test environment. The trials in the test phase consisted of two sessions: First we put the animals into the familiar arena, and time spent with interaction with a 4-week-old male DBA/2J-$Penk1^{-/-}$ mouse was evaluated for 5 min using "The Observer" software (Noldus, Netherlands) by an observer unaware of the genotype or treatment. We selected DBA/2J-$Penk1^{-/-}$ mice as partner because these animals have a distinctive fur colour and they seldom initiate social contact (Bilkei-Gorzo et al., 2004), therefore the test animals initiated the vast majority of the interactions. In the second session after a variable interval (day 5 – 1 h, day 6 – 4 h, day 7 – 8 h, days 8 and 9 – 16 h and days 10 and 11 – 24 h) the same pairs were placed again in the arena and time of interaction was evaluated as in the first session for five minutes. Groups were compared using three-way ANOVA (between effects: genotype and duration of interval; within effect: session) followed by Bonferroni's *t*-test. Recognition index was calculated as (T2 – T1)/(T1)*100 where T1 is the time spent with social interaction in the first session and T2 is the time spent with social interaction in the second session and plotted. Additionally, duration of recognition was assessed as the longest interval when the interaction time in the second session was significantly lower as in the first session (Bilkei-Gorzo et al., 2005) according to Bonferroni's *t*-test.

2.4. Partner recognition test after drug treatment

For the pharmacological treatments the kappa opioid receptor (KOR) blocker nor-binaltorphimine (norBNI) and the KOR agonist U-50488 was purchased from Sigma–Aldrich. The experiment was carried out similarly as the partner recognition test described above with different groups of animals. Animals were tested in 9 consecutive days. After four habituation trials mice were treated intraperitoneally 30 min before the first session with 1 mg/kg norBNI (wild-type animals) or with 1 mg/kg U-50488 ($Pdyn^{-/-}$ mice). The interval between the sessions was 8 h (day 5), 16 h (days 6–7) and 24 h at days 8–9. Partner recognition ability was assessed using two-way ANOVA (between effect: duration of inter-trial; within effect: session) followed by Bonferroni's *t*-test. Recognition index, duration of recognition was calculated as described above.

2.5. Dynorphin A and B immunoreactivity

Four animals per group were killed 2 h after the first session of the object or social recognition test to determine dynorphin A and B levels in the hippocampus and amygdala. The brains were removed after transcardial fixation with 4% paraformaldehyde (PFA) solution, post-fixed at 4 °C in PFA solution for 90 min and equilibrated in 10% sucrose solution for 24 h. Subsequently, they were shock frozen and stored at −80 °C until further processing. Brains were sliced in a cryostat at 16 μm thickness. The sections were labelled using rabbit anti-dynorphin A or dynorphin B primary antibody (both Abcam, UK) and a biotinylated donkey anti-rabbit-IgG secondary antibody (Jackson Laboratories, USA). Staining was performed with the ABC-Kit (Vector Laboratories, USA). As controls we used four additional wild-type animals that were habituated to the open-field arena as animals in the object and partner recognition test groups, but on the test day they were exposed only to the empty open-field arena for five minutes. Control mice were killed 2 h after the test, their brains were fixed, prepared, sliced and stained as described above. Quantitative analysis of the sections was done by an experienced researcher blind to the experimental groups. Images of the sections were taken using a standard light microscope (Zeiss, Axioplan 2 imaging) connected to a digital camera (KY-F75K, JVC, Japan). Dynorphin levels were determined in the central and basolateral amygdala, and in mossy fibre area of the CA3 region of the hippocampus. For quantification pictures were converted to 8-bit grey scale and the mean signal intensities (calculated as total signal intensity divided by the area within the region of interest (ROI)) were determined using the ImageJ software. For statistical analysis

four-six representative slides were evaluated from each animal. Groups were compared using one-way ANOVA followed by Bonferroni test.

3. Results

3.1. Object recognition test

A significant effect for novelty ($F_{1,56} = 27.33$; $p < 0.001$) indicated that mice recognised the new object and displayed novelty preference. They spend significantly more time investigating the novel object compared to the familiar one (Suppl. Fig. 1). This novelty preference was neither influenced by the duration of the interval between trials (novelty × interval: $F_{2,56} = 2.137$; $p > 0.05$), nor by the genotype of the animals (novelty × genotype $F_{1,56} = 1.310$; $p > 0.05$) (Fig. 1). We also did not find interaction between novelty × genotype × interval ($F_{2,56} = 0.150$; $p > 0.05$). However, Bonferroni *t*-test revealed that the maximum duration of object recognition was 2 h in wild-type and Pdyn$^{-/-}$ mice (Fig. 1).

The time animals spent investigating the objects was significantly influenced by the genotype ($F_{1,56} = 18.94$; $p < 0.001$), by the duration of the interval ($F_{2,56} = 6.441$; $p < 0.01$), and we also found a significant genotype × interval interaction ($F_{2,56} = 8.184$; $p < 0.001$). As shown at Suppl. Fig. 1, wild-type mice spent more time investigating the objects as Pdyn$^{-/-}$ mice. The interaction time increased in wild-type, but decreased in Pdyn$^{-/-}$ mice animals when the duration of interval was increased from 2 h to 4 h (Suppl. Fig. 1).

3.2. Partner recognition test

Time spent with social interactions was significantly lower in the second session (session: $F_{1,89} = 120.6$; $p < 0.001$) indicating that the animals recognised their previously seen partner. The partner recognition was significantly influenced by the duration of the interval between the sessions (session × interval interaction: $F_{4,89} = 3.831$; $p < 0.01$) and differed between the genotypes (session × genotype interaction: $F_{1,89} = 3.965$; $p < 0.05$) (Fig. 2A). We did not find interaction between genotype × interval × session ($F_{4,89} = 0.594$; $p > 0.05$). The maximum duration of partner recognition was 4 h in Pdyn$^{+/+}$ mice, whereas Pdyn$^{-/-}$ animals recognised the previously seen partner up to 24 h after the first presentation (Fig. 2A).

Time spent with social interactions was significantly influenced by the genotype ($F_{1,89} = 48.80$; $p < 0.001$), by the interval duration ($F_{4,89} = 6.852$; $p < 0.001$), whereas no genotype × interval interaction was observed ($F_{4,89} = 2.270$; $p > 0.05$). As shown in Suppl. Fig. 2A, Pdyn$^{-/-}$ mice spent more time with social interactions as wild-type animals.

When we evaluated the social memory of KOR antagonist-treated Pdyn$^{+/+}$ mice, we found a significant effect for the session ($F_{1,25} = 32.68$; $p < 0.001$), but not for the duration of the interval ($F_{2,25} = 1.348$; $p > 0.05$). There was no interaction between session and interval ($F_{2,25} = 0.643$; $p > 0.05$) (Suppl. Fig. 2B). Bonferroni's *t*-test revealed that mice injected with norBNI recognised their previously shown partner up to 24 h (Fig. 2B). When the KOR agonist U-50488 was administered to Pdyn$^{-/-}$ animals, we found a significant effect of session ($F_{1,24} = 19.49$; $p < 0.001$) showing that the animals readily recognised their previously presented partner. This ability was significantly influenced by the duration of the interval ($F_{2,24} = 8.783$; $p < 0.01$) (Suppl. Fig. 2C). Bonferroni's *t*-test showed that Pdyn$^{-/-}$ mice treated with the KOR agonist U-50488 could recognise their previous partner only for 16 h but not longer (Fig. 2C). The time spent with social interaction was not influenced by the duration of session in this group ($F_{2,24} = 0.078$; $p > 0.05$).

3.3. Dynorphin A and B immunoreactivity

The intensity of dynorphin A immunoreactivity in the central and basolateral amygdala and in the mossy fibre area of the hippocampus differed significantly between control animals and animals exposed to the object or social recognition tests (central amygdala: $F_{2,58} = 84.60$; $p < 0.001$; basolateral amygdala: $F_{2,58} = 67.57$; $p < 0.001$; hippocampus: $F_{2,58} = 18.84$; $p < 0.001$). Post hoc analysis of the data with Bonferroni correction revealed that dynorphin A levels were increased after the partner recognition test in each brain area. Object recognition test itself induced a significant elevation in the central and basolateral amygdala but not in the hippocampus compared to control animals (Fig. 3A and B). Dynorphin A immunoreactivity was more intensive after partner recognition as after object recognition test situation in each three areas.

When we assessed dynorphin B immunoreactivity, we received an almost identical picture: significant difference in dynorphin B levels between the groups in each three brain regions: (central amygdala: $F_{2,63} = 31.62$; $p < 0.001$; basolateral amygdala: $F_{2,63} = 27.56$; $p < 0.001$; hippocampus: $F_{2,58} = 28.25$; $p < 0.001$). Dynorphin B levels were higher after each recognition test then after exposing the animals to an empty open-field (Fig. 4A and B). The elevation in dynorphin B levels was significantly higher in the hippocampus, but not in the amygdala after partner recognition then after object recognition test.

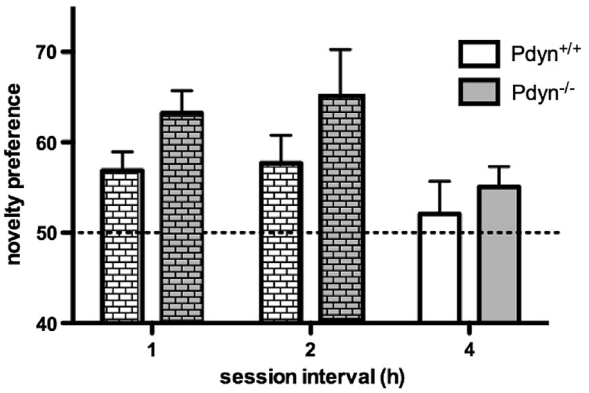

Fig. 1. No difference on novelty preference in the object recognition test between Pdyn$^{+/+}$ and Pdyn$^{-/-}$ mice. Bars show group means, errors bars represent standard error of mean (SEM) ($n = 10$–12). Stacked bar represents significant novelty preference according to Bonferroni's *t*-test.

4. Discussion

Multiple lines of evidences showed that dynorphin signalling is involved in the generation and extinction of stress-related memories (Bilkei-Gorzo et al., 2012b; Bruchas et al., 2007; Carey et al., 2009). In this work we have shown that activation of KOR by dynorphins released after an emotionally arousing situation suppressed the intensity of emotional memories unrelated from their valence.

The duration of social partner recognition was at least six times longer in animals lacking dynorphin as in wild-type mice, whereas the object recognition ability was similar between the lines. This result is surprising at the first sight, because there is a high similarity between the tests: both are models of declarative memory, the duration of habituation and the trials, the open-field arenas used for the tests are the same. However, the emotional arousal during the events – meeting an inanimate object or a con-species in a familiar environment – is clearly different. For wild-type

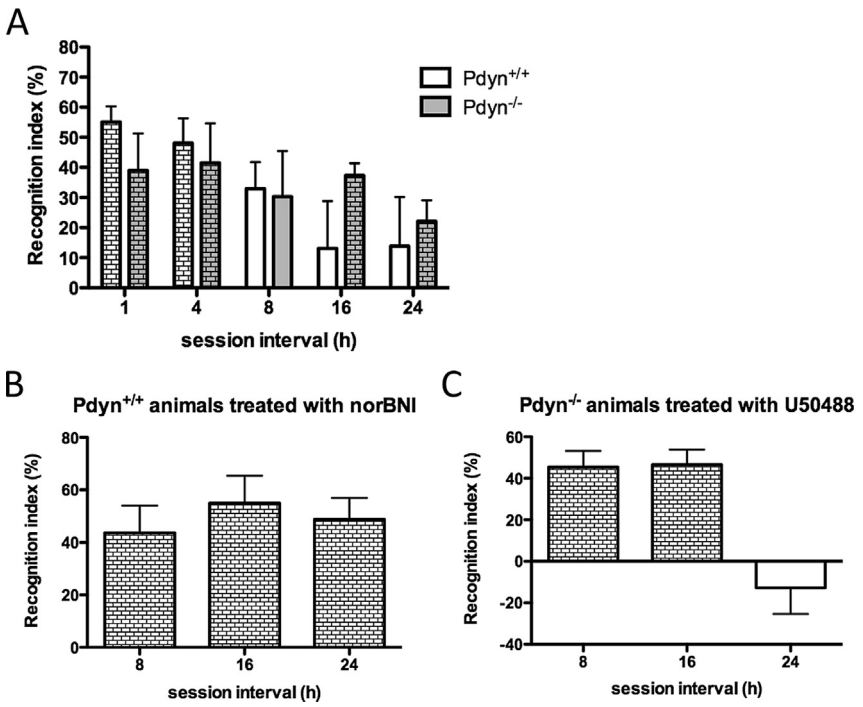

Fig. 2. Partner recognition test. **(A)** In Pdyn$^{+/+}$ mice the maximal duration of partner recognition was 4 h, Pdyn$^{-/-}$ animals remembered to their previously seen partner 24 h long. Stacked bar represents significant recognition according to Bonferroni's *t*-test. **(B)** Pdyn$^{+/+}$ animals treated with the KOR antagonist norBNI recognise the previously presented partner 24 h after the first trial. **(C)** The duration of partner recognition is 16 h in U-50488 treated Pdyn$^{-/-}$ mice. Bars show group means, errors bars represent standard error of mean (SEM) (n = 10–12).

C57BL/6 animals the interaction with an unknown partner animal is clearly rewarding: they show higher preference to a partner animal than to an object (Crawley et al., 2007). Moreover, it is possible to use social interaction as reward for place preference conditioning (Panksepp and Lahvis, 2007). The fact that dynorphin knockout animals had superior partner recognition ability and fear memory suggests that dynorphin suppresses the strength of emotional memory in general – unrelated from the valence of the experience.

The time animals spent inspecting the objects or partner animals may have an effect on the strength of memory and it was indeed influenced by the duration of the interval and by the genotype in both models. Moreover, we found a significant interaction between genotype and interval in the object recognition test reflecting the fact that the inspection time increased in wild-type whereas decreased in knockout animals during the consecutive experimental days (Suppl. Fig. 1). It is likely that the animals habituated to the presence of objects during the experiments, which can increase exploratory activity by reducing aversion against the new situation, but in the same time it may decrease exploration by diminishing curiosity and thus the exploratory drive. Our result suggests that in wild-type animals the decrease in anxiety facing repeatedly the same experimental condition dominated, whereas in Pdyn$^{-/-}$ mice rather a decrease in exploratory drive. The animals probably remembered the experimental situation, but not the objects in the following experimental days because they could not discriminate between familiar and novel object when the session interval was more than 2 h. Although the strains differed in time spent with explorations, the duration of object recognition was the same suggesting that the genotype effect on exploratory activity did not influence object recognition ability.

In the partner recognition test we found a decline in exploratory activity during consecutive experimental days in both strains and a higher exploration in Pdyn$^{-/-}$ mice (Suppl. Fig. 2A). Although we used different partner animals in each experimental day, the decline in interaction time reflects a diminishing exploratory drive. The higher time spent with interactions may indicate a lower anxiety of the Pdyn$^{-/-}$ strain in the test situation, an enhanced social drive or the combination of both factors. The longer period with partner interactions in Pdyn$^{-/-}$ mice may contribute to the enhanced partner recognition ability of the knockout strain.

Pharmacological manipulation of KORs further supported this hypothesis, because antagonism of KOR activity with norBNI improved social memory similar to what was observed in mice with genetic deletion of Pdyn. As a caveat it is important to note that for technical reasons we lost the data with vehicle treated animals. Nevertheless, it is unlikely that the injection-induced mild stress elicited a six-fold increase in the social memory of wild-type animals and decreased memory in Pdyn$^{-/-}$ mice: Previous studies showed that forced swim stress had an opposite effect as seen here: it impaired recognition ability in wild-type animals but had no effect on the memory of dynorphin deficient mice (Carey et al., 2009).

Although pharmacological activation of KORs clearly lacks the temporal and spatial resolution of the dynorphin release in the test situation, it still reduced the social memory of Pdyn$^{-/-}$ mice: the KOR agonist U-50488 diminished the partner recognition ability of Pdyn$^{-/-}$ mice. It is important to note that the pharmacological treatment with norBNI and U-50488 may alter KOR activity in the nucleus accumbens and thus may alter the rewarding effect of the social interaction (Chefer et al., 2005; DePaoli et al., 1994). This could additionally contribute to the observed drug effects in the partner recognition test. Similarly to our result previous studies generally found an amnesic effects of dynorphins (Magnusson et al., 2009) or synthetic KOR agonists (Castellano et al., 1988; Sandin et al., 1998; Schindler et al., 1987). Based on these findings it was assumed that the increase in Pdyn expression in ageing contributes to the development of age-related cognitive deficits (Jiang et al., 1989; Nguyen et al., 2005) by decreasing glutamate

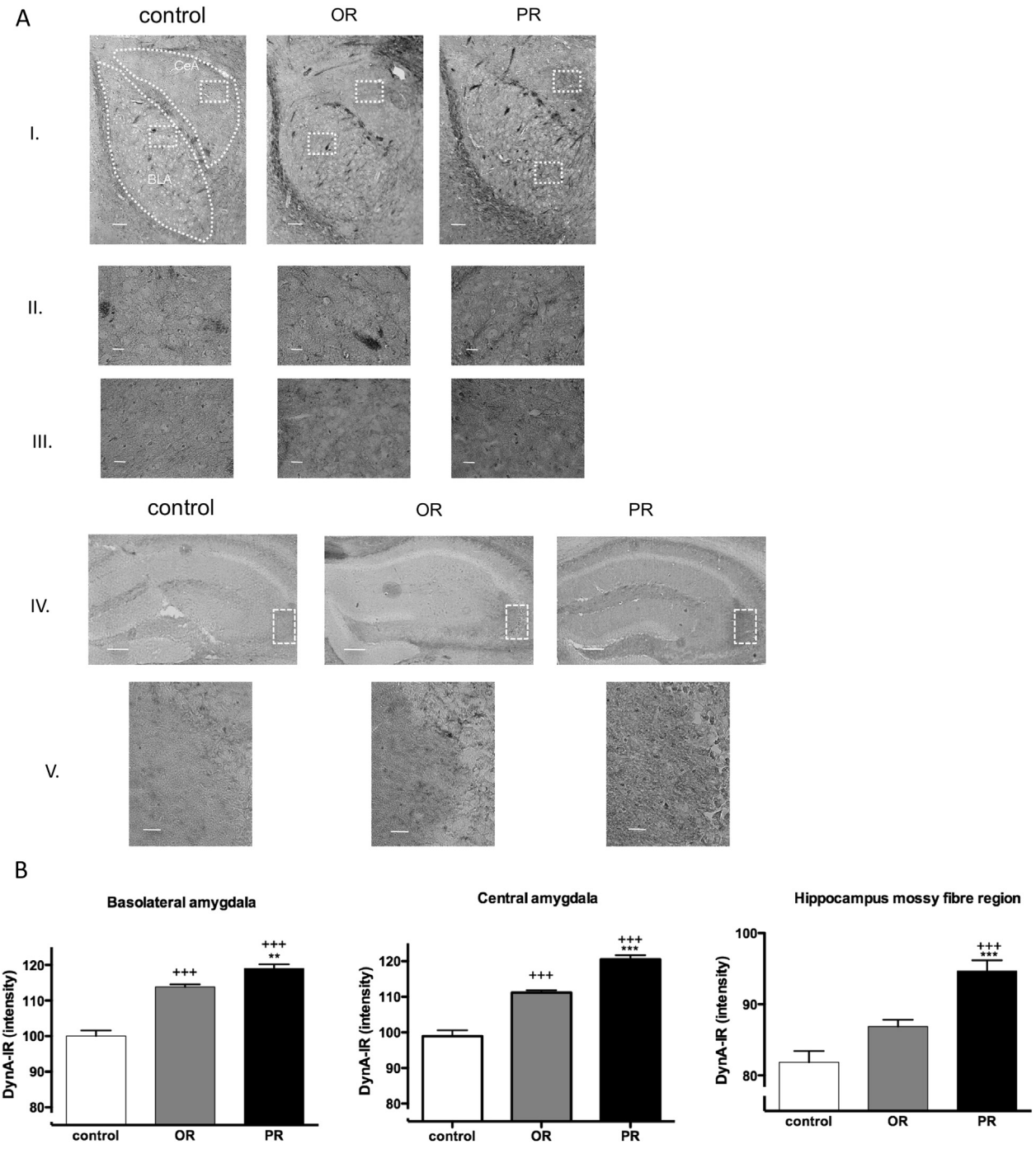

Fig. 3. Dynorphin A immunoreactivity. **(A)** Representative microphotograph of the **I**: Amygdala. Cea – central nucleus of the amygdala; BLA – basolateral amygdala **II**: Higher magnification of the indicated area within the basolateral amygdala. **III**. Higher magnification of the indicated area within the central nucleus of the amygdala. **IV**. Hippocampus. **V**. Higher magnification of the indicated area within the mossy fibre area of the CA3 region of the hippocampus. Control – animals exposed to an empty open field. OR – animals after object recognition test. PR – animals after partner recognition test. Scale bars represent at panel I: 200 μm; at panel II, III and V: 20 μm and at panel IV. 400 μm. **(B)** Semiquantitative analysis of dynorphin A signal intensities. Dyn A levels were higher in mice from OR or PR groups in the amygdala and in the PR group in the hippocampus as in animals from the control group. PR elicited significantly higher dynorphin A immunoreactivity in each three areas as OR. Bars show group means, errors bars represent standard error of mean (SEM). $^{**}p < 0.01$ $^{***}p < 0.001$ OR vs. PR; $+++p < 0.001$ control vs. OR or PR by one-way ANOVA followed by Bonferroni's *t*-test ($n = 3–4$).

signalling (Zhang et al., 1991). The possible relation between dynorphin signalling and cognitive abilities was supported by a human genetic study (Kolsch et al., 2009). It is unclear, however, how decreased KOR activity influences memory, because in previous studies each improvement (Colombo et al., 1993; Jamot et al., 2003), deficit (Fanselow et al., 1991) and no change (Magnusson et al., 2009) was reported.

We considered also two alternative hypotheses to explain the higher partner recognition ability of Pdyn$^{-/-}$ mice. Time spent with social interaction in the first session of the partner recognition test was higher in Pdyn$^{-/-}$ mice (Suppl. Fig. 2A), which may contribute to the observed differences in the social memory between the strains. However, pharmacological blockade of KOR also increased social memory but did not influence social time in the first session

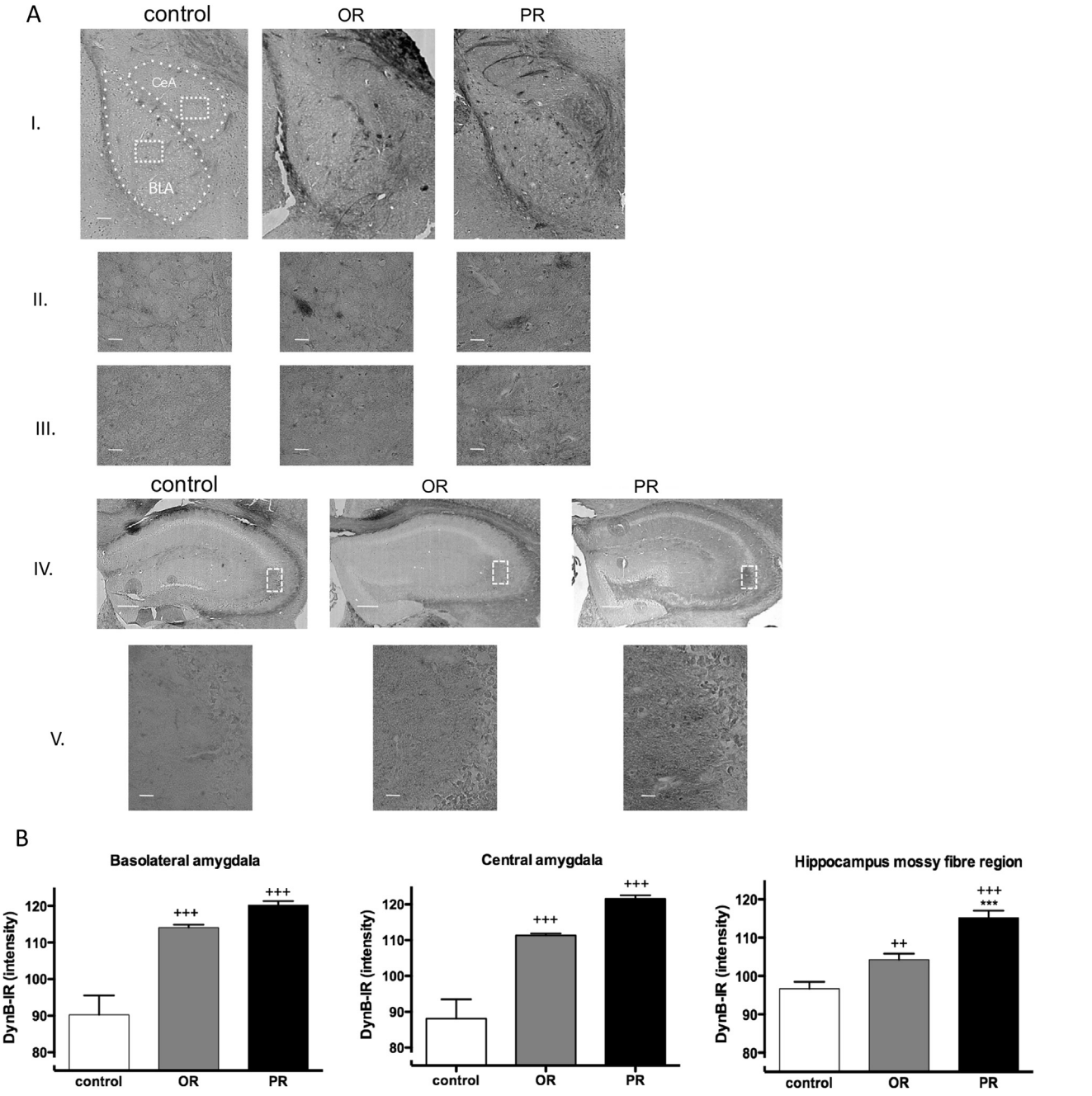

Fig. 4. Dynorphin B immunoreactivity. **(A)** Representative microphotograph of the **I**: Amygdala. Cea – central nucleus of the amygdala; BLA – basolateral amygdala **II**: Higher magnification of the indicated area within the basolateral amygdala. **III**. Higher magnification of the indicated area within the central nucleus of the amygdala. **IV**. Hippocampus. **V**. Higher magnification of the indicated area within the mossy fibre area of the CA3 region of the hippocampus. Control – animals exposed to an empty open field. OR – animals after object recognition test. PR – animals after partner recognition test. Scale bars represent at panel I: 200 μm; at panel II, III and V: 20 μm and at panel IV. 400 μm. **(B)** Semiquantitative analysis of dynorphin B signal intensities. Dynorphin B levels were higher in animals from OR or PR groups as in animals from the control group. PR elicited significantly higher dynorphin B immunoreactivity in the mossy fibre region of the hippocampus. Bars show group means, errors bars represent standard error of mean (SEM). $^{***}p < 0.001$ OR vs. PR; $+++p < 0.001$ control vs. OR or PR by one-way ANOVA followed by Bonferroni's *t*-test ($n = 3–4$).

(Suppl. Fig. 2B). Thus, decreased activity of KOR is enough to improve partner recognition ability. Another alternative possibility is that dynorphins influence the formation of olfactory memories. Olfactory signals are essential for partner recognition in rodents (Hurst et al., 2001), therefore altered olfactory abilities may influence the performance of mice in the partner recognition test. It is known that dynorphins are expressed in the olfactory bulb and olfactory tubercle (Lin et al., 2006), therefore they may influence olfaction. It was shown that Pdyn$^{-/-}$ mice fail to develop aversion against stress-paired odorant, which might suggest the contribution of dynorphin signalling in olfactory learning. However, conditioned place preference to reward (cocaine) associated odours was developed normally in norBNI-treated animals, suggesting that dynorphin encodes aversive component of the stressful experience but does not influence olfactory learning per se (Land et al., 2008).

Our histological study suggests that the higher the emotional component of the event, the more dynorphin released in key areas for the emotional memory: Dynorphin A levels were higher after

partner recognition compared to object recognition in the hippocampus and amygdala, whereas dynorphin B expression was higher in the hippocampus after partner recognition compared to object recognition. A similar increased dynorphin A and B level in the hippocampus was reported after stress (Shirayama et al., 2004). This elevation probably plays a significant role in the memory-impairing effect of dynorphin, because its hippocampal release impaired memory performance (McDaniel et al., 1990; Sandin et al., 1998). In the hippocampus, the source of the increased dynorphin levels is most probably the granular neurons in the dentate gyrus, which express Pdyn on a relatively high level. Dynorphin released by this neuronal population suppresses mossy fibre signalling and decreases the activity of pyramidal neurons of the CA3 region (Salin et al., 1995). Nevertheless, dynorphins inhibit long term potentiation also at the perforant-path granule cell synapse (Terman et al., 1994), which may further contribute to the inhibitory effect of dynorphins on hippocampal activity. The source of dynorphin in the amygdala is less clear. The central nucleus of the amygdala contains relatively high levels of Pdyn, therefore it is possible that dynorphin released by local neurons is the source of the increased immunoreactivity (Schwarzer, 2009). Although in this area the KOR levels are rather low, this locally released dynorphin may influence amygdalar activity. Besides the central nucleus of the amygdala we also found enhanced dynorphin levels in the basolateral amygdala, area containing high KOR levels. It is unlikely that this dynorphin is released locally or from the CeA, because in the BLA Pdyn levels are low and the main intraamygdaloid signal pathway runs from the BLA to the CeA (Ehrlich et al., 2009; Pitkanen et al., 1997). Most probably dynorphin in the BLA is originated from the cortex through the cortico-amygdaloid pathway (Bilkei-Gorzo et al., 2012b; Yakovleva et al., 2006). The presence of dynorphin, like in the hippocampus, decreases synaptic transmission and inhibits the long-term potentiation induction also in the basolateral amygdala (Huge et al., 2009).

Our results altogether suggest that dynorphin signalling will be activated and suppresses memory formation not only in fearful (Bilkei-Gorzo et al., 2012b; Cole et al., 2011) but also in emotionally arousing situations unrelated from its valence (LaBar and Cabeza, 2006), thus regulating the strength of emotional memories.

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Appendix A. Supplementary data

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