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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