Glucocorticoid signaling in a rat model of post-traumatic stress disorder

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The role of β-arrestin-2 on Fear/anxious-related memory in a rat model of post-traumatic stress disorder

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Abstract

Background: Post-traumatic stress disorder (PTSD) can be categorized as a disorder of dysregulated fear processing. In the formation and development of PTSD, whether fear/anxious-related memory is involves changes in β-arrestin-2, and its associated signal transduction pathways remains unknown.

Method: We used the single prolonged stress (SPS) as a rat model of PTSD. Next, the elevated plus maze test (EPM) was performed to examine fear/anxious memory- related behaviors. Then, we determined β-arrestin-2, PDE-4, and signal transduction pathways with immunofluorescence, co-immunoprecipitation, immune-histochemistry, Elisa, western blot, and real-time PCR in the basolateral amygdala.

Results: Our data indicated that SPS enhanced fear/anxious memory-related behaviors. This was associated with low expression of β-arrestin-2, PDE-4 and their complex, and high activity of signal transduction pathways 7 days after SPS.

Conclusions: The data indicate that β-arrestin-2 may be involved in the formation of abnormal fear/anxious memory in PTSD; through activation the signal transduction pathways. This may be relevant for the formation and development of PTSD.

1. Introduction

Post-traumatic stress disorder (PTSD) is an anxiety disorder arising as a certain severe psychological consequence of exposure to, or confrontation with, stressful events that a person experiences as highly traumatic. PTSD can be categorized as a disorder of dysregulated fear processing [1]. Fear/anxious memory is a form of emotional memory that recruits the amygdala [2-4] and is often disturbed in individuals suffering from PTSD [5-7].

The amygdala has been implicated in the storage and expression of fear/anxious memory in both animal [2, 3, 8] and human studies [9]. The amygdala can be divided into three distinct subgroups: central nucleus (CeA), corticomedial nucleus (MeA) and basolateral nucleus (BLA) [10]. BLA is the largest among these three and is the key region for the initiation of fear/anxiety.

Many signal molecules, such as protein kinase A (PKA), are involved in fear memory consolidation. Accumulating evidence revealed that the formation of associative fear/anxious memory involved multiple signal cascades, including cAMP- PKA and ERK- MAPK. It was revealed that perfusion of the PKA or ERK inhibitor into lateral amygdala (LA) before fear conditioning results in the impairment of fear memory [11, 12]. This PKA signal transduction pathway is necessary for the formation of long-term memory. Various activated signaling cascades converge upon transcription factors within the nucleus. cAMP response element binding protein (CREB), a key target of PKA, is one particular transcription factor that is responsible for regulating protein synthesis. Phosphorylation of CREB at Ser133 occurs when upstream signaling cascades get activated. CREB is also activated in the amygdala after fear conditioning [13]. Therefore, the cAMP- PKA- CREB signal transduction pathway is involved in the physiological processing of fear/anxious memory.

β-arrestins, including β-arrestin-1 and β-arrestin-2, play a critical role in a wide variety of physiological and pathophysiological cellular processes [14] and are found in high abundance in the immune and central nervous systems [15, 16]. Of the two types, β-arrestin-2 is widely

distributed but functions in PTSD are remains unknown. Emerging evidence implicates that β-arrestin-2 may play an important role in regulating basic brain functions, particularly fear/anxious memory formation and in the synaptic plasticity of the amygdala. β-arrestin-2 was reported to be a key molecule of feedback regulating cAMP signal transduction pathways [17]. In the mechanism regulating stress and anxiety responses, β-arrestin-2 recruitment also play an important role [18].

PDE-4 can interfere with the formation of long-term memory by its mechanism of degradation of specific enzymes of cAMP; this leads to a decrease in cAMP levels and alteration of the cAMP- PKA- CREB signaling pathways. Therefore, CREB-dependent gene expression and the synthesis of the associated proteins that involved in learning and memory are attenuated after PDE-4 activation. Therefore, an optimum PDE-4 activity is required for normal conditioning of fear memory [19]. β-arrestins are known to recruit PDE-4, thus controlling PKA activity at the membrane [20, 21]. Accordingly, PDE-4 a role in both memory and anxiety, and several lines of evidence suggest specific inhibition of PDE4B as a promising therapeutic approach for disorders of memory and anxiety [22].

PTSD likely involves changes in the amygdala, leading to enhanced fear/anxious memory. However, to date, in the process of formation of PTSD, the roles of β-arrestin-2 and PDE-4 in the regulation of fear/anxious memory remain unknown. It is also uncertain whether changes in signal transduction pathways are part of the PTSD pathophysiology.

Here we evaluate the activity of β-arrestin-2 and PDE-4 as essential modulators of regulating amygdala PKA activity, in response to fear/anxious memory formation in the SPS model of PTSD. Our results suggest that β-arrestin-2, PDE-4 and signal transduction pathways may be involved in the formation and development of PTSD.

2. Materials and methods

2.1 Animals

Male Wistar rats (China medical university, about 8 weeks old, weighing 150-180 g) were used

for all experiments. All rats were housed in the experimental animal facility for a week to let them acclimate to their new environment (temperature: (22±1 °C, humidity: 50~60%, lights on: 07:00~19:00). Standard food pellets and tap water were available ad libitum. All procedures followed the National Guidelines on Animal Care.

The SPS procedure is internationally recognized method for the preparation of an animal model of PTSD [23]. SPS is one of the animal models proposed for PTSD [24]. The SPS rats show enhanced inhibition of the HPA axis, which has been frequently demonstrated in patients with PTSD. In brief, the SPS model consisted of a 2 h whole body restraint in an acrylic animal holder, which was followed immediately by 20 min forced swimming (temperature: 25 °C, water depth: 40 cm). These rats were then allowed to recuperate for 15 min. Next, the rats were exposed to ether vapor until loss of consciousness [25, 26] and then placed to their home cages and left undisturbed until the behavioral testing. The rats were divided randomly into four groups (15/group), including three SPS groups (1d, 7d, and 14d) and the control group. For each group, three rats were used for histological analysis, three for Elisa, three for Western blotting, and three for Real-Time PCR.

2.2 Behavioral test -Elevated Plus Maze (EPM) test

All rats of each group underwent the behavioral test (EPM test) at two hours before being killed. The EPM apparatus consists of a plus-shaped maze elevated above the floor with 2 oppositely positioned closed arms (50 cm × 10 cm), 2 oppositely positioned open arms (50 cm × 10 cm), and a center area (10 × 10 cm). At the beginning, rats were placed in the central area of the maze, facing a closed arm. Behavior was recorded with a video camera during 5 min. The number of entries into open arms, into closed arms and the time spent in the open arms, in the closed arms were measured. The percentage of open arm entries (number of entries into the open arm/total number of entries in both arms), and the percentage of time in the open arms (time in the open arms /the time in both arms) were calculated. The measures of fear/ anxiety are the percentage (%) of open arm entries and the percentage (%) of time spent

on the open arms.

2.3 Fixation and sections making

Rats of each group were anaesthetized with 50 mg/kg body weight sodium pentobarbital and then infused with 500 ml of 0.01 M PBS (pH 7.4) including 4% paraformaldehyde. The brains were rapidly removed and put into the same fixative for 24 h at 4 °C. The brains were immersed in 30% sucrose in 0.1 M PB for 3 days for cryoprotection. The brain tissue was cut into slices of 14 um thickness using a cryostat (Leica CM 3050, Germany).

2.4 Double immunofluorescent labeling for β-arrestin-2 and PDE-4

The sections were incubated with mouse monoclonal antibody against β-arrestin-2 (Santa Cruz, USA; 1:200) plus rabbit polyclonal antibody against PDE-4 (Santa Cruz, USA; 1:200) overnight at 4 ℃. After three times washing, the sections were incubated with FITC anti-mouse IgG (Company of Zhongshan Goldenbridge, Beijing, China; 1:1000) plus CY3 anti-rabbit IgG (Company of Zhongshan Goldenbridge, Beijing, China; 1:1000) for 0.5 h at room temperature. After being washed in PBS and mounted. Confocal laser scanning microscope was applied for colocalization observation.

Six slides were randomly selected from each group. Each slide was randomly selected five visual fields in BLA (×40). The immunoreactivity of β-arrestin-2 and PDE-4- immuno-positive cells were collected using an EZ-C1 Thumbnailler morphology image analysis system.

2.5 Western blotting used to detect β-arrestin-2, PDE-4 and PKA

Rats were decapitated, and the brain were removed and immediately placed in an ice-cold dish. Then BLA was dissected according to the atlas (Paxinos and Watson, 1998) by use of a stereomicroscope. Fresh BLA tissue samples of control rats and SPS rats were respectively homogenized with a sample buffer and were denatured by boiling for 3 min. Samples were loaded on a 10%SDS- polyacrylamide gel, and electroblotted onto a PVDF membrane (Millipore Corp., Bedford, MA) from the gel by a semi-dry blotting apparatus (Bio-Rad Laboratories, Inc,

Hercules, CA). The blotted membrane was then blocked with 1.5% skim milk, 0.05% Tween-20 in TBS (TBST) at 4 ℃ overnight, and then incubated with 1:500 mouse monoclonal antibody against β-arrestin-2 (Santa Cruz, USA), 1:500 rabbit polyclonal antibody against PDE-4 (Santa Cruz, USA) and 1:500 rabbit polyclonal antibody against PKA (Santa Cruz, USA) at 4 ℃ for 24 h. Blots were washed three times with TBST, and then incubated with a second antibody (anti-mouse or anti-rabbit IgG-HRP from Santa Cruz, USA; 1:5000) for 2 h at room temperature. After incubation, blots were washed three times with TBST before visualization by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech). To confirm equal protein loading, the same blots were incubated with antibodies specific for GAPDH (Abcam, British; 1:1,000). The protein levels of β-arrestin-2, PDE-4 and PKA were determined by calculating the OD ratio of β-arrestin-2 /GAPDH, PDE-4 /GAPDH and PKA/GAPDH. The OD of β-arrestin-2, PDE-4 and PKA were analyzed on the Gel Image Analysis System (Tanon 2500R, Shanghai, China). The procedures were repeated 3 times to obtain the average value.

2.6 Assessing the interaction of β-arrestin-2 and PDE-4 using Co-immuno- precipitation

The protein samples were extracted from the fresh BLA tissues, and then mixed with non-specific mouse or rabbit immunoglobulin G and the fully resuspended Protein A+G Agarose (Beyotime Institute of Biotechnology) and slowly shaken at 4 °C for 2 h. 2500 rpm for 5 min and the supernatant was used for subsequent immunoprecipitation. Mouse antibody against β-arrestin-2 or rabbit antibody against PDE-4 (Sata Ltd.) was added at 4 °C, the mixture was slowly agitated overnight and then fully resuspended in Protein A+G Agarose with 4 °C with gentle agitation for 2 h, 2500 rpm for 5 min, PDE-4 or β-arrestin-2 protein was immunoprecipitated from the whole cell lysates. Immuno-precipitates were washed, and subsequently subjected to western blot analysis using anti- PDE-4 or anti- β-arrestin-2 antibody.

2.7 Using Elisa to detect the concentration of cAMP

After the evaporation of liquid nitrogen, the frozen BLA tissue was weighed and homogenized

in 10 volumes of 0.1 M HCl. Pipet all the liquid in plate wells reference manual (cAMP Elisa kit, ewEast, China). The plate was incubated at room temperature for 2 h on a plate shaker at 250-500 rpm. The contents of the wells were emptied and each well was washed three times with 400 µL of solution; after the final wash, any remaining wash buffer was removed. Next, 200 µL of the Substrate solution was add in each well and then incubated at room temperature for 5-30 min without shaking. Finally, 50 µL of stop solution was added to every well to halt the reaction; the plates were read immediately with an optical density of 450 nm.

2.8 CREB immunohistochemistry

Cryosections sections were washed 3 times (5 min each) with 0.01 M PBS, and then treated with 2% BSA in PBS for 2 h at RT for blocking nonspecific reactions. The sections were treated with mouse monoclonal anti-CREB antibody (diluted to 1:200; Santa Cruz; CA, USA) in PBS solution for 24 h at 4 °C. The sections were washed 3 times with PBS, and then incubated with two-step IHC detection reagent (PV6001 and PV6002, Company of Zhongshan Golden bridge, Beijing, China) at 37 °C for 30 min. A brown color appeared in the slices after 3, 3'-diaminobenzidine colorization. Slices were then dehydrated and mounted with neutral gum. Five slides were randomly selected from each rat. For each slide, 5 randomly selected visual fields in the amygdala were chosen (×40 magnification). We recorded the optical density (OD) of positive cells in each field to evaluate the average OD. The OD of CREB immunopositive cells were analyzed using a Meta Morph/DPIO/BX41 morphology image analysis system.

2.9 Using real-time PCR to detect β-arrestin-2, PDE-4 and CREB

After decapitation, rat brains were dissected and BLA was removed. Total RNA was extracted using TRIzol (Invitrogen, Japan) according to the manufacturer's instructions. Reverse transcription of 1 µg of total RNA was into cDNA, and was performed with an RNA PCR Kit (AM Ver.3.0, TaKaRa bio, Otsu, Japan). The primers were designed and synthesized by Sangon Biotech Limited Company (Shanghai, China).

The primer sequences used for PCR amplification are shown in Table 1. The levels of β-arrestin-2, PDE-4 and CREB mRNA were determined from the ratio of β-arrestin-2/β-actin, PDE-4/β-

actin and CREB/β-actin.

Table 1. Primers respectively used for PCR.

Name		Primer	Product size
β-arrestin-2	Sense:	5'-CCA CAA AAG GAA CTC CGT GC-3'	185
	Antisense:	5'-GGA CGT TGA CAT TGA GGG GT-3'	
PDE-4	Sense:	5'-GAT GCG CTT GGA ACT TGA GC-3'	173
	Antisense:	5'-CCA CAT CAA AGC ATG TAT GAG CC-3'	
CREB	Sense:	5'-ATG CTG CGT CCA AAC ATA AAC AC-3'	121
	Antisense:	5'-CTG GCA CTC ACA TTG CCT ATC-3'	
β-actin	Sense:	5'-CGG AAA GAA GAT GAC GCA GAT A-3'	159
	Antisense:	5'-ACC AGA GTC CAA GAC AAT GC-3'	

2.10 Statistics

The results were expressed as Mean± S.D. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test using SPSS 17.0 software. A level of P <0.05 was considered to be statistically significant.

3. Results

3.1 Animal behavioral test

In the EPM Test (Table 2), the percentage of time in the open arms and the percentage of open arm entries were calculated. Rats showed a significant reduction in the percentage of time spent in the open arm ($F_{(3,8)} = 24.64$, $P < 0.05$) and percentage of the number of entries into open arms ($F_{(3,8)} = 23.65$, $P < 0.05$) on SPS 1d, SPS 7d and SPS 14d in comparison with control group. These results indicated SPS induced increased fear/anxiety-related behaviors.

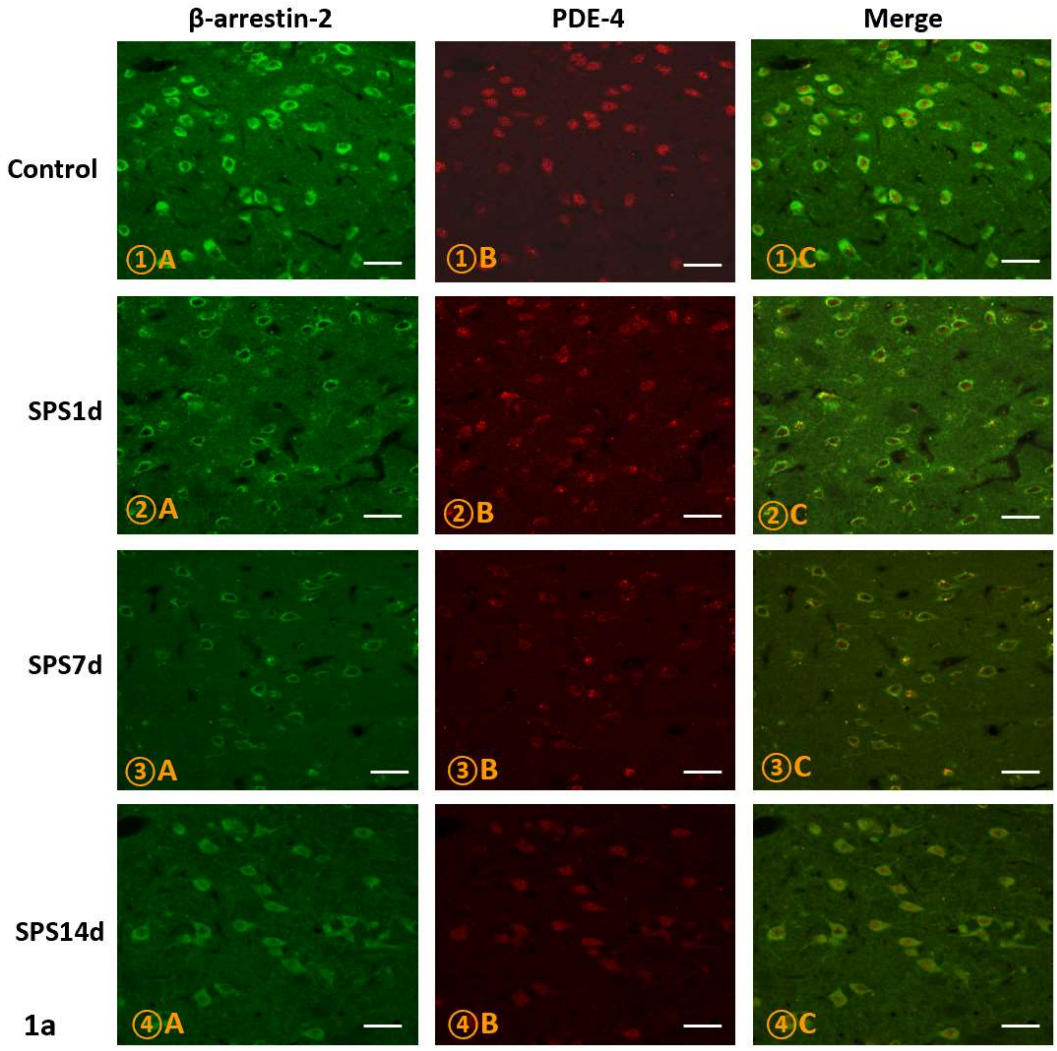
Table 2. The results of EMP test

Group	Time spent in open arm (%)	Number of open arm entries (%)
Control	30.92±2.17	45.69±3.48
SPS 1d	22.67±2.22*	38.04±2.57*
SPS 7d	16.99±2.08*	28.39±2.00*
SPS 14d	20.91±1.67*	35.52±1.77*

Statistical analysis was carried out by ANOVA test (*P < 0.05 compared with control group).

3.2 Immunofluorescent observation of β-arrestin-2 and PDE-4 expression

The concentrations of FITC- and CY3- labelled β-arrestin-2 and PDE-4 were measured in the BLA. The immunofluorescence staining results are shown in Figure 1. In the control group, immunoreactivity of β-arrestin-2 was mainly distributed in the cytoplasm of BLA neurons. At all the time points after SPS, the expression of β-arrestin-2 decreased significantly in comparison with the control group. In addition, at 7d after SPS, β-arrestin-2-signal was mainly distributed near the cell membrane (Fig. 1a) suggesting that after SPS, β-arrestin-2-positive products may transfer from the cytoplasm to the cell membrane. PDE-4 signal was mainly distributed in the nucleus and the cytoplasm of BLA neurons. BLA neurons showed strong positive reactions in the control group with relatively heavier staining. Like β-arrestin-2, PDE-4 was significantly decreased in the BLA region of SPS 7d rats compared with control rats, and then gradually increased to normal (Fig. 1b).



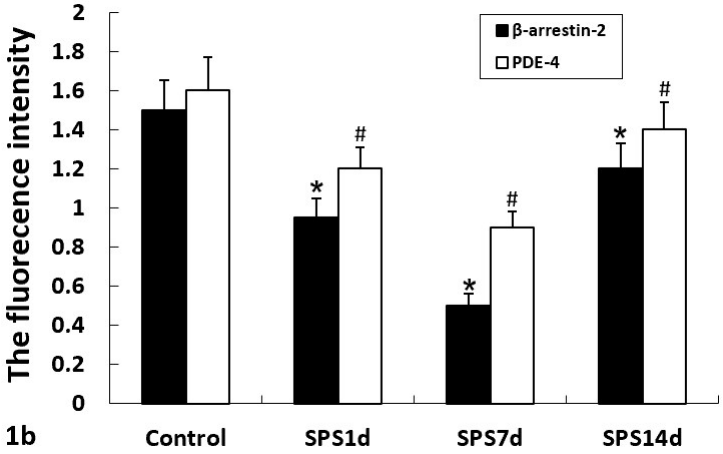


Fig. 1 Immunofluorescent positive result and quantitative analysis of β-arrestin-2 and PDE-

4 in the amygdala. 1a: Positive images (×400). The merged images show that β-arrestin-2 (green) and PDE-4 (red) were co-located. ①A →③A, showed that β-arrestin-2-positive products may transfer from the cytoplasm to the cell membrane. Bar = 50 μm. 1b. Quantitative analysis. The intensity of β-arrestin-2 and PDE-4 decreased after SPS, with a minimum at SPS 7d. *P < 0.05 compared with rats in the control group. # P < 0.05 compared with rats in the control group.

3.3 Western blot analysis protein expression levels for β-arrestin-2, PDE-4 and PKA

Similar findings were observed in the results of the western blot for β-arrestin-2 and PDE-4, as shown in Figure 2. Molecular weights of β-arrestin-2, PDE-4, PKA and GADPH were 55, 90, 42 and 36 kDa, respectively, showing clear bands (Fig. 2a). After SPS, the density of β-arrestin-2 ($F_{(3, 8)}$ = 93.82) and PDE-4 ($F_{(3, 8)}$ = 37.55) bands showed a significant decrease on SPS 1d and a further decrease on SPS 7d (Fig. 2b, P < 0.05). The levels of PKA significantly increased on SPS 1d and peaked on SPS 7d (Fig. 3), and then decreased on SPS 14d ($F_{(3, 8)}$ = 52.20, P < 0.05).

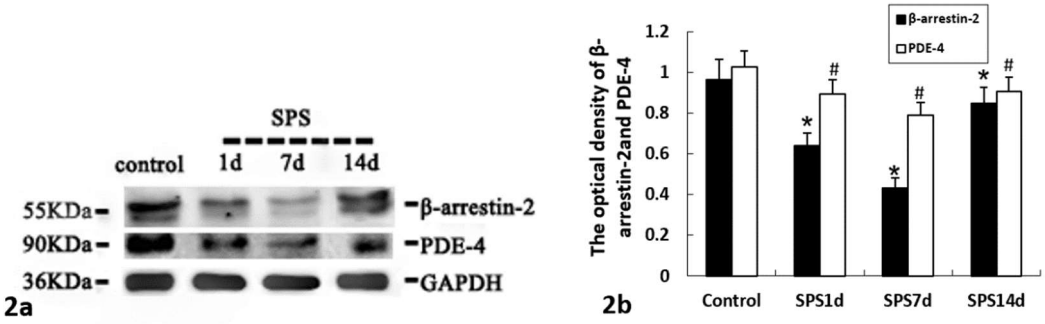


Fig. 2. Protein expression in the BLA detected by western blot. Fig 2a: Presentation of representative bands of β-arrestin-2 and PDE-4 protein levels. Fig. 2b. Quantitative results. A decrease in β-arrestin-2 and PDE-4 protein expression was observed in SPS rats. *P < 0.05 compared with rats in the control group. # P < 0.05 compared with rats in the control group.

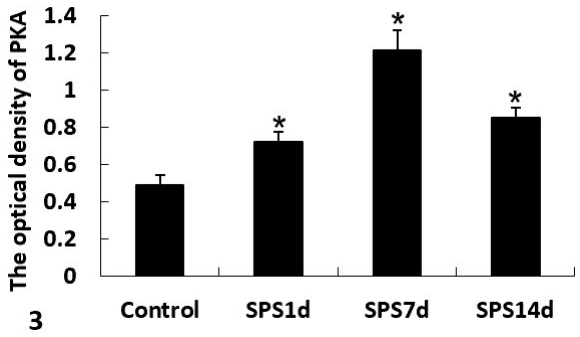


Fig. 3. Quantitative analysis for PKA based on western blot results. The level of PKA was peaked on SPS 7d. *P < 0.05 compared with the control group.

3.4 The results of co-immunoprecipitation for β-arrestin-2 and PDE-4

The results of co-immunoprecipitation showed that β-arrestin-2 and PDE-4 were present as a complex in the amygdala. The amount of the complex decreased in SPS7d (Fig. 4).

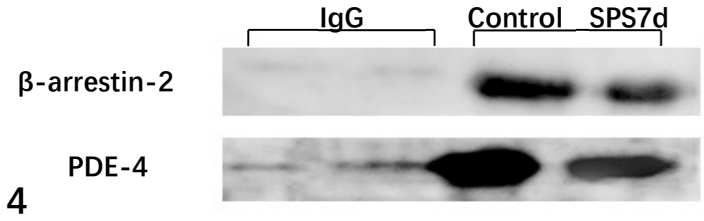


Fig. 4 Co- immunoprecipitation of β-arrestin-2 and PDE-4 in amygdala. Above bands β-arrestin-2: Homogenates were treated with antibody against β-arrestin-2, and presence of the partner protein PDE-4 was determined by Western for blot. Below band PDE-4: Homogenates were treated with antibody against PDE-4, and presence of the partner protein β-arrestin-2 was determined by Western for blot. Normal IgG as negative control which is non-specific interference.

3.5 cAMP levels were increased in SPS rats

A significant increase in cAMP levels in the amygdala was observed at 1 day, 7 days and 14 days after SPS exposure in comparison with the control group. The levels of cAMP began to increase on SPS 1d, and peaked on SPS 7d and then returned towards normal ($F_{(3, 8)} = 196.72$, $P < 0.05$, Fig. 5).

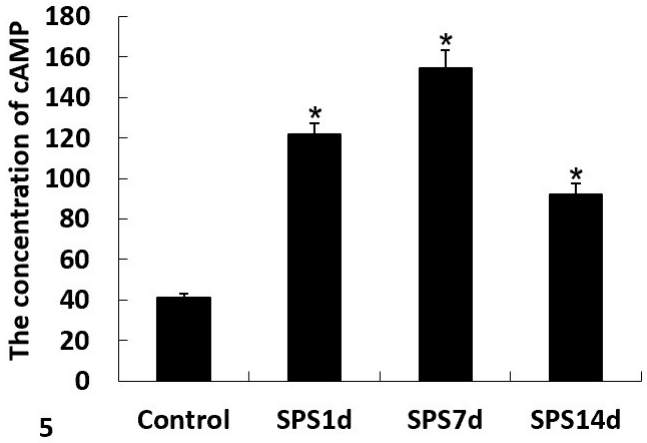


Fig.5. cAMP levels in the amygdala based on Elisa results. The concentration of cAMP began to increase on SPS 1d and peaked on SPS 7d. *P < 0.05 vs. the control group.

3.6 Increase of CREB in the BLA neurons after SPS exposure in immunohistochemical assay

Because CREB is downstream of cAMP signaling, we performed immunohistochemical staining in the BLA (Fig. 6). The immunoreactivity of CREB was localized in the nucleus (Fig. 6a). We observed an upregulation of the immunoreactivity of CREB on 1d after SPS. It peaked on 7d after SPS and then declined on 14d after SPS (Fig. 6b) ($F_{(3, 8)} = 41.83$, Fig. 7, $P < 0.05$).

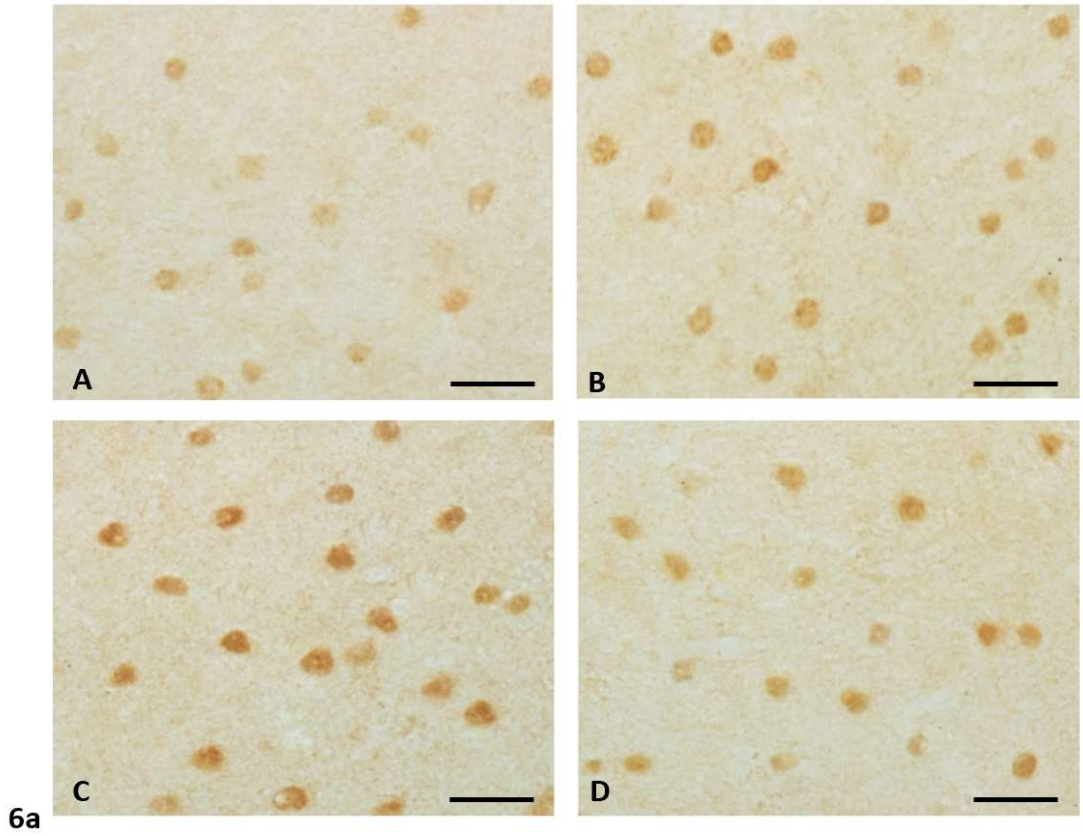


Fig.6. Immunoreactivity and quantitative analysis of CREB in the BLA (×400). 6a: is the expression of CREB in the SPS groups was increased compared to the control group. A: Control group; B: SPS 1d group; C: SPS 7d group; D: SPS 14d group. Bar=50μm. 6b: Quantitative analysis results. The intensity of CREB increased at the SPS 1d, and peaked at SPS 7d. *P< 0.05 compared with the control group.

3.7 Real-time PCR results of mRNA for β-arrestin-2, PDE-4 and CREB

The expressions of β-arrestin-2/β-actin ($F_{(3, 8)} = 16.51$, $P < 0.05$), PDE-4/β-actin ($F_{(3, 8)} = 21.37$, $P < 0.05$) decreased significantly after SPS stimulation and began to come towards normal on SPS 14d (Fig. 7a), which was consistent with the results of immunofluorescence and western blot.

The expression of CREB mRNA analyzed by real-time PCR showed a significant increase in the SPS group compared with that in the control group (Fig. 7b). The ratio of CREB/β-actin peaked on SPS 7d and then gradually decreased ($F_{(3, 8)} = 26.22$, $P < 0.05$).

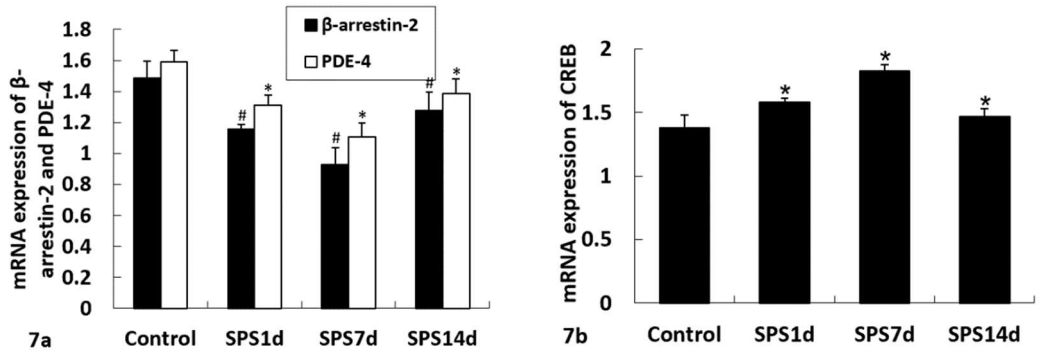


Fig. 7. Real-time PCR was used to detect changes in the mRNA expression of β-arrestin-2, PDE-4 and CREB. 7a: β-arrestin-2 and PDE-4 mRNA expression. 7b: CREB mRNA expression. *P < 0.05 and # P < 0.05 compared with the control group.

4. Discussion

PTSD is an anxiety disorder caused by a life-threatening traumatic experience, which affects a patient's quality of life and social stability. PTSD can be categorized as a disorder of dysregulated fear processing [1]. Aberrant fear learning is one of the central features of this disorder as demonstrated by cue-induced re-experiencing responses (e.g. flashback) that are slow to extinguish in humans [27]. Different types of memory depend on different parts of the

brain; for example, space location memory is associated with the hippocampus and fear/anxious emotional memories are associated with the amygdala. It is well-known that the amygdala and prefrontal cortex are key sites of synaptic plasticity that mediates aspects of fear learning and memory [28].

Many animal studies have suggested that molecular mechanisms of synaptic plasticity in the amygdala play a key role in fear extinction and ultimately in the PTSD symptoms. Recent studies have found that the morphology and arborization of dendritic spines (small protrusions that receive the majority of excitatory synapses) change as a result of fear conditioning and extinction in the cortical areas of the brain that are central to these learning processes [29-32]. On the basis of these findings, it is hypothesized that the amygdala, particularly BLA may be the key region of fear initiation.

The amygdala has also been directly implicated in PTSD. Evidence from clinical studies comparing individuals with PTSD to healthy controls showed that those with PTSD have increased amygdala activity to both negative stimuli and to trauma-specific stimuli [33]. The amygdala is a key brain structure in emotional processing and plays a critical role in the acquisition, consolidation and behavioral response to associative fear [34]. Thus, we aimed to detect changes in BLA.

In the present study, we used elevated plus maze tests to examine fear/anxious -related behaviors and then to confirm the main symptom of PTSD was abnormal fear memory. We found that SPS induced fear/anxious memory enhancement, and peaked on SPS 7d. After SPS, β-arrestin-2, PDE4 and the complex of β-arrestin-2/PDE4 were reduced. In line with a reduced containment of stimulatory G-protein signaling we found that the amygdala cAMP levels gradually increased after PTSD and peaked at SPS 7d. The enzyme immediately downstream of cAMP is PKA, and so is predicted to show higher activity. PKA levels were also increased after SPS stimulus and peaked on SPS 7d. Thus, our data suggest that response to fear conditioning, cAMP/PKA signaling is increased for 2 weeks, perhaps as a consequence of lower

activity of the β-arrestin-2/ PDE-4 pathway.

According to literature, the activity of PKA change is a necessary signal for fear memory consolidation [35]. Because it can bring about a change in the activity of nuclear transcription factors, such as CREB, to cause a new protein synthesis. CREB activation can lead to structural change of dendritic spines in BLA to promote and to maintain long-term fear/anxious memory. Experimental results showed that phosphorylation of CREB had a regulatory role in the synaptic plasticity of hippocampal neurons [36-38]. Thus, CREB can be considered molecular master switch of fear memory/anxious mechanism. Our results showed that CREB increased after SPS, peaked on SPS 7d, and then decreased to normal, and we expect that elevated CREB signaling leads to an abnormal amygdala-driven fear/anxious memory of PTSD. A caveat is that we quantified total CREB levels, and not the specific phosphorylated protein that is linked more directly to transcriptional activity.

Therefore, β-arrestin-2 and PDE-4 may act through the cAMP-PKA signaling pathways and further influence CREB phosphorylation, which further affect changes in neuron synaptic plasticity in BLA. Taken together, our data demonstrate that the reductions in β-arrestin-2, PDE-4 and the complex of β-arrestin-2/PDE-4 may lead to fear/anxious memory enhancement after SPS.

5. Conclusions

Our results suggest that β-arrestin-2 and PDE-4 may be involved in the formation of PTSD; low β-arrestin-2 and PDE-4 expression may cause or maintain high signal transduction pathway activity promote the formation and development of PTSD by influencing BLA in fear/anxious memory. β-arrestin-2 and PDE-4 may provide alternative intervention targets for more effective treatment for PTSD.

Acknowledgments

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