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Social interaction reward decreases p38 activation in the nucleus accumbens shell of rats

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ABSTRACT

We have previously shown that animals acquired robust conditioned place preference (CPP) to either social interaction alone or cocaine alone. Recently it has been reported that drugs of abuse abnormally activated p38, a member of mitogen-activated protein kinase family, in the nucleus accumbens. In this study, we aimed to investigate the expression of the activated form of p38 (pp38) in the nucleus accumbens shell and core of rats expressing either cocaine CPP or social interaction CPP 1 h, 2 h and 24 h after the CPP test. We hypothesized that cocaine CPP will increase pp38 in the nucleus accumbens shell/core as compared to social interaction CPP. Surprisingly, we found that 24 h after social interaction CPP, pp38 neuronal levels were decreased in the nucleus accumbens shell to the level of naïve rats. Control saline rats that received saline in both compartments of the CPP apparatus and cocaine CPP rats showed similar enhanced p38 activation as compared to naïve and social interaction CPP rats. We also found that the percentage of neurons expressing dopaminergic receptor D2R and pp38 was also decreased in the shell of the nucleus accumbens of social interaction CPP rats as compared to controls. Given the emerging role of p38 in stress/anxiety behaviors, these results suggest that (1) social interaction reward has anti-stress effects; (2) cocaine conditioning per se does not affect p38 activation and that (3) marginal stress is sufficient to induce p38 activation in the shell of the nucleus accumbens.

1. Introduction

Switching the preference of the substance dependent individual toward non-drug related activities remains one of the great challenges that confront drug dependence therapy. Beneficial social interaction can positively affect personal relationships and may serve as an alternate natural reward to drug use (Iglesias et al., 2014). We developed a rat experimental model based on the conditioned place preference (CPP) paradigm in which only four social interaction episodes (15 min each) with a male early-adult conspecific rat as an alternative (i.e., non-drug-associated) stimulus completely reversed CPP for cocaine (15 mg/kg i.p.) and were even able to inhibit cocaine-induced reinstatement of cocaine CPP (Fritz et al., 2011a). These protective effects of social interaction were paralleled by effects on the brain circuitry known to be involved in drug reinforcement and reward. Social interaction during extinction of cocaine CPP reversed cocaine CPP-reinstatement-associated Zif268 expression in the nucleus accumbens shell, the central and basolateral amygdala, and the ventral tegmental area (Fritz et al., 2011a). In the nucleus accumbens, cocaine CPP-induced Zif268 expression was found to be reversed by social interaction preferentially in dopaminergic D1 receptor-expressing GABAergic medium spiny neurons than D2 receptor-expressing GABAergic medium spiny neurons (Prast et al., 2014). Furthermore, social interaction during extinction of cocaine CPP reduced cocaine-CPP-stimulated FosB expression in the nucleus accumbens shell and core; and increased pCREB (cAMP response element binding protein) expression in the nucleus accumbens shell and the cingulate cortex area 1 (Cg1) (El Rawas et al., 2012b). These findings suggest that social interaction, if offered in a context that is clearly distinct from the previously drug-

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associated one, may profoundly decrease the incentive salience of drug-associated contextual stimuli.

As for cocaine, rats acquire and express CPP for social interaction (Calcagnetti and Schechter, 1992; Douglas et al., 2004; Thiel et al., 2008; Trezza et al., 2009; Fritz et al., 2011a; Kummer et al., 2011; El Rawas et al., 2012a). Recently we showed that cocaine CPP and social interaction CPP activated almost the same brain regions (El Rawas et al., 2012a). However, the granular insular cortex and the dorsal part of the agranular insular cortex were more activated after cocaine CPP, whereas the prelimbic cortex and the core subregion of the nucleus accumbens were more activated after social interaction CPP. These results suggest that the insular cortex appears to be potently activated after drug conditioning learning while activation of the prelimbic cortex—nucleus accumbens core projection seems to be preferentially involved in the conditioning to non-drug stimuli such as social interaction.

P38 (also called SAPK, for stress-activated protein kinase) is a member of mitogen-activated protein kinase (MAPK) family that regulates both physiological and pathological processes (Ji et al., 2009). Recent reports have shown that p38 is involved in effects of drugs of abuse. Indeed, it has been shown by western blot that p38 activation was abnormally induced in the nucleus accumbens microglia following morphine CPP (Zhang et al., 2012). The same group has also found that bilateral nucleus accumbens core injections of SB203580, an inhibitor of p38, prior to morphine administration inhibited p38 activation in the microglia and impaired the acquisition of morphine CPP (Zhang et al., 2012). Also, injections of SB203580 in the nucleus accumbens dose dependently impaired amphetamine CPP (Gerdjikov et al., 2004). Therefore, our aim was to assess the expression of p38 activation into the nucleus accumbens shell and core sub-regions at different time points (1 h, 2 h and 24 h) after cocaine (drug) CPP, social interaction (non-drug) CPP or in control saline rats. We hypothesized that cocaine CPP will enhance the activation of p38 in the nucleus accumbens as compared with social interaction CPP.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (SD) aged 6–8 weeks (weighing 150–200 g) were obtained from the Research Institute of Laboratory Animal Breeding of the Medical University Vienna (Himberg, Austria). All animals were housed at a constant room temperature of 24 °C and had *ad libitum* access to tap water and pelleted chow (Tagger, Austria). Experiments were performed during the light phase of a continuous 12-h light/dark cycle with the lights on from 08:00 h to 20:00 h. Animals were singly housed 7 days before the start of the behavioral experiments. The present experiments were approved by the Austrian National Animal Experiment Ethics Committee (BMWF-66.011/0095-ll/3b/2013). All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Conditioned place preference

Conditioning of SD rats was conducted in a custom-made three-chamber CPP apparatus (64 cm wide × 32 cm deep × 31 cm high) made of unplasticized polyvinyl chloride. The middle (neutral) compartment (10 × 30 × 30 cm) had white walls and a white floor. Two doorways led to the two conditioning compartments (25 × 30 × 30 cm each) with walls showing either vertical or horizontal black-and-white stripes of the same overall brightness and with stainless steel floors containing either 168 holes (diameter 0.5 cm) or 56 slits (4.2 × 0.2 cm each). Time spent in each compartment was digitally recorded with a video camera and analyzed offline with hand timers. The CPP apparatus was cleaned with a 70% camphorated ethanol solution after each session. All experiments were performed under neon ceiling light (58 W, 1 m distance).

2.2.1. Acquisition of cocaine CPP

The conditioning procedure comprised a pretest session on day one, eight consecutive training days (alternate-day-design, one training session per day, a total of four training sessions each for cocaine or saline), and a CPP test on day 10. Pretest-, training-, and CPP test session lengths were of equal duration, i.e., 15 min = 900 s. The initially non-preferred chamber was subsequently paired with intraperitoneal (i.p.) injections of cocaine (pure base) or saline immediately before placing the rat into the closed dedicated chamber. The CPP test was performed 24 h after the last conditioning trial by placing the rat in the middle (neutral) compartment of the CPP apparatus and allowing it to move freely between the three compartments (n = 20).

2.2.2. Acquisition of social interaction CPP

The conditioning procedure comprised a pretest session on day one, eight consecutive training days (alternate-day-design, one training session per day, a total of four training sessions each for social interaction or saline), and a CPP test on day 10. Pretest-, training-, and CPP test session lengths were of equal duration, i.e., 15 min = 900 s. The initially non-preferred chamber was subsequently paired with social interaction. If a compartment was paired with social interaction during CPP training, each rat received an i.p. injection of saline and was placed in the compartment to allow for social interaction with a conspecific of the same weight and gender (male) during the whole conditioning session. Each rat was assigned a different partner, which stayed the same for the whole duration of the experiment. All animals remained singly housed. The CPP test was performed 24 h after the last conditioning trial by placing the rat in the middle (neutral) compartment of the CPP apparatus and allowing it to move freely between the three compartments (n = 20).

2.2.3. Control rats

The conditioning procedure comprised a pretest session on day one, eight consecutive training days, and a CPP test on day 10. Pretest-, training-, and CPP test session lengths were of equal duration, i.e., 15 min = 900 s. Each rat received an i.p. injection of saline in both compartments. The CPP test was performed 24 h after the last conditioning trial by placing the rat in the middle (neutral) compartment of the CPP apparatus and allowing it to move freely between the three compartments (n = 12).

Preference was defined as the difference in time spent in the compartment where cocaine or social interaction were given on the test day minus the time spent in the same stimulus-associated compartment on the pre-test day and was reported as preference score in Fig. 1. As the initially non-preferred chamber was subsequently paired with social interaction for social CPP rats or cocaine for cocaine CPP rats, preference scores for control rats receiving saline in both compartment was defined as the difference in time spent on the test-day minus the time spent on the pre-test day in the less-preferred compartment during the pre-test.

At the end of the experiments, rats from different treatments were randomly assigned to the three time points: 1 h (saline control n = 4, cocaine CPP n = 7, social interaction CPP n = 8); 2 h (saline control n = 4, cocaine CPP n = 7, social interaction CPP n = 6) or 24 h (saline control n = 4, cocaine CPP n = 6, social interaction CPP n = 6) in order to investigate early and late activation after the CPP test.

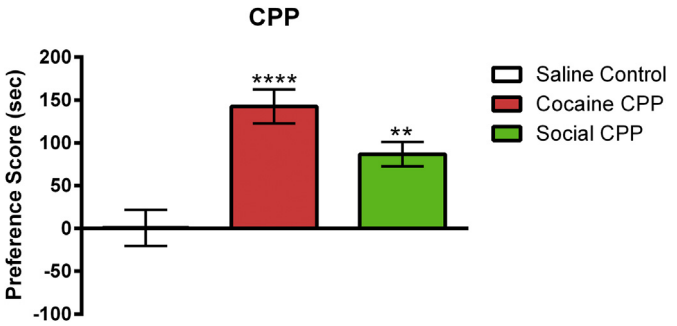

Fig. 1. Conditioned place preference to cocaine and social interaction. Preference scores in seconds (time spent in the stimulus associated chamber during the test minus the pre-test) were significantly increased after conditioning with cocaine (15 mg/kg) or with social interaction as compared to saline control rats receiving saline injections in both compartment of the CPP. ****$p < 0.0001$; **$p < 0.01$ different from saline control.

2.2.4. Naïve rats

The naïve rats are untreated rats that served for investigating the effects of saline injection to the control rats on p38 phosphorylation in the nucleus accumbens shell and core ($n = 5$).

2.3. Tissue preparation

Rats were deeply anesthetized using isoflurane and transcardially perfused with 0.1 M phosphate buffered saline followed by 4% paraformaldehyde (PFA) dissolved in 0.1 M phosphate buffered saline (PBS; pH 7.4). Brains were then removed and postfixed in 4% PFA overnight, then stored in 30% sucrose at 4 °C until the brain sank, and then at −80 °C until sectioning. All serial brain sections (40 μm) were cut using a Cryostat (Leica). Sections were stored in an assorter buffer (Tris buffer, NaN3 0.1%) at 4 °C until processed for immunolabeling (Fritz et al., 2011a; El Rawas et al., 2012a, 2012b).

2.3.1. Immunohistochemistry

Free-floating sections from rats in different groups (Naïve, saline control, cocaine CPP and social interaction CPP) were processed simultaneously for protein expression. Sections were washed in PBS 0.1 M and incubated in 0.3% hydrogen peroxide/PBS 0.1 M. Then they were washed in PBS 0.1 M and incubated in the blocking solution containing 0.3% Triton X-100 and 3% normal goat serum (Vector Laboratories, # S-1000) in PBS 0.1 M. Subsequently, sections were incubated in the primary antibody (phospho-p38 MAPK thr180/tyr182: rabbit, cell signaling #9211, dilution 1:200) diluted in the blocking solution containing 0.3% Triton X-100 and 1% normal goat serum in PBS 0.1 M. Sections were then washed in PBS and incubated in the secondary biotinylated anti-rabbit antibody (Vector Laboratories # BA-1000, dilution 1:200). Afterward, the tissue was incubated in avidin-biotinylated horseradish peroxidase complex (ABC Elite kit, Vector Laboratories) diluted in PBS 0.1 M. Then, sections were incubated in 3,3-diaminobenzidine tetrahydrochloride (DAB tablets, Sigma #D4418). This reaction was terminated by rinsing the tissue in PBS 0.1 M. Finally, sections were then mounted onto Leica Extra adhesive micro slides, dried, and dehydrated before coverslipping.

Brain sections were scanned using a Zeiss optical microscope set at x 20 magnification equipped with a camera (Axioplan 2 Imaging) interfaced to a PC. Intensity of DAB staining as described by (Nguyen et al., 2013) was evaluated by an experimenter who was blind to treatment conditions using Fiji imaging software. For each brain region, three sections per animal were counted.

2.3.2. Immunofluorescence

Double immunofluorescence was performed as described previously (Prast et al., 2014). Sections were washed three times for 5 min each in TBS-T (50 mM Tris-buffered saline (TBS; pH 7.4) containing 0.1% Triton-X-100) and were incubated at 90–100 °C in a 10 mM citrate buffer, pH 6.0, for 6 min for antigen retrieval. After a TBS-T wash, slices were incubated for 30 min in TBS containing 50 mM glycine, followed by another wash in TBS-T (3 × 5 min) and a 1 h incubation in TBS-T containing 2% BSA and 10% normal goat serum. In order to investigate the type of phospho-P38 stained cells, we performed double immunohistochemistry for pp38 (phosphor-p38 MAPK thr180/tyr182: rabbit, cell signaling #9211, dilution 1:50) and a polyclonal antibody against the neuron-specific nuclear protein NeuN (NeuN: mouse, Millipore, # MAB377, dilution 1:200) or Dopaminergic D2 receptor (D2R: mouse, santa cruz biotechnology, # sc-5303, dilution 1:100). Sections were washed in 50 mM TBS-T for 1 h and incubated for 2 h in 50 mM TBS-T containing 2% BSA, anti-rabbit Alexa Fluor 488-conjugated secondary antibody (Invitrogen, # A21441, dilution 1:400) for pp38 staining and anti-mouse Alexa Fluor 555-conjugated secondary antibody (Invitrogen, # A31570, dilution 1:400) for NeuN staining. After an additional wash in 50 mM TBS for 1 h, sections were mounted onto Leica Extra adhesive micro slides and coverslipped using Vectashield (Vector Laboratories, # H-1000). We tried also to perform double immunofluorescence with pp38 (rabbit, cell signaling, # 9211) and dopaminergic D1 receptor. We tried three different antibodies but none of them worked.

Representative images for the double staining were taken with a laser scanning confocal microscope (Zeiss LSM 510 Meta) at a magnification of 100×Cells stained for pp38, NeuN, D2R and co-localized pp38/NeuN and D2R/pp38 were counted by an experimenter who was blind to treatment conditions using Fiji imaging software. For each brain region, three sections per animal were counted.

2.4. Statistical analyses

All data were expressed as mean ± SEM (Standard Error of the Mean). Differences in preference scores and in intensity of pp38 staining between groups were assessed by one or two -way analysis of variance (ANOVA). Direct comparison between naïve and saline control rats in pp38 intensity or between saline control and social CPP in the double immunofluorescence data was performed using two-sided unpaired students T-test. Results showing significant overall changes were subjected to Tukey's Multiple Comparison post-hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Acquisition of cocaine CPP and social interaction CPP

In rats, social interaction alone and cocaine alone produced a significant CPP (Fig. 1; $n = 52$, one-way ANOVA, $p < 0.0001$, $F_{2,47} = 13.16$). Conditioning with cocaine (cocaine CPP, $p < 0.0001$) or social interaction (saline control vs social interaction CPP, $p < 0.01$) yielded to a significantly higher preference score as compared to saline control. Also, the preference score for cocaine CPP did not differ from social CPP ($p > 0.05$) suggesting that both cocaine and social interaction reward have equal strength as we have previously shown (Fritz et al., 2011a, 2011b; Kummer et al., 2014).

3.2. Expression of pp38 in the nucleus accumbens after CPP

In the nucleus accumbens core, pp38 expression did not differ between the saline control, cocaine CPP and social interaction CPP

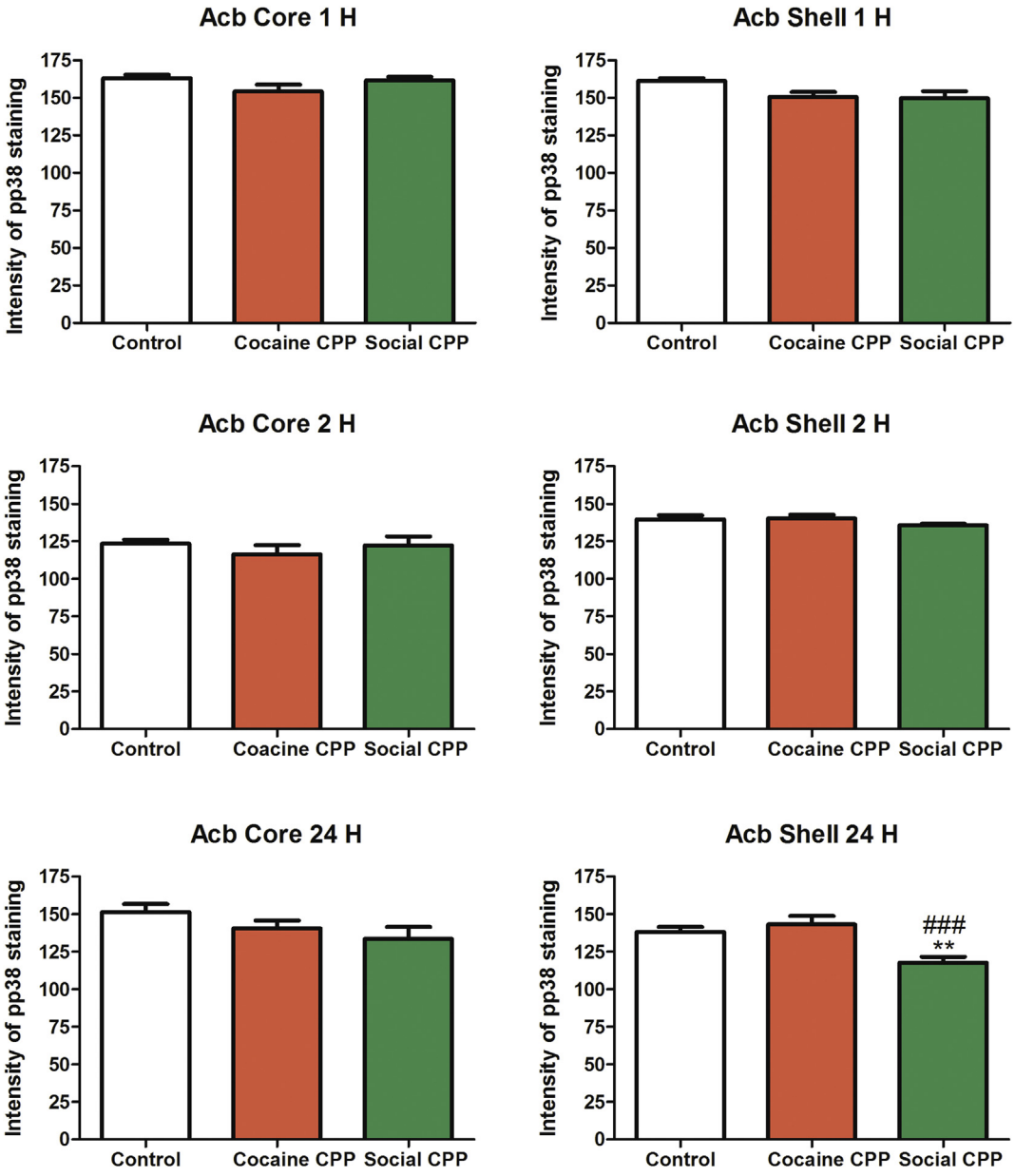

Fig. 2. Pp38 expression at 1, 2 and 24 after the CPP test. Pp38 levels in the nucleus accumbens core (Acb Core) and the nucleus accumbens shell (Acb Shell) after 1 h, 2 h and 24 h after cocaine CPP, social interaction CPP and control saline rats. 24 h after CPP, social interaction decreased pp38 levels in the nucleus accumbens shell. **p < 0.01 different from control; ###p < 0.001 different from cocaine CPP.

groups (Fig. 2). Two-way ANOVA (factors: treatment groups, i.e. saline control, cocaine CPP and social interaction CPP; and time points, i.e. 1 h, 2 h and 24 h after the CPP test) revealed a significant main effect of time points, but not treatment groups on the intensity values of pp38 expression (time points, $F_{2,43} = 39.73$, $p < 0.001$; treatment groups, $F_{2,43} = 1.93$, $p = 0.16$), with no interaction between both factors (time points x treatment groups, $F_{4,43} = 0.93$, $p = 0.45$). Post-hoc analysis of main effects showed that pp38 expression differed significantly over the different time points (Tukey post-hoc test, 1h − x = 159.6, 2h − x = 120.8, 24h − x = 141.9, 1h vs 2h $p < 0.001$, 1h vs 24h $p < 0.001$, 2h vs 24h $p < 0.001$).

In the nucleus accumbens shell, social interaction CPP decreased pp38 expression 24 h after the CPP test as compared to cocaine CPP and saline control-rats (Fig. 2). Two-way ANOVA (factors: treatment groups, i.e. saline control, cocaine CPP and social interaction CPP; and time points, i.e. 1 h, 2 h and 24 h after the CPP test) revealed a significant main effects of time points and treatment groups on the intensity values of pp38 expression (time points, $F_{2,41} = 20.87$, $p < 0.001$; treatment groups, $F_{2,41} = 8.35$, $p = 0.001$). Further, there was a significant interaction between time points and treatment groups on pp38 intensity values (time points x treatment groups, $F_{4,41} = 3.54$, $p = 0.014$). Testing the simple main effects of the treatment groups within each time point revealed that after 24 h, the pp38 expression differed significantly for the different treatment groups (saline control − x = 138.3, cocaine CPP − = 143.2, social interaction CPP − = 117.8, $F_{2,41} = 11.53$, $p < 0.001$; pair wise comparisons: saline control vs. cocaine CPP $p = 1.0$, saline control vs. social interaction CPP $p = 0.005$, cocaine CPP vs. social interaction CPP $p < 0.001$).

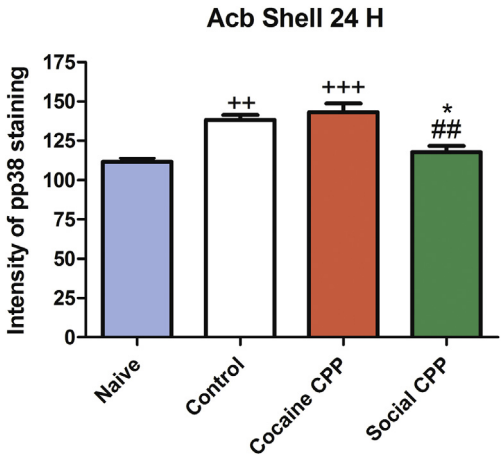

Fig. 3. Pp38 expression at 24 h after the CPP test in the nucleus accumbens shell. Social interaction CPP decreased pp38 to the levels of the naïve group. Control and cocaine CPP rats show similar enhanced pp38 levels as compared to naïve and social CPP rats. *p < 0.05 different from control; ##p < 0.01 different from cocaine CPP; +++ p < 0.001, ++ p < 0.01 different from naïve.

In order to investigate whether a marginal stress, such as injecting the animals with saline and putting them into the CPP apparatus as we treat control saline rats, can affect p38 activation; we added the naïve untreated group (n = 5) to the comparison (Fig. 3). One-way ANOVA yielded to a p = 0.0001 and F 3,16 = 13.69. We found that pp38 expression was increased in control-rats (saline control vs naive, p < 0.01) and cocaine CPP rats (cocaine CPP vs naive, p < 0.001) in the shell of the nucleus accumbens as compared to naïve rats. Interestingly, we also found that social interaction 24 h after the CPP test decreased pp38 expression levels in the shell of the nucleus accumbens to the levels of the naïve rats (Naïve vs social interaction CPP, p > 0.05). This difference in pp38 levels between naïve and saline control rats was specific to the shell of the nucleus accumbens as these groups showed similar pp38 intensity in the core of the nucleus accumbens (pp38 intensity ± SEM; saline control 151.5 ± 5.393; naïve 137.6 ± 8.477; two-sided unpaired students T-test, t (7) =1.2972, p = 0.2357, ns).

In order to know what is the type of cells expressing pp38, we performed a double immunostaining for pp38 and NeuN (neuronal marker). We found that most of the cells expressing pp38 were neuronal in the shell and the core of the nucleus accumbens (Fig. 4, percentage of pp38/NeuN positive cells ±SEM; Acb shell 92 ± 1.958; Acb Core 87.8 ± 3.929).

As the majority of neurons in the nucleus accumbens are GABAergic medium spiny neurons expressing D1 dopaminergic receptor or D2 dopaminergic receptor, we investigated the percentage of neurons expressing both D2R and pp38 in the saline control and social CPP groups (Fig. 5A and B). We have found that the percentage of neurons expressing dopaminergic receptor D2R and pp38 is similar in the core (two-sided unpaired students T-test, t (10) = 0.6671, p = 0.519, ns) but decreased in the shell of the nucleus accumbens of social CPP rats as compared to saline control (Fig. 5B, two-sided unpaired students T-test, saline control vs social CPP, t (7) = 3.339, p = 0.0124).

4. Discussion

In the present study we could show that, 24 h after social interaction CPP, pp38 neuronal levels were decreased in the nucleus accumbens shell to the level of naïve rats. Control saline and cocaine CPP rats showed similar enhanced p38 activation as compared to naïve and social interaction CPP rats.

Pp38 expression in the nucleus accumbens (shell and core unseparated) was found to be increased after morphine CPP as compared to naïve rats (Zhang et al., 2012). Consistent with this finding, we have also found that cocaine CPP also increased pp38 levels specifically in the nucleus accumbens shell as compared to naïve rats. These results suggest that, compared to naïve rats, drug conditioning increased p38 activation in the nucleus accumbens shell. However, we opine that control saline rats that received the same amount of handling, injections and exposure to the CPP apparatus are a more appropriate control for the study. Interestingly, cocaine CPP and control saline rats showed similar pp38 levels in the nucleus accumbens shell. Consequently, cocaine conditioning per se did not affect pp38 expression. Therefore, the choice of the “control” to compare with different treatment groups is of great importance for results interpretation.

Using pharmacological approaches, p38 MAPK activity has been identified as a critical mediator of stroke-induced apoptosis, osmotic shock response, and in the regulation of transcriptional pathways responsible for cell death and differentiation (Coulthard et al., 2009). Beyond the cellular level, p38 activity was also found to be increased after stress/anxiety exposure. Indeed, p38 was activated (phosphorylated) after acute cold exposure stress in the pre frontal cortex (Zheng et al., 2008) and after repeated swim stress in the nucleus accumbens, the cortex and the hippocampus (Bruchas et al., 2007). Also, p38 activation was found to be associated to stress/anxiety behaviors (Peng et al., 2013) and to increased maternal rejection and poor maternal care (Sanchez et al., 2007). In

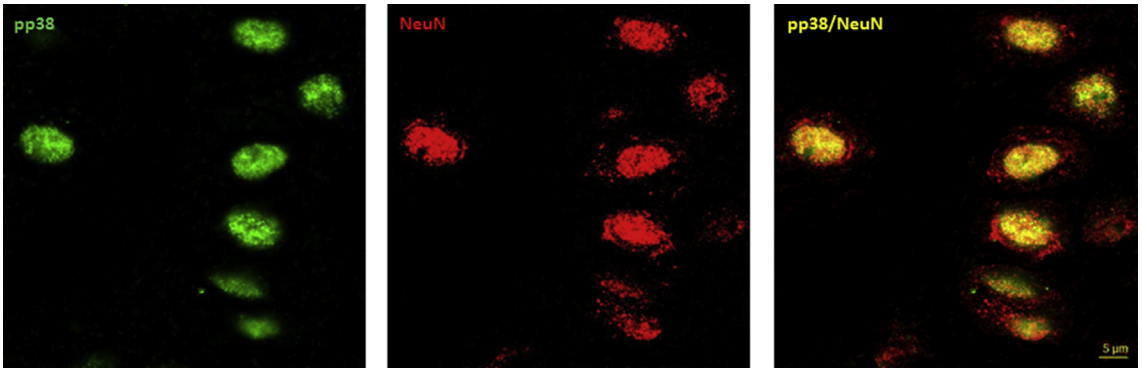

Fig. 4. Double immunofluorescence pp38, NeuN. Representive images of pp38 staining (green) and NeuN staining (red) and co-localized pp38, NeuN cells (yellow) in the nucleus accumbens show that the majority of pp38 cells are co-labelled with NeuN. Objective: 100×. Scale bare 5 µm.

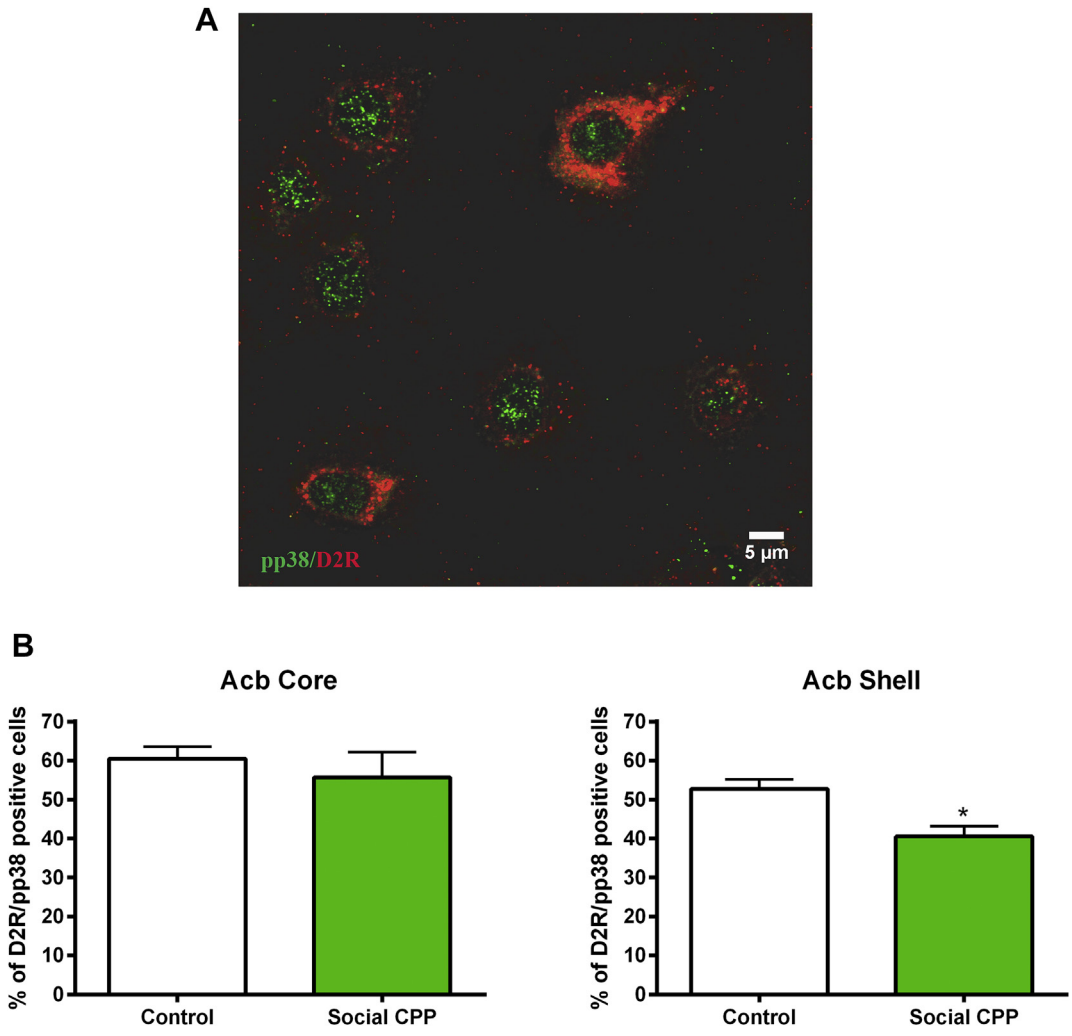

Fig. 5. Double immunofluorescence pp38, D2R in the nucleus accumbens shell (Acb Shell) and Core (Acb Core) 24 h after the CPP test. Figure 5A: Representive image of co-localized D2R (red), pp38 (green) in the nucleus accumbens. Objective: 100×. Scale bare 5 µm. Figure 5B: Percentage of D2R, pp38 positive cells was decreased in the shell of the nucleus accumbens after social CPP as compared to control rats. *$p < 0.05$ different from control.

this study, we found that social interaction reward decreased pp38 expression in the nucleus accumbens shell to the level of naïve untreated rats. These results suggest that social interaction reward may have anti-stress effects. This is consistent with our previous analysis of behavioral components of social interaction reward (Kummer et al., 2014). We did not find any episodes of hostile behavior, i.e., boxing or biting, suggesting that the rats fully engaged in friendly ("agonistic") social interaction from the first training session onwards (Kummer et al., 2014). Importantly, the findings of this study also highlight the role of the nucleus accumbens shell in mediating social interaction reward as we have previously shown (Fritz et al., 2011b). Indeed, when rats were concurrently conditioned for place preference by pairing cocaine with one compartment and social interaction with the other (concurrent paradigm), pre-acquisition lesioning of the nucleus accumbens shell shifted the preference toward cocaine CPP (Fritz et al., 2011b). In comparison with control saline animals, naïve rats showed decreased levels of pp38 in the shell but not the core of the nucleus accumbens. This finding suggests that even a marginal stress, such as injecting animals with saline and placing them into the CPP apparatus, is sufficient to induce p38 activation in the nucleus accumbens shell.

We have found that social interaction reward has anti-stress effects in the shell but not the core of the nucleus accumbens. The shell of the nucleus accumbens has been previously reported to play an important role in the emotional responses. Indeed, mild footshock stress selectively increased extracellular dopamine levels in the shell while the levels of dopamine remained unaltered in the core of the nucleus accumbens (Kalivas and Duffy, 1995). Consistently, psychological stress for 20 min significantly increased extracellular dopamine levels in the shell but not the core of the nucleus accumbens (Wu et al., 1999). Moreover, short duration immobilization stress selectively increased dopamine utilization in the shell of the nucleus accumbens (Deutch and Cameron, 1992). Interestingly, glucocorticoide hormones, increasing in response to stressful stimuli, are shown to modify dopamine transmission in only the shell of the nucleus accumbens without modifying it in the core (Marinelli and Piazza, 2002).

Our results also show that pp38 is in majority co-localized with neurons similarly in the shell and the core of the nucleus accumbens. 95% of the neurons in the nucleus accumbens are GABAergic medium spiny neurons, which can be further differentiated into those that express the D1 dopamine receptor along with dynorphin and substance P, and those that express the D2 dopamine receptor

along with enkephalin. In addition, about 1–2% of the nucleus accumbens neurons are spiny large cholinergic interneurons, and a similar number are GABAergic interneurons (Robison and Nestler, 2011). We therefore assumed that most of neurons expressing pp38 might be GABAergic medium spiny neurons expressing either D1 or D2 dopamine receptors. Our results show that decreased pp38 levels in the nucleus accumbens shell occurred in neurons expressing D2 dopamine receptor. It seems that D2 receptors are more sensitive in response to stress than D1 receptors. Indeed, prenatal stress was found to produce a significant increase in D2 receptor binding in the nucleus accumbens in the adult offspring without modifying D1 receptor binding (Henry et al., 1995). Also, decreased D2 receptor mRNA in the nucleus accumbens (shell and core) was reported after chronic mild stress, whereas no significant changes in D1 receptor mRNA were found (Dziedzicka-Wasylewska et al., 1997). Moreover, treatment with anti-depressant drugs increased the D2 receptor mRNA in the shell, but not the core, of the nucleus accumbens (Dziedzicka-Wasylewska et al., 1997). Nevertheless, in the present study we cannot rule out the possibility of involvement of D1 dopaminergic receptor in mediating the anti-stress effects of social interaction as we could not assess double immunohistochemistry with D1 dopaminergic receptor and pp38. Recently, we have shown that in the nucleus accumbens, cocaine CPP-induced Zif268 expression was reversed by social interaction preferentially in D1R-positive than D2R-expressing GABAergic medium spiny neurons (Prast et al., 2014). On the other hand, (Zhang et al., 2012) have shown that morphine CPP increased pp38 levels in nucleus accumbens microglia in comparison with naïve animals. It seems that microglial activation is involved in chronic morphine treatment effects. Previous reports have also shown that chronic morphine induced microglia activation (Zhang et al., 2011) and the expression of the microglial marker Iba1 (Zhang et al., 2012) in the nucleus accumbens.

We propose in this study that social interaction reward may have anti-stress effects. Given that social stress worsens drug abuse and relapse (Aguilar et al., 2013), social interaction as alternative to drugs may have protective and possibly therapeutically relevant effects against drug dependence.

Conflicts of interest

None.

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