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Changes in the level of calcyon mRNA in the brain of rats exposed to cocaine, self-administered or received passively

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ABSTRACT

The level of mRNA encoding calcyon (measured by *in situ* hybridization), one of the dopamine receptor interacting proteins, has been examined in the rat brain in the established animal model used to study the mechanisms of cocaine addiction (cocaine self-administration involving a yoked procedure). Two weeks of cocaine self-administration (maintenance) did not affect the level of calcyon mRNA, regardless of the way cocaine was delivered, except for tuberculum olfactorium, where calcyon mRNA was increased after cocaine treatment. In the reinstatement phase of the experiment cocaine alone induced an increase in the calcyon mRNA expression in most of the brain region studied (caudate putamen; tuberculum olfactorium; paraventricular thalamic nucleus; ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus) but only in the yoked saline control group. In other words, these results show that the single dose of cocaine (10 mg/kg) was able to induce an alteration in the level of calcyon mRNA in these rats which never before experienced any cocaine administration. The most significant effects were observed in the ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus. Interestingly, a similar effect was observed when the reinstatement of cocaine-seeking behaviour was evoked by cue (conditioned stimuli) that indicates that no cocaine was necessary to induce the changes in the level of calcyon mRNA expression. This effect was significant in tuberculum olfactorium, ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus. Such a result together with the brain areas involved in these effects might suggest the role of calcyon similar to the CART peptides and special vulnerability of calcyon expression rather to acute than chronic stimuli.

1. Introduction

Calcyon is a transmembrane protein with a predicted single transmembrane segment, mainly localized to intracellular vesicles of dendritic spines (Lezcano et al., 2000). Recently Zelenin et al. (2002) have shown that rat calcyon mRNA is expressed only in the brain and not in other tissues studied (as for example kidney cortex and medulla, lung, heart, liver). Brain-specific expression of calcyon is strong and distinct in various areas, especially in the hippocampus and hypothalamus (Oakman and Meador-Woodruff, 2004).

It has been shown that calcyon modulates the binding properties of the dopamine D_1 receptor and may play a role in intracellular calcium signalling mediated by that receptor (Lezcano et al., 2000). Calcyon protein has been also indicated as a modulator of dopamine D_1 receptor sensitivity for ligands (Lidow et al., 2001). Among the various brain functions in which the dopamine D_1 receptor has been involved, its role in cocaine addiction has been also strongly indicated (Xu et al., 1994; Hummel and Unterwald, 2002; Anderson and Pierce, 2005). It has been shown that the D_1 receptor mediates cocaine-induced reinforcement and reward (Weed and Woolverton, 1995), behavioural sensitization and also changes in gene expression (Zhang et al., 2002).

Calcyon has been shown to be co-expressed with the dopamine D_1 receptor in various brain regions (Zelenin et al., 2002), which enables the interaction between these two proteins. It suggests, in turn, the potential role of calcyon in various D_1-mediated processes, including – among others – reinforcement and reward, behavioural sensitization and locomotor activity. Therefore we decided to study the changes in the level of mRNA encoding calcyon in three phases of cocaine self-administration in the well established model involving a "yoked" procedure (Frankowska et al., 2008a, b) in which each experimental animal (working actively to get cocaine) was paired with 2 rats serving as a "yoked" control — one receiving cocaine passively and the other one receiving saline.

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2. Materials and methods

2.1. Animals

The experiments were carried out on rats (male Wistar, weighing 280–300 g) delivered by a licensed breeder (T. Górzkowska, Warsaw, Poland). The animals had free access to food and water during the 7-day habituation period. Rats were then maintained on limited water during the initial training session (Frankowska et al., 2008a,b). All experiments were conducted during the light phase of the light/dark cycle (between 8.00 a.m. and 3.00 p.m.) and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with approval of the Bioethics Commission as compliant with the Polish Law (21 August 2007). The animals were experimentally naïve.

2.2. Behavioural experiments

2.2.1. Drug

Cocaine hydrochloride (Sigma-Aldrich, USA) was dissolved in sterile 0.9% NaCl. Cocaine was given either *iv* (0.05 ml/infusion) or *ip* (1 ml/kg).

2.2.2. Cocaine self-administration

Rats were trained to press the lever of standard operant conditioning chambers (Med-Associates, USA) under a fixed ratio 5 schedule of water reinforcement. Two days following "lever-press" training and free access to water, the rats were chronically implanted with a silastic catheter in the external right jugular vein, as described previously (Filip, 2005). Catheters were flushed every day with 0.1 ml of saline solution containing heparin (70 U/ml, Biochemie, Austria) and 0.1 ml solution of cephazolin (10 mg/ml; Biochemie GmbH, Austria). Catheter patency was tested periodically with the ultrashort-acting barbiturate anesthetic methohexital (10 mg/kg, *iv*; loss of consciousness within 5 s) (Table 1).

After a 10-day recovery period, all animals were water deprived for 18 h and trained to lever press to fixed ratio 5 schedule of water reinforcement over a 2-h session. Subjects were then given access to cocaine during 2-h daily sessions performed 6 days/week (maintenance) and from that time they were given *ad libitum* water. Rats were tested simultaneously in groups of three, with two rats serving as yoked controls that received an injection of either 0.5 mg/kg cocaine or saline which was not contingent on responding each time a response-contingent injection of 0.5 mg/kg cocaine was self-administered by the third paired rat. In addition, similarly like rats with self-administered cocaine, the yoked animals received presentations of the stimulus complex.

The house light was illuminated throughout each session. Each completion of five presses on the "active" lever complex (fixed ratio 5 schedule) resulted in a 5-s infusion of cocaine (0.5 mg/kg per 0.1 ml) and a 5-s presentation of a stimulus complex (activation of the white stimulus light directly above the "active" lever and the tone generator, 2000 Hz; 15 dB above ambient noise levels). Following each injection, there was a 20-s time-out period during which response was recorded but had no programmed consequences. Response on the "inactive" lever never resulted in cocaine delivery. Acquisition of the conditioned operant response lasted a minimum of 10 days until the subjects met the following criteria: minimum requirement of 22 reinforcements with an average of 6 days and active lever presses with an average of 6 consecutive days and a standard deviation within those 6 days of <10% of the average; this criterion was selected based on our prior experiments (Filip, 2005). Once stable rates of response were established, the extinction procedure was carried out on the following day. During extinction sessions subjects had 2-h daily training sessions with no delivery of cocaine or the presentation of the conditioned stimulus. Once they reached the extinction criteria (a minimum of 10 extinction days with responding on the active lever below 10% of the level observed during maintenance, during at least 3 consecutive days), the separate groups of rats ($n = 7$–8 rats/group) were tested for response reinstatement induced by either a non-contingent presentation of the self-administered reinforcer (10 mg/kg cocaine, *ip*) or discrete contextual cue (tone + light previously paired with cocaine self-administration). During the reinstatement tests (2-h sessions), active lever presses on the fixed ratio 5 schedule resulted only in an intravenous injection of saline. The results of behavioural experiments are presented elsewhere (Frankowska et al., 2008a,b).

Table 1
Experimental design.

Maintenance	Extinction	Reinstatement
18 days	10 days	
Cocaine self-administration		
"Yoked" saline		
"Yoked" cocaine		
Cocaine self-administration	Saline	
"Yoked" saline	Saline	
"Yoked" cocaine	Saline	
Cocaine self-administration	Saline	Cocaine
Cocaine self-administration	Saline	Saline
Cocaine self-administration	Saline	Cue
Cocaine self-administration	Saline	–
"Yoked" saline	Saline	Cocaine
"Yoked" saline	Saline	Saline
"Yoked" saline	Saline	Cue
"Yoked" saline	Saline	–

2.3. Biochemical experiments

2.3.1. Tissue preparation

Immediately following the behavioural experiments, the rats were killed by decapitation. The brains were rapidly dissected, frozen by immersion in *n*-heptan dry-ice bath and stored at −70 °C until sectioned. Consecutive coronal sections (12 μm) were cut on the cryostat microtome (Leica CM 1850, Germany) at −22 ± 2 °C and mounted on gelatin-coated slides and stored at −70 °C. The identification and nomenclature of the brain structures were based on the Paxinos and Watson Rat Brain Atlas (1998).

2.3.2. Measurements of mRNA encoding calcyon — in situ hybridization

The sections were fixed in 4% paraformaldehyde/phosphate-buffered saline (PBS) for 15 min, rinsed in PBS and treated with 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0) for 10 min to reduce nonspecific hybridization of the probes. The sections were washed twice for 5 min in 2× SSC (300 mM NaCl/30 mM sodium citrate, pH 7.0). Following dehydration in increasing concentration of ethanol (70–100%), the sections were dilapidated in chloroform for 5 min, rinsed in ethanol and dried. For measurements of mRNA encoding calcyon oligonucleotide probes were designed: 5′CTTTGG CTGGTCCGCAGAGGTAAGTTGATATTCTGTCTGTGTT 3′ complementary to bp: 308–350 of rat calcyon mRNA. The oligonucleotide probes were labelled at the 3′ end with [^{35}S] dATP (ICN) using terminal transferase (Roche). The sections were hybridized for 18 h at 38 °C with 1×10^6 dpm of the labelled probe in 50 μl of the hybridization solution (50% formamide, 4× SSC, 1× Denhardt's solution, 10% dextran sulfate, 0.5 mg/ml herring sperm DNA, 0.25 mg/ml yeast tRNA, 25 mM sodium phosphate (pH 7.0) and 50 mM dithiothreitol). After hybridization, each slide was washed twice for 5 s in 1× SSC (room temperature), three times for 15 min in 1× SSC at 55 °C, then for 30 min in 1× SSC at room temperature and rinsed in deionized water. The sections were dehydrated in increasing concentration of ethanol (70–100%) and air dried.

2.3.3. Image analysis

The slides processed for *in situ* hybridization were exposed to plates BioMax (Kodak) for 3 weeks before being developed. Autoradiograms

were analyzed using MCID imaging analysis system (Imaging Research, Ontario, Canada). Data are expressed as a mean of optical density (O.D.) ± S.D.

2.3.4. Statistical analysis

Data from biochemical experiments for the cocaine self-administration group and the withdrawal phase group were analyzed using one-way or two-way analysis of variance (ANOVA). A *post hoc* Bonferroni test was performed to locate differences between group means. Data obtained in the reinstatement phase were analyzed using a two-way analysis of variance (ANOVA) with a *post hoc* Bonferroni test to locate differences between group means. The criterion for statistically significant differences for all experimental groups was set at $P<0.05$.

3. Results

The level of calcyon mRNA was observed in various brain regions in the "yoked" saline control group. It was especially high in the paraventricular thalamic nucleus, posterior part, arcuate nucleus, ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus. That localization was similar to the data provided by Zelenin et al. (2002). In the hippocampus the level of calcyon mRNA was relatively lower but well pronounced in the CA1, CA3 and dentate gyrus (DG) (Fig. 1).

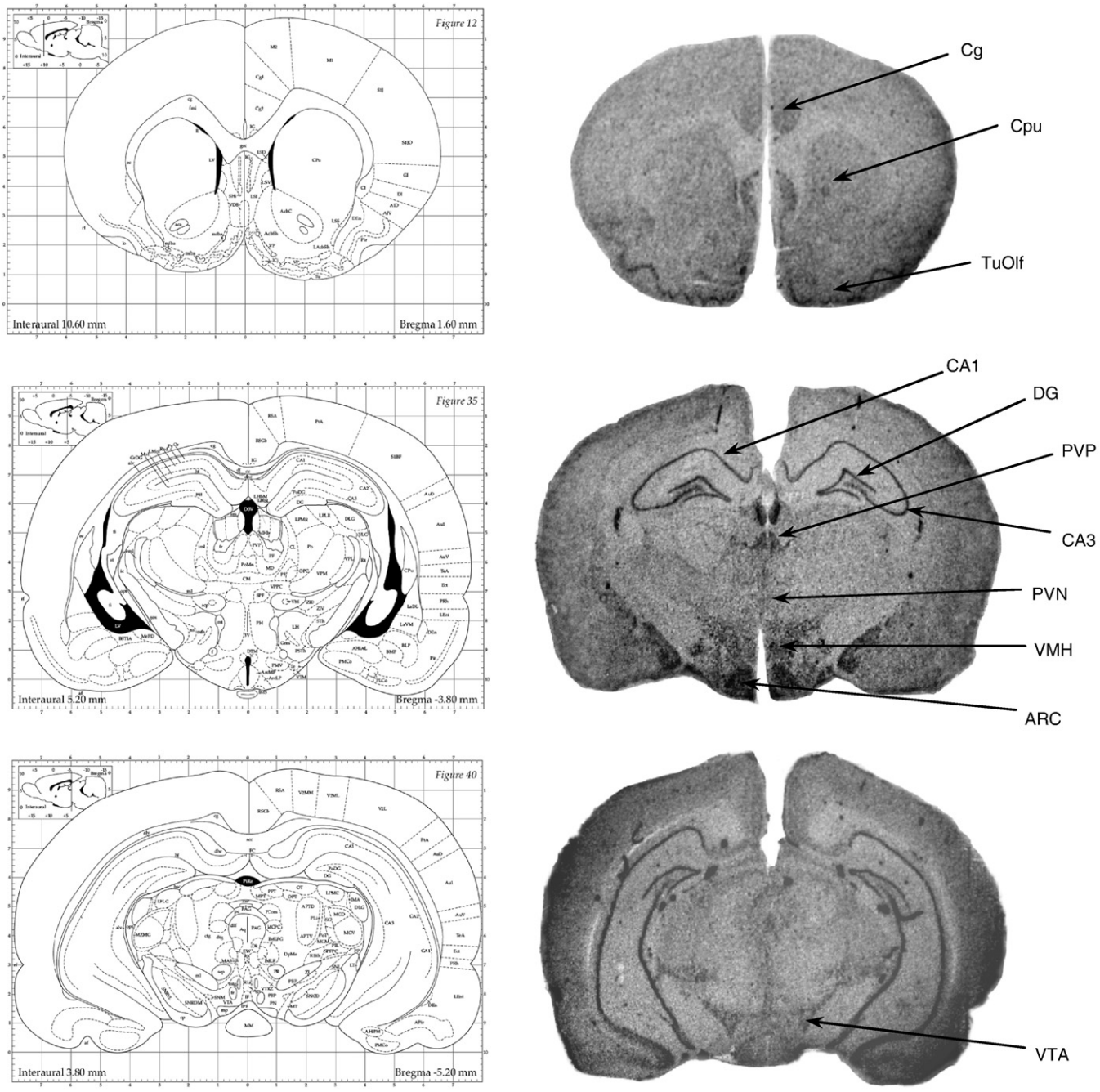

Fig. 1. Representative autoradiograms of mRNA encoding for calcyon in the rat brain. The brain regions used for quantitative analysis were chosen according to Paxinos and Watson (1998). Cingular cortex (Cg); caudate putamen (Cpu); tuberculum olfactorium (TuOlf); paraventricular thalamic nucleus (PVP); arcuate nucleus (ARC); ventromedial hypothalamic nucleus (VMH); paraventricular hypothalamic nucleus (PVN); ventral tegmental area (VTA); field CA1 of hippocampus (CA1); field CA3 of hippocampus (CA3); dentate gyrus (DG).

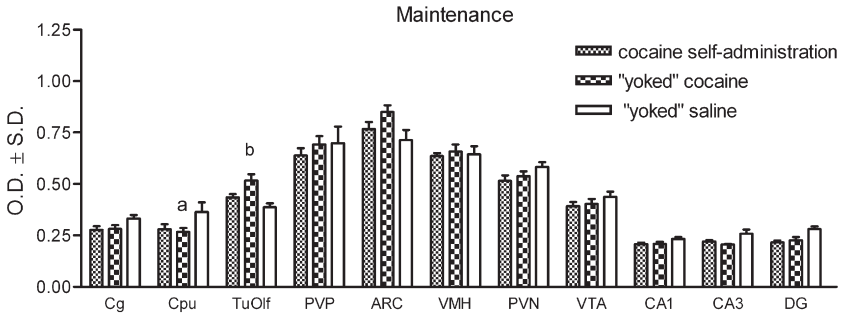

Fig. 2. The level of calcyon mRNA in the rat brain in the maintenance phase. Data are expressed as a mean of optical density (O.D.) ± S.D. For statistical analysis a one-way ANOVA test was used with a Bonferroni *post hoc* test. [a]$P<0.05$; [b]$P<0.01$ vs. "yoked" saline group. Cingular cortex (Cg); caudate putamen (Cpu); tuberculum olfactorium (TuOlf); paraventricular thalamic nucleus (PVP); arcuate nucleus (ARC); ventromedial hypothalamic nucleus (VMH); paraventricular hypothalamic nucleus (PVN); ventral tegmental area (VTA); field CA1 of hippocampus (CA1); field CA3 of hippocampus (CA3); dentate gyrus (DG).

3.1. Cocaine self-administration

Cocaine self-administration (either active or passive, "yoked") did not alter the level of calcyon mRNA in the studied brain regions. Only in the animals passively receiving cocaine ("yoked" cocaine control group), a significant increase was observed in the tuberculum olfactorium (0.51 ± 0.10 "yoked" cocaine control group vs. 0.39 ± 0.06 "yoked" saline control group; $P<0.01$), while in the caudate putamen a slight decrease of calcyon mRNA was observed (0.27 ± 0.07 "yoked" cocaine control group vs. 0.36 ± 0.12 "yoked" saline control group; $P<0.05$) (Fig. 2).

3.2. Withdrawal from cocaine self-administration

The level of calcyon mRNA in the paraventricular hypothalamic nucleus, arcuate nucleus, ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus was higher after the 10 day withdrawal period in the cocaine self-administration group receiving cocaine, both actively and passively, as compared to yoked saline controls (Fig. 3).

However, in that experimental phase the level of calcyon mRNA in the yoked saline control group was significantly decreased in these brain regions as compared to that group in the maintenance phase. The level of calcyon mRNA did not significantly change in these brain regions in comparison to the level observed in rats receiving cocaine (actively or passively) examined after the maintenance phase. The statistical significance of the differences was due to the decreased level of calcyon mRNA in the yoked saline control group. Such result may be interpreted as if it was the experimental procedure, involving many intravenous administrations, which enhanced the level of calcyon mRNA. During the withdrawal phase that level decreased in a saline group, but it stayed elevated in groups receiving cocaine.

It is also interesting to note that – although no significant differences between the three groups of animals (yoked saline control vs. cocaine received actively or passively) were observed in the hippocampus – the level of calcyon mRNA was generally increased in comparison to cocaine self-administration phase. While comparing these two phases of the experiment using a two-way ANOVA analysis it has been noted that in the hippocampus it was rather the effect of the experimental procedure than the cocaine itself which had an influence on the level of calcyon mRNA.

On the other hand, in the paraventricular hypothalamic nucleus and ventromedial hypothalamic nucleus the statistical analysis (two-way ANOVA) indicated the interaction experimental procedure × administration (i.e. saline or drug). For paraventricular hypothalamic nucleus $F(2,59)=5.15$; $P<0.01$; for ventromedial hypothalamic nucleus $F(2,62)=3.72$; $P<0.01$.

3.3. Reinstatement

Reinstatement of cocaine-seeking behaviour was evoked either by cocaine alone (Table 2) or by contextual cue (tone + light previously associated with the conditioned stimuli). In that phase of the experiment cocaine alone — given here as a reinforcement – induced alterations in the calcyon mRNA expression in most of the brain region studied (caudate putamen, tuberculum olfactorium, paraventricular thalamic nucleus, posterior part, ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus), but only in the yoked saline control group (Table 2). In other words, these results show that the single dose of cocaine (10 mg/kg) was able to induce alteration in the level of calcyon mRNA in these rats which never before experienced any cocaine administration, although they have gone through the entire experimental procedure, receiving always saline. The most significant effects were observed in the ventromedial

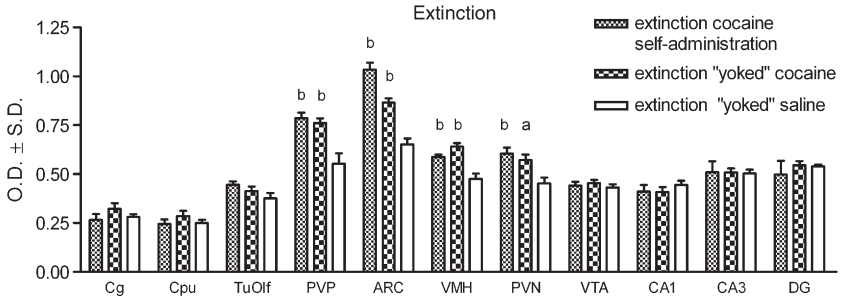

Fig. 3. The level of calcyon mRNA in the rat brain in extinction phase. Data are expressed as a mean of optical density (O.D.) ± S.D. For statistical analysis a one-way ANOVA test was used with a Bonferroni *post hoc* test. [a]$P<0.05$; [b]$P<0.01$ vs. extinction "yoked" saline group. Cingular cortex (Cg); caudate putamen (Cpu); tuberculum olfactorium (TuOlf); paraventricular thalamic nucleus (PVP); arcuate nucleus (ARC); ventromedial hypothalamic nucleus (VMH); paraventricular hypothalamic nucleus (PVN); ventral tegmental area (VTA); field CA1 of hippocampus (CA1); field CA3 of hippocampus (CA3); dentate gyrus (DG).

Table 2
Effect of cocaine (10 mg/kg *ip*)-induced reinstatement of cocaine-seeking behaviour on calcyon mRNA in the rat brain. For statistical analysis one-way ANOVA test was used with Bonferroni *post hoc* test.

Brain area	"Yoked" saline/saline (*ip*)	"Yoked" saline/cocaine (10 mg/kg, *ip*)	Cocaine self-administration/ saline (*ip*)	Cocaine self-administration/ cocaine (10 mg/kg, *ip*)
Cingular cortex (Cg)	0.39 ± 0.07	0.34 ± 0.08	0.30 ± 0.10	0.32 ± 0.08
Caudate putamen (Cpu)	0.36 ± 0.07	0.33 ± 0.09[a]	0.30 ± 0.11	0.26 ± 0.06
Tuberculum olfactorium (TuOlf)	0.61 ± 0.16	0.51 ± 0.08[a]	0.67 ± 0.22	0.48 ± 0.22
Paraventricular thalamic nucleus (PVP)	0.73 ± 0.20	0.72 ± 0.18[a]	0.72 ± 0.15	0.67 ± 0.06
Arcuate nucleus (ARC)	0.76 ± 0.19	0.85 ± 0.22[b]	0.89 ± 0.23	0.68 ± 0.16
Ventromedial hypothalamic nucleus (VMH)	0.64 ± 0.16	0.69 ± 0.18	0.65 ± 0.07	0.61 ± 0.07
Paraventricular hypothalamic nucleus (PVN)	0.61 ± 0.18	0.65 ± 0.17[b]	0.53 ± 0.07	0.53 ± 0.05
Ventral tegmental area (VTA)	0.45 ± 0.14	0.47 ± 0.12	0.48 ± 0.18	0.47 ± 0.13
Field CA1 of hippocampus (CA1)	0.58 ± 0.07	0.48 ± 0.07[a]	0.45 ± 0.06	0.43 ± 0.02
Field CA3 of hippocampus (CA3)	0.64 ± 0.08	0.54 ± 0.06[a]	0.46 ± 0.03	0.48 ± 0.08
Dentate gyrus (DG)	0.64 ± 0.09	0.55 ± 0.05	0.44 ± 0.05	0.52 ± 0.09

[a] $P<0.05$ vs. "yoked" saline/saline group.
[b] $P<0.001$ vs. "yoked" saline/saline group.

hypothalamic nucleus ($F(1,38)=5.37$; $P<0.001$) and paraventricular hypothalamic nucleus ($F(1,38)=7.07$; $P<0.001$).

Interestingly, a similar effect was observed when the reinstatement of cocaine-seeking behaviour was evoked by cue (conditioned stimuli), which indicates that no cocaine was necessary to induce the changes in the level of calcyon mRNA expression (Table 3). This effect was significant in tuberculum olfactorium ($F(1,60)=12.92$; $P<0.01$), ventromedial hypothalamic nucleus ($F(1,48)=6.26$; $P<0.01$) and paraventricular hypothalamic nucleus ($F(1,48)=16.73$; $P<0.001$).

A different situation was observed in the hippocampus — in all regions studied (CA1, CA3 and dentate gyrus) cocaine administered as a reinforcement affected the level of calcyon mRNA, but only in the saline yoked group (however, a statistical significance was observed in CA1 and CA3 areas). It is also interesting that in the hippocampus – in contrast to the previously described brain regions – cue-evoked reinstatement did not induce any changes in the yoked saline group, but increased the calcyon mRNA level in the cocaine self-administering group. This effect was significant in all studied regions of hippocampus (for CA1 $F(1,20)=28.73$; $P<0.01$; for CA3 $F(1,20)=37.27$; $P<0.01$; and for dentate gyrus $F(1,20)=24.89$; $P<0.01$).

4. Discussion

In the present study the level of calcyon mRNA – measured by *in situ* hybridization – was observed in various brain regions; it was especially high in the paraventricular thalamic nucleus, posterior part, arcuate nucleus, ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus. That localization was similar to the data provided by Zelenin et al. (2002) and happened to overlap with the distribution of CART (cocaine- and amphetamine-regulated transcript) peptides (Rogge et al., 2008). In the hippocampus the level of calcyon mRNA was relatively lower but well pronounced in the CA1, CA3 and dentate gyrus. Also the tuberculum olfactorium seems enriched in mRNA encoding calcyon.

Since the role of the dopamine D_1 receptor has been postulated in the mechanisms of cocaine addiction (Xu et al., 1994), therefore we decided to study the changes in the level of mRNA encoding calcyon in three phases of cocaine self-administration in the highly advanced and well established model involving the "yoked" procedure (Frankowska et al., 2008a,b), in which each experimental animal (working actively to get cocaine) was paired with 2 rats serving as a "yoked" control — one receiving cocaine passively and the other one receiving saline.

Two weeks of self-administration of cocaine did not affect the level of calcyon mRNA regardless of the way cocaine was delivered, whether animals were actively working on it or were yoked, receiving cocaine passively. The exception was tuberculum olfactorium, where calcyon mRNA was increased after cocaine treatment, and that effect was even stronger in yoked animals. This result is very interesting, especially in the light of data provided by Ikemoto (2003), who has shown the strongest effects of intra-cerebral cocaine self-administration, postulating the critical role of this brain region in mediating rewarding action of cocaine. It is also worth noting that tuberculum olfactorium has been shown to play an important role in the expression of ethanol-induced behavioural sensitization in the mice (de Araujo et al., 2009). On the other hand, in the present study the observed decrease in the calcyon mRNA level in caudate putamen in this stage of the experiment might point to one of the molecular mechanism underlying the loss of signalling of the D_1 receptor upon cocaine self-administration, which has

Table 3
Effect of cocaine associated cue (tone + light)-induced reinstatement of cocaine-seeking behaviour on calcyon mRNA in the rat brain. For statistical analysis one-way ANOVA test was used with Bonferroni *post hoc* test.

Brain area	"Yoked" saline/–	"Yoked" saline/cue	Cocaine self-administration/–	Cocaine self-administration/cue
Cingular cortex (Cg)	0.39 ± 0.07	0.33 ± 0.06	0.30 ± 0.10	0.34 ± 0.08
Caudate putamen (Cpu)	0.36 ± 0.07	0.32 ± 0.04	0.30 ± 0.11	0.32 ± 0.09
Tuberculum olfactorium (TuOlf)	0.61 ± 0.16	0.55 ± 0.12[a]	0.67 ± 0.22	0.52 ± 0.14
Paraventricular thalamic nucleus (PVP)	0.73 ± 0.20	0.63 ± 0.16	0.72 ± 0.15	0.59 ± 0.08
Arcuate nucleus (ARC)	0.76 ± 0.19	0.87 ± 0.25	0.89 ± 0.23	0.84 ± 0.24
Ventromedial hypothalamic nucleus (VMH)	0.64 ± 0.16	0.68 ± 0.21[a]	0.65 ± 0.07	0.66 ± 0.14
Paraventricular hypothalamic nucleus (PVN)	0.61 ± 0.18	0.63 ± 0.14[b]	0.53 ± 0.07	0.61 ± 0.18
Ventral tegmental area (VTA)	0.45 ± 0.14	0.43 ± 0.14	0.48 ± 0.18	0.49 ± 0.16
Field CA1 of hippocampus (CA1)	0.58 ± 0.07	0.57 ± 0.06	0.45 ± 0.06	0.51 ± 0.05[c]
Field CA3 of hippocampus (CA3)	0.64 ± 0.08	0.64 ± 0.05	0.46 ± 0.03	0.56 ± 0.03[c]
Dentate gyrus (DG)	0.64 ± 0.09	0.66 ± 0.09	0.44 ± 0.05	0.58 ± 0.03[c]

[a] $P<0.01$ vs. "yoked" saline/– group.
[b] $P<0.001$ vs. "yoked" saline/– group.
[c] $P<0.01$ vs. cocaine self-administration/– group.

been suggested by various studies using dopamine D_1 receptor agonists and antagonists (Dias et al., 2004; Khroyan et al., 2000; Alleweireldt et al., 2002; Edwards et al., 2007; Caine et al., 1995; Maldonado et al., 1993).

The lack of the effect of chronic cocaine exposure on the level of calcyon mRNA in other brain regions examined is very interesting in the light of the data obtained in the third phase of the experiment, when the reinstatement of cocaine-seeking behaviour was examined after the period of withdrawal. When the single dose of cocaine was used as a non-contingent reinforcer, it did not influence the level of calcyon in the groups of animals with prior exposure to the drug, but it induced a strong increase in the expression of calcyon in the saline yoked control group, never exposed to cocaine before. A similar effect in that particular group of animals was evoked by a discrete contextual cue (tone + light), previously associated with cocaine self-administration.

Cocaine self-administration in the maintenance phase did not alter the level of calcyon mRNA in the studied brain regions. However, as described above, in the animals passively receiving cocaine ("yoked" cocaine control group), a significant increase was observed in the tuberculum olfactorium. The level of calcyon mRNA in the paraventricular thalamic nucleus, arcuate nucleus, ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus was higher after the 10 day withdrawal period in the cocaine self-administration group receiving cocaine passively as compared to yoked saline controls. It should be noted that in this experimental phase the level of calcyon mRNA in the yoked saline control group was significantly decreased in these brain regions as compared to the group in the maintenance phase. In the reinstatement phase of the experiment cocaine alone induced an increase in the calcyon mRNA expression in most of the brain region studied, but only in the yoked saline control group. In other words, these results show that a single dose of cocaine (10 mg/kg) was able to induce alteration in the level of calcyon mRNA in these rats which never before experienced any cocaine administration. The most significant effects were observed in the ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus. Interestingly, a similar effect was observed when the reinstatement of cocaine-seeking behaviour was evoked by cue (conditioned stimuli), which indicates that no cocaine was necessary to induce the changes in the level of calcyon mRNA expression. This effect was significant in the tuberculum olfactorium, ventromedial hypothalamic nucleus and paraventricular hypothalamic nucleus. Such a result together with the brain areas involved in these effects might suggest the role of calcyon similar to the CART peptides (Vicentic and Jones, 2007) and special vulnerability of calcyon expression rather to acute than chronic stimuli.

In the hippocampus no significant differences were observed in the level of calcyon mRNA between three groups (yoked saline control vs. cocaine received actively or received passively) — both in the maintenance as well as the withdrawal phases of the experiment but in the latter phase it was generally increased in comparison to the cocaine self-administration phase. In the reinstatement phase of the experiment in all hippocampal regions studied (CA1, CA3 and dentate gyrus) cocaine used as a reinforcer decreased the level of calcyon mRNA in the saline yoked group but – in contrast to other brain regions described above – cocaine-evoked reinstatement increased the level of calcyon mRNA in the cocaine self-administering group (in the CA1). It is also interesting that in the hippocampus – in contrast to the previously described brain regions – cue-evoked reinstatement did not induce any changes in the yoked saline group, but increased the calcyon mRNA level in the cocaine self-administering group. The dependence of the alterations in the level of calcyon mRNA in hippocampus in the reinstatement phase on the previous experience with cocaine may point to the role of memory mechanisms operating in that brain region. Such notion is justified, since calcyon has been shown to localize near excitatory synapses (Xiao et al., 2006) and to play a specialized role in regulating activity-dependent removal of synaptic AMPA receptors (Davidson et al., 2009), which role in the synaptic plasticity, a proposed neural correlate of learning and memory, is well established (Malinow, 2003).

In conclusion, the obtained results implicate the role of calcyon gene expression as an interesting indicator of neuronal activity in various phases of cocaine addiction. It seems that expression of calcyon might serve as a response to novel stimuli associated with passive administration of cocaine, however further studies are necessary to elucidate that working hypothesis.

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