

# **Enhanced secretory activity of midbrain Ucn1 neurons in two different mouse models with reduced alcohol consumption**

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## **Abstract**

To date the neurocircuitry regulating alcohol consumption and dependence is not well understood. Several experimental studies have focused on the role of CRF peptide; however, recent studies also implicate another CRF-related neuropeptide, urocortin 1 (Ucn1) in alcohol consumption. Ucn1 is highly expressed in the non-preganglionic Edinger-Westphal nucleus (npEW), and our group has previously shown that npEW Ucn1 is involved in various physiological responses including regulation and modulation of food consumption, stress adaptation and depression.

In order to understand better the role of this neuropeptide in alcohol consumption and dependence, we used two different models: PKC-epsilon null mice and the chromosome 2 substitution strain (CSS-2) mice. Both lines voluntarily consume less ethanol, because they derive less reward from ethanol and are more sensitive to its aversive effects. Using fluorescence immunocytochemistry and *in situ* hybridization, we measured changes in the secretory activity of npEW Ucn1 neurons in these mice. Compared with wild type mice, PKC-epsilon null mice exhibited increased secretory activity of npEW Ucn1 neurons. Next we compared the secretory

activity of npEW Ucn1 neurons between CSS-2 and C57BL/6J mice in a chronic alcohol consumption paradigm (they consumed alcohol for two hours a day for 3 weeks). FosB/deltaFosB immunocytochemistry revealed a similar activation in the npEW of CSS-2 and C57BL/6J mice in response to chronic alcohol consumption. For CSS-2 mice, however, the expression of npEW Ucn1 mRNA was significantly higher than in C57BL/6J mice. Our results further implicate Ucn1 in the modulation of consumption and responses to alcohol, and suggest that Ucn1 plays a role in the neuroadaptive changes driving excessive consumption of alcohol.

**Keywords:** Urocortin 1, non-preganglionic Edinger-Westphal nucleus; alcohol.

## **Introduction**

Alcohol use disorders are a major health problem, and besides the psychological problems that result from dependence itself, excessive alcohol use affects numerous physiological systems, which may contribute to certain co-morbid behavioural disorders such as major depression (Lynskey, 1998; Davis et al. 2008). In the United States alone, 4.5% of the population is affected from alcohol abuse and associated secondary complications (Shalala, 2000). Furthermore, the World Health Organization predicted that 3.2% of global mortality is attributable to alcohol abuse and dependence problems (Rehm et al. 2003). Consequently, the discovery of effective treatments which cause fewer side effects represents a major challenge for the scientific community.

To date numerous studies have focused on the role of the major stress hormone; corticotropin-releasing hormone (CRF) in alcohol consumption and dependence. Indeed now it is well established that CRF activity in the amygdala through enhancement of GABAergic transmission drives excessive alcohol self-administration (Funk et al. 2006; Nie et al. 2004).

The accumulating evidence for the involvement of CRF neuropeptide in alcohol consumption and stress responses and the wide and differential distribution of CRF1 and CRF2 receptors throughout the mammalian brain raises the intriguing possibility that other members of this family of peptide may be implicated in similar behavioral manifestations (Ryabinin & Weitemier, 2006; Kozicz, 2007). Urocortin 1, a member of CRF family of neuropeptides is mostly expressed in the midbrain non-preganglionic Edinger-Westphal nucleus (Vaughan et al. 1995; Kozicz et al. 1998). Ucn1 and CRF are closely related and Ucn1 binds to both CRF receptors (CRF1 and CRF2) with the highest affinity for the CRFR2 (Lewis et al. 2001; Kozicz et al. 2004).

An increasing number of experimental studies implicate Ucn1 activity in alcohol consumption. Many of these studies however have provided contradictory results, partially, due to the fact that this is a new line of research and in many cases the role of Ucn1 is underestimated. Studies investigating the expression of activity-dependent inducible transcription factors (ITF) such as c-Fos, FosB and  $\Delta$ FosB, after alcohol administration have shown an increased sensitivity of npEW nucleus neurons to alcohol (Ryabinin& Weitemier, 2006; Chang et al. 1995;Bachtell et al. 2002a, 2002b). Furthermore, comparative analysis between genetically modified animals strains specifically bred for aversion or preference for alcohol confirmed the involvement of Ucn1 (Weitemier& Ryabinin, 2005).

In order to understand better the role of Ucn1 neuropeptide in alcohol consumption, we investigated Ucn1 levels in two genetically modified mice strains; PKC-epsilon null and chromosome 2 substitution (CSS-2) mice. PKC-epsilon is one of the seven isozymes of PKC protein which phosphorylate other proteins changing their function. The first indications for the involvement of PKC-epsilon in regulating consumption of ethanol were provided by in vitro cell cultures studies. These studies showed that ethanol treatment of different cell lines causes translocations of PKC-epsilon to different subcellular locations (Gordon et al. 1997). But the confirmation for PKC-epsilon implication in ethanol consumption came from studies with PKC-epsilon null animals. These studies showed that PKC-epsilon null mice exhibit reduced preference and consumption of alcohol and reduced levels of anxiety-like behavior (Hodge et al. 2002; Olive et al. 2000). In addition, rescuing PKC-epsilon expression in key structures implicated in responses to ethanol such as amygdala and nucleus accumbens restores ethanol intake and sensitivity to the wild-type littermates levels (Choi et al. 2002).

Genetic studies using whole genome linkage analysis and quantitative trait loci mapping have identified numerous genes that may explain predisposition and biological basis of high ethanol consumption and dependence. Several chromosome 2 loci have been repeatedly implicated in as risk factors in these studies (Wiener et al. 2005; Agrawal et al. 2008). Similarly to PKC-epsilon null strain, CSS-2 mice exhibit reduced preference and consumption of alcohol. In these type of mice, chromosome 2 from an A/J strain of mice (in this case a low drinking strain) is transferred to a C57BL/6J (a high drinking strain) resulting in the observed behavior in terms of consumption and preference for alcohol. Chromosome substitution strain method is very useful in identifying risk genes for drug dependence and other pathological conditions since it allows investigation of single chromosomes manipulations effects on behavior (Singer et al. 2005).

The similar behavioral manifestations in these two different mice strains motivated us to hypothesize that the observed consumption and preference for alcohol is a result of an altered secretory activity of Ucn1 containing neurons in the midbrain npEW nucleus.

## **Materials and Methods**

### *Animals*

PKC-epsilon null mice were generated by homologous recombination in embryonic stem cells of the mouse PKC-epsilon gene (Khasar et al. 1999). Male and female PKC-epsilon (+/-) mice were maintained on inbred 129S4 and backcrossed for more than 10 generations. F1 hybrid mice were generated by C57BL/6J x 129S4 mating. Subsequently these mice were intercrossed to produce F2 hybrid PKC-epsilon (+/+) and PKC-epsilon (-/-) used for the experiments (Wallace et al. 2007). The

animals were housed in standard Plexiglas cages in 12hr light/dark cycle, lights on at 6pm. and had *ad libidum* access to food and water. All animals were males and aged 4-5 weeks at the date of sacrifice.

CSS-2 mice were generated by transferring chromosome 2 from an A/J strain (donor) to a C57BL/6J strain (host) as described in Nadeau et al. (2000). Animals were housed in standard Plexiglas cages in 12hr light/dark cycle, lights on at 7pm. and had *ad libidum* access to food and water.

CSS-2 and C57BL/6J controls were tested in a daily (each day for two hours in the beginning of the dark cycle) alcohol (15% weight/volume) paradigm for 3 weeks. 24-28 hr after the last drinking session the mice were sacrificed and brains were rapidly removed and stored in 4% PFA in PBS for 3-4 days. Thereafter the brains were transferred to 30% sucrose solution in PBS until the day of experiment.

All procedures were conducted in accordance with the Declaration of Helsinki and the guidelines for animal experimentation approved by the Ernest Gallo Clinic and Research Center institutional animal care and use committee and the ethical committee for animal experiments of the University Medical Centre Utrecht.

### *Histology*

All animals were deeply anesthetized with Nembutal (sodium-pentobarbital; Sanofi-Synthélabo, Maassluis, The Netherlands; 100 mg/kg body weight), their chest cavity was opened, and thereafter transcardially perfused with 20 ml of 0.1 M sodium phosphate-buffered saline (PBS) (pH 7.4), for 2 min, followed by perfusion with 100 ml of ice-cold 4% paraformaldehyde in 0.2 M Millonig sodium phosphate buffer (pH 7.4), for 20 min. Then, mice were decapitated and their brains quickly dissected and postfixed in mixture of 4%

PFA and 30% sucrose in 0.1M PBS.. After complete submergence of the brains, 25 µm thick coronal sections with 100-µm intervals were cut at the level of the midbrain npEW nucleus (Bregma-2.80 to -4.04mm; Paxinos and Franklin, 2001) on a MICROM HM 440 E freezing microtome (MICROM GmH, Germany). Thereafter, the sections were saved in sterile antifreeze solution (0.05 M PBS, 30% ethylene glycol, 20% glycerol) at -20 °C, until further histological processing.

All subsequent staining procedures were carried out simultaneously, to assure that brains of all experimental groups were treated in the same way.

### *Immunocytochemistry*

For fluorescent immunocytochemistry, 3x20 min. rinses in PBS and consecutive incubations in: 0.5% Triton x 100, in PBS, 2% normal donkey serum (NDS) for 30 min. in PBS-B and in a mixture of primary antiserum (anti-Ucn1 rabbit 1: 25000 ) in 2% NDS for 24hr in room temperature. Subsequently, sections were rinsed 2x15min. in PBS and incubated in a mixture of secondary antiserum (Cy3-conjugated anti-rabbit IgG 1:100; Jackson Immunoresearch Labs. USA) in 2% NDS for 3 hr. After 2x 15 min. rinses, sections were mounted on gelatine coated slides and coverslipped with Fluorosave. Visualization was performed in Leica confocal laser scanning microscope (Leica Microsystems TCS SP2 AOBS system; Germany)

For ABC-DAB immunolabeling, 4x15min. rinses in PBS were followed by consecutive incubations in: 0.5% Triton x 100 in PBS for 30 min., 2% NDS for 60 min. and in a mixture of primary antiserum (anti-ΔFosB rabbit 1:2000) in PBS-B for 24hr in room temperature. Subsequently, sections were rinsed 2x15min. in PBS and incubated in: in a mixture of secondary antiserum (anti-rabbit Vector ABC Elite Kit;

PK-6101; Vector Labs, Burlingame, CA, USA) in PBS-B for 60 min., and in ABC reagent (1:200) with PBS-B for 60 min. Immunolabeling was visualized by implementing the sections in 10mg 3-3'-diaminobenzidine (DAB; D 5637; Sigma–Aldrich) in 50 ml Tris buffer for 10 min. the signal intensification was controlled under a stereomicroscope and the reactions suspended in Tris buffer. After 10min rinse in Tris buffer, sections undergone gradual steps of incubations at alcohol, isopropanol and xylene and were mounted in gelatine coated slides covered with Entellan. Finally, immunolabeling was studied under a Leica DMRBE microscope connected to a Leica DC 500 (Leica Microsystems, Germany).

#### *Non-radioactive in situ hybridization*

Hybridization was carried out using antisense and sense (control; no hybridization signal was seen) cRNA probes transcribed from a linearized 550 bp Ucn1 cDNA, and labeled with DIG-11-UTP using a labeling kit (Roche Molecular Biochemicals, Basel, Switzerland). *In situ* hybridization steps were carried out at room temperature unless stated otherwise. First, sections were fixed in 0.1 M borax-buffered 4% paraformaldehyde (pH 9.5), at 4 °C for 30 min. Then, sections were rinsed four times for 7 min with 0.1 M PBS followed by pre-incubation in proteinase K medium (0.1 M Tris/HCl, 0.05 M EDTA, 0.1 mg proteinase K), for 10 min at 37 °C. After rinsing in autoclaved MQ water, acetylation was performed with 0.25% acetic acid anhydride in 0.1 M tri-ethanolamine buffer (pH 8.0), for 10 min, followed by rinsing in 2 times concentrated (2×) standard saline citrate buffer (SSC; pH 7.0) for 5 min. Hybridization mixture (50% deionized formamide, 0.3 M NaCl, 0.001 M EDTA, Denhardt's solution, 10% dextran sulfate), together with 0.5 mg/ml tRNA and the mRNA digoxigenin (DIG) probe (ca. 2.5 ng/ml), was placed into a water bath at 80 °C for 5 min and then on ice for another 5 min. Sections were incubated in



hybridization solution for 16 h at 58 °C, rinsed four times for 7 min with 4× SSC, incubated for 30 min at 37 °C in preheated RNase medium (0.5 M NaCl, 0.01 M Tris/HCl, 0.001 M EDTA; pH 8.0) containing 0.01 mg/ml RNase A, which had been added just before the start of incubation, and stringently washed in decreasing SSC concentrations (2×, 1×, 0.5×, 0.1×), for 30 min at 58 °C. The alkaline phosphatase method with nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate, toluidine salt (NBT/BCIP) as substrate, was used for the detection of the DIG label. Briefly, after rinsing four times for 5 min with buffer A (0.1 M Tris/HCl, 0.15 M NaCl; pH 7.5), sections were pre-incubated in buffer A containing 0.5% blocking agent (Roche) for 1 h, followed by 3 h of incubation with sheep anti-DIG-AP (Roche; 1:5000) in buffer A containing 0.5% blocking agent. Subsequently, sections were rinsed four times for 5 min in buffer A, followed by two times of 5 min rinsing in buffer B (0.1 M Tris/HCl, 0.15 M NaCl, 0.05 M MgCl<sub>2</sub>; pH 9.5). After 8 h of incubation in NBT/BCIP medium (10 ml buffer B, 2.4 mg levamisole, 175 µl NBT/BCIP mixture; Roche) in a light-tight box, the reaction was stopped by placing the sections in buffer C (0.1 M Tris/HCl, 0.01 M EDTA; pH 8.0). After rinsing twice for 5 min, sections were mounted on gelatin-coated glass slides, dried for 16 h at 37 °C, rinsed in distilled water, dehydrated, cleared in xylene and coverslipped with Entellan (Derks et al. 2007).

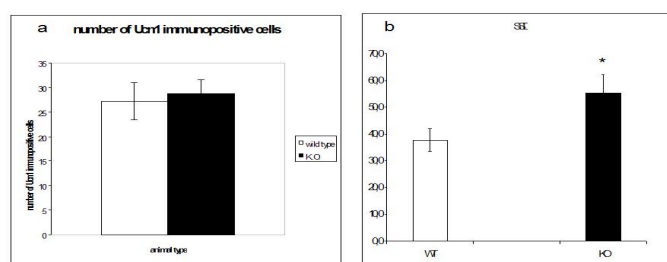
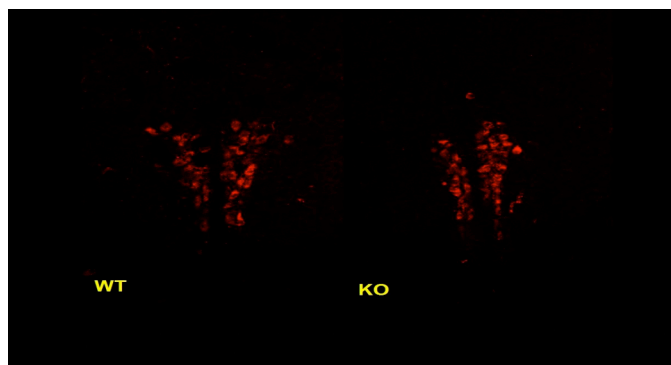
#### *Statistical analysis*

All statistical analysis was done in Excel (Microsoft Corporation, USA) and SPSS 15.0 for Windows (SPSS Inc. USA) by the use of student's t-test. The accepted level of significance was set at  $p < 0.05$ . All data are presented as mean  $\pm$  standard error of mean.

## Results

### *PKC-epsilon mice*

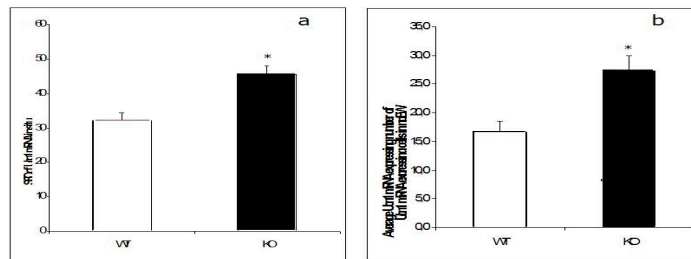
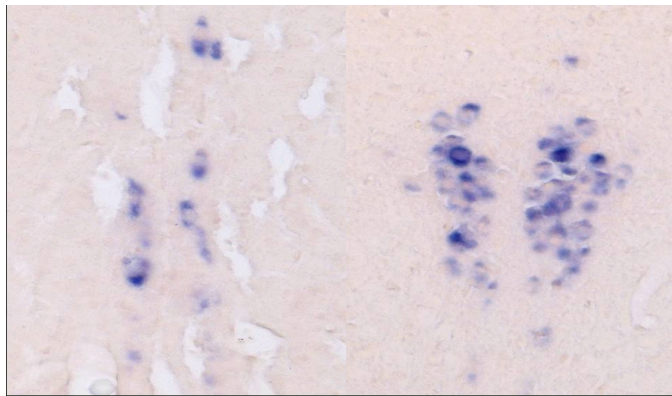
The levels of Ucn1 in terms of number of Ucn1 expressing cells and optical density of the npEW nucleus cells were compared between PKC-epsilon null mice and their wild-type littermates. The number of Ucn1 expressing cells in the npEW was not significantly different [t (7)  $p > 0.05$ ] between PKC-epsilon null mice [ $28.7 \pm 2.9$ ] and their wild-type littermates [ $27.1 \pm 3.9$ ]. However there was a significant difference [t (7)  $p < 0.05$ ] in optical density; which is a measure of protein abundance, of Ucn1 expressing cells. Namely, in PKC-epsilon null mice Ucn1 cells showed a higher labeling intensity [ $51 \pm 2.8$ ] compared to their wild type littermates [ $38 \pm 2.7$ ].



**Fig. 1.** Representative immunostaining in PKC-epsilon null and wild type mice. a. Non-significant difference between PKC-epsilon null mice and wild type littermates, in terms of number of Ucn1 immunopositive cells. b. Higher signal specific density (SSD) of Ucn1 protein in Ucn1 immunopositive cells of PKC-epsilon null mice. All data are presented as mean  $\pm$  S.E.M.

Next we investigated the levels of Ucn1 mRNA expressing cells in the npEW with non-radioactive *in situ* hybridization technique. The results revealed a significant

difference [t(7)  $p < 0.05$ ] between PKC-epsilon null [ $27.3 \pm 2.6$ ] and their wild type littermates, [ $16.6 \pm 1.9$ ] in terms of the number of Ucn1 m RNA expressing cells. Furthermore, in PKC-epsilon null mice [ $45.5 \pm 2.6$ ] cells showed a significant higher [t(7),  $p < 0.05$ ] optical density labeling per cell compared to their wild type littermates [ $32 \pm 2.2$ ]. Similarly, the comparison of the total optical density of the npEW nucleus revealed a higher intensity labeling for the PKC-epsilon null [ $11.3 \pm 1.15$ ] compared to wild type mice [ $3.9 \pm 1.45$ ], [t(7),  $p < 0.05$ ].

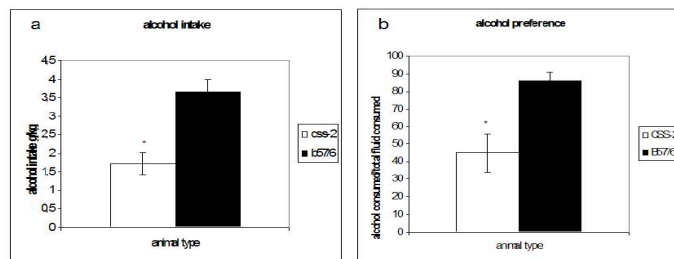


**Fig. 2** Representative labeling of Ucn1 m RNA in the npEW nucleus of PKC-epsilon null (right) and wild type (left). **a.** Significantly more Ucn1 m RNA expressing cells in npEW of PKC-epsilon null mice. Higher SSD of Ucn1 m RNA in npEW nucleus of PKC-epsilon null mice. All data are presented as mean  $\pm$  S.E.M.

#### *CSS-2 vs C57BL/6J*

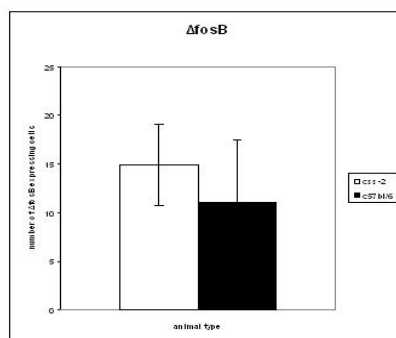
Behavioral results revealed a significant reduction in alcohol consumption for CSS-2 [ $1.72 \pm 0.30$ ; t(10),  $p < 0.05$ ] compared to C57BL/6J controls [ $3.67 \pm 0.31$ ]. Similarly, the CSS-2 mice exhibited a reduced preference for alcohol, measured as the

ratio of alcohol consumed/total fluid consumed [ $44.97 \pm 10.60$ ;  $t(10), p < 0.05$ ], compared to their C57BL/6J littermates [ $86.08 \pm 4.62$ ].



**Fig. 3. Behavioral results showing the significant reduced consumption (a) and preference (b) for alcohol of CSS-2 mice compared to C57BL/6J controls. Apparently, the transfer of chromosome-2 from a low-drinking strain to a high-drinking strain had a significant effect on the alcohol consumption and preference of the high-drinking strain (C57BL/6J). All data are presented as mean  $\pm$  S.E.M.**

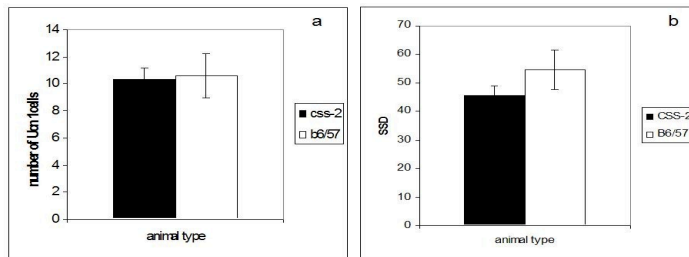
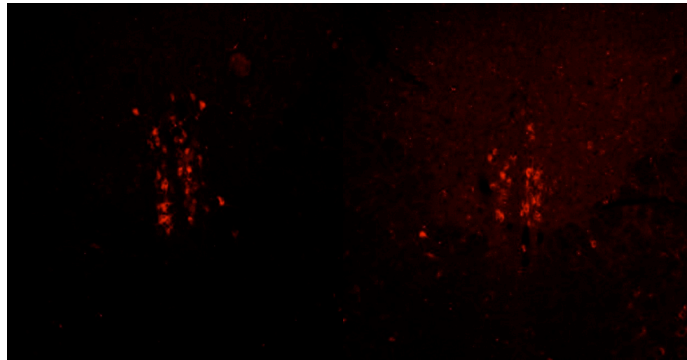
$\Delta$ FosB an activity-dependent inducible transcription factor immunocytochemistry was performed to determine the levels of chronic alcohol activation of Ucn1 cells. The statistical analysis revealed similar chronic activation levels for the two groups [CSS-2,  $14.95 \pm 4.15$ ; C57BL/6J,  $11.03 \pm 6.53$ ;  $t(4), p > 0.05$ ]



**Fig. 4. Results show similar levels of chronic activation by alcohol in CSS-2 and C57BL/6J controls, as revealed by  $\Delta$ fosB immunoreactivity. All data are presented as mean  $\pm$  S.E.M.**

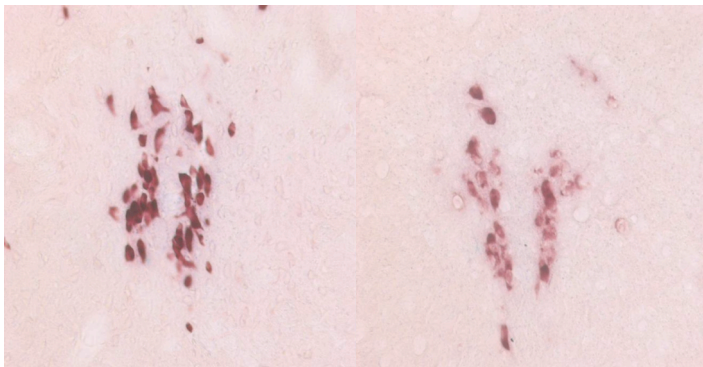
Fluorescence immunocytochemical labeling of Ucn1 expressing cells analysis revealed a non-significant difference between the two groups [CSS-2,  $10.3 \pm 0.87$ ; C57BL/6J,  $10.6 \pm 1.59$ ;  $t(4), p > 0.05$ ]. Similarly, optical density per cell analysis

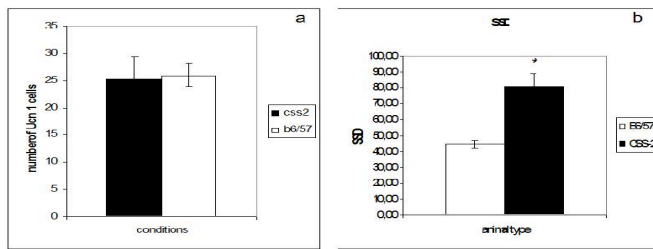
revealed a non-significant difference between the groups [CSS-2,  $45.57 \pm 3.29$ ; C57BL/6J,  $54.58 \pm 6.83$ ;  $t(4), p > 0.05$ ].



**Fig. 5. Representative immunostaining in CSS-2 (left) and C57BL/6J controls (right). There were no significant differences in terms of number of Ucn1 immunopositive cells (a) and Ucn1 protein abundance in these cells (b). All data are presented as mean  $\pm$  S.E.M.**

Non-radioactive in situ hybridization analysis of Ucn1 mRNA levels between the two groups revealed a non-significant difference in terms of number of cells [CSS-2,  $25.26 \pm 4.12$ ; C57BL/6J,  $25.9 \pm 2.21$ ;  $t(4), p > 0.05$ ]. But there was a significant difference when the two groups were compared in terms of optical density





**Fig.6. Representative labeling of Ucn1 m RNA in npEW nucleus of CSS-2 (left) and C57BL/6J (right). a. Similar levels of Ucn1 m RNA expressing cells in npEW nucleus of CSS-2 and C57BL/6J controls. b. Higher SSD of Ucn1 m RNA in npEW nucleus of CSS-2 mice. All data are presented as mean  $\pm$  S.E.M.**

per cell. Namely, the CSS-2 mice cells exhibited a higher optical density per cell compared to C57BL/6J mice [CSS-2,  $80.54 \pm 8.23$ ; C57BL/6J,  $44.62 \pm 2.40$ ;  $t(4), p < 0.05$ ].

## Discussion

In the present study we investigated the involvement of Ucn1 neuropeptide as a potential explanatory mechanism for the behavior of two different mouse models of alcohol consumption. We choose the PKC-epsilon null and chromosome substitution strain (CSS-2) animal models. Both models have shown a significant reduction in alcohol consumption in different types of alcohol drinking paradigms (Hodge et al. 1999).

The majority of previous studies on Ucn1 involvement in alcohol consumption have reported higher Ucn1 levels in npEW nucleus of mice bred for high alcohol consumption (Bachtell et al., 2002b; Weitemier and Ryabinin, 2005). One of the major caveats of those studies is that their inferences are based solely on immunocytochemical analyses. Immunocytochemistry gives an indication of the amount of a protein present in a cell at a particular moment; however conclusions regarding protein secretion and/or production would be anecdotal in this case. In order

to overcome this problem we used *in situ* hybridization; in addition to immunocytochemistry; which allowed us to have a more complete view of cell's activity.

Our measurements revealed differential secretory activity in both types of animal models. In particular, PKC-epsilon null mice showed increased levels of Ucn1 neuropeptide compared to their wild-type littermates, by means of immunocytochemistry; which measures Ucn1 content of immunopositive neurons, and *in situ* hybridization; which shows the mRNA levels of Ucn1 producing neurons. Consequently, we can be confident that indeed Ucn1 levels are differentially regulated in the two groups.

The epsilon form of PKC isozymes has been repeatedly shown to inhibit GABA signaling via phosphorylation of specific subunits (Hodge et al. 1999; Qi et al. 2007). Therefore, the absence of PKC-epsilon in the PKC-epsilon null mice results in an oversensitive GABA system which acts to reduce alcohol consumption by increasing the sensitivity of animals to the aversive effects of alcohol. The sensitivity to a single exposure to alcohol is highly correlated with the predisposition for high alcohol consumption (Newlin and, Thomson, 1990; Schuckit, 1994). Furthermore, it was shown that Ucn1 enhances the release of GABA in rat amygdalar slices after electrical stimulation (Bagosi et al. 2008). Consequently, the increased level of Ucn1 in PKC-epsilon null mice raises the intriguing possibility that Ucn1 further facilitates GABA signaling, which subsequently reduce alcohol consumption in these mice.

Another explanation would implicate the role of lateral septum in these processes. Lateral septum is one of the major projection sites of npEW Ucn1 neurons (Bittencourt et al. 1999; Ryabinin, Weitemier, 2006). Previous studies have reported that lesions of the lateral septum increases water and food consumption (Iovino,

Steardo, 1985; Oliveira et al., 1990), presumably suggesting an inhibitory effect of lateral septum in consumatory- related behaviors. Moreover, it is now established that lateral septum neurons are mainly GABAergic and possibly project to the hypothalamus, having an inhibitory effect on it. Furthermore, Ucn1 injections into the lateral septum suppress alcohol but not water consumption (Ryabinin et al. 2008). Therefore, in accordance with the previous findings, the increased Ucn1 levels in the npEW nucleus in our study may mean increased Ucn1 release into lateral septum; there the increased levels of Ucn1 would further enhance GABA signaling which subsequently will cause a stronger inhibition of hypothalamic centers regulating consumption of alcohol. In addition, lateral septum is rich in CRF2 receptors (Skelton et al. 2000). CRF2 receptors have been shown to mediate the anxiolytic effects that oppose the anxiogenic effects of CRF1 receptors (Kishimoto et al. 2000). Furthermore, Ucn1 shows a much higher affinity for the CRF2 receptors compared to CRF1 and is more potent activator of both receptors types than CRF itself (Skelton et al. 2000). Therefore, in accordance with these findings our results partially explain the fact that PKC-epsilon null mice in addition to their reduced consumption and preference for alcohol exhibit a reduced anxiety-like behavior (Hodge et al. 2002). In particular, the increased Ucn1 expression and content of npEW neurons drive the increased release of Ucn1 into the lateral septum; subsequently Ucn1 binds to the preferred CRF2 receptors, thus resulting in the observed reduced anxiety-like behavior.

In CSS-2 mice the results were not clear. We compared Ucn1 producing neurons activation levels between CSS-2 and C57BL/6J control in a chronic alcohol consumption paradigm. The number of positive cells for  $\Delta$ fosB activity-inducible transcription factor revealed similar activation levels by alcohol in both groups. We did not find any significant difference in terms of Ucn1 content of immunopositive



neurons. Subsequently, in situ hybridization revealed similar levels of m RNA expression in both groups. The small number of mice (3) in each group may have masked any significant difference between the two groups. However, when the specific optical densities per cell were compared; CSS-2 mice showed an increased m RNA expression levels.

In chromosome substitution strains the insertion of a single chromosome from a different strain may affect a number of physiological systems which are difficult to control and delineate their specific actions. On the other hand, the enhanced signal specific density of Ucn1 m RNA expressing cells in CSS-2 mice raises the possibility that alcohol differentially affect the gene expression patterns in these cells. This is supported by the fact that we found a similar level of chronic activation by chronic alcohol administration in the two groups, as revealed by  $\Delta$ fosB measurements.

Our results reveal an altered secretory activity of Ucn1 containing cells in the npEW nucleus of PKC-epsilon null and partially of CSS-2 mice. This suggests that these neurons play a role in the modulation of alcohol preference and consumption, via GABA-signaling and by acting on the lateral septum.

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