ΔFosB in the supraoptic nucleus contributes to hyponatremia in rats with cirrhosis

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Abstract

Bile duct ligation (BDL), a model of hepatic cirrhosis, is associated with dilutional hyponatremia and inappropriate vasopressin release. ΔFosB staining was significantly increased in vasopressin and oxytocin magnocellular neurosecretory cells in the supraoptic nucleus (SON) of BDL rats. We tested the role of SON ΔFosB in fluid retention following BDL by injecting the SON (n = 10) with 400 nl of an adeno-associated virus (AAV) vector expressing ΔJunD (a dominant negative construct for ΔFosB) plus GFP (AAV-GFP-ΔJunD). Controls were either uninjected or injected with an AAV vector expressing only GFP. Three weeks after BDL or sham ligation surgery, rats were individually housed in metabolism cages for 1 week. Average daily water intake was significantly elevated in all BDL rats compared to sham ligated controls. Average daily urine output was significantly greater in AAV-GFP-ΔJunD-treated BDL rats as compared to all other groups. Daily average urine sodium concentration was significantly lower in AAV-GFP-ΔJunD-treated BDL rats than the other groups, although average daily Na excretion was not different among the groups. SON expression of ΔJunD produced a diuresis in BDL rats that may be related to decreased circulating levels of vasopressin or oxytocin. These findings support the view that ΔFosB expression in SON magnocellular secretory cells contribute to dilutional hyponatremia in BDL rats.
Introduction

Hyponatremia is the most frequently occurring clinical electrolyte disorder and the cost of treating patients with hyponatremia is estimated to be 1.6 to 3.6 billion dollars per year (3). It also is associated with negative outcomes in many chronic disease states such as congestive heart failure and hepatic cirrhosis (3, 9, 14, 43, 47). In cirrhosis, inappropriate release of vasopressin contributes to the accumulation of fluid in the abdominal cavity, or ascites, and dilutional hyponatremia (9, 22, 47, 50).

The concentration of circulating vasopressin is primarily determined by the activity of magnocellular neurosecretory cells located in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus that project to the posterior pituitary. The activity of these cells is regulated by plasma osmolality, blood volume, and blood pressure (1, 5, 17, 20, 23). The increases in circulating vasopressin that occur during cirrhosis are reported to be the result of a functional decrease in effective plasma volume and systemic hypotension (9, 10, 37, 47, 50). This reduced effective circulating volume is thought to result in non-osmotically mediated vasopressin release (9, 10, 37, 47, 50). The CNS mechanisms that stimulate and maintain vasopressin release during this disorder are not completely understood.

Synaptic activation of vasopressin neurones in the hypothalamus is associated with increased expression of Fos and Fos-related proteins. Fos, the protein product of c-fos, and ΔFosB, a splice product of fosB, are both members of the AP-1 family of transcription factors (17, 18, 25, 40, 42). The expression of Fos has been used to map regions of the CNS that are activated following acute physiological and supraphysiological stimulation (17-19, 25). ΔFosB has a much longer half-life than Fos,
resulting in its accumulation with chronic or intermittent stimulation of the CNS (17, 25, 42) and has been linked to changes in neuronal morphology, receptor expression, and cell signalling related to depression, drug addiction and other motivated behaviours (24, 38, 42, 45, 54). AP-1 transcriptional activity has also been shown to play a critical role in the adaptive control of sympathetic outflow during dehydration (16) indicating that ΔFosB and other AP-1 proteins may play similar roles in neural networks that regulate vegetative function.

Therefore, increased expression of ΔFosB in the SON of cirrhotic rats could mediate cellular adaptations which contribute to increased vasopressin release. This hypothesis was tested by using a dominant negative construct cloned into a neurotropic AAV vector (52, 54) to inhibit the transcriptional effects of ΔFosB in SON of rats subjected to chronic bile duct ligation (BDL) and measuring resulting changes in fluid balance.
Methods

Animals. Adult male Sprague-Dawley rats (Charles River, 250-350g) were individually housed and maintained on a 12/12 h light cycle and provided with ad libitum access to food and water. Rats were provided with food containing 0.4% NaCl. All procedures using animals were conducted according to NIH guidelines, and were reviewed and approved by the Institutional Animal Care and Use Committees at UTHSCSA and UNTHSC.

Bile duct ligation surgery. Each rat was anesthetized with isoflurane (2-3%) and its abdomen was shaved and cleaned. A midline abdominal incision was performed and the common bile duct was isolated and cut between two ligatures. Sham ligated rats received the same surgical procedure except the bile duct was not ligated or cut. Each animal was returned to its cage and monitored for 1 week of recovery. Liver/body weight ratio was determined for verification of cirrhosis. Visual inspection of the abdomen to evaluate ascitic fluid in the peritoneal cavity was performed daily after surgery.

ΔFosB Immunohistochemistry. Separate groups of sham ligated (n = 6) and BDL rats (n = 6) were used in immunohistochemistry studies to determine the effects of BDL on ΔFosB staining in the SON. Four weeks after BDL or sham ligation surgery, rats were anesthetized with thiobutabarbitral (Inactin, 100 mg/kg ip) and perfused transcardially with 50-100 ml of PBS followed by 300-400 ml of 4% paraformaldehyde in PBS for immunohistochemistry as previously described (29). The descending aorta and vena cava were clamped below the heart with haemostats and each liver was removed and weighed during the perfusion. Forty µm coronal sections containing the SON were processed for FosB (goat anti-FosB(102), sc-48-G Santa Cruz Biotechnology, Santa Cruz, CA; 1:5000) and vasopressin (polyclonal guinea pig anti-(Arg8)-Vasopressin,
Peninsula Laboratories, San Carlos, CA; 1:5000) immunohistochemistry. The primary antibody used in this study does not discriminate between ΔFosB and full length FosB, however, it will be referred to as ΔFosB staining in the text since ΔFosB is the only species expressed outside of acute stimulation conditions. After incubation in the anti-FosB antibody for 72 h at 4°C, the sections were rinsed and incubated with biotin-conjugated horse anti-goat IgG (1:200; Vector Laboratories, Berlingame, CA), an avidin-peroxidase conjugate (Vectastain ABC Kit; Vector Laboratories), and PBS containing 0.04% 3,3′-diaminobenzidine hydrochloride and 0.04% nickel ammonium sulphate as previously described (29). Next, the sections were incubated with the vasopressin antibody for 72 h at 4°C followed by rinsing and incubation with a Cy3-conjugated Affinipure Donkey Anti-Guinea Pig IgG (1:250; Jackson ImmunoResearch, West Grove, PA).

Alternate sets of forebrain sections were processed for oxytocin and ΔFosB immunohistochemistry. The sections were first stained for ΔFosB as described above. After rinsing, the sections were incubated with a mouse anti-oxytocin antibody (courtesy of A.J. Silverman) using a dilution of 1:1000 for 5 days at 4°C. After 60 min of rinsing, the sections were incubated with a Cy3-conjugated Donkey Anti-Mouse IgG (1:250; Jackson ImmunoResearch, West Grove, PA) for 3 h at room temperature.

Confocal Imaging: An Olympus IX-2 DSU confocal microscope equipped for epifluorescence and appropriate excitation/emission filter sets was used for imaging ΔFosB and AVP or OXY positive cells in the SON. Images were captured using a Retiga-SRV camera (Q-imaging, Surrey, British Columbia, Canada). Brain areas were
identified using Paxinos and Watson (44). Sections containing the SON located -0.80 mm to -1.80 mm posterior to bregma were analysed.

**Laser Capture Microdissection:** Separate groups of animals were used for the laser capture microdissection protocol. Four weeks after BDL (n = 8) or sham ligation surgery (n = 6), each rat was anesthetized with Inactin (100 mg/kg ip) and quickly decapitated. The brains were removed and immediately snap frozen in cooled isopentane. Ten µm serial coronal sections containing the SON were cut using a cryostat (CM1950 Leica Microsystems, Buffalo Grove, IL). These sections were mounted onto PEN membrane coated slides (LCM0522- Arcturus Bioscience, Mountain View, CA) with 2-3 forebrain sections on the membrane part of a slide. These slides were then stored in -80°C until further processing.

Each slide was processed for vasopressin immunohistochemistry following fixation in ice cold 100% methanol as previously described (12). All reagents were prepared with DEPC-water and contained RNAse inhibitor. Higher concentrations of the guinea pig anti-AVP primary antibody (1:50) and Cy3-conjugated donkey anti-guinea pig secondary antibody (1:50) were used for 3 min incubations at room temperature. An Arcturus Veritas Microdissection instrument, equipped with IR capture and UV cutting lasers, was used to laser capture the AVP labelled neurones from the SON. At least 7-10 vasopressin-positive SON neurones were collected from each rat for RNA extraction and amplification. RNA was extracted and purified from each sample using 30µl of ArrayPure Nano-Scale Lysis Solution with 5.0 µg of proteinase K and ArrayPure Nano-Scale RNA Purification Kit reagents (Epicentre Biotechnology, Madison, WI). The quality of each RNA sample was evaluated using a Nanodrop Spectrophotometer to
measure RNA content and identify contamination. Samples yielding less than 20 ng/µl RNA and/or a 260/280 ratio lower than 1.8 were not used for amplification. A 1–2 µl aliquot of cellular RNA from each sample was amplified with TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit materials (Epicentre Biotechnology), in accordance with the manufacturer’s instructions.

**qRT-PCR:** Less than 50 ng of the synthesized aminoallyl-aRNA from each sample was reverse-transcribed to cDNA with Sensiscript RT Kit reagents (prod. no.205213; Qiagen Inc., Valencia, CA, USA) as previously described (12). Forward and reverse primers for target genes (Table 1) were obtained from Integrated DNA Technologies (Coralville, IA). For polymerase chain reaction, samples consisted of 2µl of cDNA, 8.3µl of RNase / DNase-free water, 2µl of each primer, and 12.5µl of iQ SYBR Green Supermix (prod. no. 170-8880; Bio-Rad). PCR reactions were performed in a Bio-Rad iQTM5 iCycler system, with the following cyclic parameters: initial denaturation at 95 ºC for 3 min, followed by 50 cycles of 1.1 min each (40 s at 94ºC, followed by 30 s at 60 ºC for ΔFosB and AVPs and 30s at 95 ºC followed by 1mt at 65 ºC for GAPDH). No-template and -RT controls were performed for each analysis. For AVP hnRNA, an additional reaction was also performed in the absence of reverse transcriptase to account for DNA contamination in the sample. The housekeeping gene, GAPDH, was used for normalization of mRNA expression. Melt curves generated were analysed to identify nonspecific products and primer-dimers. The data were analysed by the $2^{-\Delta\Delta CT}$ method (36, 46).

**Stereotaxic surgery.** Rats received bilateral stereotaxic injections of AAV-GFP or AAV-GFP-ΔJunD in the SON. Each rat was anesthetized with isoflurane (2%) and
placed in a stereotaxic apparatus equipped with an isoflurane delivery system that was used to maintain anaesthesia during surgery. The injections were made using a 30 gauge injector that was connected to a 5 µl Hamilton syringe with calibrated polyethylene tubing. The viral vectors were back loaded into the injection system that had been previously filled with distilled water and was separated from the distilled water by a 0.2-0.5 µl bubble. Movement of the bubble in the calibrated tubing was used to verify the injection volume. Stereotaxic coordinates for the injections were 1.3 mm posterior; 2.2 mm lateral and 9.6 mm ventral at 8° from vertical based in the atlas of Paxinos and Watson (44). Each SON was injected with 400 nl over a 10 minute period and the injector was left in place for an additional 5 min. The AAV vectors (AAV2) and ΔFosB constructs were generated as previously described (27, 52).

Experimental Protocol. There were six separate groups used for metabolism cage studies: uninjected sham ligated (n = 10), uninjected BDL (n = 7), AAV-GFP sham ligated (n = 7); AAV-GFP BDL (n = 8); AAV-GFP-ΔJunD sham (n = 7), and AAV-GFP-ΔJunD BDL (n = 10). Stereotaxic surgery was performed one week prior to sham ligation or BDL. Two weeks after BDL or sham ligation surgery, the rats were moved into commercially available metabolism cages (Lab Products Inc., Seaford, DE). After one week of habituation, water intake and urine output was measured once per day for seven days. Water intake was measured using calibrated water bottles (Lab Products Inc., Seaford, DE). Urine was collected into 50 ml centrifuge tubes containing 2-3 ml of mineral oil. After the volume was measured, the urine samples were centrifuged and a 1-2 ml aliquot was reserved for determining urine sodium concentrations and daily sodium excretions using a flame photometer (PFP7 flame photometer, Jenway,
Burlington, NJ). At the end of the experiment, each rat was anesthetized with Inactin (100 mg/kg ip) and perfused for immunohistochemistry. A 2-3 ml blood sample was taken by cardiac puncture before each perfusion for measuring plasma osmolality, haematocrit, and plasma protein. Plasma osmolality was measured on a vapour pressure osmometer (Wescor Inc., Logan, UT). Two heparinized capillary tubes (Fisher) were filled for measuring haematocrit (Micro-Hematocrit capillary tube reader, Lancer, St. Louis, MO) and plasma protein by refractometry (National Protometer, National Instruments, Baltimore, MD). Each rat’s liver was removed and weighed as described above. The brain from each rat was processed for vasopressin immunohistochemistry using a Cy3-conjugated secondary antibody as described above. Sections containing the SON were analysed for GFP and vasopressin staining using epifluorescence microscopy to determine location of the injection sites and transfection of vasopressin SON neurones.

**Statistical Analysis:** All results are presented as means ± SEM. Data were analysed by one-factor or two-factor ANOVA and Student-Newman-Keuls post hoc analysis (SigmaPlot v. 12, Systat Software Inc., San Jose, CA). P < 0.05 was considered statistically significant.
Results

Effects of BDL on ΔFosB in the SON

BDL was associated with increased ΔFosB staining in the SON and colocalization of ΔFosB with vasopressin (Figure 1A-F). The numbers of ΔFosB positive cells in SON was significantly increased 4 weeks after BDL as compared to sham ligated controls (Figure 1G). We also observed increases in the numbers of vasopressin positive cells as well as the numbers of ΔFosB and vasopressin labelled cells in the SON of BDL rats (Figure 1G). In BDL rats, 59% of the ΔFosB positive cells also were vasopressinergic as compared to 14% in sham ligated controls. ΔFosB gene expression was significantly increased in vasopressin neurones of the SON from BDL rats as was hnRNA for vasopressin (Figure 1H).

The results obtained from sections stained for ΔFosB and oxytocin are shown in Figure 2. Although we did not observe an increase in the numbers of oxytocin positive cells associated with BDL, the numbers of ΔFosB positive and ΔFosB + oxytocin labelled neurons were significantly increased in the SON of BDL rats (Figure 2). In BDL rats, 43% of the ΔFosB positive cells also were stained for oxytocin while 17% of ΔFosB positive cells were also oxytocinergic in sham ligated controls.

Effects of SON ΔFosB inhibition

Injections of the AAV constructs produced GFP labelling in the SON in both vasopressin positive and non-vasopressin labelled magnocellular cells (Figure 3). GFP positive cells were also observed in the surrounding perinuclear zone (Figure 3) and along the cannula tracks. GFP positive cells were not typically observed intermingled with vasopressin positive dendrites in the ventral glial lamina (Figure 3). All rats included in the study contained bilateral GFP labelling in the SON. Three rats were excluded
from the study due to injection sites that were too dorsal to produce GFP labelling in the SON.

BDL was associated with significant and comparable increases in liver weight to body weight ratio in all three BDL groups compared to sham (Table 2). Uninjected and AAV-GFP treated BDL rats had plasma osmolalities and haematocrits that were significantly lower than sham ligated groups. BDL rats injected in the SON with AAV-GFP-ΔJunD had plasma osmolalities that were significantly higher than the other two BDL groups and were not different from the sham ligated groups (Table 2). Similar results were obtained for haematocrit (Table 2). There were no significant differences observed among any of the groups for plasma protein measurements.

BDL ligated rats drank significantly more water per day as compared to sham ligated control (Figure 4). This increase in water intake was not affected by SON injections with either the vectors in either sham ligated controls or BDL rats (Figure 4). The average daily urine volume of uninjected BDL rats and BDL rats injected with AAV-GFP was not different from sham ligated controls (Figure 4). However, the urine volume of BDL rats injected with AAV-GFP-ΔJunD was significantly greater than the other groups (Figure 4). In addition, the average daily urine sodium concentrations of BDL rats injected with AAV-GFP-ΔJunD were significantly lower than all other treatment groups but there were no differences in average daily sodium excretion among the treatment groups (Figure 5). Although there was a trend for decreased food intake in BDL rats injected with AAV-GFP, food intake was not significantly different among the groups (Sham (n = 5) 25.6 ±0.6 g; Sham + AAV-GFP (n = 7) 23.0 ± 1.4; Sham + AAV-GFP-ΔJunD (n = 7) 21.0 ±
0.9; BDL (n = 6) 25.1 ± 1.6; BDL + AAV-GFP (n = 8) 18.1 ± 2; BDL + AAV-GFP-ΔJunD (n = 7) 23.2 ± 2.7; P > 0.05).
Discussion

Rats with BDL demonstrated increased ΔFosB staining and gene expression in vasopressin neurones of the SON and increased expression of vasopressin hnRNA. BDL also increased ΔFosB staining in oxytocin SON neurones although we did not observe an increase in the numbers of oxytocin positive cells. Based on these observations, we employed an AAV vector that produced a dominant negative construct against ΔFosB, ΔJunD, in order to determine if increased expression of ΔFosB in the SON contributes to the changes in body fluid balance associated with BDL. Previous studies using this vector have shown that its effects are primarily neurotropic and that it produces stable overexpression of ΔJunD or other constructs for up to 10 weeks (27, 51, 52, 54). Overexpression of ΔJunD has been shown to block ΔFosB-dependent behavioral adaptations associated with cocaine administration in the orbitofrontal cortex (52) and an animal model of posttraumatic stress disease in the nucleus accumbens (51). Dominant negative inhibition of ΔFosB in the NTS prevents changes in the network control of sympathetic nerve activity that occur during dehydration (16). Therefore, we used the same approach to test the hypothesis that ΔFosB may have a similar role in activity dependent changes of magnocellular neurosecretory cells of the SON following BDL.

Rats subjected to BDL and injected in the SON with AAV-GFP-ΔJunD did release a significantly larger average daily volume of urine as compared to uninjected and AAV-GFP treated BDL rats. Plasma osmolality and haematocrit were significantly reduced to control levels in AAV-GFP-ΔJunD treated BDL rats. Although daily average sodium excretion was not influenced by dominant negative inhibition of SON ΔFosB in BDL rats,
urine sodium concentration was significantly decreased in these rats. This decrease in urine sodium concentration occurred without a significant change in average daily food intake in BDL rats injected in the SON with AAV-GFP-ΔJunD. Injections of the control virus had no effects on these variables in either sham ligated or BDL rats indicating that these effects were specific to the vector containing the dominant negative construct. Also, inhibition of SON ΔFosB did not affect urine volume or urine sodium concentration in sham ligated control rats. This is consistent with the lack of translational activation of SON neurones in sham ligated controls as compared to the significant increases in ΔFosB immunohistochemistry and gene expression observed in the SON of BDL.

BDL rats drank significantly more water despite lower than normal plasma osmolalities as previously reported (11). Previous studies using a different strain of rats have reported an increase in sodium appetite in BDL rats with no change in water intake and that the increase in sodium intake is independent of the renin-angiotensin system (21, 34). This suggested that other non-osmotic mechanisms may contribute to increased fluid intake in BDL rats. The increase in water intake that was observed in the present study was not affected by dominant negative inhibition of ΔFosB in the SON. This result is consistent with the role of the SON in neurohypophysial function and suggests that SON ΔFosB does not contribute to changes in fluid intake associated with BDL. The mechanism responsible for elevated water intake in BDL rats remains to be determined.

These results suggest that inhibition of SON ΔFosB prevented or delayed the progression of dilutional hyponatremia in BDL rats presumably due to blocking changes in gene expression in magnocellular neurosecretory neurones in the SON. Based on the
changes in urine volume and urine sodium concentration, inhibition of SON ΔFosB likely affected vasopressin magnocellular neurosecretory cells resulting in diuresis due to possible changes in circulating vasopressin.

The AAV vectors also transfected SON oxytocin neurones as well as neurones in the surrounding perinuclear zone of the SON (PNZ). It is possible that ΔFosB inhibition in SON oxytocin magnocellular neurosecretory cells or PNZ contributed to the diuresis observed in BDL injected with the dominant negative construct. The PNZ was initially defined by neuroanatomical studies that demonstrated the existence of a putative set of interneurons dorsal and lateral to the SON (28, 49) although a sequent characterization of these neurones described them as anatomical heterogeneous with extensive axonal collateral projections (2). Electrophysiological studies using coronal slice preparations have provided evidence that the PNZ may be a source of local excitatory and inhibitory synaptic regulation for magnocellular neurosecretory cells in the SON (4). Therefore, it is possible that PNZ ΔFosB inhibition may have influenced the network regulation of magnocellular neurosecretory cells in the SON and contributed to the diuresis observed in BDL rats injected with AAV-GFP-ΔJunD.

Circulating oxytocin can contribute to hyponatremia due to its structural similarity to vasopressin and its ability to stimulate renal vasopressin receptors (15, 35). In a previous study, experimental cirrhosis in the rat was not associated with increased oxytocin gene expression in the hypothalamus, although the study used a different model of cirrhosis (33). We did not observe a significant increase in the numbers of oxytocin positive neurones following BDL in the current study or a previous publication (41). However we did observe a significant increase in ΔFosB and oxytocin double
labelling in the SON of BDL rats. This is consistent with a recent study of hepatic ischemia-reperfusion (7) that did report translational activation of SON oxytocin neurones. Although a majority of the ΔFosB positive SON neurones in BDL rats appear to be vasopressinergic, translational activation also is evident in oxytocin SON neurones. If circulating oxytocin contributes to the development of dilutional hyponatremia in BDL rats, ΔFosB inhibition of SON oxytocin neurosecretory cells may have contributed to both the diuresis and normalization of plasma osmolality following AAV-GFP-ΔJunD SON injections. Since we did not directly measure plasma vasopressin or oxytocin, additional experiments will be required to determine the contribution of vasopressin and oxytocin to the diuretic effects of SON ΔFosB inhibition.

We observed an increase in the numbers of vasopressin positive neurones and an increase in hnRNA for vasopressin in the SON of BDL rats. The role of c-fos and Fos related proteins in the regulation of vasopressin and oxytocin gene expression remains controversial (8). Recent studies have suggested that the rat vasopressin gene promoter contains a functional AP1 regulatory cite (53) and that c-fos gene expression and vasopressin hnRNA expression in both SON and PVN are significantly correlated with changes in plasma osmolality (32). However, direct SON injections of putative neurotransmitter agonists produced differential effects on c-fos mRNA and vasopressin hnRNA expression (31). The failure to observe concurrent increases in c-fos and vasopressin hnRNA expression led the authors to suggest the induction of these genes following acute osmotic stimulation likely involves different mechanisms. Therefore, dominant negative inhibition of ΔFosB in the SON may not have directly affected vasopressin or oxytocin gene expression in BDL rats.
There are many other possible target genes that may have been influenced by dominant negative inhibition of ΔFosB. In the nucleus accumbens, GluR2, NR1, Cdk5, NF-κB, and dynorphin have been identified as putative gene targets of ΔFosB that are related to changes in neural function associated with cocaine or morphine administration or the actions of antidepressants in this regions (39, 51). In SON, ΔFosB could be regulating similar targets that influence the synaptic responsiveness of magnocellular secretory cells following BDL. Dynorphin is an important autocrine factor that regulates phasic activity in vasopressin magnocellular neurosecretory cells (6, 48). Overexpression of ΔFosB decreases dynorphin expression in the nucleus accumbens (54) and, if ΔFosB similarly regulates dynorphin in the SON, increased ΔFosB expression could have a profound effect on the activity of vasopressin magnocellular neuroendocrine cells and vasopressin release. It is also possible that the downstream targets of ΔFosB are involved in other cellular processes necessary for sustained activation and/or hormone release. It has previously been reported that BDL is associated with significant changes in TRPV2 and TRPV4 expression in SON neurones (11, 41). However, few studies have characterized the transcriptional regulation of these channels (13, 26, 30), and therefore it is not known if their expression is regulated by AP-1 transcription factors.

Perspectives and Significance

These results indicate that ΔFosB and AP-1 transcriptional regulation plays a role in neurohypophysial function in an animal model of dilutional hyponatremia. Understanding the cellular mechanisms underlying this effect and the role of ΔFosB in the normal physiological function of magnocellular neurosecretory cells may provide
new insight to how activity dependent transcription factors contribute to synaptic homeostasis and pathophysiology.

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References


16. **Colombari DSA, Colombari E, Freiria-Oliveira AH, Antunes VR, Yao ST, Hindmarch C, Ferguson AV, Fry M, Murphy D, and Paton JFR.** Switching control of


Figure 1: Representative digital images of ΔFosB/FosB staining (A & B), vasopressin immunofluorescence (C & D), and merged pseudocoloured images (E & F) of the supraoptic nucleus from a bile duct ligated rat (left column) and a sham ligated control (right column). Scale bar is 50 µm for each image. Abbreviations: ot, optic tract; SON, supraoptic nucleus. G: Effects of bile duct ligation on the numbers of cells stained for ΔFosB, vasopressin (AVP) and ΔFosB and vasopressin in the supraoptic nucleus of sham ligated (Sham, n = 6) and bile duct ligated rats (n = 6). H: Effects of bile duct ligation on ΔFosB mRNA (Sham n = 5; BDL n = 8) and vasopressin hnRNA (Sham n = 6; BDL n = 8) expression from 7-10 laser microdissected vasopressin cells from the SON from each rat. The data depict mean ± SEM mRNA levels as calculated by the 2-ΔΔCT method. * is p< 0.05 compared to Sham.

Figure 2: Representative digital images of ΔFosB/FosB staining (A & B), oxytocin immunofluorescence (C & D), and merged pseudocoloured images (E & F) of the supraoptic nucleus from a bile duct ligated rat (left column) and a sham ligated control (right column). Scale bar is 100 µm for each image. G: Effects of bile duct ligation on the numbers of cells stained for ΔFosB, oxytocin (OXY) and ΔFosB and oxytocin in the supraoptic nucleus of sham ligated (Sham, n = 5) and bile duct ligated rats (n = 7). * is P < 0.05 from sham.

Figure 3: Representative example of GFP fluorescence (A), vasopressin immunofluorescence (B), and a merged image (C) of the supraoptic nucleus from a bile duct ligated rat injected with AAV-GFP-ΔJunD. Scale bar is 100 µm for each image.

Figure 4: The effects of bile duct ligation (BDL) and dominant negative inhibition of ΔFosB in the SON on average daily water intake and urine volume in uninjected sham ligated rats (Sham), sham ligated rats injected with the control AAV (Sham + GFP), sham ligated rats injected with the dominant negative construct for ΔFosB (Sham + ΔJunD), uninjected bile duct ligated rats (BDL), and bile duct ligated rats injected the control virus (BDL + GFP) or the dominant negative construct against ΔFosB (BDL + ΔJunD). The number of rats for each group is given in Table 2. ** is P < 0.05 from all sham groups. # is P < 0.05 from all other groups.

Figure 5: Average daily urine sodium concentration (A) and average daily sodium excretion (B) in uninjected sham ligated rats (Sham), sham ligated rats injected with the control AAV (Sham + GFP), sham ligated rats injected with the dominant negative construct for ΔFosB (Sham + ΔJunD), uninjected bile duct ligated rats (BDL), and bile duct ligated rats injected the control virus (BDL + GFP) or the dominant negative construct against ΔFosB (BDL + ΔJunD). # is P < 0.05 from all other groups.

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Table 1: qRT-PCR primer sequences.

AVP hnRNA forward: 5' - GCCCTCACCTCTGCCTGCTA 3'
AVP hnRNA reverse: 5' - CCTGAACGGACCACAGTGGT 3'
Δ FosB forward: 5' - AGGCAGAGCTGGAGTGGAGAT-3'
Δ FosB reverse: 5' - GCCGAGGACTTGAACTTCACTCG-3'
GAPDH forward: 5' - CTCATGACCACAGTCGAGC -3'
GAPDH reverse: 5' - TACATTGGGGGTAGGAACAC -3'
Table 2: Plasma osmolality, haematocrit, plasma protein and liver weight to body weight ratios in uninjected sham ligated rats (Sham), sham ligated rats injected with the control AAV (Sham + GFP), sham ligated rats injected with the dominant negative construct for ΔFosB (Sham + ΔJunD), uninjected bile duct ligated rats (BDL), and bile duct ligated rats injected the control virus (BDL + GFP) or the dominant negative construct against ΔFosB (BDL + ΔJunD).

<table>
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<th></th>
<th>n</th>
<th>Osmolality (mOsm/kg)</th>
<th>Haematocrit (%)</th>
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<td>Sham</td>
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<td>42.7 ± 1.3</td>
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<td>45.8 ± 1.0</td>
<td>6.6 ± 0.2</td>
<td>0.03 ± 0.001</td>
</tr>
<tr>
<td>BDL</td>
<td>7</td>
<td>291.2 ± 1.1*</td>
<td>39.6 ± 1.1*</td>
<td>6.6 ± 0.2</td>
<td>0.08 ± 0.005**</td>
</tr>
<tr>
<td>BDL + GFP</td>
<td>8</td>
<td>288.4 ± 1.9*</td>
<td>37.0 ± 2.1*</td>
<td>6.5 ± 0.1</td>
<td>0.07 ± 0.006**</td>
</tr>
<tr>
<td>BDL + ΔJunD</td>
<td>7</td>
<td>295.9 ± 2.1</td>
<td>41.4 ± 1.6</td>
<td>6.1 ± 0.2</td>
<td>0.07 ± 0.003**</td>
</tr>
</tbody>
</table>

Values are means + SEM. * is P < 0.05 from sham groups and BDL + ΔJunD. ** is P < 0.05 from all sham groups.